ASRM 2014
SCIENTIFIC ABSTRACTS to be presented at the 70th Annual Meeting of the American Society for Reproductive Medicine, October 18-22, 2014, Honolulu, Hawaii.

(e2) ORAL SESSION
(e141) POSTER SESSION
(e357) AUTHOR INDEX
(e376) TOPIC INDEX
(e378) AUTHOR AND SPOUSE/PARTNER DISCLOSURES INDEX

October 18-22, 2014
Honolulu, HI

These abstracts of research studies, printed as submitted by the authors, are presented in the ASRM 2014 meeting sessions and are published in the order of their presentation. Abstracts of plenary lectures, symposia and interactive sessions are not included.

Copyright ©2014 American Society for Reproductive Medicine,
1209 Montgomery Highway, Birmingham, Alabama 35216-2809
The following papers are candidates for the ASRM Scientific Program Prize Paper Awards. Additional candidates will be presented during the Prize Paper Candidates’ Session on Tuesday.

PRIZE PAPER SESSIONS 1

SPECIAL RESEARCH PRESENTATION: DEFINING THE ROLE OF GHRELIN IN WOUND HEALING AND THE INFLAMMATORY RESPONSE IN THE POST-OPERATIVE SETTING. K. Hwang, a E. Bianchi, a M. Sigman, a D. J. Lamb, b K. Boekelheide, a Department of Surgery (Urology), Brown University, Providence, RI; aDepartment of Urology, Baylor College of Medicine, Houston, TX.

OBJECTIVE: Peritoneal adhesion formation is a well-recognized consequence of abdominal and pelvic surgery, causing infertility, chronic pelvic pain and intestinal obstruction. We hypothesized that ghrelin, a 28 amino acid peptide predominantly found in the stomach, can play an important role in reducing adhesion formation in order to establish a novel approach to prevent post-operative surgical adhesions. The purpose of the study is to define the role that ghrelin plays in wound healing in a post-surgical animal model and to determine the molecular mechanism by which ghrelin impacts the inflammatory response and adhesion formation.

DESIGN: Three mouse models were used: C57BL/6, Ghrelin-KO and growth hormone secretagogue receptor-KO (GHSR-KO). The mice were subjected to a median laparotomy in order to establish peritoneal adhesion model and were randomly separated into two groups: treatment group (ghrelin) and control group (saline). Alzet minipumps were implanted in the peritoneal cavity providing a continuous infusion of ghrelin or saline for 14 days post-surgery.

MATERIALS AND METHODS: Peritoneal adhesions were created using an ischmic button model with cecal abrasions and monolateral abdominal cryptorchidism. Peritoneal tissue from ischemic buttons was analyzed for histological appearance by hematoxylin/eosin staining. The tissue was pulled at a rate of 8 mm/min until breakage occurred. Surgically induced undescended right testis was harvested to determine gene/protein expression of ghrelin. The testis was pulled at a rate of 8 mm/min until breakage occurred. Surgically induced undescended right testis was harvested to determine gene/protein expression of ghrelin.

RESULTS: The ghrelin treatment significantly reduced adhesion formation in Wild type and Ghrelin-KO mice. We found a similar post-surgical adhesion score in treated and control GHSR-KO mice. Ghrelin's adhesion reduction seems to be mediated via GHSR receptor. We found a significant difference in expression of reduced inflammatory markers and histological evidence of decreased collagen deposition after ghrelin treatment in Wild type and Ghrelin-KO mice.

CONCLUSION: Our work indicates that ghrelin may improve surgical outcomes by reducing peritoneal adhesion formation and inflammatory response. GHSR receptor shows a significant involvement in the ghrelin’s peritoneal adhesion reduction.

Supported by: ASRM Research Grant and Lifespan Seed Grant Program for Research.

EFFECTS OF PRECONCEPTION INTERVENTION ON THE PCOS PHENOTYPE, OVULATION, AND LIVE BIRTH RATES: A MULTI-CENTER, MULTI-PHASE RCT. R. S. Legro, a W. C. Dodson, a A. R. Kunselman, a P. M. Kris-Etherton, a K. C. Allison, b D. B. Sarwer, a A. Dokras, a C. Coutifaris. aPenn State, Hershey, PA; bUniversity of Pittsburgh, Philadelphia, PA.

OBJECTIVE: To determine the relative efficacy of varying preconception interventions that improved hyperandrogenism or achieved weight loss/fitness or both on the PCOS phenotype and live birth rates(LBR) with clomiphene.

DESIGN: Randomized, 2 site study in overweight/obese infertile women with PCOS(BMI 27-42) of 3 preconception interventions of 16 weeks duration-Phase 1: 1) Lifestyle Modification(LS) consisting of caloric restriction with the use of meal replacements, increased physical activity, and use of a weight loss medication. 2) Continuous oral contraceptive pills(OCP) and 3) Combined OCP/LS(COMB). After Phase1, all subjects received 4 monitored cycles of ovulation induction with clophamide-Phase 2. Pregnancies were followed to delivery with trimester visits-Phase 3. The study was powered for 248 subjects(with 20% dropout) to detect this trend in live birth rates: LS:20% vs OCP:30% vs COMB:43%.

RESULTS: We screened 1241, consented 216, and randomized 149 women(LS = 50, OCP = 49, COMB =50) with a 19% dropout rate through the end of Phase 2. Ovulation rates were OCP:46%, LS:60%, COMB:67%(P < .05) and LBR were OCP:12%, LS:26%, COMB:24%(P<.05 OCP vs LS). The study was stopped after an interim analysis because of a low likelihood of showing a clinically meaningful difference between LS and COMB with continued enrollment.

CONCLUSION: We developed a simple and effective weight loss intervention for obese women with PCOS that erases adverse metabolic OCP effects on the PCOS phenotype, and compared OCP alone is associated with higher ovulation rates and trends towards improved LBR.

Supported by: This project was supported by the Eunice Kennedy Shriver National Institutes of Child Health and Human Development, National Center for Research Resources, and the National Center for Advancing Translational Sciences at the National Institutes of Health, through Grants R01 HD056510, UL1 TR000127 and U54 HD29834 (UVA Core Ligand Assay Core of the Specialized Cooperative Centers Program in Reproduction). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

O-3 Monday, October 20, 2014 11:45 AM

DO COMMON UNDERLYING MECHANISMS MAKE THE OVARY A WINDOW ONTO GENERAL HEALTH RISK FOR DISEASE DEVELOPMENT? M. I. Cedars, a M. Bleil, b C.-N. Kao, a T. Carranza, a E. Epel, b J. Lin, a E. Blackburn, a M. P. Rosen. aDepartment of Obstetrics, Gynecology and Reproductive Sciences, UCSF, San Francisco, CA; bDepartment of Psychiatry, UCSF, San Francisco, CA; cDepartment of Biochemistry, UCSF, San Francisco, CA.

OBJECTIVE: To determine if ovarian aging, assessed by ovarian reserve markers (anti-mullerian hormone-AMH/antral follicle count-AFC), is associated with biomarkers of cellular senescence and increased cardiovascular (CV) risk.

DESIGN: Longitudinal cohort study.

MATERIALS AND METHODS: 1100 healthy, ovulatory women (25-45yo) were identified from a community-based cohort study. A subset (250) has returned for longitudinal follow-up. All subjects had AMH and AFC along with fasting glucose and insulin, fasting lipids, body mass index (BMI), waist circumference, and blood pressure. Framingham score was calculated at the follow-up visit. Telomere length (TL) and mtDNA were used as biomarkers of cellular senescence. Peripheral lymphocytes were isolated and analyzed by PCR to assess for TL, and mtDNA was further assessed by comparing relative copy number. T-tests were used to test if the baseline top and bottom 10th percentiles of age-adjusted AMH/AFC were associated with TL and mtDNA. ANOVA models were used to determine if baseline age-adjusted AFC and AMH were associated with Framingham scores at the follow-up visit.

RESULTS: The upper 10th percentile of AMH/AFC was associated with longer TL (p < 0.05) and lower mtDNA copy number (p = 0.03). The

MATERIALS AND METHODS: After meeting the diagnosis of PCOS, couples were screened to exclude male or tubal factor. Subjects were randomized and underwent baseline testing of psychosocial measures, TV U/S, serum hormones, 75g OGTT, DXA body composition scan and submaximal VO2 exercise testing. These were repeated at the end of the preconception intervention(table below) and the end of the ovulation induction phase. Subjects also underwent monthly visits for weight/compliance monitoring during Phase 1 or follicular monitoring in Phase 2.

Mean Change from Baseline (% CI) in Select Parameters

<table>
<thead>
<tr>
<th></th>
<th>OCP</th>
<th>LS</th>
<th>COMB</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Weight loss</td>
<td>-1.0(-2.2,0.6)</td>
<td>-6.2(-7.4,-5.0)</td>
<td>-6.5(-7.7,-5.2)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>AMH(ng/mL)</td>
<td>-3.2(-4.4,-1.9)</td>
<td>-0.8(-2.1,0.5)</td>
<td>-2.9(-4.2,-1.6)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>AUC Glucose</td>
<td>24(9,39)</td>
<td>-17(-30,-4)</td>
<td>-1(-14,13)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Odds Ratio of New Metabolic Syndrome</td>
<td>2.5(1.5,4.4)</td>
<td>1.2(0.6, 2.3)</td>
<td>0.7(0.4,1.2)</td>
<td>.001</td>
</tr>
</tbody>
</table>

Supported by: ASRM Abstracts

Vol. 102, No. 3, Supplement, September 2014
follow-up (at 3-5 years) Framingham scores for the high, mid and low AFC tertiles were 1.1%, 1.3%, and 2.1%, respectively. For both AFC and AMH, the overall test for significance was <0.01, and pairwise comparisons were significant between the high-low (<0.01) and mid-low (<0.05) AFC tertiles and 0.01, <0.01, 0.04 for low-mid, low-high, mid-high, respectively for AMH.

CONCLUSION: These findings suggest the ovary can be a window to systemic cellular aging and that lower markers of ovarian reserve may be the first objective finding to identify women at increased long-term CV risk. We hypothesize the sensitivity of the ovary to oxidative stress and TL shortening, with the need for functional mtDNA in the oocyte, underlies this process. As women continue to be diagnosed later in the course of their disease and have a worse prognosis then men, earlier identification of women at risk may be an initial step in improving outcome for women with CV disease.

Supported by: NICHD: HD 054956 (MIC, MPR); UCSF Bridge Funding (MIC).

O-4 Monday, October 20, 2014 12:00 PM


OBJECTIVE: Obesity is associated with a pro-inflammatory state and relative hypogonadotropic hypogonadism. Estrogen inhibits production of pro-inflammatory cytokines. We examined gonadotropin sensitivity and circulating cytokines after transdermal estrogen (E2) administration.

DESIGN: Prospective Study.

MATERIALS AND METHODS: 21 regularly menstruating women underwent frequent blood sampling q 10 min for 8 hours, with a 75 ng/kg bolus of GnRH given at 6 hours. Testing was done in the early follicular phase BEFORE and AFTER transdermal E2. At the completion of baseline studies, a 0.1 mg/day transdermal E2 patch was applied starting with day 1 of the subsequent menses. Patches were applied for the entire subsequent menstrual cycle or up to 40 days (if there was no menses). LH and FSH were assayed by immunofluorometric assay (DELFIA). A custom 10 cytokine array kit (Raybiotech) was used [1].

RESULTS: Obese women had significantly higher baseline IL-6, IL-10, TGF-B, and IL-12 compared to normal weight women. After E2, obese but not normal weight women had a significant increase of LH pulse amplitude and FSH response to GnRH.

Inflammatory Cytokines* and Improvement of Gonadotropin Sensitivity in Obesity

<table>
<thead>
<tr>
<th>Normal weight, n=10</th>
<th>Obese, n=11</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs</td>
<td>29.4 (1.9)</td>
<td>32.5 (1.8)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>21.2 (1.5)</td>
<td>36.7 (1.3)</td>
</tr>
<tr>
<td>FSH response to GnRH, % change after E2</td>
<td>-45</td>
<td>+55</td>
</tr>
<tr>
<td>LH pulse amplitude, % change after E2</td>
<td>-15</td>
<td>+29</td>
</tr>
<tr>
<td>IL-6, baseline</td>
<td>34.1 (13.8)</td>
<td>46.3 (10.3)</td>
</tr>
<tr>
<td>IL-10, baseline</td>
<td>30.5 (11.5)</td>
<td>40.2 (6.5)</td>
</tr>
<tr>
<td>TGF-B, baseline</td>
<td>52.3 (14.0)</td>
<td>65.2 (10.0)</td>
</tr>
<tr>
<td>IL-1B, baseline</td>
<td>43.0 (17.3)</td>
<td>55.0 (9.7)</td>
</tr>
<tr>
<td>IL-2, baseline</td>
<td>25.8 (9.6)</td>
<td>32.8 (4.9)</td>
</tr>
</tbody>
</table>

Data as mean (standard deviation) unless otherwise stated. *Measured as intensity per mm²

Most cytokines were significantly reduced after E2 in obese women (~6% for IL-1B, p=0.020, -4% for IL-12, p=0.018), but not in normal weight women.

CONCLUSION: Obesity modulates responsiveness to GnRH in women. Following E2, a significant increase in LH pulses and FSH responsiveness to GnRH was demonstrated in obese women. Improved pro-inflammatory cytokine profiles by E2 administration in obese women were associated with improved gonadotropin sensitivity. Medical and/or nutritional strategies directed at decreasing chronic inflammation may decrease the burden of obesity on fertility.

Supported by: Bayer Drogemann Group in Clinical Research (grant# 7347); U54 HD058155 Center for the Study of Reproductive Biology; UL1 RR025780 (University of Colorado CTRC).

O-5 Monday, October 20, 2014 12:15 PM

WNT AND MTOR PATHWAYS IN THE G-PROTEIN COUPLED RECEPTOR 10 (GPR10) TRANSGENIC MOUSE MODEL OF UTERINE FIBROIDS. F. Kooshesti, M. M. McWilliams, R. A. Wertenberger, V. M. Chennathukuzhi. Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS.

OBJECTIVE: Uterine fibroids (UFs) represent the most common pelvic tumors in women of reproductive age. Despite being benign, these tumors are associated with high morbidity including pelvic pain and pressure, bleeding, and complications in fertility and pregnancy. However, due to our poor understanding of their pathogenesis, available treatment options are limited. In addition, there are currently no relevant animal models for UF which can mimic the human condition. In an effort to address these issues, we generated a transgenic (Tg) mouse model expressing human GPR10, which is not only the most dysregulated GPRs in UF, but also upstream of mTOR as one of the dysregulated genes in UFs. This model was used to investigate the role of two major signaling pathways, mTOR and WNT, in developing cellular characteristics of UF with tumors with altered tissue architecture and cellular proliferation.

DESIGN: Gene expression profiling data available from the GEO database (GSE13319) was used to identify GPR10 as the most dysregulated GPR upstream of mTOR in UF. The cDNA from this gene was used to generate GPR10-Tg mice. Uteri from Tg and wild type (Wt) animals were analyzed for development of UFs, changes in cellular characteristics, tissue architecture, and alterations in components of mTOR and WNT pathways.

MATERIALS AND METHODS: Fixed uterine tissues from Tg and Wt animals were subjected to H&E and immunostaining. RNA and protein were analyzed with qPCR and western blotting, respectively. Primers and antibodies specific to genes and molecules of interest were used for all experiments. T-test and ANOVA was used for statistical analysis with a cutoff level of P<0.05 (n=10).

RESULTS: Histological analysis of mice uteri showed that Tg mice developed UF-like tumors in myometrium with an increased expression of Ki67, COL1A1, COL3A1, ACTA2 and TGFβ3 similar to human fibroids. qPCR analysis showed similar changes at transcript level. Compared to Wt, AKT, RPS6KB1 and mTOR were activated in Tg uteri. While expression of CTNNB1 was found to be cytoplasmic in both Tg and Wt uteri, there was an increase in phosphorylated form of CTNNB1 in Tg uteri with no change in the activation of the receptor LRPS6. Activation of CDC42 and expression of RHO-A were also found to be different between Tg and Wt mice.

CONCLUSION: The GPR10-Tg mice uteri show altered activation of mTOR and WNT pathways leading to increased cell proliferation and altered tissue architecture. This animal model reflects molecular phenotypes of human UF.

Supported by: P20 RR016475, P20 GM103418 and 1R01HD076450-01A1.

O-6 Monday, October 20, 2014 12:30 PM

ONLY THREE Y CHROMOSOME GENES ARE ENOUGH FOR OBTAINING SPERM FUNCTIONAL IN ASSISTED FERTILIZATION AND YIELDING LIVE OFFSPRING IN THE MOUSE. Y. Yamuchi. N. Vernet, J. M. Riel, P. S. Burgoyne, M. A. Ward. Institute for Biogenesis Research, Department of Anatomy, Biochemistry and Physiology, University of Hawaii, John A. Burns School of Medicine, Honolulu, HI; Stem Cell Biology and Developmental Genetics, The National Institute for Medical Research, Mill Hill, London, United Kingdom.

OBJECTIVE: Recently, we have shown that live mice can be obtained from males with the Y chromosome contribution limited to only two genes, the testis determinant factor Sry and the spermatogonial proliferation factor Eif2s3y from males with round spermatid injection (ROSI) is used. ROSI efficiency, measured as proportion of offspring from embryos transferred was <10%. When ROSI was attempted with males, in which Sry was substituted for sex reversal factor Sxr encoding Zfy2/Zf, Sry, H2al2y and Rbny genes, the
efficiency increased 2-fold. Spermatogenesis progression in males carrying Sox9 was more advanced, with clear spermatid elongation. This suggested that a gene(s) encoded within Sox9 provides benefit for spermatogenesis and ROSI success. In this study our objective was to identify the gene responsible. We hypothesized that this gene might be Zfy2, a promoter of which drives the expression of Sox9 encoded Zfy2/1 fusion gene.

**DESIGN:** Research study.

**MATERIALS AND METHODS:** Mice lacking Y chromosome and transgene for Y-derived transgenes, Sry, Eif2s3y, and Zfy2 (‘SEZ’ males), provided testes for the histological analyses and assisted reproduction trials.

**RESULTS:** Spermatogenesis progression in ‘SEZ’ males was more advanced than in males with Sry and Eif2s3y only, with many seminiferous tubules containing elongated and condensed spermatids. Four ‘SEZ’ males provided testicular cells for injection. Three had testicular sperm, which when injected yielded live offspring. The efficiency of intracytoplasmic sperm injection (ICSI) measured as a proportion of offspring from embryos transferred was 23% (18/79), lower than that obtained after ICSI with testicular sperm from wild-type controls (57%, 32/56, P<0.001). The same ‘SEZ’ and control males provided round spermatids for injections. ROSI efficiency was 53% (48/90) for ‘SEZ’ and 23% (18/79) for control males (P<0.01).

**CONCLUSION:** Zfy2 is essential for the formation of mature testicular sperm fundamental in assisted fertilization. In the presence of Zfy2, mice with testis determination driven by Sry and initiation of spermatogenesis warranted by Eif2s3y, are capable of producing sperm, which yield live offspring in ICSI. Therefore, in the mouse, only three Y encoded genes, Sry, Eif2s3y and Zfy2, constitute the minimum Y chromosome complement compatible with successful ICSI.

**Supported by:** NIH HD072380 to MAW.

O-7 Monday, October 20, 2014 12:45 PM

**THE OCCURRENCE OF BIRTH DEFECTS IN RELATION TO ASSISTED REPRODUCTIVE TECHNOLOGIES IN THE MASSACHUSETTS OUTCOMES STUDY OF ASSISTED REPRODUCTIVE TECHNOLOGY DATABASE.** K. D. Getz, a R. F. Liberman, a B. Luke, a J. E. Stern, a E. Declercq, a M. T. Anderka, a Center for Birth Defects Research and Prevention, Massachusetts Department of Public Health, Boston, MA; aDepartment of Obstetrics, Gynecology, and Reproductive Biology, Michigan State University, East Lansing, MI; aDepartment of Obstetrics and Gynecology, Geisel School of Medicine at Dartmouth, Lebanon, NH; aDepartment of Community Health Sciences, Boston University School of Public Health, Boston, MA.

**OBJECTIVE:** To estimate and compare the prevalence of several specific cardiac and non-cardiac birth defects among deliveries to mothers who conceived using assisted reproductive technologies (ART) and those who conceived spontaneously.

**DESIGN:** A longitudinal cohort study using a database of deliveries (live births and stillbirths) to Massachusetts residents during 2004-2008 created via a linkage of data from the Society for Assisted Reproductive Technology (SART) and the Massachusetts Perinatal Quality Collaborative (PQC) reporting system to the Massachusetts Department of Public Health, Boston, MA.

**MATERIALS AND METHODS:** Deliveries were classified as spontaneous or ART cycles (live births and stillbirths) to Massachusetts residents during 2004-2008 created via a linkage of data from the Society for Assisted Reproductive Technology (SART) and the Massachusetts Perinatal Quality Collaborative (PQC) reporting system to the Massachusetts Department of Public Health, Boston, MA.

**RESULTS:** The prevalence of cardiac defects was 82 per 10,000 among ART deliveries compared to 52 per 10,000 among spontaneous deliveries (PR=1.60, 95% CI: 1.30, 1.96). The prevalence of non-cardiac defects was 180 per 10,000 among ART deliveries compared to 130 per 10,000 among spontaneous deliveries (PR=1.33, 95% CI: 1.16, 1.52). Preliminary analyses of specific defects suggested elevated rates of tetralogy of Fallot, hypoplastic left heart syndrome, esophageal atresia, and rectal and large intestinal atresia among ART deliveries. Ongoing analyses will account for potential confounding, and evaluate biologic interaction with maternal age as well as potential mediation of observed associations through an increase in plural birth rates.

**CONCLUSION:** ART use is unlikely to be a major risk factor for birth defects. Preliminary analyses suggest that there may be modest associations between ART use and some birth defects; however, the prevalence of birth defects is low, even among ART-assisted conceptions.

**Supported by:** NIH Grants R01HD064595 and R01HD067270.

---

**REPRODUCTIVE ENDOCRINOLOGY AND INFERTILITY FELLOWS RESEARCH I**

O-8 Monday, October 20, 2014 04:15 PM

**THE EFFECT OF DONOR OOCYTE RECIPIENT OBESITY ON LIVE BIRTH: AN ANALYSIS OF 3,922 SHARED DONOR OOCYTE ASSISTED REPRODUCTIVE TECHNOLOGY (ART) CYCLES.** S. M. Zarek, a E. M. Mitchell, a A. H. DeCherney, b K. S. Richter, c K. Devine, c P. E. Browne, c J. E. O’Brien, b NIH, Bethesda, MD; bShady Grove Fertility, Rockville, MD.

**OBJECTIVE:** Data regarding the effect of recipient obesity on donor oocyte cycle outcome are mixed. Prior analyses have not fully accounted for intrinsic variation between donors. Our objective was to isolate the impact of body mass index (BMI) on live birth in donor egg sharing cycles in which oocytes from the same donor are shared by recipients with differing BMI.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** Live births were analyzed in fresh, shared donor ART cycles from 2004-2012 in which recipients of oocytes from the same donor oocyte retrieval fell into disparate BMI categories. Recipients had normal endometrial cavities by both saline infusion sonography and hysterosalpingogram and all donors were of normal BMI. Analysis was via generalized estimating equations with adjusted standard errors for repeated measures and nesting of recipients with differing BMI receiving oocytes from the same donor retrieval. Recipients were compared using relative risk (RR) and 95% confidence intervals (CIs) adjusted for age of the recipient.

**RESULTS:** There were 3,922 cycles identified with 2,425 (63%) with recipient BMI below 25 kg/m2 (control group), 961 (24%) with recipient BMI between 25-30 kg/m2, 344 (8%) with recipient BMI between 30-35 kg/m2 and 192 (5%) with recipient BMI above 35 kg/m2. Increasing recipient BMI was associated with decreased live birth rate, and the association persisted when adjusted for age of recipient, number of embryos transferred, embryo stage, embryo grade and presence of severe male factor (0.990, 95% CI 0.981-0.998). Live birth was further impacted in the subset of recipients with BMI greater than 35 kg/m2, (RR 0.790, 95% CI 0.626-0.977) compared to normal weight recipients. Significant differences were not appreciated in recipients with BMI between 25-30 kg/m2 (0.958, 95% CI 0.872, 1.053) and in recipients with BMI between 30-35 kg/m2 (0.931, 95% CI 0.807, 1.072).

**CONCLUSION:** To our knowledge, this is the first report of a unique analysis of shared donor oocyte cycles demonstrating that increasing BMI of donor oocyte recipients is negatively associated with live birth. These findings persist after controlling for potential confounders, suggesting diminished uterine receptivity. A clinically significant, 21% reduction in live birth was demonstrated when BMI exceeded 35 kg/m2. This large dataset suggests a benefit to counseling on weight modification in obese recipients, with the greatest potential benefit for recipients with morbid obesity.

**Supported by:** Intramural Research, PRAE.

O-9 Monday, October 20, 2014 04:30 PM

**DIET-INDUCED OBESITY IMPAIRS ENDOCRINAL STEM CELL DECELLULARIZATION.** J. S. Rhee, a J. L. Saben, a V. Klenov, a K. H. Moley. OB/Gyn, Washington University in St. Louis, St Louis, MO.

**OBJECTIVE:** There is strong evidence that decreased pregnancy rates and higher miscarriage rates exist in obese women. Whether these effects are secondary to abnormal implantation, embryo development, or poor oocyte quality are unknown, although all of these factors may contribute. Our study objective was to investigate, via an in vivo and in vitro model, whether exposure to endometrial stromal cells (ESC) to saturated fatty acids or a high fat environment impairs decellularization.

**DESIGN:** To test the effects of diet-induced obesity on ESC decellularization in vivo, artificial deciduomas were induced in high fat-fed versus Chow-fed control mice. In vitro cell culture models were also used to determine the impact of saturated fatty acids on ESC differentiation.

**MATERIALS AND METHODS:** Artificial Deciduomas: Six-week old C57BL/6 female were fed either a high fat diet (59.4% Kcal from fat, HFD) or chow (CON) for twelve weeks. To induce decellularization in vivo, a silk thread was placed in the left uterine horn of pseudopregnant CON (n=5) and HFD (n=6) mice on dpc 3.5. Three days after thread placement, uterine horns were collected, and deciduoma formation was evaluated by comparing the uterine masses and morphologic changes via histology.
In Vitro Decidualization: Human immortalized endometrial stromal cells (hESC-T) and human primary endometrial stromal cells were cultured in L-15 media supplemented with 17-β-estradiol and 0.5mM dibutyryl adenosine cAMP for 9 days to induce decidualization. During this time, cells were also treated with either saturated fatty acids (100μM palmitic acid) or vehicle (BSA). RNA was isolated and RT-qPCR was performed to analyze expression of decidual markers, IGFBP1 and PRL.

RESULTS: HFD mice were significantly heavier, had 100% increase in fat mass, and were glucose intolerant. Furthermore, we observed a 36% decrease in decidua weights and impaired ESC differentiation via histological analysis in the HFD vs. CON mice. In vitro studies also revealed that hESC-T cells differentiated in the presence of saturated fatty acids had significantly lower gene expression of IGFBP-1 and PRL, indicating impaired decidualization. This observation was confirmed in human primary endometrial stromal cells. CONCLUSION: Taken together, these data indicate that a diet high in saturated fatty acids may impair uterine receptivity via altered ESC decidualization. Although the mechanism by which this occurs needs to be further investigated, our preliminary data suggests that poor decidualization may contribute to implantation defects in obesity.

Supported by: T32HD040135-12 (JXK).

O-10 Monday, October 20, 2014 04:45 PM

PREIMPLANTATION GENETIC SCREENING (PGS) OF EMBRYOS PRIOR TO TRANSFER: A COST ANALYSIS OF SINGLE EMBRYO TRANSFERS (SET) AND DOUBLE EMBRYO TRANSFERS (DET).

DESIGN: Cost analysis.

OBJECTIVE: Determine at what cost PGs of embryos prior to embryo transfer is cost-effective on a per live birth basis in DET and SET cycles.

MATERIALS AND METHODS: Fresh and frozen IVF cycle costs were based on published US averages. The cost of a fresh IVF cycle was estimated at $10,500. The cost of a freeze-all cycle was estimated at $14,250 and included the costs of the initial fresh cycle without embryo transfer, freezing of embryos, and subsequent FET. Based on published estimates, the delivery rates of SET of singletons with PGS were 61% and 67%, respectively; the delivery rates of SET of twins and triplets was 34%, 30%, and 1.3%, respectively. The costs of care during pregnancy and through 1 year of life for a singleton, twin, and triplet gestation were $21,458, $104,831, and $407,199, respectively, based on published estimates. Models were constructed for the following situations: fresh DET with PGs vs without PGs, frozen DET with PGs vs frozen DET without PGs, fresh DET with PGs vs without PGs, and DET with PGs vs DET without PGs including birth costs. A sensitivity analysis was performed across a range of costs to account for variations in the cost of procedures.

RESULTS: It was more cost-effective to perform PGS for the fresh DET cycles when PGS cost less than 25% of that of a fresh IVF cycle, which was $2,625. For the fresh DET vs frozen DET with PGS, there was no cost at which PGs was cost-effective, due to the cost of freezing and thawing the embryos. For the fresh DET vs SET with PGs, it was cost-effective to perform PGS when it cost less than $6,900. PGs became more cost-effective in the DET vs SET with PGs when including birth costs when PGS cost less than $27,450 on a per delivery event and $10,450 on a per infant born basis.

CONCLUSION: PGS cycles requiring freezing of all embryos was not found to be cost effective, which highlights the importance of PGS platforms with rapid turnaround times. Fresh transfers with PGs appeared to be cost effective in both SET and DET cycles. When the cost of care of twins and triplets was included in the model, the cost-effectiveness of PGS increased considerably. Published data suggest DET without PGs and SET with PGs have similar live birth rates. PGS is most cost effective when used as a screening tool to promote single embryo transfer.

Supported by: This work was supported, in part, by the Program in Reproductive and Adult Endocrinology, NICHD, NIH, Bethesda, MD.

O-11 Monday, October 20, 2014 05:00 PM

T-CELL INTRACELLULAR ANTIGEN (TIA-1) MODULATES THE EXPRESSION OF IMMUNE FACTORS IN ENDOMETRIAL CELLS AND MAY CONTRIBUTE TO ENDOMETRIOSIS.

DESIGN: Experimental.

OBJECTIVE: To investigate the role of TIA-1 in endometriosis.

MATERIALS AND METHODS: H19 expression was decreased in the eutopic endometrial tissues from women with endometriosis (n=30) and eutopic and ectopic endometrial tissues from women with endometriosis (n=17) were immunostained for TIA-1. Staining intensities were evaluated by HSCOREs. Regulation of endometrial TIA-1 expression by immune factors and steroid hormones was studied by treating primary cultured human endometrial stromal cells (HESCs) with vehicle, lipopolysaccharide (LPS), tumor necrosis factor-α (TNF-α), estradiol or progesterone, followed by protein blot analyses. HESCs were engineered to over- or under-express TIA-1 using a lentiviral system in order to test whether TIA-1 regulates interleukin-6 (IL-6) or TNF-α expression in these cells.

RESULTS: TIA-1 is expressed in endometrial stromal and glandular cells throughout the menstrual cycle and TIA-1 expression is significantly higher in the peri-menstrual phase. TIA-1 expression in eutopic and ectopic endometrium was reduced in women with endometriosis compared to TIA-1 expression in eutopic endometrium of unaffected controls. LPS and TNF-α increased TIA-1 expression in HESCs in vitro, while steroid hormones had no effect. In HESCs, down-regulation of TIA-1 resulted in elevated IL-6 and TNF-α expression while TIA-1 overexpression resulted in decreased IL-6 and TNF-α expression.

CONCLUSION: Endometrial TIA-1 is regulated throughout the menstrual cycle, with increased expression during peri-menstrual phase. The reduced expression of TIA-1 during the peri-menstrual period in women with endometriosis is predicted to permit a pro-inflammatory environment in these women potentially contributing to the pathogenesis of the disorder.

O-12 Monday, October 20, 2014 05:15 PM

THE NONCODING RNAs H19 AND LET-7 ALTER IGF SIGNALING AND STROMAL CELL GROWTH IN THE ENDOMETRIUM OF WOMEN WITH ENDOMETRIOSIS.

DESIGN: Experimental.

OBJECTIVE: Endometriosis is a major cause of infertility in reproductive age women and can reduce endometrial receptivity. H19 is a long noncoding RNA that sequesters microRNA let-7 and prevents its inhibition of target genes essential to endometrial development, such as IGF1R, which play an important role in endometrial development. Our aim was to investigate the role of H19 and expression of its downstream targets in women with and without endometriosis.

MATERIALS AND METHODS: We evaluated 21 women undergoing surgery for suspected endometriosis or idiopathic infertility (10 women without endometriosis and 11 women with endometriosis). Endometrial biopsies were obtained during the proliferative phase of the menstrual cycle. Primary endometrial stromal cells were transfected with siRNA to knockdown H19 expression and a let-7 inhibitor was used for rescue experiments. Cells were also transfected with an H19 expression construct. Total RNA was isolated and cDNA was synthesized using reverse transcription. Relative quantification of the expression of H19 and IGF1R were analyzed using quantitative real-time PCR. RNA levels were normalized to those of beta tubulin and shown as relative expression levels using the delta CT method. Western blot was performed to analyze protein levels of IGF1R in conditions of H19 overexpression and knockdown. Cell viability and apoptosis assays were performed. Statistical analysis was performed using the Student’s t-test.

RESULTS: H19 expression was decreased in the eutopic endometrial tissue of women with endometriosis compared to women without endometriosis (p=0.03). H19 knockdown in primary endometrial stromal cells was associated with decreased expression of IGF1R and addition of a let-7
inhibitor rescued this effect. When H19 was overexpressed there was an associated increase in IGF1R mRNA and protein expression compared to control. Cell viability and apoptosis assays showed that when H19 was decreased, cell viability and apoptosis were decreased and when H19 was overexpressed, cell viability was increased.

CONCLUSION: Women with endometriosis have decreased endometrial expression of H19 compared to women without endometriosis. Decreased H19 prevents the sequestration of let-7, increasing let-7 repression of targets such as IGF1R. Altered expression of noncoding RNAs impacts endometrial cell growth and function and thus is a potential mechanism of infertility in patients with endometriosis.

Supported by: NIH.

O-13 Monday, October 20, 2014 05:30 PM

THE ROLE OF MONOCYTES IN ENDOMETRIOSIS. C. E. Bedient, D. C. Rodriguez, C. Roberts, N. Sidell, S. C. Schutte. Department of Gynecology and Obstetrics, Emory University, Atlanta, GA.

OBJECTIVE: Endometriosis impacts approximately 10% of women with symptoms of debilitating pain or infertility (1). Women with endometriosis (endo) have been shown to have altered peritoneal macrophage activation (2) and increased peritoneal recruitment (3). The primary aim of this study is to determine if the increase in macrophage recruitment in endo is due to abnormal chemotactic signals originating in the peritoneal fluid (PF) or inherent differences in monocyte response. To address this question, we used peripheral blood monocytes (PBM) to eliminate potential influences of the peritoneal environment.

DESIGN: This is a translational, cross-sectional, prospective study utilizing primary cells.

Monocyte Invasion: Average Cell Number (Cells Per Frame)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Control PF</th>
<th>Endometriotic PF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Monocytes</td>
<td>30.8 ± 8.6</td>
<td>15.0 ± 4.3†</td>
</tr>
<tr>
<td>Endometriotic Monocytes</td>
<td>308.8 ± 163.2†</td>
<td>264.9 ± 81.2</td>
</tr>
</tbody>
</table>

† p=0.045 between these conditions

Regardless of PF source as chemoattractant, a >10 fold increase in invasion was seen using PBM from endo patients.

MATERIALS AND METHODS: Suspected endo and control patients undergoing benign reproductive surgery were consented with IRB approval, clinical symptoms were recorded and peripheral blood and PF was collected. Endometriosis was confirmed by pathology. PF was centrifuged and stored at -80°C until use. PBM were isolated and quantified using CD14+ magnetic beads and magnetic activated cell sorting. Monocyte recruitment was assessed via a transwell invasion assay; 50,000 normal or endometriotic monocytes were placed in the top well above a thin layer of Matrigel. The chemoattractant utilized was PF with normalized protein content from endo patients or controls. After 48 hours the number of cells invading through the transwell were averaged from 3 random fields of view. Statistical analysis was completed using a t-test or a one-way ANOVA with a post hoc t-test using a 95% confidence interval.

RESULTS: An n of 5 was obtained, with similar average age of control vs. endo patients (36.6 ± 2.3 years vs. 34.2 ± 3.2 years, p:NS). Protein content in PF was normalized, with endometriotic PF containing increased protein levels prior to normalization (35.2 ± 6.2 mg/ml vs. 24.5 ± 6.9 mg/ml, p:NS).

CONCLUSION: The PBM source was a more significant factor in invasion than PF source. PBM from endo patients have inherently abnormal responses to chemotactic signals, suggesting that a significant component of the pathophysiology of endometriosis resides in the monocytes and not solely with differences seen in endometriotic peritoneal fluid.

Supported by: This research was supported by the Atlanta Center for Translational Research in Endometriosis via a grant from the Eunice Kennedy Shriver NICHD (U01HD66439).

O-14 Monday, October 20, 2014 05:45 PM

DIMINISHED OVARIAN RESERVE (DOR) IN THE US ART POPULATION: DIAGNOSTIC TRENDS AMONG 183,555 CYCLES FROM THE SOCIETY FOR ASSISTED REPRODUCTIVE TECHNOLOGY – CLINICAL OUTCOMES REPORTING SYSTEM (SART-CORS). K. Devine, a M. Mumford, a M. J. Hill, b M. Wu, b A. H. DeCherney, a A. Propst, c NICHHD, NIH, Bethesda, MD; bWalter Reed, Bethesda, MD; cTX Fertility Center, Austin, TX.

OBJECTIVE: DOR diagnosis is on the rise despite vaguely defined diagnostic criteria. Possible explanations include the following: a trend towards older patients seeking ART, additional diagnostic modalities such as antral follicle count (AFC) and anti-mullerian hormone (AMH), and clinics’ desire to attract patients and/or explain suboptimal success rates. DOR has generally been associated with poorer ART outcomes, and over-diagnosis causes unnecessary distress to patients. ESHRE defined poor ovarian response (POR) as cycle cancellation for poor response or fewer than four oocytes at retrieval following conventional gonadotropin stimulation (>149 IU FSH daily)1. We sought to evaluate trends in DOR diagnosis and accuracy of DOR assignment in predicting POR by this definition using SART-CORS data.

DESIGN: Retrospective.

MATERIALS AND METHODS: We analyzed all fresh, stimulated, autologous cycles from 2004 and 2011 (earliest and most recent reporting years available). DOR assignment was the primary exposure. POR was the primary outcome. Prevalence of DOR and POR, power of DOR and FSH (<7≥12 IU/L) to predict POR, and correlation of DOR and POR with live birth (LB) were calculated. Associations were tested using X2 or ANOVA as appropriate.

RESULTS: Though DOR prevalence increased from 19 to 26% from 2004 to 2011, prevalence of POR decreased somewhat from 16 to 15%. LB among patients with DOR improved slightly (Table). Comparing basal FSH ≥12 versus a clinical diagnosis of DOR, basal FSH had a higher specificity (91.9% versus 81.4%) and PPV (37.5% versus 30.4%) for predicting POR.

CONCLUSION: DOR diagnosis is increasing, but accuracy has not improved with the availability of additional parameters such as AFC and AMH. Though POR entails very poor outcomes, the majority of patients clinically assigned as DOR will not experience POR. Development and utilization of more accurate predictors of POR are needed to minimize patient distress resulting from over-diagnosis.

Supported by: Program in Reproductive and Adult Endocrinology, Eunice Kennedy Shriver National Institute of Child Health and Human Development. National Institutes of Health, Intramural Research.

-80°C until use. PBM were isolated and quantified using CD14+ magnetic beads and magnetic activated cell sorting. Monocyte recruitment was assessed via a transwell invasion assay; 50,000 normal or endometriotic monocytes were placed in the top well above a thin layer of Matrigel. The chemoattractant utilized was PF with normalized protein content from endo patients or controls. After 48 hours the number of cells invading through the transwell were averaged from 3 random fields of view. Statistical analysis was completed using a t-test or a one-way ANOVA with a post hoc t-test using a 95% confidence interval.

CONCLUSION: DOR diagnosis is increasing, but accuracy has not improved with the availability of additional parameters such as AFC and AMH. Though POR entails very poor outcomes, the majority of patients clinically assigned as DOR will not experience POR. Development and utilization of more accurate predictors of POR are needed to minimize patient distress resulting from over-diagnosis.

Supported by: Program in Reproductive and Adult Endocrinology, Eunice Kennedy Shriver National Institute of Child Health and Human Development. National Institutes of Health, Intramural Research.
OBJECTIVE: Use of a gonadotropin-releasing hormone (GnRH) antagonist protocol with GnRH agonist (GnRHa) trigger for final oocyte maturation is the most effective strategy to reduce the rate of ovarian hyperstimulation syndrome (OHSS) during IVF. However, concerns persist regarding GnRHa trigger and ART outcomes. Use of a combination GnRHa and low-dose human chorionic gonadotropin (hCG) co-trigger has been reported to reduce OHSS and preserve IVF outcomes. The objective of this study was to examine the impact of final oocyte maturation method (GnRHa vs co-trigger) on OHSS, oocyte yield and maturity.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: Autologous IVF cycles using a GnRH antagonist protocol at Penn Fertility Care from 1/2008 through 4/2014 with either GnRHa alone or GnRHa and low-dose hCG for oocyte maturation were included. Primary outcome was OHSS by Golan criteria; secondary outcomes were total oocytes retrieved and oocyte maturity. Logistic, linear and Poisson regression models were used to compare outcomes by trigger group and predict OHSS, adjusting for confounders.

RESULTS: Demographics and ovarian reserve measures were similar in the groups. After adjusting for age, AFC and PCOS diagnosis, more mature oocytes were retrieved after co-trigger v GnRHa alone (RR 1.18 [CI 1.1-1.27], p<0.01 and 1.14 [CI 1.02-1.29], p=0.03). Early OHSS was more common after co-trigger (8.6% v 0%, p<0.01); with the majority (6/7, 67%) developing severe OHSS. Age<35, BMI and AFC were predictive of OHSS. Clinical pregnancy rate after day 5 transfer did not differ.

IVF Cycle Characteristics & Outcomes By Trigger Group

<table>
<thead>
<tr>
<th>Co-Trigger (n=71)</th>
<th>GnRHa Trigger (n=106)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age* (32-37)</td>
<td>32 (30-35)</td>
<td>0.02</td>
</tr>
<tr>
<td>Antral Follicle (12-25)</td>
<td>23 (15-31)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Count (AFC)*</td>
<td>3.2 (1.7-5.0)</td>
<td>4 (2.5-6.5)</td>
</tr>
<tr>
<td>Hormone Level [ng/mL]*</td>
<td>13 (16)</td>
<td>41 (38)</td>
</tr>
<tr>
<td>PCOS(%)</td>
<td>4 (44)</td>
<td>6 (14)</td>
</tr>
<tr>
<td>History of OHSS(%)</td>
<td>2650 (1850-3700)</td>
<td>2350 (1800-2963)</td>
</tr>
<tr>
<td>Total Gonadotropin Use [IU]*</td>
<td>3542 (2936-3014)</td>
<td>4333 (3501-5653)</td>
</tr>
<tr>
<td>Max E2 [pg/mL]*</td>
<td>27.5 (21-35)</td>
<td>31 (25-40)</td>
</tr>
<tr>
<td>Oocytes Retrieved*</td>
<td>18 (12-24)</td>
<td>17 (11-22)</td>
</tr>
<tr>
<td>Maturity*</td>
<td>82 (73-91)</td>
<td>68 (55-86)</td>
</tr>
<tr>
<td>Any OHSS(%)</td>
<td>6 (8.6)</td>
<td>0</td>
</tr>
<tr>
<td>Severe OHSS(%)</td>
<td>4 (6)</td>
<td>0</td>
</tr>
<tr>
<td>Freeze All for OHSS(%)</td>
<td>4 (9.3)</td>
<td>1 (1)</td>
</tr>
</tbody>
</table>

*Median (Interquartile Range), Mann-Whitney U test. ±Chi-square test

CONCLUSION: Although use of a co-trigger is associated with greater oocyte yield and maturity compared to GnRHa trigger alone, it is accompanied by an elevated risk of severe OHSS. Predictors of OHSS were limited and therefore co-trigger should be used with caution.

Supported by: T32 PD10032525 (KO), 5K12HD001265-14 (SS).

SOCIETY FOR MALE REPRODUCTION AND UROLOGY TRAVELING SCHOLARS

O-16 Monday, October 20, 2014 04:15 PM

INFERTILE MEN WITH NON-OBSTRACTIVE AZOSPERMIA EXHIBIT DEFECTS IN THE DNA MISMATCH REPAIR PATHWAY. A. D. Ridgeway, L. Gomez, R. Ramasamy, L. Lipshultz, D. Lamb, Lester and Sue Smith Chair Urologic Research, Baylor College of Medicine, Houston, TX. A. Dokras. Department of Obstetrics & Gynecology, University of Pennsylvania, Philadelphia, PA.

OBJECTIVE: To examine the global DNA methylation status of NOA patients and fertile controls using the Infinium HumanMethylation450 microarray. DNA was isolated from fibroblasts cultured from the testicular biopsy of NOA patients (n=21) and fertile controls (n=5).

MATERIALS AND METHODS: Genes with the most significant changes in DNA methylation (p<0.05) were identified in NOA patients vs. controls using the R statistical suite. Bisulfite clonal sequencing validated the microarray results and provided a platform to screen additional men (10 NOA and 15 controls). MMP gene expression was quantified using qPCR, immunofluorescence and Western blot. The functional consequences of MMP defects were tested using a cell proliferation assay of growth after exposure to the DNA alkylating agent N-Nitroso-N-methylurea (MNU) or the radiomimetic drug Neocarzinostatin (Neo).

RESULTS: DNA methylation was increased in a cohort of NOA patients (6/31) at a meiosis-associated gene in the MMR family - MSH5. MSH5 is implicated in the repair of double-stranded breaks (DSB) and in the resolution of the holiday junction during meiosis. The NOA cohort also exhibited down-regulation of MSH5 gene and reduced MSH5 protein expression. Cells with methylated MSH5 ceased to proliferate in response to Neo and did not up-regulate MSH5 expression. This was in contrast to control cells that had a 5-fold increase after Neo treatment. Defects in MMR genes that did not display abnormal patterns of DNA methylation (potentially due to genetic causes) were defined using the Sn1-type alkylating agent MNU. A subset of testicular fibroblasts from 30 NOA men were resistant to the cytotoxic effects of MNU. This finding was in contrast to fertile control cells (n=10) that were sensitive to MNU treatment.

CONCLUSION: A cohort of NOA men with defects in an important DNA repair pathway for spermatogenesis -MMP were identified. Defects included the deleterious DNA methylation of MSH5 and an abnormal response of NOA fibroblasts to cytotoxic drugs. These defects may affect genomic stability and DNA recombination in germ cells and contribute to impaired spermatogenesis in NOA.

Supported by: Funding Supported by a Male Reproductive Health Research Career (MHRH) Development Physician Scientist Award (K12 HD073917-01) from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) Program and by NIH grants P01HD36289 from the Eunice Kennedy Shriver NICHD and 1R01DK078121 from the National Institute of Kidney and Digestive Diseases.

O-17 Monday, October 20, 2014 04:30 PM

A SPERM-DERIVED WW DOMAIN-BINDING PROTEIN INITIATES ZYGOTIC DEVELOPMENT IN HUMAN AND MOUSE AND DETERMINES MALE-FACTOR INFERTILITY. M. Aarabi, H. Balakier, S. Bashar, S. L. Moskovtsev, P. Sutton, C. L. Librach, R. Oko, *Department of Biomedical and Molecular Sciences, Queen’s University, Kingston, ON, Canada; *Department of Human Genetics, McGill University, Montreal, QC, Canada; †CreAle Fertility Centre, Toronto, ON, Canada; ‡Department of Obstetrics & Gynaecology, University of Toronto, Toronto, ON, Canada; †Department of Animal Sciences, University of Missouri, Columbia, MO; †Departments of Obstetrics, Gynecology & Women’s Health, University of Missouri, Columbia, MO.

OBJECTIVE: To examine whether sperm-derived postacrosomal WW-domain-binding protein (PAWP) is required for initiation of calcium oscillations and zygotic development after human and mouse fertilization; to determine PAWP levels in the spermatozoa of men undergoing intracytoplasmic sperm injection (ICSI) and to correlate them with infertility treatment outcomes.

DESIGN: Prospective clinical and laboratory study.

MATERIALS AND METHODS: Following Research Ethics Board approvals, recombinant human PAWP protein or complementary RNA (cRNA) was injected into mouse and human oocytes. PAWP-induced calcium oscillations were measured and compared to sperm–induced oscillations in a time-lapse trial. Competitive peptides derived from the PAWP sequence were co-injected with sperm/PAWP for inhibition trials. Surplus sperm after use for ICSI cycle was collected from 110 men to determine the levels of PAWP by flow cytometry and its correlation to treatment outcomes.

FERTILITY & STERILITY®
RESULTS: PAWp recombinant protein/cRNA triggered calcium oscillations in human and mouse oocytes similar to those triggered by sperm factors released during ICSI. Furthermore, sperm-induced calcium oscillations were blocked by co-injection of the competitive peptide derived from the WWI domain-binding motif of PAWp, implying the requirement of sperm PAWp for successful fertilization. Strong positive correlations were found between sperm PAWp levels and fertilization rates as well as normal preimplantation embryonic development in couples undergoing ICSI.

CONCLUSION: Our functional and developmental data strongly suggest that sperm-delivered PAWp is a unique protein which has a non-redundant role during human and mouse fertilization, is required to trigger zygotic development and has potential applications in the diagnosis and treatment of infertility.

Supported by: Canadian Institutes of Health Research (CIHR); Food for the 21st Century Program, University of Missouri.

O-18 Monday, October 20, 2014 04:45 PM

OUTCOMES OF MICRODISSECTION TESTICULAR SPERM EXTRACTION IN MEN WITH EARLY VERSUS LATE MATURATION ARREST. A. M. Bernie, a C. Bryson, a R. Ramasamy, b B. Robinson, c P. N. Schlegel. d urology, New York Presbyterian/Weill Cornell, New York, NY; bUrology, Baylor College of Medicine, Houston, TX; cPathology, New York Presbyterian/Weill Cornell, New York, NY.

OBJECTIVE: Maturation arrest (MA) histology on testis biopsy has been associated with worse prognosis for sperm retrieval in men with nonobstructive azoospermia (NOA). Men with early MA have worse outcomes compared to men with late MA. The objective of this study was to evaluate sperm retrieval outcomes for men with NOA and MA who underwent microdissection testicular sperm extraction (micro-TESE).

DESIGN: Retrospective study.

MATERIALS AND METHODS: A retrospective review of charts for 1068 consecutive patients with NOA, confirmed by analysis of 2 centrifuged semen samples, who underwent micro-TESE at a single center after a sample obtained on the day of planned micro-TESE confirmed absolute azoospermia, was performed. Men with complete AZFs or AZFB microdeletions were excluded. Clinical factors including age, FSH, testis volume, varicocele, history of cryptorchidism or Klinefelter syndrome and sperm retrieval rate (SRR) were analyzed. Patients were considered to be MA on their pathology if the most advanced pattern was consistent with MA, which included both patients with mixed pathology and with 100% MA. Slides were re-reviewed by a uropathologist who was blinded to outcome of the micro-TESE to determine early versus late MA, with early including germ cell and primary spermatocyte arrest and late including arrest at the spermatid level. Differences in SRR were compared using a chi-square analysis.

RESULTS: 175 patients with mean age 35.6 ± 6.4 years were analyzed. Mean testis volume was 11.0 ± 5.4 cc. Mean FSH was 19.5 ± 14.6 IU/L. Overall SRR was 44.8%. SRR was similar in the early MA group compared to the late MA group (47.8% vs 33.3%, p=0.7). SRR trended towards significance among patients with testis volume <10cc compared to ≥10cc (62.7% vs 47.8%, p=0.07). Patients with FSH ≥10 IU/L had a higher SRR compared to patients with an FSH <10 IU/L (62.5% vs 38.2%, p<0.003).

CONCLUSION: MA on diagnostic biopsy in men with NOA is associated with a sperm retrieval rate of 45% using micro-TESE at our center. Despite previous findings, the SRR was similar amongst men with either early or late MA. Men with larger volume testis and low FSH had the worse SRR of any men with MA. These observations are paradoxical to the typical assumptions that larger testis volume and lower FSH are associated with better testicular function.

O-19 Monday, October 20, 2014 05:00 PM

MALE CAFFEINE AND ALCOHOL INTAKE IN RELATION TO IN VITRO FERTILIZATION OUTCOME AMONG FERTILITY PATIENTS. A. E. Karmom, a T. L. Toth, a A. J. Gaskins, b M. C. Afeiche, c B. Robinson, a C. Tanrikut, c R. Hauser, c J. C. Chavarro. b Pathology, New York Presbyterian/Weill Cornell, New York, NY; cObstetrics and Gynecology, Massachusetts General Hospital, Boston, MA; aDepartment of Environmental Health, Harvard School of Public Health, Boston, MA.

OBJECTIVE: To examine the relation of male caffeine and alcohol intake with their partner’s clinical pregnancy rates following in vitro fertilization (IVF).

DESIGN: Prospective cohort study.

MATERIALS AND METHODS: The Environment and Reproductive Health Study is an on-going prospective cohort study enrolling subfertile couples presenting at Massachusetts General Hospital (2007-2013). Information on pre-treatment dietary intake was collected from 105 men who underwent 214 IVF cycles. Logistic regression models using generalized estimating equations (GEE) were fit to investigate the relationship between male beverage intake and clinical pregnancy after IVF. Results were adjusted for male and female age at BMI, male smoking status, male total calorie and macronutrient intake, infertility diagnosis, and female caffeine and alcohol intakes.

RESULTS: Mean male age was 37 years and median male caffeine and alcohol intakes were 169 mg/day and 13g/day, respectively. Clinical pregnancy rate per initiated cycle was 55%. In multivariable regression, male caffeine intake was negatively associated with clinical pregnancy per initiated cycle (p-trend=0.04), while male alcohol intake was positively associated with clinical pregnancy per initiated cycle (p-trend<0.01). Compared to men consuming <88mg/day of caffeine, adjusted odds ratios (95% CI) for clinical pregnancy per initiated cycle were 1.4(0.5-3.8), 1.7(0.6-4.8), and 0.4(0.1-1.0) for men consuming 88-168mg/day, 169-264mg/day, and ≥265mg/day of caffeine, respectively. Compared to men who consumed <3g/day of alcohol, adjusted odds ratios (95%CI) for clinical pregnancy per initiated cycle were 1.1(0.4-3.1), 3.1(1.8-5.7), and 4.5(1.5-13.6) for men consuming 3-12g/day, 13-21g/day, and ≥22g/day of alcohol, respectively.

CONCLUSION: Although there is extensive literature on the relationship between beverage intake and semen parameters, little data exist on male caffeine and alcohol intake and pregnancy outcomes. Our results suggest that male caffeine and alcohol intake impact in vitro fertilization outcome. Supported by: NIH grants R01ES009718, R01ES000002, P03DK46200, T32DK007073, T52-HD060454.

O-20 Monday, October 20, 2014 05:15 PM

FRUIT AND VEGETABLE INTAKE AND THEIR PESTICIDE RESIDUES IN RELATION TO SEXUAL QUALITY AND FERTILIZATION RATES AMONG SUBFERTILE MEN. Y.-H. Chiu, a M. C. Afeiche, a A. J. Gaskins, a P. L. Williams, b D. L. Wright, c J. C. Petrozza, a,b,c Tanrikut, c R. Hauser, c J. E. Chavarro. a,b Harvard School of Public Health, Boston, MA; bDepartment of Obstetrics and Gynecology, Massachusetts General Hospital, Boston, MA; cDepartment of Urology, Massachusetts General Hospital, Boston, MA.

OBJECTIVE: To examine the relation of fruit and vegetable (FV) intake, taking into consideration their pesticide residue status, with semen quality and fertilization rates.

DESIGN: Prospective cohort study at an academic hospital.

MATERIALS AND METHODS: Between April 2007 and June 2012, 155 men presenting to a fertility center completed a food frequency questionnaire and produced a total of 338 semen samples. Of these men, 105 and their female partners underwent a total of 190 in vitro fertilization (IVF) cycles. FVs were dichotomized as high or low pesticide residue based on the USDA annual reports. The intakes of high and low residue FVs were then summed annually reports. The intakes of high and low residue FVs were then summed.

RESULTS: Intake of total FVs and low pesticide residue FVs were unrelated to semen quality. However, men in the top quartile of high pesticide residue FV intake had 64% (95% CI: 41%, 78%) lower total morphologically normal sperm count and 70% (95% CI: 43%, 85%) lower total motile sperm count than men in the lowest quartile of intake (p trend=0.01 and 0.01, respectively). Total FV intake was positively related to fertilization rates among couples undergoing IVF with conventional insemination (p trend=0.03), but not Intra-cytoplasmic sperm injection (p trend=0.71). This association was mainly driven by intake of low pesticide residue FVs. Specifically, the adjusted IVF fertilization rates by increasing quartile of male pesticide residue FV intake were 0.60 (0.34, 0.81), 0.60 (0.40, 0.77), 0.75 (0.56, 0.87), and 0.88 (0.72, 0.95) (p trend=0.04).
CONCLUSION: Intake of high pesticide residue FVs may negatively affect semen quality while intake of low pesticide residue FVs may have a positive impact on fertilization. 

Supported by: NIH grants ES009718 and T32DK007703.

O-21 Monday, October 20, 2014 05:30 PM

INFLAMMASOME ACTIVATION IN MEN WITH ABNORMAL SEMEN QUALITY. M. Jurewicz, a E. Ibrahim, b G. Attia, c S. Roberge, a C. Lynne, a N. Brackett, a Dept of Urology, University of Miami, Miami, FL; bMiami Project to Cure Paralysis, University of Miami, Miami, FL; cDept of OB/Gyn, University of Miami, Miami, FL.

OBJECTIVE: Our previous work has shown that inflammasome activation and the consequent elevation of semen cytokines leads to impaired sperm motility in men with spinal cord injury (SCI). a The present study sought to determine if inflammasome activation was evident in non-SCI men with abnormal semen parameters.

DESIGN: Prospective Study.

MATERIALS AND METHODS: Semen was obtained by masturbation from men presenting for infertility workup (n=48) and healthy controls (n=9). Infertile patients were divided into 3 groups. Group 1: patients with normal sperm concentration (≥ 20 million/cc) and low sperm motility (<40%) (LM, n=7); Group 2: patients with normal sperm motility and oligozoospermia (Oligo, n=7); Group 3: patients with combined low motility and oligozoospermia (LM+Oligo, n=34). After liquefaction, semen was centrifuged at 3000X and the resulting seminal plasma (SP) was analyzed for caspase-1, IL-18, and IL-1β concentrations using ELISA. T-tests were used to compare Groups 1, 2 and 3 to the control group using Prism software. P value <0.05 was considered statistically significant.

RESULTS: See Table 1. In the two groups in which oligozoospermia was a major component (Oligo and LM+Oligo), statistically significant elevated SP concentrations of caspase-1, IL-18 and IL-1β were found vs controls. No such elevations were found in the LM only group. None of the groups showed elevations in IL-1β.

TABLE 1.

<table>
<thead>
<tr>
<th>Caspase-1 (pg/ml)</th>
<th>Control</th>
<th>LM</th>
<th>Oligo</th>
<th>LM+Oligo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caspase-1 (pg/ml)</td>
<td>46.3 ± 8.1</td>
<td>58.4 ± 6.1 (p&lt;0.30)</td>
<td>92.6 ± 53.0 (p&lt;0.05)</td>
<td>213.4 ± 26.6 (p&lt;0.05)</td>
</tr>
<tr>
<td>IL-18 (pg/ml)</td>
<td>33.2 ± 3.6</td>
<td>44.1 ± 10.9 (p&lt;0.33)</td>
<td>67.0 ± 9.9 (p&lt;0.05)</td>
<td>71.2 ± 5.2 (p&lt;0.05)</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>12.9 ± 3.6</td>
<td>18.8 ± 6.4 (p&lt;0.41)</td>
<td>48.9 ± 9.7 (p&lt;0.05)</td>
<td>52.3 ± 3.2 (p&lt;0.05)</td>
</tr>
<tr>
<td>IL-1β (pg/ml)</td>
<td>21.3 ± 1.3</td>
<td>26.9 ± 5.3 (p&lt;0.24)</td>
<td>36.8 ± 18.0 (p&lt;0.34)</td>
<td>21.5 ± 4.8 (p&lt;0.99)</td>
</tr>
</tbody>
</table>

Values are means ± standard error of the mean; p-value=comparison to the control group within the same row

CONCLUSION: Our study shows a novel and significant elevation of caspase-1 and multiple cytokines, and thus likely activation of the inflammasome complex in patients presenting for an infertility workup. Further investigation of these elevations may lead to options for therapeutic interventions.

ENDOMETRIOSIS I

O-22 Monday, October 20, 2014 04:15 PM

MUTATIONS IN THE GNRH SIGNALING PATHWAY ARE RISK FACTORS FOR ENDOMETRIOSIS. K. Ward, R. Chettier, P. Farrington, H. M. Albertsen. Juneau Biosciences, LLC, Salt Lake City, UT.

OBJECTIVE: Some but not all patients with endometriosis have abnormal functioning of their GnRH axis and some but not all endometriosis patients respond to GnRH agonist therapies. The clinical variation observed may be caused by intrinsic genetic variation in these pathways. We tested 177 genes for multiple testing (p<4.4E-05), we discovered 10 strongly associated variants representing 10 distinct genes as shown in Table 1. The top 2 significantly associated variants (CRTC1 and ARHGAP32) are predicted to be damaging to the encoded protein.

TABLE 1.

<table>
<thead>
<tr>
<th>CHR</th>
<th>Position</th>
<th>Var Allele</th>
<th>Endm Freq</th>
<th>Population Freq</th>
<th>Gene</th>
<th>Fisher p</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>18876309</td>
<td>G</td>
<td>0.16099</td>
<td>0.08759</td>
<td>CRTC1</td>
<td>9.51E-37</td>
<td>2.00</td>
</tr>
<tr>
<td>12</td>
<td>128938540</td>
<td>A</td>
<td>0.04207</td>
<td>0.02242</td>
<td>ARHGAP32</td>
<td>8.09E-09</td>
<td>1.77</td>
</tr>
<tr>
<td>18</td>
<td>77170479</td>
<td>A</td>
<td>0.00359</td>
<td>0.00037</td>
<td>NFATC1</td>
<td>2.62E-08</td>
<td>10.73</td>
</tr>
<tr>
<td>11</td>
<td>2161530</td>
<td>A</td>
<td>0.00015</td>
<td>0.000048</td>
<td>P2</td>
<td>2.37E-07</td>
<td>40.37</td>
</tr>
<tr>
<td>6</td>
<td>36075326</td>
<td>A</td>
<td>0.00130</td>
<td>0.000029</td>
<td>MAPK14</td>
<td>2.28E-05</td>
<td>44.83</td>
</tr>
</tbody>
</table>

CONCLUSION: Gene variants affecting the human GnRH signaling pathway are significant risk factors for predisposition to endometriosis. These gene variants are likely to contribute to the clinical variation observed in women with endometriosis.

Supported by: Juneau Biosciences.

O-23 Monday, October 20, 2014 04:30 PM

RARE MUTATIONS IN WNT SIGNALING PATHWAYS ARE RISK FACTORS FOR ENDOMETRIOSIS. R. Chettier, H. M. Albertsen, K. Ward. Juneau Biosciences, LLC, Salt Lake City, UT.

OBJECTIVE: Wnt proto-oncogenes are believed to play a role in endometriosis. Recently, genetic association studies have shown that common variants near Wnt4 are associated with endometriosis across different ethnicities. In order to test the hypothesis that variants in other genes involved in Wnt signaling pathways contribute to the pathogenesis of endometriosis, we tested exome variants found in 250 candidate genes involved in Wnt signaling for genetic association with endometriosis.

DESIGN: A list of candidate genes involved in Wnt signaling were obtained using PANTHER database. 1537 Caucasian endometriosis patients were genotyped for 1138 rare exome variants in candidate genes. The number of heterozygous subjects observed was compared with published population data (n=50,000).

MATERIALS AND METHODS: 1537 patients with surgically confirmed endometriosis were tested using the Infinium HumanExome BeadChip (Illumina, San Diego, CA). Single marker association was tested using Fisher’s exact Test. In silico prediction of protein function was estimated using polyphen 2 database.

RESULTS: We tested a total of 1138 variants representing 250 candidate Wnt genes. 88 showed suggestive/deleterious effect for endometriosis. After adjusting for multiple testing (p<4.4E-05), we discovered 10 strongly associated variants in 10 distinct genes. None of these associated variants were predicted to be protein altering. The average OR among these associated variants is 10.7.

CONCLUSION: Wnt signaling proteins, particularly the protocadherins, may play a major role in the pathogenesis of endometriosis.

Supported by: Juneau Biosciences.

TABLE 1.

<table>
<thead>
<tr>
<th>CHR</th>
<th>Position</th>
<th>Var Allele</th>
<th>Endm Freq</th>
<th>Population Freq</th>
<th>Gene</th>
<th>Fisher p</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>140238124</td>
<td>A</td>
<td>0.0656</td>
<td>0.0131</td>
<td>PCDHA10</td>
<td>7.54E-70</td>
<td>5.27</td>
</tr>
<tr>
<td>14</td>
<td>140797212</td>
<td>A</td>
<td>0.0008</td>
<td>0.0008</td>
<td>PCDHA10</td>
<td>4.84E-20</td>
<td>15.61</td>
</tr>
<tr>
<td>5</td>
<td>140238124</td>
<td>A</td>
<td>0.0009</td>
<td>0.0020</td>
<td>PCDHA9</td>
<td>1.94E-10</td>
<td>4.73</td>
</tr>
<tr>
<td>22</td>
<td>469296692</td>
<td>A</td>
<td>0.0644</td>
<td>0.0399</td>
<td>CELSR1</td>
<td>3.75E-10</td>
<td>1.65</td>
</tr>
<tr>
<td>18</td>
<td>77170479</td>
<td>A</td>
<td>0.0039</td>
<td>0.0004</td>
<td>NFATC1</td>
<td>2.62E-08</td>
<td>10.71</td>
</tr>
<tr>
<td>140250471</td>
<td>A</td>
<td>0.0020</td>
<td>0.0000</td>
<td>PCDHA11</td>
<td>2.37E-07</td>
<td>40.37</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>10212619</td>
<td>G</td>
<td>0.0033</td>
<td>0.0004</td>
<td>MYH13</td>
<td>1.83E-06</td>
<td>8.13</td>
</tr>
<tr>
<td>140802374</td>
<td>A</td>
<td>0.0036</td>
<td>0.0006</td>
<td>PCDHA11</td>
<td>8.45E-06</td>
<td>5.91</td>
<td></td>
</tr>
<tr>
<td>140581543</td>
<td>A</td>
<td>0.0049</td>
<td>0.0012</td>
<td>PLCB2</td>
<td>1.74E-05</td>
<td>3.97</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>68687265</td>
<td>G</td>
<td>0.0010</td>
<td>0.0000</td>
<td>CDH1</td>
<td>2.41E-05</td>
<td>Inf</td>
</tr>
</tbody>
</table>
Endometriosis-like cystic lesions had grown in the abdominal cavity of all mice. The monolayer epithelial cell lining of the cyst was shown. Administering LPS for 4 weeks significantly increased the total number (1.7 vs. 2.9/mouse), the average weight (1.3-fold vs. control), and surface area (1.4-fold vs. control) of endometriosis-like lesions compared with the control. LPS did not influence the weight of uteri and ovaries. LPS enhanced Cox-2, Vegf and Il-6 mRNA expression in endometriosis-like lesions evaluated by real time RT-PCR, Ki67 and PECAM immunohistochemical staining, respectively. The degree of inflammation was assessed by CD3 (T-cell marker) and F4/80 (macrophage cell marker) immunostaining.

RESULTS: Endometriosis-like cystic lesions had grown in the abdominal cavity of all mice. The monolayer epithelial cell lining of the cyst was shown. Administering LPS for 4 weeks significantly increased the total number (1.7 vs. 2.9/mouse), the average weight (1.3-fold vs. control), and surface area (1.4-fold vs. control) of endometriosis-like lesions compared with the control. LPS did not influence the weight of uteri and ovaries. LPS enhanced Cox-2, Vegf and Il-6 mRNA expression in endometriosis-like lesions evaluated by real time RT-PCR, Ki67 and PECAM immunohistochemical staining, respectively. The degree of inflammation was assessed by CD3 (T-cell marker) and F4/80 (macrophage cell marker) immunostaining.

CONCLUSION: Using this mouse endometriosis model, we found that the bacterial endotoxin enhances the development of endometriosis-like lesions by stimulating the peritoneal inflammatory status.

Supported by: KAKENHI(Japan Society for the Promotion of Science Grant-in-Aid).

O-25 Monday, October 20, 2014 05:00 PM

DOXYCYCLINE CAUSES REGRESSION OF ENDOMETRIOTIC IMPLANTS IN A RAT ENDOMETRIOSIS MODEL: A PRELIMINARY STUDY. R. Attar,1 O. Kızilkale Yıldırım,2 G. Yıldırım,2 S. Bostancı,1 M. Bakacak,1 F. Özkan,1 E. Attar.1 1Obstetrics and Gynecology, Yeditepe University, Istanbul, Turkey; 2Obstetrics and Gynecology, Sakarya University Medical School, Kocaeli, Turkey.

OBJECTIVE: The aim of this study is to evaluate the effect of doxycycline on surgically induced endometriosis lesions in a rat endometriosis model.

DESIGN: This is a prospective, randomized, controlled experimental animal study carried out at the Experimental Research Center of Yeditepe University (YUDTEM). MATERIALS AND METHODS: Endometriosis was surgically induced in twenty ovariectomized, nulligravid Sprague Dawley rats. Two weeks after the induction operation second laparotomies were performed and volumes of the endometriotic implants were measured and biopsies were done. Then the rats were randomly divided into two groups: Group 1 and group 2. Group 1 was treated with oral 5mg/kg/day doxycyclin for two weeks. Group 2 was not given medication and served as the control group. Es- trogen was administered throughout the study. Two weeks after the 2nd operation all rats were sacrificed. We measured the volumes of the endometriotic implants again and did the biopsies. We compared the volumes of the endometriotic lesions on 2nd and 4th week. We used Mann-Whitney U test for statistical analysis.

RESULTS: The mean volumes in the control group were 117.39±18.58 and 137.02±17.7 on the 2nd and 4th week, respectively. There was an insignific- ant increase in the volumes of the endometriotic lesions between the 2nd and 4th weeks in the control group (P = 0.32). The mean volumes in doxycyclin group were 159.37±36.64 mm³ and 25.87±9.65 mm³ on the 2nd and 4th week, respectively. Doxycycline caused regression on the volumes of the endometriotic lesions between the 2nd and 4th weeks and it was statistically significant(P = 0.001).

CONCLUSION: Doxycycline caused regression of endometriotic lesions in our experimental study. Our study suggests that doxycycline can be a novel treatment modality for the treatment of endometriosis.
deep infiltrating disease) lesions were collected at surgery. Transcripts for BDNF, its receptors (NTRK2, NGFR, SORT1), and the estrogen receptor 1 (ESR1) were quantified by PCR. Mice (N=30) were ovariectomized and given estradiol (E2), progesterone (P4), E2+P4, or saline for 4 days. Uterine horns were collected, protein was assessed by western blot. Human endometrial epithelial cells (CRL-1671) were treated for 24 or 48 hours with E2 (0.1nM-1µM) or P4 (10nM-100µM) in the presence of letrozole to suppress endogenous aromatase. BDNF secretion was measured by ELISA. Results were compared by ANOVA.

RESULTS: Transcription of SORT1, a co-receptor for BDNF secretion, was elevated in ovarian cysts compared with the endometrium of cases and controls, and deep infiltrating disease (P<0.007). ESR1 was higher in ovarian cysts versus peritoneal foci and deep infiltrating disease (P=0.016). In the mouse uterus, E2 increased mature BDNF 2 fold (P=0.019), pro-BDNF 5 fold (P=0.012) and BDNF 5 fold (P<0.001) versus other treatments. BDNF secretion by endometrial epithelial cells in culture was increased by several E2 and P4 treatments within 24 hours (P<0.05), and by all concentrations of E2 and P4 at 48 hours (P<0.05).

CONCLUSION: Here we provide further evidence for the dysregulation of BDNF and its receptors in endometriosis and expose the profound effect of estrogen in their uterine regulation. We show that endometrial secretion of BDNF is controlled by E2 and P4, and its uterine expression is increased by E2. We postulate that endometriotic lesions express and release BDNF in response to hormones, and could be important in the pathophysiology and treatment of disease. Further, because of the participation of BDNF in nerve growth, proliferation, adhesion, and angiogenesis, its expression in ectopic lesions may contribute to disease progression and pain.

Supported by: Canadian Institutes of Health Research (CIHR), Natural Sciences and Engineering Council of Canada, and the Vanier Canada Graduate Scholarship, CIHR (JMW).

O-28 Monday, October 20, 2014 05:45 PM

B-CELL LYMPHOMA PROTEIN 6 (BCL-6): A NOVEL DIAGNOSTIC MARKER FOR ENDOMETRIOSIS. S. L. Young,1 E. A. Evans-Hoeker,1 J. Jeong,1 J.-Y. Yoo,1 R. Savarís,2 B. A. Lessey.2

1Reproductive Endocrinology & Infertility, UNC School of Medicine, Chapel Hill, NC; 2Michigan State University College of Human Medicine, Grand Rapids, MI; 3Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil; 4University of South Carolina School of Medicine, Greenville, SC.

OBJECTIVE: To date, the only clinically validated diagnostic for endometriosis is surgical exploration. BCL-6 is proto-oncogene whose expression is associated with inflammation and cell proliferation. In this study, we examined eutopic endometrial BCL-6 immunostaining as a novel diagnostic marker.

DESIGN: Prospective, controlled study.

MATERIALS AND METHODS: Midsecretory (urine LH+7 to +10) endometrial sampling was performed in Group A and B subjects. Group A (n=81) samples were obtained from an infertility clinic population prior to undergoing laparoscopy to treat suspected endometriosis. Presence of endometriosis was detected by visualization of lesions (49/58 cases were confirmed by pathologic examination). Group B (n=28) samples were taken from healthy, fertile volunteers who did not undergo laparoscopy. Sections of formalin fixed and paraffin-embedded endometrial samples were immunostained using an automated clinical laboratory method and a monoclonal BCL-6 antibody (Clone LN22, Vector Labs). Immunostaining was assessed by a single examiner, blinded to diagnosis and group assignment, using H-SCORE. A positive test was defined as an H-SCORE of 0.9, based on an ROC analysis of initial staining of 68 women with endometriosis and 12 fertile controls. Area under the curve, 0.96; sensitivity, 95.5%; specificity, 93%.

RESULTS: In Group A, 61/81 women had endometriosis and 12/81 did not. BCL-6 staining yielded a sensitivity of 94%, specificity of 75%, and a positive and negative predictive value of 96% and 69%, respectively. Of the 3 false positives, one had hydrosalpinx and adhesions and another had benign teratomas. Given that only 15% of Group A women in Group B lacked endometriosis, we further investigated the specificity of the test using samples from 28 fertile controls who were presumed to be free of endometriosis (Group B): 2/26 (7.7%) of Group B samples had positive BCL-6 staining, similar to endometriosis incidence (3-10%) in the general population. Combining Group B (all positives taken as false positives) and Group A yielded an improved specificity of 87.5% and negative predictive value of 90%. Age and BMI were not different between groups A and B (chi square).

CONCLUSION: Eutopic endometrial BCL6 is a promising new biomarker for the detection of endometriosis.

Supported by: This work was supported by NIH R01 HD067721 to S.L.Y and B.A.L.

O-29 Monday, October 20, 2014 06:00 PM


OBJECTIVE: This is a study to test the efficacy of fenretinide for inducing apoptosis in human endometriotic cells in vitro and for decreasing endometriotic lesions volume in vivo. Fenretinide is a synthetic retinoid analogue with decreased toxicity when compared to other natural and synthetic retinoids. Fenretinide is also a differentiation inducing agent that promotes apoptosis in variety of cell and tissue types. We have previously shown that RA production in endometriotic tissue is decreased, resulting in blunted estrogen metabolism and apoptotic resistance. We hypothesize fenretinide may reduce lesion burden.

DESIGN: In vitro and in vivo experiments.

MATERIALS AND METHODS: Primary endometriotic cells were collected using an IRB approved protocol, isolated, cultured and treated with fenretinide using doses from 0-10 µM. Cell count, cell viability and immunoblotting were performed to examine apoptosis. Quantitative RT-PCR on mRNA isolated from endometriotic cells treated with fenretinide was used to examine the expression of genes involved in retinoic acid signaling including STRA6, CRABP2 and FABP5. Endometriotic tissue was also xenografted in both the flanks of mice. Mice were given 120 mg/kg of fenretinide at a volume of 10 ml/kg of body weight for 2 weeks, after which the mice were sacrificed. The lesion volume was measured and compared between the control and the fenretinide group. Statistical analysis was performed for all the applicable experiments using t-test and ANOVA.

RESULTS: Treatment with fenretinide significantly decreased total cell count and the viability of the cells. Fenretinide also increased protein levels of the apoptotic marker PARP and decreased the proliferation marker PCNA. Examination of genes involved in retinoid uptake and action showed that fenretinide treatment induced STRA6 expression, which is involved in retinoid uptake, while expression of CRABP2 and the FABP5, genes involved in retinoid action, remained unchanged. When compared to controls, fenretinide also significantly decreased the endometriotic lesion xenograft volume.

CONCLUSION: Fenretinide appears to increase retinol uptake in endometriotic stromal cells by increasing STRA6 expression, and potentially reversing the pathological loss of retinoid availability. Treatment with this compound induces apoptosis. Targeting the retinoic acid signaling pathway may be a promising novel treatment for women suffering with endometriosis.

Supported by: K12HD050121, Friends of Prentice, R37HD038691.

CONCEPTION

O-30 Monday, October 20, 2014 04:15 PM


OBJECTIVE: To measure the rate of unplanned pregnancies in users of IUDs and to characterize complications associated with these pregnancies.

DESIGN: This large-scale, prospective, multinational, non-interventional study involved two cohorts: new users of LNG-IUDs and new users of copper IUDs.

MATERIALS AND METHODS: Recruitment began in 2006 and was conducted in six European countries (Austria, Finland, Germany, Poland, Sweden, UK). A ‘non-interference’ approach was used to provide standardized, comprehensive, reliable information under routine medical conditions. Women and physicians completed questionnaires at baseline and again 12 months later. All patient-reported outcomes of interest were validated by obtaining objective information, based on professional opinion and, where
necessary, by direct contact with those health care providers that treated the respective conditions. Low loss to follow-up rates were ensured by a comprehensive 4+ level follow-up procedure. Multivariate techniques such as Cox regression were used to compare IUD user cohorts. The study ended in 2013.

RESULTS: Of the 61,448 women recruited, 70% used an LNG-IUD and 30% a copper IUD. The mean age of LNG-IUD users was higher (37.4 yrs) compared to copper IUD users (35.3 yrs). The 118 contraceptive failures in 26 LNG-IUD users and 92 copper IUD users resulted in a Pearl Index of 0.06 and 0.52, respectively. The risk of contraceptive failure was significantly lower in LNG-IUD users than copper IUD users, with a hazard ratio of 0.16 (95% CI: 0.10-0.25) (adjusted for age, BMI and parity). The statistically significant lower risk of contraceptive failure with LNG-IUD use was evident across all age groups except in women aged 40 to 50. A total of 21 confirmed ectopic pregnancies were reported (in 7 LNG-IUD users and 14 copper IUD users). The associated hazard ratio adjusted for Age, BMI and parity was 0.26 (95% CI: 0.10-0.66).

CONCLUSION: IUD use is associated with a low risk of contraceptive failure, and the risk is significantly lower in users of LNG-IUDs compared to copper IUDs. When pregnancy despite IUD use is suspected, physicians should consider that an extra-uterine pregnancy is not improbable.

O-31 Monday, October 20, 2014 04:30 PM

OBJECTIVE: Interest in vasectomy may vary based on economics factors, given the financial impact of child rearing. With the most recent economic downturn, an increase in vasectomy volume has been suggested by some high volume centers. These associations have not been evaluated at a population based level. We hypothesized that men were more likely to have had a vasectomy as a result of the recession and evaluated data from the National Survey for Family Growth (NSFG) to assess this relationship.

DESIGN: Retrospective analysis of NSFG.

MATERIALS AND METHODS: The NSFG surveyed 10,403 men from across the United States during June 2006 to June 2010 on topics such as birth rates and sterilization. We analyzed the data for vasectomy rates and plans for further children in relation to the Great Recession that lasted the 31 months from December 2007 to June 2009 per the National Bureau of Economic Research. We performed a multivariable logistic regression of all men given the financial impact of child rearing. With the most recent economic downturn, an increase in vasectomy volume has been suggested by some high volume centers. These associations have not been evaluated at a population based level. We hypothesized that men were more likely to have had a vasectomy as a result of the recession and evaluated data from the National Survey for Family Growth (NSFG) to assess this relationship.

RESULTS: Of the 61,448 women recruited, 70% used an LNG-IUS13.5mg for whom placement was attempted (successful in 279), and 281 women randomized to COC who took ≥ 1 pill. In the LNG-IUS13.5mg and COC groups, respectively, the mean age was 23.7 and 23.9 years, and 77.4% and 73.3% were nulliparous. Among LNG-IUS13.5mg and COC users, respectively, 36.6% and 15.3% reported study drug-related AEs. LNG-IUS13.5mg users were more likely than COC users to report acne (9.0% vs 0.4%), dysmenorrhea (8.2% vs 1.1%), ovarian cyst (5.7% vs 0.0%), and abdominal pain (5.0% vs 0.0%). There were 3 drug-related serious AEs in the LNG-IUS13.5mg group; ectopic pregnancy, spontaneous abortion, and ovarian cyst. There were 2 spontaneous abortions in the COC group; these were not considered drug-related. 9.7% and 0.7% of LNG-IUS13.5mg and COC users, respectively, reported AEs related to protocol-required procedures; mainly insertion-related discomfort. At Month 18/end of study (EOS), 268 and 251 women in the LNG-IUS13.5mg and COC groups, respectively, completed a general satisfaction questionnaire; 82.1% and 81.7%, respectively, reported they were either ‘very satisfied’ or ‘satisfied’ with study treatment, with 38.6% and 46.6%, respectively, reporting they were ‘very satisfied’. At Month 18/EOS, in the LNG-IUS13.5mg and COC groups, 263 and 250 women, respectively, completed a satisfaction and bleeding questionnaire; 91.3% and 92.8% rated administration of study treatment as ‘acceptable with/without some inconvenience/discomfort’; 63.1% and 70.0% reported being ‘very/ somewhat satisfied’ with their bleeding pattern; and 66.2% and 48.8% reported that, given the choice, they would continue using study treatment after the study. CONCLUSION: LNG-IUS13.5mg and a COC were both associated with high user satisfaction and were well tolerated. Although the LNG-IUS13.5mg group reported a higher incidence of AEs, they were more likely to prefer to continue their method after the study.

Supported by: Study and abstract funded by Bayer HealthCare.

O-32 Monday, October 20, 2014 04:45 PM
A MULTICENTER, RANDOMIZED PHASE III STUDY COMPARING A 13.5 MG LEVONORGESTREL INTRAUTERINE CONTRACEPTIVE SYSTEM WITH A COMBINED ORAL CONTRACEPTIVE: ANALYSIS OF USER SATISFACTION AND SAFETY. L. Borgatta, K. Roth, S. Rybowski, R. Rosen. Boston University School of Medicine, Boston, MA; Bayer Pharma AG, Berlin, Germany; Bayer HealthCare, Whippany, NJ.

OBJECTIVE: To compare user satisfaction and adverse events (AEs) with use of the 13.5 mg total content levonorgestrel intrauterine contraceptive system (LNG-IUS13.5mg) and a 30ug ethinyl estradiol/3mg drospirenone combined oral contraceptive (COC).

DESIGN: Phase III study at 42 centers.

MATERIALS AND METHODS: Nulliparous and parous women (aged 18-29 years) with regular menstrual cycles (21-35 days), requesting contraception, were randomized to use LNG-IUS13.5mg or a COC for 18 months. Primary outcome: user satisfaction.

RESULTS: The full analysis set included 279 women randomized to LNG-IUS13.5mg for whom placement was attempted (successful in 279), and 281 women randomized to COC who took ≥ 1 pill. In the LNG-IUS13.5mg and COC groups, respectively, the mean age was 23.7 and 23.9 years, and 77.4% and 73.3% were nulliparous. Among LNG-IUS13.5mg and COC users, respectively, 36.6% and 15.3% reported study drug-related AEs. LNG-IUS13.5mg users were more likely than COC users to report acne (9.0% vs 0.4%), dysmenorrhea (8.2% vs 1.1%), ovarian cyst (5.7% vs 0.0%), and abdominal pain (5.0% vs 0.0%). There were 3 drug-related serious AEs in the LNG-IUS13.5mg group; ectopic pregnancy, spontaneous abortion, and ovarian cyst. There were 2 spontaneous abortions in the COC group; these were not considered drug-related. 9.7% and 0.7% of LNG-IUS13.5mg and COC users, respectively, completed a general satisfaction questionnaire; 82.1% and 81.7%, respectively, reported they were either ‘very satisfied’ or ‘satisfied’ with study treatment, with 38.6% and 46.6%, respectively, reporting they were ‘very satisfied’. At Month 18/end of study (EOS), 268 and 251 women in the LNG-IUS13.5mg and COC groups, respectively, completed a general satisfaction questionnaire; 91.3% and 92.8% rated administration of study treatment as ‘acceptable with/without some inconvenience/discomfort’; 63.1% and 70.0% reported being ‘very/somewhat satisfied’ with their bleeding pattern; and 66.2% and 48.8% reported that, given the choice, they would continue using study treatment after the study. CONCLUSION: LNG-IUS13.5mg and a COC were both associated with high user satisfaction and were well tolerated. Although the LNG-IUS13.5mg group reported a higher incidence of AEs, they were more likely to prefer to continue their method after the study.

Supported by: Study and abstract funded by Bayer HealthCare.

A MULTICENTER, OPEN-LABEL, SINGLE-ARM STUDY EXPLORING THE SAFETY OF A NEW 13.5 MG TOTAL DOSE LEVORNESTREL INTRAUTERINE CONTRACEPTIVE SYSTEM IN POSTMENARCHEAL ADOLESCENTS. K. Gemzell-Danielsson, K. Buhl, E. Lukkari-Lax, E. Montegriffo, S. Rybowski, D. Apert. Karolinska Institutet, Stockholm, Sweden; University Hamburg-Eppendorf, Hamburg, Germany; Bayer Oy, Espoo, Finland; Bayer HealthCare, Newbury, United Kingdom; Bayer HealthCare, Whippany, NJ; Sexual Health Clinic, Västöliitto, Finland.

OBJECTIVE: To assess the safety profile of the 13.5 mg total content levonorgestrel intrauterine contraceptive system (LNG-IUS13.5mg; Skylla in the US, Jaydess elsewhere) over 1 year of use in postmenarcheal adolescents aged <18 years.

DESIGN: Phase III study conducted at 36 centers in Europe. Primary outcome: incidence of any treatment-emergent adverse event (TEAE).

Supported by: Study and abstract funded by Bayer HealthCare.

TABLE. Economic characteristics and family planning before and after the Great Recession

<table>
<thead>
<tr>
<th>Factor</th>
<th>Before Recession</th>
<th>After Recession</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annual income</td>
<td>$273,000 ± $155,600</td>
<td>$257,100 ± $154,500</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Working full time</td>
<td>205 (66%)</td>
<td>337 (56%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Have medical insurance</td>
<td>2245 (72.1%)</td>
<td>4057 (69.1%)</td>
<td>0.003</td>
</tr>
<tr>
<td>Want more children</td>
<td>2210 (67.7%)</td>
<td>4007 (68.2%)</td>
<td>0.467</td>
</tr>
<tr>
<td>Underwent vasectomy</td>
<td>123 (3.9%)</td>
<td>257 (4.4%)</td>
<td>0.350</td>
</tr>
<tr>
<td>Number of children intend to have</td>
<td>0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (%)</td>
<td>0 (0)</td>
<td>2 (0.2)</td>
<td></td>
</tr>
<tr>
<td>1 (%)</td>
<td>254 (46.0%)</td>
<td>354 (51.8%)</td>
<td></td>
</tr>
<tr>
<td>2 (%)</td>
<td>187 (33.9%)</td>
<td>353 (43.2%)</td>
<td></td>
</tr>
<tr>
<td>3 (%)</td>
<td>56 (10.1%)</td>
<td>104 (10.1%)</td>
<td></td>
</tr>
<tr>
<td>≥ 4 (%)</td>
<td>43 (7.8%)</td>
<td>25 (2.4%)</td>
<td></td>
</tr>
</tbody>
</table>

*As percentage of federal poverty line. †Student’s t test. ‡Chi squared test. §Kendall tau c test.

e12  ASRM Abstracts Vol. 102, No. 3, Supplement, September 2014
O-34 Monday, October 20, 2014 05:15 PM


OBJECTIVE: Infertility is a common reproductive disease, with a prevalence of 9% to 18% of the general population. To date, no studies have attempted to examine the prevalence and experience of infertility among resident physicians in the United States. In female OB/Gyn residents of age where infertility becomes more prevalent, ability to seek fertility may be influenced by rigorous professional demands and low remuneration. We seek to understand the prevalence, experience, and utilization of infertility services among OB/Gyn residents.

DESIGN: Cross-sectional survey.

MATERIALS AND METHODS: A survey was emailed via Qualtrics to 215 OB/Gyn residency programs nationwide. Demographics, intentions to conceive during residency, fertility problems if any, fertility treatment and outcome if any, affordability of care and perception of support were surveyed.

RESULTS: 253 responses were received in an equal distribution between junior (n=126) and senior (n=127) residents. The majority of respondents were female (91%), 25–35 years old (94%) and married (54%), 85% (195/230) did not actively pursue fertility during residency. 29% (67/234) considered fertility preservation, but only 2% sought consultation. 29% (227/75) experienced infertility of some degree. 63% felt low or no support from the program. 35% reported stigma associated with their infertility.

CONCLUSION: Infertility is prevalent reproductive health impairment among OB/Gyn residents. The majority of residents defer childbearing during residency despite being of advanced reproductive age. Majority felt little or no support from training programs in addressing their fertility care. Further studies are indicated to understand the barriers and impact among resident trainees.

O-35 Monday, October 20, 2014 05:30 PM

EXPOSURE TO POLIDOCANOL FOAM IS TOXIC TO MOUSE AND MONKEY GAMETES AND EMBRYOS. S. Yao, C. Hanna, O. Shalaby, J. Jensen. Division of Reproductive & Developmental Sciences, Oregon National Primate Research Center, Beaverton, OR; Department of Ob/Gyn, Oregon Health & Sciences University, Portland, OR.

OBJECTIVE: Previously we have shown that transcervically administered 5% polidocanol foam (PF) will obstruct the fallopian tubes of Rhesus macaques and baboons. The objective of this study was to examine the toxicity effects of PF on zygotes, cleavage stage pre-implantation embryos, and fresh collected monkey sperm in vitro.

DESIGN: In vitro toxicity study on mouse and monkey gametes and embryos.

MATERIALS AND METHODS: Mouse zygotes were collected from the fallopian tubes of mated PMSG-treated females 16-18 hr after an ovulatory stimulus. Rhesus monkey zygotes were obtained from animals that underwent controlled ovarian stimulation and in vitro fertilization protocols. Zygotes and cleavage stage embryos were exposed to 3% polidocanol foam (PF) or 1% methylcellulose foam (MCF) for 0.5 minutes; controls received no further treatment. Morphology of zygotes and oocytes was assessed by microscopy at the end of incubation and at 24 hrs. Fresh collected Rhesus sperm (2X10^7/ml) were exposed to 3% PF or 1% MCF (0.1ml sperm to 1ml foam) for 0.5 minute. Viability was evaluated and viability was determined by propidium iodine (PI) staining.

RESULTS: A total of 12 mice and 5 monkeys were used in the experiments. Most of the mouse zygotes (51/52 98%) and monkey zygotes (18/18, 100%) exposed to 3% PF underwent lysis, and none showed normal development after 24 hrs. Monkey cleavage-stage cell embryos initially survived PF treatment, but all (16/16, 100%) underwent lysis by 24 hrs. In contrast, most zygotes exposed to 1%MCF (mouse: 29/44, 66%; monkey: 2/14, 14%) and non-treated controls (mouse 42/46, 91%; monkey 3/8, 37.5%) continued to show normal morphology and development at 24hours. Rhesus sperm (95% baseline motility) exposed to 3% PF for 0.5min were completely dead with 0% motility and 100% PI labeling. In contrast, treatment with 1% MCF decreased motility to 50% with 96% viability at 0.5 min. Non-treated sperm maintained 85% motility with 98% or greater viability after treatment.

CONCLUSION: Polidocanol foam is highly toxic to early embryos and gametes. This study provides reassurance that pregnancy is unlikely following inadvertent post-ovulatory or luteal phase exposure to polidocanol foam for permanent contraception.

Supported by: OPP 1025233, NICHD RR00163, P51OD011092 and by RR019386.

O-36 Monday, October 20, 2014 05:45 PM


OBJECTIVE: According to the World Health Organization, the developing world accounts for 99% of the 980 women who die daily from pregnancy related causes, Sub-Saharan Africa alone accounts for half of these deaths. This inverse relationship between contraceptive use and maternal deaths highlights the need for increased contraceptive utilization in sub-Saharan Africa and Nigeria with low contraceptive prevalence. This study was carried out to determine the factors influencing knowledge and usage of "MCMs" by pregnant women in Nigeria.

DESIGN: The cross sectional hospital based study was carried out in three regions (North – East, North – Central and South- west) of Nigeria.

MATERIALS AND METHODS: Women accessing antenatal care completed standardized structured questionnaire in accordance with ethical regulations. Logistic regression was applied to find the determinants of knowledge and use of “MCMs”. Statistically significant results were determined from calculated odds ratios with 95% confidence intervals.

RESULTS: 1418 women participated in the study; average age was 27.70 years ± 5.39 SD with 75.03% response rate. 50% of respondent knew of “MCMs” and 40.34% use “MCMs”. Factors found to positively influence knowledge of “MCMs”: health services availability (doctor for regular gynaecological check OR=1.43; 1.03-2.00 and access to skilled health workers OR=1.55; 1.14-2.12), family status (single compared to monogamic OR=2.38; 1.03-5.50), educational level (secondary OR=2.59; 1.57-4.28 and tertiary OR=2.89; 1.70-4.93 compared to none educated) as well as urban residence (OR=1.72; 1.07-2.75). Attributable risk (AR) for knowledge of “MCM” explained by the studied socio-cultural determinants was 69.68%. Factors associated with use of “MCMs” were family status (single compared to monogamic OR=2.23; 1.04-4.77), religion (Christian religion compared to Muslims OR=1.50; 1.01-2.24) and education (secondary OR=1.96; 1.17-3.27 and tertiary OR=1.86; 1.08-3.20 compared to none.
O-37 Monday, October 20, 2014 06:00 PM

EMERGENCY CONTRACEPTION USE AND UNINTENDED PREGNANCY IN YOUNG CANCER SURVIVORS: A FIRST STUDY. M. R. McLean, a S. A. Dominick, b W. Whitecomb, b H. I. Su, b J. M. Bouknight. a Dept OB/GYN, University of Alabama at Birmingham, Birmingham, AL; b Dept Reproductive Medicine, University of California, San Diego, La Jolla, CA; Division of Biostatistics and Epidemiology, University of Massachusetts Amherst, Amherst, MA.

OBJECTIVE: Female cancer survivors are at risk for unintended pregnancy as estimating fertility after cancer is challenging and family planning (FP) counseling in the context of comorbidities is complex. The rates of unintended pregnancy and emergency contraception (EC) use in cancer survivors are not well characterized. We assessed the frequency of unintended pregnancy and EC use in this population and sought to identify clinical factors associated with EC use.

DESIGN: Retrospective Cohort.

MATERIALS AND METHODS: The Fertility Information Research Study (FIRST) is a cohort study of reproductive outcomes after cancer. Participants are recruited through social media outreach by cancer advocacy groups and six university-based fertility preservation programs. FIRST participants aged 20-44 years were included if they had not undergone hysterectomy or bilateral oophorectomy (N=267). All had a history of cancer and/or exposure to gonadotoxic therapies. Multivariable regression was used to evaluate associations of clinical factors with EC use as prevalence ratios (PR).

RESULTS: Mean age of the cohort was 31.6 years (SD 5.7). Median years since cancer diagnosis was 2.4 (IQR 4.0). The most common cancer type was breast (33%). Seven women (3%) reported unintended pregnancy since cancer diagnosis; 5 (71%) resulted from birth control failure. 29 women (11%) used EC after cancer diagnosis. In the unadjusted models participants <31yrs were more likely to use EC compared to >=31yrs (RR 3.55, p < 0.01) as were non-breast cancer subjects compared to those with breast cancer (RR 3.07, p < 0.05). Other factors associated with EC use included family planning (FP) counseling since cancer diagnosis (RR 3.22, p < 0.01) and unintended pregnancy (RR 4.29, p < 0.01). In adjusted models, age (RR 2.53, p=0.03) and FP counseling (RR 2.72, p=0.01) were associated with EC use.

CONCLUSION: Female cancer survivors face greater risks of adverse pregnancy outcomes related to cancer status and co-morbidities, which underscores the need for FP. FP counseling after cancer diagnosis was related to younger survivors’ EC use and contributes to potential avoidance of unintended pregnancy. Female cancer survivors should receive FP counseling as part of their survivorship care.

Supported by: UL 1 RR024926, HD-058799, MRSG-08-110-01-CEC.

MENTAL HEALTH

O-38 Monday, October 20, 2014 04:15 PM

HEALTH-RELATED QUALITY OF LIFE IMPROVEMENT IN WOMEN WITH ENDOMETRIOSIS-ASSOCIATED PAIN DURING TREATMENT. O. Muneeyiric-Delale, a C. Charles, a N. Sinai, b M. Dalloul, a P. Stratton, a Obstetrics and Gynecology, SUNY Downstate Medical Center, Brooklyn, NY; a Obstetrics and Gynecology, Kings County Hospital Center, Brooklyn, NY; a Biostatistics & Clinical Epidemiology Service, NIH Clinical Center, Bethesda, MD; a Program in Reproductive and Adult Endocrinology, NICHD/NIH, Bethesda, MD.

OBJECTIVE: To assess changes in health-related quality of life (HRQoL) of women with endometriosis-associated pain undergoing treatment with Leuprolide Acetate Depot form (LD) vs Norethindrone Acetate (NA).

DESIGN: Prospective randomized double-masked clinical trial.

MATERIALS AND METHODS: 62 women with endometriosis-associated pain were randomized to receive LD 11.2mg every 3 months or NA 5mg daily for 24 weeks (24W) in Phase I then all were given NA for another 28 weeks (52W) in Phase II. HRQoL was assessed at entry, 24W and 52W using Endometriosis Health Profile Questionnaire-30 (EHP-30) Core Questions.

Data are delta mean ±SD in dimension scores. **p<0.0001; *p<0.001; p<0.02

CONCLUSION: The subjects’ responses were scored individually, and summarized according to the EHP-30 algorithm for dimensions of pain, control & powerlessness, emotional well-being, social support and self-image. Data were analyzed using paired or two sample t-test, Wilcoxon rank-sum test and Wilcoxon signed rank test.

RESULTS: Women were predominantly Black (82%), between age 21 and 47 years with 31 NA and 31 LD. All EHP-30 dimensions significantly improved from entry to 24W in both groups for Phase I. During Phase II, there were no statistically significant changes in summary scores between 24W and 52W in either group. Throughout treatment, there were no statistically significant differences in any of the HRQoL dimensions between treatment groups.

CONCLUSION: HRQoL significantly improved both with norethindrone acetate and leuprolide treatment. Improvements in the EHP-30 dimensions of pain, control & powerlessness, emotional well-being, social support and self-image were maintained on norethindrone only treatment.

Supported by: NIH: R01-HD043281, Intramural Program, PRAE/NICHD and BCES/CC.

O-39 Monday, October 20, 2014 04:30 PM

TOWARD AN IDEAL PATIENT EXPERIENCE: LONGITUDINAL ASSESSMENT OF PATIENT KNOWLEDGE, EXPERIENCE, AND REASONS FOR DISCONTINUATION OF INFERTILITY TREATMENT. K. J. Childress, a A. K. Lawson, a M. S. Ghant, G. Mendoza, a M. L. Steinberg, a E. Confinio, a E. E. Marsh. Obstetrics and Gynecology-ReI Division, Northwestern University Feinberg School of Medicine, Chicago, IL.

OBJECTIVE: To determine patient understanding of and satisfaction with infertility treatment, and reasons for discontinuation of treatment after the first infertility visit.

DESIGN: Prospective survey study.

MATERIALS AND METHODS: English speaking, non-pregnant, 18-50 year old women completed pre and post-visit surveys at their first infertility clinic visit (T1) and follow up surveys at 1 (T2) and 3 (T3) months. Surveys queried patients on their understanding of fecundity, personal treatment plan, likelihood of successful pregnancy, satisfaction with treatment and associated anxiety, and reasons for discontinuation of treatment.

RESULTS: Surveys were completed by 234, 127, and 79 eligible patients at T1, T2, and T3 respectively. Participants were 34.2±4.6 (mean±SD) years old, 83.8% married, 79.7% White, 6.8% Black, 9.5% Asian, and >90% had ≥4 years college education. Neither participants’ understanding of fecundity nor personal beliefs of their chances of conception significantly changed over time. 95.6% and 80.4% of participants were satisfied with treatment at T2 and T3. Reasons for dissatisfaction with treatment included poor understanding of the treatment process, dissatisfaction with provider communication, and/or complexity and length of the treatment process. 45 participants discontinued treatment over the 3-month period. Only 45.4% of participants were in touch with a financial counselor at T2. 98.2% of those participants not in touch with a financial counselor discontinued treatment (p=.008). Other reasons for discontinuation of treatment included pregnancy (48.9% of those who discontinued), life events, personal health, and desire to conceive naturally. Anxiety was reported by >90% of responders at T2 and T3, with treatment-related factors (e.g. side effects, length of treatment) being the most cited reason.

CONCLUSION: While most patients were satisfied with their fertility experience, reasons for dissatisfaction and discontinuation centered around challenges in patient-staff communication and patient education. These findings highlight an opportunity to increase patient satisfaction by improving individualized patient counseling and provider-patient communication.

e14 ASRT Abstracts Vol. 102, No. 3, Supplement, September 2014
ABNORMAL STRICT MORPHOLOGY EVOSES ANXIETY IN INFERTILE MALES. J. R. Kovac, a R. P. Smith, a M. Cajipe, c R. Ramasamy, c J. Scovell, d J. D. Lamb, b L. I. Lipshultz, a "Urology of Indiana, Carmel, IN; bUniversity of Virginia, Charlottesville, VA; bBaylor College of Medicine, Houston, TX.

OBJECTIVE: Males undergoing infertility testing face anxiety and stress. Indeed, given recent studies linking infertility with cancer, an association between semen parameters and health outcomes has been drawn. We sought to determine whether the findings of abnormal strict morphology (SM) contributed to a patient's anxiety and generated health concerns.

DESIGN: A cohort study on patients from a high volume, tertiary, academic infertility clinic (2010-2013).

MATERIALS AND METHODS: A total of 156 men with a SM of <4% normal forms (NF, Kruger strict criteria) were contacted (22 refused participation), <4% SM was stratified into subgroups of 0.5-4% and 0% NF. Randomly selected men >4% NF were controls (n=18). Anxiety was measured using a 10-point Licker scale & concern for general health was quantified via an IRB-approved questionnaire.

RESULTS: Average age of men <4% (n=134) was similar to controls. Patients with <4% NF rated their levels of anxiety at 4.8±0.2 (1=no anxiety; 10=severe anxiety). 9.8% (n=13/133) of these men rated their anxiety at the highest level (10/10). This was significantly higher (p<0.05) than controls (anxiety=3.2±0.5) where no men rated their anxiety at >5. In addition, 25% of men <4% NF (n=34/134) expressed significant concerns about their general health compared to 0% of control men (n=0/18). Subgroup analysis compared men with 0.5-4% NF to those with 0% NF. A total of 9.3% (n=10/107) of men with 0.5-4% NF rated anxiety at 10/10 with 25.9% (n=28/108) having health concerns. Men with only 0% NF were not significantly different with 12.5% (n=3/24) rating their anxiety at 10/10 and 25% (n=6/24) having major concerns about their general health.

CONCLUSION: Men are anxious and concerned about their overall health as a direct consequence of their abnormal SM. The presence of abnormal SM corresponded to increased rates of anxiety and health concerns. The severity of the abnormal SM did not affect patient perception. A discussion concerning the abnormal semen analysis in general, and SM in particular, is an important part of the counseling process.

Supported by: JRK is Supported by a Male Reproductive Health Research (MRHR) Career Development Physician Scientist Award (k12) (HD073917-01) from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) Program to DIL.

O-41 Monday, October 20, 2014 05:00 PM

EGG DONORS WORKING WITH GESTATIONAL SURROGACY AGENCIES: MOTIVATIONS AND PERSONALITY TRAITS. Z. Brew, a K. Bergman, a R.-J. Green, a K. Katuzy, b Rockway Institute at CSSP/Alliant International University, San Francisco, CA; aFertility Counseling Services, Inc, Los Angeles, CA.

OBJECTIVE: This study investigates motivations and personality characteristics of egg donors working with gestational surrogacy agencies. The personality traits of egg donors and non-donors from a demographically matched sample of women were examined.

DESIGN: Researchers compared the personality characteristics of 100 randomly selected egg donors and a normative control group of 100 women (non-egg donors) matched for race/ethnicity, education, and age (20-29 years) on the Minnesota Multiphasic Personality Inventory (MMPI-2). Major themes from egg donors responses to an intake question regarding motivations were also analyzed.

MATERIALS AND METHODS: De-identified interview transcripts and MMPI-2 data were obtained from an egg donor agency's archival database. Similar MMPI-2 data on a non-egg donor sample of women were obtained from an independent research facility specializing in personality assessment. Mean scores of the two groups (egg donors versus non-egg donors) were compared on MMPI-2 clinical and nonclinical scales using a series of independent t-tests.

RESULTS: Comparisons on MMPI-2 revealed that the control group scored significantly higher (p < .01) on Hypochondriasis, Depression, Psychopathic Deviant, Psychoticism, Schizophrenia, Social Introversion, Psychoticism, Constraint, Negative Emotionality/Neuroticism, Introversion/Low Pos.

FERTILITY & STERILITY® e15

O-42 Monday, October 20, 2014 04:15 PM

FACTORS THAT INFLUENCE REJECTION AND NON-COMPLETION FOR MEXICAN OVUM DONOR CANDIDATES. C. Palachu, a A. M. Braverman, a J. Rosales, a R. Melendez, a J. I. Obeso, a "IECH Fertility CenterIECH Fertility Center, Monterrey, Nuevo Leon, Mexico; aObstetrics & Gynecology, Thomas Jefferson University, Philadelphia, PA.

OBJECTIVE: To explore why Mexican ovum donor candidates are not accepted to psychological, medical and non-medical reasons.

DESIGN: A retrospective chart review of women who applied to be egg donors at a large Mexican center to identify the reasons for inclusion or exclusion in the program.

MATERIALS AND METHODS: Demographic information, evaluation data, and laboratory results were reviewed. Frequencies and percents were calculated; total response was reported for each item. Differences were tested using an ANOVA.

RESULTS: Women ages 18-25 were solicited to be ovum donors. 457 applicants to the egg donor program were reviewed, of those a total 344 were rejected due to psychological, medical and non-medical reasons. Three groups of non-accepted donors were formed. Group I included donors rejected due to medical reasons (89), group II due to non-medical reasons (139) and group III due to psychological issues (116). There were no significant demographic differences among the 3 groups. Donors with children were less likely to be rejected (p < .05). Further analysis was performed to determine if the presence of children in rejected donors due to psychological reasons was statistically relevant. Group III was analyzed according to the reason the donor was rejected. Of a total of 116 candidates, 75 were rejected due to emotional instability, 19 due to a personality disorder, 8 due to the presence of depression, and 14 due to a family psychiatric history. In each case, rejected donors were subdivided into two groups according to the presence of children. Statistical analysis showed that rejected donors due to emotional instability were the ones with no children but higher levels of education (p < .005), currently unemployed (p < .03), and single (p < .006).

CONCLUSION: Psychological issues and non-medical issues contributed most to Mexican women not becoming donors. Donors who do not have children were more likely to not complete for reasons other than medical or psychological. Donors with children may be more motivated for financial reasons as culturally women tend to have children younger which may contribute more to a financial incentive. The women with more education, who have no children and are single may not be the most successful egg donor candidate as they are more likely to be emotionally unstable. Another influence may be that women who have children may have more empathy for the recipients and, therefore, more likely to complete the process.

O-43 Monday, October 20, 2014 05:30 PM

THE EFFECT OF COPING STYLES ON MENTAL HEALTH OUTCOMES FOR WOMEN RECEIVING IVF TREATMENT. S. R. Holley, a,b L. A. Pasch, a b A. L. Belohlavek, a,b N. E. Adler, a P. Katz, a "Psychology, San Francisco State University, San Francisco, CA; a Psychiatry, University of California, San Francisco, San Francisco, CA; a,bSchool of Medicine, University of California, San Francisco, San Francisco, CA.

OBJECTIVE: People cope with the stress of fertility treatment in different ways. Some use approach-oriented strategies (e.g., planning), whereas others use avoidance-oriented strategies (e.g., denial). This study evaluated whether these respective coping styles predict mental health outcomes for women seeking fertility treatment.
DESIGN: 157 women in a prospective cohort study over an 18-month period.

MATERIALS AND METHODS: Participants completed questionnaires at baseline and at the 18-month follow-up. At baseline, coping strategies were assessed via the Brief Cope Inventory and perceived stress via the Perceived Stress Scale. At baseline and follow up, depression was assessed via the Center for Epidemiologic Studies Depression scale and anxiety via the State-Trait Anxiety Inventory. "Coping style" was operationalized as the difference between approach and avoidance coping scores (approach - avoidance). We hypothesized a negative association such that low coping style scores (i.e., an avoidance coping style) would be associated with greater depression and anxiety.

RESULTS: Multiple regression models were used; all models controlled for age, time trying to conceive, and perceived stress. At baseline, coping style scores were negatively associated with depression and anxiety. For the longitudinal models, the covariates of IVF failures and baseline depression and anxiety scores were added. Coping style predicted depression and anxiety at follow up; again with low scores associated with greater psychological symptomatology.

### Table 1

<table>
<thead>
<tr>
<th>Depression</th>
<th>Anxiety</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline Models</strong></td>
<td></td>
</tr>
<tr>
<td>Step 1: Covariates</td>
<td>$R^2$</td>
</tr>
<tr>
<td>Age</td>
<td>.05</td>
</tr>
<tr>
<td>Years Trying to Conceive</td>
<td>.02</td>
</tr>
<tr>
<td>Perceived Stress</td>
<td>.64***</td>
</tr>
<tr>
<td><strong>Step 2: Coping Style</strong></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>.02</td>
</tr>
<tr>
<td>Years Trying to Conceive</td>
<td>.05</td>
</tr>
<tr>
<td>Perceived Stress</td>
<td>.21*</td>
</tr>
<tr>
<td>IVF Failures</td>
<td>.06</td>
</tr>
<tr>
<td>Baseline Depression</td>
<td>.21*</td>
</tr>
<tr>
<td>Baseline Anxiety</td>
<td></td>
</tr>
<tr>
<td><strong>Step 2: Coping Style</strong></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-.18**</td>
</tr>
</tbody>
</table>

CONCLUSION: The way patients cope with the stress of fertility treatment appears predictive of psychological distress. Specifically, an avoidance coping style was associated with concurrent and future depression and anxiety. Interventions that shift coping strategies from avoidance-oriented ones to approach-oriented ones may help reduce the distress associated with treatment.

Supported by: Support provided by NICHD/NIH Grant # PO1 HD37074.

O-44 Monday, October 20, 2014 05:45 PM


OBJECTIVE: In infertility treatment, patients experience loss following non pregnancy test result as well as after miscarriage. Moreover, such losses are frequently repeated, tending to enhance patient anxieties surrounding pregnancy. And yet the stationing of psychological counselors remains atypical within fertility treatment facilities in Japan, and access to grief care is not yet sufficiently widespread. In this study, an attempt was made to introduce a system whereby support cards were created for use in grief counseling after miscarriage, with physicians, nurses, and psychological counselors working together to provide care.

DESIGN: Retrospective study.

MATERIALS AND METHODS: Between December 2012 and March 2014, support cards were given to 103 patients determined to have experienced miscarriage within 9 weeks of becoming pregnant by in vitro fertilization. These cards carried explanations by counselor that can be experienced after a miscarriage and ways to deal with such feelings, instructions on how to make appointments for grief counselling. After the physician had explained the miscarriage assessment, the cards were handed to patients by nurses.

RESULTS: The support cards were used for clients to receive psychological counselling in 15 of 103 cases (14.2%). The average age of the subjects in this User Group (n=15) was 36.5 ± 4.6 years; these subjects had undergone Embryo transfer (ET) an average of 4 ± 1.6 times, experienced non pregnancy test results an average of 2.6 ± 1.6 times, miscarriage an average of 1.4 ± 0.6 times. The average age of the subjects in the Non-User Group (n=88) was 36.6 ± 4.2 years; they had undergone ET an average of 2.6 ± 1.7 times, experienced non pregnancy test results an average of 1.3 ± 1.6 times, miscarriage an average of 1.2 ± 0.5 times. The numbers of ET and non pregnancy test results were both significantly higher in the User Group (p<0.05). Card usage occurred variably between the date of miscarriage assessment and 2.5 months later.

CONCLUSION: Post-miscarriage support card users had experienced more ET and non pregnancy test results than non-users. Therefore, it appears that psychological support interventions would seem to be particularly effective for patients who have repeatedly experienced loss through seeking infertility treatments. Additionally, given the existence of patients who made use of support cards not only immediately after the assessment of miscarriage but even months afterwards, grief care needs to incorporate a long-term perspective.

O-45 Monday, October 20, 2014 06:00 PM

COMPARISON OF SOCIAL ADAPTATION IN PRESCHOOL SINGLETON CHILDREN BORN AFTER FROZEN AND FRESH EMBRYO TRANSFER IN ART. L.-F. Xing, L.-T. Chen, Y.-L. Qian. Reproductive Medicine, Women’s Hospital, School of Medicine, Zhejiang University, Hangzhou, Zhejiang, China.

OBJECTIVE: Investigate whether there is a difference in social adaptation between preschool full-term singleton children born after frozen and fresh embryo transfer in IVF/ICSI.

DESIGN: The controlled study followed up 250 preschool singleton children born after IVF/ICSI with or without FET and 152 strictly matched NC children, in order to measure their social adaptation abilities.

MATERIALS AND METHODS: In order to eliminate interference of other difficulties associated with multiple births, low birth weight and prematurity, all of which were known to adversely affect children’s early development, inclusion criteria for children born after IVF/ICSI were as follows: 1) without PGD, 2) singleton, 3) full-term delivery, 4) normal birth weight, 5) no mortal disease, 6) pass the routine IQ test (measured by the Chinese Revised Version of the Wechsler Intelligence Scale for Children). 352 eligible children as well as their parents were invited for the study and a total of 250 children (response rate of 71%, including 99 IVF-conceived with fresh embryo transfer, 49 IVF-conceived with FET, 58 ICSI-conceived with fresh embryo transfer and 44 ICSI-conceived with FET) were recruited finally. After strictly matching all socio-demographic variables, 152 NC children as controls were randomly recruited from four randomly selected kindergartens in our province.

RESULTS: 1. After strictly matching all socio-demographic variables, children conceived through ART did not show significantly lower social adaptation abilities in all dimensions of the scale (P>0.05). 2. Children born after ART (whether or not with FET) showed significantly better performances than children born after ART with a fresh embryo. The difference is much more significant in children born after IVF than ICSI.

### Table 2

<table>
<thead>
<tr>
<th>IVF fresh</th>
<th>IVF with FET</th>
<th>ICSI with fresh embryo transfer</th>
<th>ICSI with FET</th>
</tr>
</thead>
<tbody>
<tr>
<td>15.3</td>
<td>17.1*</td>
<td>16.1</td>
<td>16.5</td>
</tr>
<tr>
<td>7.4</td>
<td>7.6</td>
<td>7.6</td>
<td>7.3</td>
</tr>
<tr>
<td>6.9</td>
<td>7.0*</td>
<td>7.2</td>
<td>7.6</td>
</tr>
<tr>
<td>10.0</td>
<td>11.0*</td>
<td>10.5</td>
<td>10.3</td>
</tr>
<tr>
<td>9.3</td>
<td>10.2*</td>
<td>9.1</td>
<td>10.3*</td>
</tr>
<tr>
<td>5.1</td>
<td>6.0*</td>
<td>5.2</td>
<td>5.5</td>
</tr>
<tr>
<td>10.2</td>
<td>10.5*</td>
<td>10.1</td>
<td>10.3</td>
</tr>
</tbody>
</table>

* means significantly different compared with its fresh embryo group.

CONCLUSION: Children born after IVF/ICSI showed similar social adaptation level with their NC counterparts. Children born after FET in IVF/ICSI cycles demonstrated better performances in major dimensions of social adaptation scale compared with children born after fresh embryo transfer.

Supported by: The research was Supported by the National NSFC Program of P. R. China (No.81371244).
**ART OUTCOME PREDICTORS - CLINICAL I**

O-46 Monday, October 20, 2014 04:15 PM

ASSOCIATION OF ASSISTED REPRODUCTIVE TECHNOLOGY (ART) TREATMENT AND PARENTAL INFERTILITY DIAGNOSIS WITH AUTISM IN ART-CONCEIVED CHILDREN. D. M. Kissin, Y. Zhang, S. L. Boulet, C. Fountain, P. Bearman, L. Schieve, M. Yeargin-Allsopp, D. J. Jamieson. 1National Center for Chronic Disease Prevention and Health Promotion, Centers for Disease Control and Prevention, Atlanta, GA; 2Department of Sociology, Fordham University, New York, NY; 3Department of Sociology, Columbia University, New York, NY; 4National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention, Atlanta, GA.

OBJECTIVE: To assess whether assisted reproductive technology (ART) treatment factors or infertility diagnoses are associated with autism among ART-conceived children.

DESIGN: Retrospective population-based cohort study.

MATERIALS AND METHODS: We linked National ART Surveillance System (NASS) data for 1996-2006, California Birth Certificate data for 1997-2006, and California Department of Developmental Services Autism Caseload data, which include information on the vast majority of persons with autism in California, for 1997-2011 to allow 5 years of follow-up for each child in the study. Among ART-conceived children born between 1997 and 2006, we calculated trends in autism diagnosis and adjusted hazard risk ratios (aHRR) and 95% confidence intervals (CI) for the association of autism diagnosis with ART treatment factors and parental infertility diagnoses; we adjusted for infant sex, plurality, gestational age, birth weight, maternal and paternal age at delivery, number of previous births, mode of delivery, male factor infertility and the interaction between plurality and mode of delivery.

RESULTS: Among ART-conceived infants born in California between 1997 and 2006, the annual incidence of autism diagnosis remained at about 1.0%, ranging from 0.9 to 1.3% (P for trend 0.66). Incidence of autism diagnosis in offspring was lower when ART patients had unexplained (aHRR 0.4; CI 0.2-0.8) or tubal factor (aHRR 0.5; CI 0.3-0.9) infertility, and higher when intracytoplasmic sperm injection (ICSI) was used (aHRR 1.9; CI 1.1-3.2), when compared to cases without these patient and treatment characteristics.

CONCLUSION: Our study suggests that unexplained and tubal factor parental infertility is associated with decreased risk, and use of ICSI is associated with increased risk of autism diagnosis in ART-conceived children during the first 5 years of life. More studies are needed to examine possible mechanisms of these associations.

Supported by: NIH Director’s Pioneer Award program, part of the NIH Roadmap for Medical Research, through grant number 1 DPI OD003635-01 and the National Institutes of Mental Health award number R21MH096122.

O-47 Monday, October 20, 2014 04:30 PM

PREGNANCY OUTCOMES DECLINE WITH INCREASING RECIPIENT BMI: AN ANALYSIS OF 22,317 DONOR/RECIPIENT CYCLES FROM THE 2008-2010 SART REGISTRY. M. P. Provost, K. S. Acharya, C. R. Acharya, J. S. Yeh, R. G. Steward, J. L. Eaton, J. M. Goldfarb, S. J. Muasher. 1Division of Reproductive Endocrinology and Infertility, Duke University Medical Center, Durham, NC; 2Department of Biostatistics and Bioinformatics, Duke University Medical Center, Durham, NC; 3University Hospitals Fertility Center, Beachwood, OH.

OBJECTIVE: To examine the effect of recipient BMI on IVF outcomes in donor oocyte cycles.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: Previous studies looking at recipient BMI have demonstrated no effect of recipient obesity and IVF success, while others have been limited by sample size. A total of 22,317 donor oocyte cycles from the 2008-2010 SART registry were stratified into cohorts based on WHO BMI guidelines and analyzed (Table). This cohort represents the largest study on this topic to date. Cycles reporting normal recipient BMI (18.5-24.9) were used as the reference group (REF). Main outcomes measured were clinical pregnancy rate, live birth, implantation rate and miscarriage rate. All pregnancy outcomes were analyzed using multivariate logistic regression.

RESULTS: Success rates and adjusted odds ratios (OR) with 95% confidence intervals for all pregnancy outcomes were most favorable in cohorts with recipients of low and normal BMI, but progressively worsened as BMI increased.

<table>
<thead>
<tr>
<th>BMI (n)</th>
<th>Clinical Pregnancy Rate % [OR]</th>
<th>Live Birth Rate % [OR]</th>
<th>Implantation Rate % [OR]</th>
<th>Miscarriage % [OR]</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;18.5 (637)</td>
<td>59.7 [1.05]*</td>
<td>51.2 [1.07]</td>
<td>49.3 [1.00]</td>
<td>8.5 [0.90]</td>
</tr>
<tr>
<td>18.5-24.9 (13,058)</td>
<td>59.6 [REF]</td>
<td>51.4 [REF]</td>
<td>49.3 [REF]</td>
<td>8.6 [REF]</td>
</tr>
<tr>
<td>25-29.5 (5,394)</td>
<td>57.7 [0.92]*</td>
<td>48.6 [0.88]*</td>
<td>47.9 [0.99]</td>
<td>10.2 [1.19]*</td>
</tr>
<tr>
<td>30-34.9 (2,016)</td>
<td>53.6 [0.77]*</td>
<td>43.9 [0.75]*</td>
<td>42.6 [0.94]*</td>
<td>11.2 [1.26]</td>
</tr>
<tr>
<td>35-39.9 (823)</td>
<td>56.9 [0.84]*</td>
<td>46.7 [0.84]*</td>
<td>45.6 [0.97]</td>
<td>12.3 [1.31]</td>
</tr>
<tr>
<td>40-44.9 (280)</td>
<td>52.9 [0.81]</td>
<td>40.0 [0.63]</td>
<td>40.9 [0.94]</td>
<td>15.9 [2.03]*</td>
</tr>
<tr>
<td>45-49.9 (77)</td>
<td>48.0 [0.63]</td>
<td>42.9 [0.72]</td>
<td>39.3 [0.94]</td>
<td>8.3 [0.97]</td>
</tr>
<tr>
<td>&gt;50 (32)</td>
<td>53.1 [0.41]*</td>
<td>40.6 [0.37]*</td>
<td>39.7 [0.84]*</td>
<td>7.1 [1.20]</td>
</tr>
</tbody>
</table>

* = P < 0.05. OR = Adjusted Odds ratio compared to REF

CONCLUSION: Success rates in donor/recipient IVF cycles are more favorable in recipients with low and normal BMI. There is a progressive and statistically significant worsening of outcomes in groups with higher BMI. More research is needed to determine the relationship between BMI and pregnancy outcomes after IVF in recipients of oocyte donation.

O-48 Monday, October 20, 2014 04:45 PM

BIRTH OUTCOMES BY INFERTILITY TREATMENT: ANALYSES OF THE MASSACHUSETTS OUTCOMES STUDY OF ASSISTED REPRODUCTIVE TECHNOLOGIES (MOSART). B. Luke, J. B. Stern, M. Kotelchuck, M. D. Hornstein, E. Declercq, B. Cohen, H. Diop, Michigan State University, East Lansing, MI; 1Geisel School of Medicine at Dartmouth, Lebanon, NH; 2MassGeneral Hospital for Children, Boston, MA; 3Brigham & Women’s Hospital, Boston, MA; 4Boston University School of Public Health, Boston, MA; 5Massachusetts Department of Public Health, Boston, MA.

OBJECTIVE: To evaluate ART pregnancy outcomes by infertility treatments.

DESIGN: Historical cohort.

MATERIALS AND METHODS: ART data on women who were treated and gave birth in Massachusetts between 2004 and 2008 were linked to vital records and hospital data. Live births were categorized by ART treatment parameters (oocyte and semen sources, intracytoplasmic sperm injection, assisted hatching, embryo state, embryos transferred, fetal heartbeats). Risks of preterm birth (PTB), low birthweight (LBW), small-for-gestation (SGA), pregnancy hypertension (PIH), gestational diabetes (GDM), prenatal admissions, and primary cesarean delivery were modeled by plurality at birth for each treatment parameter using logistic regression, adjusted for parental ages, race/ethnicity, education; infertility diagnoses; and maternal preexisting medical conditions (chronic hypertension and diabetes mellitus) as adjusted odds ratios, AORs, and 95% confidence intervals.

RESULTS: Among the 6,526 singleton and 4,422 twin pregnancies, risks for adverse outcomes were significantly higher among twins, including PIH (AOR 2.58, 2.27-2.42), GDM (AOR 1.30, 1.10-1.54), prenatal admissions (AOR 3.65, 3.12-4.26), primary cesarean delivery (AOR 5.83, 5.17-6.57), PTB (AOR 11.84, 10.56-12.77), LBW (AOR 10.68, 9.45-12.08), and SGA (AOR 2.17, 1.86-2.53); the risks were only slightly modified with adjustment for each treatment parameter. Oocyte source (donor), embryo state (thawed), and fetal heartbeats (>one) were each associated with increased risks: donor oocytes (PIH, AOR 1.87, 1.45-2.42; primary cesarean delivery and PTB, AOR 1.43, 1.14-1.78 and AOR 1.43, 1.11-1.83, respectively); thawed embryos (PIH, AOR 1.30, 1.08-1.57); two versus one fetal heartbeat (PTB, AOR 1.49, 1.16-1.91; LBW, AOR 1.57, 1.19-2.08); three versus one fetal heartbeat (PTB, AOR 2.07, 1.39-3.09; LBW, AOR 2.30, 1.52-3.49; SGA, AOR 2.04, 1.25-3.34). The risks for LBW and SGA were significantly lower with the use of thawed embryos (AOR 0.79, 0.65-0.96 and AOR 0.38, 0.28-0.53, respectively).

CONCLUSION: Plurality was the predominant ART treatment risk factor for adverse pregnancy and birth outcomes, associated with substantial excess morbidity for both mother and infants.

Supported by: NIH R01 HD 064595 and R01 HD067270.

FERTILITY & STERILITY® e17
O-49 Monday, October 20, 2014 05:00 PM

PREGNANCY OUTCOMES DECLINE WITH INCREASING BMI: AN ANALYSIS OF 239,127 CYCLES FROM THE 2008-2010 SART REGISTRY, M. P. Provost, a K. S. Acharya, a C. R. Acharya, a J. S. Yej, a R. G. Steward, a J. L. Eaton, a J. M. Goldfarb, a S. J. Muasher. b d
Division of Reproductive Endocrinology and Infertility, Duke University, Durham, NC; Department of Biostatistics and Bioinformatics, Duke University, Durham, NC; a University Hospitals Fertility Center, Beachwood, OH.

OBJECTIVE: To examine the effect of BMI on IVF outcomes in autologous patients as well as other diagnostic subgroups.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: This represents the largest and most recent cohort study on this issue to date. A total of 239,127 fresh IVF cycles from the 2008-2010 SART registry were stratified into cohorts based on WHO BMI guidelines and analyzed (Table). Cycles reporting normal BMI (18.5-24.9) were used as the reference group (REF). Sub-analyses were performed on cycles reporting purely male factor (MF) infertility as well as those with PCOS (89,354 and 34,137 cycles, respectively). Main outcomes measured were clinical pregnancy rate, live birth, implantation rate and miscarriage rate. All pregnancy outcomes were analyzed using multivariable logistic regression.

RESULTS: Success rates and adjusted odds ratios (OR) with 95% CIs for all pregnancy outcomes were most favorable in cohorts with low and normal BMI, and progressively worsened as BMI increased (Table). Obesity also had a statistically significant impact on IVF outcomes in cycles performed for pure MF and PCOS, which has not been previously reported.

Pregnancy Outcomes with Increasing BMI

<table>
<thead>
<tr>
<th>BMI (n)</th>
<th>Clinical Pregnancy Rate (OR)</th>
<th>Live Birth Rate (OR)</th>
<th>Implantation Rate (OR)</th>
<th>Miscarriage Rate</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;18.5 (7,149)</td>
<td>37.7 (0.99)*</td>
<td>31.2 (0.93)</td>
<td>30.4 (0.99)*</td>
<td>11.4 (1.10)*</td>
<td></td>
</tr>
<tr>
<td>18.5-24.9 (134,588)</td>
<td>37.9 (REF)</td>
<td>31.4 (REF)</td>
<td>29.5 (REF)</td>
<td>11.3 (REF)</td>
<td></td>
</tr>
<tr>
<td>25-29.9 (54,882)</td>
<td>36.8 (0.96)</td>
<td>29.8 (0.94)</td>
<td>28.3 (0.99)</td>
<td>12.7 (1.13)</td>
<td></td>
</tr>
<tr>
<td>30-34.9 (24,922)</td>
<td>35.7 (0.90)</td>
<td>28.0 (0.84)</td>
<td>26.9 (0.99)</td>
<td>14.6 (1.32)</td>
<td></td>
</tr>
<tr>
<td>35-39.9 (11,747)</td>
<td>33.7 (0.81)</td>
<td>26.3 (0.75)</td>
<td>25.8 (0.96)</td>
<td>15.3 (1.41)</td>
<td></td>
</tr>
<tr>
<td>40-44.9 (4,084)</td>
<td>32.0 (0.78)</td>
<td>24.3 (0.71)</td>
<td>23.6 (0.95)</td>
<td>14.8 (1.28)</td>
<td></td>
</tr>
<tr>
<td>45-49.9 (1,292)</td>
<td>30.6 (0.73)</td>
<td>22.8 (0.66)</td>
<td>22.9 (0.95)</td>
<td>17.6 (1.62)</td>
<td></td>
</tr>
<tr>
<td>&gt;50 (463)</td>
<td>30.0 (0.68)</td>
<td>21.2 (0.53)</td>
<td>20.3 (0.92)</td>
<td>20.3 (1.92)</td>
<td></td>
</tr>
</tbody>
</table>

*p = not statistically significant. All other values P < 0.05. OR = Adjusted OR compared to REF

CONCLUSION: Success rates in autologous cycles, including those done for specifically MF or PCOS, are highest in those with low and normal BMI. Furthermore, there is a progressive and statistically significant worsening of outcomes in groups with higher BMI. More research is needed to determine the causes and extent of how BMI affects IVF success rates in other patient populations.

O-51 Monday, October 20, 2014 05:30 PM

FEWER PRETERM BIRTHS IN UNSTIMULATED VERSUS STIMULATED IN VITRO FERTILIZATION (IVF), W. Mak, a L. A. Kondapalli, b M. DiMattina, c J. D. Gordon, c G. Celia, c M. Puyson, c Division of REI, Yale School of Medicine, New Haven, CT; bDivision of REI, University of Colorado Denver Anschutz Medical Campus, Aurora, CO; cDominion Fertility, Arlington, VA.

OBJECTIVE: To compare birth outcomes of infants conceived by natural cycle (unstimulated) IVF to those conceived by stimulated IVF cycles.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: Non-smoking women undergoing fresh IVF-ET cycles from 2007-2011 at a single private practice center were stratified by stimulated (n=120) or unstimulated (n=113) exposure status. IVF cycle and birth outcomes were analyzed using appropriate parametric and non-parametric tests.

<table>
<thead>
<tr>
<th>TABLE 1. Cycle Characteristics and Birth Outcomes α</th>
</tr>
</thead>
<tbody>
<tr>
<td>STIMULATED IVF (n=120)</td>
</tr>
<tr>
<td>-------------------------------------------</td>
</tr>
<tr>
<td>No of oocytes retrieved</td>
</tr>
<tr>
<td>ICSI (n, %)</td>
</tr>
<tr>
<td>Day of embryo transfer</td>
</tr>
<tr>
<td>No of embryos transferred</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
</tr>
<tr>
<td>Birth weight (grams)</td>
</tr>
<tr>
<td>Low birth weight (&lt;2500g)</td>
</tr>
<tr>
<td>Large gestational age (&gt;4000g)</td>
</tr>
</tbody>
</table>

α Values are mean ± SD or n (%)

CONCLUSION: This is the first report of improved implantation rates in patients receiving day 3 ET based on the adjunctive use of a time-lapse enabled test and traditional morphology. The adjunctive Eeva Test day 3 implantation rate appears equivalent to published day 5 blastocyst rates. Our findings confirm that the non-invasive Eeva Test can be used to increase the clinician’s confidence in recommending eSET on day 3, thereby achieving the benefits of higher pregnancy rates, lower multiple pregnancy rates and improved clinical outcomes. The current study is ongoing and will further evaluate the impact on pregnancy rates and multiple rates with increased sample size.

O-50 Monday, October 20, 2014 04:15 PM


OBJECTIVE: A recent publication described development of a test that automatically measures key time-lapse parameters and provides quantitative information regarding embryo development (Eeva® Test) (Conaghan et al. 2013). The objective of this study was to determine if use of the test as an adjunct to traditional morphology for day 3 embryo transfer (ET) results in improved clinical outcomes.

DESIGN: Pilot cohort study using concurrent controls.

MATERIALS AND METHODS: IRB approval was obtained. 51 patients consented to using the Eeva Test as an adjunct to morphology for day 3 ET. The Eeva Test generates a High or Low score based on the automatic extraction of key cell division timings from time-lapse videos. Inclusion criteria were: maternal age < 41 years, < 3 previous failed IVF attempts, and ≥4 zygotes available for image analysis. In the Eeva Test group, preference in selection for transfer was given to embryos that had good morphology and exhibited a High score. In the control group, embryos were selected for transfer based on morphology only. Clinical characteristics, implantation and pregnancy were compared. χ² test was used for statistical analysis.

RESULTS: Positive hCG and implantation rates were significantly higher when Eeva Test scores were used as an adjunct to morphology to select embryos for transfer on Day 3. There were no significant differences in clinical characteristics between groups.

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Eeva Test</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>51</td>
<td>131</td>
</tr>
<tr>
<td>Age (mean ± sd)</td>
<td>34.5 ± 3.8</td>
<td>34.4 ± 3.4</td>
</tr>
<tr>
<td>Eggs #</td>
<td>16.5 ± 6.7</td>
<td>16.5 ± 6.1</td>
</tr>
<tr>
<td>ET #</td>
<td>1.9 ± 0.6</td>
<td>2.0 ± 0.6</td>
</tr>
<tr>
<td>2PN #</td>
<td>8.5 ± 3.4</td>
<td>8.8 ± 3.3</td>
</tr>
<tr>
<td>Positive hCG</td>
<td>73%</td>
<td>60%*</td>
</tr>
<tr>
<td>Clinical pregnancy rate</td>
<td>55%</td>
<td>40%</td>
</tr>
<tr>
<td>Ongoing pregnancy rate</td>
<td>49%</td>
<td>35%</td>
</tr>
<tr>
<td>Multiple rate</td>
<td>44%</td>
<td>28%</td>
</tr>
<tr>
<td>Implantation rate</td>
<td>43%</td>
<td>28%**</td>
</tr>
</tbody>
</table>

*p=0.04, **p=0.01
non-parametric comparisons by STATA 11.1. Cycles resulting in a multiple gestation were excluded. Natural cycle IVF (NCIVF) was performed without the use of exogenous ovarian stimulation but included hCG trigger prior to oocyte retrieval.

RESULTS: Maternal age, parity and history of prior ART cycles were comparable between the groups. Infertility diagnoses were similar except greater prevalence of PCOS in the stimulated group (16.7 vs. 0.88%, p<0.001). Time to retrieval was shorter in the NCIVF group (11.0 vs. 12.8 days, p<0.001). While gestational age at delivery was comparable between groups, preterm and very preterm birth was significantly reduced in the NCIVF group.

CONCLUSION: Our study is the first to investigate birth outcomes of singleton pregnancies in NCIVF compared to stimulated IVF. Infants conceived by NCIVF had a lower prevalence of preterm and very preterm birth, with a trend toward lower prevalence of low birth weight. We propose that impact on preterm delivery rates may be due to effects of superovulation on oocyte quality or a supraphysiological estrogen milieu during implantation. In this study the data suggests improved perinatal outcomes in NCIVF versus stimulated IVF, notably a lower incidence of preterm birth with natural cycle IVF.

O-52 Monday, October 20, 2014 04:55 PM

PROGESTERONE CONCENTRATION PER RETRIEVED OOCYTE FOR IVF IS DIRECTLY RELATED TO PATIENT AGE, BUT NOT TO OVERALL PREGNANCY OUTCOME. S. Purcell, a B. Tilley, b G. Navarrete, a M. Thomas, a K. Lee, a S. Chantilis, a R. Gada, a M. Meintjes. a,b Dallas Fertility Center, Dallas, TX; 3Frisco Institute for Reproductive Medicine, Frisco, TX.

OBJECTIVE: It has long been suggested that elevated progesterone (P4) at the time of hCG-trigger for IVF is inversely related to pregnancy outcome. More recently, some interest has been expressed to correlate P4 levels at oocyte retrieval (OR) with clinical outcomes. It has been observed (Niu et al., 2008) that the serum P4 level at OR is directly correlated with the number of follicles and, consequently, with the number of quality embryos. When adjusting for the number of oocytes retrieved by considering the P4/oocyte ratio, one may see a true P4 effect on clinical outcomes. Therefore, the purpose of this study was to evaluate the effect of P4/oocyte on pregnancy outcomes.

DESIGN: Prospective, non-randomized study.

MATERIALS AND METHODS: Serum P4 levels were measured at the time of OR in patients with a fresh transfer after standard ovarian stimulation. Implantation and ongoing pregnancy (heartbeat at 8 weeks) were the primary outcomes. These primary outcomes were analyzed within five P4/oocyte groups: 0.25-0.5 (n=67), 0.51-0.75 (n=95), 0.76-1.0 (n=62), 1.01-1.25 (n=40) and >1.25 ng/oocyte (n=49); respectively. Differences between P4/oocyte ratios in pregnant and non-pregnant patients were compared with a paired t-test. The effect of P4/oocyte ratio at TVOR on age, and their interaction on implantation and ongoing pregnancy were compared using an ANOVA.

RESULTS: No difference was found in the P4/oocyte between the pregnant (0.86 ng) and non-pregnant (0.95 ng) groups. However, the P4/oocyte ratio significantly increased with patient age. Even though the expected age-related decline in implantation and ongoing pregnancy rates were observed, there was no difference in the P4/oocyte ratio within age groups.

O-53 Monday, October 20, 2014 06:00 PM

URINARY CONCENTRATIONS OF BISPHENOL A AND THEIR ASSOCIATION WITH INDICATORS OF IMPLANTATION IN IVF COUPLES. K.-R. Kim, a H.-K. Kim, a E.-K. Chun, a S. Chun, a W.-K. Min. a b i-Dream Clinic, Dep. of Obs. & Gyn., MizMedi Hospital, Seoul, Republic of Korea; 3Division of Clinical Chemistry, Dep. of Lab. Med., University of Ulsan College of Medicine and Asan Medical Center, Seoul, Republic of Korea.

OBJECTIVE: Bisphenol A (BPA) is a synthetic chemical widely used in the production of polycarbonate plastics and epoxy resins found in numerous consumer products and lead to human exposure. There was a report suggesting positive linear dose-response association between BPA urinary concentrations and implantation failure in women undergoing in vitro fertilization (IVF) (1, 2, 3). We evaluated the association of female and male urinary BPA concentrations with various indicators of IVF.

DESIGN: Prospective observational study.

Patients recruited: 113 couples undergoing IVF during August 2013-February 2014 in MizMedi Hospital.

MATERIALS AND METHODS: We collected urine samples from 113 couples undergoing IVF at the day of oocyte retrieval. We used high performance liquid chromatography–tandem mass spectrometry to measure urinary BPA concentrations after the extraction process including the addition of β-glucuronidase and incubation. The quantification of BPA was done by using Multiple Reaction Monitoring (MRM), MRM transitions and collision energies (CE) were 227.2->212.2 (CE 20V) and 227.2->133.2 (CE 20V) for BPA. The retention time of BPA was 2.51 min at the flow rate of 0.35 mL/min. Along with implantation result, D3 FSH level, peak E2, Number of retrieved oocytes, sperm concentration and motility, percentage of fertilization and percentage of good quality embryo were also assessed as indicators of IVF.

RESULTS: 105 couples excluding 8 couples with unusual BPA exposure were analyzed. The average concentration of female urinary BPA was 3.1 ng/mL (range, N.D.-26.9), That of male urinary BPA was 3.9 ng/mL (range, N.D.-24.6). The implantation results showed significant difference between low male urinary BPA concentration group and high male urinary BPA concentration group (cut-off = 3.0 ng/mL; P-value 0.021). Other indicators of IVF were not associated with both female and male urinary BPA concentrations. When patients were divided into 4 groups accounting both female and male urinary BPA concentrations, low female urinary BPA and high male urinary BPA concentration group showed significantly poor implantation result: only 4 implantations among 24 patients (16.7%).

CONCLUSION: There was a significant difference in implantation results of IVF depending on male urinary BPA concentration. It suggests that BPA exposure to male shows significantly more harmful effect to IVF outcome rather than BPA exposure to female.

Supported by: Department of Laboratory Medicine, Asan Medical Center.

EMBRYO BIOLOGY

O-54 Monday, October 20, 2014 04:15 PM

TIME-LAPSE MICROSCOPIC ANALYSIS TO VERIFY HOW BLASTOMERE BIOSPY FOR PGD AFFECTS THE DYNAMICS OF EMBRYONIC DEVELOPMENT. D. Ben-Yosef, L. Bar-El, T. Shwartz, T. Cohen, A. Carmon, N. Mey Raz, S. Raviv, M. Malcov, B. Almog, F. Azem, A. Amit, Racine IVF Unit, Lis Maternity Hospital, Tel-Aviv Sourasky Medical Center, Tel Aviv, Israel.

OBJECTIVE: Morphokinetik by time-lapse analysis was recently added to embryo scoring to assist in their selection. To date, selection of embryos following PGD was based mainly on their dynamic development following biopsy, as assessed by static evaluation on day 4-5, i.e., addition of cells following cleavage and/or compaction. The aim of this study was to explore the dynamics of the developmental events following blastomere biopsy, by determining their percise timings following culture in the EmbryoScope concomitantly with time-lapse analysis.

DESIGN: The study group included 234 embryos from PGD treatments (Feb.-Aug. 2013) cultured in the EmbryoScope, and the control group included 71 embryos from standard ICSI cycles performed during the same period and cultured in the EmbryoScope until day 5. Time-points of key embryonic events before and after embryo biopsy were registered.

FERTILITY & STERILITY®
O-55 Monday, October 20, 2014 04:30 PM

BLASTOCYSTS FROM WOMEN OF ADVANCED MATERNAL AGE HAVE COMPROMISED TRANSCRIPTION OF KEY IMPLANTATION AND DEVELOPMENT GENES. A. Strieby, a B. R. McCallie, b J. C. Parks, a W. B. Schoolcraft, b, c M. G. Katz-Jaffe, a, c National Foundation for Fertility Research, Lone Tree, CO; cColorado Center for Reproductive Medicine, Lone Tree, CO.

OBJECTIVE: Maternal age is the most significant risk factor associated with human infertility. This is due to the declining number and quality of oocytes as a woman approaches menopause. Women in their forties exhibit significantly lower implantation rates, less than 10% with their own oocytes, compared to >60% with fertile donor oocytes. The aim of this study was to investigate the impact of maternal age on blastocyst transcription throughout successive time points during the window of implantation.

DESIGN: Research study.

MATERIALS AND METHODS: Surplus cryopreserved human D5 blastocysts of equivalent good morphology were donated with IRB and patient consent. Individual blastocysts were warmed for co-culture on a monolayer of fertile endometrial epithelial cells (one blastocyst per well) to simulate an in vivo like environment: blastocysts from fertile oocyte donor cycles with no male factor (n=24) and blastocysts from infertile AMA women with no male factor and no other infertility diagnosis (≥39 years; n=24). Blastocyst gene transcription was assessed after 6, 12 and 24 hours of co-culture for 22 implantation genes using quantitative real-time PCR relative to a constant internal housekeeping gene. Analysis of mRNA profiles was performed with REST® statistical software, with a P value of <0.05 considered significant.

RESULTS: Blastocyst growth and development was assessed after 6, 12 and 24 hours of co-culture with no significant differences noted between the two maternal age groups. Analysis of gene transcription after both 6 and 12 hours of co-culture revealed no expression changes between time points or maternal age groups. In contrast, after 24 hours of co-culture on D6 of embryonic development closer to the time of implantation, significant decreased expression was observed for key implantation and developmental genes in association with AMA. These genes included, BRCA1 (DNA repair), MAD2L1 (spindle checkpoint), TRIML1 (ubiquitin pathway), HCF1 (cell cycle regulation) and TEAD4 (trophodectom development) (P<0.05).

CONCLUSION: A viable competent blastocyst is essential for effective apposition and adhesion to the maternal endometrium and the facilitation of successful implantation. Even though infertile women in their forties produced morphologically good grade embryos, these blastocysts displayed compromised transcription. Comparison with the control group demonstrated that blastocyst transcription rates were significantly lower compared to those of blastocysts from fertile women, thus leading to significantly lower implantation potential.

O-56 Monday, October 20, 2014 04:45 PM

LIBRARY OF PHENOTYPES ELICITED BY RNAI DURING MOUSE EMBRYOGENESIS. AVALUABLE ARCHETYPE FOR HUMAN EMBRYO DEVELOPMENT. Y. G. Kramer, a M. Gutwein, a C. McCaffrey, b J. Buldo-Licciardi, a K. Gansalus, c N. Noyes, a bNYU Fertility Center, New York, NY; cCenter for Genomics and Systems Biology, New York, NY.

OBJECTIVE: Our goal is to develop non-invasive diagnostic methods to predict the developmental potential of pre-implantation mammalian embryos that could be applied to evaluate the quality of in vitro fertilized (IVF) human embryos in a clinical setting. Toward this end, we have coupled time-lapse microscopy of mouse embryos with targeted gene knock-downs using RNA interference (RNAi) to catalog high-content phenotypic data of embryos up to the blastocyst stage.

DESIGN: Animal research.

MATERIALS AND METHODS: Female C57Bl6J mice were super-ovulated using standard gonadotropin (PMSG and HCG) superovulation protocols and then mated with fertile male mice. At the control group demonstrated that blastomere biopsy significantly delays the timing of compaction (by 4-5h) and the start of blastulation (by 5-10h); (p<0.01).

CONCLUSION: Analysis of morphokinetic parameters enabled us to explore the first time, how blastomere biopsy interferes with the dynamic sequence of developmental events. It demonstrates the importance of time-lapse microscopy for determining the optimal timing for blastomere biopsy for improving PGD outcomes.

O-57 Monday, October 20, 2014 05:00 PM

THE TIMING OF EARLY CLEAVAGE EVENTS AND SEVERITY OF FRAGMENTATION IS CORRELATED WITH GENE EXPRESSION PATTERNS IN CUMULUS CELLS. E. R. Hammond,a,b E. W. Benjamin,a E. R. Britton,a,b aDepartment of Obstetrics and Gynecology, Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand; bFertility Associates, Auckland, New Zealand.

OBJECTIVE: The aim of our study was to identify relationships that exist between gene expression in cumulus cells and time-lapse imaging analysis of early cleavage events in the developing embryo.

DESIGN: A cohort of 80 oocytes had their individual cumulus cell masses collected prior to fertilisation, and the resulting embryos underwent time-lapse imaging analysis up to day 3, and were subsequently cultured to day 5.

MATERIALS AND METHODS: Individual cumulus cell masses underwent gene expression analysis for 27 genes that are important indicators of follicular microenvironment. Specifically, these genes were involved in cumulus cell expansion, signalling and energy metabolism. All embryos underwent time-lapse imaging analysis for early cleavage events using the Primoscan monitoring system, with pictures taken every 10 minutes. Time to 2PN breakdown, the first cleavage furrow and cleavage to 2, 3, 4 and 5 cells were annotated. Additionally, embryos with mild (≤ 10%), moderate (10-25%) and severe (≥ 25%) fragmentation were annotated.

RESULTS: The expression levels of some of our candidate genes in cumulus cells correlated with the timings of early cleavage events and the severity of fragmentation of the developing embryo. Additionally, gene expression profiles in cumulus cells and time-lapse imaging analysis of the developing embryo were correlated to embryo quality on day 5.

CONCLUSION: This study provides further evidence that the developmental potential of the embryo is reflective of the follicular environment from which the oocyte developed. Our findings suggest that some of the variation observed in the timings of early cleavage events and severity of fragmentation can be attributed to cumulus cell gene expression and thus the follicular microenvironment, providing further evidence that cumulus cell...
gene expression can be a marker of embryo quality. Additionally, it is possible that combining time-lapse analysis of early cleavage events with gene expression profiling in cumulus cells may provide a more reliable marker of embryo quality.

Supported by: The University of Auckland and Fertility Associates, Auckland, New Zealand.

O-58 Monday, October 20, 2014 05:15 PM

HYPMETHYLATION OF MONOSOMY CHROMOSOMES MAY REFLECT THE DECREASED IMPLANTATION POTENTIALS: B. R. McCamie, a,b,c J. C. Parks, a A. Strzyb, d W. B. Schoolcraft, e M. G. Katz-Jaffe, b,c d National Foundation for Fertility Research, Lone Tree, CO; eColorado Center for Reproductive Medicine, Lone Tree, CO.

OBJECTIVE: With the exception of Turner’s syndrome, the implantation potential of chromosomal monosomies is extremely low compared to corresponding trisomies, which often develop in utero. DNA methylation is an essential epigenetic control mechanism responsible for gene regulation, chromatin remodeling and genome stability. Decreased expression and activity of DNA methyltransferases (DNMTs) has been shown in our lab to be associated solely with chromosomal monosomy. The aim of this study was to investigate the blastocyst methylome in association with chromosomal constitution and potential implantation outcome.

DESIGN: Research study.

MATERIALS AND METHODS: Surplus cryopreserved euploid (Group A, n = 20), trisomy 15 (Group B, n = 20), trisomy 22 (Group C, n = 20), monosomy 15 (Group D, n = 20) and monosomy 22 (Group E, n = 20) blastocysts were donated with patient and IRB consent. All blastocysts were warmed, pooled in groups of 10 for bisulfite conversion prior to hybridization to the Illumina Infinium Human Methylation450 BeadChip, which covers 486,428 CpG sites. Analysis was performed using Illumina GenomeStudio software for normalization, beta value calculation (0 = no methylation to 1 = complete methylation) and differential methylation analysis with P < 0.05 for significance.

RESULTS: Comparable methylation profiles were observed for beta values of all groups irrespective of chromosome constitution: A = 0.21, B = 0.20, C = 0.20, D = 0.20 and E = 0.21. Individual chromosome methylation profiles were evaluated and no significant differences were observed between euploid (Group A) and trisomy blastocysts (Groups B and C), including for the chromosome associated with the trisomy. In contrast, significant hypomethylation was detected in monosomy blastocysts for their respective monosomy chromosomes; chromosome 15 (A = 0.22, B = 0.22, C = 0.22, D = 0.19 and E = 0.22) and chromosome 22 (A = 0.18, B = 0.17, C = 0.18, D = 0.17 and E = 0.14; P < 0.05).

CONCLUSION: This novel study has shown that the global methylene and individual chromosome methylation profiles of trisomy blastocysts is similar to euploid blastocysts. Evidence from fetal and trisomy cell lines suggests that the gained chromosome mimics the epigenomic signature of X chromosome inactivation reflecting a potential cellular adaptation to the additional chromosome. In contrast, monosomy chromosomes demonstrate a hypomethylated state that may impact chromatin remodeling and genome stability resulting in implantation failure.

O-59 Monday, October 20, 2014 05:30 PM

PREDICTION MODEL FOR ANEUPLOIDY IN EARLY HUMAN EMBRYOS BASED ON THE TRANSCRIPTOMIC SIGNATURE: M. Vera,a b,c S. Chavez,a,b C. Rubio,a,b,c,d R. Reijo Pera,a,b,c d Institute for Stem Cell Biology and Regenerative Medicine, Stanford University, CA; bDepartment of Obstetrics, Stanford University, CA; cFundación Instituto Valenciano, (FIVI), Valencia, Spain; dInstituto Universitario, Icliva, Valencia, Spain.

OBJECTIVE: To analyze in the same human embryo the complete chromosomal status, gene expression, and morphokinetic, in order to establish an aneuploidy prediction model for early human embryo development.

DESIGN: Human zygotes donated to research were thawed and cultured from the zygote stage to the third mitotic division and development monitored by time-lapse imaging. Embryos were then disassembled into individual blastomeres for chromosome and gene expression analysis at the single-cell level.

MATERIALS AND METHODS: Morphology and kinetic parameters were evaluated using the PN disappearance (PNd) as a reference point. Half of the cells of each embryo were analyzed by array comparative genomic hybridization (aCGH) (Illumina Inc, UK). The other half were analyzed via high-throughput qPCR (Fluidigm Co, USA) by studying the expression of 86 genes selected from literature. R and Babelomics software packages were implemented for functional and expression analysis, and SPSS was used for statistical tests.

RESULTS: From a total of 85 embryos (Female age: 33.7 ± 4.3 y.o), gene expression data was obtained in 78 embryos. Complete aCGH results were collected in 57 embryos, with an aneuploidy rate of 50.9%. Further, comparative genome and expression results were obtained in 53 embryos. Interestingly, time from PNd to start of first cytokinesis was the only kinetic parameter significantly different in aneuploid versus euploid embryos (2.8 vs. 2.4 h, respectively) (p = 0.02). In addition, the time frame between PNd and the subsequent 30 hours was the single period in which the genetic landscape was significantly different between euploid and aneuploid embryos. A total of twenty genes were differentially expressed in aneuploid versus euploid embryos (p < 0.05). The twelve genes that showed the greatest differences were selected to define the transcriptomic signature of early human embryo aneuploidy generation. Finally, an aneuploidy predictor model was developed based on this gene expression signature with a proven accuracy of 85.2%.

CONCLUSION: This is the first study to analyze chromosomal status, gene expression, and morphokinetic simultaneously in the same human embryo. The transcriptomic signature of 12 genes allowed the development of a highly accurate model for aneuploidy prediction in early human embryo development with the potential for clinical translation.

O-60 Monday, October 20, 2014 05:45 PM

INVESTIGATION OF ANEUPLOIDY IN HUMAN BLASTOCYSTS THROUGH UTILIZATION OF SINGLE NUCLEOTIDE POLYMORPHISM (SNP) ARRAYS AND ARRAY COMPARATIVE GENOMIC HYBRIDIZATION (aCGH): M. Konstantinidis,a,b D. Tortoriciello,a J. Hill,a R. Kiltz,a,b G. Letterie,a C. Laskin,a B. Collins,b d Wells,b,e; f Reproductive Genetics, Livingston, NJ; bSher Institute for Reproductive Medicine, New York, NY; cFertility Centers of New England, Reading, MA; dNCY Fertility Center, Syracuse, NY; eSeattle Reproductive Medicine, Seattle, WA; fLifeQuest Centre for Reproductive Medicine, Toronto, ON, Canada; gReprogenetics, Oxford, Oxfordshire, United Kingdom.

OBJECTIVE: To study parental and meiotic/mitotic origin of aneuploidy in human blastocysts using a combination of techniques.

DESIGN: Blastocysts undergoing preimplantation genetic diagnosis (PGD) for single gene disorders and aneuploidy were screened by Karyomapping and aCGH. The frequency of recombination events was also studied.

MATERIALS AND METHODS: This biopsied sample was amplified with multiple displacement amplification and aliquots were used to perform the different tests. Karyomapping was employed to carry out genome-wide SNP genotyping (300K SNPs), aneuploidy detection and recombination analysis. Additionally, aCGH was performed for chromosome copy number analysis.

RESULTS: 144 blastocysts from 33 couples were assessed. 64 (44%) were found to be aneuploid by aCGH comprising a total of 113 aneuploidies (44 monosomies, 69 trisomies). Quantitative and qualitative (genotyping) Karyomapping analysis was possible for 48 of these samples. For this set of samples, Karyomapping was able to detect 78.4% (29/37) of the aneuploidies detected by aCGH. SNP genotyping alone was possible for all 144 blastocysts and was able to detect 21/69 (30.4%) of trisomies identified by aCGH, designating these to be of meiotic and of maternal origin. Investigation of recombination events revealed that each of the 23 chromosomes inherited from father and mother had experienced at least one instance of recombination failure, with some chromosomes showing no recombination events at a rate as high as 45%.

CONCLUSION: Meiotic trisomies detected in the blastocysts assessed in this study were all found to be maternally derived. SNP genotyping by Karyomapping was unable to detect a large portion of trisomies detected by aCGH, indicating these to be of mitotic origin. Recombination failure theses an explanation for some of the trisomies detected by aCGH but missed by SNP microarray. Failure of recombination can increase the risk of premature separation of homologues or sister chromatids at meiosis I, potentially producing a disomic gamete with identical chromatid copies at the end of meiosis II, not distinguishable by SNP genotyping.
CONFOCAL LIVE CELL IMAGES WITH FLUORESCENT PROTEINS REVEAL MULTINUCLEATION OF MORPHOLOGICALLY GOOD DAY 3 EMBRYOS IMPAIRS SUBSEQUENT DEVELOPMENT. Y. Nakaoka, a S. Hashimoto, a A. Amo, a K. Yamagata, a C. Takaya, a H. Iwahata, a T. Himeno, a T. Inoue, a K. Ito, a Y. Yamauchi, a TVF Namba Clinic, Osaka, Japan; Osaka University, Suita, Osaka, Japan.

OBJECTIVE: Serial observations of morphological changes using time-lapse cinematography have improved the outcome of assisted reproductive technology. However, the relationship between morphological changes and nuclear dynamics is not fully understood.

In this study, we observed the nuclear status of morphologically good day 3 embryos by confocal microscope and investigated the relationship between their morphological changes and their nuclear dynamics from the pronucleus to the blastocyst stage. Chromosomal analysis was also conducted.

DESIGN: Laboratory assessment.

MATERIALS AND METHODS: Seventy-two frozen-thawed pronuclear embryos intended for disposal were used, after obtaining the informed consent of the patients and the approval of ethics committees. A mixture of mRNAs encoding enhanced green fluorescent protein coupled with α-tubulin and monomeric red fluorescent protein fused with histone H2B was injected into the cytoplasm of pronuclear embryos. These embryos were cultured in KSOMaa medium under 5% O2, 5% CO2, and 90% N2 atmosphere. Time-lapse images were captured at 15-min intervals for 5 days using a confocal microscope. Morphology of day 3 embryos was assessed 48 h after initiation of culture. Chromosomal analysis of blastocysts was conducted by array comparative genomic hybridization.

RESULTS: Of the 41 morphologically good day 3 embryos, 28 (68.3%) had multinuclei. Abnormal mitosis in which one cell divided into three or more cells was observed in 7 embryos at the first cleavage (17.1%) and in 6 embryos at the second cleavage (14.6%). Only 13 embryos (31.7%) showed normal nuclear status, and 15 embryos (36.6%) showed multinucleation without abnormal mitosis. Blastocyst formation rate of embryos with abnormal mitosis was 14.3% (1/7) at the first cleavage and 33.3% (2/7) at the second cleavage. Of the embryos without abnormal mitosis, blastocyst formation rate was significantly higher in embryos with normal nuclear status (84.6%, 11/13) than in those showing multinucleation (26.7%, 4/15). Chromosomal abnormalities were observed in 50.0% (2/4) of the multinucleated embryos and 14.3% (1/7) of the embryos without multinuclei.

CONCLUSION: Serial confocal images with fluorescent proteins revealed that abnormal nucleation was high in morphologically good day 3 embryos. Thus, multinucleation is a crucial determinant for selection of viable embryos on day 3.

ELEVATED ANTI-MÜLLERIAN HORMONE (AMH) LEVELS DECREASE ODDS OF RESPONSE TO OVULATION INDUCTION WITH LETROZOLE OR CLOMIPHENE CITRATE. M. S. M. Lanham, E. C. Prochaska, Y. Smith. Department of Obstetrics and Gynecology, University of Michigan, Ann Arbor, MI.

OBJECTIVE: To evaluate the effect of elevated Anti-Müllerian Hormone (AMH) levels on response to oral ovulation induction with letrozole or clomiphene citrate.

DESIGN: Retrospective case-control study.

MATERIALS AND METHODS. All women who presented to an academic fertility center between January 2009 and September 2012, who had a serum AMH level drawn during their evaluation, and who underwent oral ovulation induction with clomiphene citrate or letrozole as their first treatment regimen after presentation were eligible for inclusion in the study.

The endpoint for each treatment cycle was response to ovulation induction, defined as presence of ultrasound documentation of at least one follicle over 17 mm, or documentation of a positive home urine luteinizing hormone (LH) kit. The data was analyzed using a marginal model with logit link function, time-varying cycle medication type, dose, and monitoring method, and time-invariant baseline AMH quartile and diagnosis of polycystic ovary syndrome (PCOS).

RESULTS: Oral ovulation induction was prescribed to 209 women with known AMH levels through 606 cycles during the study timeframe. Fifty-six cycles were classified as “unknown response”, leaving 570 cycles from 203 patients available for analysis. Analyzed cycles included 347 with clomiphene citrate, 223 with letrozole; 151 with home LH kit monitoring and 419 with ultrasound monitoring. Subjects had a median age of 33 years, range 21 to 43. AMH levels (ng/ml) ranged from undetectable (limit of detection 0.3ng/ml) to 37, with median 2.3 and interquartile range 1 to 4. The diagnosis of polycystic ovary syndrome (PCOS) was documented in 54 women (25.8%). After controlling for cycle medication, dose, and monitoring method, AMH level >4ng/ml (4th quartile of study population) was associated with a lower odds of response to oral medications both in women without PCOS (OR 0.10, 95%CI 0.03 – 0.38, p=0.0007) and in women with PCOS (OR 0.26, 95%CI 0.09 – 0.75, p=0.0122). Diagnosis of PCOS was associated with a lower odds of responding to oral ovulation induction medications (OR 0.20, 95%CI 0.09 – 0.47, p=0.0002). Use of letrozole was associated with a higher odds of response than use of clomiphene citrate (OR 2.64, 95%CI 1.04 – 6.68, p=0.041).

CONCLUSION: Women with elevated AMH levels, with or without PCOS, have lower odds of response to their prescribed letrozole or clomiphene citrate than women without elevated AMH levels. On average, letrozole is more likely to lead to ovulation than clomiphene citrate, when controlling for AMH level and PCOS diagnosis.

Supported by: Michigan Institute for Clinical & Health Research Grant (CTSA: UL1RR024986).

O-64 Monday, October 20, 2014 04:45 PM


O-61 Monday, October 20, 2014 06:00 PM

450 IU VS. 600 IU IN POOR RESPONDERS: NO DIFFERENCES IN CUMULATIVE PREGNANCY RATES. J. Lefebvre, a,b R. Antaki, a,b I.-J. Kadoch, a,b C. Sylvestre, a,b F. Bissonnette, a,b J. Benoit, a,b L. Lapensee, a,b S. Menard, a,b Clinique OVO, Montreal, QC, Canada; Department of Obstetrics and Gynecology, University of Montreal, Montreal, QC, Canada.

OBJECTIVE: Assess if the cumulative pregnancy rates are similar in women at risk of poor ovarian response undergoing a microdose agonist flare-up protocol IVF / ICSI cycle with a daily administration of 600 IU or 450 IU of gonadotropins.

DESIGN: In a prospective randomized (by sequential number) controlled non-blinded study in a university-affiliated private IVF clinic, 356 women were recruited from October 2009 to September 2013. Sample size was calculated by started cycle with a tolerance margin of 1.6 mature oocytes, with a 90% power and alpha error of 0.025. The original study showed that 600 IU daily of gonadotropins does not significantly increase the number of oocytes or pregnancy rates compared to 450 IU. In a secondary analysis of the study, we wanted to evaluate if cumulative biochemical and clinical pregnancy rates (fresh and frozen transfers) would be similar between the 450 IU and 600 IU groups.

MATERIALS AND METHODS: Participants were female < 41 years old at risk of poor ovarian response (< 5 oocytes or < 8 follicles or a cancellation in a previous IVF cycle with ≥ 300 IU gonadotropins/day, basal FSH > 10 IU/L or AMH < 1 ng/ml or antral follicle count ≤ 8). They were randomized in two groups: receiving 450 IU or 600 IU of gonadotropins (Menopur® and Bravelle®). The secondary analysis was performed using Chi-squared tests.

RESULTS: In the group 450 IU, 62/124 frozen embryos were transferred and in the group 600 IU 67/112 frozen embryos were transferred. Groups were similar for embryo quality and embryo survival. No statistically significant differences were found between both groups in cumulative biochemical pregnancy rates (24.6 % (450 IU) and 32.6% (600 IU) P=0.13), cumulative clinical pregnancy rates (20.5% (450 IU) and 25.7% (600IU) P=0.51). Implantation rates were also similar in both groups.

CONCLUSION: This secondary analysis confirms that cumulative pregnancy rates are similar in poor ovarian responders using a dosage of 600 IU of gonadotropins compared to those using 450 IU.

Supported by: For the study, Ferring Pharmaceuticals provided some of the gonadotropins, free of charge. There was no financial remuneration of any member of the research team.
OBJECTIVE: Human chorionic gonadotropin (hCG) is usually used at the end of controlled ovarian hyperstimulation (COH), as a surrogate LH surge, to induce final oocyte maturation and resumption of meiosis. Recently, the co-administration of GnRH agonist and hCG for final oocyte maturation - 40 and 34 hours prior to OPU, respectively (double trigger) was suggested to improve IVF outcome in patient with genuine empty follicle syndrome. In the present study, we aim to evaluate whether the double trigger might improve the proportions of metaphase-II (MII) oocytes in patients with low proportion of mature oocytes (<66%) per number oocytes retrieved.

DESIGN: A cohort historical study.

MATERIALS AND METHODS: Two consecutive IVF cycles were compared per each patient, one triggered with hCG and the other utilized double triggering. Stimulation characteristics and IVF outcome were compared. We compared the stimulation characteristics of 34 IVF cycles, which included the cycle with the double trigger to the patients’ previous IVF attempt, triggered with hCG only.

RESULTS: In the double triggered cycles (study group) there were significantly higher number of mature oocytes - MII (7 ± 3.3 vs. 3.6 ± 2.2, p < 0.001), number of eggs transferred (2.2 ± 1.0 vs. 1.1 ± 1.0, p = 0.01), a significantly higher proportions of MII oocytes per number of oocytes retrieved (52.4% ± 23.7% vs. 52.4% ± 23.1%, p = 0.01) and a higher number of top quality embryos (3.1 ± 2.7 vs. 0.8 ± 1.5, p = 0.008), as compared to their previous control cycles (hCG-only trigger).

Eleven pregnancies were recorded in the study group and none in the control group.

CONCLUSION: Co-administration of GnRH-agonist and hCG for final oocyte maturation, 40 and 34 hours prior to OPU, respectively (double trigger) improves IVF outcome in patients with high proportion of immature oocytes.

O-65 Monday, October 20, 2014 05:00 PM


OBJECTIVE: In an ongoing effort to reduce multiple pregnancies, gonadotropins use and ovarian hyper-stimulation syndrome (OHSS), minimal stimulation IVF was employed in our center while conventional IVF is a routine approach at most other centers. We aimed at evaluating the differences in clinical outcomes between minimal stimulation IVF with single embryo transfer and conventional IVF with double embryo transfer, including ongoing pregnancy rates, administration of drugs, OHSS and multiple pregnancy rates.

DESIGN: An open-label, randomized controlled non-inferiority trial was completed among 564 infertile women between 2009 and 2013 at a single fertility center.

MATERIALS AND METHODS: Infertile women under the age of 38 who were undergoing their first IVF cycle were randomly allocated to either conventional (long agonist protocol, blastocyst culture with fresh/vitrified embryo transfers) or minimal stimulation (extended clomiphene regime with additional low-dose gonadotropins, blastocyst culture, vitrification and delayed vitrified embryo transfers) IVF treatments. The study was registered at clinicaltrials.gov NCT01253587.

RESULTS: A total of 564 women were included, of whom 285 were allocated to minimal stimulation IVF and 279 to conventional IVF. As expected the number of obtained eggs (4.3 ± 3.2 vs 12.8 ± 8) and blastocysts (2.6 ± 1.9 vs 5.9 ± 4.3) was significantly higher in the conventional arm. The cumulative ongoing pregnancy rate was 51% (144/285) for minimal stimulation IVF and 66% (185/279) for conventional IVF (RR 0.76, 95% CI 0.66-0.88). The ongoing multiple pregnancy rate was 6.3% in minimal stimulation IVF compared to 36% in conventional IVF (RR 0.18, 95% CI 0.09-0.34). There were no cases of OHSS in minimal stimulation IVF compared to 16% (5.7%) moderate/severe OHSS cases in the conventional arm. Gonadotropin consumption was significantly lower with minimal stimulation IVF than in conventional IVF (459 ± 131 versus 2079 ± 389 IU; p < 0.0001).

CONCLUSION: Minimal stimulation IVF with single embryo transfer resulted in relatively lower ongoing pregnancy rates but multiple pregnancy rates were almost 6 fold lower. The gonadotropin use in minimal stimulation IVF was decreased by 78% and OHSS was completely prevented. The results of this study argue for minimal stimulation IVF as the intervention of choice to produce a successful pregnancy while reducing the complication rate associated with conventional IVF protocol.

O-66 Monday, October 20, 2014 05:15 PM

A RANDOMIZED CLINICAL TRIAL COMPARING CLOMID MINIMAL STIMULATION IVF TO CONVENTIONAL IVF. J. J. Zhang, L. Chang, A. Wang, H.-T. Pan, M. van Wely, New Hope Fertility Center, New York, NY; Academic Medical Center, University of Amsterdam, Amsterdam, North Holland, Netherlands.

OBJECTIVE: In an ongoing effort to reduce multiple pregnancies, gonadotropins use and ovarian hyper-stimulation syndrome (OHSS), minimal stimulation IVF was employed in our center while conventional IVF is a routine approach at most other centers. We aimed at evaluating the differences in clinical outcomes between minimal stimulation IVF with single embryo transfer and conventional IVF with double embryo transfer, including ongoing pregnancy rates, administration of drugs, OHSS and multiple pregnancy rates.

DESIGN: An open-label, randomized controlled non-inferiority trial was completed among 564 infertile women between 2009 and 2013 at a single fertility center.

MATERIALS AND METHODS: Infertile women under the age of 38 who were undergoing their first IVF cycle were randomly allocated to either conventional (long agonist protocol, blastocyst culture with fresh/vitrified embryo transfers) or minimal stimulation (extended clomiphene regime with additional low-dose gonadotropins, blastocyst culture, vitrification and delayed vitrified embryo transfers) IVF treatments. The study was registered at clinicaltrials.gov NCT01253587.

RESULTS: A total of 564 women were included, of whom 285 were allocated to minimal stimulation IVF and 279 to conventional IVF. As expected the number of obtained eggs (4.3 ± 3.2 vs 12.8 ± 8) and blastocysts (2.6 ± 1.9 vs 5.9 ± 4.3) was significantly higher in the conventional arm. The cumulative ongoing pregnancy rate was 51% (144/285) for minimal stimulation IVF and 66% (185/279) for conventional IVF (RR 0.76, 95% CI 0.66-0.88). The ongoing multiple pregnancy rate was 6.3% in minimal stimulation IVF compared to 36% in conventional IVF (RR 0.18, 95% CI 0.09-0.34). There were no cases of OHSS in minimal stimulation IVF compared to 16% (5.7%) moderate/severe OHSS cases in the conventional arm. Gonadotropin consumption was significantly lower with minimal stimulation IVF than in conventional IVF (459 ± 131 versus 2079 ± 389 IU; p < 0.0001).

CONCLUSION: Minimal stimulation IVF with single embryo transfer resulted in relatively lower ongoing pregnancy rates but multiple pregnancy rates were almost 6 fold lower. The gonadotropin use in minimal stimulation IVF was decreased by 78% and OHSS was completely prevented. The results of this study argue for minimal stimulation IVF as the intervention of choice to produce a successful pregnancy while reducing the complication rate associated with conventional IVF protocol.

O-67 Monday, October 20, 2014 05:30 PM

CARDIOVASCULAR DYSFUNCTION IN CHILDREN BORN TO WOMEN WITH OVARIAN HYPERSTIMULATION SYNDROME: A RETROSPECTIVE COHORT STUDY AND PROTEOMICS ANALYSIS. G.-F. Xu, J.-Y. Zhang, H.-T. Pan, T.-T. Yu, J. Sheng, H.-F. Huang, Key Laboratory of Reproductive Genetics, Ministry of Education (Zhejiang University), Hangzhou, Zhejiang, China; 2Reproductive Medicine Unit, Obstetrics and Gynecology, Tiberias, Israel; Department of Pathology and Pathophysiology, School of Medicine, Zhejiang University, Hangzhou, Zhejiang, China.

OBJECTIVE: Ovarian hyperstimulation syndrome (OHSS) is a complication of assisted reproductive technologies (ARTs). The cardiovascular dysfunction in children born with ARTs has been widely concerned. However, whether OHSS is associated with worse cardiovascular functions and the mechanisms underlying the association remain unknown.

DESIGN: A prospective randomized controlled study.

MATERIALS AND METHODS: Sixty three consecutive infertile women above 35 years of age and/or with a previous low ovarian response admitted for IVF/ICSI treatment were prospectively recruited. All women were similarly treated employing the recombinant FSH (Gonal-F) 300 IU/day and the flexible GnRH antagonist (Cetrotide) 0.25 mg/day protocol. On the same day of the start of the antagonist, r-LH (Luveris) 150 IU/day was administered to the study group and continued till the hCG day.

RESULTS: Patients’ characteristics including basal ovarian reserve studies were similar in both the study and control groups. Although r-LH addition has reduced the GnRH antagonist duration from 5.0±1.5 to 4.0±1.6 days (p < 0.5), it did not change total dosage of rFSH administered, maximal E2 level and follicular phase duration. Moreover, number of ≥ 14 mm follicles, oocytes, MII oocytes, 2PN zygotes, embryos and top graded embryos did not change between the two groups. Endometrial thickness, implantation and pregnancy rates were also similar between the groups. Serum FSH, LH, E2 and P levels did not show significant difference throughout the follicular phase.

CONCLUSION: Primary evaluation of our results does not reveal an obvious advantage regarding rLH addition to the rFSH/GnRH antagonist flexible protocol in the advanced reproductive age women.
2. Statistically significant differences were found between CS and MS in older group when three Ampoles or more of hMG (3A hMG) were used after frozen-thawed embryo transfer.

**O-69 Monday, October 20, 2014 06:00 PM**

**GONADOTROPIN DOSE IS INVERSELY CORRELATED WITH LIVE BIRTH RATE: AN ANALYSIS OF 541,967 ART CYCLES.**

V. L. Baker, a, B. Brown, b, B. Luke, a, G. W. Smith, b J. J. Ireland. c

aObstetrics and Gynecology, Stanford University, Palo Alto, CA; bAnimal Science, Michigan State University, East Lansing, MI; cAnimal Science, Michigan State University, East Lansing, MI.

**OBJECTIVE:** To examine the correlation between total gonadotropin dosage and live birth rate.

**DESIGN:** Retrospective analysis of fresh autologous cycles reported to the Society for Assisted Reproductive Technology from 2004 to 2011.

**MATERIALS AND METHODS:** Logistic regression models were created using categorical values for total gonadotropin dose as the primary predictor variable and live birth rate as the primary outcome variable. Differences were evaluated based on number of oocytes retrieved.

**RESULTS:** Live birth rate decreased with increasing gonadotropin dose, regardless of the number of oocytes retrieved (see Table, p<0.0001 across all oocyte and dose categories). The statistically significant decrease in live birth rate with increasing gonadotropin dose remained regardless of female age and total number of days of ovarian stimulation, as well as in subgroup analyses limited to cycles with diminished ovarian reserve or body mass index>30, except for cycles for women >40 years of age with 1-5 oocytes retrieved.

**Effect of gonadotropin dose on live birth rate**

<table>
<thead>
<tr>
<th>Number of oocytes retrieved</th>
<th>All</th>
<th>1-5</th>
<th>6-10</th>
<th>11-15</th>
<th>16-20</th>
<th>21-25</th>
<th>&gt;25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gonadotropin dose (IU)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1000</td>
<td>34.9</td>
<td>14.3</td>
<td>35.6</td>
<td>42.2</td>
<td>46.2</td>
<td>47.1</td>
<td>43.1</td>
</tr>
<tr>
<td>1001-2000</td>
<td>44.3</td>
<td>24.5</td>
<td>41.3</td>
<td>46.2</td>
<td>48.6</td>
<td>48.0</td>
<td>44.8</td>
</tr>
<tr>
<td>2001-3000</td>
<td>38.9</td>
<td>21.9</td>
<td>36.4</td>
<td>42.0</td>
<td>43.8</td>
<td>43.2</td>
<td>40.7</td>
</tr>
<tr>
<td>3001-4000</td>
<td>31.5</td>
<td>18.6</td>
<td>30.7</td>
<td>36.0</td>
<td>36.4</td>
<td>38.0</td>
<td>35.2</td>
</tr>
<tr>
<td>4001-5000</td>
<td>26.4</td>
<td>15.9</td>
<td>27.2</td>
<td>30.8</td>
<td>32.9</td>
<td>32.2</td>
<td>30.6</td>
</tr>
<tr>
<td>≥5001</td>
<td>19.2</td>
<td>12.1</td>
<td>21.3</td>
<td>25.8</td>
<td>26.1</td>
<td>25.2</td>
<td>26.7</td>
</tr>
<tr>
<td>Total</td>
<td>33.0</td>
<td>16.5</td>
<td>30.9</td>
<td>38.2</td>
<td>41.6</td>
<td>42.5</td>
<td>40.5</td>
</tr>
</tbody>
</table>

**CONCLUSION:** Gonadotropin dose is inversely correlated with live birth rate, regardless of the number of oocytes retrieved or female age. Supported by: NIH P01 HD 065647.

**PREIMPLANTATION GENETIC DIAGNOSIS I**

**O-70 Monday, October 20, 2014 04:15 PM**

**IMPROVEMENT OF CLINICAL OUTCOME IN SEVERE MALE FACTOR INFERTILITY WITH EMBRYO SELECTION BASED ON ARRAY-CGH:**

A. Guillen, a, b, d, E. Rodriguez, c, J. Bélíver, a, M. Guirao, a, b, d, J. Remohi, b, d, A. Pellicer, b, d, C. Simón, b, d, a, b, d, IVIOMICS, Paterna, Valencia, Spain; bFundación Instituto Valenciano de Infertilidad (FIVI)/INCLIVA, Paterna, Valencia, Spain; cIVI Barcelona, Barcelona, Spain; dInstituto Universitario IVI, Valencia, Spain; eIVI Madrid, Aravaca, Spain; fDepartment of Obstetrics and Gynecology, School of Medicine, Stanford University, Palo Alto, CA.

**OBJECTIVE:** To assess the value of comprehensive chromosome screening (CCS) by array CGH in severe male factor infertility.

---

**TABLE:**

<table>
<thead>
<tr>
<th>Gonadotropin dose (IU)</th>
<th>1-5</th>
<th>6-10</th>
<th>11-15</th>
<th>16-20</th>
<th>21-25</th>
<th>&gt;25</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1000</td>
<td>34.9</td>
<td>14.3</td>
<td>35.6</td>
<td>42.2</td>
<td>46.2</td>
<td>47.1</td>
</tr>
<tr>
<td>1001-2000</td>
<td>44.3</td>
<td>24.5</td>
<td>41.3</td>
<td>46.2</td>
<td>48.6</td>
<td>48.0</td>
</tr>
<tr>
<td>2001-3000</td>
<td>38.9</td>
<td>21.9</td>
<td>36.4</td>
<td>42.0</td>
<td>43.8</td>
<td>43.2</td>
</tr>
<tr>
<td>3001-4000</td>
<td>31.5</td>
<td>18.6</td>
<td>30.7</td>
<td>36.0</td>
<td>36.4</td>
<td>38.0</td>
</tr>
<tr>
<td>4001-5000</td>
<td>26.4</td>
<td>15.9</td>
<td>27.2</td>
<td>30.8</td>
<td>32.9</td>
<td>32.2</td>
</tr>
<tr>
<td>≥5001</td>
<td>19.2</td>
<td>12.1</td>
<td>21.3</td>
<td>25.8</td>
<td>26.1</td>
<td>25.2</td>
</tr>
<tr>
<td>Total</td>
<td>33.0</td>
<td>16.5</td>
<td>30.9</td>
<td>38.2</td>
<td>41.6</td>
<td>42.5</td>
</tr>
</tbody>
</table>

**CONCLUSION:** Gonadotropin dose is inversely correlated with live birth rate, regardless of the number of oocytes retrieved or female age. Supported by: NIH P01 HD 065647.
OBJECTIVE: To evaluate the capability of next-generation sequencing (NGS) to detect whole chromosome and segmental aneuploidies in trophectoderm biopsy and to define the concordance rate with the results obtained with the current platform of array comparative genomic hybridization (aCGH).

DESIGN: Validation study of a NGS platform using amplified DNA from trophectoderm biopsies in which whole or partial chromosome aneuploidies were previously detected by aCGH using comprehensive chromosome screening.

MATERIALS AND METHODS: Trophectoderm samples from each embryo underwent whole genome amplification using the Sureplex Kit (Blue-Gnome, UK). For aCGH, samples and reference DNA were labeled and co-hybridized in 24× arrays. After washing, slides were scanned and analyzed by BlueFuse Multi software. Main outcomes were ongoing pregnancy rates per transfer and per cycle. Statistical comparisons were performed using Fisher's exact test.

RESULTS: In the group of trophectoderm transfer, 35 cycles were completed with 33 transfers and 15 ongoing pregnancies (45.4% ongoing pregnancy rate per transfer and 42.8% per cycle). In the CCS group, 33 cycles were performed with 31 transfers and 22 ongoing pregnancies (71.0% ongoing pregnancy rate per transfer and 66.7% per cycle). One-side Chi-square test showed significant differences for ongoing pregnancy rates per transfer (p=0.0345) and per cycle (p=0.0417). In the blastocyst transfer group, miscarriage rate was 28.6%, whereas no miscarriage was observed in the CCS group.

CONCLUSION: Trophectoderm biopsy could be considered as a valuable clinical tool to assess embryo viability in severe male factor patients, since significantly improved clinical outcome.

Supported by: Blue-Gnome (IllumiVa), IVI, IVIOMICS.

O-73 Monday, October 20, 2014 05:00 PM

CLINICAL EXPERIENCE WITH KARYOMAPPING FOR PREIMPLANTATION GENETIC DIAGNOSIS (PGD) OF SINGLE GENE DISORDERS. R. Prates,a R. Konstantinidisa, a N.-N. Goodall,a J. Fischer,a W. Hummela,b J. Grifo,c C. Laskina,d J. Hesla,a D. Tourgermana,b D. Wills,b S. Munne.b Reprogenetics, Livingston, NJ; aSan Diego Fert Center, San Diego, CA; bSan Diego Fert Center, San Diego, CA; cNYU Fert Center, New York, NY; dLifeQuest Centre Reprod Medicine, Toronto, ON, Canada; bOregon Reprod Medicine, Portland, OR; cHRC Fert, Encino, CA; dReprogenetics UK, Oxford, Oxfordshire, United Kingdom.

OBJECTIVE: To clinically assess the efficacy of Karyomapping (Kmap) for PGD of single gene disorders.

DESIGN: A total of 43 clinical cases for PGD of single gene disorders were carried out using Kmap. For the first 23 of the cases, a conventional PGD test [short tandem repeat (STR) linkage analysis and direct mutation detection] was carried out in parallel with Kmap. For the rest of the cases, Kmap was performed with direct mutation detection alone.

MATERIALS AND METHODS: 181 blastocysts were biopsied and the obtained cells amplified with multiple displacement amplification. Separate aliquots of product were used to carry out the Kmap protocol and the validated STR and direct mutation tests.

RESULTS: Successful amplification was detected in 91.7% (166/181) of the biopsied samples. 86 embryos were given a diagnosis by Kmap and conventional PGD testing. Results obtained were in complete concordance over 95% of the cases. It was able to provide diagnosis on an additional 5 embryos that successfully amplified but received an inconclusive diagnosis using the conventional test due to allele dropout (ADO) or recombination. For the rest of the cases where Kmap was used in parallel with direct mutation detection, results agreed between the two methods for 74/75 (98.7%) embryos (discordance observed was due to ADO affecting the
AN EMBRYO COHORT WHICH CONTAINS ALL ANEUPLOID EMBRYOS IS NOT INDICATIVE OF FUTURE EMBRYO COHORT ANEUPLOIDY.

OBJECTIVE: Use of comprehensive chromosome screening (CCS) has enhanced embryo selection. However, some patients will have 100% of their cohort return as aneuploid. This varies from 5% in women in their late 20s to 55% in women >45. Beyond the initial disappointment, patients and clinicians are left with the difficult decision about whether or not to attempt another IVF cycle. Is a cohort which is 100% aneuploid indicative of future aneuploidy rates? The data presented here seek to answer those questions and assist in patient counseling and clinical decision making.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: All patients undergoing their first IVF cycle with CCS who had complete embryonic aneuploidy were selected for inclusion. This cohort was then followed through subsequent IVF/CCS cycles to determine how often the embryo cohort resulted in all aneuploid embryos. The total number of euploid and aneuploid embryos was recorded for each patient as well as the percent of complete embryonic aneuploidy per subsequent IVF/CCS cycle.

RESULTS: In 2012-2014, 316 patients had complete embryonic aneuploidy and 128 of these patients pursued a subsequent cycle. Of all embryos screened in a subsequent cycle, 43.8% were euploid. For all patients undergoing a subsequent cycle, 59.4% eventually had at least 1 euploid embryo. Of those patients that had complete embryonic aneuploidy in the 2nd consecutive cycle, 20 pursued an additional cycle for an overall euploid rate of 39.9%. Within this group, 60% had at least 1 euploid embryo in a subsequent cycle. Of those with 3 consecutive cycles with complete embryonic aneuploidy, only 3 patients completed a subsequent cycle with an overall euploid rate of 20%.

CONCLUSION: In patients who undergo IVF/CCS which results in an all aneuploid embryo cohort, they are likely to have a euploid embryo in subsequent cycles. These data are limited by the drop-out rate due to patients who do not have a euploid embryo and do not cycle again. However, it clearly demonstrates that presence of an all aneuploid embryo cohort should not prevent a repeat cycle of IVF/CCS.

O-75 Monday, October 20, 2014 05:15 PM

AN EMBRYO COHORT WHICH CONTAINS ALL ANEUPLOID EMBRYOS IS NOT INDICATIVE OF FUTURE EMBRYO COHORT ANEUPLOIDY.

OBJECTIVE: The research was funded by a grant from Brigham and Women's Department of Obstetrics and Gynecology.

O-76 Monday, October 20, 2014 05:45 PM

INCREASING PATERNAL AGE IS NOT ASSOCIATED WITH ANEUPLOIDY IN PREIMPLANTATION EMBRYOS.

OBJECTIVE: To determine whether the incidence of aneuploidy as determined by preimplantation genetic screening (PGS) is associated with increasing paternal age.

DESIGN: Retrospective cohort.

MATERIALS AND METHODS: 658 PGS cycles between 10/2010 and 2/2014 were evaluated. In all cases, PGS occurred after trophectoderm biopsy on day 5 or 6, and chromosomal evaluation was performed by array comparative genomic hybridization. Each PGS cycle was categorized according to the SART criteria for maternal age (<35, 35-37, 38-40, 41-42, >42 years). Within each SART group, a linear regression analysis was performed to evaluate whether increasing paternal age was associated with the percentage of euploid embryos in a given cohort. Within the <35 year old maternal group, euploidy rates were also compared in men <30 years versus men >45 years by student’s t-test.

RESULTS: There was no consistent correlation between increasing paternal age and euploidy rate among the SART groups.

Correlation between Paternal Age and Euploidy Rate

<table>
<thead>
<tr>
<th>SART</th>
<th>Maternal Age Category (years)</th>
<th>n</th>
<th>Average Maternal Age (ranges)</th>
<th>Pearson Correlation Coefficient between Paternal Age and Euploidy Rate</th>
<th>Pearson Correlation Coefficient between %PNS that made it to biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;35</td>
<td>148</td>
<td>36.8 (22-71)</td>
<td>0.1 (p=0.22)</td>
<td>-0.02 (p=0.8)</td>
<td></td>
</tr>
<tr>
<td>35-37</td>
<td>115</td>
<td>38.9 (29-59)</td>
<td>-0.131 (p=0.16)</td>
<td>0.104 (p=0.27)</td>
<td></td>
</tr>
<tr>
<td>38-40</td>
<td>181</td>
<td>42.1 (31-62)</td>
<td>-0.021 (p=0.78)</td>
<td>-0.102 (p=0.17)</td>
<td></td>
</tr>
<tr>
<td>41-42</td>
<td>135</td>
<td>43.6 (33-71)</td>
<td>0.045 (p=0.6)</td>
<td>-0.02 (p=0.82)</td>
<td></td>
</tr>
<tr>
<td>&gt;42</td>
<td>79</td>
<td>44.8 (34-68)</td>
<td>0.135 (p=0.24)</td>
<td>0.038 (p=0.73)</td>
<td></td>
</tr>
</tbody>
</table>

O-74 Monday, October 20, 2014 05:15 PM

AN EMBRYO COHORT WHICH CONTAINS ALL ANEUPLOID EMBRYOS IS NOT INDICATIVE OF FUTURE EMBRYO COHORT ANEUPLOIDY.

OBJECTIVE: Use of comprehensive chromosome screening (CCS) has enhanced embryo selection. However, some patients will have 100% of their cohort return as aneuploid. This varies from 5% in women in their late 20s to 55% in women >45. Beyond the initial disappointment, patients and clinicians are left with the difficult decision about whether or not to attempt another IVF cycle. Is a cohort which is 100% aneuploid indicative of future aneuploidy rates? The data presented here seek to answer those questions and assist in patient counseling and clinical decision making.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: All patients undergoing their first IVF cycle with CCS who had complete embryonic aneuploidy were selected for inclusion. This cohort was then followed through subsequent IVF/CCS cycles to determine how often the embryo cohort resulted in all aneuploid embryos. The total number of euploid and aneuploid embryos was recorded for each patient as well as the percent of complete embryonic aneuploidy per subsequent IVF/CCS cycle.

RESULTS: In 2012-2014, 316 patients had complete embryonic aneuploidy and 128 of these patients pursued a subsequent cycle. Of all embryos screened in a subsequent cycle, 43.8% were euploid. For all patients undergoing a subsequent cycle, 59.4% eventually had at least 1 euploid embryo. Of those patients that had complete embryonic aneuploidy in the 2nd consecutive cycle, 20 pursued an additional cycle for an overall euploid rate of 39.9%. Within this group, 60% had at least 1 euploid embryo in a subsequent cycle. Of those with 3 consecutive cycles with complete embryonic aneuploidy, only 3 patients completed a subsequent cycle with an overall euploid rate of 20%.

CONCLUSION: In patients who undergo IVF/CCS which results in an all aneuploid embryo cohort, they are likely to have a euploid embryo in subsequent cycles. These data are limited by the drop-out rate due to patients who do not have a euploid embryo and do not cycle again. However, it clearly demonstrates that presence of an all aneuploid embryo cohort should not prevent a repeat cycle of IVF/CCS.
Within the <35 year old maternal group, euploidy rates were actually higher when men were >45 years old compared to <30 years old, though this was not statistically significant (58% vs. 41%, p = 0.08). Furthermore, there was no consistent correlation between paternal age and the percentage of two pronuclear embryos (2PN) that made it to biopsy.

CONCLUSION: Consistent with previous observations, there is no significant significant association between increasing paternal age and aneuploidy rates. Previous studies have demonstrated that increasing male age is not associated with sperm aneuploidies. However, this observation has never been followed through to the preimplantation embryo. The findings presented here suggest that the slight decline in natural fertility and increase in spontaneous abortion rates with advancing paternal age does not appear to be attributable to an increase in embryonic aneuploidy with advancing paternal age. These findings also confirm that the vast majority of embryo aneuploidy is due to maternal meiotic errors.

O-77 Monday, October 20, 2014 06:00 PM

ANALYSIS OF PRODUCTS OF CONCEPTION (POC) BY ARRAY-CGH, NEXT GENERATION SEQUENCING AND COMPARISON TO CLASSIC KARYOTYPE APPROACH. P. Colls, 1 A. Kung, 1 M. Alikani, 1 S. Oskowitz, 2 B. Acacio, 2 B. Berger, 2 L. Ribustello, 4 Reprogenetics LLC, Livingston, NJ; 3 The Center for Human Reproduction, Manhattan, NY; 2 Boston IVF, Waltham, MA; 4 Acacio Fertility Center, Laguna Niguel, CA.

OBJECTIVE: Cytogenetic analysis is indicated for products of conception (POC). The benefits are undermined by limitations of the G-banded karyotyping on cultured cells method. In this study we aimed to assess the use of array comparative genomic hybridization (aCGH) and validate it by next generation sequencing (NGS) for analysis of POC in detection of Trisomy 19, an abnormality seldom reported by karyotyping (<1%).

DESIGN: Comparison of aCGH and NGS analysis of POC to published results by karyotype analysis.

MATERIALS AND METHODS: POC and maternal bucal or blood samples were referred to a single reference laboratory. Following DNA isolation, POC samples were analyzed by aCGH. Normal samples by aCGH were fingerprinted by STRs for 21 markers for maternal cell contamination (MCC) and polyploidy. Results were obtained in 24-72 hours. POC samples diagnosed with Trisomy 19 by aCGH were reanalyzed by NGS.

RESULTS: A total of 438 POC samples were analyzed. Of these, 110 (25%) were inconclusive due to MCC, 130 (29.6%) were normal, 7 (1.5%) were polyploid, 186 (42.4%) were aneuploid (168 trisomies and 18 monosomies) and 5 (1.1%) contained structural chromosome abnormalities. Of the 168 trisomies, 11 (6.5%) were trisomy 19. The analysis by NGS confirmed the diagnosis of trisomy 19 in all samples.

CONCLUSION: More abnormalities are detected by aCGH (60%) compared to karyotyping (50%) due to MCC elimination and because 10-25% of karyotyping attempts fail due to culture failures or no results. Because aCGH does not require tissue culture, abnormal samples that may not survive culture, can be detected by aCGH. This might be the case for POCs involving trisomy 19, which are often detected by aCGH but seldom reported by karyotyping. POC analysis by aCGH is faster, yields higher proportion of analyzable and of abnormal samples and is able to detect some aneuploidies not detectable by karyotype analysis.

POLYCYSTIC OVARY SYNDROME

O-78 Monday, October 20, 2014 04:15 PM

POPULATION BASED COHORT ANALYSIS REVEALS PERSISTENTLY HIGH DEPRESSION AND ANXIETY RATES AT THE AGE OF 46 IN WOMEN WITH PCOS AND/OR HIRSUTISM – A 15-YEAR FOLLOW-UP STUDY. T. T. Piltonen, 1 P. Colls, 1 S. Karjula, 1 J. Auvinen, 1 L. Morin-Papunen, 1 J. Miettunen, 1 J. S. Tapanainen, 1, 2, 4 Department of Obstetrics and Gynecology, Oulu University Hospital, University of Oulu, Oulu, Finland; 3 Institute of Health Sciences, University of Oulu, Oulu, Finland; 4 Department of Psychiatry, Oulu University Hospital, Oulu, Finland; 5 Department of Obstetrics and Gynecology, University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland.

OBJECTIVE: Polycystic ovary syndrome (PCOS) is associated with increased mental stress. Data on larger population based studies with follow-up are however missing. We have previously shown that PCOS (self reported oligo/amenorrhea (OA) and/or hirsutism (H)) associates with increased rate of depression and anxiety at the age of 31 independent of BMI. The aim was to investigate whether psychosomatic stress persists or even enhances in these women at the age of 46.

DESIGN: A population based cohort analysis of Northern Finland Birth Cohort 1966 (NFBC66) with 15-year follow-up.

MATERIALS AND METHODS: A postal questionnaire on OA, H, depression and anxiety [Horpects Symptoms Checklist-25 (HSCL-25) and Patient Health Questionnaire-9 (PHQ-9)] was sent to women in the NFBC66 at the age of 31 [2188 asymptomatic (ASYM), 331 OA, 323 H, 125 PCOS]. The same depression and anxiety-related questions were also asked at the age of 46. The follow-up included 1576 ASYM, 239 OA, 231 H and 85 PCOS. The associations were analyzed using ANOVA/Pearson Chi-square test/Fishers exact test, as appropriate. The results were adjusted for BMI, history of infertility and socio-economic status (SES).

RESULTS: In line with 31-year data, women with PCOS or H presented with increased anxiety score in HSCL at the age of 46 compared to ASYM (PCOS: 1.42 vs. 1.31 P = 0.023; H: 1.38 vs. 1.31, P = 0.028). A sub-analysis with cutoff score 1.55 Supported this (PCOS: 27.1% vs. 16.5%, P = 0.01; H: 23.4% vs. 16.3%, P = 0.008). Moreover, H was associated with increased depression compared to ASYM (P = 0.027). The diagnosis for depression tripled both in PCOS and ASYM from 31 to 46 years, however PCOS women reported more often diagnosed depression compared to ASYM (31yrs: 9.6% vs. 5.3%, P = 0.041; 46yrs: 26.2% vs. 14.0, P = 0.002). The PCOS women lost at the 15-year follow-up were more depressive at age 31 than the rest of the group, suggesting that the differences between PCOS and ASYM at 46 yrs are even greater than presented herein. After adjusting for infertility, BMI and SES the association of anxiety and depression increased with PCOS and/or H persisted.

CONCLUSION: The study reveals that women with PCOS or hirsutism alone have persistently high depression and anxiety rates at the age of 46.

Supported by: The Academy of Finland (project grants 252799, 104781, 120315, 129269, 1114194, 268336, SALVIE), Sigrid Juselius Foundation, the North Ostrobothnia Regional Fund, Northern Finland Health Care Support Foundation, University Hospital Oulu, Biocenter, University of Oulu, Finland (75617), the European Commission (EURO-BLCS, Framework 5 award QLG1-CT-2000-01643) and the Medical Research Council, UK (PreMetSyn/SALVIE).

O-79 Monday, October 20, 2014 04:30 PM

INCREASED WNT5A EXPRESSION UPREGULATES INFLAMMATORY VIA PI3K/AKT/ NF-κB SIGNALING IN THE GRANULOSA CELLS OF PCOS PATIENTS. Y. Zhao, J. Qiao, C. Zhang, Y. Huang, P. Liu. Reproductive Medical Center, Department of Obstetrics and Gynecology, Peking University Third Hospital, Beijing, China.

OBJECTIVE: Our aim was to investigate the action of Wnt5a in the development of chronic inflammation in polycystic ovary syndrome (PCOS), as well as explore the related molecular signaling pathways.

DESIGN: This was a prospective study on the evaluation of Wnt5a expression change in ovarian granulosa cells of PCOS women and the analysis of cross-talk between Wnt5a signaling and inflammatory response using human granulosa-like tumor cell line, KGN.

MATERIALS AND METHODS: Muratal granulosa cells from follicular fluids of 35 PCOS patients and 37 controls were collected during oocyte retrieval, mRNA was extracted and gene expressions were analyzed. Human KGN cells were cultured and treated with lipopolysaccharide (LPS, 1 μg/ml) and distinct inhibitors of specific pathways (10μM). The following techniques were used: construction and transfection of Wnt5a expression vector, small RNA Interference of Wnt5a, gene expression analysis by quantitative RT-PCR, dual-luciferase reporter assay of NF-κB activation, flow cytometric assay of reactive oxygen species (ROS) levels, flow cytometric assay of cytokine expression, and western blot analysis.

RESULTS: Our data demonstrated significant increased Wnt5a expression at both mRNA and protein level in the mural granulosa cells of PCOS women compared with controls. Besides, Wnt5a mRNA level in granulosa cells of all subjects was positively correlated with the expression levels of inflammation-related genes, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL6, IL8, monocyte chemotactic protein-1 (MCP-1), and C-reactive protein (CRP). Moreover, LPS stimulation dramatically increased Wnt5a mRNA expression in human KGN cells, and BAY-117082 (NF-κB inhibitor) treatment could suppress Wnt5a mRNA below the control level. In addition, Wnt5a overexpression upregulated pro-inflammatory cytokines and chemokines and promoted the intracellular ROS levels, IL-1β treatment (50 ng/ml)

FERTILITY & STERILITY® e27

OBJECTIVE: To determine if an elevated fasting insulin in the setting of normal fasting glucose is predictive of metabolic abnormalities in women with PCOS.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: Women seen at a multidisciplinary PCOS clinic between 2006 and 2013 and diagnosed with PCOS by Rotterdam 2003 criteria were considered for inclusion. Data were collected on fasting glucose and insulin, glucose and insulin after 2-hour glucose tolerance test, fasting lipids, body mass index (BMI), waist circumference, diastolic blood pressure (BP), total testosterone and sex hormone binding globulin (SHBG). Patients with an elevated fasting glucose >100 mg/dL were excluded from analysis. Patients with normal fasting glucose were grouped by fasting insulin level (fasting insulin: <12 mU/L and metabolic parameters were compared after controlling for age. Data were analyzed using SAS, and ANOVA was used for comparisons.

RESULTS: 255 women met criteria for inclusion in the study. Of these, 170 (67%) had normal fasting insulin and 85 (33%) had abnormal fasting insulin. After controlling for age, patients with abnormal fasting insulin had higher BMI, larger waist circumference, higher 2-hour glucose/insulin, total cholesterol, LDL and diastolic BP, and lower HDL and SHBG when compared to patients with normal fasting insulin (see Table). No differences were seen in total testosterone level. Even when controlling for BMI, fasting insulin remained a predictor of HDL and 2-hour insulin.


OBJECTIVE: To determine if an elevated fasting insulin in the setting of normal fasting glucose is predictive of metabolic abnormalities in women with PCOS.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: Women seen at a multidisciplinary PCOS clinic between 2006 and 2013 and diagnosed with PCOS by Rotterdam 2003 criteria were considered for inclusion. Data were collected on fasting glucose and insulin, glucose and insulin after 2-hour glucose tolerance test, fasting lipids, body mass index (BMI), waist circumference, diastolic blood pressure (BP), total testosterone and sex hormone binding globulin (SHBG). Patients with an elevated fasting glucose >100 mg/dL were excluded from analysis. Patients with normal fasting glucose were grouped by fasting insulin level (fasting insulin: <12 mU/L and metabolic parameters were compared after controlling for age. Data were analyzed using SAS, and ANOVA was used for comparisons.

RESULTS: 255 women met criteria for inclusion in the study. Of these, 170 (67%) had normal fasting insulin and 85 (33%) had abnormal fasting insulin. After controlling for age, patients with abnormal fasting insulin had higher BMI, larger waist circumference, higher 2-hour glucose/insulin, total cholesterol, LDL and diastolic BP, and lower HDL and SHBG when compared to patients with normal fasting insulin (see Table). No differences were seen in total testosterone level. Even when controlling for BMI, fasting insulin remained a predictor of HDL and 2-hour insulin.

O-80 Monday, October 20, 2014 04:45 PM

THE IMPACT OF PREOPERATIVE FSH ON THE LONG-TERM OUTCOMES OF LAPAROSCOPIC OVARIAN DRILLING IN WOMEN WITH POLYCYSTIC OVARIAN SYNDROME. R. Fujikura, A. Fukuji, K. Fuchinoue, Y. Sasaki, R. Nakamura, H. Mizumuma. Obstetrics and Gynecology, Hiroaki University Graduate School of Medicine, Hiroaki, Aomori, Japan.

OBJECTIVE: Laparoscopic ovarian drilling (LOD) is currently recommended as a second-line treatment for clomiphene citrate (CC)-resistant infertile women with polycystic ovary syndrome (PCOS). Many authors have reported high ovulation and pregnancy rates following LOD. However, the duration of the positive effect of LOD on ovulation is unknown and some anovulatory PCOS women fail to respond to LOD. The purpose of this study was to identify the factors that may predict the long-term outcome of LOD.

DESIGN: Retrospective study.

MATERIALS AND METHODS: Cases of CC resistant PCOS (n = 59) treated with LOD were retrospectively investigated. Preoperative and postoperative changes in the parameters measured were examined, as well as the factors that contributed to maintaining spontaneous ovulation after surgery, using logistic regression analysis. The parameters measured were serum levels of LH, FSH, prolactin, testosterone (T), free-T, and dehydroepiandrosterone sulfate, as well as fasting blood glucose, insulin and the homeostasis model assessment (HOMA) index as an indicator of insulin resistance.

RESULTS: The preoperative FSH value showed significantly higher in the group that maintained spontaneous ovulation at least 4 cycles after LOD compared to the group that transitioned to CC resistant. The logistic regression analysis showed that preoperative FSH value was a significant predictive factor for the effectiveness of LOD (p = 0.02; OR, 1.8; confidence interval, 1.1–2.9). Of the 22 cases in which 5-year postoperative

O-81 Monday, October 20, 2014 05:00 PM

ENDOPHASIC RETICULAR STRESS PATHWAY GENE EXPRESSION IS ALTERED IN THE CUMULUS CELLS OF PCOS PATIENTS UNDERGOING IVF. S. C. Collins, E. Babayev, E. Seli. Department of Obstetrics, Gynecology, and Reproductive Sciences, Yale University School of Medicine, New Haven, CT.

OBJECTIVE: The endoplasmic reticulum (ER) stress pathway responds to accumulation of unfolded proteins in the ER by either restoring cellular homeostasis or mediating cell death. ER stress response is implicated in the pathogenesis of insulin resistance in type 2 diabetes and obesity. The expression of genes in the ER stress pathway is altered in granulosa cells of obese women. Given the role of insulin resistance in polycystic ovary syndrome (PCOS), we sought to examine whether the expression of genes in the ER stress response pathway is also altered in the cumulus cells of women with PCOS undergoing IVF.

DESIGN: Experimental.

MATERIALS AND METHODS: Cumulus cells were obtained from sixteen infertile couples undergoing IVF, eight with infertility secondary to PCOS and eight with male factor infertility. Total RNA was extracted from the cumulus cells and cDNA was synthesized by reverse transcription. Expression of five genes in the ER stress response pathway (ATF4, ATF6, CHOP, GRP78, and XBP1s) was assessed with quantitative real-time PCR. ER stress response gene RNA levels were normalized to GAPDH RNA levels, and relative gene expression was calculated by the ddCt method. Statistical analysis was performed with Student’s t-test.

RESULTS: There was a 2.4-fold increase in ATF4 expression (p = 0.01) and a 2.0-fold increase in ATF6 expression (p = 0.03) in cumulus cells of subjects with PCOS when compared to women undergoing IVF for male factor infertility. There were no significant differences in these subjects in the expression of CHOP, GRP78, or XBP1s. Of note, the two study groups had no significant differences in median age (30.5 in PCOS vs. 31.0 in male factor), BMI (25.4 kg/m² vs. 24.4 kg/m²), or day 3 FSH level (5.26 mIU/mL vs. 5.46 mIU/mL).

CONCLUSION: This study is the first to demonstrate altered expression of genes in the ER stress response pathway in cumulus cells of women with PCOS undergoing IVF. While ATF4 and ATF6 have parallel expression in many cellular contexts, prior in vitro studies in goat granulosa cells revealed that sustained ER stress over time leads to increasing amounts of ATF6 expression but attenuation of ATF4 expression. Thus, the concurrent upregulation of ATF6 and downregulation of ATF4 we observe in this study may reflect a sustained increase in ER stress in the cumulus cells of women with PCOS when compared to women undergoing IVF for male factor infertility. Future studies can investigate whether the ER stress response pathway mediates the ovarian dysfunction of PCOS.
follow-up was possible, approximately 70% remained CC sensitive; after 10 years of follow-up, 7 (50%) of 14 patients were CC sensitive, demonstrating the continuous effect of LOD. Of all the preoperative test values for the patients who experienced a long-term continuous effect, only the FSH values were found to be significantly higher.

CONCLUSION: FSH values were significantly related to maintaining long-term spontaneous ovulation after LOD, suggesting that examining preoperative FSH values would be useful in predicting prognosis when deciding whether LOD should be indicated. Moreover, the effect was maintained for >10 years in at least 50% of cases in which long-term follow-up was possible.

O-83 Monday, October 20, 2014 05:30 PM
PSYCHOSOCIAL DISTRESS, COPING, AND HEALTH-RELATED QUALITY OF LIFE (HRQL) IN ADULT WOMEN WITH POLYCYSTIC OVARY SYNDROME (PCOS). R. C. Caron,1 R. I. Alvero,1 Fay W. Whitney School of Nursing, University of Wyoming, Laramie, WY; 2Reproductive Endocrinology and Infertility, University of Colorado, Aurora, CO.

OBJECTIVE: The objective of this research study was to determine the health-related quality of life (HRQL) in women with PCOS by ascertaining depressive and anxiety symptoms, perceived stress, and coping strategies.

DESIGN: A descriptive, correlational study using an online survey with five validated questionnaires.

MATERIALS AND METHODS: Descriptive, correlational, and multiple regression procedures were used to analyze the relationships among the variables using the Statistical Package for the Social Sciences, Version 21. A sample of 72 women with PCOS clinically confirmed by the Rotterdam criteria, aged 18-40, not pregnant, and able to read and write in English was recruited from an urban metro area. The study sample was adequate to show significance with a power of 0.8, an alpha of 0.05, and a 0.2 effect size. Variables were measured with the Beck Depression Inventory-II, Spielberger’s Trait Anxiety Scale, Perceived Stress Scale-10, the Ways of Coping Questionnaire, and the Polycystic Ovary Syndrome Questionnaire (PCOSQ).

RESULTS: 30.6% of the sample had severe levels of depressive symptoms, anxiety symptoms, suicidal ideation. Women with severe psychosocial distress used significantly less adaptive coping processes (p < .05) and more maladaptive coping (p < .05). Weight was PCOSQ subscale with LOD in women with PCOS (p < .01), but not in controls. The odds of clinical pregnancy was significantly diminished in overweight (OR = 0.23, CI = 0.09-0.58), obese (0.45, CI = 0.22-0.90) and morbidly obese (21%) women. Rates of spontaneous abortion, ectopic pregnancy, and multiple gestation were similar across BMI categories although individual comparisons were limited by the rarity of the outcomes. Adjusting for age and duration of infertility, the odds of clinical pregnancy was maintained.

CONCLUSION: Women with PCOS who have higher BMI after combined oral and injectable OI as compared to their overweight, obese, and morbidly obese counterparts. Patients with a lean PCOS phenotype may preferentially benefit from this treatment approach.

O-85 Monday, October 20, 2014 06:00 PM
ACUPUNCTURE INCREASES WHOLE BODY GLUCOSE UPTAKE DURING AND AFTER STIMULATION IN WOMEN WITH POLYCYSTIC OVARY SYNDROME. E. Stener-Victorin,1 A. Bengtsson,1 M. Kokosar,1 M. Malilqua,1 C. Behrens,2 K. Holjum,1 A. Sazonova,11 Department of Physiology, Institute of Neuroscience and Physiology, Göteborg, Sweden; 2Department of Cardiology, Institute Medicine, Göteborg, Sweden; 3Department of Endocrinology, University Hospital Odense, Odense, Denmark; 4Department of Obstetrics and Gynecology, Institute of Clinical Science, Göteborg, Sweden.

OBJECTIVE: Impaired glucose regulation, hyperinsulinemia and insulin resistance (IR) are common features of polycystic ovary syndrome (PCOS). A single acupuncture treatment increases whole body glucose uptake during and after stimulation in IR PCOS rats. If acupuncture has such an effect in women with PCOS is unknown. The aim of the present study was to investigate whether a single acupuncture treatment increase whole body glucose uptake during and after stimulation in women with and without PCOS.

DESIGN: A prospective experimental study.

MATERIALS AND METHODS: Twenty-one women with PCOS and 21 controls matched for age, weight and BMI were included. After an overnight fast, a euglycemic-hyperinsulinemic clamp was performed. In brief, insulin was infused (40mU/min/kg) for 120 min to reach steady state glucose infusion rates (GIR). At steady-state, acupuncture needles were placed in the abdominal and quadriceps muscles and below the knee in somatic segments corresponding to the innervation of the ovaries and pancreas. Needles were stimulated by manual rotation every 10 min and by 2 Hz electrical stimulation during 45 min. Clamp continued for 60 min after end of acupuncture. The GIR during the steady-state, during acupuncture (last 20 min) and after end of acupuncture (last 30 min) were used to assess insulin sensitivity (M value), the insulin sensitivity index (M/I value) for each period was also calculated.

RESULTS: The M value was higher during and after acupuncture compared to the controls during and women with PCOS (P < .01). The insulin sensitivity index (M/I value), did not increase during acupuncture. After acupuncture the M/I was increased compared with the effect of insulin per se in women with PCOS (P < .01), but not in controls. The lack of changes in the M/I value during acupuncture may be explained by increased insulin concentrations during acupuncture which decreased after stimulation in women with PCOS but not controls. There were no differences between cases and controls.

CONCLUSION: This is the first study to demonstrate that a single acupuncture treatment with combined manual and electrical stimulation of the needles improves whole body glucose uptake during and after stimulation in women with and without PCOS.

Supported by: Swedish Research Council, Jane and Dan Olsson Foundation, the Swedish government under the LUA/ALF agreement, and the Regional Research and Development Agreement.
ART - IN VITRO FERTILIZATION

O-86 Monday, October 20, 2014 04:15 PM

OBJECTIVE: To explore the association of endometrial thickness on the day of hCG (human chorionic gonadotropin) administration and ongoing pregnancy rate in IVF-ET cycles.

DESIGN: Large sample size, single-center retrospective cohort study.

MATERIALS AND METHODS: All patients were divided into three groups according to the endometrial thickness on the day of hCG administration: Group A, thin endometrial thickness (≤ 7 mm); Group B, medium endometrial thickness (8-13 mm); Group C, thick endometrial thickness (≥ 14 mm). In addition, patients were divided into poor (≤ 5 oocytes), medium (6-14 oocytes), and high (≥ 15 oocytes) ovarian responders based on the number of oocyte retrieved. Ongoing pregnancy rate (OPR) was separately compared among those three endometrial thickness groups in poor, medium, and high ovarian responders, respectively.

RESULTS: A total of 10,406 women undergoing their first IVF cycles were included into this study (Group A: 159 cycles; Group B: 7,403 cycles; Group C: 2,844 cycles). The incidence of thin endometrial thickness was 1.53% in total, and was highest in poor responders (2.81%, 55/1957). In addition, OPR was significantly lower in group A than that in the other two groups (29.6% versus 49.7%, and 58.8%, P<0.01). (1) For poor responders, OPRs were significantly different in the three endometrial thickness groups (20.0%, 36.2%, and 46.1%, P<0.01). The association between thin endometrial thickness and OPR was significant after controlling for age, number of embryos transferred by multivariate logistic regression analysis [adjusted OR: 0.40, 95%CI (0.20-0.83), P<0.05, Reference=thin endometrial thickness]. (2) For medium responders, OPRs were 37.5%, 53.8%, and 62.5% (P<0.01) in the three groups. Adjusted OR for thin endometrial thickness was 0.38 [95% CI (0.23-0.61), P<0.01]. (3) For high responders, OPRs were also significantly different in the three endometrial thickness groups (28.1%, 50.7%, and 58.8%, P<0.01). The association between thin endometrial thickness and OPR was also significant in multivariate logistic regression analysis [adjusted OR: 0.27, 95% CI (0.12-0.60), P<0.01].

CONCLUSION: For patients undergoing IVF with different ovarian response, a thin endometrium on the day of hCG administration adversely affects ongoing pregnancy rate.

O-87 Monday, October 20, 2014 04:30 PM
DOES TIMING OF HYSTEROSCOPIC ENDOMETRIAL BIOSPY (HEB) IN RELATION TO TIMING OF EMBRYO TRANSFER (ET) AFFECT PREGNANCY OUTCOMES IN PREVIOUS IN VITRO FERTILIZATION (IVF) FAILURES? M. Chawla, M. Fakih, A. Shunnar, J. Diwakaran, Y. Alhelou. Infertility and Reproductive Endocrinology, Fakih-IVF Fertility Center, Abu Dhabi, United Arab Emirates.

OBJECTIVE: To assess the impact of the timing of endometrial local biopsy by hysteroscopic endometrial biopsy (HEB) relative to the timing of embryo transfer on outcomes of previous in vitro fertilization (IVF) failures.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: 239 women with previous IVF failures underwent hysteroscopy, cavity check and appropriate procedures depending on findings. Women needing operative intervention for uterine pathology were excluded from analysis. 217 women (25 -38 years of age) with normal cavity who underwent hysteroscopy and endometrial biopsy (HEB) for local injury were included. Women were scheduled for HEB irrespective of the phase of the preceding menstrual cycles (after exclusion of inadvertent pregnancy) and in the initial stimulating phase of the ongoing IVF cycle. Clinical pregnancy rates were calculated based on timing of the HEB in relation to timing of embryo transfer (ET). Group 1 - Less than 30 days from ET (114 patients) and Group 2 - More than 30 days (103 patients). Chi square test was used to compare the pregnancy rates between groups.

RESULTS: The two groups did not differ in their mean age (34.4 ±4.68 years), duration of infertility (4.72 ± 1.36 years), number of embryos transferred (2.57 ± 0.56) and number of previous failed cycles (2.4 ± 0.81). The pregnancy rate in group 1 was found to be 62.57% versus 25.4% in the group 2, the difference being statistically significant (p <0.05). Further evaluation of the timings of HEB from the timing of ET showed that the highest clinical pregnancy rates were achieved when HEB was done within 11 to 15 days of ET (72.4%).

CONCLUSION: The implantation enhancing effect of endometrial injury seems to be the maximum if HEB is performed within 11-15 days of embryo transfer. Hysterscopies for local endometrial injury / scratching should be scheduled in the current treatment cycle for optimal results.

O-88 Monday, October 20, 2014 04:45 PM

OBJECTIVE: Embryo transfer is one of the critical steps in an IVF cycle, and contributes to improved implantation and pregnancy rates. Many factors such as the use of the ultrasound guidance, type of the catheter, presence of blood or cervical mucus in the catheter, manipulation of the cervix may play role in the success of the procedure. Limited data are available regarding the waiting time of the catheter inside the uterine cavity following embryo transfer. We aimed to compare outcome of IVF cycles between a 20-second wait with immediate withdrawal of the embryo transfer catheter after deposition of blastocyst.

DESIGN: Prospective randomized study was undertaken in a fertility center from March 2012 to November 2013. Patients were randomized by a computer program before embryo transfer.

MATERIALS AND METHODS: A total of 277 patients undergoing blastocyst transfer were prospectively randomized into two groups. Embryo transfer catheter was gently withdrawn immediately in 141 patients (Group I), and after a 20-second wait in 136 (Group II). Controlled ovarian hyperstimulation was performed using GnRH-antagonist protocol and maximum of two blastocysts were transferred into mid cavity. ICSI was carried out for fertilization in all patients.

RESULTS: Average age (33.7 ±5.3 vs. 32.7±5.6 years), duration of infertility (5.9±2.3 vs. 5.7±2.5 years), etiology of infertility, number of oocytes collected (11.4±5.4 vs. 11.6±5.1), fertilization rates (81.4% vs. 79.2%) and the number of blastocysts transferred (1.5±0.3 vs. 1.5±0.2) were similar between Group I and Group II, respectively. Implantation (25.8% vs. 25.7%), clinical pregnancy (38.2% vs. 38.9%), multiple pregnancy (18.5% vs. 16.7%) and miscarriage rates (9.7% vs. 9.8%) did not differ significantly between Group I and Group II, respectively.

CONCLUSION: A 20-second delay of the embryo transfer catheter in the uterine cavity following blastocyst transfer does not result in a better outcome than immediate withdrawal. The advantage of the prolonged time of retention of the catheter does not seem to exist between the two different waiting times used in this study.

Supported by: Duration of the keeping embryo transfer catheter inside the uterus has been questioned in very limited number of studies. Our study will provide additional information regarding embryo transfer procedure.

O-89 Monday, October 20, 2014 05:00 PM
TRANSPLANTATION OF ZONA-FREE CLEAVAGE STAGE EMBRYOS INTO AN EMPTY ZONA WITH SUCCESSFUL BLASTULATION, A CASE SERIES. J. Ding, Y. Yang, P. Brezina, R. Ke, W. Kutteh. Kutteh Ke Fertility Associates of Memphis, Memphis, TN.

OBJECTIVE: To evaluate the blastulation potential of cleavage stage zona-free embryos transplanted into an empty zona. DESIGN: Case series.

MATERIALS AND METHODS: Zona-free oocytes were obtained incidentally during cumulus removal using the standard treatment with hyaluronidase followed by strand away with 140 μm ID (Cook Medical Inc, Bloomington IN, USA). Zona-free eggs were fertilized with intracytoplasmic sperm injection (ICSI) and were cultured to 8-cell stage on day-3. Then the
blastomeres were transplanted into an empty zona-pellucida to form a reconstructed embryo.

RESULTS: Two patients (patient A: age 32 and patient B: age 35) underwent in vitro fertilization (IVF) with a diagnosis of male factor infertility. GnRH antagonist protocol was used in both patients. Patient A had 20 eggs retrieved, 15 of which were mature, and patient B had 10 eggs retrieved, 7 of which were mature. Cumulus was removed using hyaluronidase treatment followed by repeated pipetting using a 140µm stripping pipette (Cook Medical, Bloomington IN, USA) in each case. In both patients, a zona-free oocyte was inadvertently obtained during the stripping procedure. The zona-free eggs were then fertilized using intracytoplasmic sperm injection (ICSI). In both cases, the oocytes fertilized and grew to the 8-cell stage on day 3. Unfertilized sibling oocytes from the same patient with intact zonas were then identified and cyttoplasm was removed with a blastomere biopsy pipet (55 µm ID, Humagen/Origio) following laser compromise of zona. Cells from the zona-free cleavage stage embryos were then transferred into the empty zona shells. In each case, the reconstructed embryo developed normally and formed a good quality blastocyst, both grade BB. These embryos were then cryopreserved and have not yet been used for embryo transfers.

CONCLUSION: The results of this report have important implications for assisted reproductive technologies (ART). Currently, ART laboratories may discard zona-free oocytes. Transplantation of zona-free compacting embryo into the zona of an immature egg represents a modality that may maximize success rates for many couples, particularly couples with low numbers of oocytes obtained at time of oocyte retrieval. These findings also are important for further understanding the processes associated with early human embryo genesis.

O-90 Monday, October 20, 2014 04:15 PM

A MULTICENTRIC RETROSPECTIVE STUDY TO DEFINE AND VALIDATE AN ALGORITHM FOR EMBRYO SELECTION BASED ON KINETIC MARKERS. N. Basile,a P. Vime,a M. Riquerob,c B. Aparicio Ruiz,c M. Meseguer,c IVI Madrid, Madrid, Spain; IVI Sevilla, Sevilla, Spain; IVI Barcelona, Barcelona, Spain; IVI Valencia, Valencia, Spain.

OBJECTIVE: To define and validate an algorithm for embryo selection based on morphokinetic parameters and to correlate it with implantation rates.

DESIGN: Retrospective observational study.

MATERIALS AND METHODS: Patients undergoing their first or second cycle of intracytoplasmic sperm injection (ICSI) using their own or donated oocytes were included. Embryo development was analyzed with time-lapse imaging system. Variables studied included the timing to two (t2), three (t3), four (t4) and five (t5) cells, the length of the second cell cycle (cc2 = t3 - t2) and the synchrony from two to four cells (s2 = t4 - t3). Data to develop the new algorithm was obtained from 799 couples with 6424 oocytes, 4630 embryos, 1640 transferred embryos, and 764 embryos with known implantation data (KID). Only KID embryos with either full implantation (n = 228) or no implantation (n = 526) were included. Once developed, the algorithm was validated in 865 cycles with 1620 transferred embryos. Implantation was confirmed by the existence of a fetal heart beat at ultrasound after 7 weeks of gestation.

RESULTS: Development of the algorithm: a logistic regression analysis identified t3 (34-40h) OR = 1.542 (95% CI 1.094-2.173), followed by cc2 (9-12h) OR = 1.425 (95% CI 1.025-1.981) and t5 (45-55h) OR = 1.210 (95% CI 0.841-1.731) as the most relevant variables related to implantation. Based on these results a hierarchical model classifying embryos into four categories was obtained: grade ‘A’ (32 % n = 129/407), ‘B’ (28 % n = 52/185), ‘C’ (26 % n = 37/140), ‘D’ (20 % n = 38/193) and ‘E’ (17 % n = 34/197).

CONCLUSION: A new algorithm for embryo selection has been elaborated and validated prospectively in a multicentre study. This classification method is strongly correlated with implantation rates and can be applied to improve embryo selection in different clinical settings with the expectancy of increasing outcome results.

O-91 Monday, October 20, 2014 04:30 PM

“NO DIAGNOSIS” EMBRYOS AFTER PGS SHOULD NOT BE DISCARDED: REBIOPSY AND REANALYSIS DEMONSTRATE THE MAJORITY ARE EUPLOID. M. Browera,d D. Hill,e,f H. Danzere,f M. Surrey,e,f S. Ghadird, W. Chang,f,c C. Wambach,c,b C. Alexander,c J. Bartt. Obstetrics and Gynecology, University of California Los Angeles, Los Angeles, CA; ART Reproductive Center, Beverly Hills, CA; Southern California Reproductive Center, Beverly Hills, CA; Obstetrics and Gynecology, Cedars-Sinai Medical Center, Los Angeles, CA.

OBJECTIVE: Preimplantation genetic screening (PGS) is an increasingly common practice among fertility clinics. Not all embryos that have been biopsyed for PGS will receive a genetic diagnosis. The “no diagnosis” result can be due to failure of DNA amplification, degraded DNA, or failure to load cell(s) into the tube. The chromosomal makeup of these embryos is unclear and there have been no reports on their genetic viability. The aim of our study was to determine the likelihood that an embryo without a diagnosis after initial biopsy and array comparative genomic hybridization (aCGH) will be chromosomally balanced, if it is rebiopsied for reanalysis.

DESIGN: Retrospective data analysis from a single large private fertility clinic.

MATERIALS AND METHODS: Data for all patients (n = 536) undergoing aCGH testing of embryos (n = 3,207) between 2010 and 2013 were analyzed. All embryos that did not have a diagnosis after the initial biopsy and were subsequently rebiopsied for repeat aCGH testing were included in the analysis (n = 44). Embryo biopsy was performed originally on day 3 or day 5/6 with subsequent rebiopsy of a fresh or thawed blastocyst.

RESULTS: At least one embryo with “no diagnosis” was found in 4.3% of cases in which embryo biopsy with PGS was performed, and 0.86% of all biopsied embryos had “no diagnosis”. Of the 44 embryos that were rebiopsied for reanalysis 100% were assigned a genetic diagnosis. Twenty-four (54.5%) were diagnosed euploid. Of the 20 embryos that were abnormal the most common diagnosis was trisomy (n = 7). Other abnormalities included 5 monosomies, 4 double, 3 complex, and 1 chaotic.

CONCLUSION: The majority of embryos with no diagnosis after genetic screening are diagnosed euploid when rebiopsy and reanalysis is performed. In addition, 100% of the rebiopsied embryos were assigned a diagnosis with repeat aCGH testing. The willingness of most reproductive genetic analysis labs to subsequently reanalyze a “no diagnosis” embryo at no additional cost to the patient will allow for better outcomes by preventing normal embryos from being discarded and obviating transfers of abnormal ones.

O-92 Monday, October 20, 2014 04:45 PM

THE IMPACT OF VARIATIONS OF TSH WITH THE “LOW NORMAL RANGE” ON EUPLOID BLASTOCYST IMPLANTATION RATE (IR). K. Anderson,a M. D. Wernera,b J. M. Franasiak,e K. H. Hong,e R. T. Scott, Jr.,f “REI, RWJ, Rutgers, Basking Ridge, NJ; bBeaumont School of Medicine, Royal Oak, MI; cRMA NJ, Basking Ridge, NJ.

OBJECTIVE: Supplementation of thyroid hormone during the first trimester is recommended by the American Thyroid Association for TSH values >2.5mIU/L as they may be associated with adverse outcomes. This standard may also be applied to infertile patients attempting conception, but there has been no analysis on whether variation of TSH values within this “low normal range” have an impact on IR. The goal
FERTILITY PRESERVATION I

O-94 Monday, October 20, 2014 04:15 PM

SAFETY OF FERTILITY PRESERVATION BY OVARIAN STIMULATION WITH LETROZOLE AND GONADOTROPIN IN PATIENTS WITH BREAST CANCER: A PROSPECTIVE CONTROLLED STUDY WITH SUBGROUP ANALYSIS. J. Kim, a,b V. Turan, a,b K. Oktyab

OBJECTIVE: Breast cancer is the most common malignant neoplasm encountered in reproductive age women. Aromatase inhibitor protocols have been developed with a view to increase the safety margin of controlled ovarian stimulation (COS) for fertility preservation by oocyte or embryo cryopreservation before chemotherapy. The objective of this study was to investigate the safety of letrozole-gonadotropin COS protocol in breast cancer patients including various subgroup analysis (estrogen receptor (ER) positive vs. negative cancer and pre- vs. post-operation).

DESIGN: Prospective cohort study.

MATERIALS AND METHODS: Three-hundred and three women diagnosed with stage 0–3 breast cancer were prospectively evaluated for fertility preservation by embryo or oocyte cryopreservation before chemotherapy. Of these, 151 (COS group) elected to pursue COS and 157 (control group) did not. Patients were followed annually for breast cancer recurrence.

RESULTS: COS and control groups were similar at enrollment (Table 1). The mean follow-up after diagnosis was 4.9 years in COS and 6.2 years in control group. In the COS group, the hazard ratio for recurrence after IVF was 0.69 (95% CI: 0.27, 1.76) and the survival was not compromised compared with controls (P = 0.24). Among patients who pursued letrozole-FSH COS, the hazard ratio for recurrence in ER-negative group was 0.63 (95% CI: 0.06, 5.31) and the survival was not compromised compared with ER-positive group (log rank P = 0.75). The survival was not different between patients who pursued COS after and before tumor resection (P = 0.56).

<table>
<thead>
<tr>
<th>Table 1. Patient and Tumor Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment Group (n=151)</td>
</tr>
<tr>
<td>------------------------</td>
</tr>
<tr>
<td>Age at FP consultation</td>
</tr>
<tr>
<td>Age at cancer diagnosis</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
</tr>
<tr>
<td>Positive node (%)</td>
</tr>
<tr>
<td>Tumor size (cm)</td>
</tr>
<tr>
<td>&lt;2</td>
</tr>
<tr>
<td>2-5</td>
</tr>
<tr>
<td>&gt;5</td>
</tr>
<tr>
<td>Lymphovascular space invasion (%)</td>
</tr>
<tr>
<td>Histologic grade (%)</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>Estrogen receptor (%)</td>
</tr>
<tr>
<td>HER-2/neu positive (%)</td>
</tr>
</tbody>
</table>

CONCLUSION: We have reported the largest prospective study on the safety of fertility preservation by oocyte or embryo cryopreservation after COS with letrozole-gonadotropins. COS with letrozole and gonadotropins is unlikely to cause substantially increased recurrence risk in breast cancer, even when the stimulation is performed before the resection of the tumor.

Supported by: NIH ROI HD053112 and R21 HD061259.
O-95 Monday, October 20, 2014 04:30 PM

PREVIVO UTERINE LAVAGE CATHETER: A NOVEL DEVICE FOR THE RECOVERY OF INVIVO DERIVED HUMAN EMBRYOS BY NON-SURGICAL UTERINE LAVAGE. K. Pagidas, C. Nezhat, S. Carson, A. Nezhat, M. Cesario, S. Woodward, A. Nadi, J. E. Buster, Previvo Genetics, LLC, San Jose, CA; Warren Alpert Medical School, Brown University, Providence, RI; Center for Special Minimally Invasive and Robotic Surgery, Palo Alto, CA; Alacriti Medical, San Jose, CA.

OBJECTIVE: Develop a novel device for the safe recovery of in vivo human derived embryos by non-surgical uterine lavage for pre-implantation genetic screening and diagnosis.

DESIGN: Pre-Clinical Laboratory Test of New Medical Device.

MATERIALS AND METHODS: The Previvo catheter was fabricated by Previvo Genetics LLC, San Jose, CA and consists of a co-axial uterine catheter, a distal supply port attached to a supply line that is regulated by an electrical controller managing a consistent flow and withdrawal of lavage fluid.

The Previvo catheter was tested using a series of experiments to verify its ability to recover blastocysts. (1) 350 lavages were conducted using a simulated silicon uterus to test variable settings on the catheter for optimal fluid dynamics to prevent fluid loss. The lavage cycle included fluid supplied and vacuumed in alternating pulse cycles of approximately 0.5 to 4.0 sec using low flow and vacuum conditions with a total volume of 100-300 ml per lavage cycle. (2) 24 uterine lavages were conducted in 12 extirpated human uteri to assess that the device will not cause harm to the uterine anatomy. After two lavage cycles, each uterus was dissected and inspected for mechanical damage. (3) 129 mouse blastocystcs were placed in silicon uteri and lavages were performed to recover the blastocysts, which were then cultured and scored for cell divisions and morphology.

RESULTS: 98% of lavage fluid was recovered from the silicon uterus models. (2) No visible tissue abrasion, pinching or puncture occurred after 2 lavage cycles in the extirpated uteri. (3) 96.7% of lavage-recovered mouse blastocysts were viable one day after the lavage cycle as indicated by ongoing cell divisions and morphological assessment.

CONCLUSION: The Previvo catheter successfully recovers blastocysts non-surgically without damage to the embryos or uterus. These results indicate the device is ready for human trials for recovery of human embryos derived in vivo to afford the opportunity for pre-implantation genetic diagnosis without undergoing IVF.

Supported by: Research Sponsored by Previvo Genetics, LLC.

O-96 Monday, October 20, 2014 04:45 PM

THE IMPORTANCE OF CRYOPROTECTANT EXPOSURE TIME ON SURVIVAL OF ISOLATED SECONDARY FOLLICLES VITRIFIED IN A CLOSED SYSTEM AFTER SHORT TERM 3-DIMENSIONAL (3D) CULTURE. D. L. Bulgarelli, A. Y. Ting, M. B. Zelinski, Obstetrics and Gynecology, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil; Division of Reproductive & Developmental Sciences, Oregon National Primate Research Center, Oregon Health & Sciences University, Beaverton, OR; Division of Reproductive & Developmental Sciences; Department of Obstetrics & Gynecology, Oregon National Primate Research Center, Oregon Health & Sciences University, Beaverton, OR.

OBJECTIVE: The current study evaluated the influence of two vitrification procedures, vary by their cryoprotectant agent exposure time, on follicle survival during short-term 3D culture.

DESIGN: Rhesus macaque follicles were studied individually during vitrification and encapsulated 3D follicle culture.

MATERIALS AND METHODS: Ovaries were obtained from adult rhesus macaques (n=8, 8-11 years, normal cycles), the cortex was cut into 1x1x0.5mm3 fragments, and secondary follicles were isolated mechanically without enzyme digestion. Follicles were incubated sequentially with shaking in vitrification solution containing 5% glycerol, 7.5% glycerol+7.5% ethylene glycol (EG), 15% glycerol+15% EG and 25% glycerol+25% EG+12.5% polymers (PXZ) for 2 exposure times; VT1 group: 1 min in each solution and 10 sec in the final solution, and VT2 group: 5 min, 3 min, 2 min and 10 sec in each solution, respectively. Follicles were transferred into 0.25 cc straws that were heat sealed then cooled in liquid nitrogen (LN2) and stored. Thawed follicles were transferred into holding media containing 1, 0.5, 0.25M sucrose for 3 min each and 0M sucrose for 5 min.

Fresh and vitrified-thawed follicles were encapsulated into alginate (0.25% w/v), and cultured for seven days at 5% O2 in cMEM with FSH. Follicle survival was analyzed.

RESULTS: Survival rate in the VT1 group was reduced (P<0.05) in vitrified follicles (25 ± 13%) compared to fresh follicles (87 ± 1%). However, follicle survival in the VT2 group was similar between fresh and vitrified follicles (80 ± 7%; 68 ± 12%, respectively). Vitrified follicles from VT2 had a greater (P=0.05) survival rate compared to those from VT1.

CONCLUSION: Vitrification of isolated secondary follicles using shorter cryoprotectant exposure times (VT2) followed by 3D culture for one week maintained follicle morphology and survival. The shorter exposure times prevented major osmotic changes that are detrimental to preserving follicular integrity. Further studies are necessary to investigate the function of vitrified-thawed secondary follicles during long-term 3D culture.


O-97 Monday, October 20, 2014 05:00 PM

MESENCHYEMAL STEM CELLS ENHANCE ANGIOGENESIS AND FOLLICLE SURVIVAL IN HUMAN CRYOPRESERVED OVARIAN CORTEX TRANSPLANTATION. X. Xu, J. Qiao, T. Yin, L. Yan, C. L. Yu, Peking University Third Hospital, Beijing, China; Affiliated Shenzhen Nanshan Hospital, Guangdong Medical College, Shenzhen, Guangdong, China; Peking University Shenzhen Hospital, Shenzhen, Guangdong, China.

OBJECTIVE: Since the main obstacle of the cryopreserved ovarian tissue transplantation is massive follicle loss as a result of ischemia in the process of transplantation, this study is aimed to investigate whether mesenchymal stem cells (MSCs) could be applied to enhance the angiogenesis and follicle survival in human cryopreserved ovarian cortex transplantation.

DESIGN: We transplanted human cryopreserved ovarian cortex tissues with MSCs in the subcutaneous of female athymic BALB/c mice. At different time points, vascular formation, functional blood perfusion, follicle count and apoptosis were measured to assess the effects of MSCs on the cryopreserved ovarian tissue transplantation.

MATERIALS AND METHODS: Human MSCs were isolated from the bone marrow of normal individuals and ovarian tissues were obtained from a consenting 21-year-old female-to-male transsexual and slow-frozen and thawed using ethylene glycol (EG) (Sigma) as a cryoprotectant. Thirty six 8-week-old female athymic BALB/c mice were used for the subcutaneous transplantation in the study. 3, 7 and 21 days after the transplantation, in vivo grafts perfusion measurement using infrared camera, histological evaluation to evaluate follicle count, immunohistochemistry to measure vascular formation and follicle apoptosis and statistical analyses performed by ANOVA analysis followed by pairwise comparison test were applied in the study.

RESULTS: Here we show that human MSCs could significantly stimulate neovascularization and increase blood perfusion of the grafts in the cryopreserved ovarian tissue transplantation. Further studies reveal that MSCs could notably reduce primordial follicles apoptosis rates and decrease follicle loss in the grafted ovarian tissues.

CONCLUSION: In summary, our findings demonstrate a previously unrecognized function of MSCs in improving human ovarian tissue transplantation and provide a useful strategy to optimize fertility preservation and restoration.

Supported by: This work was funded by the National Basic Research Program of China (2011CB944500), the National Science Foundation (No. 3120047, No. 81000275, No. 81130971; No. 81200470; No. 81370729; No. 81070493) and the China Postdoctoral Function (No.2013M540027).

O-98 Monday, October 20, 2014 05:15 PM

OVARIAN PERFORMANCE, IVF RESULTS, PREGNANCIES AND LIVE BIRTHS INDICATE; FERTILITY PRESERVATION USING OVARIAN TISSUE HARVESTING AND TRANSPLANTATION OF THAWED OVARIAN STRIPS IS EFFECTIVE. D. Meirov, H. Raanani, M. Brenghausen, O. Lebovitz, R. Orvieto, J. Dor, Fertility Preservation IVF Unit, Sheba Medical Center, Tel Hasomer, Israel.

OBJECTIVE: The field of fertility preservation (FP) in females has extended with increasing medical and public awareness. Significant scientific
O-99 Monday, October 20, 2014 05:30 PM


OBJECTIVE: Random-start COH has been reported as an alternative method for initiating oocyte/embryo cryopreservation cycles for the purpose of fertility preservation, with the benefit of a shortened interval from presentation to retrieval and thus to cancer treatment. We evaluated our experience with random start COH antagonist cycles, compared outcomes in luteal and non-luteal cycles, and measured the time from initial consultation to COH start.

DESIGN: Retrospective cohort study.

Comparison between non-luteal and luteal start

<table>
<thead>
<tr>
<th></th>
<th>Non-luteal start (n=12)</th>
<th>Luteal start (n=9)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>31.5±7.0</td>
<td>32.8±6.6</td>
<td>NS</td>
</tr>
<tr>
<td>AMH</td>
<td>3.18±2.8</td>
<td>2.24±1.8</td>
<td>NS</td>
</tr>
<tr>
<td>AFC</td>
<td>15.6±7.9</td>
<td>16.1±11.3</td>
<td>NS</td>
</tr>
<tr>
<td>Cycle Cancellations</td>
<td>1±</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Days of stimulation</td>
<td>10.5±1.3</td>
<td>11.2±1.0</td>
<td>NS</td>
</tr>
<tr>
<td>Total dosage (IU)</td>
<td>4086±1850</td>
<td>5492±1308</td>
<td>NS (.056)</td>
</tr>
<tr>
<td>Oocytes retrieved</td>
<td>18.2±15.4</td>
<td>17.2±10.7</td>
<td>NS</td>
</tr>
<tr>
<td>MII Oocytes</td>
<td>13.8±8.4</td>
<td>13.2±10.0</td>
<td>NS</td>
</tr>
<tr>
<td>Days from intake to COH start*</td>
<td>6.7±4.8 (0-16)</td>
<td>na</td>
<td></td>
</tr>
</tbody>
</table>

* 1 cycle canceled after 7 days of stimulation due to poor response (AMH 0.39, AFC-7); 1° reported for both groups combined; data presented as mean ± SD; (range)

RESULTS: Seventeen patients underwent transplantation of cryopreserved ovarian tissue. The diagnosis was Hodgkin’s lymphoma, Non-Hodgkin’s lymphoma, Leukemia (CML), sarcoma and breast cancer. Patient’s age at transplantation was 22-45 years. In 2 patients a secondary malignancy was identified and fertility treatments were withheld. Recovery of menstruation and endocrine function was documented in 13/14 patients. Repeated IVF cycles (1-10 cycles per patient) using modified natural protocols resulted in 1-2 mature oocyte retrieval per-cycle. In 25% of the cycles there were no eggs at retrieval which was not related to the time period from grafting. Nine pregnancies were reported, 6 post IVF and 3 spontaneous pregnancies. Six healthy children were born and one of the patients conceived 3 times.

CONCLUSION: The results of this study performed in one center using the same laboratory and surgical procedure indicate that the use of stored ovarian tissue to restore fertility is effective and results with repeated pregnancies and live birth of normal babies, both following IVF and with natural conception. The ability of the graft to produce mature oocytes in repeated cycles indicates the long term survival of ovarian grafts. Correlation of transplantation results with the published literature on egg and embryo freezing for FP indicates that the total number of eggs collected following ovarian tissue transplantation is higher and as well as the chances to conceive.

O-100 Monday, October 20, 2014 05:45 PM


OBJECTIVE: To explore disparities in counseling of adult female cancer patients for fertility preservation.

DESIGN: Retrospective study of female patients with a diagnosis of breast, gynecologic, or hematologic cancer from 2009-2013 at an academic medical center.

MATERIALS AND METHODS: Electronic medical record data for demographics, counseling for fertility preservation, and cancer diagnosis and treatment was obtained for 587 female patients with breast, gynecologic, or hematologic cancer. Retrospective analysis of 353 patients (19-42y; x 35.0, SD 5.31) with available chemotherapeutic treatment data was performed.

RESULTS: 262/353 (74%) women were exposed to a gonadotoxic chemotherapeutic agent; 181 women were diagnosed with breast cancer, 53 with a hematologic (i.e., leukemia/lymphoma) cancer, and 28 with a gynecologic cancer. 161/262 (62%) chemotherapy exposed women had documented counseling for fertility preservation; 77 (29%) women were not counseled, and counseling was not documented in 24 (9%) charts. Younger women were more likely to be counseled than older women; the average age of those counseled was (x 34.0, SD 5.6yrs), not counseled (x 35.0, SD 4.7yrs), and not appropriate for counseling (x 35.5, SD 4.4 yrs) (p < .05). Racial differences in counseling were also found (p < .05) with 86% of Hispanic women, 65% of Asian women, 62% of white women, and 53% of black women with documented counseling. Divorced women were less likely to be counseled than women of any other marital status (p < .05). Women with gynecological or hematological cancer or who had a lower cancer stage were more likely to be counseled that those with other cancers or higher stages (p < .05). Logistic regression resulted in no unique variance contribution in a model including age, marital status, race, cancer diagnosis, and stage.

CONCLUSION: In the current study, demographic and diagnostic disparities were evident in the counseling of cancer patients for fertility preservation. Patients who are not offered fertility preservation prior to cancer treatment have previously been found to experience significant regret and poorer quality of life. In addition to emotional harm, disparate counseling for fertility preservation could result in an unnecessary decreased ability for some women to fulfill their future reproductive desires. Equality in the counseling of female cancer patients for fertility preservation is imperative in order to reduce the risk of emotional harm and future infertility.

Supported by: (EEM) NIH Women’s Reproductive Health Research Scholar Award; Robert Wood Johnson Foundation, Friends of Prentice and the Evergreen Invitational.
RESULTS: A total of 140 thaw cycles have resulted in 74 (53%) pregnancies; 61 (44%) are currently either ongoing (21) or delivered (40). See the Table for full results.

CONCLUSION: OC is proving itself a valuable means to preserve/prolong female fertility, regardless of indication. Improved successes will continue to move this technology into mainstream ART.

**CLINICAL FEMALE INFERTILITY I**

O-102 Monday, October 20, 2014 04:15 PM

DNA BINDING AND GENE EXPRESSION ANALYSIS OF THE PROGESTERONE RECEPTOR TARGET FOS-LIKE ANTIGEN 2 (FOSL2) IN DECIDUALIZING HUMAN ENDOMETRIAL STROMAL CELLS, E. C. Mazur, a E. Eklund, b J. S. Rhee, a N. Noyes, a Obstetrics and Gynecology, Baylor College of Medicine (ECM) and R01 HD042311 and 60K microarray. Microarray expression changes were confirmed by qPCR.

RESULTS: FOSL2 was found to bind in the vicinity (±10 kilobases) of 8586 distinct genes. FOSL2-bound genes include 80% of the genes bound by PGR under the same conditions with a great degree of overlap (i.e., FOSL2 and PGR are bound at the same sites). Microarray analysis revealed 1465 differentially regulated genes in HESCs after 72 hours exposure to a decidual stimulus between cells transfected with siRNA targeting FOSL2 versus non-targeting scrambled siRNA. Genes with altered expression after knockdown of FOSL2 were enriched in pathways of development, cell proliferation, and cell migration. These genes differentially expressed in decidualized HESCs after FOSL2 knockdown are largely distinct from genes with expression change after PGR knockdown.

CONCLUSION: Progesterone receptor (PGR) is essential for human endometrial stromal cell (HESC) decidualization, though the mechanisms through which PGR mediates this critical process remain elusive. We previously validated FOSL2 as a transcription factor induced and directly regulated by PGR during HESC decidualization. Here we describe that the genomic binding of FOSL2 encompasses 80% of the genes bound by PGR under the same conditions. While FOSL2 and PGR occupy the same intervals of DNA, the gene signature of FOSL2 knockdown is largely distinct from that of PGR knockdown. The role of FOSL2 as a potential pioneer factor for PGR in HESC decidualization requires further investigation.

Supporting by: Research Supported by the Department of Obstetrics and Gynecology, Baylor College of Medicine (ECM) and R01 HD042311 and U54 HD007495 (FID).
newborn studies, most cord blood samples had detectable AMH levels on this more sensitive assay. Increased maternal age was highly associated with decreased fetal cord blood AMH levels (p<0.001) and increased fetal weight was associated with increased AMH values (p<0.007). Mean cord blood AMH in singleton pregnancies (0.87±1.3 ng/mL) is higher than that from twins (p=0.018), though zyosity was unknown. In a multivariate analysis controlling for maternal age, BMI and fetal weight, increasing maternal pre-pregnancy AMH was associated with decreasing newborn cord blood AMH values (p<0.025).

CONCLUSION: Cord blood AMH values appear to be regulated by maternal characteristics. Our findings suggest that pathologic processes related to higher maternal AMH values could lead to an untoward affect on oocyte pool endowment and early antral follicle development in utero. Differences between singleton and twin female neonates warrant further investigation.

Supported by: 1K12HD063086-01 (ARC), Beckman Coulter Inc support (ARC) and grant funding (GLM), 5T32HD040135-12 (JSR).

O-104 Monday, October 20, 2014 04:45 PM

DIFFERENTIAL CONTRIBUTIONS OF AGE AND BASAL FOLLICLE STIMULATING HORMONE (FSH) LEVELS TO ODDS OF ANEUPLOIDY. J. Rodríguez-Purata, A. J. A. Lee, a E. Cervantes, a M. Luna, a H. V. Karvīr, a J. Klein, b P. Yurttas Beim, A. B. Copperman. a b b

RESULTS: A total of 462 patients with 2207 embryos were analyzed. Overall, patients with normal ploidy were younger (35.5±4.0 vs. 38.1±4.4) and had a lower basal FSH level (7.56±3.6 vs. 8.1±3.5) compared to those with aneuploidy. Our study demonstrated that the odds of aneuploidy increased by 10% for each year of a woman’s reproductive lifespan (OR=1.1, p<0.0001). We found no independent contribution of FSH levels to odds of aneuploidy, either when assessed as a continuous variable or above/below a threshold of 13 mIU/mL (p=0.02). We did observe that for women with FSH levels above 13 mIU/mL, their odds of aneuploidy increased at a substantially higher rate (50%) for each additional year (OR=1.52, p<0.0001) of life.

Factor OR p-value
Age 1.1 <0.0001
FSHMax 1.01 0.75
FSHMax>13 0.84 0.45
FSHMax>13:Age 1.52 <0.0001

CONCLUSION: Our findings suggest that equivalent FSH levels should not be directly equated with egg quality in women of different age. This has significant implications for the management of infertility in younger women with elevated FSH levels. Also, these women might benefit from earlier treatment intervention and egg/embryo banking, given that their odds of aneuploidy rise more rapidly over time than women of the same age without elevated FSH levels.

O-105 Monday, October 20, 2014 05:00 PM

EFFICACY OF TNF-ALPHA BLOCKERS ON POSTOPERATIVE INTRA ABDOMINAL ADHESIONS. S. Demirbag, a B. Uysal, b I. Surer, a N. Yesildaglar. a b

OBJECTIVE: Various studies have been performed to find out novel treatment strategies to prevent postoperative adhesion formation. Tumor necrosis factor -alpha (TNF-a) is a pro-inflammatory agent and induce the acute phase reaction with local and systemic inflammation. We reported significant elevation in TNF-a level in the peritoneal washing fluids in rats with peritoneal adhesion, before. Therefore, we aimed to evaluate the efficacy of TNF – a blockers in experimental model of postoperative adhesions.

MATERIALS AND METHODS: Thirty female Wistar rats (200–250 g) were divided into three groups: sham, control, and TNF-a blocker groups. The control and TNF-a blocker groups were subjected to the postoperative adhesion procedure created by bipolar coagulation set at 10 watts per second on the uterine horns and corresponding pelvic sidewall parietal peritoneum. In the control and TNF-a blocker groups, approximately 2 cm of the both uterine horns and 4 cm2 of the opposing pelvic sidewall peritoneum of the rats were electrocoagulated using bipolar forceps and a power setting of 10 W/sec. The rats in the TNF-a blocker (Infliximab®) group were administered a single dose of 5 mg/kg mg/kg/day Infliximab® intraperitoneally (i.p.) after adhesion induction. The animals were killed on the 7th day and uterine adhesions were evaluated according to the scoring system published by Leach et al. (1998). The adhesions, electrocoagulated tissues, and samples of peritoneal washing fluid were collected for biochemical evaluation.

RESULTS: In the control group, a significant elevation in TNF-a level in the peritoneal washing fluids, increased levels of Malondialdehyde (MDA) in the uterine tissues, and decreased antioxidant enzyme activities Superoxide dismutase (SOD) and Glutathione peroxidase (GSH-Px) in the uterine tissues were observed. None of these changes were found in the TNF-a blocker group. Macroscopic adhesion scores were significantly higher in the control group than those observed in the other groups (p<0.05, control vs. sham and control vs. TNF-a blocker group).

CONCLUSION: TNF-a blocker therapy prevents postoperative uterine adhesions by modulating TNF-a levels and oxidative/antioxidative status in an experimental uterine adhesion model.

Supported by: Funding was provided by Gulhane Military Medical Academy Research Center.

O-106 Monday, October 20, 2014 05:15 PM

INFERTILITY DIAGNOSIS HAS A SIGNIFICANT IMPACT ON THE DEVELOPING BLASTOCYST’S TRANSCRIPTOME. M. G. Katz-Jaffe, a b J. C. Parks, a B. R. McCalle, a S. A. Strieby, b W. B. Schoolcraft, a b

OBJECTIVE: Limited studies have investigated the impact of infertility diagnosis on the viability of human embryos. A competent blastocyst is a key contributor to successful implantation, placentation and ongoing viable fetal development. The aim of this novel study was to explore the transcriptome of human blastocysts and uncover the biological pathways that may be influenced by infertility diagnosis.

MATERIALS AND METHODS: Surplus cryopreserved human blastocysts of equivalent good morphology were donated under IRB consent and grouped according to their distinct infertility diagnosis: Fertile donor oocyte controls with no male factor (n=50), Polycystic ovaries (PCO; n=50), Male factor (MF; n=50). Unexplained infertility (n=50). Total RNA was isolated from pooled blastocysts, and transcriptomes were assessed by the SurePrint G3 Human Gene Expression Microarray with 50,739 gene transcripts (Agilent Technologies). Analysis was performed using GeneSpring software (Agilent Technologies), including principal component analysis (PCA), unsupervised hierarchical clustering, one way ANOVA and unpaired t-test with Benjamini-Hochberg correction (significance at P value of <0.05).

CONCLUSION: Our findings suggest that equivalent FSH levels should not be directly equated with egg quality in women of different age. This has significant implications for the management of infertility in younger women with elevated FSH levels. Also, these women might benefit from earlier treatment intervention and egg/embryo banking, given that their odds of aneuploidy rise more rapidly over time than women of the same age without elevated FSH levels.
RESULTS: The human blastocyst transcriptome contained 33,587 gene transcripts including numerous splicing variants and isoforms resulting in 13,156 annotated genes. PCA and unsupervised hierarchical clustering completely separated each of the four infertility diagnoses groups by their transcriptomes. Significant differences in gene transcription were observed compared to fertile controls (PCO = 1,129, MF = 495 and Unexplained = 650 altered transcripts; >2 fold; P<0.05). Notably, the most significant transcriptome variation was observed in blastocysts derived from infertile PCO patients. Analysis recognized enriched pathways with decreased transcripts for gap junction proteins, p53 and calcium signaling, histidine metabolism, ECM receptor interaction and cytokine-cytokine receptor interactions (P<0.05).

CONCLUSION: Infertility diagnosis, specifically a PCO environment, has a significant impact on the developing blastocyst’s transcriptome, including alterations in key signaling pathways. This study opens new perspectives for understanding the impact of infertility diagnosis on the molecular signature of embryonic development and implantation potential. In addition, it emphasizes the importance of accurate and consistent clinical diagnosis for patient management.

O-107 Monday, October 20, 2014 05:30 PM

ROBOTICALLY ASSISTED OVARIAN TISSUE TRANSPLANTATION WITH HUMAN EXTRACELULAR MATRIX: OVARIAN STIMULATION AND IN VITRO FERTILIZATION OUTCOMES. K. H. N. Kennedy, a,b F. B. Bedoschi, a,b R. Stoezki, a V. Turan. a,b Innovation Fertility Preservation and IVF, New York, NY; aNew York Medical College, Valhalla, NY.

OBJECTIVE: One of the major limitations of ovarian transplantation (OT) is the loss of follicle numbers to initial ischemia until full revascularization occurs. We hypothesized that robotic assistance and the utility of a revascularizing extracellular matrix scaffold (Alloderm®) may enhance OT and in vitro fertilization outcomes.

DESIGN: A prospective study of two subjects (P-A and P-B) at an academic center who underwent robotically assisted orthotopic autologous OT using Alloderm® as the scaffold.

MATERIALS AND METHODS: One ovary was cryopreserved as 5x5-mm ovarian cortical pieces via a slow freezing method prior to preconditioning chemotherapy+radiotherapy for bone marrow transplantation at the ages of 22 (P-A) and 23 (P-B) for Hemophagocytic Lymphohistiocytosis and Hodgkin Lymphoma, respectively. One vial from each patient was thawed prior to OT to assess primary follicle density.

RESULTS: Both experienced menopausal post-chemotherapy as shown by high FSH, undetectable AMH and cessation of menses. Pre-OT follicle density was higher in P-A (1.66 ±0.37) than P-B (0.625 ±0.32 follicles/mm²) (P=0.05). Five of 10 (P-A) and 6 of 12 (P-B) vials, containing 10 and 12 cortical pieces respectively, were thawed. The pieces were sown onto Alloderm® under a surgical microscope and sutured onto the bivalved in situ ovary with robotic assistance. Ovarian follicle development was observed 10 (P-A) and 8 (P-B) weeks after grafting. Participants wished to cryopreserve as many embryos as possible before attempting pregnancy, against the possibility of early cessation of graft function. Following 6 and 4 cycles of IVF, five D3 embryos were cryopreserved from each (P-A: 8-cell-A, 6-cell-A and three 6-cell-B; P-B: 8-cell-A, two 6-cell-A, 10-cell-B and 5-cell-B) at the time of this report. Peak E2 levels reached 808 pg/mL in P-A and 543 pg/mL in P-B. While the baseline FSH (range: 8.0-15.4 mIU/mL) and E2 (7.7-70 mIU/mL) levels reached 808 pg/mL in P-A and 543 pg/mL in P-B. While the baseline FSH (range: 8.0-15.4 mIU/mL) and E2 (7.7-70 mIU/mL) levels

OBJECTIVE: To evaluate the effective role of beta human chorionic gonadotropin (β-hCG) levels in days 1, 4 and 7 as predictor for second dose Methotrexate requirement and success for women with ectopic pregnancies.

DESIGN: Retrospective study.

MATERIALS AND METHODS: Women with extra-uterine pregnancies treated with Methotrexate “single dose” protocol were included. β-hCG levels in days 1, 4 and 7 were used to evaluate Methotrexate second dose requirement and success. Surgical intervention was performed in cases of Methotrexate second dose failure or suspected tubal rupture.

RESULTS: During the study period 1703 patients were admitted for ectopic pregnancy. Four-hundred and nine received Methotrexate of whom 73 women required second dose of Methotrexate. The requirement of second dose of Methotrexate was associated with significantly higher day 1 β-hCG levels (1425 vs. 781 respectively, p<0.001). Of those women who required second dose of Methotrexate (n=73), 58 (79.4%) were treated successfully (success group), while 15 women (20.6%) required surgical intervention (failure group). The medians of β-hCG level in days 1, 4 and 7 were significantly higher in the “failure group” compared to the “success group” (2844 vs. 1601 IU/mL, p<0.01, 3225 vs. 2164 IU/mL p<0.05, and 3745 vs. 1915 IU/mL, p<0.05 respectively). Using logistic regression analysis, we found that day 1 β-hCG levels were the only significant independent variable for second dose treatment outcome. Receiver operator characteristic curve for β-hCG levels in day 1 was 0.727, and at a cutoff value of 2234 IU/mL the sensitivity and specificity reached the optimum for treatment success (77.5% and 73.3% respectively).

CONCLUSION: Day 1 β-hCG levels were the only predictors for Methotrexate second dose requirement and success. While the β-hCG levels on Days 5 and 7 did not predict treatment outcome. The cut-off value of β-hCG on day 1 with the optimal treatment results was found to be 2234 mIU/mL.

O-109 Monday, October 20, 2014 06:00 PM

UTILITY OF MAGNETIC RESONANCE IMAGING (MRI) IN THE EVALUATION OF INTRAOPERATIVELY CONFIRMED PELVIC ADHESIONS: A RETROSPECTIVE ANALYSIS. M. L. Meier, M. Spektor, M. Mathur, S. Gysler, S. M. McCarthy, P. H. Kodaman Yale-New Haven Hospital, New Haven, CT.

OBJECTIVE: To evaluate the effectiveness of MRI in predicting intraoperative pelvic adhesions.

DESIGN: An IRB approved retrospective analysis of 98 patients referred to a reproductive endocrinology and infertility practice at a single academic hospital between 2008 and 2013. MRI images and operative reports were reviewed specifically for pelvic adhesions and findings were compared.

MATERIALS AND METHODS: 600 patients who underwent MRI pelvic imaging were reviewed. Inclusion criteria were patients with MRI and subsequent gynecologic surgery within 6 months. Imaging was reviewed by two independent radiologists (A and B) examining specifically for pelvic adhesions. Adhesions were classified into 5 locations: right pelvic sidewall, left pelvic sidewall, posterior cul de sac, anterior cul de sac, and not otherwise specified. Operative reports were reviewed for the presence of pelvic adhesions and similarly classified in the above locations. The MRI and intraoperative findings were then compared to calculate MRI sensitivity and specificity for pelvic adhesions.

RESULTS: 98 patients fit inclusion criteria. Patient ages ranged from 11-51 years. Of these, 54/98 (55%) had intraoperative findings of pelvic adhesions. For radiologist A, sensitivity and specificity analysis is as follows: any pelvic adhesion: 62% and 91%; right pelvic sidewall: 38% and 78%; right pelvic sidewall: 40% and 90%; posterior cul-de-sac: 73% and 78%; and anterior cul-de-sac: 38% and 95%. For radiologist B, sensitivity and specificity analysis is as follows: any pelvic adhesion: 44% and 91%; left pelvic sidewall: 5% and 95%; right pelvic sidewall: 0% and 99%; posterior cul-de-sac: 64% and 84%; and anterior cul-de-sac: 31% and 97%.

CONCLUSION: Our study demonstrates that preoperative MRI is useful for the evaluation of pelvic adhesions. The specificity of MRI in the detection of pelvic adhesions is consistently over 90% in all locations with the exception of the posterior cul-de-sac. Furthermore, the results are consistent between two independent radiologists. These findings demonstrate the utility of preoperative MRI in evaluating adhesive disease. If pelvic adhesions are seen on MRI, they will likely be encountered intraoperatively, which is helpful for surgical planning. The specificity, which is low in all locations, which makes MRI a poor screening tool, but may be attributed to MRI slice thickness and adhesion characteristics. Future studies with a prospective design will be useful in further defining the utility of preoperative MRI.
The following papers are candidates for the ASRM Scientific Program Prize Paper Awards. Additional candidates will be presented during the Prize Paper Candidates’ Session on Monday.

**PRIZE PAPER SESSIONS 2**

**O-110 Tuesday, October 21, 2014 11:15 AM**

**SPECIAL RESEARCH PRESENTATION: MOLECULAR SIGNATURES OF FIBROID STEM CELLS (SCS) IMPLICATE IGF2 AS A POTENTIAL NEW TARGET.** M. B. Moravek, P. Yin, M. Ono, V. Vidimar, J. Kim, S. E. Butun. Northwestern University, Chicago, IL.

**OBJECTIVE:** We previously identified 3 populations of fibroid cells—CD34+CD49b+ (+/+, SCs), CD34+CD49- (+/-), and CD34-CD49b- (-/-). We sought to determine differential gene expression among the populations and explore critical pathways for SC function and fibroid pathogenesis.

**DESIGN:** Laboratory Study.

**MATERIALS AND METHODS:** Fibroid cells isolated from 8 subjects were sorted based on CD34 and 49b and microarray performed. Differential expression was defined as fold change >1.5 and FDR <5%. Cells were cultured and treated with IGF2 or vehicle for 0-90 minutes to determine activation of AKT or ERK or for 48 hours to measure proliferation, cell viability, mRNA expression by real-time PCR, protein expression by Western blot, and cell survival and differentiation by flow cytometry. All experiments were performed in triplicate.

**RESULTS:** Microarray revealed that the 3 populations are distinct, with >1500 differentially expressed genes, and suggested a transition from +/+ SCs through +/- cells to -/- cells. Pathway analysis indicated a significant role for IGF signaling. Insulin receptor (IR) and IGFBP3 were significantly overexpressed in +/- SCs. IGF1 and IGFBP2 were overexpressed in +/- cells (5-fold and 150-fold, respectively, p<0.05). Of the four IGF2 isoforms, two (3 and 4) were expressed in the fibroid cells and overexpressed in +/- cells. IGF2 treatment activated both Akt and ERK signaling and resulted in increased protein levels of proliferating cell nuclear antigen compared to vehicle. Total cell number was increased by a factor of 1.5-2 in IGF2-treated cells compared to vehicle, but there were no significant differences in percent cell survival or cell differentiation. There was a trend toward increased cell viability in +/- SCs treated with IGF2.

**CONCLUSION:** These data suggest that IGF2 signaling is important for fibroid cell proliferation and growth and may increase SC viability. The overexpression of ligand in +/- cells and receptor in +/- SCs suggests paracrine interaction by IGF2, resulting in increased proliferation. Targeting pathways necessary for fibroid SC function may lead to new treatments that can both address existing fibroids and prevent the development and growth of new tumors.

**Supported by:** ASRM In-Training Grant in Heavy Menstrual Bleeding (MBM), NIH/NICHD P01-HD057877 (SBD).

**O-111 Tuesday, October 21, 2014 11:30 AM**


**OBJECTIVE:** The Department of Health and Human Services (DHHS) recommends 75 minutes of vigorous or 150 minutes of moderate activity per week. It is unclear if these options afford equivalent health benefits. We sought to determine the relative health benefits of moderate vs vigorous exercise compared to inactivity in women with PCOS.

**DESIGN:** Prospective cross-sectional.

**MATERIALS AND METHODS:** 326 women with PCOS-Rotterdam completed the International Physical Activity Questionnaire and were systematically evaluated for evidence of metabolic dysfunction. Women were categorized as those who achieved at least 75 minutes of vigorous activity or 150 minutes of moderate activity per week (Vigorous), those who did not, but did achieve 150+ minutes moderate intensity (Moderate), and those who did not achieve either threshold (Inactive). Metabolic outcomes were compared using non-parametric test equations for correlated outcomes within clinics to calculate unadjusted and adjusted risk ratios and 95% confidence intervals for the association between ectopic pregnancy and selected patient characteristics and ART treatment factors.

**RESULTS:** A total of 9,480 of the 553,577 (1.7%) pregnancies included in our study were ectopic pregnancies. From January 1, 2001 to December 31, 2011 that resulted in a clinical intrauterine pregnancy, an ectopic pregnancy, or a heterotopic pregnancy. We calculated overall and annual ectopic pregnancy rates for all types of embryo transfers during the study period; the Cochran-Armitage test was used to assess temporal trends. We further restricted the analysis to fresh, non-donor cycles and used log-binomial regression models with generalized estimating equations for correlated outcomes within clinics to calculate unadjusted and adjusted risk ratios and 95% confidence intervals for the association between ectopic pregnancy and selected patient characteristics and ART treatment factors.

**CONCLUSION:** Exercise therapy is routinely recommended as a primary treatment for women with PCOS. Women who met DHHS guidelines for exercise were afforded a variety of health benefits, with vigorous activity yielding additional benefits compared to moderate activity, independent of total energy expenditure.

**O-112 Tuesday, October 21, 2014 11:45 AM**

**RISK OF ECTOPIC PREGNANCY ASSOCIATED WITH ASSISTED REPRODUCTIVE TECHNOLOGY (ART), UNITED STATES, 2001-2011.** K. M. Perkins, S. L. Boulet, D. M. Kissin, D. J. Jamieson. Centers for Disease Control and Prevention, Atlanta, GA.

**OBJECTIVE:** To assess national trends in the incidence of ectopic pregnancy among the ART population and to identify patient characteristics and ART treatment factors associated with increased risk for ectopic pregnancy.

**DESIGN:** Retrospective population-based cohort study.

**MATERIALS AND METHODS:** The data used for our study were obtained from the National ART Surveillance System (NASS). We included all in vitro fertilization, transcervical embryo transfer procedures performed from January 1, 2001 to December 31, 2011 that resulted in a clinical intrauterine pregnancy, an ectopic pregnancy, or a heterotopic pregnancy. We calculated overall and annual ectopic pregnancy rates for all types of embryo transfers during the study period; the Cochran-Armitage test was used to assess temporal trends. We further restricted the analysis to fresh, non-donor cycles and used log-binomial regression models with generalized estimating equations for correlated outcomes within clinics to calculate unadjusted and adjusted risk ratios and 95% confidence intervals for the association between ectopic pregnancy and selected patient characteristics and ART treatment factors.

**RESULTS:** A total of 9,480 of the 553,577 (1.7%) pregnancies included in our study were ectopic pregnancies. From 2001 to 2011, there was a decline in the incidence of ectopic pregnancy from 2.0% to 1.6% (P for trend <0.0001). Fresh, non-donor cycles had the highest rate of ectopic pregnancy (2.0%) and fresh, donor cycles had the lowest rate (1.0%). Among fresh, non-donor cycles, the risk for ectopic pregnancy was increased for maternal age 29 to 43 years (aRRs ranged from 1.18-1.23), those with more than one prior ART cycle (aRR 1.21, 95% CI 1.1-1.31), tubal factor infertility (aRR 1.25, 95% CI 1.16-1.35), and the transfer of more than 2 embryos (aRR ranged from 1.33-1.49), after adjusting for patient characteristics and ART treatment factors.

**CONCLUSION:** The risk of ectopic pregnancy was lower among those with 1 or more prior live births (aRR ranged from 0.55-0.71) and male factor infertility (aRR 0.85, 95% CI 0.79-0.92).

---

**Patient Characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Inactive (N=144)</th>
<th>Moderate (N=31)</th>
<th>Vigorous (N=151)</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI, kg/m²</td>
<td>31.7 (8.7)</td>
<td>30.9 (8.7)</td>
<td>29.0 (7.7)</td>
<td>0.007</td>
</tr>
<tr>
<td>Age, yrs</td>
<td>28.3 (6.7)</td>
<td>28.3 (5.5)</td>
<td>27.7 (5.8)</td>
<td>0.478</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>36.9 (7.5)</td>
<td>36.4 (7.5)</td>
<td>34.7 (8.3)</td>
<td>0.006</td>
</tr>
<tr>
<td>HDL, mg/dL</td>
<td>53.5 (15.1)</td>
<td>54.3 (19.1)</td>
<td>59.6 (17.6)</td>
<td>0.005</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>185.2 (37.7)</td>
<td>193.6 (61.7)</td>
<td>185.5 (36.8)</td>
<td>0.937</td>
</tr>
<tr>
<td>Fasting glucose, mg/dL</td>
<td>89.2 (14.9)</td>
<td>90.0 (16.3)</td>
<td>88.7 (19.1)</td>
<td>0.476</td>
</tr>
<tr>
<td>2-hr glucose</td>
<td>112.8 (39.9)</td>
<td>108.5 (42.2)</td>
<td>99.3 (39.5)</td>
<td>0.002</td>
</tr>
<tr>
<td>Fasting insulin, mU/L</td>
<td>21.0 (40.2)</td>
<td>13.4 (12.5)</td>
<td>12.9 (14.6)</td>
<td>0.028</td>
</tr>
<tr>
<td>2-hr insulin</td>
<td>105.8 (109.2)</td>
<td>70.9 (46.8)</td>
<td>83.8 (102.5)</td>
<td>0.056</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>5.1 (10.6)</td>
<td>3.2 (3.5)</td>
<td>3.1 (4.2)</td>
<td>0.035</td>
</tr>
<tr>
<td>% with acanthosis</td>
<td>40.2</td>
<td>30.0</td>
<td>24.2</td>
<td>0.021</td>
</tr>
<tr>
<td>% with metabolic syndrome</td>
<td>46.9</td>
<td>35.5</td>
<td>32.5</td>
<td>0.034</td>
</tr>
</tbody>
</table>
CONCLUSION: The incidence of ectopic pregnancy has decreased between 2001 and 2011. The risk for ectopic pregnancy is increased by unmodifiable risk factors such as older maternal age, previous ART cycles, and tubal factor infertility. The transfer of multiple embryos, however, is a modifiable risk factor that increases the risk of ectopic pregnancy.

O-113 Tuesday, October 21, 2014 12:00 PM
SPHINGOSINE-1-PHOSPHATE, A PROTECTOR AGAINST CHEMOTHERAPY-INDUCED OVARIAN FOLLICLE APOPTOSIS, DOES NOT DIMINISH THE EFFECTIVENESS OF CHEMOTHERAPY. S. Titus, a b R. Stobiecki, a V. Turan, a D. Halicka, a P. De Sutter, a K. Oktay, a b aNew York Medical College, Valhalla, NY; bInstitute for Fertility Preservation and IVF, New York, NY; Gent University, Gent, Belgium.

OBJECTIVE: It has recently been reported that S1P (sphingosine-1-phosphate), a ceramide-induced death pathway inhibitor, prevents the human primordial follicles from apoptosis during chemotherapy. We conducted this study to address the concern that Sphingosine-1 Phosphate (S1P) may shield the tumor, along with the primordial follicles, from cytotoxic effects of chemotherapy drugs.

DESIGN: Experimental study.

MATERIALS AND METHODS: A breast cancer cell line (met-1) was injected under the mammary fat pad of 3-4 months old immunodeficient (SCID) mice and 2 weeks were allowed for tumorigenesis. Mice then received either the vehicle (control, n=5), single dose of cyclophosphamide at 100 mg/kg (cyclo) alone (n=5), Cyclo+S1P (n=5 or S1P alone (n=5). S1P (200M) was administered via continuous infusion using a mini-osmotic pump beginning 24 h prior to and ending 72 h post-chemotherapy. To determine the effects of S1P on chemotherapy induced apoptosis and cell cycle behavior of cancer cells, the met-1 cells were treated with 500ng/ml of doxorubicin (Doxo) for 16h with and without S1P (20uM). Apoptosis and the cell cycle effects were measured by TUNEL assay and flow cytometry.

RESULTS: As compared to the control group, S1P+cyclo treatment resulted in a significant decrease in the amount of the tumor (0.55±0.06 mm vs 0.; p<0.002). S1P alone had no effect on the tumor size (0.55±0.1) vs. the controls (p=0.2). There was no difference in the tumor size when cyclo alone was compared to cyclo+S1P (0.025±0.025) vs 0 (p=0.3). In Met-1 cells, there was no significant difference between the percentage of apoptotic cells after treatment with Doxo alone (19%) vs. Doxo+S1P (22%), (p=NS). In both groups, a similar percentage of cells arrested in G2/M phase (Doxo: 50.1±3.09) and (Doxo+S1P: 38.15±5.9), (p=0.1) with no difference in the distribution in other cell cycle stages.

CONCLUSION: S1P does not negate the impact of major chemotherapeutic agents on tumor cells in vivo or in vitro nor does it have an effect on the cell cycle stages in tumor cells. On the contrary, S1P enhances the anti-tumor effects of cyclophosphamide in vivo. These findings suggest that S1P is a promising fertility preservation agent which can be tested in clinical trials.

Supported by: This research is Supported by NIH’s NICHD and NCI (5R01HD055925-06 and 5R21HD061259-02) and the Flemish Foundation for Scientific Research (FWO-Vlaanderen, grant number FWO G0.065.11N10).

O-115 Tuesday, October 21, 2014 12:30 PM

OBJECTIVE: Studies have demonstrated differences in ovarian steroid production among women with PCOS when exposed to GnRH agonist (GnRHa) or human chorionic gonadotropin (hCG). Approximately 50% PCOS patients exhibited exaggerated 17-hydroxyprogesterone (17-OHP) responses (hyper-responders, HR-PCOS) while others (normal responders, NR-PCOS) had responses equivalent to normal controls following stimulation. These findings raised the consideration that adrenal androgens may contribute to excess androgen production in NR-PCOS individuals. To assess this possibility, adrenal androgen responses to a step-wise infusion of adrenocorticotropic hormone (ACTH) was determined in women with PCOS and normal controls. We hypothesized that NR-PCOS women would have exaggerated adrenial responses to ACTH compared to HR-PCOS subjects.

DESIGN: Prospective study in an academic center.

MATERIALS AND METHODS: Women with PCOS (age, 19-34 yr; n=14) and normal controls (age, 19-35; n=18) were enrolled. PCOS women were categorized as either NR-PCOS (n=6) or HR-PCOS (n=8) based on serum 17-OHP levels 24 hours after exposure to hCG (25 mcg). All participants received 1 mg dexamethasone the night before and morning of the study, followed by continuous infusion of incrementally increasing doses of ACTH, 0.25-25 mcg/hr, over 6 hours. Blood samples were obtained every 30 minutes during the infusion. Serum levels of cortisol (C), androstenedione (A4), testosterone (T), dehydroepiandrosterone (DHEA), and DHEA sulfate (DHEAS) were measured by RIA. Individual time points and the total area-under-curve (AUC) were tested for signific ance using ANOVA followed by post-hoc analysis with the Tukey-Kramer test.

RESULTS: HR-PCOS subjects had significantly higher changes in the AUC for DHEA and A4 production compared to NR-PCOS or controls. At the highest ACTH infusion rates, HR-PCOS women produced higher absolute levels of 17-OHP, A4, DHEA, and DHEAS compared to controls.

O-114 Tuesday, October 21, 2014 12:15 PM
OUTCOMES OF THE NICHD’S COMPARATIVE EFFECTIVENESS ASSESSMENT OF MULTIPLE INTRAUTERINE GESTATIONS FOLLOWING OVARY STIMULATION (AMIGOS) TRIAL. THE NICHD COOPERATIVE REPRODUCTIVE MEDICINE NETWORK. M. P. Diamond. OB/Gyn, Georgia Regents University, Augusta, GA.

OBJECTIVE: To examine whether treatment of women with unexplained infertility (UI) with an aromatase inhibitor, Letrozole, however is a modifiable risk factor that increases the risk of ectopic pregnancy.

RESULTS: Among women receiving Gn, CC, and Letrozole, conception occurred in 46.8%, 35.7% and 28.4% respectively. Live birth occurred in 32.2%, 23.3% and 18.7% of cycles respectively; Letrozole pregnancy rates were significantly less than Gn (p<0.0001) and CC (p<0.045). Rate of multiple gestations among all women treated with Gn (21%), were approximately eight fold higher than with CC (1.3%) or four fold higher than with Letrozole (2.7%). Among pregnancies with fetal heart rates identified, women treated with Letrozole had fewer multiple pregnancies (9/67, 13.4%) than women treated with Gn (34/107, 31.8%, p < 0.006) with no difference compared to women treated with CC (8/85, 9.4%, NS). All multiples in the CC and Letrozole groups were twins; in the Gn group there were 24 twins and 10 triplet gestations. There were no differences in the rates of infants with congenital anomalies (3.1%, 4.2%, and 3.6% respectively) or other fetal and neonatal complications.

CONCLUSION: Although ovarian stimulation with Letrozole was overall safe, live births were reduced compared to CC or Gn, and the multiple pregnancy rate was intermediate between CC and Gn. Thus, CC/IUI remains first line therapy for couples with unexplained infertility.

Supported by: This work was Supported by National Institutes of Health (NIH)/Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) Grants U10 HD59605 (to M.P.D.), U10 HD38992 (to R.S.L.), U10 HD27049 (to C.C.), U10 HD38998 (to R.A.), U10 HD55594 (to R.D.R.), HD055944 (to P.R.C.), U10 HD05936 (to G.M.C.), U10HD055925 (to H.Z.); and U10 U54 HD29834 (to the University of Virginia Center for Research in Reproduction Ligand Assay and Analysis Core of the Specialized Cooperative Centers Program in Reproduction and Infertility Research). Most importantly, this research was made possible by the funding by American Recovery and Reinvestment Act. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NICHD or NIH.
and/or NR-PCOS individuals. Additionally, ovarian steroid production in response to hCG compared to adrenal steroid AUC in response to ACTH revealed significant correlations for the production of 17-OHP, A, and DHEA.

CONCLUSION: HR-PCOS women demonstrate heightened responses to ovarian theca stimulation, and have exaggerated responses to adrenal stimulation. Furthermore, the degree of responsiveness to ovarian stimulation correlates with the degree of responsiveness to adrenal stimulation. These results suggest that hyper-responsiveness in women with PCOS reflects a common alteration of steroid producing cells.

Supported by: Eunice Kennedy Shriver NICHD/NIH (U54 HD012303-29) and NIH grant MO1 RR08277

O-116 Tuesday, October 21, 2014 12:45 PM

TIME-TO-PREGNANCY AND SECONDARY SEX RATIO IN A PRE-CONCEPTION COHORT, K. J. Sapra, J. Bae, S. Kim, G. M. Buck Louis. Division of Intramural Population Health Research, Eunice Kennedy Shriver Institute for Child Health & Human Development, Rockville, MD.

OBJECTIVE: Folklore suggests couples who conceive quickly are more likely to have boys than girls, yet previous studies using retrospectively reported time-to-pregnancy (TTP) and secondary sex ratio (SSR) are equivocal (1-3). No prior study has used the gold-standard prospectively observed TTP to evaluate this relationship, serving as the impetus for this study.

DESIGN: Prospective preconception cohort with follow up through delivery.

MATERIALS AND METHODS: 501 couples planning to discontinue contraception for purposes of becoming pregnant were followed until hCG pregnancy or 12 months of unsuccessful trying; pregnant women were followed through pregnancy loss or delivery. TTP is defined by the number of menstrual cycles fully observed. Logistic regression models for the odds of a male infant (OR>1 excess male, OR<1 excess female) were constructed using discrete TTP and categorical TTP (>3 cycles compared to ≤3 cycles). Analyses were conducted accounting for time off contraception before study entry and for several putative confounders, including maternal age, BMI, parity, income, smoking and paternal age and BMI.

RESULTS: Of 400 pregnancies, 245 (61%) resulted in a live born singleton; infant’s sex was reported for 234 (96%). Prospectively observed discrete TTP was not associated with SSR (OR: 0.94, 95% CI: 0.83, 1.05), adjusting for maternal age, parity, income, and paternal age. However, TTP >3 months was associated with an excess of female infants (OR: 0.53, 95% CI: 0.29, 0.98), though this was not significant when accounting for time off contraception before study entry (OR: 0.88, 95% CI: 0.51, 1.53). An excess of female births was observed in the most fecund couples who conceived in the enrollment cycle (57.7% female), while an excess of males was noted in couples conceiving in the first 3 fully observed cycles (53.8-57.9% male). No consistent pattern of SSR emerged for couples conceiving in later cycles.

CONCLUSION: Despite popular folklore, we observed no consistent evidence that TTP is associated with infant sex. To our knowledge, this is the first study with preconception enrollment to investigate this lore and our findings do not support it. Previous positive findings may reflect errors in retrospective report of TTP, differences in TTP modeling or confounder selection rather than an underlying biologic mechanism.

Supported by: NICHD Intramural funding (contracts N01-HD-3-3355; N01-HD-3-3356; NOH-HD-3-3358; HHSN27520001)

REPRODUCIVE ENDOCRINOLOGY AND INFERTILITY FELLOWS RESEARCH II

O-118 Tuesday, October 21, 2014 04:30 PM


OBJECTIVE: Phosphatase and tensin homolog (Pten) negatively regulates the activity of phosphatidylinositol 3-kinase signaling. Selective deletion of the Pten gene in mouse theca cells results in androgen excess, enlarged ovaries and ovulatory defects, mimicking human PCOS. Changes in growth factor signaling within the ovary may lead to structural changes, promoting functional changes such as increased androgen production within theca and biomechanical restriction preventing follicle expansion into dominant follicles. We assessed for differences in growth factors and potential ovarian rigidity related proteins in iPtenMT mice compared to wild type (WT).

DESIGN: Laboratory study using a conditional gene knockout mouse model.

MATERIALS AND METHODS: Ovaries from 75-day iPtenMT and WT littermates were prepared for real-time quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR), immunohistochemistry (IHC), or Western Immunoblotting (WB).

RESULTS: Comparisons are tPtenMTovaries to WT. Growth factors FGF-7, TGF-β1, TGF-β2, Bone Morphogenetic Protein-4 (BMP4), and BMP7 were evaluated with qRT-PCR. FGF-7 and TGF-β1 mRNA levels were significantly increased while TGF-β2, BMP4, and BMP7 were not significantly altered. FGF-7 protein was significantly increased in the theca and granulosa using IHC and WB. TGF-β1 protein was increased in the theca and granulosa using IHC. Potential ovarian rigidity related proteins occludin (OCLN) and nectin-2 were elevated while β-catenin and α-E-catenin proteins were decreased using WB.

OBJECTIVE: To evaluate the relationship between various anesthetic and analgesic agents used at the time of oocyte retrieval and pregnancy outcome.

DESIGN: Cohort study.

MATERIALS AND METHODS: Data were obtained from electronic IVF and anesthesia databases. Records from consecutive oocyte retrievals which occurred between 2007-2013 at Massachusetts General Hospital Fertility Center were reviewed. Oocyte donation cycles, embryo or oocyte cryopreservation cycles, and cycles with incomplete anesthesia records were excluded. The analysis included 2184 women who underwent oocyte retrievals. Logistic regression models using generalized estimating equations (GEE) were fit to investigate the relationship between anesthetic and analgesic agents used during oocyte retrieval and clinical pregnancy per oocyte retrieval. Agents included propofol, meperidine, fentanyl, midazolam, and inhaled anesthetics. Multivariate results were adjusted for agents used as well as age, BMI, day 3 FSH, and year of retrieval.

RESULTS: Mean age was 35 and mean BMI was 24. After adjusting for potential confounders including the agents used at the time of surgery, propofol, inhaled anesthetics, fentanyl, and midazolam had no relationship with clinical pregnancy per oocyte retrieval. Meperidine was negatively associated with clinical pregnancy per oocyte retrieval. Clinical pregnancy rates per retrieval were 49.1%, 55.9%, and 46.7%, for cycles where women received 0mg, 10-30mg, and ≥30mg of meperidine, respectively. Adjusted odds ratios (95% CI) for clinical pregnancy per retrieval were 1.2(0.98-1.56) and 0.84(0.71-0.99), for cycles where women received 10-30mg and ≥30mg meperidine, respectively, as compared to women who received 0mg of intraoperative meperidine (p, linear trend=0.02).

CONCLUSION: Most of the anesthetic and analgesic agents used at the time of oocyte retrieval are not associated with pregnancy after IVF. Meperidine, however, may be negatively related to IVF success. Further investigation assessing for potential mechanisms, additional exposures, and additional pregnancy outcomes is ongoing.

O-117 Tuesday, October 21, 2014 04:15 PM


OBJECTIVE: To evaluate the relationship between various anesthetic and analgesic agents used at the time of oocyte retrieval and pregnancy outcome.

DESIGN: Cohort study.

MATERIALS AND METHODS: Data were obtained from electronic IVF and anesthesia databases. Records from consecutive oocyte retrievals which occurred between 2007-2013 at Massachusetts General Hospital Fertility Center were reviewed. Oocyte donation cycles, embryo or oocyte cryopreservation cycles, and cycles with incomplete anesthesia records were excluded. The analysis included 2184 women who underwent oocyte retrievals. Logistic regression models using generalized estimating equations (GEE) were fit to investigate the relationship between anesthetic and analgesic agents used during oocyte retrieval and clinical pregnancy per oocyte retrieval. Agents included propofol, meperidine, fentanyl, midazolam, and inhaled anesthetics. Multivariate results were adjusted for agents used as well as age, BMI, day 3 FSH, and year of retrieval.

RESULTS: Mean age was 35 and mean BMI was 24. After adjusting for potential confounders including the agents used at the time of surgery, propofol, inhaled anesthetics, fentanyl, and midazolam had no relationship with clinical pregnancy per oocyte retrieval. Meperidine was negatively associated with clinical pregnancy per oocyte retrieval. Clinical pregnancy rates per retrieval were 49.1%, 55.9%, and 46.7%, for cycles where women received 0mg, 10-30mg, and ≥30mg of meperidine, respectively. Adjusted odds ratios (95% CI) for clinical pregnancy per retrieval were 1.2(0.98-1.56) and 0.84(0.71-0.99), for cycles where women received 10-30mg and ≥30mg meperidine, respectively, as compared to women who received 0mg of intraoperative meperidine (p, linear trend=0.02).

CONCLUSION: Most of the anesthetic and analgesic agents used at the time of oocyte retrieval are not associated with pregnancy after IVF. Meperidine, however, may be negatively related to IVF success. Further investigation assessing for potential mechanisms, additional exposures, and additional pregnancy outcomes is ongoing.

O-116 Tuesday, October 21, 2014 12:45 PM
CONCLUSION: Selective deletion of the Pten gene in theca cells leads to changes in growth factors TGF-β1 and FGF-7 along with changes in ovarian proteins OCLN, nectin-2, β-catenin, and α5-β-catenin. Dysregulation of these growth factors may contribute to the PCOS-like ovarian phenotype of tPtenMT mice by causing an imbalance in structural proteins that may affect ovarian rigidity. When combined with recent studies suggesting the TGF-β signaling pathway may have the strongest genetic link to the etiology of PCOS, the tPtenMT model may give insight into the pathogenesis of and treatment for PCOS in humans.

DESIGN: Cohort study.

MATERIALS AND METHODS: Records from 220 consecutive fresh IVF donor-oocyte cycles which occurred between 2004–2013 at the Massachusetts General Hospital Fertility Center were reviewed. There were 186 oocyte donors who underwent 215 oocyte donation cycles with complete donor thyroid stimulating hormone (TSH) data included in our analysis. The most recent TSH obtained prior to but within 12 months of a cycle was considered for analysis. Logistic regression models using generalized estimating equations (GEE) were fit to investigate the relationship between donor TSH and clinical pregnancy per oocyte retrieval while controlling for confounders and within-person correlations in cycle outcome. Multivariate results were adjusted for recipient TSH, donor age, recipient age, donor BMI, recipient BMI, donor antral follicle count, donor number of prior cycles, male factor infertility, and donor status (known vs. anonymous).

RESULTS: Mean age of oocyte donors was 26.9 years and mean donor TSH was 1.9mIU/L at the time of first cycle. Donor TSH ranged from 0.4-9.0mIU/L in our sample. Crude clinical pregnancy rate per oocyte retrieval was 116/172 (67%) and 19/43 (44%) among cycles with donor TSH levels of < 2.5mIU/L and ≥ 2.5mIU/L, respectively. There were no significant differences in rate of male factor or recipient uterine factor infertility among the two TSH groups (16% vs. 6% p=0.17 and 3% vs. 0% p=0.99, respectively, for TSH < and ≥ 2.5mIU/L). In multivariate regression, odds of clinical pregnancy per oocyte retrieval were lower among cycles with donor TSH levels ≥ 2.5mIU/L as compared to cycles with donor TSH levels < 2.5mIU/L (age-adjusted odds ratio 0.39, 95% CI 0.19-0.79, p<0.01, and multivariate odds ratio 0.37, 95% CI 0.3-0.8, p<0.01). Recipient TSH was not related to pregnancy outcome.

CONCLUSION: Although a correlation between TSH concentration and pregnancy outcome has been well documented, its role prior to fertilization remains unclear. This analysis of oocyte donation cycles suggests that there may be relationship between TSH prior to fertilization and IVF outcome.

SUPEROVULATION DOES NOT INCREASE EMBRYONIC ANEUPLOIDY: A PROSPECTIVE EVALUATION OF ANEUPLOIDY IN NATURAL IVF CYCLES WITH COMPARISON TO 15,169 EMBRYS FROM AGE CONTROLLED PEERS WHO HAD SUPEROVULATION. K. H. Hong, a,b M. D. Werner, a,b J. M. Franasiak, a,b E. J. Forman, a,b D. A. Gabriele, a M. Cheng, a R. T. Scott, Jr., a,b 1RMANI, Basking Ridge, NJ 2Division of Reproductive Endocrinology, Rutgers-RWJ Medical School, Basking Ridge, NJ.

OBJECTIVE: The use of superovulation during IVF provides a greater number of mature oocytes and embryos per cycle and an overall increase in delivery rates when compared with natural cycles. However, some data suggest that superovulation may diminish oocyte quality. Of particular concern are limited animal and human data indicating increasing embryonic aneuploidy with increasing gonadotropin dose. Unfortunately, no data are available which systematically address aneuploidy rates in natural cycles where neither exogenous LH/FSH nor GnRH analogs prior to the mid-cycle surge have been administered. This study seeks to determine the embryonic aneuploidy rate during natural cycles and to compare them to rates observed in embryos from cycles employing superovulation.

DESIGN: Prospective observational study.

MATERIALS AND METHODS: All infertile patients with maternal ages 18 to 49 and regular ovulatory cycles lasting <39 days were offered study participation. Serial endocrine and ultrasound monitoring was performed. When a follicle reached ≥ 15mm in size, both hCG and GnRH...
agonist were administered. Oocyte retrieval was performed 36 hours later. No LH, FSH, or GnRH antagonist was used. All mature oocytes underwent ICSI and the resulting embryos were cultured until they arrested or became blastocysts. Viable blastocysts were biopsied for aneuploidy screening. The aneuploidy data were then compared with a retrospective cohort of 15,169 embryos from stimulated IVF cycles employing aneuploidy screening.

RESULTS: Sixty four of 119 (56.3%) embryos obtained from natural IVF cycles were aneuploid. Logistic regression controlling for maternal age demonstrated that the aneuploidy rates in natural and stimulated cycles were equivalent (P=0.81).

CONCLUSION: Embryonic aneuploidy rates in natural and stimulated IVF cycles do not differ. These data do not support a causative role for gonadotropin stimulation in embryonic aneuploidy. Data collection is ongoing.

O-122 Tuesday, October 21, 2014 05:30 PM


OBJECTIVE: To evaluate the difference in survival, fertilization, and pregnancy rates between internal-freeze-external thaw, internal freeze-external thaw, and external freeze-internal thaw of donor oocytes that were cryopreserved and banked at participating fertility centers.

DESIGN: Retrospective analysis.

MATERIALS AND METHODS: Frozen donor oocyte banks used internally at our center, exported from our center, or imported to our center were identified between 2012-2013. The frozen donor egg banks were categorized into 3 groups based on freeze-thaw locations: internal freeze-internal thaw (II), internal freeze-external thaw (IE), and external freeze-internal thaw (EI). Outcomes evaluated were: thaw survival, fertilization rates, cleavage rates, clinical pregnancy/embryo transfer (CP/ET), and ongoing pregnancy per thaw (OP/Thaw).

RESULTS: While comparison of cleavage rates amongst II versus IE and II versus EI showed statistical significance, the survival of thaw and pregnancy outcomes from these comparisons showed no statistical significant difference (Table 1). There was a trend towards higher pregnancy rates in the II group overall at 52.63% compared to IE at 41.92% and EI at 42.75%.

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outcomes</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>Survival %</td>
</tr>
<tr>
<td>Fert %</td>
</tr>
<tr>
<td>Cleavage %</td>
</tr>
<tr>
<td>CP/ET</td>
</tr>
<tr>
<td>OP/Thaw</td>
</tr>
</tbody>
</table>

CONCLUSION: As donor egg banking becomes more popular, the transport of banked oocytes will also increase. Despite efforts to standardize oocyte vitrification and thaw techniques across the egg bank network, the variation seen in our results undoubtedly reflects variations in oocyte vitrification and culture conditions. This preliminary data shows a trend toward higher internal-freeze-internal thaw pregnancy rates yet not statistically significant, possibly due to low numbers. However, this data also shows a relatively small decrease in success rates with oocyte importation and export, which is reassuring. While initial results are promising, further studies are warranted.

Supported by: In part by PRAE/NICHD/NIH and Donor Egg Bank USA.

O-123 Tuesday, October 21, 2014 05:45 PM


OBJECTIVE: With an increasing number of patients seeking medical information on the Internet, there are concerns about information quality and content on patient-oriented websites. We sought to assess ethical issues related to information presented by fertility clinic websites, and evaluated them for completeness and adherence to ASRM guidelines.

DESIGN: Cross-sectional evaluation.

MATERIALS AND METHODS: Between 2/2014-4/2014, 396 Society of Assisted Reproductive Technologies (SART) member websites were evaluated by two independent researchers. Websites were surveyed for the following parameters: practice location, type and size, risks of IVF, egg donor traits, experimental procedures, advertising presence, sex selection, and presentation of success rates. X^2 tests were used to assess for differences between groups.

RESULTS: 98% (387/396) of clinics had a website, of which 80% were private and 20% academic. 26% of practices performed ≥ 500 cycles/year while 74% performed <500 cycles/year. Only 36% of websites discussed the risks of IVF, with no differences between academic and private practices (35 vs 39%, p<.49), or between small- and large-volume centers (35 vs. 45%, p=.07). Of the 259 practices that have egg donor availability, 14% provided public information on egg donor traits, however all of these websites abided by ASRM guidelines regarding the commodification of donors, and no additional fees were advertised for specific traits. Academic centers were more likely than private to offer experimental procedures such as ovarian tissue cryopreservation (44 vs.14%, p<.0001). Only 7% of practices had industry advertisements. ASRM guidelines suggest that success rates be reported as live births per cycles initiated, however, only 54% of websites reported both with large volume practices more likely to report both as compared to small practices (76 vs 50%, p<.0001). Meanwhile, there was no difference in success rate reporting between academic and private practices (55 vs 54%, p=.6). 25% of websites advertise elective sex selection. Private practices were more likely to advertise sex selection than their academic counterparts (31.2 vs. 2.5%, p<.0001), with no difference between large and small practices (26 vs 27%, p=.2).

CONCLUSION: The majority of websites abide by ASRM advertising guidelines, however, there is still limited information regarding the risks of IVF and other experimental procedures. Moreover, there is considerable heterogeneity in success rate reporting along with promotion of controversial services such as elective sex selection on patient-oriented websites.


OBJECTIVE: To determine if alterations in DNA methylation following in-vitro fertilization (IVF) can be attributed to modifiable aspects of ART procedures.

DESIGN: Prospective cohort study.

MATERIALS AND METHODS: Full-term placentas from singleton deliveries were analyzed, 49 from in-vivo conceptions and 62 from IVF/ICSI pregnancies: 40 from fresh embryo transfer (ET) cycles, 13 donor oocyte cycles, and 9 frozen embryo transfer (FET) cycles. Placental methylation was analyzed at 45 cytosine-phosphate-guanine (CpG) sites, representing 22 genes, by pyrosequencing. Methylation of an additional 51 placentas, 42 fresh and 9 donor, was analyzed for two placenta and growth related genes, MEST and GRB10. Percentage methylation at each CpG site was compared for the outcome measures, including fresh ET, FET, length of gonadotropin stimulation, and length of embryo culture.
RESULTS: Methylation of GRB10 was significantly associated with type of ET; FET cycles were differentially methylated compared to fresh, and were more similar to control cycles (mean methylation difference 10%, p<0.01). Methylation of MEST was significantly associated with length of gonadotropin stimulation, with higher levels of methylation associated with longer time of stimulation (mean methylation difference 4.6%, p<0.03). This effect was modified by peak estradiol level (E2), with the highest levels of methylation seen in patients with low E2 levels (<50%) and longer stimulations (12-15 days) (mean difference from control 11%, p<0.006). Length of embryo culture was associated with differential methylation in 2 genes involved in neuronal development, GRIN2C and PCDHB9. In both cases, day 5 ET was associated with methylation differences compared to day 3 ET, with day 3 ET more similar to control (mean methylation difference GRIN2C 3.5% p=0.004, PCDHB9 14%, p<0.03).

CONCLUSION: Methylation differences have been observed between in-vivo and IVF conceived pregnancies and may be associated with differences in fetal growth and development. This study demonstrates, for the first time, methylation differences that are associated with specific, modifiable aspects of the IVF procedure. Further studies are necessary to elucidate the mechanisms and significance of these observed changes.

Supported by: U54-HD06157 NIH/NICHD.

MENOPAUSE

O-125 Tuesday, October 21, 2014 04:15 PM


OBJECTIVE: Mid-reproductive age women exposed to gonadotoxic therapy are known to have impaired ovarian reserve, but little is known about the impact of cancer treatment on follicular development in this population. We aim to evaluate ovulatory function in cancer survivors.

DESIGN: Prospective cohort.

MATERIALS AND METHODS: Mid-reproductive age cancer survivors with normal menstrual cycles were compared to two control populations: similarly aged and late reproductive age women. Subjects collected daily urine samples for one cycle. Integrated urinary pregnanediol glucuronide (PDG), follicle stimulating hormone (FSH), and luteinizing hormone (LH) were normalized to creatinine and compared between groups. Serum Anti-Mullerian Hormone (AMH), FSH, and antral follicle counts (AFC) were measured. Multivariable linear regression models were utilized to control for confounders.

RESULTS: 38 women (13 survivors, 11 same-age and 14 late reproductive age controls) were enrolled and provided 1082 urine samples. Cycle length, luteal phase length, and luteal activity were similar between groups. Ovarian reserve was impaired in cancer survivors compared to same-age controls but similar between survivors and late reproductive age controls. In contrast, total and peak PDG were higher in mid-reproductive age survivors and controls compared to late reproductive age controls. There was no difference in total and peak PDG when survivors were compared to same age controls. AMH was not associated with PDG in unadjusted or adjusted models.

CONCLUSION: Despite impaired ovarian reserve, women exposed to alkylating agents have progesterone excretion similar to same-age peers rather than late reproductive age women. This finding suggests that the dynamics of follicular development in survivors are more dependent on age rather than ovarian reserve. Our results may help explain the better-than-expected pregnancy rates in survivors with decreased ovarian reserve compared to late reproductive age women.

Supported by: LR01HD062797 (CG, MS), K01 L:1-CA-133839-03 (CG), T32 HD007440 (LJ).


OBJECTIVE: To identify the possible risk factors for falls among Saudi postmenopausal women, in a population-based study with a mean follow-up period of 5.2 ± 1.3 years.

FERTILITY & STERILITY®
DESIGN: Prospective cohort study.
MATERIALS AND METHODS: 707 postmenopausal women aged ≥50 years were studied. Participants included demographic characteristics, medical history, lifestyle factors, past-year history of falls, and physical activity (PA) scores were assessed. We recorded single and multiple falls, anthropometric parameters, five special physical performance tests, hormone levels, and bone mineral density measurements. Data on knee osteoarthritis (OA), lumbar spondylosis and osteopenia were collected. Knee and lower back pain were assessed by interview and cognition was assessed by mini-mental state examination.

RESULTS: During ~5 years of follow-up, 164 women (23.2%) reported at least one fall, of whom 73 (10.3%) reported multiple falls. Six independent predictors of all falls were identified: past-year history of falls; relative risk estimate (RR) 3.53; (95% CI confidence interval [CI]: 2.61-4.76); PA-score ≤2.46; 95% CI:1.50-4.01; age ≥65 years (RR=2.16, 95% CI:1.30-3.12); presence of knee OA (RR=1.76; 95% CI:1.32-2.39); hand grip strength ≤ lowest quartile, (RR=1.60; 95% CI:0.8-2.40); and serum 25(OH)D ≤ lowest quartile, (RR=1.48; 95% CI:1.16-1.84).

CONCLUSION: These results identified six risk factors for all falls including past-year history of falls, poor physical activity score, age ≥65 years, presence of knee OA, poor handgrip strength, and vitamin-D deficiency among Saudi postmenopausal women.

Supported by: This study was Supported by the Center of Excellence for Osteoporosis Research.

O-128 Tuesday, October 21, 2014 05:15 PM

MEDICAL SPECIALTY AFFECTS WHEN AND WHY WOMEN ARE PRESCRIBED MENOPAUSAL HORMONE THERAPY. C. Yondon, S. E. Pollack, J. Bauer, G. Neal-Perry. Obstetrics & Gynecology and Women’s Health, Albert Einstein College of Medicine, Bronx, NY.

OBJECTIVE: The goal of this study is to assess attitudes, menopausal medicine knowledge, and prescribing patterns of menopausal hormone therapy (MHT) by healthcare providers who render health care to women.

DESIGN: An anonymous survey designed to assess attitudes, MHT prescribing patterns, and general menopausal medicine knowledge was administered to residents and faculty of the Departments of Family Medicine (FM) and of Obstetrics and Gynecology (OB/GYN) at Montefiore Medical Center- Albert Einstein College of Medicine. We tested the hypothesis that training in a non-OB/GYN discipline and training after the paradigm shifting 2002 WHI study reduces the likelihood clinicians will prescribe MHT for bothersome vasomotor symptoms.

MATERIALS AND METHODS: Chi squared analysis and Fisher’s exact test were used to determine differences in the proportion of FM and OB/GYN clinicians who responded to survey questions. P<0.05 is considered statistically significant.

RESULTS: We received 184 responses, 130 OB/GYN and 54 FM physicians. Significantly more OB/GYN than FM physicians prescribe MHT (p<0.01). When data were stratified by when physicians trained relative to the landmark WHI report in 2002, nearly twice as many OB/GYNs reported prescribing MHT if he/she trained before compared to after WHI (p=0.018; OR 2.6). Additionally, as much as 8-times more FM physicians reported that they prescribed MHT if they trained prior to WHI (p<0.01; OR 45.6). OB/GYNs were more likely to prescribe systemic MHT than FM physicians who typically used local MHT. FM and OB/GYN physicians cited vasomotor symptoms and vaginal dryness as common indications for MHT. However, OB/GYNs were twice as likely to prescribe MHT for cardioprotection and cognitive health compared to FM doctors (P<0.01). Among physicians who do not prescribe MHT, 96% FM compared to 26% OB/GYN physicians expressed fear regarding adverse effects of MHT on patient health (P<0.01) as a limiting factor.

CONCLUSION: Medical discipline (OB/GYN vs FM) and timing of training relative to the publication of the primary WHI study (before vs after) significantly affects prescribing patterns of MHT. Overall, our findings suggest a need to educate OB/GYN and FM physicians regarding the indications and utility of MHT. Education of the primary healthcare providers of women about the use of MHT will ensure that aging women receive comprehensive and current evidence-based care.

Supported by: Department of Obstetrics and Gynecology.

O-129 Tuesday, October 21, 2014 05:15 PM


OBJECTIVE: Few investigations have studied sleep quality in surgically menopausal women. We aimed to evaluate clinical factors associated with disordered sleep quality in BRCA 1 and 2 carriers after risk-reducing BSO (RRBSO).

DESIGN: A cross-sectional investigation of 594 women after RRBSO.

MATERIALS AND METHODS: The Pittsburgh Sleep Quality Index was completed as a subjective measure of sleep quality. A score of 6 or greater indicates poor sleep quality. Vasomotor symptoms were assessed using the Green Climacteric Scale. Depression and anxiety were assessed with the Hospital Anxiety Depression Scale. Univariate tests of association (Wilcoxon ranksum test and χ2) and logistic regression modeling were used to evaluate associations of selected variables with poor sleep.

RESULTS: The majority of participants reported poor sleep quality (61.2%, n=364). Vasomotor symptoms were significantly associated with poor sleep even when accounting for hormone replacement therapy (HRT) and antidepressant use. Anxiety and depression were also associated with poor sleep after adjusting for antidepressant use. In women without night sweats, the presence anxiety was significantly associated with poor sleep.

CONCLUSION: Depressed mood, anxiety, and vasomotor symptoms are significantly associated with poor sleep quality in surgically menopausal BRCA 1 and 2 carriers even when controlling for use of HRT and antidepressants. Our results suggest that these conditions are inadequately treated in this population, contributing to the poor sleep quality observed.

Supported by: Susan G. Komen, grant Sac10003 (SD), Basser Research Center for BRCA (SD), NIH T32 HD007440 (LJ), NIEHS 5P30ES015308-07 (SB).

Factors Associated with Poor Sleep Quality

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Univariate Associations</th>
<th>Multivariable Regression</th>
<th>Regression Excluding Subjects with Night Sweats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds Ratio (OR)</td>
<td>P value</td>
<td>Adjusted OR*</td>
</tr>
<tr>
<td>Vasomotor Symptoms</td>
<td>2.14 (1.45-3.15)</td>
<td>&lt;.0001</td>
<td>1.68 (1.13-2.5)</td>
</tr>
<tr>
<td>Obesity</td>
<td>1.61 (1.07-2.51)</td>
<td>.02</td>
<td>1.46 (0.89-2.42)</td>
</tr>
<tr>
<td>Age &gt;50</td>
<td>0.83 (0.59-1.17)</td>
<td>.3</td>
<td>1.03 (0.69-1.55)</td>
</tr>
<tr>
<td>HRT Use</td>
<td>0.72 (0.48-1.08)</td>
<td>.09</td>
<td>0.79 (0.51-1.25)</td>
</tr>
<tr>
<td>Depressed Mood</td>
<td>4.69 (3.14-7.06)</td>
<td>&lt;.0001</td>
<td>2.88 (1.8-4.61)</td>
</tr>
<tr>
<td>Antidepressants</td>
<td>3.62 (2.43-5.44)</td>
<td>&lt;.0001</td>
<td>2.04 (1.26-3.5)</td>
</tr>
<tr>
<td>Taking Antidepressants</td>
<td>2.46 (1.64-3.73)</td>
<td>&lt;.0001</td>
<td>2.09 (1.34-3.28)</td>
</tr>
</tbody>
</table>

*Adjusted for Age, Depressed Mood (borderline or clinical), Anxiety (borderline or clinical), Vasomotor Symptoms, HRT Use, Antidepressant Use

OBJECTIVE: The aim of the study is to analyse the effect of sun exposure as a consequence of lifestyle and outer clothing over bone health in postmenopausal women with normal dual energy x-ray absorptiometry (DEXA).

DESIGN: Cross sectional study of 190 postmenopausal women with normal DEXA values of femoral neck and L1-L4 and not taking supplemental Ca or vitamin D.

MATERIALS AND METHODS: Blood samples were obtained for 25-OHD, calcium, phosphorus, osteocalcin, deoxypyridinoline, thyroid stimulating hormone, HDL, LDL, TG, fasting insulin, fasting glucose, HbA1C, FSH and estradiol levels. Daily sun exposure and lifestyle were questioned for every participant. The examination of femoral neck, total femur and total L1-L4 bone were established with DEXA. Participants were grouped into three categories in relation to daily sun exposure (A: no exposure, B: daily exposure for 10-30 minutes and C: daily exposure more than 30 minutes). The groups were compared with Kruskal-Wallis and posthoc analysis.

RESULTS: 25-OHD was found to be higher in group C in comparison to group A (21.96+12.62 vs 12.18+10.96, p=0.005). Deoxypyridinoline was found to be higher in group B in comparison to group C (12.61+3.88 vs 11.17+2.76, p=0.035). Calcium was found to be higher in group C in comparison to group A (9.96+0.41 vs 9.62+0.42, p=0.005). After partial correlation analysis, 25-OHD and calcium were positively and serum deoxypyridinoline was negatively correlated with daily sun exposure time after adjustment for age and body mass index (r=0.234, p=0.001; r=0.241, p=0.001; r=-0.157, p=0.031, respectively).

CONCLUSION: Exposure to ultraviolet rays in the sunlight through skin is the main source for vitamin D besides the supply from food. Serum 25-hydroxyvitamin D is a good marker for vitamin D which is crucial for calcium and bone metabolism. Serum 25-hydroxyvitamin D (25-OHD) and calcium levels are positively and serum deoxypyridinoline levels are negatively correlated with daily sun exposure time after adjustment for age and body mass index in postmenopausal women without osteoporosis. Serum 25-hydroxyvitamin D levels were within the insufficiency range in the postmenopausal population without osteoporosis even in the sun exposure >30 minutes group. Due to nature of a cross sectional we can only demonstrate an association not a cause effect relationship. Further studies with a large population size are required to investigate the relationship between sun exposure and bone health.

GAPS BETWEEN PHYSICIANS’ PERCEPTIONS OF THE IMPORTANCE OF SHARED DECISION MAKING AND THEIR PRACTICE IN MENOPAUSAL SYMPTOM MANAGEMENT. S. Kiatponsan,* S. Feibelman,** K. Srupsacha,*-d Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand; bHarvard Interfaculty Initiative in Health Policy, Cambridge, MA; cMassachusetts General Hospital, Boston, MA; dHarvard Medical School, Boston, MA.

OBJECTIVE: To evaluate physicians’ perceptions of the importance of shared decision making (SDM) in menopausal symptom management and their practice.

DESIGN: Survey.

MATERIALS AND METHODS: 200 U.S. physicians who had seen patients with menopause symptoms within the past year were invited to participate. The participants were identified and randomly sampled through the American Medical Association Master File. Participants were assessed for their knowledge about menopause symptom management. Participants rated the importance of (1) having well-informed patients and of (2) explicitly discussing patients’ preferences. Participants also self-rated (1) how well-informed their patients are and (2) frequency of explicit discussions of their patients’ preferences. Multivariable ordinal regression models, Chi-Square tests and Median’s tests were used for statistical analyses. Statistical significance was determined at p < 0.05.

RESULTS: 108 physicians completed the survey. 12 physicians were ineligible or did not receive the survey. The response rate was 57.4% (108 out of 188). 57 (52.8%) respondents were female, 75 (69.4%) were White, and 55 (50.9%) were obstetricians/gynecologists. Participants’ mean age was 51.2 years. Median duration of years in practice was 20.0. Median number of patients with menopausal symptoms encountered per year was 100.0. Median knowledge score was 84.6%. 98 (90.7%) participants reported that it is very or extremely important that their patients are well-informed about treatments for menopause, but only 52 (48.2%) reported that their patients actually were. Most participants, 102 (94.4%), reported that it is very or extremely important to explicitly discuss patients’ preferences before a treatment decision is made while 88 (81.5%) reported that they always do so. 38 (69.1%) obstetricians/gynecologists reported that their patients were very or extremely well-informed while 14 (26.4%) primary care physicians or internists reported the same (p < 0.01).

CONCLUSION: There are significant gaps between the perceptions of the importance of SDM and self-reported SDM practice in menopausal symptom management.

Supported by: The study was funded by the Informed Medical Decisions Foundation.
MALE REPRODUCTION AND UROLOGY - CLINICAL

O-133 Tuesday, October 21, 2014 04:15 PM


OBJECTIVE: We provide the first long-term followup on sexual function in post-reconstruction CME patients.

DESIGN: We administered a sexual-health telephone survey to CME patients who underwent reconstructive surgery at our institution.

MATERIALS AND METHODS: We screened a prospective institutional database of 1216 exstrophy-epispadias patients for CME patients currently ≥18 years of age who underwent reconstruction since 1969. Patients who could be contacted were asked to complete a phone survey regarding sexual function. Reconstructive history and clinical details were obtained by chart/database review. Patient-perceived importance of fertility was assessed using a five-point Likert-like scale (1 = least important, 5 = most important). Descriptive data is presented as median (range).

RESULTS: Of 132 CME patients, 74 met inclusion criteria, and 14 (19%) completed the telephone questionnaire (Table 1). Of the 14 patients, 50% reported currently being in a relationship. Although 86% reported satisfactory sexual intercourse, 71% of patients admitted to problems with sexual function including unsatisfactory/difficulty ejaculating (50%), diminished sensation (21%), and difficulty maintaining an erection (21%). When questioned regarding the importance of fertility, 71% of patients responded with ≥4. Five patients reported having impregnated a sexual partner. Although four patients described suspicions of fertility problems, only two had confirmed abnormal semen analysis findings.

<table>
<thead>
<tr>
<th>Patient Demographics and Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at First Repair</td>
</tr>
<tr>
<td>Current Age</td>
</tr>
<tr>
<td>Epispadias Repair Type</td>
</tr>
<tr>
<td>Young/Cantwell-Ransley Repair</td>
</tr>
<tr>
<td>Pts Requiring Second Epispadias Repair</td>
</tr>
<tr>
<td>Pts Requiring Bladder Neck Reconstruction</td>
</tr>
<tr>
<td>Continent via Urethra/Continent</td>
</tr>
<tr>
<td>via Stoma/Incontinent</td>
</tr>
<tr>
<td>Length of Follow-Up</td>
</tr>
<tr>
<td>Relationship Status: Single</td>
</tr>
<tr>
<td>Partner/Engaged</td>
</tr>
<tr>
<td>Married</td>
</tr>
<tr>
<td>Engage in Regular Sexual Intercourse</td>
</tr>
<tr>
<td>Satisfactory Sexual Intercourse</td>
</tr>
<tr>
<td>Issues Regarding Sexual Intercourse</td>
</tr>
<tr>
<td>Fertility Importance: ≥ 4</td>
</tr>
<tr>
<td>≤ 3</td>
</tr>
<tr>
<td>Impregnated Partner (Natural/ART)</td>
</tr>
<tr>
<td>Suspected Fertility Issue</td>
</tr>
<tr>
<td>Abnormal Semen Test</td>
</tr>
</tbody>
</table>

CONCLUSION: To the best of the authors’ knowledge, this is the first report on sexual health in post-reconstruction CME. Patients are able to engage in relationships, enjoy sexual intercourse, and sometimes impregnate their partners. Still, these results highlight sexual concerns and issues that can aid in counseling CME patients.

O-135 Tuesday, October 21, 2014 04:45 PM

CLOMIPHENE CITRATE IS SUPERIOR TO ANASTRAZOLE IN RAISING TESTOSTERONE IN HYPOGONADAL INFERTILE MEN: A PROSPECTIVE RANDOMIZED DOUBLE BLIND COMPARISON TRIAL. S. Helo,1 C. Mechlin,1 C. A. Alkaram,1 B. F. Feustel,1 E. Ditkoff,1 M. Grossman,1 A. McCullough,1 A. Y. Center for Neuropharmacology & Neuroscience, Albany Medical Center, Albany, NY; 1Center for Neuropharmacology & Neuroscience, Albany Medical College, Albany, NY; 1CNY Fertility Center, Latham, NY.

OBJECTIVE: In this prospective double-blinded randomized trial we set out to show non inferiority of anastrozole (AZ) to clomiphene citrate (CC) with the objectives of raising testosterone (T) and improving testosterone to estradiol (T/E2) ratio in hypogonadal infertile men (HM).

DESIGN: In this randomized double blind study patients were randomized to either CC or AZ. The effects on several hormone levels were then measured. Patient reported measure were also recorded.

MATERIALS AND METHODS: Our cohort consisted of 23 men presenting with infertility and hypogonadotrophic hypogonadism defined as serum T less than 300 ng/dL, and normal gonadotropins. Patients were randomized to either AZ (1 mg/day) or CC (25 mg/day) for 12 weeks. Hormones assayed were total and free, E2, luteinizing hormone (LH), follicle stimulating hormone (FSH), and sex hormone binding globulin (SHBG). Safety labs included complete blood count and hepatic profile. Patients reported measures were quantified using the International Index of Erectile Function, Erection Hardness Scale, and the Androgen Deficiency in the Aging Male questionnaires. Blood tests and questionnaires were recorded at baseline, 6 and 12 weeks. Semen analyses were performed at baseline and 12 weeks.

RESULTS: The mean age was 34 years. The baseline T increased significantly from baseline (AZ 249 ng/dL, CC 250 ng/dL) to 12 weeks (AZ 419 ng/dL, CC 577 ng/dL) in both groups (p < 0.007). Mean increase from baseline total T at 12 weeks was less in the AZ group (71%) than the CC group (130%) (p < 0.003). The T/E2 ratio increased from baseline (AZ 10.2, CC 9.3) to 12 weeks (AZ 17.2, CC 12) (p < 0.003). LH and FSH levels also increased in both groups. Neither group demonstrated a significant change in safety measures, seminal parameters or patient reported measures.

CONCLUSION: While mean T levels increased in both groups at 6 and 12 weeks, CC demonstrated clear superiority. Although both drugs resulted in a...
T/E2 ratio greater than 10, AZ was superior to increasing the T/E2 ratio. Superiority in seminal parameters was not demonstrated.

Supported by: Capital Region Medical Research Foundation and Urological Institute of Northeastern New York.

O-136 Tuesday, October 21, 2014 05:00 PM

ASSOCIATION BETWEEN TESTOSTERONE THERAPY AND THROMBOTIC EVENTS IN ELDERLY MEN. R. Ramasamy, J. M. Scovell, M. Mederos, R. Ren, S. Besada, L. I. Lipshultz. Urology, Baylor College of Medicine, Houston, TX.

OBJECTIVE: The association between testosterone therapy and thrombotic risk in elderly men remains controversial. We evaluated the prevalence of cardiovascular and atherosclerotic events in men older than 65 years with symptomatic hypogonadism treated with testosterone therapy. We compared men treated with testosterone to an age and comorbidity matched cohort of men without hypogonadism.

DESIGN: Retrospective study.

MATERIALS AND METHODS: We retrospectively reviewed the charts of 232 men older than 65 years. Of the 232 men, 172 men were on testosterone therapy (injections n = 59; gel n = 71; pellets n = 37; cream n = 3; patch n = 2) and 60 men did not have symptomatic hypogonadism. We evaluated age and Charlson Comorbidity Index. In addition, we compared the serum total testosterone (T), free testosterone (fT), estradiol (E), sex hormone binding globulin (SHBG), dihydrotestosterone (DHT), prostate specific antigen (PSA), hemoglobin, and lipids (triglycerides, HDL, LDL) between men treated with testosterone and those not treated with testosterone. We evaluated the prevalence of myocardial infarction (MI), transient ischemic attack (TIA), cerebrovascular accident (CVA), or stroke, and pulmonary embolism (PE). Cardiovascular and thrombotic events prior to initiation of testosterone therapy were included in the treatment group so as to not bias prevalence rates.

RESULTS: Mean age and Charlson Comorbidity Index of men on testosterone therapy (72y; 1.9) was similar to men without hypogonadism (73y, p = 0.82, 1.5, p = 0.11). The mean duration of testosterone therapy was 4.4 ± 4.3 years. The prevalence of MI (8% vs. 13%, p = 0.23), TIA/CVA (8% vs. 3%, p = 0.37), and PE (0% vs. 2%, p = 0.26) were similar between men treated with testosterone and men not on testosterone therapy. Serum testosterone (524 ng/dL vs. 338, p = 0.005), IT (15.7 ng/dL vs. 5.7, p = 0.01), and DHT (448 ng/dL vs. 269, p = 0.01) were greater in men on testosterone therapy compared to men not on testosterone therapy. There was no difference in SHBG, E, and lipid parameters in men on testosterone therapy and men not on testosterone therapy.

CONCLUSION: Testosterone supplementation appears to be a safe and effective therapy for symptomatic hypogonadism in elderly men. There was no difference in prevalence of MI, TIA/CVA, or PE between patients who were treated with testosterone and men not treated with testosterone. Despite reassuring data from our cohort study, testosterone should be used with caution in elderly men with comorbidities until larger randomized trials are performed.

O-137 Tuesday, October 21, 2014 05:15 PM

WHAT ARE INFERTILITY TREATMENT CENTER WEBSITES TELLING COUPLES ABOUT MALE FACTOR INFERTILITY? A. Leung, Z. Khan, D. Patil, A. Mehta. Urology, Emory University School of Medicine, Atlanta, GA.

OBJECTIVE: To evaluate patient-directed website contents of infertility treatment centers.

DESIGN: Cross-sectional.

MATERIALS AND METHODS: 428 infertility treatment centers were identified from the 2011 Centers for Disease Control Fertility Clinic Success Rates Report. Each center’s website was evaluated for the presence of terms related to the contribution, etiology, evaluation, and treatment of male factor infertility using a standardized data abstraction form. Specific variables included seminal analysis, karyotype, Y-chromosome microdeletion, azo-ospermia, hypogonadism, varicocele, hormone therapy, surgical sperm extraction, and referral to a urologist. Websites in languages other than English were excluded. Descriptive and inferential statistical analysis with Chi-square and Fischer's exact tests was performed using SAS 9.3, to evaluate the frequency with which websites included information pertaining to male factor infertility. Differences in the specified variables were examined with respect to academic center affiliation, as well as geographic distribution within four regions demarcated by the U.S. Census Bureau.

RESULTS: The majority (76%) of treatment centers were non-academic practices. Overall, only 78% of websites acknowledged a male factor contribution to infertility, with 86% mentioning any evaluation of the male partner, 63% mentioning any treatment options for male factor infertility, and 23% discussing referral to a urologist. Of websites that mentioned a male factor contribution, the most commonly described etiologies were azoospermia (72%), varicocele (48%), cystic fibrosis (32%), hypogonadism (27%), Klinefelter syndrome (15%), and Y-chromosome microdeletion (11%). Of websites that mentioned treatment for male factor infertility, 39% discussed medical and 97% discussed surgical options. Statistically significant regional differences were found in the distribution of academic vs. non-academic treatment centers (p = 0.001), and in the mention of any treatment for male infertility (p = 0.034).

CONCLUSION: Patient-directed information pertaining to the contribution, etiology, work-up and treatment of male factor infertility on the websites of infertility treatment centers is variable at best, and completely lacking in more than 20% of websites. Even amongst websites that acknowledged the contribution of male factor infertility, specific referral for urologic evaluation is mentioned less than 25% of the time. It is likely that couples undergoing infertility evaluation and treatment are not well informed about the importance of a male factor evaluation.

O-138 Tuesday, October 21, 2014 06:30 PM

AGE DOES MATTER IN SPERM FUNCTION TO ACTIVATE OO-CYTES IN SUBFERTILE MEN. T. Shin, M. Fukushima, T. Iwahata, K. Suzuki, Y. Kobori, H. Okada. Department of Urology, Dokkyo Medical University Koshigaya Hospital, Koshigaya, Saitama, Japan.

OBJECTIVE: To investigate sperm function in subfertile men with normal semen analysis and to examine the question of whether advancing male age impact sperm function.

DESIGN: Observational study.

MATERIALS AND METHODS: We analyzed oocyte activation ability of spermatozoa in subfertile men and in the volunteers proven fertility. Mouse oocyte activation test, which was first proposed in 1995 by Rybicki et al., was used in the present study. After obtaining institutional review board approval, spermatozoa were obtained from 100 male subfertile patients with normal semen analysis whose wives reportedly no gynecologic factor. As the control, fertile spermatozoa were obtained from 30 donors with proven fertility. Human spermatozoa were injected into mouse oocytes by a piezo-driven unit and these oocytes were incubated at 37° under 5% CO2 in air. Approximately 10 hours after injection, the oocytes were observed under an inverted microscope and were rotated to determine whether or not a second polar body was present. An oocyte with the second polar body was recorded as ‘activated’. We evaluated the activation rates of oocytes in male subfertile patients and the controls, comparing the activation rates of the two groups in six consecutive age subgroup (<30 years, 30-34 years, 35-39 years, 40-44 years, 45-50 years, >50 years). Multiple linear regression was performed to study the influence of male age on the activation rate. The results were expressed as the regression coefficient (RC) value and p value.

RESULTS: Over all activation rates of the subfertile group and the control group were 62.0% and 78.2%, respectively, and this difference was statistically significant (p = 0.001). After 35 years, in each age subgroup, the activation rate of the subfertile group significantly decreased compared to that of the control group. Multiple linear regression analysis showed that male age had a negative influence on the activation rate only in the subfertile patients (p = 0.016, RC = −1.234) but not in the control volunteers (p = 0.126, RC = −0.597).

CONCLUSION: Sperm function to activate oocytes drops in subfertile patients after 35 years. The etiology of sterility in older couples cannot solely be attributed to the aging of eggs. Aging of sperm, which cannot be detected by the ordinary semen analyses, may affect the fecundity of them. We have to enlighten this fact to all the people and encourage men of subfertile couples to consult male infertility clinic as soon as possible.

ENDOMETRIOSIS II

O-139 Tuesday, October 21, 2014 04:15 PM

SIMVASTATIN REDUCES VOLUME OF ACTIVE ENDOMETRIOTIC LESIONS IN PRIMATE MODEL OF ENDOMETRIOSIS. A. Duleba, A. Fuzileabas, A. Nyachieie, D. Chai, T. D’Hooogh, H. S. Taylor. L. Jolla. CA; 2Michigan State University, Grand Rapids, MI; 3IPR, Karen, Nairobi, Kenya; 4Leaven University, Leuven, Belgium; 5ObGyn, Yale University, New Haven, CT.

OBJECTIVE: Endometriosis is characterized by ectopic attachment and growth of endometrial tissues. Previous studies have shown that statins

FERTILITY & STERILITY®

reduce adhesiveness and inhibit proliferation of human endometrial stromal cells in vitro. Furthermore, administration of statin decreased the size and the number of endometriotic-like lesions in the nude mouse model of endometriosis. Since baboons are phylogenetically, anatomically and physiologically close to humans, this study was designed to determine whether treatment with statin (simvastatin) affects experimental endometriosis in a well-established baboon model of this disorder.

DESIGN: In vivo study.

MATERIALS AND METHODS: Endometriosis was induced in sixteen baboons by inoculation of autologous menstrual endometrial tissues into peritoneal cavity. Subsequently the animals were either not treated (Control, n=8) or treated with simvastatin (20 mg po daily, n=8). Laparoscopies performed at 3 months documented sizes, volumes and type of endometriotic lesions as well as the number and length of adhesions. Lesions were considered "active" when categorized as red, orange or white. Volumes of lesions were estimated based on the volume of prolate ellipsoid. Statistical analysis was performed using Wilcoxon sign rank test.

RESULTS: Table summarizes findings at laparoscopy: each value represents mean ± SEM per animal.

<table>
<thead>
<tr>
<th>Control</th>
<th>Simvastatin</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of all lesions</td>
<td>12.1±2.8</td>
<td>10.9±2.5</td>
</tr>
<tr>
<td>Number of active lesions</td>
<td>10.6±2.4</td>
<td>7.6±2.4</td>
</tr>
<tr>
<td>Volume of all lesions (mm³)</td>
<td>11.2±6.4</td>
<td>12.7±5.9</td>
</tr>
<tr>
<td>Volume of active lesions (mm³)</td>
<td>117.4±64.6</td>
<td>26.4±6.9</td>
</tr>
<tr>
<td>Number of all adhesions</td>
<td>4.4±1.0</td>
<td>3.8±0.8</td>
</tr>
<tr>
<td>Length of all adhesions (mm)</td>
<td>51.6±15.8</td>
<td>15.2±5.4</td>
</tr>
</tbody>
</table>

CONCLUSION: This is the first report demonstrating that statin reduces volume of active endometriotic lesions in primates. Significant animal-to-animal variability in the number and size of endometriotic lesions was noted, consistent with genetic diversity of studied animals. Present findings provide rationale for consideration of clinical trial using statin as a potential novel treatment of endometriosis.

Supported by: NIH U54HD052668 (AID and HST) and NIH U54HD049009 (ATF).

O-140 Tuesday, October 21, 2014 04:30 PM

INVOLVEMENT OF GLUCOSYLCERAMIDES IN ENHANCING ENDOMETRIOTIC ESCS PROLIFERATION. J. K. Y. Chan, C. Tan, L. Griffith, Y. H. Lee. SMART, SGF, Singapore; MIT, Cambridge, MA; KK Hospital, SGF, Singapore.

OBJECTIVE: Glucosylceramides (GlcCers), are one of the bioactive glycosphingolipids widely distributed in cells and are the precursors of over 200 glycosphingolipids. GlcCer have been implicated in cell growth, differentiation, oncogenic metastasis and migration. However, its role in endometrial stromal cells (ESC) and endometriosis is unknown. The Src-related protein kinase Lyn is implicated in GlcCer synthase-induced cellular migration. Here, we determined the cellular effects of GlcCer on cell migration in ESC and downstream Lyn signaling. Understanding plausible signaling mechanisms enhancing ESC migration may enhance our understanding of lesion development in endometriosis.

DESIGN: Telomerase-immortalized human ESC (T-HECs) were exposed to GlcCer to determine safety profiles and functional concentrations. Co-incubation of Lyn inhibitory peptide and GlcCer was used to functionally dissect the role of Lyn in GlcCer-induced migration.

MATERIALS AND METHODS: To examine the role of GlcCer in migration, T-HECs were incubated with GlcCer with varying acyl chain lengths. GlcCer C8:1, C16:1 and C24:1 for 12 hr. The migratory extent of T-HECs with GlcCer, Lyn-specific inhibitory inhibitioty peptide βc 450-465 (YGGYRLRKKWKPEPFPF-NH2) or a combination of GlcCer and Lyn inhibitory peptide treatment was done. Multiple concentrations of GlcCer and βc 450-465 were tested to determine safety profiles and functional concentrations Lyn and phosphorylated Lyn protein expression in both cell supernatants and lysates during the GlcCer treatment were quantified via ELISA and Western Blot. Student’s t-test or 1-way ANOVA was used where appropriate.

RESULTS: GlcCer induced enhanced migration in an acyl chain length dependent manner. The long chain and very chain GlcCer C16:1 and C24:1 induced significant migration (2.4 and 2.9 fold, p<0.005 and p<0.001 respectively), while GlcCer C8:1 did not. Western blot showed basal Lyn activation after 12 hours was 20%. However, Lyn underwent significant phosphorylation and activation in cells treated with GlcCer as measured by phosphorylated-Lyn/Lyn ratio (1.4 fold rise, p<0.05). Inhibiting Lyn using Lyn-specific inhibitory peptide, βc 450-465 significantly suppressed GlcCer-induced migration (1.6 fold decrease; p<0.0005) and this was coupled with a 52% decrease in Lyn activation (p<0.05).

CONCLUSION: Long and very long-chain GlcCer induced cellular migration through the activation of Lyn. This suggests that GlcCer-Lyn signaling axis may be a target to reduce cell migration in endometriosis development.

Supported by: This work was funded by the National Research Foundation, Singapore, through the Singapore-MIT Alliance for Research and Technology's BioSystems and Micromechanics Inter-Disciplinary Research Programme. J.C.K.Y. received salary support from National Medical Research Council, Singapore (CSA/043/2012). C.W.T. is a recipient of the Singapore-MIT Alliance scholarship.

O-141 Tuesday, October 21, 2014 04:45 PM


OBJECTIVE: A non-invasive diagnostic test for endometriosis is needed to shorten the current diagnostic delay of 8-11 years. The goal of this study was to discover new biomarkers for endometriosis to improve existing diagnostic models.

DESIGN: A total of 103 plasma samples from patients with laparoscopically confirmed presence (n = 68, follicular n = 26, luteal n = 26, menstrual n = 16) or absence (n = 35, follicular n = 12, luteal n = 12, menstrual n = 11) of endometriosis was selected. All disease samples were pooled and all control samples were pooled. Pooled samples were examined by antibody array.

MATERIALS AND METHODS: Pooled samples were 5-fold diluted and dialysed before being biotinylated. Biotinylated samples were incubated onto the RayBio L-series Human Antibody Array L.507 and L.493 (Ray Biotechnology) at a 1:5 dilution and visualized with a streptavidin-Cy3 conjugate using a Genepix 4000B scanner (Molecular Devices), equipped with GenePix Pro software. A 1.5-fold increase or decrease in signal intensity for a given analyte among groups was considered a measurable and significant difference, provided that the signals were 2 standard deviations above the mean background (according to manufacturer’s manual).

RESULTS: A total of 74 proteins was upregulated (1.5-7.1 fold change) and 125 proteins were downregulated (1.5-5.4 fold change) in endometriosis patients compared with healthy controls. The top 10 of most differentially expressed markers consisted of 5 upregulated proteins (IGF2BP1 (7.1-fold), IL23p19 (6-fold), PP43CXCL4 (4.5-fold), Osteopontin (3.7-fold), GASP-1/ WIK2NRP ((3.2-fold)) and 5 downregulated proteins (SSTR2 (5.4-fold), VGF (4.7-fold), BNP2 (3.8-fold), PTN (3.4-fold), Thymidine Kinase 1 (3.3-fold)).

CONCLUSION: The plasma proteome is fundamentally different between women with and without endometriosis. The top 10 differentially regulated proteins can be integrated into existing plasma biomarker platforms (Vodolazka et al., 2012) to improve their sensitivity and specificity for the diagnosis of endometriosis.

Supported by: This study was Supported by grants from the Leuven University Hospital Fund and by the Flemish Foundation for Scientific Research (FWO, Fonds voor Wetenschappelijk Onderzoek).

O-142 Tuesday, October 21, 2014 05:00 PM


OBJECTIVE: To assess the impact of endometriosis alone, or with other factors, on IVF outcomes.

DESIGN: Population-based retrospective cohort.

MATERIALS AND METHODS: Autologous IVF cycles from the SART Database (2008-2010) were analyzed by diagnosis: endometriosis only (EO), endometriosis plus other diagnoses (EP), tubal factor only (TF), unexplained...
infertility (UN), and all other diagnostic groups (AO). Logistic, linear, poisson, and negative binomial regression models assessing the impact of diagnosis on ongoing pregnancy (OP), live birth (LB), oocyte yield, fertilization, and implantation were adjusted for age, FSH, year, prior cycles, smoking, BMI, parity, and micromanipulation as appropriate.

RESULTS: 353,022 cycles were included (Table 1). Within EP, Male factor (41%), tubal factor (29%), and diminished ovarian reserve (22%) were most common. Endometriosis was associated with lower oocyte yield than UN, TF, and AO (EO: RR 0.86 [0.84-0.87], 0.89 [0.88-0.90], 0.89 [0.88-0.92]; EP: RR 0.83 [0.81-0.84], 0.89 [0.87-0.91], 0.89 [0.88-0.91], all p<.0001). Women with EO had similar OP and LB compared to women with UN and AO, but had higher LB than women with TF in fresh cycles (OR 1.12[1.06-1.18], p=.0001). Women with EP had lower OP compared to TF and AO (fresh cycles OR 0.75 [0.72-0.78], 0.82[0.78-0.86], 0.86[0.82-0.88], frozen cycles OR 0.82[0.76-0.89], 0.81 [0.5-0.88], 0.86 [0.81-0.91], all p<.0001).

CONCLUSION: Endometriosis alone is associated with similar or better pregnancy outcomes compared to other diagnoses. When observed with confounding diagnoses, pregnancy outcomes are inferior suggesting a particularly poor prognosis group. Further studies are needed to assess the role of peri-implantation environment in endometriosis and ART outcomes.

Supported by: K24HD006067 (KB), 5K12HD001265-14 (SS,MDS,KB).

O-144 Tuesday, October 21, 2014 05:15 PM

IAP INHIBITOR REPRESSES INFLAMMATION AND THE DEVELOPMENT OF ENDOMETRIOSIS-LIKE LESIONS IN A MURINE MODEL. F. Taniguchi, T. Uegaki, M. Izawa, N. Terakawa, T. Harada. Ob&Gyn, Tottori University Faculty of Medicine, Yonago, Tottori, Japan.

OBJECTIVE: Inhibitor of apoptosis proteins (IAPs) promote pro-survival signaling pathways and prevent activation of the effector phase of apoptosis by interfering with the activation of caspases. Aberrant expression of the IAP family in endometriotic tissues may sustain their abnormal survival in ectopic sites. We previously demonstrated that the IAP family (cIAP-1, cIAP-2, XIAP, and survivin) in human endometriotic tissues derived from ovarian endometriomas are highly expressed compared with those in eutopic endometrial tissues, and that survivin plays a critical role in the susceptibility of human endometrial stromal cells (ESCs) to drug-induced apoptosis. We also showed that an IAP inhibitor (BV6) could inhibit cell proliferation in human ESCs. In the present study, we sought to evaluate the effect of the IAP inhibitor on a murine endometriosis model.

DESIGN: Experimental study.

MATERIALS AND METHODS: A homologous murine endometriosis model was established by uterine tissue transplantation in the abdominal cavity. Estradiol-treated ovariectomized BALB/c mice were injected intraperitoneally with endometrial fragments from the donor mice. After 4 weeks of BV6 injections (10mg/kg, thrice weekly), the extent of endometriosis-like lesions was analyzed. Gene expressions of the several inflammatory cytokines (Vegf, Il-6, Mcp-1, and Lf) in the lesions were assessed by real time RT-PCR. The proliferative or angiogenic activity in the lesions was evaluated by Ki67 and PECAM immunohistochemical staining. The degree of inflammation was also assessed by CD3 (T-cell marker) or F4/80 (macrophage cell marker) immunohistochemical staining.

RESULTS: All four IAPs proteins were expressed in the murine endometriosis-like implants. After BV6 treatment, the total number, average weight, and surface area of the endometriosis-like lesions were markedly repressed compared with the control. Administering BV6 significantly reduced the level of Vegf, Il-6, Mcp-1, and Lf gene expression in the lesions. The percentage of Ki67 positive cells in the lesions decreased after BV6 treatment (epithelium: 26.8 vs 8.8 %, stroma: 15.2 vs. 5.0 %). In addition, the intensity of PECAM, CD3, and F4/80 immunoreactive cells in the lesions was repressed.

CONCLUSION: These results suggest that IAPs could be critically involved in the pathogenesis and progression of endometriosis. We found that IAP inhibitor could repress inflammatory reaction and the development of endometriosis-like lesions in the murine model. IAPs may be effective therapeutic targets for treating endometriosis.

Supported by: KAKENHI (Japan Society for the Promotion of Science Grant-in-Aid).

O-144 Tuesday, October 21, 2014 05:30 PM

NEW BIOMARKERS OF ADENOMYSOSIS IN ENDOMETRIUM. H. Dechaud,1 D. Haouzi,2 C. Vincens,2 N. Bersinger,3 M. D. Mueller,4 S. Hamamah.5 Gynecologie, Montpellier, Languedoc-Roussillon, France; 2CHU Montpellier, IRB, Inserm U1040, Montpellier, Languedoc-Roussillon, France; 3Département de Biologie de la Reproduction, CHU Montpellier, ART/PGD Division, Montpellier, Languedoc-Roussillon, France; 4Département de Gynécologie - Obstétrique de l'Hôpital de l'Ile, Hôpital Universitaire de Berne, Inselspital, Berne, Switzerland.

OBJECTIVE: The aim of this study was to improve molecular characterisation of adenomyosis and identify potential biomarkers of this pathology. For this purpose, transcriptomes of myometrium containing adenomyosis lesions and endometrium from patients with versus without adenomyosis were investigated.

DESIGN: Endometrial and myometrial biopsies were obtained from hysterectomy specimens of patients with (n=21) and without (n=13) adenomyosis. RNAs were extracted and gene expression profiles were investigated using the affymetrix microarrays. The differential gene expression profile was evaluated with bioinformatics.

MATERIALS AND METHODS: The differential gene expression profiles between endometrial and myometrial samples were investigated in patients with adenomyosis (paired sample analysis) and then, in patients without adenomyosis. The lists of significant genes were cross-identified to identify of list of genes exclusive to the pathological group. Endometrial biomarkers of adenomyosis were validated by RT-qPCR.

RESULTS: In the control group, 3265 genes were differentially expressed with 1580 and 1685 genes up- and down-regulated respectively in endometria compared with myometria. In patients with adenomyosis, this differential was characterized by 2570 genes with 1298 and 1272 genes up- and down-regulated, respectively, in patients without adenomyosis.

CONCLUSION: These results suggest that IAPs could be critically involved in the pathogenesis and progression of endometriosis. We found that IAP inhibitor could repress inflammatory reaction and the development of endometriosis-like lesions in the murine model. IAPs may be effective therapeutic targets for treating endometriosis.

Supported by: KAKENHI (Japan Society for the Promotion of Science Grant-in-Aid).
**SHZD3A (x32), KLHL31 (x15), ADAMTS16 (x14), FOXP2 (x-31), FZRL2 (x-29), DGBK (-14) and play roles in several functions including migration and extracellular remodeling.

**CONCLUSION:** These results could contribute for improving our understanding of the physiological origin of adenomyosis and should open new perspectives in the diagnosis of this pathology.

**Supported by:** This work was partially Supported by a grant from the Ferring Pharmaceutical.

---

**O-145 Tuesday, October 21, 2014 04:55 PM**

**RADICAL LAPAROSCOPY (LSC) FOR DEEP INFLTRATIVE ENDOMETRIOSIS (DIE) RESTORES PROGESTERONE (P) RESPONSIVENESS IN THE EUOTIC ENDOThRIM (EUE) BY DECREASING MIR-29C AND INCREASING FKBP52. E. H. Miyadahira,a,b  P. C. Serafín,a,b  N. R. Joshi,c  L. P. Chami, D. M. R. Ribeiro, A. T. Fazleabas,a  Dep of Gyn, Faculdade de Medicina da USP, São Paulo, SP, Brazil; Huntington Medicina Reprodutiva, São Paulo, SP, Brazil;  Dep of Obst/Gyn & Reprod Biol, Michigan State University, Grand Rapids, MI;  Clinica Dr. Duarte Miguel Ribeiro, São Paulo, SP, Brazil;  Chamiê Imagem da Mulher, São Paulo, SP, Brazil.

**OBJECTIVE:** To compare molecular changes in the eutopic and ectopic endometrium of women with DIE before and after surgery due to pelvic pain.

**DESIGN:** Case control study at single Infertility Center.

**MATERIALS AND METHODS:** 14 patients diagnosed with DIE by transvaginal ultrasound with bowel preparation (TVSBP) underwent surgical excision of endometriosis by an experienced surgeon. Eutopic and ectopic secretory endometrium were collected before and after 6 months following surgery. TVSBP from 12 women without DIE served as controls and had normal eutopic endometrium. Samples were grouped as: a) normal EuE (n=8); b) Eosis EuE Pre-op = eutopic endometrium from patients with DIE before LSC (n=9); c) Lesions = peritoneal ectopic endometrium (n=9); d) Eosis EuE Post-op = eutopic endometrium after LSC (n=4).

Total RNA was isolated from the samples and quantitative real time polymerase chain reaction analysis (qRT-PCR) was performed to evaluate the expression of microRNA 29c (miR-29c) and, one of its targets, FK506-Binding Protein 52 (FKBP52) which plays a crucial role in progesterone (P) signaling. Statistical analysis was performed using the Graphpad Prism 5.0 software.

**RESULTS:** The qRT-PCR analysis showed significantly higher expression of miR-29c (p=0.016) in the Eosis EuE Pre-op when compared to Normal EuE. Expression of miR-29c was significantly decreased (p=0.037) in Eosis EuE Post-op when compared to Eosis Pre-op tissues. The miR-29c target FKBP52 showed an inverse trend and its expression was significantly decreased (p=0.0005) in Eosis EuE Pre-op compared to Normal EuE. LSC treatment significantly reversed the expression of FKBP52 (p=0.011) which correlated with the decrease in miR-29c.

**CONCLUSION:** To our knowledge this is the first study to report on the reversal of P-resistance in the EuE of women with DIE following radical LSC resection. Our data clearly demonstrates that presence of DIE causes a significant increase in miR-29c expression and a significant decrease of FKBP52. FKBP52 regulates P-responsiveness in the uterus and suppression of P regulated genes which probably contribute to endometriosis-induced infertility. Further, radical LSC resection of DIE appears to reverse P resistance associated with pelvic endometriosis.

**Supported by:** This work was Supported by NIH U54 HD40093 to A. T. F.

---

**O-146 Tuesday, October 21, 2014 06:00 PM**

**COMPARISON OF LONG TERM OUTCOMES IN ROBOTIC VERSUS CONVENTIONAL LAPAROSCOPY FOR TREATMENT OF ADVANCED-STAGE ENDOMETRIOSIS: IS THERE ANY DIFFERENCE?**  I. Sirota, F. Nezhat. Obstetrics and Gynecology, Mount Sinai Roosevelt Hospital, New York, NY.

**OBJECTIVE:** To determine long term outcome differences in patients undergoing robotic-assisted versus conventional laparoscopic surgery for advanced-stage endometriosis.

**DESIGN:** Prospective cohort study in a community hospital setting.

**MATERIALS AND METHODS:** Cases were collected from our prospectively maintained computerized database of robotic-assisted (RALS) and conventional laparoscopic surgical (CLS) treatments for endometriosis. Women treated between July 2009 and October 2012 for endometriosis stage III or IV (ASRM) were included in the study. A questionnaire composed of questions ranging from assessing post operative pain to subsequent medical or surgical treatment, followed by future pregnancies was distributed to all patients six months after the time of surgery and compared between the groups. In addition, patients were divided into sub-groups of those who had hysterectomy and those who did not. A Chi-square test was used to compare presence of long-term outcomes between CLS and RALS groups. The level of significance was set as 5%.

**RESULTS:** Included were 54 CLS and 23 RALS subjects. No significant differences were noted between RALS and CLS groups when comparing post operative pain, subsequent medical or surgical treatment, and future pregnancies. In the sub-group analyses, 39 CLS patients who did not have a hysterectomy needed additional medical treatment for endometriosis after the surgery mainly for unresolved pain compared to the 15 CLS patients who had hysterectomy (p<0.01 and p<0.05, respectively). In contrast, no statistical differences were found between the 14 RALS patients who did not have a hysterectomy and the 9 RALS patients who did have.

**CONCLUSION:** RALS patients who had surgery for advanced-stage endometriosis demonstrated similar long-term outcomes as compared to CLS patients. However, when stratifying for hysterectomy versus no hysterectomy, in contrast to the RALS group, hysterectomy in the CLS group seemed to play a protective role in the long term management of pain symptoms compared to those patients who did not have a hysterectomy.

---

**CANCER**

**O-147 Tuesday, October 21, 2014 04:15 PM**

**COLORECTAL CANCER RISK AFTER OVARian STIMULATION FOR IN VITRO FERTILIZATION.**  M. Spaan, A. W. van den Belt-Dusebout, C. W. Burger, F. E. van Leeuwen. Epidemiology, Netherlands Cancer Institute, Amsterdam, NH, Netherlands; Obstetrics and Gynecology, Erasmus University Medical Center, Rotterdam, ZH, Netherlands.

**OBJECTIVE:** Colorectal cancer is the most common cancer worldwide. Apart from life-style factors, sex hormones may also play a role in its etiology. Lower risks have been observed in parous women and after hormone replacement therapy. This raises interest in the possible effects of fertility drugs. While associations between ovarian stimulation for in vitro fertilization (IVF) and reproductive tract cancers have been examined in depth, little is known about the association between IVF treatment and colorectal cancer risk.

The aim of this study was to investigate the association between ovarian stimulation for IVF and colorectal cancer in a large subfertile cohort.

**DESIGN:** In 1996, a nationwide cohort study (the OMEGA-cohort) was set-up to examine the risk of cancer in a subfertile population receiving ovarian stimulation for IVF. The cohort includes 19,158 women who received IVF and a comparison group of 5,950 women not treated with IVF.

**MATERIALS AND METHODS:** Detailed information on subfertility cause and treatment (agents and doses) was collected from the medical records. Data on reproductive and life-style factors were obtained from the women through a questionnaire. Cancer incidence was ascertained through linkage with the Netherlands Cancer Registry (1989-2009). Colorectal cancer risk in the cohort was compared with the incidence in the general population.
population by calculating Standardized Incidence Ratios (SIRs) and between the IVF group and non-IVF group using multivariate time-dependent Cox regression analyses.

RESULTS: After a median follow-up of 17 years 71 colorectal cancers were observed. The SIRs in both the IVF group (SIR: 1.27; 95% Confidence Interval [CI] 0.80-1.39) and the non-IVF group (SIR: 0.78; 95% CI 0.45-1.24) were not different from the risk in the Dutch general population. In preliminary analyses, the risk of colorectal cancer in the IVF group was not statistically significantly different from the non-IVF group (adjusted Hazard Ratio: 1.58; 95% CI 0.82-2.77). No trend emerged with a higher number of IVF cycles or number of ampoules of gonadotropins used. The risk did also not differ by cause of subfertility.

CONCLUSION: This is the first study that could investigate colorectal cancer risk in a large subfertile population after long follow-up. No association was found between ovarian stimulation for IVF and colorectal cancer risk.

Supported by: This research is Supported by the Dutch Cancer Society, grant nr NKI 2006-3631.

O-148 Tuesday, October 21, 2014 04:30 PM

ART AND THE RISK OF CHILDHOOD CANCER – PRELIMINARY RESULTS. L. G. Spector,1 B. Luke,2 M. B. Brown,3 E. Wantman,1 M. Richardson,2 V. L. Baker,2 G. S. Letterie,1 M. J. Schymura,4 University of Minnesota, Minneapolis, MN;1 Michigan State University, East Lansing, MI;1 University of Michigan, Ann Arbor, MI;1 Redshift Technologies, Inc., New York, NY;1 Stanford University School of Medicine, Palo Alto, CA;4 Seattle Reproductive Medicine, Seattle, WA;4 New York State Cancer Registry, Albany, NY.

OBJECTIVE: To compare incidence of cancer in ART to non-ART children.

DESIGN: Population-based cohort study in CO, CT, MA, MI, NY, NC, and PA.

MATERIALS AND METHODS: Women with records of live birth following ART in participating states were identified in SART CORS during 2004-2009. Their children were identified in their respective states’ cancer registries (ART children); the ten births following each ART birth served as the comparison with the general population. Multivariable Cox regression analysis was used to quantify effects of treatment and cause of subfertility on endometrial cancer risk.

RESULTS: After a median follow-up duration of 16.9 years, 37 endometrial cancers were observed (SIR: 1.22; 95% confidence interval [CI], 0.86-1.68). Endometrial cancer risk was comparable after IVF (SIR: 1.13; 95% CI, 0.72-1.69) and after other fertility treatments (SIR: 1.41; 95% CI, 0.77-2.37). The SIR for nulliparous women was 1.9 (95% CI, 1.9-2.88), whereas the SIR for parous women was 0.76 (95% CI, 0.42-1.28). The risk after IVF was not increased (HR, 0.71; 95% CI, 0.36-1.41), comparing the IVF-group with the non-IVF group. The risk did not significantly differ for 1-3, or 4 or more IVF-cycles compared with no IVF, and for different causes of subfertility, adjusted for confounders.

CONCLUSION: After a median follow-up duration of 17 years after treatment, the risk of endometrial cancer was not significantly increased after ovarian stimulation for IVF treatment.

Supported by: This research is Supported by the Dutch Cancer Society, grant nr NKI 2006-3631 and a Departmental grant from the Department of Obstetrics and Gynecology of Erasmus University Medical Center.

O-150 Tuesday, October 21, 2014 05:00 PM

CANCER TREATMENT NEGATIVELY IMPACTS ANTI-MULLERIAN HORMONE IN PEDIATRIC AND ADOLESCENT FEMALES. L. M. Cookingham,1 A. J. Hubbs,1 J. L. de la Garza,5 D. K. Fleener,1 M. K. Santillan,1 S. K. Hunter,6 E. M. Smith,1 B. J. Steigmann,4 D. A. Santillan,4 Obstetrics & Gynecology, University of Iowa, Iowa City, IA;5 Pediatrics, University of Iowa, Iowa City, IA;1 Epidemiology, University of Iowa, Iowa City, IA;6 Merck & Co, Rahway, NJ.

OBJECTIVE: The study objective was to determine if Anti-Mullerian Hormone (AMH) levels decline following gonadotoxic therapy in pediatric and adolescent females with cancer as compared to the reported decline demonstrated in adult women. We compared AMH levels in newly diagnosed cancer patients to healthy controls.

DESIGN: Prospective cohort study.

MATERIALS AND METHODS: Consent and/or assent were obtained via the University of Iowa IRB. Data and samples were collected from control and affected cases at the University of Iowa Hospitals & Clinics over 16 months. Enzyme-linked immunosorbent assays (ELISA) of AMH were performed using the GenII assay (Becton Dickinson). AMH levels were measured in affected cases at baseline and 3 months post-treatment (chemo- or radiation). Baseline AMH levels were compared to healthy controls recruited from the pediatric clinic. Linear regression models were constructed using regression identified and clinically significant confounding variables with AMH as the dependent variable. Chi square was used for categorical variables and Student’s t-test or ANOVA was utilized for continuous variables. All variables were tested at significance level of 0.05.

CONCLUSION: This preliminary analysis detected no strong associations of ART with childhood cancer(s). Findings are based on small numbers of cases and may change as data from the remaining study states (CA, FL, GA, IL, MN, NJ, OH, TX, VA) are included.

Supported by: NIH K01 CA151973.
RESULTS: Samples were collected from 104 healthy controls, 10 cases at baseline, and 5 cases at 3 months post-treatment. No statistically significant difference was observed at baseline between cases and controls. The average AMH concentration (ng/ml) was 2.3 +/- 0.21 in controls, 1.8 +/- 0.3 in affected cases at baseline, and 0.60 +/- 0.36 at 3 months post-treatment. No significant differences in between groups were observed in age at sample collection, race, body mass index, age at menarche, menarche status or Tanner stage.

CONCLUSION: Cancer alone does not result in a statistically significant decrease in AMH levels in pediatric and adolescent females; however, post-treatment AMH levels are reduced significantly, as expected. These findings are similar to that seen in the adult female cancer population. Results from this study will contribute to determining the use of AMH as a biomarker of fertility for female pediatric and adolescent cancer patients. Validating a viable biomarker will be critical to identifying fertility preservation methods in young female oncology patients.

Supported by: Aiming for a Cure.

O-151 Tuesday, October 21, 2014 05:15 PM


OBJECTIVE: To characterize the essential role of oocyte-specific gene, Uromodulin-like 1 (Umodl1) in female reproduction.

DESIGN: Umodl1 is one of the candidate genes implicated in Kallmann syndrome. Previously, we've demonstrated that increased Umodl1 in vivo desensitizes the responsiveness of ovary to the neuroendocrine signals, thus leading to accelerated ovarian aging. The present study reports the overgrowth of ovaries in mice lacking Umodl1. Cellular and molecular underlyng the tumorigenesis were investigated.

MATERIALS AND METHODS: Mice carrying null Umodl1 allele (knockouts) were generated. Fertility parameters including litter size were monitored. Ovarian tissues of the mutants were collected and processed for RNA-sequencing to examine global changes in gene expression. Age-matched WT control tissues were included in all experiments. Unpaired student’s t-test was used for statistical analysis.

RESULTS: All of the mutant females exhibited reduced fertility (litter size, WT, 8.2±1.6, n=16; KOs, 4.1±1.3, n=36; p < 0.01) from young age. Among the aging females (≥ 6 month; n=36), 56% of them became infertile. Moreover, enlarged ovaries were observed in 30% of the mutant females examined (WT, 8.01±0.61, n=11 vs KO, 206±240 mg/ovary in weight, n=11; p < 0.001). Histological analysis showed that the mutant ovaries had transformed into cancerous tissue composed of a highly proliferated cortex region of epithelial origin and a core area filled with adipocytes, in which no ovarian follicular structures were disenable. RNA-sequencing clearly demonstrates a profound disturbance in the expression of genes involved in cell cycle progression, metabolism and extracellular matrix integrity. For instance, the rodent specific reproductive homeobox (Rhox) gene cluster on the X-chromosome, including Rhox2 and Rhox4, were drastically upregulated. Furthermore, IHC showed a notable increase in ERα/β expression in the tumors. More interestingly, despite a decrease in FSH, the FSHR was abundantly mis-expressed in the highly vascularized cortex, suggesting that, as result of Umodl1 knockout, the proliferative signaling pathway mediated by FSHR has become FSH-independent and constitutively active.

CONCLUSION: Balanced Umodl1 levels are crucial for ovarian function. Deficiency in Umodl1 in mice increases the propensity for ovarian cancer; whereas elevated Umodl1 expedites ovarian degeneration.

O-152 Tuesday, October 21, 2014 05:30 PM

CANCER IN WOMEN BEFORE ART. B. Luke, a M. B. Brown, b L. G. Spector, c S. A. Misserm, d R. Leach, e M. Williams, f L. Koch, g Y. Smith, h J. E. Stern, g G. D. Ball, h M. J. Schymura, i Michigan State University, East Lansing, MI; University of Michigan, Ann Arbor, MI; University of Minnesota, Minneapolis, MN; You Medical School, Boston, MA; Texas Cancer Registry, Austin, TX; Illinois State Cancer Registry, Springfield, IL; Geisel School of Medicine at Dartmouth, Lebanon, NH; Seattle Reproductive Medicine, Seattle, WA; New York State Cancer Registry, Albany, NY.

OBJECTIVE: To compare the incidence of cancer among women prior to ART treatment to the general population.


MATERIALS AND METHODS: Women with their first ART treatment in 2004-09 were identified from the SART CORS and linked to their respective Cancer Registries. Years were rounded; i.e., year 1 = 6-18 months before treatment. This study used data from 5.5 years before to 6 months after treatment; women with cancer prior to 5.5 years before ART were excluded. Standardized incidence ratios (SIR) and their 95% confidence intervals (CI) were calculated between observed cancer cases and age-specific expected cancer rates.

RESULTS: The study included 54,589 women and 326,705 person-years of follow-up; 717 women were diagnosed with cancer, 12 were subsequently diagnosed with a second cancer. Mean age at diagnosis was 33.1 ± 5.8 years; age at start of ART treatment was 34.3 ± 5.9 for women with cancer compared to 36.0 ± 6.8 for the women without cancer. Embryo banking was used by 37.7% of women with cancer compared to 1.7% of women without cancer. Among women with cancer, 55% (392 women) had ART treatment the same year as their cancer diagnosis (58% did embryo banking) and another 15% (106 women) had ART treatment within one year after their diagnosis (31% did embryo banking).

SIRs and 95% CIs for All Cancers by Age and Parity at Cycle Start

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>SIR (95% CI)</th>
<th>Parity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>1.59, 1.48-1.71</td>
<td></td>
</tr>
<tr>
<td>18-29</td>
<td>6.96, 5.95-8.09</td>
<td></td>
</tr>
<tr>
<td>30-34</td>
<td>2.04, 1.75-2.36</td>
<td></td>
</tr>
<tr>
<td>35-37</td>
<td>1.66, 1.41-1.95</td>
<td></td>
</tr>
<tr>
<td>38-40</td>
<td>1.33, 1.11-1.57</td>
<td></td>
</tr>
<tr>
<td>41-43</td>
<td>0.64, 0.49-0.83</td>
<td></td>
</tr>
<tr>
<td>44-64</td>
<td>0.63, 0.45-0.86</td>
<td></td>
</tr>
<tr>
<td>Parity 0</td>
<td>1.78, 1.64-1.92</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.23, 0.98-1.52</td>
<td></td>
</tr>
<tr>
<td>≥2</td>
<td>0.75, 0.51-1.05</td>
<td></td>
</tr>
</tbody>
</table>

CONCLUSION: Women with cancer are more likely to seek ART treatment sooner (i.e., at a younger age) than those without cancer. Among women who have ART treatment, those with a prior cancer diagnosis are more likely to be younger, nulliparous, and banking embryos.

Supported by: NIH RO1 CA151973.

O-153 Tuesday, October 21, 2014 05:45 PM

CANCER IN WOMEN AFTER ASSISTED REPRODUCTIVE TECHNOLOGY. B. Luke, a M. B. Brown, b L. G. Spector, c S. A. Misserm, d R. Leach, e M. Williams, f L. Koch, g Y. Smith, h J. E. Stern, g G. D. Ball, h M. J. Schymura, i Michigan State University, East Lansing, MI; University of Michigan, Ann Arbor, MI; University of Minnesota, Minneapolis, MN; Harvard Medical School, Boston, MA; Texas Cancer Registry, Austin, TX; Illinois State Cancer Registry, Springfield, IL; Geisel School of Medicine at Dartmouth, Lebanon, NH; Seattle Reproductive Medicine, Seattle, WA; New York State Cancer Registry, Albany, NY.

OBJECTIVE: To compare cancer incidence among women with ART treatment to the general population.

DESIGN: Population-based cohort study in New York (NY), Texas (TX), and Illinois (IL).

MATERIALS AND METHODS: Women with initial ART treatment in 2004-09 were identified from the SART CORS and linked to their respective State Cancer Registries. Years of follow-up were rounded (i.e., 1 year = 6-18
months after initial ART); end of follow-up was 12/2010 for NY and 12/2012 for TX and IL. Standardized incidence ratios (SIR) and 95% confidence intervals (CI) were calculated.

RESULTS: The study included 53,872 women and 263,457 person-years of follow-up; 450 women were diagnosed with cancer; 10 were subsequently diagnosed with a second cancer. No significant excess cancer risk was observed with ART treatment overall, by age at cycle start, parity, cause of infertility, number of cycles, cumulative gonadotropin dose, or ART outcome. Relative to all cancers, the SIR for melanoma was elevated (with non-overlapping CI). There was a nonsignificant increase in cancer within two years after ART treatment, generally consistent regardless of ART outcome.

CONCLUSION: Although limited by short duration of follow-up, these results show no significant excess risk of cancer after ART, with the possible exception of melanoma.

Supported by: NIH R01 CA151973.

O-154 Tuesday, October 21, 2014 06:00 PM


OBJECTIVE: As malignancy survivorship has increased, fertility preservation (FP) has become a major concern for cancer patients of reproductive age. Although embryo cryopreservation has long been the standard of care, improved technology is moving OC into the mainstream FP arena. Thus, hoping to maximize OC efficacy, we assessed pre-treatment variables & OC cycle outcomes in females newly diagnosed with malignancy.

DESIGN: Retrospective, university-based.

Pre-treatment variables and OC cycle outcomes in cancer patients.

<table>
<thead>
<tr>
<th>Cancer type n=200</th>
<th>Subdiagnosis (n cycles)</th>
<th>Age (y)</th>
<th>Peak E2 (pg/mL)</th>
<th>No. oocytes retrieved</th>
<th>No. oocytes frozen</th>
<th>No. MII oocytes frozen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast (n=83 pts; 85 cycles)</td>
<td>Invasive (82), DCIS (3)</td>
<td>35±5</td>
<td>1457±1357</td>
<td>17±13</td>
<td>14±10</td>
<td>11±9</td>
</tr>
<tr>
<td>Gynecologic (n=57 pts; 59 cycles)</td>
<td>Ovarian (33; 27 cancer + 8 LMP), Cervical (17), Uterine (8), Vaginal (1)</td>
<td>29±6</td>
<td>2796±1809</td>
<td>17±11</td>
<td>14±9</td>
<td>12±8</td>
</tr>
<tr>
<td>Hematologic (n=35)</td>
<td>Hodgkins (24), Non-Hodgkins (6), Leukemia (5)</td>
<td>24±6</td>
<td>3164±1779</td>
<td>23±14</td>
<td>20±12</td>
<td>17±11</td>
</tr>
<tr>
<td>Other (n=25)</td>
<td>CNS (10), GI (6), GU (2), Thymic/ Carcinoid/Melanoma/Ewing’s (7)</td>
<td>29±7</td>
<td>2457±1555</td>
<td>20±10</td>
<td>14±9</td>
<td>13±9</td>
</tr>
</tbody>
</table>

MATERIALS AND METHODS: All cancer patients referred to our center for FP received counseling compliant with current ASRM guidelines. Treatment protocols were adjusted for cancer diagnoses.

RESULTS: 200 patients (mean age 31y) completed 204 OC cycles (4 pts had 2 treatments) from 2005-4/2014. Day 2 FSH & estradiol (E2) were normal. Mean time between request & consult was 2 days & consult & oocyte retrieval 13 days when emergent. Ovarian stimulation lasted 10±2 days. Avg peak stimulation E2 was 2000 pg/ml; a mean of 20 oocytes were retrieved [14 mature (MII) frozen]/cycle. 14 women also froze embryos. As it became the standard over time, 40 breast cancer cycles had letrozole co-treatment, resulting in a peak E2 of 1165 pg/ml & 11 MII frozen. 5 pts (6 cycles) returned to thaw oocytes & 7 (11 cycles) to use embryos, resulting in 3 & 4 live births, respectively.

CONCLUSION: This review demonstrates that OC is a practical FP option for women of childbearing age who require gonadotoxic therapies. Novel ART & tailored stimulation protocols have advanced the field of oncofertility & now allow such patients to routinely cryopreserve oocytes. Continued research & education, in addition to communication across disciplines, will allow appropriate candidates to be offered FP & the opportunity for family after cure, a noteworthy quality-of-life factor for cancer survivors. Thus, FP not only safeguards cancer patient’s reproductive rights, but also ensures that their dreams of motherhood are respected.

EARLY PREGNANCY I

O-155 Tuesday, October 21, 2014 04:15 PM

COST-EFFECTIVENESS ANALYSIS OF PREIMPLANTATION GENETIC SCREENING (PGS) AND IN VITRO FERTILIZATION (IVF) VERSUS EXPECTANT MANAGEMENT IN PATIENTS WITH UNEXPLAINED RECURRENT PREGNANCY LOSS (RPL). G. Murugappan, M. S. Ohno, R. B. Lathi. Department of Obstetrics & Gynecology, Stanford University Medical Center, Stanford, CA.
OBJECTIVE: Due to the high frequency of aneuploidy in first trimester miscarriages, IVF/PGS (PGS) are increasingly performed in RPL patients with limited clinical evidence. Our objective is to determine if PGS is cost-effective compared to EM in achieving live birth (LB) and preventing clinical miscarriage (CM) for patients with unexplained RPL.

DESIGN: A decision analysis of cost incurred and clinical outcomes with PGS versus EM.

MATERIALS AND METHODS: A decision-analytic model was constructed using TreeAge Pro 2013. Probabilities for IVF/PGS were derived from published data of outcomes of IVF with 24-chromosome PGS involving 2,282 embryos and 287 PGS cycles followed by fresh embryo transfer (ET) in patients with unexplained RPL.2 Probabilities for EM were obtained from a prospective longitudinal study of 325 patients with unexplained RPL who underwent clinical evaluation and attempted spontaneous conception with close follow-up.4 Cost data was obtained from literature and adjusted to 2014 US dollars.3,4,5,6 For both groups, the average cost of pre-conception counseling and baseline RPL workup including parental karyotyping, maternal anti-phospholipid antibody testing and uterine cavity evaluation was $4,577.3,4,5,6 No additional cost was incurred in the EM group. The average cost of IVF with one ET was $18,227 and the average cost of PGS was $4,268.3,4,5,6 Cost to achieve live birth and cost to prevent one CM were the primary outcomes of the analysis. In the first model, all patients underwent one PGS cycle and ET, if a euploid embryo was produced. In the second model, patients did a second cycle if they did not conceive with the first. Base case, threshold, and one-way sensitivity analyses and a Monte Carlo simulation were performed to assess the robustness of the model.

RESULTS: Cost effectiveness analysis favored EM over PGS. The CP rate per attempt is 35.6% with IVF/PGS compared to 69.5% with EM. Clinical outcomes, cost per LB, and cost to prevent one CM using one and two cycles of PGS compared to EM is shown in Table 1. The model was robust to one-way sensitivity analysis and Monte Carlo simulation.

CONCLUSION: PGS is a very costly way to reduce miscarriage without a significant improvement in LB rate compared to EM in a fertile RPL population. Cost effectiveness in an infertile RPL population is yet to be determined.

O-156 Tuesday, October 21, 2014 04:30 PM

RANDOMIZED CLINICAL TRIAL OF PRECONCEPTION LOW DOSE ASPIRIN USE AND PREGNANCY LOSS: RESULTS FROM THE EAGER TRIAL. S. L. Mumford, a L. L. Lesher, b M. V. White, c N. J. Perkins, d E. F. Schisterman, e R. M. Silver, b NIH, Rockville, MD; U. Utah, Salt Lake City, UT; The Commonwealth Medical College, Scranton, PA.

OBJECTIVE: Low dose aspirin (LDA) initiated post-conception is commonly prescribed to prevent pregnancy loss despite its proven efficacy. As preconception LDA may affect endometrial vascularization and placentation, post-conception LDA initiation may miss the critical window for intervention. Our objective was to evaluate the effect of LDA on pregnancy loss among women with 1-2 prior losses.

DESIGN: Multi-site prospective block-randomized double-blind placebo-controlled trial of preconception LDA (81 mg/day).

MATERIALS AND METHODS: Women aged 18-40 actively trying to conceive were stratified as: 1) restricted: women with 1 documented loss <20 weeks gestational age (GA) during the past year, or 2) general: women with 1-2 prior losses regardless of GA or time since loss. Randomization was stratified by site and restricted/general strata. Participants were treated/followed for up to 6 menstrual cycles or if they conceived, throughout pregnancy with treatment discontinued at 36 weeks GA. An intent-to-treat approach with sensitivity analysis for compliance was used to estimate effects.

RESULTS: 1228 women were randomized: 615 LDA and 613 placebo. 1078 (87.8%) women completed the trial and 792 women had a hCG detected pregnancy (64.4%). Overall there were 133 clinical losses (12.6% LDA vs. 11.8% placebo, p=0.7) and 56 chemical losses (5.4% LDA vs. 4.9% placebo, p=0.7). There were no differences observed by eligibility strata; specifically in the restricted strata (n=492), clinical pregnancy loss rates were 12.3% LDA vs. 11.1% placebo (p=0.7), and in the general strata (n=586), 12.8% LDA vs. 12.4% placebo (p=0.9).

CONCLUSION: Daily LDA initiated preconception was not associated with clinical or chemical pregnancy losses among women with a history of 1-2 prior losses.

Supported by: Intramural Research Program, Eunice Kennedy Shriver National Institute of Child Health and Human Development.

O-157 Tuesday, October 21, 2014 04:45 PM


OBJECTIVE: Previous studies recommend cytogenetic analysis of fresh POC at the time of a second pregnancy loss. When it is not performed or fails to yield a result, ASRM recommends a RPL workup (parental karyotypes, antiphospholipid antibodies (APA), TSH, Prolactin and uterine cavity evaluation). New SNP technology may determine karyotype from PPOC, with the added ability to exclude maternal cell contamination (MCC). This study investigated whether PPOC testing is a suitable alternative to the ASRM RPL workup for the initial evaluation of RPL without prior cytogenetic data.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: We reviewed all PPOCs sent for SNP at a large fertility center from 1/2012-12/2013. We included women with 2 or more first trimester losses who had the ASRM RPL workup and 1 or more PPOCs tested. We assessed the yield of PPOC testing for determining a cause of pregnancy loss compared to the ASRM RPL workup. We then compared women with at least 1 euploid loss to those who had aneuploid-only results, with respect to findings on RPL workup. Associations were analyzed using χ2 or Fisher’s Exact test. SNP was performed by Natera.

RESULTS: 41 women met inclusion criteria. SNP determined 13(32%) women had at least 1 euploid loss. 21(51%) had aneuploid-only results and 7(17%) women had no results (insufficient sample or MCC). RPL workup identified pathology in 7(17%) women (6 uterine abnormalities, 1 APA). SNP identified a cause for pregnancy loss in more women than the ASRM RPL workup. We then compared women with at least 1 euploid loss to those with aneuploid-only results, with respect to findings on RPL workup. Associations were analyzed using χ2 or Fisher’s Exact test. SNP was performed by Natera.

CONCLUSION: This study demonstrates that SNP may play a critical role in determining a cause for pregnancy loss. Furthermore, detecting a euploid loss identified a population at high risk for additional pathology. Aneuploid-only results eliminated the need for additional testing, except perhaps a cavity evaluation. We propose a step-wise evaluation with first-line SNP when PPOC are available, followed by a cavity evaluation for a euploid loss. If no pathology is found, further evaluation of hormonal or immunologic etiologies is warranted. This method would have avoided unnecessary invasive testing in nearly half the patients in this study.
ASSOCIATION OF ANTI-MULLERIAN HORMONE (AMH) AND LIVE BIRTH: RESULTS FROM THE EFFECTS OF ASPIRIN ON GESTATION AND REPRODUCTION (EAGER) TRIAL.

E. F. Schisterman, a S. M. Zarek, b E. M. Mitchell, a N. Galai, a J. M. Townsend, a S. L. Mumford, a NIH, Rockville, MD; aUniversity of Haifa, Haifa, Israel; bThe Commonwealth Medical College, Buffalo, NY.

OBJECTIVE: Although AMH has been studied as a predictor of live birth in assisted reproductive technology (ART), less is known about the association in women with normal fertility. The objective was to assess a possible association between preconception AMH levels with live births.

DESIGN: Secondary analysis of a multicenter, block-randomized, double-blind, placebo-controlled clinical trial.

MATERIALS AND METHODS: 1228 women attempting pregnancy, aged 18–40 years, with one to two prior pregnancy losses and no history of infertility, pelvic inflammatory disease, tubal occlusion, endometriosis, anovulation, uterine abnormality, or polycystic ovarian syndrome were included. Women were block-randomized by center and eligibility stratum in a 1:1 ratio to preconception-initiated daily low-dose aspirin or placebo. The primary outcome was live birth (defined as a living infant born after 23 weeks’ gestation). AMH was assayed using the Gen II ELISA assay (Beckman-Coulter) at the baseline visit before randomization. AMH was clinically categorized and verified by data analysis into low (<1.25 ng/mL), normal (1.25 to 4.0 ng/mL), and high (>4.0 ng/mL). The Mann Whitney test was utilized to evaluate differences in AMH levels by live birth status. Relative Risk (RR) and 95% confidence intervals (CIs) for live birth by AMH categories were estimated using generalized linear models adjusted for age.

RESULTS: Women were followed for up to six menstrual cycles with N=776 (72%) achieving pregnancy and N=582 (55%) with a live birth. Women were predominately of white race (95.6%) with a mean age of 28.9 years (standard deviation (SD) 4.7) and body mass index (BMI) of 26.1 (SD 6.5). Among women with a single recent loss, the live birth rate was 53.6% among women with low AMH, 53.9% among normal AMH, and 65.9% among high AMH (p=0.04). After adjusting for age, women with a single recent loss and high (>4.0 ng/mL) AMH levels had an increased risk of live birth (RR=1.2, 95% CI 1.1, 1.4) compared to women with normal levels, whereas women with low AMH (<1.25) were not significantly different from women with normal AMH levels (RR=. 1.04, 95% CI 0.81, 1.3).

CONCLUSION: High AMH was associated with live birth in women with normal fertility. These findings are consistent with previous studies in the ART population, confirming that AMH is associated with live birth in women with normal and subfertility.

Supported by: Intramural Research Program, DIPHR, PRAE.

CE defined as positive CD138 staining on IHC.

RESULTS: The use of CD138 staining to identify women with CE resulted in a significantly higher prevalence of CE compared to the use of H&E staining and morphology alone, 56% (60/107) vs 13% (14/107) (p <0.01). 11 women received antibiotic treatment for CE were excluded, leaving 51 women with untreated CE and 45 women without CE for outcome analysis. At least one subsequent pregnancy was documented in 71% (36/51) of women with untreated CE and 73% (33/45) of women without CE (NS). 6 pregnancies were lost to follow up in each group. The miscarriage rate in the next clinical intrauterine pregnancy was 36.7% (11/30) in women with untreated CE vs 14.8% (4/27) in women without CE (NS). Age, BMI, results of RPL evaluation and number of prior losses were not significantly different.

CONCLUSION: There was a high prevalence of CE when it was defined as the presence of CD138 positive plasma cells. CD138 may provide increased sensitivity when screening for CE compared to H&E staining and morphologic evaluation alone. Although we did not see a statistically significant difference in miscarriage rates between women with untreated CE and women without CE, this subject deserves further study with a larger study sample.

O-159 Tuesday, October 21, 2014 05:15 PM
MISCARRIAGE RATES IN WOMEN WITH RECURRENT PREGNANCY LOSS AND UNTREATED CHRONIC ENDOMETRITIS. D. McQueen, a C. Perfetto, b F. Hazard, b R. Lathi, b aObstetrics and Gynecology, University of Chicago, Chicago, IL; bObstetrics and Gynecology, Stanford University, Stanford, CA.

OBJECTIVE: To evaluate the prevalence of chronic endometritis (CE) and subsequent pregnancy outcomes in women with recurrent pregnancy loss (RPL).

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: 107 women with 2 or more pregnancy losses received an endometrial biopsy as part of their work up for RPL. Hematoxylin and eosin (H&E) staining was performed and treatment decisions were made based on the identification of plasma cells by morphology alone. Immunohistochemical staining was later applied to archived paraffin samples in order to detect CD138 (syndecan-1), an antibody marker of plasma cells. A single blinded pathologist reviewed all slides. CE was defined as the presence of at least 1 plasma cell per high power field by CD138 staining. Charts were reviewed to evaluate the outcome of the next clinical intrauterine pregnancy.

RESULTS: The use of CD138 staining to identify women with CE resulted in a significantly higher prevalence of CE compared to the use of H&E staining and morphology alone, 56% (60/107) vs 13% (14/107) (p <0.01). 11 women received antibiotic treatment for CE were excluded, leaving 51 women with untreated CE and 45 women without CE for outcome analysis. At least one subsequent pregnancy was documented in 71% (36/51) of women with untreated CE and 73% (33/45) of women without CE (NS). 6 pregnancies were lost to follow up in each group. The miscarriage rate in the next clinical intrauterine pregnancy was 36.7% (11/30) in women with untreated CE vs 14.8% (4/27) in women without CE (NS). Age, BMI, results of RPL evaluation and number of prior losses were not significantly different.

CONCLUSION: There was a high prevalence of CE when it was defined as the presence of CD138 positive plasma cells. CD138 may provide increased sensitivity when screening for CE compared to H&E staining and morphologic evaluation alone. Although we did not see a statistically significant difference in miscarriage rates between women with untreated CE and women without CE, this subject deserves further study with a larger study sample.

O-160 Tuesday, October 21, 2014 05:30 PM
TROPHOBLAST RETRIEVAL AND ISOLATION FROM THE CERVIX (TRIC) TO PREDICT RISK FOR SPONTANEOUS ABORTION. R. Fritz, a J. Bolnick, a A. Bolnick, a M. Modi, a B. Kilburn, a M. P. Diamond, a D. R. Arman, a aWayne State University, Detroit, MI; bGeorgia Regents University, Augusta, GA; cNational Institute of Child Health & Human Development, Bethesda, MD.

OBJECTIVE: Spontaneous abortion (spAb) is one of the most common pregnancy complications and is associated with abnormal trophoblast invasion. Our objective was to evaluate whether abnormal protein expression occurs in first trimester extravillous trophoblast cells isolated from
the cervix of pregnant patients with spAb, as compared to normal pregnancies.

DESIGN: Case Control Study.

MATERIALS AND METHODS: Trophoblast retrieval and isolation from the cervix (TRIC) was performed with PAP specimens collected in the first trimester from 8 patients with known spAb's and 8 patients with uncomplicated term pregnancies, isolating trophoblast cells with anti-human leukocyte antigen (HLA)-G antibody coupled to magnetic nanoparticles. Purity of the isolated trophoblast cells was assessed by staining the β subunit of human chorionic gonadotropin. Fifty trophoblast cells from each specimen were examined by semi-quantitative immunofluorescence for cytoketatin 7, placental protein 14 (PP14), PP13, placental growth factor, alpha-fetoprotein (AFP), endoglin (ENG), pregnancy associated plasma protein A, and vascular endothelial growth factor receptor (FLT-1). Images were captured and fluorescence intensity was measured utilizing Simple PCI image analysis software on at least 10 isolated trophoblast cells from each specimen. Statistical analysis was performed with an independent t test using SPSS 21.0.

RESULTS: In the control and spAb groups, respectively, the average trophoblast purity was 99.1% and 94.8%, trophoblast recovery was 678.8 and 267.75, and average gestational age was 8.1 weeks and 7.3 weeks. Significant elevations were found in ENG, FLT-1, and AFP, as well as a significant decrease in PP14 in spAb vs. control patients.

CONCLUSION: During the first trimester, abnormal protein expression is observed in trophoblast cells obtained by TRIC from spAb patients. TRIC could provide a platform for early diagnosis of patients at risk of spAb, which could permit therapeutic interventions.

Supported by: NIH (R21HD071408), W.K. Kellogg Foundation, Women's Reproductive Health Research Program (K12HD001254), and the Division of Intramural Research of NICHD.

O-161 Tuesday, October 21, 2014 05:45 PM

IDENTIFICATION OF EARLY PLACENTAL STEROIDOGENESIS. R. Setton, B. A. Levine, S. Karipcin, Z. Rosenwaks, S. Spandorfer. The Ronald O. Perelman & Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medical College, New York, NY; Department of Obstetrics & Gynecology, Weill Cornell Medical College, New York, NY.

OBJECTIVE: There is a dearth of literature describing the onset of early placental steroidogensis. In this study we sought to identify the initiation of placental hormonal production as defined by the production of estradiol (E2) and progesterone (P4).

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: All oocyte donation charts from 1/2004 to 4/2013 were reviewed to identify patients who had undergone a programmed recipient cycle at our institution and had a resultant live born singleton. All patients were treated with E2 patch (changed every other day) and P4 50mg daily. Main outcome measures were serial E2 and P4 with mean values calculated for cycle days 28 (baseline) through 70. The baseline mean value was compared to each daily mean cycle day value using an unpaired t-test.

RESULTS: A total of 1,650 cycles met inclusion criteria. Serum E2 and P4 levels remained low but continued to increase daily. Compared to baseline E2 (478.7 pg/mL), on cycle-day 35, the serum E2 was significantly elevated 574.3 pg/mL (P<0.0022). With respect to baseline P4 (31.1 ng/mL), on cycle-day 42, the serum P4 was significantly elevated 35.6 ng/mL (P<0.0001).

CONCLUSION: These results demonstrate that endogenous placental estradiol and progesterone production occur by cycle day 35 and 42 respectively. This also shows that the luteo-placental shift occurs earlier than previously reported. Taken together, these results suggest that modifications to luteal support paradigms need to be considered given that most programmed oocyte recipient cycles are steroidogenic by the sixth week of gestation.

O-162 Tuesday, October 21, 2014 06:00 PM


OBJECTIVE: To evaluate if local endometrial injury (endometrial scratch) could improve the rates of clinical pregnancy and ongoing pregnancy after frozen-thawed blastocyst transfer in hormone replacement cycles.

DESIGN: Prospective randomized controlled trial.

MATERIALS AND METHODS: A total of 77 patients, less than 40 years of age, who had a history of at least two unsuccessful embryo transfers and received blastocyst transfer during hormone replacement cycle between February and June 2013, were enrolled in this study. The patients were allocated into two groups according to the last digit of their clinical record number: an experimental group (n=22) that excluded patients who disagreed or couldn’t undergo in the appropriate period, and a control group (n=55). In the experimental group, the endometrial scratch was performed once with an Endocyte® endometrial sampler during the luteal phase of the cycle preceding the blastocyst transfer. The primary outcome measure was the clinical pregnancy rate. The secondary outcome measure was the ongoing pregnancy rate. All the patients in the experimental group provided written informed consent. The study procedures were approved by the Institutional Review Board of the Hanabusa Women’s Clinic.

RESULTS: No significant differences were found between the groups in terms of age or previous number of failed embryo transfers. The clinical pregnancy rate in the experimental group was 45.5%, while that of the control group was 21.8% (p<0.05). The ongoing pregnancy rate was 100% in the experimental group, while that in the control group was 50.0% (p<0.05).

CONCLUSION: To the best of our knowledge, this is the first report on the effect of endometrial local injury (endometrial scratch) on frozen-thawed blastocyst transfer in hormone replacement cycles.

In this study, the clinical and ongoing pregnancy rates in the experimental group were significantly higher than in the control group. In recent studies, the mechanical manipulation of the endometrium in the cycle preceding ovarian stimulation for in vitro fertilization and embryo transfer was shown to improve implantation rates in patients with unexplained recurrent implantation failure. We consider that, even for frozen-thawed blastocyst transfer, endometrial injury may increase pregnancy rates, and decrease the risk of miscarriage in patients less than 40 years of age with a history of at least two unsuccessful embryo transfers.

<table>
<thead>
<tr>
<th>Cycle Day</th>
<th>Estradiol (pg/mL)</th>
<th>P-value</th>
<th>Progesterone (ng/mL)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>28</td>
<td>478.7</td>
<td>-</td>
<td>31.1</td>
<td>-</td>
</tr>
<tr>
<td>30</td>
<td>533.8</td>
<td>N.S.</td>
<td>33.2</td>
<td>N.S.</td>
</tr>
<tr>
<td>32</td>
<td>529.2</td>
<td>N.S.</td>
<td>32.7</td>
<td>N.S.</td>
</tr>
<tr>
<td>34</td>
<td>530.3</td>
<td>N.S.</td>
<td>34.0</td>
<td>N.S.</td>
</tr>
<tr>
<td>35</td>
<td>574.2</td>
<td>P&lt;0.0022</td>
<td>33.3</td>
<td>N.S.</td>
</tr>
<tr>
<td>42</td>
<td>746.8</td>
<td>P&lt;0.0001</td>
<td>35.6</td>
<td>P&lt;0.0001</td>
</tr>
</tbody>
</table>

N.S.- not statistically significant
SHOULD WE PERSIST WITH OVULATION INDUCTION AND INSEMINATION IN WOMEN WITH MALE FACTOR INFERTILITY? 
C. Chatzichanalampous, D. Patel, N. Virji, J. R. Stelling, M. A. Bray, Obstetrics and Gynecology, The Brooklyn Hospital Center, Brooklyn, NY; 
Reproductive Specialists of New York, Mineola, NY; Obstetrics and Gynecology, Stony Brook University School of Medicine, Stony Brook, NY.

OBJECTIVE: The FORT-T trial (1, 2) has demonstrated that moving quickly to IVF in women over forty leads to shorter time to pregnancy. Similar results were found on the FAST-T trial for younger women (3). However, for a variety of reasons (financial, geographic or personal) not all women have access to IVF. We sought to determine if persistence with Ovulation Induction and Intrauterine Insemination (OI/IUI) beyond three cycles was other than an exercise in futility in women diagnosed with or without male factor infertility.


MATERIALS AND METHODS: Women undergoing IUI/OI were grouped by age category (20-37 and 38-42) and by ovulation induction cycle group (1-3 and 4-6). The primary outcome was clinical pregnancy rate (CPR) which required the presence of a gestational sac on sonogram. For statistical analysis we used Chi-square, Fisher’s Exact test and Life-Table Analysis. A P-value <0.05 was considered statistically significant.

RESULTS: For women without male factor infertility the cumulative CPR was, as expected, higher in young vs. older women (p<0.0001) but not significantly different between cycle groups (1-3 vs. 4-6), regardless of age. For women with male-factor only infertility, older women had a significantly lower cumulative CPR in the first three OI/IUI cycles. However, in cycles 4-6, the older women attain equal success as the younger ones.

Clinical Pregnancy Rates by Age Group and Male-Factor Status

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Cycles</th>
<th>Male Factor CPR(%)</th>
<th>Non-male Factor CPR(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-37</td>
<td>1-3</td>
<td>9.5</td>
<td>19.4</td>
</tr>
<tr>
<td></td>
<td>4-6</td>
<td>8.1</td>
<td>17.2</td>
</tr>
<tr>
<td>38-42</td>
<td>1-3</td>
<td>4.5*</td>
<td>12.3i</td>
</tr>
<tr>
<td></td>
<td>4-6</td>
<td>8.3</td>
<td>9.4</td>
</tr>
</tbody>
</table>

CPR: Clinical Pregnancy Rate. * Statistically significant (p<0.05). iStatistically significant (p<0.001)

CONCLUSION: Cumulative success rates with OI/IUI are lower in all cases of male-factor only infertility. In an ideal world, IVF would be the starting point for these couples. However, the equivalent success rates of older and younger women with male factor infertility in cycles 4-6 suggests that persistence with IUI is not an exercise in futility in women with limited or no access to IVF. Future study is needed to determine if the improvement in cycles 4-6 is secondary to more aggressive ovulation induction.

O-164 Tuesday, October 21, 2014 04:30 PM
EFFECTIVENESS OF VIDEO-BASED TEACHING IN THE USE OF INJECTABLE FERTILITY MEDICATIONS: A RANDOMIZED CONTROLLED PILOT STUDY. 
A. B. Patel, M. Matthews. Obstetrics & Gynecology, Carolinas Medical Center, Charlotte, NC.

OBJECTIVE: To determine if video-based teaching is effective for patient education of injectable infertility medications.

DESIGN: Prospective randomized controlled pilot study.

MATERIALS AND METHODS: English-speaking infertility patients requiring Gonal-F injections who presented to Carolinas Medical Center Women’s Institute were included. Patients with prior experience using injectable medications and medical personnel were excluded. Study participants were randomized to a video-based teaching modality (n=24) or a standard one on one didactic session with a pharmacist experienced in injection teaching (n=20). After the teaching session, participants were asked to complete a post-test to assess their knowledge regarding Gonal-F injections. On the third day after starting home injections, participants completed a follow up survey to evaluate their satisfaction with their teaching modality. The primary outcome assessed was performance on the post-test. Secondary outcomes were time required for teaching and patient preference of teaching method.

A chi-square or Fisher’s exact test with a 95% confidence interval was used to analyze categorical data. For interval data, the t-test, was employed. If interval data was not normally distributed or the data was ordinal, the Wilcoxon rank sum test was employed. SAS Enterprise Guide, version 5.1, was used for statistical analysis. A two-tailed p-value of less than 0.05 was considered statistically significant.

RESULTS: Video-based learners scored significantly higher on the post-test compared with those randomized to a standard didactic session (92% correct compared with 82%, P=0.0277). On average, video-based teaching requiring less time than a one-on-one didactic session (6.9 minutes compared with 10.1 minutes, P=0.0056). Despite their performance on the post-test, 72% of video-based learners would prefer a one-on-one didactic teaching, 85% of video-based learners would prefer additional teaching compared to 25% of those randomized to a didactic teaching session.

CONCLUSION: Although patients preferred one-on-one injection training, video-based teaching was more effective in educating patients on appropriate injectable administration and a more time efficient teaching modality.

O-165 Tuesday, October 21, 2014 04:45 PM
IN VITRO FERTILIZATION (IVF) PATIENTS ONLINE BEHAVIORS: HOW DO THEY BROWSE THE WEB ABOUT INFERTILITY ISSUES AND WHAT ARE THEY LOOKING FOR ONLINE? 
A. Futer,a L. Dugay,b M. Stafford Bell,c J. Greindl,c L.-J. Kadoch,a Ajay,a S. Patel,b G. Baccino,b R. Nunezc, S. L. Tan, Medical Safari, Phillip, ACT, Australia; Fertility North, Joondalup, Western Australia, Australia; Canberra Fertility Centre, Canberra, Australia; PM'A Chirec, Hôpital de Braine-l'Alleud, Braine-l’Alleud, Wallonia, Belgium; Centre PMA, Centre Hospitalier de l’Université de Montréal, Montréal, QC, Canada; Nordica Fertility Centre, Ikyoi, Lagos, Nigeria; Vita Fertility Clinic, Mumbai, Maharashtra, India; IVF Madrid, Madrid, Spain; Clinica Tambre, Madrid, Spain; Originelle, Montréal, QC, Canada.

OBJECTIVE: International study on patients online behaviors to better understand and match their quest for information about infertility.

DESIGN: Specific survey including 24 questions assessing participants online behavior. Significance was set at P<0.05.

MATERIALS AND METHODS: Retrospective, non-randomized, cross-sectional study stratified across 9 centres in 6 countries from October 2013 till April 2014. 744 consecutive participants answered the survey.

RESULTS: 72% of participants searched online about infertility. 63% of respondents browsed for 1 hour or more. Overall, 37% used mobile devices with significant difference between Australia (51%) and India (20%). The main source of online information came from Google (59%). Content was the most important factor while browsing (66%). Overall, when looking for information about infertility, description about treatments and emotional/psychological aspects were the leading 2 topics searched, 28 and 25%, respectively. Participants across the 6 countries consistently expressed interest for additional information about complementary treatments (35%) and emotional aspects/psychological advices during treatment (17%). The 2 most appreciated illustrations for infertility were medical pictures (24%) and pictures of babies (21%).

CONCLUSION: Results showed relatively consistent online behaviors across countries. Majority of respondents spent time looking for information. Infertility being a topic not necessarily familiar to many, 63% of participants were apparently willing to dedicate time getting more familiarized with it. Among the most common subjects searched about infertility, emotional and psychological aspects were quite significant. This could indicate that many patients look for alternative approaches to cope with the emotional burden often associated with IVF. Two factors should be influential while developing patient communication: the need for valuable content and the mobile responsiveness of information.

O-166 Tuesday, October 21, 2014 05:00 PM
MANY COUPLES STILL DECLINE ESET DESPITE FINANCIAL INCENTIVES: AN UPDATE. 
F. L. Sharara,a,b Virginia Center for Reproductive Medicine, Reston, VA; OB/Gyn, George Washington University, Washington, DC.

OBJECTIVE: Multiple pregnancies remain the most common cause of maternal, fetal, and perinatal morbidities. Strategies to increase eSET use
in the US have been frustrating especially in states with no ART mandates, and patient resistance remains a major barrier to significant implementation. We sought to increase eSET adoption by offering significant financial incentives to offset some of the costs associated with ART.

**DESIGN:** Prospective, pilot study.

**MATERIALS AND METHODS:** All women < 38 yo proceeding with ART were consented for participation in a novel program to incentivize them to undergo eSET, including couples with prior failed cycles, women with uterine fibroids or prior uterine surgery, and women with diminished ovarian reserve. Only couples with very severe male factor (testicular sperm or counts < 1 million/cc) were excluded. To date, 68 women (< 38 yo) have participated in the study. All couples had an extensive consultation stressing the perinatal, neonatal, and maternal morbidities associated with twin gestation before starting ART. Couples were provided with free gonadotropins (Menopur), free embryo freezing of all extra blastocysts, and free storage for the first year if they agreed to have an eSET.

**RESULTS:** Between October 2012 and April 2014, 68 couples were consented for their participation in this novel program. Of the 68 couples, 41 (60.3%) agreed to have an eSET after counseling. 27 couples (39.7%) declined to participate despite an extensive review of the increased morbidities associated with twins. Of the 68 couples, 59 cycles were completed to date, and the clinical PR was 72.9% (43/59), and ongoing/delivery PR were 55.9% (33/59). The clinical and ongoing/delivered PR in the eSET group were 72.3% (27/37) and 56.8% (21/37), compared to 72.7% (16/22) and 54.5% (12/22) for the DET group (P = NS). The incidence of twin gestation in the DET group was 30% (6/20). Couples in the DET group were counseled again at the time of ET, and three couples underwent eSET. One case of dichorionic-diamniotic twin gestation was encountered to date in the eSET group.

**CONCLUSION:** Despite significant financial incentives and high ongoing PR, nearly 40% of couples eligible declined to have an eSET, not out of fear of lower success rate but because they want twins. By using this innovative trial and extensive counseling before ART start, and again at the time of ET, we were able to increase our eSET rate to over 60%. Short of mandating trial and extensive counseling before ART start, and again at the time of ET, we were able to increase our eSET rate to over 60%. Short of mandating eSET, it seems many couples in the US will remain reluctant to have an eSET despite an excellent pregnancy rate.

Supported by: Ferring.

O-167 Tuesday, October 21, 2014 05:15 PM

**INFERTILITY PATIENTS’ MOTIVATIONS FOR AND EXPERIENCES OF CROSS BORDER REPRODUCTIVE SERVICES (CBRS): A PARTIAL TRANS-THEORETICAL MODEL.** L. Lui, E. Blyth, K. Chiroma. School of Human and Health Sciences, University of Huddersfield, Huddersfield, West Yorkshire, United Kingdom.

**OBJECTIVE:** Cross border reproductive services (CBRS) is a growing phenomenon. CBRS is the travel by infertile patients from one country or jurisdiction where access to treatment is limited or unavailable to another country or jurisdiction to seek infertility services. Both the American Society for Reproductive Medicine (ASRM, 2013) and European Society of Human Reproduction and Embryology (Pennings et al., 2008) have provided guidance and recommendations for CBRS. There are numerous reasons for CBRS (Pennings et al., 2008) and CBRS is an under-researched and under-theorised area (Inhorn and Gurtin, 2011) of health research. This study provided themes on the decision making process of CBRS patients and contextualised them within a partial trans-theoretical model (Prochaska and DiClemente, 1983).

**DESIGN:** Data were collected via asynchronous email interviews.

**MATERIALS AND METHODS:** Data regarding CBRS were collected from 26 participants by means of asynchronous email in-depth semi-structured interviews via Infertility Networks between April and November, 2010. SPSS v20 was used to analyse the quantitative descriptive data whereas NVivo 10 software aided the systematic thematic coding method within a Straussian Interpretative Grounded Theory methodological perspective.

**RESULTS:** Participants’ motivations for and experiences of CBRS are complex. Seven stages emerged to describe patients’ CBRS journey. 1. Pre-contemplation: participants had no awareness of their own infertility; 2. Contemplation: participants became aware of their infertility and facilities at home and of the possibility of CBRS; 3. Preparation: participants actively researched information about CBRS using internet/infertility networks; 4. Action: participants took specific steps to initiate CBRS; 5. Maintenance: participants’ expectations and experiences had an important impact on whether or not they would continue on their CBRS journey; 6. Exit: Some participants successfully built their family. Others’ overall experience was negative, their expectations were not met and they decided to quit treatment; 7. Relapse: some participants re-engaged with infertility treatment to build their family; some participants reconsidering their decision regarding infertility treatment either at home or using CBRS again.

**CONCLUSION:** Participants have diverse motivations for and experiences of CBRS. A partial trans-theoretical model could explain some of the decision making process in seeking CBRS.

O-168 Tuesday, October 21, 2014 05:30 PM


**OBJECTIVE:** Single Embryo Transfer (SET) after Comprehensive Chromosome Screening (CCS) assists in multiple gestation risk reduction. Development of a clinical program.

**DESIGN:** Retrospective.

**MATERIALS AND METHODS:** A private IVF clinic developed a program to reduce multiple gestation risk. After program leaders committed to the concept, a consistent message, educational materials, a lecture series, and clinical and laboratory protocols were developed encouraging SET after CCS. Frozen embryo transfers from 2012 and 2013 were analyzed. Embryo transfer number was at physician and patient’s discretion. Primary group was all FETs, CCS, and non-CCS. Secondary was age groups <35, 35-37, 38-40, 41-42, >42, and OD (Ovum Donation). Comparison of transfers per year assessed patient acceptance.

**RESULTS:** 1034 FETs were performed, CCS=345, non-CCS=689. Pregnancy rate was all FET=56%, CCS=64%, non-CCS=52%. Average number of embryos transferred was all FET=1.27, CCS=1.1, non-CCS=1.35. Multiple gestation risk was all FET=17.6%, CCS=11.8%, non-CCS=21.1%. From 2012 to 2013, all FETs increased 17%, CCS transfers increased 260%, non-CCS transfers decreased 19%. Multiple gestation risk declined in most groups.

**FET Results**

<table>
<thead>
<tr>
<th>All FETs</th>
<th>&lt;35</th>
<th>35-37</th>
<th>38-40</th>
<th>41-42</th>
<th>&gt;42</th>
<th>OD</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transfers</td>
<td>280</td>
<td>153</td>
<td>151</td>
<td>40</td>
<td>12</td>
<td>398</td>
<td>1034</td>
</tr>
<tr>
<td>CPR all FET</td>
<td>53.6%</td>
<td>64.7%</td>
<td>66.9%</td>
<td>55.0%</td>
<td>41.7%</td>
<td>51.3%</td>
<td>56.2%</td>
</tr>
<tr>
<td>IR all FET</td>
<td>51.6%</td>
<td>63.6%</td>
<td>61.4%</td>
<td>43.1%</td>
<td>31.3%</td>
<td>44.9%</td>
<td>51.6%</td>
</tr>
<tr>
<td>#ET all FET</td>
<td>1.22</td>
<td>1.20</td>
<td>1.37</td>
<td>1.28</td>
<td>1.33</td>
<td>1.29</td>
<td>1.27</td>
</tr>
<tr>
<td>Multiple Rate</td>
<td>19.3%</td>
<td>16.2%</td>
<td>22.8%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>16.7%</td>
<td>17.6%</td>
</tr>
<tr>
<td>CCS Transfers</td>
<td>90</td>
<td>72</td>
<td>77</td>
<td>23</td>
<td>10</td>
<td>73</td>
<td>345</td>
</tr>
<tr>
<td>CPR CCS</td>
<td>51.1%</td>
<td>69.4%</td>
<td>74.0%</td>
<td>65.2%</td>
<td>50.0%</td>
<td>65.8%</td>
<td>64.1%</td>
</tr>
<tr>
<td>IR CCS</td>
<td>52.0%</td>
<td>73.3%</td>
<td>72.9%</td>
<td>65.2%</td>
<td>50.0%</td>
<td>65.5%</td>
<td>64.6%</td>
</tr>
<tr>
<td>#ET CCS</td>
<td>1.13</td>
<td>1.04</td>
<td>1.10</td>
<td>1.00</td>
<td>1.00</td>
<td>1.15</td>
<td>1.10</td>
</tr>
<tr>
<td>Multiple Rate</td>
<td>17.4%</td>
<td>10.0%</td>
<td>8.8%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>16.7%</td>
<td>11.8%</td>
</tr>
<tr>
<td>non-CCS Transfers</td>
<td>190</td>
<td>81</td>
<td>74</td>
<td>17</td>
<td>2</td>
<td>325</td>
<td>689</td>
</tr>
<tr>
<td>CPR non-CCS</td>
<td>54.7%</td>
<td>60.5%</td>
<td>59.5%</td>
<td>41.2%</td>
<td>0.0%</td>
<td>48.0%</td>
<td>52.2%</td>
</tr>
<tr>
<td>IR non-CCS</td>
<td>51.5%</td>
<td>56.9%</td>
<td>53.3%</td>
<td>25.0%</td>
<td>0.0%</td>
<td>40.9%</td>
<td>46.4%</td>
</tr>
<tr>
<td>#ET non-CCS</td>
<td>1.26</td>
<td>1.35</td>
<td>1.65</td>
<td>1.65</td>
<td>3.00</td>
<td>1.32</td>
<td>1.35</td>
</tr>
<tr>
<td>Multiple Rate</td>
<td>20.2%</td>
<td>22.4%</td>
<td>40.9%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>16.7%</td>
<td>21.1%</td>
</tr>
</tbody>
</table>

**CONCLUSION:** A program in SET requires a program commitment, a consistent message, patient and staff education, and optimized clinical and laboratory services. Patients accept this program in increasing numbers. Pregnancy rate was improved after CCS in all ages over 35. Fewer embryos were transferred, and multiple gestation risk was reduced, after CCS in all ages. In women over 35, CCS improved the implantation
rate. In women under 35, CCS did not change implantation or pregnancy rates, but there was a trend to fewer embryos transferred after CCS. Patient acceptance, with appropriate education, is accelerating. CCS PFT increased over the two-year period.

O-169 Tuesday, October 21, 2014 05:45 PM

IMPROVING ICSI OUTCOMES BY ADDING MYO-INOSITOL TO THE SEMEN PREPARATION PROCEDURES: A PROSPECTIVE, RANDOMIZED SINGE BLIND TRIAL ON SIBLING-OOCYTE. P. Rubino, a G. Carlomagno, b S. Chigioni, a G. Galofre Balles-ton, a A. Quaghiariello, a A. G. Baglioni, a Tecnolab s.r.l., Milano, MI, Italy; 1R&D, Lo.Li. Pharma, Rome, RM, Italy.

OBJECTIVE: The aim of the study was to evaluate whether myo-inositol (MI) treatment during semen preparation procedures is able to improve fertilization rate (FR) and embryo quality (EQ) after ICSI.

DESIGN: The study was designed as a prospective randomized single blind controlled trial on sibling-oocyte and was performed from March to December 2013 (NCT02050672). Before recruiting, the sample size was calculated via a power analysis and the FR was designed as primary outcome. In particular, based on a difference of 15% on the FR, and power of 90% and confidence of 95%, a minimum of 210 oocytes per group were required.

MATERIALS AND METHODS: Inclusion criteria: 1) couple counseled for ICSI due to male factor; 2) fresh cycles 3) men with ejaculated spermatozoa; 3) women age of >35 yrs; 4) ≥ 2 MI oocyte retrieved. At ovum pick up, oocytes were randomly divided in two groups. Mature oocytes of one group (MI) were injected with spermatozoa prepared with routinely used media enriched with 2mg/ml of MI (Androstiol1LAB Lo.Li. Pharma), mature oocytes of the other group (CTR) were injected with spermatozoa prepared with routinely used media. Secondary outcome was the EQ. A blinded embryologist performed ICSI procedure and embryo evaluation.

RESULTS: In summary, FR cleavage rate (CR) and EQ were significantly higher in MI group. Furthermore, significantly more embryos of the MI group were transferred at day 3; noteworthy, the blind researcher has chosen to transfer only embryos belonging to the MI group with a significant higher frequency.

<table>
<thead>
<tr>
<th>ICSI Outcomes</th>
<th>MI</th>
<th>CTR</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oocyte injected</td>
<td>262</td>
<td>238</td>
<td></td>
</tr>
<tr>
<td>Oocyte Fertilized</td>
<td>205</td>
<td>146</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FR</td>
<td>78%</td>
<td>61%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>N Emb D2</td>
<td>205</td>
<td>120</td>
<td>0.0292</td>
</tr>
<tr>
<td>CR</td>
<td>100%</td>
<td>97%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Grade 1 Emb(%)</td>
<td>117(57%)</td>
<td>62(44%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Grade 2 Emb(%)</td>
<td>60(29%)</td>
<td>59(42%)</td>
<td>0.0221</td>
</tr>
<tr>
<td>Grade 3 Emb(%)</td>
<td>19(9%)</td>
<td>40(28%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ETD3(%)</td>
<td>71(35%)</td>
<td>34(23%)</td>
<td>0.0047</td>
</tr>
<tr>
<td>N BL</td>
<td>53</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>BL rate</td>
<td>40%</td>
<td>38%</td>
<td></td>
</tr>
<tr>
<td>TOT ET</td>
<td>87</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>PURE ET</td>
<td>46</td>
<td>18</td>
<td></td>
</tr>
</tbody>
</table>

CONCLUSION: It is known that the FR and the EQ directly depend on the mitochondrial membrane potential (MMP) of the sperm cells that fertilize the oocyte[1]. Having this in mind the drop of the MMP caused by the semen preparation procedures has to be considered as one of the main issue that need to be addressed in order to improve ART efficiency[2]. In previously published paper, it has been reported that MI is able to increase the Sperm MMP in both normospermic and OAT patients[3, 4]. According to our findings, MI treatment by increasing the sperm MMP, increases the FR and EQ allowing the ART to likely perform a step towards the increase of the implantation rate.

Supported by: Lo.Li. Pharma provided the product for the study free of charge.

O-170 Tuesday, October 21, 2014 06:00 PM


OBJECTIVE: Currently there is a lack of published studies (Verberg et al, 2008) evaluating treatment discontinuation following the use of mild IVF approaches. We aimed to calculate overall and age-specific drop-out rates in a large cohort of mild IVF treatment cycles from our center.

DESIGN: A single-center retrospective review performed between November 2008 and December 2011.

MATERIALS AND METHODS: Natural cycle IVF or clomiphene-based minimal ovarian stimulation was coupled with a universal single embryo transfer (SET) policy and increased use of delayed frozen-thawed blastocyst transfer. Per cycle and cumulative drop-out rates (after 9 cycles) were calculated for the entire cohort and according to six female age categories (26-34, 35-37, 38-40, 41-42, 43-44, 45-52 years).

RESULTS: 727 consecutive infertile patients (mean age 38.4±4.5 range: 26-52 years) underwent 2876 treatment cycles (median: 4, range: 1-26). Cumulative live birth rates (conservative estimate) were 65, 60, 39, 15 and 5% in 26-34, 35-37, 38-40, 41-42 and 43-44 years old patients, respectively. No live births occurred in ≥ 45 year old patients. For the entire cohort per cycle drop-out rates varied between 13-25% whereas 56% of the initial cohort discontinued treatment after 9 cycles. Age-specific cumulative drop-out rates gradually increased with advancing female age and were 35, 40, 57, 73, 82 and 83% for 26-34, 35-37, 38-40, 41-42, 43-44 and 45-52 years old patients, respectively.

CONCLUSION: Per cycle drop-out rate was considerably lower than published discontinuation rates following conventional IVF treatment (usually above 50%). This suggests decreased physical/ psychological burden and improved patient tolerance which might play an important role in compensating for the lower per cycle success rate associated with mild IVF protocols. Despite the lack of active censoring from the medical staff cumulative drop-out rates were steadily increasing with advancing female age.

ART OUTCOME PREDICTORS - CLINICAL II

O-171 Tuesday, October 21, 2014 04:15 PM

DON'T WAIT TO FREEZE YOUR EGGS: AS AGE INCREASES SIGNIFICANTLY MORE EGGS ARE NEEDED TO GENERATE A NORMAL EMBRYO. J. M. Brower,a b,c M. Bower,a D. Hill,d e H. Danzer, b,c M. Surrey, c S. Ghadir, b,c B. Lim, b,c S. Munne, d J. Barritt, b,c PhGyn, University of California Los Angeles, Los Angeles, CA; aART Reproductive Center, Beverly Hills, CA; cSouthern California Reproductive Center, Beverly Hills, CA; dReprogenetics, Los Angeles, CA.

OBJECTIVE: In recent years the outcomes after oocyte cryopreservation (OC) have been significantly improved due to vitrification. However, most of the literature on OC outcomes is in women under the age of 35. Little is known about the usefulness of OC as a means of fertility preservation in older women. The aim of this study was to calculate the number of oocytes a woman would need to have retrieved in order to create a single euploid embryo based on her age.

DESIGN: Retrospective data analysis from a single private fertility clinic.

MATERIALS AND METHODS: Data for all patients (n=350) undergoing in vitro fertilization for gender selection through preimplantation genetic screening (PGS) by array comparative genomic hybridization between 1/2008 and 3/2014 were analyzed. Patients undergoing PGS for gender selection were used as a proxy for fertile couples. The subjects were divided into 5 groups based on the SART age groups. The total number of oocytes retrieved, number of embryos available for biopsy, and number of euploid embryos were compared between the groups using ANOVA. Within each age group we calculated the number of retrieved oocytes needed to generate a single euploid embryo by dividing the mean number of oocytes retrieved by the mean number of euploid embryos. The calculation thus took into account the women who did not make any euploid embryos.

RESULTS: The numbers (mean ± SE) of oocytes retrieved, embryos available for biopsy, and euploid embryos decreased significantly with advancing age.
CONCLUSION: The average number of oocytes that would be expected to yield a normal embryo increases significantly after the age of 37 and almost exponentially after the age of 42. A 42-year-old would need to undergo approximately 11 stimulation cycles in order to produce the 103 oocytes that would be expected to generate a single normal embryo. Women should be made aware of the challenges of fertility preservation with advancing age so that they can seek treatment at ages that will allow them the greatest opportunity for success.

O-172 Tuesday, October 21, 2014 04:30 PM

EGG DONATION PRODUCES SIMILAR PREGNANCY RATES WITH THE BLASTOCYST TRANSFER FROM FRESH OR VITRIFIED OOCYTES. T. S. Domingues, a,b T. Criscuolo, a M. Mazetto, a M. Nicolielo, a T. C. S. Bonetti, a,b E. L. A. Motta. a,b a Huntington Reproductive Medicine, Sao Paulo, SP, Brazil; b Gynecology, Federal University of Sao Paulo, Sao Paulo, SP, Brazil.

OBJECTIVE: Advances in reproductive techniques have provided the opportunity to concept from oocytes banks. However, initial trials on oocyte cryopreservation have presented limited outcomes, probably due to the spindle misalignment and errors in the chromosomal arrangement, as a consequence to the ice formation during the process. Recently, improvements in method and the introduction of the vitrification allow better clinical outcomes and oocyte vitrification was included in the in vitro fertilization (IVF) routine. The aim of this study was to evaluate the clinical outcomes in an egg donation program, comparing the results from fresh or vitrified oocytes following the blastocyst transfer.

DESIGN: Prospective cohort study.

MATERIALS AND METHODS: 122 oocyte donation cycles were carried out in 2013-2014. All donor patients were younger than 30 years of age and cycles of oocyte receptors were split into two groups according with oocyte origin: Fresh (n=54) or Vitrified (n=68). All oocytes were fertilized by ICSI using ejaculated sperm and blastocyst transfer placed on days 5-6. Endometrium preparation was performed with 4 mg of estradiol valerate plus 800mg of micronized progesterone according to standard protocols.

RESULTS: Recipients presented similar age (Fresh 41.4±3.5 vs Vitrified 42.4±4.5; p=0.157) and ovarian insufficiency was diagnosed as the main infertility factor in both groups (Fresh: 83.8% vs Vitrified: 79.6%; p=0.551). Fresh group received mainly more mature oocytes to be injected compared to the vitrified (10.1±2.8 vs 8.5±1.5; p=0.001). 2PN rate (79.5% vs 78.8%; p=0.770), blastocyst rate (50.4% vs 53.9%; 0.318) and number of blastocyst transferred (2.1±0.5 vs 2.2±0.6; p=0.918) were similar between Fresh and Vitrified groups, respectively. Clinical pregnancy rates were 55.6% in Fresh and 60.0% in Vitrified groups (p=0.625).

CONCLUSION: Our findings suggest that vitrified oocytes result in similar pregnancy rates compared to fresh oocytes with the blastocyst transfer in an egg donation program. Moreover, vitrified oocytes could allow a better cycle schedule and starting with a lower number of oocytes to be fertilized. Hence, we speculate the egg bank with vitrified oocytes could be safely carried out in an egg donation program.

O-173 Tuesday, October 21, 2014 04:45 PM

CERVICAL CONISATION DOUBLES THE RISK OF PRETERM AND VERY PRETERM DELIVERY IN ART TWINS - NATIONAL CONTROLLED COHORT STUY. A. Pinborg, G. Ortoft, A. Loft, H. J. Ingerslev, Hvidovre Hospital, University of Copenhagen, Hvidovre, Copenhagen, Denmark; a Fertility Clinic, Rigshospitalet, University of Copenhagen, Århusbroen, Copenhagen, Denmark; b Department of Obstetrics and Gynaecology, Skejby Hospital, University of Aarhus, Skejby, Aarhus, Denmark.

OBJECTIVE: The primary aim of this study was to assess the prevalence of cervical dysplasia and conisation in ART singleton and twin deliveries compared with naturally conceived deliveries. The second aim was to explore the risk of preterm and very preterm birth in women conceiving after ART with a history of cervix dysplasia or conization to elucidate, if these women should be recommended single embryo transfer.

DESIGN: National controlled cohort study comparing the risk of preterm birth in ART pregnancies, where the mother had a previous conisation with mothers without previous conisation. Stratification was performed for singleton and twin pregnancy and statistical adjustments were made for maternal age and parity.

MATERIALS AND METHODS: We assessed the preterm birth rate in all ART singleton (n=16,923) and ART twin deliveries (n=4,829) identified through the Danish national registers from 1995-2009. A random sample of spontaneously conceived (SC) singletons, two-fold the size of the ART singleton group matched by date and year of birth (n=33,835) and all SC twin deliveries was also extracted (n=15,112). Data on cervical diagnoses were obtained from the Danish Registry of Pathology (DRP) further we used the IVF register and the birth register.

RESULTS: In ART singleton deliveries the preterm birth rate (PTB) rate was 13.1% vs 8.2% in women with and without conisation respectively with an adjusted odds ratio (aOR) 1.56 (95%CI 1.21-2.01). In ART twin deliveries the prevalence of PTB was 58.0% vs 41.3% in women with and without conisation respectively with aOR 1.94 (95%CI 1.36-2.77), and the risk of very PTB was also doubled. Previous dysplasia also increased the risk of very PTB in ART twins (aOR 1.74, 95%CI 1.04-2.94). Cervical dysplasia did not increase the risk for any of the other outcomes in ART singletons or twins.

CONCLUSION: In ART twin pregnancies with prior conisation in the mother, 58% are born preterm, and the risk of PTB and very PTB is doubled. Irrespective of female age and prior number of ART cycles, single-embryo transfer should be recommended in women with prior conisation since the prevalence of PTB is reduced from 58 % in twins to 13 % in singletons, where the mother had conisation. There is an additive effect of conisation and twin pregnancy on the risk of preterm birth in ART pregnancies.

O-174 Tuesday, October 21, 2014 05:00 PM


OBJECTIVE: A variety of mediators interact with the fetus and endometrium to influence implantation and embryo survival. We investigated serum concentrations of 13 biomarkers in IVF patients to determine their possible association with PTD in singleton IVF pregnancies.

DESIGN: We evaluated a retrospective cohort (matched by age, prior pregnancy history, BMI, IVF response and number of embryos replaced) of 103 singleton pregnancies after IVF, utilizing sera from days 28 (initial pregnancy test) and 35 (1 week after the initial pregnancy test). There were 35 (34%) with PTD (< 37 WGA) and there were 68 (66.0%) without term deliveries.

MATERIALS AND METHODS: Serum levels of 13 different compounds were determined by quantitative ELISAs by investigators blinded to pregnancy outcome. Data was analyzed by Fisher’s exact test for categorical variables and non-parametric tests for continuous variables.

RESULTS: On day 28, the following compounds independently predicted PTD of a singleton IVF pregnancy – Interleukin (IL)-13, Huntington’s Disease Protein 4 (HE4), IGF-2 and Secretory Leukocyte Protease Inhibitor (SLPI). On day 35, the following biomarkers independently predicted PTD of a singleton IVF pregnancy – IL-6, IGF-1, IL-13 and IGF-2. There were no associations between levels of these biomarkers and cause of infertility. Given that IGF-2 was significant over both time periods, we evaluated the change of
IGF-2 as a predictor of PTD. When IGF-2 was stable over the 2 time frames, there was significantly more likely to be a full term delivery (p = 0.02).

CONCLUSION: Circulating biomarker levels as early as the day of the pregnancy test (CD28) are highly predictive of PTD of a singleton IVF pregnancy.

Supported by: Institutional.

O-175 Tuesday, October 21, 2014 05:15 PM

SERUM 17-HYDROXYPROGESTERONE (17-OHP) LEVEL ON DAY 7 OF EMBRYO TRANSFER IS A STRONG INDICATOR OF IMPLANTATION AND A RELIABLE MARKER TO DISTINGUISH BETWEEN SINGLETON AND TWIN PREGNANCY IN IVF CYCLES.


OBJECTIVE: To evaluate 17-hydroxyprogesterone (17-OHP) levels in post embryo transfer serum samples as a determinant of endometrial receptivity and conception in IVF cycles.

DESIGN: Prospective clinical study without randomization.

MATERIALS AND METHODS: Standard ovarian stimulation with antagonist protocol was followed in 336 fresh IVF cycles involving day 3/day5 embryo transfer (ET). Serum levels of 17-OHP estradiol (E2) and progesterone were measured by radioimmunoassay on days7 and 5 post ET. Luteal phase was supported with micronized Progesterone injection. Clinical Pregnancy Rate (CPR) was the main outcome measure. Statistical analysis was done using Graph-pad prism V software.

RESULTS: 17-OHP levels on day 7 (7.26±0.43 to 2.63±0.11 ng/ml, p<0.0001) and day14 (10.31±0.72 vs. 2.41±0.22 ng/ml, p<0.0001) of ET were significantly higher in pregnant (n=98) vs. non-pregnant (n=238) cycles (CPR: 29.17%). The level not only showed a significant rise from day 7 to day 14 (7.26±0.43 to 10.31±0.72 ng/ml, p=0.0002) within the pregnant group but day 7 levels were also significantly elevated in cases of twin (n=25) vs. singleton (n=75) pregnancies (11.15±0.86 vs. 5.91±0.39 ng/ml, p<0.0001). Clinical Pregnancy rate below and above the median value 2.8 ng/ml was 6.87% (11/160, all singletons) and 49.43% (87/176, 62 singletons, 25 twins) respectively. Lower cutoff (25th percentile) value of >1.7 ng/ml increased likelihood of pregnancy (ROCAUC: 96.43%, Sensitivity:92.56%, Specificity:75.89%) whereas an upper cutoff (75th percentile) of >4.9 ng/ml enhanced the chances of twin pregnancy by 26.96%. 17-OHP levels did not differ significantly between singleton vs. twins pregnancies. Serum Progesterone levels on d7 and day 14 of ET did not differ significantly between non-pregnant group; its level on day 7 of ET did not differ significantly between singleton vs. twins pregnancies. Serum Progesterone levels on d7 and day 14 of ET did not differ significantly between non-pregnant and pregnant group (singleton as well as twins groups).

CONCLUSION: Serum level of 17-hydroxyprogesterone (17-OHP) on day7 of embryo transfer is a robust indicator of enhanced endometrial response favorable for pregnancy. It is also a distinctive marker to clearly distinguish between a singleton and twin pregnancy early on in an IVF cycle even before the ultrasonographic visualization of an embryo sac is possible.

O-177 Tuesday, October 21, 2014 05:45 PM

ARE EUPLOIDY RATES DIFFERENT IN BLASTOCYSTS FROM DONOR OOCYTES AND THOSE FROM YOUNG INFERTILE PATIENTS?

M. Alkani, D. McCulloh, J. Barratt, W. Ilwoc, A. Penzias, M. Kettle, S. Munne, North Shore University Hospital, Manhasset, NY; New York Univ. Fertility Center, New York, NY; ART Reprod. Center & S. California Reproductive Center, Beverly Hills, CA; Huntington Reproductive Center, Pasadena, CA; Boston IVF; Waltham, MA; Reproge nets, Livingston, NJ.

OBJECTIVE: To compare euploidy rates in blastocysts from donor oocytes and those from young infertile patients.

DESIGN: Egg donation cycles and cycles from infertile patients <35 years were included in the study. Euploidy rates were compared and stratified according to maternal age. Egg donation cycles and cycles involving infertile patients <35 years. Indications for PGS included male factor, RIF, RPL, or patient’s request. For egg donation cycles, PGS was performed based on patient request or the presence of severe male factor. Biopsied samples were sent from 86 centers to the same reference laboratory and analyzed by array CGH. The data were examined using multiple logistic regression analysis considering the average incidence of euploidy per cycle, maternal age and cohort size.

RESULTS: Average number of blastocysts biopsied was higher in egg donors than in young infertile patients (66% vs. 61%; p<0.001). Results are shown in the below table. Overall euploidy rate was significantly higher in egg donors than in young infertile patients (66% vs. 61%; p<0.001). The incidence of euploidy was independent of maternal age within egg donors (avg. age 25.8yrs) and young infertile patients (avg age 31.4yrs) and numbers of biopsied blastocysts in both young infertile patients and egg donors.

| # blastocysts % euploid in Egg donors % euploid in Young Infertile |
|---|---|---|
| 1-3 | 58% | 61% |
| 4-6 | 62% | 60% |
| 7-10 | 65% | 62% |
| 11-15 | 68% | 63% |
| >15 | 66% | 61% |
| TOTAL | 66% | 61% |

CONCLUSION: Euploidy rates are higher in blastocysts from donor oocytes than those from young infertile patients. This is both reassuring and consistent with expectations. Although age is a confounder with respect to ploidy, in this young population, it was not found to have an effect. Blastocyst
euploidy was also independent of another confounder, namely, the number of biopsied blastocysts. The rates were the same whether one or >15 blastocysts were biopsied for the same patient. The independence of euploidy rate from cohort size was previously demonstrated in a large population of infertile patients of all ages. The finding is now confirmed for oocyte donors.

O-178 Tuesday, October 21, 2014 06:00 PM
BLASTOCYSTS AVAILABLE FOR BIOPSY ON DAY 5 ARE MORE LIKELY TO RESULT IN A VAILABLE PREGNANCY. O. O. Barash, K. A. Ivani, S. F. Willman, N. Huen, L. N. Weckstein, S. C. Lefko. Reproductive Science Center of the San Francisco Bay Area, San Ramon, CA.

OBJECTIVE: Comprehensive chromosomal screening has become a routine test in many IVF practices around the globe. Reliable information about euploidy status of the embryos created a background for significant improvement in IVF treatment outcomes (Munne et. al., 2010). Many patients have euploid embryos with similar morphological characteristics biopsied on day 5 and day 6. The objective of this study was to evaluate pregnancy rates in frozen embryo transfer cycles in which blastocyst were biopsied on day 5 versus day 6.

DESIGN: A retrospective study of PGS outcome data from blastocysts biopsied on day 5 or day 6 was conducted to identify differences in clinical pregnancy and euploidy rates in embryos analyzed by SNP PGS.

MATERIALS AND METHODS: 90 cycles of IVF treatment with PGS between January 2013 and March 2014 were included in the study. A total of 457 embryos were analyzed, 50.08±2.9 per case, for euploidy rates and blastocyst morphology. 187 embryos were biopsied on day 5, and 270 embryos were biopsied on day 6. All embryos were vitrified after biopsy. In 49 cycles the blastocysts selected for transfer were biopsied on day 5, and in 41 cycles blastocysts selected for transfer were biopsied on day 6. Mean age of the women (36.72±4.61 years) and average number of transferred embryos (1.22±0.42) were not different between the two groups. Pregnancy rate was defined by the presence of a fetal heartbeat at 6-7 weeks of pregnancy.

RESULTS: Our data demonstrated a statistically significant difference (p<0.05, 1.5±0.79) in pregnancy rates between study groups: 73.47% (36/49) for embryos biopsied on day 5 and 51.22% (21/41) for embryos biopsied on day 6. Euploidy rate was significantly higher for embryos available for biopsy on day 5 than on day 6 (66.9±3.17% and 52.6±3.9 percent, respectively, p<0.05). The difference in clinical pregnancy rate was even more dramatic for women over 38 years of age (mean age - 39.82±1.89 years): 86.96% (20/23) for day 5 biopsy and 50% (12/24) for day 6 biopsy. The euploidy rate distribution exhibited similar values (60.76±4.5 percent and 45.99±2.7 percent, respectively, p<0.05).

CONCLUSION: Analysis of our data supports the idea that the dynamics of embryo development in vitro, as well as the morphology and euploidy of the embryos, may contribute to the determination of IVF treatment outcomes. Preferable selection for blastocyst available for biopsy on day 5 may significantly improve outcomes of infertility treatment, especially for women over 38 years of age.

O-180 Tuesday, October 21, 2014 04:30 PM

OBJECTIVE: Embryo vitrification enables patients to undergo multiple cycles of in vitro fertilization (IVF) without repeated ovarian stimulation and oocyte retrieval. Although vitrification has been shown to have fairly high post-thaw embryo survival rates, its impact on embryo viability remains poorly understood, as damage due to vitrification is difficult to detect using routine microscopy techniques. We have constructed a non-invasive, high-resolution, label-free microscope based on optical coherence tomography (OCT) that is able to observe dramatic changes in embryo appearance immediately after vitrification and thawing that are not visible under brightfield microscopy. We have also developed complementary image processing tools that can automatically quantify these changes and may enable the identification of embryos negatively affected by the vitrification process.

DESIGN: Two experimental groups of mouse embryos were imaged at the 1-cell stage. The first group (n = 16) was imaged with our microscope. The second group (n = 26) was vitrified and thawed prior to imaging. Post imaging, both sets of embryos were incubated and evaluated for blastocyst formation after five days in order to assess whether imaging parameters observed immediately after vitrification and thawing correlated to embryo viability.

MATERIALS AND METHODS: Embryos were vitrified and thawed using the Sage vitrification and warming kits, and imaged using a custom-built microscope equipped with an integrated low coherence interferometer and stage-top incubator. Computer vision-based image processing tools were used to extract parameters related to the structure of embryos within the cytoplasm.

RESULTS: Embryos that underwent vitrification and thawing showed significant clumping of structures within the cytoplasm, while embryos that had not been vitrified exhibited very little clumping. This vitrification-induced changes were invisible under observation with a brightfield microscope. Our image processing algorithm could effectively quantify the degree of clumping (> 93% accuracy), and parameters related to clumping may be predictive of blastocyst formation.

CONCLUSION: We present a novel optical technique for imaging developing embryos that can detect vitrification-induced changes in structural morphology and embryo clumping and may enable the identification of embryos with compromised viability after vitrification.

Supported by: NSF CBET-1351891 and the Center for Biomedical Imaging at Stanford seed grant program.

O-179 Tuesday, October 21, 2014 04:15 PM

Institute for Reproductive Medicine and Biotherapy, IN- SERM U1040, CHU Montpellier, Saint Eloi Hospital, Montpellier, Language-Rousillon, France; 2Art-Pgd Department, CHU Montpellier, Arnaud de Villeneuve Hospital, Montpellier, Languedoc-Rousillon, France.

OBJECTIVE: This study was for aim, to investigate if cell-free DNA (cfDNA) in human follicular fluid (FF) could provide a new non-invasive predictive biomarker for embryo outcome.

DESIGN: In this prospective study, 100 FF samples were collected at oocyte retrieval day from individual pre-ovulatory follicles, from 43 patients who included after informed consent. Women received either long or short agonist protocol. CIDNA levels were measured in each FF samples and compared with IVF (n = 26) and intracytoplasmic sperm injection (ICSI) (n = 17) outcomes.

MATERIALS AND METHODS: The pre-ovulatory follicles were aspirated individually. Only blood-free FF samples were included. CIDNA was quantified by ALU sequence qPCR. Means ± SD, correlations and linear or logistic regressions are presented.

RESULTS: Human FF samples contained measurable amounts of cfDNA (mean ± 1.6 ± 2.1 ng/μl) and cfDNA level was significantly higher in small follicles (diameter < 8 mm) (2.7 ± 0.8 ng/μl) versus large ones (diameter >18 mm) (0.54 ± 0.4 ng/μl, respectively, p=0.007). Indeed, cfDNA concentration was negatively correlated with follicle size (r=−0.34; P=0.03). Interestingly, FF cfDNA level was significantly higher when the corresponding oocyte generated a poor-quality embryo (p=0.02) and was significantly lower in FF related to embryo with low fragmentation rate (≤ 25%), compared with those with high fragmentation rate (>25%) (p=0.02). In addition, after adjustment for confounding factors, the odds to obtain a top quality embryo reached 82%, if the cfDNA level in the corresponding FF sample was < 4.8 ng/μl (Adjusted Odd Ratio=19.5, p=0.03).

CONCLUSION: Our results suggest that cfDNA in human FF might represent an innovative, non-invasive biomarker of embryo quality. This approach can be used as a supplemental test to predict embryo outcome during IVF cycles. A larger study will be conducted to investigate the relationship between cfDNA levels and implantation and pregnancy rates.

Supported by: This work was Supported by the University-Hospital of Montpellier and by a grant from the Ferring Pharmaceutical Company. The authors of the study have no competing interests to report.
DESIGN: IRB-approved retrospective cohort study.

MATERIALS AND METHODS: The study included all single-blastocyst transfers at a private fertility center in the study period. Blastocysts were measured and graded before ultrasound-guided transfer. Pregnancy was defined by rising serum hCG titers. Ongoing pregnancies had viable fetal cardiac activity at 10 weeks gestation. Early pregnancy losses were pregnancies that did not achieve ongoing pregnancy. Stepwise multiple logistic regressions were used to identify relevant outcome predictors.

RESULTS: This study included 602 single-blastocyst transfers. Of these, 361 achieved pregnancy, 257 achieved clinical pregnancy, and 131 had early pregnancy loss. The mean age at retrieval was 33.5 ± 5.2 years, the mean BMI was 24.8 ± 5.7 kg/m², and the mean weight was 67.1 ± 16.4 kg. After controlling for age at retrieval and type of cycle, inner cell mass size had the greatest correlation with pregnancy (P = 0.0077), ongoing pregnancy (P = 0.0006), and early pregnancy loss (P = 0.0160). The other available morphologic parameters were initially correlated with each outcome, but these correlations became non-significant when controlling for age at retrieval and cycle type.

CONCLUSION: Of the available morphological parameters, inner cell mass size was the best predictor for pregnancy, ongoing pregnancy, and early pregnancy loss, after controlling for age at retrieval and type of cycle (fresh or frozen, autologous or oocyte donor).

O-182 Tuesday, October 21, 2014 05:00 PM

ASSESSING MORPHOKINETIC PARAMETERS AS FRACTIONS OF THE TIME TO FULL BLASTOCYTE TO PREDICT EUPLOIDY: TESTING A MORE SENSITIVE MEASUREMENT METHOD. Y. G. Kramer, D. H. McCulloh, J. D. Kofinas, K. Melzer, N. Noyes, C. McCaffrey, L. Krey, J. A. Grifo, NYU Fertility Center, NYU Langone School of Medicine, New York, NY.

OBJECTIVE: To determine if early embryo morphokinetic values (MKV) expressed as fractions of time from syngamy to full blastocyst development are more predictive of ploidy than MKV raw data.

DESIGN: Retrospective Cohort Analysis.

MATERIALS AND METHODS: 106 embryos from 20 patients cultured to blastocyst in the EmbryoScope™ underwent trophectoderm (TE) biopsy on days 5 or 6, and awaited analysis of ploidy by with array comparative genomic hybridization (aCGH) prior to transfer. Morphokinetic events were expressed as fractions of time to full blastocyst development according to the following equation:

\[ F(MKV) = (MKV - T(syngamy)) / (T(Full Blastocyst) - T(syngamy)) \]  

(Eq 1)

The timing of morphokinetic events expressed according to Eq 1 were analyzed to determine if they could be used to asestimate ploidy of human embryos. The actual ploidy results were compared with expectations using Receiver Operator Characteristic (ROC) curves plotting sensitivity versus specificity.

RESULTS: ROC curves testing the ability of these values to discriminate euploid from aneuploid embryos were constructed. The areas under the curves (AUC) were each no more than 0.66 (4 cell 0.60, 5 cell 0.62, starts compaction 0.60 and duration of compaction 0.66). Using a threshold of 0.0045 or less for duration of compaction enriches for euploidy, 40% of the euploid embryos are detected while 60% are not.

CONCLUSION: In order to account for overall differences in rate to the blastocyst stage, we expressed MKV values as fractions of time to full blast. Despite the fact that there were four morphokinetic events with AUC slightly higher than 0.60, incidence of euploidy prediction was not enriched. We conclude that none of the parameters will function to discriminate euploid from aneuploid embryos. Further, utilizing morphokinetic parameters to assess ploidy, even as fractions to full blast, does not approach the accuracy of preimplantation genetic screening with aCGH (97.5%). Despite their poor ability to discriminate ploidy, morphokinetics may be useful in conjunction with PGS in selecting those PGS-screened euploid embryos with the best chances of implantation and live birth.

O-183 Tuesday, October 21, 2014 05:15 PM


OBJECTIVE: Various recent studies have pointed out in the direction of the trophectoderm as one of the most reliable predictors of pregnancy but so far there is no scientific consensus about which morphological blastocyst parameter weighs more in the selection of the embryo with the greatest chance of success. We designed a study to further investigate the individual contribution of each parameter to clinical pregnancy.

DESIGN: Retrospective cohort study of 1084 fresh single blastocyst transfers in a fertility centre from January 2009 through June 2012. Blastocysts were graded according to Gardner and Schoolcraft grading system (1).

MATERIALS AND METHODS: Primary outcome was clinical pregnancy rate (CPR) and secondary outcomes implantation and early pregnancy loss rates. Chi-square test of association and univariable and multivariable logistic regressions were applied.

RESULTS: Blastocyst expansion degree was the only significant predictor of CPR in the univariable regression being 7.7 times more likely to achieve a pregnancy with the transfer of a hatching blastocyst than an early blastocyst (OR = 7.73, 95% CI 2.37 – 25.22, p = 0.001). There was a significant reduction in the likelihood of achieving a clinical pregnancy when the inner cell mass grade dropped from A to C, but not from A to B (OR 0.45, 95% CI: 0.27 – 0.75, p = 0.002). On the multivariable regression only this latter association remained significant but, of note, there was a marginal association with embryo stage (OR 0.39, 95% CI: 0.15 – 1.02, p = 0.057). Early pregnancy loss rates were found to be significantly associated with embryo stage (p-value = 0.001) and ICM grade (p-value = 0.002). Early pregnancy loss rates of embryos with ICM grade C were more than double (38.00%) compared to those of grades A (15.95 %) and B (17.17 %). There were no significant associations between trophectoderm grades and studied outcomes.

CONCLUSION: Blastocyst expansion degree and ICM were the only parameters associated with CPR in the univariable analysis. The association with blastocyst expansion degree was however only marginally significant in the multivariable analysis. The transfer of an embryo with a strong inner cell mass may reduce early pregnancy loss.

O-184 Tuesday, October 21, 2014 05:30 PM

ANTIMULLERIAN HORMONE DOES NOT PREDICT OUTCOME OF PREIMPLANTATION GENETIC SCREENING. A. W. Bostian, A. W. Bostian, G. Couchman, J. K. Park. Carolina Conceptions, Raleigh, NC.

OBJECTIVE: To determine whether Antimullerian Hormone (AMH) can predict the ability to produce euploid embryos.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: All cycles utilizing IVF/ICSI with preimplantation genetic screening (PGS) were analyzed. Stimulation protocols were typically short antagonist protocols but micro-dose flare protocols were used in patients with low ovarian reserve. Trophectoderm biopsy of embryos on day 5 and 6 of embryo culture was performed and samples were analyzed by array comparative genomic hybridization. The percentage of euploid embryos was determined for each cycle. Linear regression analysis was performed with age and AMH levels as predictors of the euploidy rate of biopsied embryos.

RESULTS: There were 99 cycles of PGS in the analysis, with 334 blastocysts biopsied on day 5 and 168 biopsied on day 6. Patients ranged from 21 to 42 years of age, with AMH levels ranging from 0.6 to 18 ng/ml. In the regression analysis with age and AMH, only age was found to be a significant predictor in the percentage of euploid embryos (P < 0.05). After stratifying patients into < 35 yo and ≥ 35 yo, AMH was still not a predictor in the percentage of embryos found to be euploid. AMH was found to be a significant predictor in the number of embryos that would be available for biopsy (P < 0.05).

CONCLUSION: AMH levels do not predict the chances of having euploid embryos, but age is confirmed to be a predictor. AMH levels are related to the number of embryos that would be available for biopsy, with higher AMH levels associated with more embryos for biopsy. These findings suggest that AMH levels provide information about egg quantity, but do not reveal much information about egg quality. While controlling for age, there was a trend between AMH and the percentage of euploid embryos, but this did not reach statistical significance. It is possible that this association may reach statistical significance with a larger sample size.

FERTILITY & STERILITY®
O-185 Tuesday, October 21, 2014 05:45 PM

MORPHOKINETICS OF EMBRYOS OBTAINED WITH NORMAL EJACULATED SPERM OR WITH TESTICULAR SPERM FROM NOA PATIENTS. M. G. Minasi,* V. Casciani,* F. Scarselli,* M. Terribile,* G. Franco,* R. P. Cotarelo,* M. T. Varrichio,* E. Greco,* *Centre for Reproductive Medicine European Hospital, Rome, Italy; †Gynecological-Obstetrical and Urological Sciences, Sapienza University, Rome, Italy.

OBJECTIVE: Time-lapse technology was used to examine the developmental kinetics of embryos obtained by ICSI performed either with ejaculated normal semen or with surgically retrieved spermatozoa. We aimed to study possible differences in cellular divisions which may be due to different maturation stages of injected sperm.

DESIGN: During spermiogenesis, the transit of spermatozoa in the epididymal tract favors nuclear maturation and DNA packaging through protamine dephosphorilation and the formation of molecular bridges. It has been suggested that the timing of embryo developmental stages after ICSI insemination may be affected by the origin of sperm. In this retrospective observational study (Sep 2012-Nov 2013), time-lapse technology allowed to observe embryos obtained by ICSI either with testicular sperm (TS-group: 25 cycles, female mean age=36.29±4.29) or with normal (WHO, 2010) ejaculated sperm (ES-group: 32 cycles, mean age=36.63±4.92).

MATERIALS AND METHODS: In each group, 224 oocytes were injected. Development up to day-3 was studied: second polar body extrusion, 2P embryo; time-lapse of the 2-cell division and intervals between divisions.

RESULTS: Respectively in TS-group and ES-group, 2PN were 118 (52.7%) and 176 (76.8%) (p=0.0001); development kinetics was studied on surviving 2PN embryos: 85 and 144. Significant differences appeared in the duration of 4- and 176 (78.6%) (p=0.035) and in the overall time to develop from 5 to 8 cells (11.97±8.19 in TS, N=38; 8.45±6.99 in ES, N=80; p=0.017).

CONCLUSION: The source of spermatozoa interferes with fertilization potential. A significant difference appeared in the duration of later cleavage stages: in embryos deriving from testicular sperm, the duration of the 4-cell stage was shorter. Conversely, the time-lapse from 5 to 6 cells and the overall time-lapse between 5 and 8 cells was longer in embryos from testicular sperm as compared to normal ejaculated sperm. Understanding the dynamics of embryo development in relation to the type of sperm injected may help defining parameters for increasing ICSI outcomes and could provide valuable insights for improving clinical results.

O-186 Tuesday, October 21, 2014 06:00 PM


OBJECTIVE: To analyze the frequency of the 919 G>A (T307A), 2039A>G (N680S) and -29 (G/A) SNPs (1) in mexican mestizo women and their association with the response to controlled ovarian hyperstimulation (COH).

DESIGN: A prospective study in 80 normal oocyte donors and 144 infertile women from the Instituto Valenciano de Infertilidad (IVI) Mexico (IVI women).

MATERIALS AND METHODS: DNA from women treated with a COH protocol was analyzed by TaqMan allelic discrimination assay, restriction fragment length polymorphisms, and DNA fragment sequencing.

RESULTS: Frequencies of the FSHR SNPs were: N/680, 42%; N680, 48%; and S/680, 10%. All women, but 19, exhibited genotypes following the T/T307-N/680 or A/A307-S/680 segregation. The frequency of the 5680 SNP was further analyzed in 98 Maya mestizo women with a low cross-breeding: we hypothesized that if the 5680 SNP originated in europe, then its prevalence in the Maya women (in Hardy-Weinberg disequilibrium) should be lower than that recorded in the IVI women (in Hardy-Weinberg equilibrium). We found that the S/680 SNP frequency in Maya women was lower (7.0%) than that in IVI women (10%). The -29 G/A SNP showed frequencies of 27, 50 and 23% for the G/G, G/A y A/A variants, respectively. The total FSH dose administered was higher and the number of oocytes recovered were lower (p<0.05) in normal IVI donors with the S/680 genotype than in donors with other genotypes (N/680 or N680). No differences in these parameters were recorded among infertile patients with different genotypes. The frequency of ovarian hyporesponsiveness to COH was higher in the N/680 (25%) and S/680 (20%) genotypes than in the N680 genotype (13.5%). There was no association between the -29 G/A SNP and the response to COH.

CONCLUSION: The N680S SNP in mexican women is lower than in other populations (europe>asian>indian>mexican mestizo). The S/680 genotype is associated to ovarian hyporesponsiveness to COH. In the population studied, the -29 A/A genotype does not impact on the response to COH. This is the first study that reports the frequency of these SNPs in hispanic mexican women.

Supported by: Study Supported by a CONACY-T-Salud grant no. 86881, Mexico.

OVARIAN STIMULATION - POOR RESPONDERS

O-187 Tuesday, October 21, 2014 04:15 PM

IMPORTANCE OF POSTPRANDIAL GLYCHEMA (PGP) IN ART: A NOVEL THERAPY WITH SITAGLIPTIN FOR ART REPEATERS INCREASES PREGNANCY RATE BY DECREASING PPG AND ADVANCED GLYCATION ENDPRODUCTS. M. Jinn,* M. Terribile,* A. Watanabe,* M. Takeuchi,* N. Suciu,* Women’s Clinic Jinno, Choufu City, Tokyo, Japan; †Department of Advanced Medicine, Kanazawa Medical University, Medical Research Institute, Kakou, Ishikawa, Japan; ‡Department of Obstetrics and Gynecology, Polizu Maternity Hospital, Bucharest, Romania.

OBJECTIVE: Resistance to and inadequate secretion of insulin increase postprandial glycemia (PPG), advanced glycation end-products (AGE) and oxidative stress, and vice versa. This pathogenic cycle seems to be involved in poor folliculogenesis. Study I analyzed effects of PPG on embryonic development and study II attempted to improve folliculogenesis in women with repeated ART failure by decreasing PPG with sitagliptin.

DESIGN: Observational study and case-control study.

MATERIALS AND METHODS: Study I analyzed correlations between oral glucose tolerance test (OGTT) and embryonic development in 881 initial ART attempts. In case-control study II, sitagliptin was given to 44 multiple ART repeaters (5.8 failures, including metformin) at 41.0 years of age with mildly elevated PPG. Forty-four women with similar PPG who underwent ART without sitagliptin were matched with subjects for age, previous ART failures, and day-3 FSH.

RESULTS: Study I: The number of day 2 embryos correlated negatively with glucose and insulin after glucose load in OGTT, but not fasting ones. Study II: Sitagliptin enhanced follicular and embryonic development. Clinical and ongoing pregnancy rates improved significantly with sitagliptin (20% and 14%) compared with controls (2.5% and 0%). Triglyceride and free testosterone before sitagliptin correlated positively with ongoing pregnancy. Sitagliptin significantly decreased glucose in OGTT. Relative decreases in serum toxic AGE from sitagliptin correlated negatively with increases in superior embryos at day 2.

CONCLUSION: Elevated postprandial glucose and insulin are related with poor folliculogenesis. Sitagliptin significantly improves follicular and embryonic development and pregnancy rates in metformin-unresponsive ART repeaters with mildly elevated PPG. Decreases in PPG and toxic AGE appear to be involved.

O-188 Tuesday, October 21, 2014 04:30 PM

DOUBLE STIMULATIONS DURING THE FOLLICULAR AND LUTEAL PHASES IN PATIENTS WITH POOR OVARIAN RESPONSE: EFFECTS ON OVARian STIMULATION - POOR RESPONDERS.
DESIGN: A pilot study was performed in university-affiliated hospital. MATERIALS AND METHODS: 38 women were collected according to Bologna criteria. Mild ovarian stimulation was initiated with letrozole, clomiphene and a low dose of hMG. After the first ovum pick-up (OPU), hMG and letrozole were administered to stimulate follicle development, second OPU was performed when dominant follicles matured. All viable embryos were extracted and cryopreserved for later transfer. The primary outcome measured was the number of oocytes retrieved.

RESULTS: The results showed that the number of oocytes retrieved was 1.7 ± 1.0 in stage one and 3.5 ± 3.2 in stage two (P<0.05).

The cycle characteristics in double stimulations in patients with POR

<table>
<thead>
<tr>
<th>cycle characteristics</th>
<th>第一OPU</th>
<th>第二OPU</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stimulation duration (days)</td>
<td>10.2±2.4</td>
<td>10.8±3.1</td>
<td>0.265</td>
</tr>
<tr>
<td>no of follicles &gt;10 mm on trigger dat</td>
<td>1.9±0.9</td>
<td>4.3±2.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>no of follicles &gt;14 mm on trigger dat</td>
<td>1.5±0.6</td>
<td>3.5±2.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>no of oocytes retrieved</td>
<td>1.7±1.0</td>
<td>3.5±3.2</td>
<td>0.001</td>
</tr>
<tr>
<td>no of MII oocytes</td>
<td>1.4±1.0</td>
<td>2.7±2.7</td>
<td>0.008</td>
</tr>
<tr>
<td>no of fertilized oocytes</td>
<td>1.0±1.0</td>
<td>2.1±2.5</td>
<td>0.019</td>
</tr>
<tr>
<td>no of cleaved embryos</td>
<td>1.0±1.0</td>
<td>2.0±2.4</td>
<td>0.045</td>
</tr>
<tr>
<td>no of top-quality embryos</td>
<td>0.7±1.0</td>
<td>1.2±1.5</td>
<td>0.155</td>
</tr>
<tr>
<td>no of viable embryos</td>
<td>0.9±1.0</td>
<td>1.3±1.4</td>
<td>0.171</td>
</tr>
<tr>
<td>mature oocyte rate (%)</td>
<td>85.5% (53/62)</td>
<td>78.1% (82/105)</td>
<td>0.167</td>
</tr>
<tr>
<td>fertilization rate (%)</td>
<td>69.8% (37/53)</td>
<td>75.6% (62/82)</td>
<td>0.292</td>
</tr>
<tr>
<td>cancel rate (%)</td>
<td>52.6% (20/38)</td>
<td>43.3% (13/30)</td>
<td>0.303</td>
</tr>
</tbody>
</table>

Out of double stimulation, a total of 167 oocytes were collected and 68.4% (26/38) succeeded in producing 1-6 viable embryos cryopreserved for later transfer. 21 women underwent 23 FETs, resulting in clinical pregnancies. The implantation rate per transfer was 36.6% (15/41). CONCLUSION: Our study showed that the number of oocytes retrieved in double stimulation is a critical parameter. The number of oocytes retrieved in stage one and two is 1.7 ± 1.0 and 3.5 ± 3.2, respectively. The number of oocytes retrieved is significantly different (P<0.05).

O-190 Tuesday, October 21, 2014 05:00 PM

EFFICACY OF CLOMIPHENE CITRATE SUPPLEMENTATION TO CONVENTIONAL GnRH ANTAGONIST PROTOCOLS IN POOR RESPONDERS UNDERGOING ART: A PROSPECTIVE RANDOMIZED TRIAL

A. Fujimoto,† M. Harada,† T. Hirata,† Y. Osuga,‡ T. Fujii,§
Obstetrics and Gynecology, Sanraku Hospital, Chiyoda-ku, Tokyo, Japan;
Obstetrics and Gynecology, University of Tokyo Hospital, Bunkyo-ku, Tokyo, Japan.

OBJECTIVE: To evaluate the effect of clomiphene citrate addition to gonadotropin releasing hormone (GnRH) antagonist protocols in poor responders undergoing assisted reproductive technology (ART).

DESIGN: A prospective randomized controlled study was conducted on poor responders undergoing ART treatment in IVF Center of University of Tokyo Hospital. This study was approved by the Institutional Review Board.

MATERIALS AND METHODS: Among the patients who visited IVF Center of University of Tokyo Hospital for the purpose of ART, those who fitted at least one of the following inclusion criteria and who fitted no exclusion criteria were eligible for this study.

Inclusion criteria: 1. over the age of 45 2. antral follicle counts <7 in early follicular phase 3. 4 previous poor response to ART treatment (<5 retrieved oocytes) Exclusion criteria: 1. lover the age of 45 2. those who underwent oocyte retrieval cycles more than 3 times The patients who gave a written informed consent form were allocated into two groups. In group (n=44), controlled ovarian stimulation was initiated on day3 with 5 days of clomiphene citrate (2 tabs daily), followed by human menopausal gonadotropin (hMG) administration. After leading follicle diameter reached 14mm, GnRH antagonist Ganirelix was administered in addition to hMG. In group 2 (n=45), hMG administration was started on day3, followed by combination with Ganirelix in the same way.

RESULTS: There were no significant differences in estradiol levels on day of hCG, number of growing follicles, number of retrieved oocytes and fertilization rate between the two groups. In group 1, total hMG dose and cost for controlled ovarian stimulation were significantly less than those in group 2. Embryo transfer cancellation rate per oocyte retrieval was significantly higher in group 1 (28.6% vs. 2.5%), which was mainly due to inadequate endometrial preparation. However, live-birth rate per fresh embryo transfer and cumulative live-birth rate per patient in the two groups were comparable (9.1% vs. 11.1% and 20.5% vs. 17.8%, respectively).

CONCLUSION: The addition of clomiphene citrate in GnRH antagonist protocols could not improve clinical outcomes of ART in poor responders, but it could reduce hMG dose and cost for controlled ovarian stimulation.

OBJECTIVE: To evaluate the effect of aromatase inhibitor (letrozole) co-treatment for patients with severe poor ovarian response: matched comparison of previous (w/o) and current (w/) cycles in the same patients.

DESIGN: Retrospective cohort analysis.

MATERIALS AND METHODS: From January 2012 to February 2014, Letrozole/GnRH-antagonist treated 77 patients were analyzed. Inclusion criteria: severe poor responder, at least two of the following three feature must be present were included; 1) ≥ 40 yrs, 2) no. of previous collected oocytes; ≤ 3, 3) level of AMH; <1.1ng/ml. Matched comparison of previous (treated with GnRH antagonist) and current (co-treated with Letrozole (5mg/day)/GnRH antagonist) cycles in the same patients was performed. Estradiol level on the day of HCG injection, the number of total and mature oocytes collected and the clinical pregnancy outcome were evaluated between two groups.

RESULTS: Mean level of AMH was 0.7±0.7 (ng/ml) and mean no. of previous collection cycles = 4.7±3.9. No. of collected oocytes and mature oocytes were significantly increased in letrozole co-treatment cycles (1.6±1.9 vs. 2.6±2.2, p<0.005 and 0.8±1.1 vs. 3.2±1.4, p<0.01). Retrieval failure rates (29.9%, 23/77 vs. 9.1%, 7/77, p<0.0001) were reduced in letrozole co-treatment cycles. However, there were no differences in the maturation rate (45.7%, 58/127 vs. 48.5%, 99/204, p=0.6123), fertilization rate (85.8%, 97/113, vs. 79.9%, 143/179, p=0.1953) and top quality embryo rate (76.3%, 45/59 vs. 76.8%, 53/69, p=0.9426). Cycles with all embryos cryopreserved were 23.4% and 29.9% respectively. Clinical pregnancy and implantation rates were higher in letrozole co-treatment cycles but were not significantly different (19.2%, 5/26 vs. 27.6%, 8/29, p=0.3367 and 8.5%, 5/59 vs. 14.1%, 9/64, 0.3296). Especially, estradiol levels and endometrium thickness on day of HCG administration were significantly low in letrozole treated patients (629.2±140.7 vs. 1411.0±1037.8, p<0.005 and 8.5±1.7 mm vs. 10.6±1.3 mm, p<0.005). Ongoing pregnancy rate (0.0%, 0/26 vs. 20.7%, 6/29, p<0.005) were significantly higher in letrozole co-treatment cycle.

CONCLUSION: The aromatase inhibitor, letrozole blocks estrogen synthesis and increases intraovarian androgen level. Although the use of letrozole presented the low levels of estradiol and endometrium thickness, the patients who received letrozole co-treatment showed a significant higher number of collected oocytes and higher ongoing pregnancy rate. The present study suggests that adding of aromatase inhibitor is a suitable protocol for patients with severe poor response.
individually, CC induced follicles as small as 16 mm will yield same proportion of mature oocytes as their larger cohorts.

O-194 Tuesday, October 21, 2014 06:00 PM


OBJECTIVE: To examine and compare cycle outcomes among patients demonstrating poor response to ovarian hyperstimulation that proceeded to oocyte retrieval to those that converted to intrauterine insemination (IUI).

DESIGN: Cohort Study.

MATERIALS AND METHODS: Comparison Groups
Women demonstrating poor ovarian response while undergoing planned assisted reproductive technology (ART) cycles who proceeded to oocyte retrieval and In Vitro Fertilization (IVF).

Women demonstrating poor ovarian response while undergoing planned ART cycles who converted to IUI.

Inclusion Criteria
- Patients received gonadotropins as part of an ART cycle
- Five or fewer follicles measuring greater than or equal to 15 mm in average diameter
- Peak estradiol (E2) level less than 1000 pg/dL at time of human chorionic gonadotropin (hCG) trigger
- Patients converted to IUI must have had at least one patent fallopian tube and were inseminated with a post-wash total motile sperm count >5 million/mL
- Underwent standard stimulation protocols

Exclusion Criteria
- Sperm counts <5 million/mL for IUI group
- Age <18 or >39
- Bilateral tubal blockage for IUI group

Primary Outcome: Live birth

Secondary Outcome: Clinical pregnancy

Demographics and Patient Information
- Baseline FSH, Anti-Müllerian hormone, TSH, prolactin, DHEA-S
- E2 levels at time of hCG trigger
- Height & weight
- Ethnicity
- Age
- Gravidity/Parity
- Prior treatment.

TABLE 1. Characteristics of Study Participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>IUI (N=29)</th>
<th>IVF (N=48)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>23.9 ± 4.3</td>
<td>28.1 ± 7.2</td>
<td>0.01</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>33.5 ± 3.9</td>
<td>33.7 ± 3.7</td>
<td>0.8</td>
</tr>
<tr>
<td>Follicle counts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline Antral&lt; 15 mm</td>
<td>11.1 ± 6.7</td>
<td>11.5 ± 6.4</td>
<td>0.8</td>
</tr>
<tr>
<td>Baseline Antral ≥ 15 mm</td>
<td>0.34 ± 0.19</td>
<td>0.04 ± 0.15</td>
<td>0.3</td>
</tr>
<tr>
<td>After Hyperstimulation&lt; 15 mm</td>
<td>7.9 ± 5.9</td>
<td>10.7 ± 7.4</td>
<td>0.09</td>
</tr>
<tr>
<td>After Hyperstimulation ≥ 15 mm</td>
<td>1.7 ± 1.2</td>
<td>3.4 ± 1.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hormone Levels</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2 at trigger</td>
<td>452.6 ± 250.2743.2 ± 181.8 &lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FSH</td>
<td>8.7 ± 3.1</td>
<td>7.6 ± 2.7</td>
<td>0.12</td>
</tr>
<tr>
<td>TSH</td>
<td>2.0 ± 1.4</td>
<td>1.7 ± 1.0</td>
<td>0.32</td>
</tr>
<tr>
<td>Prolactin</td>
<td>11.3 ± 6.0</td>
<td>12.8 ± 12.5</td>
<td>0.56</td>
</tr>
<tr>
<td>DHEA-S</td>
<td>131.4 ± 62.5</td>
<td>143.7 ± 124.9</td>
<td>0.62</td>
</tr>
</tbody>
</table>

Outcomes

| Clinical Pregnancy Rate | 7.0% (2) | 58.3% (28) | <0.001 |
| Live Birth Rate         | 0.0% (0) | 44.0% (21) | <0.001 |

RESULTS: All data reported as either percentage frequency (n) or mean ± standard deviation. Statistical p-value based on either student t-test for continuous variables or chi-square/Fisher’s Exact test for categorical variables. P value <.05 considered statistically significant.

CONCLUSION: There was a clinically and statistically significant difference in both clinical pregnancy rate and live birth rate between groups.

MALE FACTOR

Date: O-195 Tuesday, October 21, 2014 04:15 PM

TRENDS IN THE USE OF ICSI: INDICATIONS AND PREGNANCY OUTCOMES, UNITED STATES 1996-2011. S. L. Boulet, A. Melhu, D. M. Kissin, E. Warner, D. J. Jamieson. Centers for Disease Control and Prevention, Atlanta, GA; Emory University School of Medicine, Atlanta, GA.

OBJECTIVE: To assess national trends and pregnancy outcomes associated with the use of intracytoplasmic sperm injection (ICSI) for fresh embryo transfer cycles with respect to selected indications for ICSI use.


MATERIALS AND METHODS: We used linear regression models to assess trends in the rate of ICSI use during 1996-2011 for all fresh in vitro fertilization (IVF) transfer cycles and those with the following indications: male factor infertility, unexplained infertility, maternal age ≥38 years, and ≥2 prior assisted reproductive technology (ART) cycles. Using more recent data (2007-2011), we calculated rates of implantation, clinical intrauterine pregnancy, miscarriage, live birth and multiple live birth for transfers using conventional IVF (no ICSI used) and those using ICSI, stratified by male and non-male factor infertility. We used robust Poisson regression models with generalized estimating equations for repeated measures clustered by clinic to estimate adjusted risk ratios for the association between the use of ICSI and treatment outcomes.

RESULTS: From 1996-2011, there was a linear increase in use of ICSI for all transfers (N=1,208,710) from 36.5% to 75.9%. Among 434,916 transfers with male factor infertility, ICSI use increased significantly from 77.3% to 93.9%; for those with non-male factor infertility, ICSI use increased from 15.4% to 65.8%. Significant temporal increases were also noted among transfers with unexplained infertility, maternal age ≥38 years, and ≥2 prior ART cycles. After adjustment for maternal factors and ART treatment characteristics, there were no differences in implantation, pregnancy, miscarriage or live birth rates for transfers with ICSI compared with conventional IVF when male factor infertility was present; however, the risk for multiple birth was reduced (aRR 0.91, 95% CI 0.87-0.95). For non-male factor infertility, transfers with ICSI had significantly decreased rates for all outcomes (aRRs ranged from 0.92-0.94) except miscarriage when compared with conventional IVF.

CONCLUSION: The use of ICSI more than doubled between 1996 and 2011, with the largest increase noted among transfers with non-male factor infertility. Use of ICSI for non-male factor infertility was associated with reduced rates of implantation, pregnancy, and live birth.

O-196 Tuesday, October 21, 2014 04:30 PM

EFFECT OF SPERM DNA FRAGMENTATION ON IVF/ICSI CLINICAL OUTCOMES IS DIVERSIFIED IN WOMEN WITH DIFFERENT OVARIAN RESERVE. J. Jin, X. Huang, C. Pan, Q. Fei, W. Ni. Reproductive Medicine Center, The First Affiliated Hospital of Wenzhou Medical U, Wenzhou, Zhejiang, China.

OBJECTIVE: To investigate the effect of sperm DNA fragmentation (SDF) on clinical outcomes of assisted reproductive technology in women with different ovarian reserve.

DESIGN: Retrospective clinical study.

MATERIALS AND METHODS: Consecutive 3350 couples underwent their first conventional in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) cycles between April 2009 and December 2012 were respectively analyzed in this study. SDF was assessed by the sperm chromatin dispersion (SCD) test in sperm samples 1-2 months prior to treatment for each male partner. Standard leuteal down regulation long protocol (LP) was used in women with normal ovarian reserve while short flare-up protocol (SP) was adopted in one of the following conditions indicating reduced ovarian reserve: [1] the women’s basal FSH>10 IU/L; [2] the number of
O-197 Tuesday, October 21, 2014 04:45 PM

HUMAN SEX RATIO AND NUMERICAL CHROMOSOME ABNORMALITIES IN SPERMATOZOA SELECTED USING MOTILE SPERM ORGANELLE MORPHOLOGY EXAMINATION (MSOME). C. G. Petersen, a,b L. D. Vagnini, a A. Renzi, a,b G. R. Oliveira-Pelegrin, b A. L. Mauri, b,c F. C. Massaro, b,c M. Cavagna, a,b,c J. B. A. Oliveira, a,b,c R. L. R. Baruffi, a,b,c J. G. Franco, Jr., a,b,c Center for Human Reproduction Prof. Franco Jr, Ribeirao Preto, Sao Paulo, Brazil; aPaulista Center for Diagnosis Research and Training, Ribeirao Preto, Sao Paulo, Brazil; cWomen’s Health Reference Centre, Perola Byington Hospital, Sao Paulo, Brazil.

OBJECTIVE: A higher incidence of XX embryos derived from IMSI (69.9%) compared to ICSI (52.5%) has been reported. Moreover, a significant difference (P<0.05) in the sex ratio of females to males at live birth has been demonstrated between children from IMSI (1:1.17) and the general population (0.95). However, spermatozoa with normal vacuoles seem to present higher chromosome abnormalities compared to normal spermatozoa, but this result is controversial. This study evaluated the impact of selecting morphological normal spermatozoa using motile sperm organelle morphology examination (MSOME) on sex ratio and numerical chromosome abnormalities.

DESIGN: Prospective.

MATERIALS AND METHODS: Forty patients who presented both normal spermatozoa and spermatozoa with large nuclear vacuoles (LNV, vacuoles occupying >50% nuclear area) were recruited. Single normal and LNV spermatozoa were selected using MSOME at 15,000x magnification and evaluated for X:Y and 18 chromosomes using FISH. The sex ratio (X:Y chromosome) and numerical chromosome abnormalities were detected using Fisher’s test.

RESULTS: A total of 1440 single normal spermatozoa were selected. A total of 757 (52.4%) presented X chromosomes, and 683 (47.4%) presented Y chromosomes. The sex ratio of XY chromosomes was 1:1. In contrast, the overall sperm X:Y ratio for 1445 single LNV spermatozoa was 0.84 (45.7% and 54.3%, respectively). There was a significant difference (P=0.0002) between the proportions. The mean frequency of sex chromosome disomy, 18 chromosome disomy, sex chromosome nullisomy, 18 chromosome nullisomy and diplodiplo were 3.7%, 2.1%, 0.9%, 0.2% and 0.2%, respectively. The levels of aneuploidy (disomy, nullisomy and diplodiplo) were significantly lower (6.1%) in normal spermatozoa compared to LNV (8.2%) (P<0.05).

CONCLUSION: The data imply that the selection of normal spermatozoa using MSOME skews the sex ratio toward female. It may be hypothesized that alterations in the Y chromosome may lead to morphological changes that prevent spermatozoa selection under high magnification. In addition, morphological normal spermatozoa showed lower numerical chromosome abnormalities. Normal spermatozoa selected using MSOME could avoid abnormal embryo formation.

O-198 Tuesday, October 21, 2014 05:00 PM

SPERM FLUORESCENCE IN SITU HYBRIDIZATION ANALYSIS OF AZF C MICRODELETION IN SEVERE OLOGOZOSPERMIA. X. Zhu, Z. Zhu, E. Zhi, Z. Li. Renji Hospital, Department of Urology, Shanghai Human Sperm Bank, Shanghai Jiao Tong University School of Medicine, Shanghai, China.

OBJECTIVE: Microdeletions of AZF (azoospermia factor) regions in Y chromosome were a genetic risk factor of spermatogenic failure and male infertility. Most laboratories carried out the AZF microdeleletion testing by using peripheral intravenous blood, and AZF microdeletion in spermatozoa of infertile patients was sometimes not identical to that of peripheral intravenous blood due to the existence of mosaicism. The aim of this study was to summarize the data of sperm fluorescence in situ hybridization (FISH) analysis of AZFc microdeletion in patients with severe oligospermia.

DESIGN: Exploratory research.

MATERIALS AND METHODS: The experiment included 16 patients with severe oligospermia which didn’t find AZF microdeletions in peripheral intravenous blood. Additionally, 2 normozoospermic cases and 2 oligozoospermic cases with AZFc microdeletions were recruited as positive controls and as negative controls respectively. Frequency of AZFc microdeletion in spermatozoa was detected by FISH with probes for chromosomes Y and chromosome 18. SRY gene was selected as the Y chromosome-specific signal, DAZ gene as AZFc-specific signal. A total of 100 spermatozoa were observed in each group.

RESULTS: A total of 2000 spermatozoa were counted from the semen samples in three groups. The hybridization rate was 97.2%, 95.3% and 96.4% in experiment group, positive group and negative group respectively. 5 out of 16 patients with severe oligospermia was found to bear DAZ gene signal missing in sperms with SRY signal, and the average missing rate was 8.7%. The positive control group was not found signal missing of DAZ gene in sperms with SRY signal, and DAZ gene signal was not found in the negative control group.

CONCLUSION: AZF microdeletions were not found in some patients with severe oligospermia via testing the peripheral intravenous blood, but sperm analysis by FISH revealed the presence of 8.7% abnormal spermatozoa with Y chromosome signal, in which DAZ gene signal were not found. This study is important to explain the mechanism of non-vertical transmission in patients with Y chromosome microdeletions, and can help to assess the genetic risk of Y chromosome microdeletions for male offspring and can be used for appropriate genetic counseling before the employment of assisted reproduction techniques.

Supported by: This work was supported by Shanghai Municipal Health Bureau Foundation (2011Y14).

O-199 Tuesday, October 21, 2014 05:15 PM


OBJECTIVE: The introduction of ICSI has revolutionized the treatment of severe male infertility. Moreover, ICSI using testicular spermatozoa has been commonly applied in the treatment of obstructive azoosperma (OA) and non-obstructive azoospermia (NOA). However, some authors describe similar and high fertilization and pregnancy rates in NOA patients whereas others report lower outcomes in NOA than in OA patients. In this study, we evaluated the differences on ART outcome after ICSI with frozen-thawed testicular spermatozoa between NOA and OA.

METHODS: Multiple testicular biopsy or microdissection TESE was performed in NOA patients while simple testicular biopsy was performed in OA patients. ICSI was performed with frozen-thawed testicular spermatozoa from NOA or OA. The ART outcome in the NOA group (4,295 oocytes, 317 cycles) and the OA group (4,187 oocytes, 336 cycles) were compared.

RESULTS: The wife’s average age and the number of the oocytes retrieved of both groups did not have the significant difference. However, the normal fertilization rate in the NOA group (67.9%) was significantly lower than that in the OA group (77.0%). The average number of embryos transferred was comparable between the two groups. And, the pregnancy rate and the implantation rate did not have the significant difference between the two groups. However, the delivery rate in the NOA group was significantly lower than that in the OA group (32.3% vs 40.8%). The mean birth weight in the
NOA group (2953.5 ± 487.9 g) was comparable with that in the OA group (2997.3 ± 356.9 g). The malformation rate was almost same between the two groups.

CONCLUSION: The normal fertilization rate and the delivery rate in the NOA group were significantly lower than those in the OA group.

O-200  Tuesday, October 21, 2014 05:30 PM
IDENTIFICATION OF IL-7R AS A CANDIDATE PROTEOMIC MARKER FOR ASEXTHENOZOOSPERMIA BY HIGH THROUGHPUT SCREENING (HTS) OF HUMAN SPERMATOZOA. E. Shlush, a,b S. Moskovtseva, a,b S. I. Moskovtsev, a,b S. I. Moskovtsev, a,b S. I. Moskovtsev, a,b a,b c CREAtE Fertility Centre, Toronto, ON, Canada; a,b c Obstetrics and Gynecology, University of Toronto, Toronto, ON, Canada; a Gynecology, Women’s College Hospital, Toronto, ON, Canada.

OBJECTIVE: Despite advances in the field of human reproduction, the underlying etiology of most male factor infertility cases is unknown. Some de novo maturation, genetic abnormalities, and cell intrinsic factors have recently been suggested to impair sperm function. In the current work we hypothesized that dysfunction related to interactions between spermatozoon and its surrounding microenvironment might play a role in some cases of male factor infertility. The aim was to identifying novel cell surface receptors on mature human spermatozoon and compare their expression in sperm from patients with asthenozoospermia with normal controls.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: We utilized a HTS methodology based on flow cytometry (FC) for the presence of 373 cell surface markers on mature sperm. HTS FC results were compared between 9 of healthy fertile sperm donors and 5 individuals with asthenozoospermia for the presence of these 373 protein receptors.

RESULTS: Our analysis identified many novel receptors present on the surface of mature spermatozoa that were not previously described. Several protein markers were expressed at a high level across all samples: CD47 (16.8% ± 6.8), CD52 (2.42 ± 14), CD257 (8.4 ± 4), CD256 (29.2 ± 5), CD257 (5.8 ± 6). In contrast CD127, also known as interleukin 7 receptor (IL-7R) which plays an important role in T and B cell differentiation, was significantly lower (1.6 ± 1.3%) in the group of asthenozoospermia in comparison to the control group (7 ± 4.5%), P = 0.03.

CONCLUSION: Our preliminary results demonstrated several cell surface proteins not previously shown to be expressed on human spermatozoa. We identified a novel potential role for the IL-7 pathway in human sperm motility. Interestingly, it has been previously shown that IL-7 knockout mice have reduced fertility with significantly reduced litter size. Our future studies will focussed on elucidating the potential role of IL-7 and its receptor in male fertility. HTC FC analysis of sperm protein expression is an underutilized tool in reproductive medicine and our results might shed light on the function of novel human sperm receptors and their differential expression between fertile and infertile men. Moreover the role of interleukins and other cytokines and external signals in sperm motility can be explored by the suggested methods.

O-201  Tuesday, October 21, 2014 05:45 PM

OBJECTIVE: Fertilization and pregnancies can be obtained with spermatozoa recovered from the seminiferous tubules. Sertoli cell only syndrome (SCOS), maturation arrest (MA), and hypospermatogenesis (HS) and, with or without focal spermatogenesis are the commonest histological patterns of patients with non-obstructive azoospermia (NOA). To our knowledge, no study has specifically examined the results of intracytoplasmic sperm injection (ICSI) in SCOS, MA, and HS patients. The aim of this study is to assess sperm retrieval rates (SRR) by microdissection testicular sperm extraction (micro-TESE), fertilization rate, and embryonic development among patients with presumed SCOS, MA, and HS in testicular biopsy in those couples whom spermatozoa were obtained.

DESIGN: Retrospective clinical analysis.

MATERIALS AND METHODS: We retrospectively evaluated 108 patients with NOA who underwent micro-TESE between September 2013 and April 2014, and identified 108 novel spermatogenesis in whom SCOS, MA, and HS were reported at pathological examination. We excluded obstructive azoospermic (OA) patients and >39 years age of the spouses at the time of ICSI cases in this study. At the same session of micro-TESE, small tissue specimens (from upper, middle, and lower pole) were placed in Bouin’s solution and sent to the histopathology laboratory. No significant difference was shown among the ages of the spouses at the time of ICSI (33.1 ± 3.7 years in SCOS and 33.8 ± 3.2 in HS). Two pronuclei (2PN) oocytes, blastocysts development, good-quality blastocysts (Grade 3BB and above, on day 5 by the Gardner score), biochemical pregnancies, clinical pregnancies and implantation rates were examined.

RESULTS: Spermatozoa were retrieved in 67 of 108 (43.5%) patients with NOA in whom micro-TESE was performed. In 108 patients with histopathology reports, 85 (78.7%), 9 (8.3%), and 14 (13.0%) patients had presumed SCOS, MA, and HS, respectively. SRR in SCOS (31/85=36.5%) and MA (2/9=22.2%) was lower than patients with HS (14/14=100%, p<0.001). Thirty-four, 2, and 12 ICSI cycles were performed in SCOS, MA, and HS, respectively. 2 PN oocytes, blastocysts development, and good-quality blastocysts rates were 53.6%, 38.8%, and 38.5% in SCOS and 63.9%, 39.0%, and 30.4% in HS. Biochemical pregnancy, clinical pregnancy and implantation rates were 42.9% (9/21), 28.6% (6/21), and 25.0% (6/24) in SCOS and 25.0% (4/16), 18.3% (3/16), and 15.8% (3/19) in HS (no significant differences, respectively).

CONCLUSION: Good fertilization and embryonic development were achieved without significant differences even in presumed SCOS in those couples whose spermatozoa were obtained.

O-202  Tuesday, October 21, 2014 06:00 PM

OBJECTIVE: The Internet is often a primary source of information for infertility patients; however, there is little data assessing the quality of this material especially aimed at male infertility. Our objective was to evaluate SART-member fertility clinic websites for availability and context of information regarding male factor infertility.

DESIGN: Cross-sectional evaluation.

MATERIALS AND METHODS: Between 2/2014-4/4014, 396 SART-member fertility clinic websites were evaluated by two independent researchers. Websites were surveyed for the following characteristics: practice location, type, and size, along with general information on male infertility, male factor treatment options, male sexual dysfunction, fertility preservation, cost of treatment, and the presence of an onsite male infertility specialist. Chi-square tests were used to assess differences between groups.

RESULTS: 98% (387/396) of clinics had a website, of which 80% were private and 20% academic centers. 26% of practices performed ≥500 cycles/year while 74% performed <500 cycles/year. 88% of websites provided general information on male infertility and 83% reviewed male treatment options. 65% of websites discussed surgical treatment and 52% discussed microsurgery. 15% of fertility clinics advertised a male infertility specialist as part of their practice. 17% provided information on male sexual dysfunction. 55% of clinics offered male fertility preservation services. 3% of websites discussed the cost of male infertility treatments. Compared to private practices, academic centers were more likely to have a male specialist on staff (50% vs 11%, p<.001). There was no difference between academic and private practices in the presentation of general treatment options, surgical treatment, microsurgery, or discussion of sexual dysfunction (p>.05). Large volume practices were more likely to discuss male sexual dysfunction (26% vs 14%, p<.006), but there were no differences regarding general treatment options, surgery or microsurgery compared to small practices (p>.05). Practices with a male specialist were more likely to offer microsurgery (60% vs 26%, p<.001) and more likely to discuss sexual dysfunction (39% vs 13%, p<.001).

CONCLUSION: Although a male factor component can affect 50% of infertility couples, our data shows that there is still a significant lack of patient information regarding specific treatment options among SART clinic websites. While the presence of a male specialist onsite significantly improves available patient information, only 15% of clinics offer a male specialist as part of their practice.
REPRODUCTIVE BIOLOGY - RESEARCH

O-203 Tuesday, October 21, 2014 04:15 PM


OBJECTIVE: To assess the duration of the second and third cell cycles (cc) as predictors of blastocyst formation and implantation (I).

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: Monitoring of 6970 embryos revealed that 3107 developed into blastocysts (B) and 3863 did not (NB). After transfer, 328 embryos were designated as 100%, when the number of gestational sacs matches with the transferred B or 0%, when no chemical gestation was achieved. Timing of cleavage (hrs post-ICSI) from 2 to 8 cells (t2, t3, t4, t5, t6, t7, t8) was determined by time-lapse imaging. The following variables were studied: cc2a: cc length of the 1st blastomere to cleave from 2 to 3 cells; cc2b: cc length of the 2nd blastomere to cleave from 2 to 4 cells; cc3a: b, c, d, cc3: cc duration of the blastomere that cleaves to 5, 6, 7 and 8 cells; cc5: average cc3 (cc3a+cc3b+cc3c+cc3d)/4; blastomere synchrony in cleavage at cc2 (SR2 = cc2a/cc2b) and cc3 (SR3 =cc3/(t8-t3)).

RESULTS: Mean ± SD and 95% confidence interval (CI) of variables according to blastocyst (B or NB) and implantation (0 or 100) status. When significance between categories was detected, variables were studied by quartiles and the optimal range (OR) for B and I was defined when appropriate.

Results

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD according to B (95CI)</th>
<th>In/out of OR (%B)</th>
<th>ODS Ratio OR</th>
<th>Mean± SD according to I (95CI)</th>
<th>In/out of OR (%I)</th>
<th>ODS Ratio OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC2a NB</td>
<td>9.8±0.1 (9.6-10.0)/B: 10.2±3.9 (10.4-10.7)</td>
<td>9.76-12.7 (54.9)/Out (32.6%)</td>
<td>1.6</td>
<td>0: 10.9±3.6 (10.5-11.2)/100: 10.5±3.5 (10.2-10.9)</td>
<td>0: 1.6</td>
<td>0: 10.9±3.6 (10.5-11.2)/100: 10.5±3.5 (10.2-10.9)</td>
</tr>
<tr>
<td>CC2b NB</td>
<td>13.1±2.6 (12.9-13.3)/B: 12.3±3.9 (12.2-12.4)</td>
<td>11.3-14.3 (51.6)/Out (36.1%)</td>
<td>1.4</td>
<td>0: 12.4±3.6 (12.1-12.7)/100: 12.0±3.3 (11.7-12.3)</td>
<td>0: 1.7</td>
<td>0: 12.4±3.6 (12.1-12.7)/100: 12.0±3.3 (11.7-12.3)</td>
</tr>
<tr>
<td>RS2 NB</td>
<td>0.7±0.3 (0.7-0.8)/B: 0.9±0.2 (0.8-0.9)</td>
<td>≤80.5 (28.7)/&gt;80.5 (49.7)</td>
<td>0.8</td>
<td>0: 0.9±0.2 (0.8-0.9)/100: 0.9±0.2 (0.8-0.9)</td>
<td>0: 1.6</td>
<td>0: 0.9±0.2 (0.8-0.9)/100: 0.9±0.2 (0.8-0.9)</td>
</tr>
<tr>
<td>CC3a NB</td>
<td>13.4±2.2 (13.1-13.7)/B: 13.6±5.6 (13.4-13.8)</td>
<td>11.5-16.5 (55.5)/Out (39.8)</td>
<td>1.3</td>
<td>0: 13.9±4.8 (13.4-14.3)/100: 13.6±4.3 (13.1-14.0)</td>
<td>0: 2.0</td>
<td>0: 13.9±4.8 (13.4-14.3)/100: 13.6±4.3 (13.1-14.0)</td>
</tr>
<tr>
<td>CC3b NB</td>
<td>17.7±8.4 (17.4-18.0)/B: 16.5±5.8 (16.3-16.7)</td>
<td>16.3±4.8 (15.8-16.7)/100: 15.6±4.6 (15.2-16.1)</td>
<td>1.3</td>
<td>0: 17.9±6.0 (17.3-18.4)/100: 16.5±4.9 (16.0-17.0)</td>
<td>0: 2.0</td>
<td>0: 17.9±6.0 (17.3-18.4)/100: 16.5±4.9 (16.0-17.0)</td>
</tr>
<tr>
<td>CC3c NB</td>
<td>19.3±9.2 (18.9-19.6)/B: 17.8±6.7 (17.6-18.0)</td>
<td>19.9±7.3 (19.1-20.7)</td>
<td>1.4</td>
<td>0: 21.6±7.9 (20.8-22.4)/100: 19.9±7.3 (19.1-20.7)</td>
<td>0: 2.0</td>
<td>0: 21.6±7.9 (20.8-22.4)/100: 19.9±7.3 (19.1-20.7)</td>
</tr>
<tr>
<td>CC3d NB</td>
<td>23.5±10.6 (23.0-23.9)/B: 21.8±8.9 (21.5-22.2)</td>
<td>≤21.0 (48.0)/Out (34.1)</td>
<td>1.7</td>
<td>0: 17.2±4.8 (16.7-17.7)/100: &lt;18.9 (48.5)/Out (38.1)</td>
<td>0: 2.0</td>
<td>0: 17.2±4.8 (16.7-17.7)/100: &lt;18.9 (48.5)/Out (38.1)</td>
</tr>
<tr>
<td>acc3 NB</td>
<td>17.6±6.5 (17.3-17.8)/B: 17.1±5.2 (16.9-17.3)</td>
<td>0±7.0 (7.0-7.0)/B: 0.7±0.1 (0.7-0.8)</td>
<td>1.5</td>
<td>0: 0.7±0.1 (0.7-0.9)/100: 0.8±0.1 (0.7-0.8)</td>
<td>0: 1.6</td>
<td>0: 0.7±0.1 (0.7-0.9)/100: 0.8±0.1 (0.7-0.8)</td>
</tr>
</tbody>
</table>

CONCLUSION: cc2 and cc3 are associated with development to B and I, respectively.

O-204 Tuesday, October 21, 2014 04:30 PM

RELATION OF SPERM METHYLATION ABNORMALITY TO MISCARRIAGE VILLUS METHYLATION ABNORMALITY. A. Sato,* E. Otsu, T. Arima,* T. Utsunomiya.∗“St.Luke Clinic, Oita City, Oita, Japan; *Tohoku University Graduate School of Medicine, Sendai City, Miyagi, Japan.

OBJECTIVE: Genomic imprinting, which describes the allele-specific expression of certain genes, accounts for the requirement of both maternal and paternal genomes in normal development. The imprints that are initiated in the germ line persist through preimplantation development and involve the formation of an epigenetic mark at specific loci in a parent-of-origin-specific manner. DNA methylation is the most well studied epigenetic mark that distinguishes the maternal and paternal alleles of imprinted genes. In this study, we examined the methylation profile of four autosomal imprinted genes in DNA obtained from ART conceptions and matched parental sperm in order to determine whether these alterations were inherited.

DESIGN: Laboratory experimental study.

MATERIALS AND METHODS: Between January 2011 and September 2013, 238 cases which resulted in miscarriage following ART were investigated. Of the 238 cases, 48 villus were found to have normal karyotype and their origin of sperm. We examined DNA methylation at imprinted gene (H19, GTL2, PEG1 and UTF1) by bisulphate PCR methylation analysis. Our project was carried out after receiving the patients’ consent and with the approval of the institutional ethics committee.

RESULTS: We found 10/48(20.8%) samples that showed abnormal DNA methylation at one or more imprinted loci. In seven out of the ten cases where there were abnormal DNA methylation in the ART samples, identical alterations were present in the parental sperm. Six samples showed abnormality of the H19 and one sample showed a GTL2 abnormality. All abnormalities were due to paternal imprint type genes.

CONCLUSION: In our previous study of the methylation abnormality in sperm, maternal type imprint genes (83.3%(20/24)) were found to have a higher frequency of abnormality than paternal type imprint genes (58.3%(14/24)). Villus and sperm abnormality were found in the paternal imprint genes. Though sperm have both paternal and maternal imprint genes, in this study, abnormalities were seen in the paternal imprint genes, it was leading us to suggest that maternal imprint genes cannot be the cause of miscarriage. Of the 48 samples with normal karyotype examined in this research, 38 were found to also have normal methylation. Further study of the relationship between imprint abnormality and miscarriages should be conducted.

O-205 Tuesday, October 21, 2014 04:45 PM


OBJECTIVE: We have previously shown that VFBT cycles compare favorably to fresh ET. The purpose of this study was to evaluate whether trophoectoderm biopsy-preimplantation genetic screening (PGS) technology optimizes pregnancy and implantation outcomes to best support SET efforts?

DESIGN: Between 2012 and 2013, all VFBT cycles were split in two groups. Group 1 (n=122) patients electively chose to vitrify all fresh
TABLE 1.

<table>
<thead>
<tr>
<th>&lt;35y.o.</th>
<th># ET</th>
<th>% Clinical Preg</th>
<th>% Live Birth</th>
<th>% LB/ Cycle</th>
<th>% SAB</th>
<th>% Impl SET: % LB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>67</td>
<td>74.6</td>
<td>62.7</td>
<td>62.7</td>
<td>16</td>
<td>55.5</td>
</tr>
<tr>
<td>Group 2</td>
<td>53</td>
<td>88.7</td>
<td>84.9*</td>
<td>80.4</td>
<td>4.3</td>
<td>87.5**</td>
</tr>
<tr>
<td>35-37y.o.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>31</td>
<td>77.4</td>
<td>61.3</td>
<td>61.3</td>
<td>20.8</td>
<td>53.5</td>
</tr>
<tr>
<td>Group 2</td>
<td>53</td>
<td>81.1</td>
<td>69.8</td>
<td>67.3</td>
<td>11.6</td>
<td>86.8**</td>
</tr>
<tr>
<td>38-40y.o.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>16</td>
<td>43.8</td>
<td>31.3</td>
<td>31.3</td>
<td>28.6</td>
<td>27.6</td>
</tr>
<tr>
<td>Group 2</td>
<td>46</td>
<td>80.4*</td>
<td>78.3**</td>
<td>70.6*</td>
<td>2.8*</td>
<td>74.6**</td>
</tr>
<tr>
<td>41-42y.o.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>6</td>
<td>33.3</td>
<td>33.3</td>
<td>33.3</td>
<td>33.3</td>
<td>25</td>
</tr>
<tr>
<td>Group 2</td>
<td>17</td>
<td>76.5</td>
<td>76.5</td>
<td>48.2</td>
<td>0</td>
<td>69.6*</td>
</tr>
<tr>
<td>43y.o.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group 2</td>
<td>2</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100*</td>
</tr>
</tbody>
</table>

* Differences between rows within age subgroups by column are different (P<0.05; **P<0.01)

CONCLUSION: Aneuploidy determination of BL, in combination with VTF-ALL, significantly increased implantation in all age groups. The live birth per SET was higher in women ≤34y.o. and the 38-40y.o. age groups. Significance was unable to be determined for women ≥41y.o., since no SET was performed in Group 1. Interestingly, the 35-37y.o. age group did not reveal an increased live birth rate. Note, many failed cycles in Group 2 women, age 35-37y.o., repeated a second SET with success. Further studies evaluating metabolomic factors and gene sequencing may provide answers as to why some euploid embryos fail to implant or spontaneously abort.

O-207 Tuesday, October 21, 2014 05:15 PM


OBJECTIVE: Capturing time-lapse images of human embryo development appeared that human embryos sometimes cleaved abnormally from 1 cell to 3 cells or more at 1st or 2nd stage. However, the characteristics of abnormally-cleaved embryos and their subsequent development are unknown. Here the developmental competence of embryos which cleaved abnormally at 1st or 2nd cleavage was examined.

DESIGN: Retrospective clinical study.

MATERIALS AND METHODS: The study included 39 patients who underwent embryo transfer (ET) on day 3 between August 2013 and April 2014 after obtaining the informed consent. Time-lapse images of 282 embryos were taken every 10 min using a time-lapse cinematography (TLC, Vitrolife) after the confirmation of normal fertilization. After day 3 ET, surplus embryos were cultured until day 6. The abnormal cleavage was investigated using the TLC. Effects of abnormalities at 1st and 2nd cleavage on the blastulation and the implantation potential were assessed. Moreover, the time which was required from insemination until 1st cleavage was measured.

RESULTS: The abnormal cleavage was observed in 77 embryos at 1st (32.3%) and in 44 embryos at 2nd cleavages (15.6%). The blastulation, the morphologically-good blastocyst and pregnancy rates were 62.5% (80/128), 43.0% (55/128), and 50.0% (12/24) in embryos which didn’t show any abnormalities at 1st and 2nd cleavage, 25.0% (18/72), 8.3% (6/72), and 5.0% (1/24) in embryos which showed abnormalities at 1st cleavage, and 35.3% (12/34), 14.7% (5/34), and 10.0% (1/10) in embryos which showed abnormalities at 2nd cleavage, respectively. The values in all 3 parameters of embryos which showed abnormalities at 1st or 2nd cleavage were lower than those of embryos showed no abnormalities (P < 0.05). The time required from insemination until 1st cleavage of embryos which showed abnormalities at 1st cleavage (31.0±0.6h) was longer than those for others (P < 0.001; no abnormalities: 25.4±0.3h, 2nd abnormal cleavage:25.2±0.5h).

CONCLUSION: Half of embryos showed abnormal cleavage at 1st or 2nd cleavage and these embryos had much lower developmental potential. Moreover, embryos showed abnormalities at 1st cleavage took longer time to complete 1st cleavage suggesting that observation of 1st cleavage timing is a good marker of embryo selection. These abnormally-cleaved embryos had low implantation potential despite their good morphology on day 3.
THE SOURCE OF OOCYTES, FRESH OR VITRIFIED, DOES NOT AFFECT IMPLANTATION POTENTIAL BASED ON KINETIC MARKERS. M. Aragón,¹ N. Basile,¹ S. Pareja,² A. Cobó,² F. Bronet,² M. Meseguer.² ¹LAB FIV, IVI Madrid, Madrid, Spain; ²LAB FIV, IVI Valencia, Valencia, Spain.

OBJECTIVE: A published algorithm based on kinetic markers classifies embryos from A to E according to implantation potential (1). The objective of this study is to evaluate if the source of oocytes (fresh or vitrified) has an impact on the percentage of embryos falling within each category.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: Oocytes from mixed Intra Citoplasmatic Sperm Injection cycles (fresh + vitrified oocytes) were monitored in a time-lapse system (Nikon: 287 fresh and 271 vitrified). Variables studied included: exact timing to 2 (t2), 3 (t3), 4 (t4), and 5 cell (t5). We also evaluated cell division intervals such as cc2 = t3−t2 and t4−t3.

To evaluate embryo quality based in morphokinetics, the proportion of optimal embryos was determined from our previous observations, identifying embryos in a best time range with a higher implantation potential. The proposed optimal timings were as follows: t5 = 48±5–56.6 hours, cc2 = 12 hours and s2 < 0.75 hours. To further evaluate correlation between morphokinetic parameters and the type of the oocyte (fresh or vitrified), we developed a hierarchical classification: t5 (if 48.8±5≤t5≤56.6 then grade A or B, otherwise grade C or D) and secondary variable: s2 (if s2 ≤0.75 then grade A or B, otherwise B or D) divides the remaining embryos into grades A, B, C or D in order of decreasing implantation rate. With cc2, we obtained a plus/minus division in each of the previous mentioned categories. We applied a Chi-squared for comparison of percentages with p<0.05 considered to be statistically significant.

RESULTS: Significant differences were observed between fresh and vitrified oocytes for t2: 27.8h (CI 95% 27.4–28.3h) vs. 29.4h (CI95% 28.8–30.0h); t3: 38.2h (CI95% 39.0–41.7h) vs. 40.1h (CI95% 39.5–40.6h) and t4: 40.9h (CI95% 40.3–41.6h) vs. 42.9h (CI95% 41.8–43.9h) respectively. No differences were observed for the rest of the variables. Considering the variables included in the algorithm (t5, cc2 and s2), no differences were observed in the percentages of embryos falling within the optimal ranges proposed. Once classified by the algorithm the percentage of embryos within each category was similar between fresh and vitrified oocytes respectively: A- (6.7% vs. 3.7%); A+ (8.8% vs. 7.0%); B- (4.1% vs. 3.0%); B+ (6.7% vs. 4.1%); C- (4% vs. 7.4%); C+ (4.7% vs. 5.5%); D- (8.0% vs. 7.7%); D+ (4.1% vs. 3.3%); and E (36.4% vs. 35.6%), p = 0.270.

CONCLUSION: The percentages of embryos in each category is similar regardless of the type of oocytes utilized.

FREEZE ALL POLICY: FRESH VERSUS ELECTIVE FROZEN-TAWED EMBRYO TRANSFER. M. Roque, S. Geber, M. Sampaio, S. Pareja, M. Aragon, A. ³C6 a 12 years) C. Vidal, N. Basile, D. Castello, P. Alama, 12 (n=209) regardless of the type of oocytes utilized. 7.4%; C+ (4.7% vs. 5.5%); D- (8.0% vs. 7.7%); D+ (4.1% vs. 3.3%); and A- (6.7% vs. 3.7%); A+ (8.8% vs. 7.0%); B- (4.1% vs. 3.0%); B+ (6.7% vs. 4.1%); C- (4% vs. 7.4%); C+ (4.7% vs. 5.5%); D- (8.0% vs. 7.7%); D+ (4.1% vs. 3.3%); and E (36.4% vs. 35.6%), p = 0.270.

CONCLUSION: The metabolic profile in the spent culture media of day-3 embryos is different in normoweight and obese women with a higher deviation when PCOS is associated.

Supported by: IVI Valencia research fund. FD’s participation was Supported by the Spanish Ministry of Economy and Competitiveness, through the Miguel Servet Programme (CP13/00075) co-founded by FEDER.

O-208 Tuesday, October 21, 2014 05:45 PM

DAY 3 EMBRYO METABOLICOS IN THE SPENT CULTURE MEDIA IS ALTERED IN OBSENE WOMEN UNDERGOING IVF. J. Bellver,²abc M. J. de los Santos,² a P. Alama,² d Castello,² c Privetera,² D. Galliano,² E. Labarta,² C. Vidal,² a Pellicer,ab c F. Domínguez.²,² ¹Instituto Valenciano de Infertilidad, Valencia, Spain; ²Department of Pediatrics, Obstetrics and Gynecology, Faculty of Medicine, University of Valencia, Valencia, Spain; ³Fundación IVI, Paterna, Valencia, Spain; ⁴NCLIVA Biomedical Research Institute, Paterna, Valencia, Spain.

OBJECTIVE: To determine whether the metabolic profile in the spent culture media of day-3 embryos is different in obese and normoweight women undergoing IVF, also considering the association with polycystic ovary syndrome (PCOS).

DESIGN: Prospective case-control analysis.

MATERIALS AND METHODS: Twenty-eight young (≤38 years) women with normal uterus and ovaries, and absence of endometriosis, hydro- salpinx or smoking habit were recruited. Patients underwent their first IVF cycle and had the first embryo transfer after endometrial priming and embryo thawing. Data were described as the mean ± standard deviation or percent- ages. The statistical analysis was performed using Student’s t test, the chi- square test, and linear regression models. A p value of <0.05 was considered statistically significant. The main outcome measure was ongoing pregnancy rate.

O-210 Tuesday, October 21, 2014 06:00 PM

FREEZE ALL POLICY: FRESH VERSUS ELECTIVE FROZEN-TAWED EMBRYO TRANSFER. M. Roque, S. Geber, M. Sampaio, F. Guimaraes, M. Valle. Origen - Center for Reproductive Medicine, RJ, RJ, Brazil.

OBJECTIVE: Controlled ovarian stimulation (COS) may adversely affect endometrial receptivity during in vitro fertilization (IVF) cycles. It has been suggested that supraphysiologic hormonal levels during COS, mainly pro- gesterone elevation (PE) on the day of human chorionic gonadotropin admin- istration, are associated with a decreased probability of pregnancy in fresh cycles; in these cases, the patient may benefit from frozen-thawed embryo transfer (FET). The main objective of this study was to compare IVF out- comes between fresh embryo transfer and elective FET (freeze all policy), considering that fresh embryo transfers were performed only in cases without progestosterone elevation.

DESIGN: Prospective observational cohort study.

MATERIALS AND METHODS: The study was conducted between January 2012 and December 2013. Accepting an alpha risk of 0.05 and a beta risk of 0.2 in a two-sided test, at least 229 subjects in each group were necessary to recognize a relative risk ≥1.3 as statistically significant. A total of 625 patients submitted to COS with gonadotropin-releasing hormone (GnRH) antagonist protocol and day-3 embryo transfer were included. In the fresh group (n=372), embryo transfers were performed only if proges- terone levels were ≤1.5 ng/mL on the trigger day. The freeze-all group (n=253) comprised patients that had all embryos cryopreserved in the fresh cycle and had the first embryo transfer after endometrial priming and embryo thawing. Data were described as the mean ± standard deviation or percent- ages. The statistical analysis was performed using Student’s t test, the chi- square test, and linear regression models. A p value of <0.05 was considered statistically significant. The main outcome measure was ongoing pregnancy rate.

RESULTS: IVF outcomes in fresh and freeze-all groups are expressed in the table below.
O-212 Tuesday, October 21, 2014 04:30 PM

OVARIAN LESIONS VOLUMES AS A SCREEN FOR MALIGNANCY IN PEDIATRIC OVARIAN TUMORS. P. I. Abbas, a S. C. Elder, a A. R. Mehollin-Ray, b R. M. Braverman, b M. E. Lopez, a J. C. Francis, c J. E. Dietrich. a Surgery, Baylor College of Medicine, Houston, TX; b Pediatric Radiology, Baylor College of Medicine, Houston, TX; c Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX.

OBJECTIVE: Preoperative evaluation of ovarian tumors is essential to determine appropriate treatment. Benign ovarian tumors undergo an ovarian sparing surgery to preserve fertility while malignant lesions undergo oophorectomy. Our study assesses the utility of gonadal volumes and tumor markers to evaluate the risk of malignancy.

DESIGN: Retrospective chart review.

MATERIALS AND METHODS: Female patients (8-18 years) who had an ovarian operation from January 2008 to December 2012 were included. Demographics, preoperative ultrasonographic gonadal measurements, and tumor markers were collected. Volumes were calculated with the prolate ellipsoid formula. Data are presented as medians.

RESULTS: 123 females at a median age of 13.7 years (IQR 12.5-16 years) were included. 115 girls had benign pathology and 8 had malignant pathology. All patients with malignant pathology had an oophorectomy. Of those with benign pathology, 22 patients had an oophorectomy and 93 patients underwent ovarian sparing surgery. The benign lesion volume was significantly smaller than the malignant lesion volume and beta-HCG, AFP, and LDH were less likely to be elevated. There was no difference in ovarian volume ratio between malignant and benign pathology.

A ROC curve analysis of lesion volume to malignancy (AUC 0.84, p=0.001) revealed a threshold volume of <184 cm³ (100% sensitivity, 54% specificity, NPV 100%, PPV 13%) to screen for malignancy. This holds true when applied to our dataset. Of 62 girls with volumes <184 cm³, none had malignant pathology.

CONCLUSION: This study reveals the novel use of ovarian volumes as a potential preoperative screening method to define candidates for ovarian sparing surgery. Ovarian volumes <184 cm³ are more predictive of benign disease. Further validation and correlation with laboratory parameters is warranted.

O-213 Tuesday, October 21, 2014 04:45 PM

THE IN-VIVO EFFECTS OF SUPEROXIDE DISMUTASE MANDATE THE DEVELOPMENT. A. O. Aomite, a N. M. Fletcher, b M. G. Saed, c O. Nusrat, a M. S. Abusamaan, a J. Belotte, b M. P. Diamond, b G. M. Saed. a Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI; b Obstetrics and Gynecology, Georgia Regents University, Augusta, GA.

OBJECTIVE: The sequels of intra-abdominal adhesions following surgery are a cause of significant morbidity. Superoxide dismutase (SOD) protects against toxic effects of superoxide anions, which plays a fundamental role in adhesion development. We have previously showed that SOD treatment effectively reduces the adhesion phenotype biomarkers in fibroblasts isolated from adhesion tissues. The objective of this study is to evaluate the in vivo effects of SOD on the incidence and severity of post-operative adhesion development after cecal abrasion.

DESIGN: Prospective experimental study.

MATERIALS AND METHODS: A total 80 female Sprague-Dawley rats (40 in the SOD and 40 in the control group) were randomized, as directed by a power analysis to detect a 33% reduction in adhesion formation. Superoxide dismutase was delivered to rats via a single use ALZET Osmotic Pump (Cupertino, CA), inserted into the peritoneal cavity through a 1 cm midline incision under isoflurane anesthesia. Pumps were filled with 300 units of SOD in 100 μl of saline or 100 μl of saline for controls. After two weeks, cecal abrasion was performed and the animals were euthanized 7 days later whereupon the degree of adhesion development was quantified.

RESULTS: So far, 33 rats in the SOD and 31 in the control groups have been subjected to cecal abrasion. There was no significant difference in the mean (± SD) weight (g) between the groups. There was a non-significant reduction in adhesion to the abraded area (37.1% vs. 30.0%, OR 0.6 [CI, 0.2 – 1.5]), adhesion to > 25% of the added area (25.7% vs. 38.2%, OR 0.6 [CI, 0.2 – 1.6]), and the proportion of vascular and cohesive adhesions (28.6% vs. 38.2%, OR 0.7 [CI, 0.2 – 1.8]) in SOD compared with the control group. SOD almost significantly reduced the proportion of animals with de novo adhesions (5.7% vs. 23.5%, OR 0.2 [CI, 0.4 – 1.0]).

CONCLUSION: Preliminary results suggest that intra-peritoneal insertion of ALZET osmotic pump containing SOD may significantly reduce de novo adhesion development but may not reduce adhesion development at the site of cecal abrasion.

Supported by: This work is Supported in part by NIH/NICHD grant number K12HD001254.
OBJECTIVE: To compare the use of a portable digital camera based hysteroscope (pHSC) with a traditional hysteroscope (tHSC) in diagnostic procedures.

DESIGN: Prospective study.

MATERIALS AND METHODS: Two consultants and three registrars performed diagnostic hysteroscopies by using the two systems. In the pHSC group, a digital mirrorless camera (Sony NEX-3) was connected with a Betocchi hysteroscope (2.9 mm optical system and 5 mm sheath; Storz) by using a C-mount optical coupler; a portable handheld cold light source was used. The procedure was visualized on the tiling 3-inch LCD screen of the digital camera. In the tHSC group, common hysteroscopic equipment and the same hysteroscope were used. The endpoints of the study were: to perform the procedure, number of failed procedures, discomfort perceived by the patients, difficulty experienced by the physicians in performing the procedure. Two consultants blindly reviewed the videos of the procedures and judged the quality of the images.

RESULTS: 196 patients (43.3% were menopausal) were included in the study. The time required to visualize the uterine cavity by vaginoscopic approach was significantly higher in the pHSC group than in the tHSC group both when the procedures were performed by consultants (p = 0.016) and by registrars (p = 0.003). The time required to enter the uterine cavity was similar between pHSC and tHSC when the hysteroscopies were performed by consultants (p = 0.579), but it was higher in the pHSC group when the hysteroscopies were performed by registrars (p = 0.039). The time required to examine the uterine cavity was similar between pHSC and tHSC when the procedure was performed by consultant (p = 0.234) and by registrars (p = 0.453). The number of failed hysteroscopies was similar in the two study groups (p = 0.651). Patients experienced a similar discomfort in the two groups when the consultants performed the procedures (p = 0.642); there was a non-significant trend toward a high discomfort in the pHSC group when the registrars performed the hysteroscopies (p = 0.056). Compared with the tHSC, the pHSC was more difficult to be used by consultants (p = 0.024) and by registrars (p = 0.004). The quality of the images was similar between pHSC and tHSC (p = 0.893).

CONCLUSION: The pHSC allows performing diagnostic hysteroscopies; however, the procedure may be more difficult to be performed because of the visualization of the procedure on the LCD-screen of the digital camera.

CONCLUSION: Endometrial scratching performed once before ICSI-ET, increases the chance of clinical pregnancy and ongoing pregnancy in the patients with previous implantation failure.

O-216 Tuesday, October 21, 2014 05:30 PM

LAPAROSCOPIC SURGERY FOR DISTAL TUBAL OCCLUSIONS: LESSONS LEARNED FROM A HISTORICAL SERIES OF 434 CASES. A. Audebert,1 J. L. Pouly.2 1Institut Greenblatt, Bordeaux, France; 2Unite de FIV, CHU de Clermont Ferrand, Clermont Ferrand, France.

OBJECTIVE: to evaluate the success rate of laparoscopic neosalpingostomy and the factors affecting the results in terms of intrauterine pregnancy (IUP), delivery (DEL) and ectopic pregnancy (EP).

DESIGN: retrospective analysis of prospectively recorded data.

MATERIALS AND METHODS: a continuous series of 434 patients who underwent laparoscopic neosalpingostomy is analysed with a follow-up of more than 10 years. Statistical analysis includes univariate and multivariate analysis, and crude and actuarial success rates.

RESULTS: 28.8% of the patients presented an IUP, 24.4% delivered and 9% presented an EP. The 5-year actuarial rate of delivery was 37%. The crude and actuarial delivery rates are largely dependent on the tubal stage (stage 1: 53.1%, stage 2: 43.1% stage 3: 24.0% and stage 4: 23.1%).

CONCLUSION: neosalpingostomy must not be proposed in selected cases according to tubal stages adhesion stage and chlamydial serology. When neosalpingostomy is performed, fimbrial erosion must be done with sutures rather than with electrocoagulation.

O-217 Tuesday, October 21, 2014 05:45 PM

LONG-TERM FERTILITY AND BLEEDING OUTCOMES AFTER ROBOTIC, LAPAROSCOPIC, AND ABDOMINAL MYOMECTOMY. R. Flyckt, E. Soto, B. Nutter, T. Falcone, Obstetrics, Gynecology, and Women’s Health Institute, Cleveland Clinic, Cleveland, OH.

OBJECTIVE: Multiple studies indicate improved short-term outcomes following minimally invasive myomectomy, such as postsurgical pain, blood loss, and length of hospitalization (1-5). However, it is unknown whether long-term outcomes such as fertility and bleeding patterns differ between different approaches to non-hysteroscopic myomectomy. The Uterine Fibroid Symptom and Quality of Life (UFS-QOL) questionnaire is validated in assessing...
Mean VAS Scores of Patients Receiving Ropivacaine vs. Saline

<table>
<thead>
<tr>
<th></th>
<th>0-2 Hours</th>
<th>2-4 Hours</th>
<th>4-8 Hours</th>
<th>8-12 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo, n = 30</td>
<td>4.0</td>
<td>3.6</td>
<td>3.1</td>
<td>2.2</td>
</tr>
<tr>
<td>Ropivacaine, n = 25</td>
<td>2.6</td>
<td>2.5</td>
<td>2.0</td>
<td>2.2</td>
</tr>
<tr>
<td>Placebo vs. Ropivacaine p value</td>
<td>0.08</td>
<td>0.10</td>
<td>0.58</td>
<td>0.88</td>
</tr>
</tbody>
</table>

GENETIC COUNSELING

O-219 Wednesday, October 22, 2014 11:15 AM

EXPANDED CARRIER SCREENING IN AN INFERTILE POPULATION: HOW OFTEN DOES IT IMPACT CLINICAL DECISION MAKING? J. M. Fransasiak,a,b M. Olchaaa, P. A. Bergh,b K. H. Hong,b M. D. Werner,b E. J. Formana,b R. T. Scott, Jr.,a,b RWJ SOM, RU, New Brunswick, NJ; bRMANJ, Basking Ridge, NJ.

OBJECTIVE: Options for preconception genetic screening (GS) have grown dramatically. Traditionally focused on ethnicity, it is only within the last decade that the recommended GS paradigms have included universal screening for numerous disorders. High density arrays now allow individuals to determine their carrier status for hundreds of genetic mutations with a single test. Literature is available on what proportion of patients will be identified as carriers for one of the panels’ screened mutations; however, very limited data exists on how often GS alters clinical management. This study seeks to determine how often large scale expanded carrier GS goes beyond providing information and actually impacts clinical care.

DESIGN: Retrospective cohort.

MATERIALS AND METHODS: All patients completing GS at a single center from 2011-2014 were evaluated. The number of individual tests which returned with an abnormality was identified. The results were then reviewed by couple and those couples whose mutations would create a risk for a child with an abnormal phenotype calculated. Finally, the latter group was divided into those where the genetic abnormality was identified prior to expanded carrier GS (prior targeted screening) or those identified solely because of the GS.

RESULTS: A total of 6643 tests were completed, representing 3738 unique couples. 946 of the panels found ≥1 positive result. In 12 of the 3738 couples, mutations for the same disorder impacted both partners or were dominant in nature. Only 6 of these were not known prior to carrier panel screening. Thus, the expanded carrier screening impacted clinical care 6 times out of 6643 tests done in 3738 cases (0.16%). Importantly, 2/6 cases were for cystic fibrosis and would have been captured on routine screening leaving only 4 cases which resulted in single gene testing which would have gone undetected with routine GS.

CONCLUSION: GS is progressively a part of routine IVF care and the use of expanded screening panels is becoming more widespread. In a large case series, ordering of carrier panels affected clinical decision making in 0.16% of cases. This information must be weighed when utilizing these tests and may be a helpful part of patient counseling.

Expanded carrier screening often identifies disease states but rarely alters clinical practice.

<table>
<thead>
<tr>
<th></th>
<th># of Unique Cases n, (%) of total</th>
<th># of Expanded Carrier Screening Tests n, (%) of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Number</td>
<td>3738</td>
<td>6643</td>
</tr>
<tr>
<td>Positive</td>
<td>12 (0.32%)</td>
<td>946 (14.2%)</td>
</tr>
<tr>
<td>Changed Clinical Management</td>
<td>6 (0.16%)</td>
<td>12 (0.18%)</td>
</tr>
</tbody>
</table>

CONCLUSION: GS is progressively a part of routine IVF care and the use of expanded screening panels is becoming more widespread. In a large case series, ordering of carrier panels affected clinical decision making in 0.16% of cases. This information must be weighed when utilizing these tests and may be a helpful part of patient counseling.
O-220 Wednesday, October 22, 2014 11:30 AM

INFERTILITY PATIENTS WITH CHROMOSOME INVERSIONS ARE SUSCEPTIBLE TO AN INTER-CHROMOSOMAL EFFECT. D. Klepacka,1 D. Young,1 L. Brzeskiewicz,1 W. B. Schoolcraft,1,2 M. G. Katz-Jaffe.1,2 1Fertility Laboratories of Colorado, Lone Tree, CO; 2Colorado Center for Reproductive Medicine, Lone Tree, CO.

OBJECTIVE: Inversions are structural chromosome abnormalities that are associated with infertility due to the increased risk of producing unbalanced gametes from recombination events within the inverted chromosome segment. However, there is also evidence indicating the presence of an interchromosomal effect during chromosome segregation influencing the generation of other chromosome aneuploidies. The aim of this study was to evaluate the nature of chromosome errors in IVF blastocysts from carriers of balanced chromosome inversions.

DESIGN: Research study.

MATERIALS AND METHODS: Infertility patients with balanced chromosome inversions (n=30; female carriers=20, male carriers=10) and maternal age-matched infertile controls with normal karyotypes that concurrently cycled (n=30), consented with IRB approval to an IVF cycle with comprehensive chromosome screening (CCS). All embryos were cultured to the blastocyst stage with a trophectoderm biopsy performed for CCS using SNP microarray (RMA-NJ). Only euploid or balanced blastocysts were transferred in a subsequent frozen embryo transfer. Statistical analysis of aneuploidy and outcome data was performed using a two sided Fisher’s exact test with p value of 0.05 for significance.

RESULTS: Maternal ovarian reserve, paternal age, oocytes retrieved, fertilization rate and the number of blastocysts biopsied were comparable between the two groups. The chromosomes that contained the balanced inversions varied but were mostly the larger chromosomes including 1, 2, 3, 5, 9, and 17. The incidence of aneuploidy, excluding the chromosome with the inversion, was significantly higher for the inversion patients compared to the maternal age matched controls (Inversion=59% vs. Control=49%; P=0.001). However, following transfer of a euploid blastocyst there was an equivalent live birth rate (Inversion=73.7% vs. Control=78.3%; ns).

CONCLUSION: Carriers of balanced chromosome inversions exhibited higher aneuploidy rates for chromosomes that were not involved in the inversion compared to maternal age-matched controls, signifying the occurrence of an inter-chromosomal effect. However, despite a higher aneuploidy rate, carriers of balanced inversions experienced excellent ART outcomes following the transfer of euploid blastocysts. These results provide valuable information for clinical management of infertility patients carrying balanced inversions prior to infertility treatment.

O-221 Wednesday, October 22, 2014 11:45 AM

HIGH NORMAL CGG REPEAT NUMBER ON THE FRAGILE X MENTAL RETARDATION 1 (FMR1) GENE IS NOT CORRELATED WITH DIMINISHED OVARIAN RESERVE. A. Schufrider,1 M. L. Uhler,2 S. M. Lee,2 E. L. Marut,2 D. B. McQueen,2 J. Davie,2 E. C. Feinberg.3 1Obstetrics and Gynecology, The University of Chicago, Chicago, IL; 2Fertility Centers of Illinois, Chicago, IL; 3Good Start Genetics, Inc., Cambridge, MA; 4Department of Obstetrics and Gynecology, NorthShore University Health System, Evanston, IL.

OBJECTIVE: To evaluate the association between high normal number of CGG repeats in the FMR1 gene and ovarian reserve.

DESIGN: Retrospective analysis.

MATERIALS AND METHODS: All women who presented for treatment at a single center between January 2012 and February 2014 and who underwent both Fragile X testing and ovarian reserve testing were included. All Fragile X testing was done by triplet repeat PCR, with confirmation of positives by Southern blot. CGG repeat numbers from both alleles were recorded, and the allele with the higher number of repeats was used for statistical calculations. Women with <19 and >54 CGG repeats were excluded from analysis. Ovarian reserve testing was carried out on cycle day 2 or 3 and included measurements of follicle stimulating hormone (FSH), antimullerian hormone (AMH) and antral follicle count (AFC). A generalized linear regression model assuming gamma distribution and log link function that controlled for age was used to assess correlation between CGG repeat numbers and FSH, AMH and AFC.

RESULTS: 1.373 women were included in the analysis. 1.018 (74.1%) women had 29-32 repeats, while 299 (21.8%) women had ≥33 repeats. Women had a mean age of 35.3±5.0, mean FSH of 8.6 ±7.7 mIU/mL, mean AMH of 2.7 ±3.2ng/mL, and mean AFC of 15.8 ±10.4. After controlling for age, no significant correlation between FMR1 CGG repeat number and FSH (p=0.46), AFC (p=0.08) or AMH (p=0.43) was found.

CONCLUSION: In contrast to previous smaller studies, our large data set demonstrated that a high normal number of CGG repeats was not significantly correlated with decreased ovarian reserve.

O-222 Wednesday, October 22, 2014 12:00 PM

NEXT GENERATION SEQUENCING OF FAMILIES WITH ENDOMETRIOSIS IDENTIFIES NEW GENOMIC REGIONS LIKELY TO CONTRIBUTE TO HERITABILITY. R. Chettier, H. M. Albertsen, K. Ward. Juneau Biosciences, LLC, Salt Lake City, UT.

OBJECTIVE: Genome-wide association studies (GWAS) of endometriosis have explained only a small portion of the observed heritability. By design, GWAS studies focus on older gene variants, typically those present in >3-5 % of the population. Next Generation Sequencing (NGS) on the other hand gives the opportunity to study rare, recent variants with larger effect size. However, the large number of variants identified through NGS impedes the process of prioritizing those variants. In this study, we used identity by descent (IBD) methods to study13 affected sister pairs from 7 distinct nuclear families in an effort to discover candidate genomic regions with excess mutations likely to predispose to endometriosis pathogenesis.

DESIGN: Family-based whole genome sequencing (WGS) of 25 individuals including 19 surgically confirmed endometriosis patients of which 17 of them are siblings.

MATERIALS AND METHODS: Paired-end WGS was performed on HiSeq 2000 instruments (Illumina, San Diego, CA). Variant analysis was performed using the Genome-Analysis-Tool-Kit (Broad Institute). Phasing was performed using impute2 and IBD regions were identified through Germline software.

RESULTS: WGS was accomplished with a 37-fold mean coverage. Using GATK, we identified ~4 million variants per individual. After phasing and subsequent discovery of long shared segments of IBD, we found that the 13 sibling pairs shared 1734MB genome-wide on average representing 69 regions. On a loci level, the shared segment counts were normally distributed with mean of 8.27 shared segments (standard deviation of 2.19). We identified 5 shared genomic regions shared by 13 sibling pairs possibly showing Terson to endometriosis (Table 1). Within these regions we found multiple novel rare variants among the affected sister pairs and evidence of an increased mutation rate.

CONCLUSION: Multiple novel rare variants among the affected sister pairs and evidence of an increased mutation rate.

O-223 Wednesday, October 22, 2014 12:00 PM

NEW STRATEGIES FOR THE GENETIC ANALYSIS OF ENDOMETRIOSIS. M. G. Katz-Jaffe.1,2,3 1Fertility Laboratories of Colorado, Lone Tree, CO; 2Fertility Centers of Illinois, Chicago, IL; 3Fertilization Laboratories of Colorado, Lone Tree, CO.

OBJECTIVE: In the past few years, studies are emerging reporting significant familial aggregation of endometriosis while simultaneously indicating that there is likely a genetic component to its etiology. However, genetic studies have been limited due to the small sample size and sample selection bias inherent to case-control studies. In this study, we utilized whole genome sequencing to evaluate the genetic basis of endometriosis.

MATERIALS AND METHODS: Whole-genome sequencing was performed on 11 affected female index cases from a total of 19 endometriosis families of which 13 were siblings.

RESULTS: The analysis revealed 844 rare variants which were identified in at least 10% of the affected cases. We identified 11 common variants which were shared in at least 50% of the affected cases. Five of these loci were associated with the well known endometriosis associated genes (EEG1, ESR1, TGFBI, GPR1, ZNF129).

CONCLUSION: Next generation sequencing of families with endometriosis identified new genomic regions likely to contribute to heritability of endometriosis.
CONCLUSION: Genetic variants that contribute to complex diseases range from ancient-and-common polymorphisms with minimal effects to recent-and-rare mutations predicted to have larger effects on the phenotype. Applying NGS and IBD analysis using nuclear families, we were able to identify 5 candidate endometriosis regions that show excess genetic burden. A more complete picture of the genetics of endometriosis is likely to emerge as additional WGS data are analyzed.

Supported by: Juneau Biosciences.

O-223 Wednesday, October 22, 2014 12:15 PM

GOING BEYOND THE GUIDELINES: A CALL FOR EXPANDED CARRIER SCREEN BASED ON AN ANALYSIS OF 3,208 CLINICAL SAMPLES. N. Kumar,⁎ S. Rodriguez,⁎ A. Bisignano,⁎ G. Kellogg,⁎ B. Chu,⁎ S. Munne,⁎ A. Huang,⁎ M. Surrey,⁎ R. Recombine, New York, NY; Reprogenetics, Livingston, NJ; Reproductive Partners Medical Group, Los Angeles, CA; Southern California Reproductive Center, Beverly Hills, CA.

OBJECTIVE: Carrier screening guidelines published by the American Congress of Obstetricians & Gynecologists (ACOG) and the American College of Medical Genetics & Genomics (ACMG) recommend that genetic screening be performed based on ethnicity. Technological genomic advancements now allow carrier screening to be performed cost-effectively for over 100 genetic diseases in a high-throughput manner. Our goal was to assess if carrier rates for diseases not included in current guidelines are significant and/or higher than expected based on the literature across multiple ethnicities.

DESIGN: Retrospective.

MATERIALS AND METHODS: The Illumina Infinium HD Custom Genotyping platform was used to identify 1679 mutations associated with 23 recessive genetic diseases. The analysis includes data from 3208 clinical referrals from reproductive endocrinologists, obstetricians, and genetic counselors. Documented informed consent to utilize data in a de-identified manner was obtained. Carrier rates for each disease were calculated for the general population and within each reported ethnic group and compared with the literature.

RESULTS: Analyses indicate significantly higher carrier rates for several diseases compared to the literature. In our patients, we found a carrier rate of 1/17 for GJB2-related nonsyndromic hearing loss & deafness (NSHL) while the literature-reported rate is 1/43. The carrier rate for NSHL was significantly high (~1/10) for our Southeast and East Asian patients. In our European patients, we calculated a carrier rate of 1/50 for Smith-Lemli-Opitz Syndrome (SLOS) and 1/64 for Glycogen Storage Disease Type II (Pompe Disease) while the reported carrier rates in the literature are 1/70 and 1/100, respectively.

CONCLUSION: ACOG/ACMG recommended diseases are high impact and typically have high carrier rates. Our analysis shows several additional genetic diseases that meet these criteria, including SLOS, NSHL, and Pompe Disease. Carrier rates reported in the literature for these diseases are lower than what we observed, suggesting that these diseases may be under-diagnosed. These results indicate that current recommendations for carrier screening are not consistent with the observed distribution of carrier rates. Moreover, with technological advancements in genomics, testing need not be limited to diseases that have carrier rates higher than 1/100.

O-224 Wednesday, October 22, 2014 12:30 PM

GUIDANCE FOR ORDERING CHROMOSOMAL MICROARRAY (CMA) ON INDIVIDUALS CONCEIVED THROUGH GAMETE DONATION. L. Iley,⁎ M. Gillespie, J. Iger, M. Ray, P. Callum. California Cryobank, Los Angeles, CA.

OBJECTIVE: To provide guidance for ordering CMA on donor-conceived offspring based on our experience with requests for copy number variation (CNV) testing on sperm donors.

DESIGN: Qualitative information was compiled for all CNV test requests on California Cryobank (CCB) donors from 2009-2013; management issues were summarized and counseling criteria developed.

RESULTS: CCB received 11 requests for targeted CMA testing on sperm donors following identification of CNVs of unknown clinical significance in a child/fetus, or product of conception (POC) conceived using donor sperm. CCB facilitated targeted testing of 5 donors, 3 of which tested positive for the CNV. Three donors were not tested because the mother tested positive or was not tested. CCB declined testing in Case #3 due to low clinical utility; the incidentally detected CNV was identified in a first-trimester POC and the miscarriage was attributed to a different etiology. Review of the donor’s karyotype was sufficient in Case #6. The donor was unresponsive to contact in Case #8.

<table>
<thead>
<tr>
<th>Case #</th>
<th>CNV</th>
<th>Fetus/ Child</th>
<th>Indication</th>
<th>Mother’s result</th>
<th>Donor’s result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>del16p11</td>
<td>Child</td>
<td>Speech delay, seizures</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>2</td>
<td>del3p26</td>
<td>Child</td>
<td>Developmental delay (DD)</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>3</td>
<td>del2q13, del16p13</td>
<td>Fetus</td>
<td>POC</td>
<td>Negative</td>
<td>Not tested</td>
</tr>
<tr>
<td>4</td>
<td>.dup2p21.3</td>
<td>Child</td>
<td>Prenatal screening</td>
<td>Not tested</td>
<td>Negative</td>
</tr>
<tr>
<td>5</td>
<td>del1p21.2p2,23</td>
<td>Child</td>
<td>POC</td>
<td>Positive</td>
<td>n/a</td>
</tr>
<tr>
<td>6</td>
<td>del16p12.2</td>
<td>Fetus</td>
<td>Increased ICHM</td>
<td>Positive</td>
<td>n/a</td>
</tr>
<tr>
<td>7</td>
<td>del4p15.1</td>
<td>Child</td>
<td>Autism, DD, motor delay</td>
<td>Negative</td>
<td>Not tested</td>
</tr>
<tr>
<td>8</td>
<td>dupq34.3</td>
<td>Fetus</td>
<td>Prenatal screening</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>9</td>
<td>dup3p22.3</td>
<td>Fetus</td>
<td>Prenatal screening</td>
<td>Positive</td>
<td>n/a</td>
</tr>
<tr>
<td>10</td>
<td>del7q31.1, del8p11.21</td>
<td>Fetus</td>
<td>Advanced maternal age</td>
<td>Negative</td>
<td>Positive</td>
</tr>
</tbody>
</table>

CONCLUSION: Guidelines on the clinical indications for ordering CMA exist; however, patients utilizing gamete donors should be counseled about additional issues that may arise in this context:

- The gamete donor facility may decline to facilitate testing if it’s deemed clinically inappropriate.
- The donor may not be immediately available, which may hinder time-sensitive pregnancy management decisions dependent on the donor’s result.
- The donor may be unwilling or unavailable to undergo testing.
- Interpretation of a CNV of unknown clinical significance in a donor is not tested. CCB declined testing in Case #3 due to low clinical utility; the

O-225 Wednesday, October 22, 2014 12:45 PM


OBJECTIVE: A nonconcurrent, or inconclusive result, is sometimes obtained after blastocyst biopsy for CCS when an embryo karyotype is not clearly diagnosed as disomic, monosomic, or trisomic. Patients who receive such a result may experience anxiety and confusion. A nonconcurrent result may be due to complex chromosomal abnormalities, or it may result from biopsy or sample processing techniques. Using embryos that received clear results after one biopsy as the benchmark background, this study sought to create descriptive statistics for initial nonconcurrent embryos that were re-biopsied and subsequently diagnosed.

DESIGN: Retrospective analysis.

MATERIALS AND METHODS: Blastocysts undergoing CCS (n=1853) were analyzed. Aneuploid and euploid rates of embryos with 1st biopsy results were compared to those of embryos with a 1st nonconcurrent and 2nd ploidy result. Groups were stratified by age.
RESULTS: Embryos that were diagnosed after a 2nd biopsy for a nonconcurrent result showed comparable overall aneuploid (41.9%) and euploid (58.1%) rates to those that received initial aneuploid (43.9%) or euploid (56.1%) diagnoses.

CONCLUSION: Our study aimed to determine whether embryos receiving nonconcurrent results after trophoderm biopsy for CCS showed higher rates of aneuploidy than embryos with euploidy after 1st biopsy. After a 2nd biopsy and successful analysis, our study found that nonconcurrent embryos fall into the anticipated ploidy rates. This suggests that a nonconcurrent diagnosis may be related to technical issues, conservative interpretation of genetic data, or sample collection techniques, and not necessarily to chaotic chromosomal abnormalities. Patients can be reassured that an embryo receiving a nonconcurrent result does not carry an increased risk of aneuploidy. We recommend re-biopsy and re-analysis of embryos with inconclusive or non-concurrent results.

ENDOMETRIOSIS III

O-226 Wednesday, October 22, 2014 11:15 AM

SYSTEMATIC REVIEW AND META-ANALYSIS ON THE SURGICAL MANAGEMENT OF ENDOMETRIOMAS ON IN VITRO FERTILIZATION OUTCOMES: AN UPDATE. C. Q. Wu, a G. M. Alkusayer, a A. M. Abou-Setta, a J. M. Goldfarb, b T. Falcone, c J. C. Havelock, c A. Ailaire, d M. A. Bedaiwy, e McGill University, Montréal, QC, Canada; aObstetrics and Gynecology, British Columbia Women’s Hospital & Health Center/University of British Columbia, Vancouver, BC, Canada; bObstetrics and Gynecology, University of Manitoba, Winnipeg, MB, Canada; cObstetrics and Gynecology, Cleveland Clinic/Case Western Reserve University School of Medicine, Cleveland, OH.

OBJECTIVE: Controversies exist regarding the surgical management of endometriomas in infertile women prior to in vitro fertilization (IVF). Growing evidence indicates that endometrioma surgery may impair ovarian response and decrease IVF success. The objective of the present study is to compare the effect of surgical versus conservative management of endometriomas on IVF outcomes.

DESIGN: Systematic review and meta-analysis.

MATERIALS AND METHODS: We systematically searched the Cochrane Library, EMBASE, and MEDLINE databases from inception to May 2014. Prospective and retrospective controlled studies comparing fertility outcomes in infertile women with endometriomas undergoing surgical and conservative treatment prior to IVF were selected for inclusion. Study selection, data extraction and quality assessment were conducted independently by 2 reviewers. Clinical pregnancy rates and total oocytes retrieved were pooled using a random effects model.

RESULTS: Twelve studies (1 randomized controlled trial and 11 observational studies; n = 3,288) meeting the inclusion criteria were pooled in the meta-analysis. Across studies, we found similar clinical pregnancy rates between the surgically and conservatively managed groups (risk ratio [RR] = 0.91; 95% CI = 0.54 to 1.54 in the randomized trial; RR = 1.06; 95% CI = 0.88 to 1.27 in observational studies). The total number of oocytes retrieved was also comparable in both groups (mean difference [MD] = -0.37; 95% CI = -0.92 to 0.18). The differences in comparisons with respect to mature oocytes retrieved, estradiol peak, and live birth rates were equally not statistically significant.

CONCLUSION: Our meta-analysis suggests that surgical management of endometriomas prior to IVF therapy yields similar clinical outcomes as conservative management. Additional trials with more rigorous study design are required to assess potential benefits of surgery in the management of endometriomas prior to IVF.

O-227 Wednesday, October 22, 2014 11:30 AM


OBJECTIVE: To investigate the efficacy of dienogest (DNG) in treating deep dyspareunia (DD) and in improving sexual function in women with rectovaginal endometriosis.

DESIGN: Prospective open-label cohort pilot study.

MATERIALS AND METHODS: This study included premenopausal sexually active women with rectovaginal endometriosis suffering DD (more than 6 month duration and intensity > 80 mm on a 100 mm visual analogue scale). The diagnosis of rectovaginal endometriosis was based on vaginal and rectal examinations and it was confirmed by transvaginal ultrasonography. The criteria for exclusion from the study were: therapies for endometriosis other than non-steroidal anti-inflammatory drugs in the 3 months before inclusion in the study (6 months for GnRH analogues); unwillingness to tolerate menstrual changes; undiagnosed vaginal bleeding; obstructive uropathy or bowel stenosis and evidence of complex adnexal cysts. Eligible patients received DNG (2 mg/day) for 6 months. The primary end-point of the study was to assess the changes in DD during treatment. The secondary objective of the study was to evaluate the changes in sexual function, which was evaluated by using the Female Sexual Function Index (FSFI). Outcomes were assessed after 3 and 6 months of treatment.

RESULTS: 23 women were included in the study; the mean (± SD) age of the study population was 34.2 (± 3.7) years. The mean (± SD) intensity of DD was 9.1 (± 0.7) cm; it significantly decreased at 3-months of treatment (7.4 ± 1.4 cm; p<0.001). At 6-month treatment the intensity of DD (6.5 ± 1.6 cm) was lower than at baseline (p<0.001) and at 3-month treatment (p<0.001). The total FSFI score was significantly higher at 3-month treatment than at baseline (p < 0.001); there was no significant difference in the total FSFI score between 3- and 6-month treatment (p = 0.188); at both 3- and 6-month treatment the mean total FSFI score remained below the threshold for sexual dysfunction. At 6-month treatment significant improvements were observed in the following subscores: lubrication (p=0.011), orgasm (p=0.010) and pain (p=0.016).

CONCLUSION: In patients with severe DD caused by rectovaginal endometriosis, 6-month treatment with DNG causes a significant decrease in the intensity of this symptom that however persists with moderate intensity. Although an improvement was observed in the total FSFI score, it remained below the threshold for sexual dysfunction.
SYSTEMIC AND FOLLICULAR OXIDATIVE STRESS IN INFERTILE WOMEN WITH ENDOMETRIOSIS UNDERGOING CONTROLLED OVARIAN STIMULATION FOR ICSI: IS THERE A ROLE IN THE ETIOPATHOGENESIS OF INFERTILITY? M. G. Da Broi, E. O. Albuquerque, A. Z. de Andrade, R. A. Prates, A. P. A. Santos, R. A. Word, O. Bukulmez, G. R. L. Cardoso, R. A. Preto, University of Sao Paulo, Ribeirao Preto, Sao Paulo, Brazil; *Nutrition and Metabolism Laboratory, Faculty of Medicine of Ribeirao Preto, University of Sao Paulo, Ribeirao Preto, Sao Paulo, Brazil.

OBJECTIVE: Controversial studies have suggested that impaired oocyte quality and oxidative stress (OS) may be involved in the pathogenesis of endometriosis-related infertility. No study to date has evaluated jointly different pro and antioxidant markers in serum and follicular fluid (FF) of women with endometriosis-related infertility. The aim of the study was to compare eight OS markers in serum and FF of infertile women with and without endometriosis (E) undergoing controlled ovarian stimulation (COS) for intracytoplasmic sperm injection (ICSI).

DESIGN: Prospective case-control study.

MATERIALS AND METHODS: From October 2009 to October 2010, 275 patients started COS: 151 were eligible for the study, 132 signed the informed consent, 99 underwent oocyte retrieval, and 87 serum samples (43 with E and 44 without E - with male or tubal factor of infertility) and 61 FF samples (29 with E and 32 without E) were collected in the day of oocyte retrieval and had data analyzed. Total hydroperoxides (FOX1), malondialdehyde (MDA), advanced oxidation protein products (AOPP), glutathione (GSH), superoxide dismutase (SOD) and the total antioxidant capacity (TAC) were determined by spectrophotometry, vitamin E (Vit E) by high performance liquid chromatography, and S-hydroxy-2’ –deoxyguanosine (8OHdG) by ELISA. Total protein (pt) levels were determined by Labtest Kits. Significance level was set on 5%.

RESULTS: We observed higher serum concentrations of GSH (220.32±43.2 mmol/L) and TAC (679.9±282.21 uM/L), lower serum concentrations of TAC (0.34±0.17 mM Eq Trolox/L), and higher follicular concentrations of 8OHdG (25.19±6.8 ng/mL) and Vit E (13.0±5.33 mM/L) in infertile women with E compared to those without E (193.92±43.25 mmol/L g pt, 563.04±169.82 uM/L, 0.46±0.15 mM Eq Trolox/L, 17.2±5.6 ng/mL and 8.71±2.51 mM/L, respectively).

CONCLUSION: We evidenced the occurrence of systemic and follicular OS in infertile patients with E undergoing COS for ICSI. For the first time it was demonstrated the presence of higher follicular 8OHdG concentrations in women with E, a marker of DNA oxidative damage, which may be related to compromised oocyte quality. We suggest that the OS may be involved in the etiopathogenesis of infertility-related disease.

Supported by: CNPq, FAPESP (2008/58197-6), Brazil.

O-230 Wednesday, October 22, 2014 12:15 PM
COMPARISON OF OUTCOMES FOR WOMEN WITH ENDOMETRIOSIS UNDERGOING SURGICAL FERTILITY PROCEDURES. W. M. Bannister. Healthcare Analytics, Optum, Minneapolis, MN.

OBJECTIVE: To compare differences between women with and without endometriosis in maternal and newborn outcomes of pregnancies resulting from surgical fertility procedures.

DESIGN: We conducted a retrospective study of commercially insured US women using administrative data. Pregnancy and newborn outcomes were compared between women with and without endometriosis, where both groups had undergone surgical fertility procedures. Risk adjusted models were developed to compare outcomes including delivery and newborn costs as well as rates of neonatal intensive care unit (NICU) newborns, cesarean deliveries, multiple births, gestational hypertension, placenta previa, and gestational diabetes.

MATERIALS AND METHODS: The study included women undergoing surgical fertility treatments within 30 days of their final treatment cycle before delivery. The women were split into two groups: those with an endometriosis diagnosis up to one year prior to delivery (n = 126) and those without (n = 478). Propensity score models were developed to account for differences in age, demographic characteristics, socioeconomic factors, and concurrent treatment types such as in vitro fertilization, superovulation, or hysterosalphingogram. Using the resulting propensity score weights, logistic regression models were developed to estimate differences in rates of adverse outcomes and general linear models were developed to compare delivery and newborn costs.

RESULTS: Women with endometriosis were significantly less likely (p < 0.001) to have multiple births than women without endometriosis. Further controlling for that difference in multiple births, we find that women with endometriosis were significantly less likely (p < 0.05) than those without endometriosis to have pregnancies involving gestational diabetes and placenta previa and no significant difference between groups in rates of NICU newborns, cesarean deliveries, or gestational hypertension. We also find that those with endometriosis had significantly lower (p < 0.001) newborn costs than those without endometriosis and no significant difference in delivery costs.

CONCLUSION: After controlling for demographic, treatment, and socioeconomic differences it appears that women with endometriosis who undergo surgical fertility procedures have lower rates of multiple births and similar or lower rates of adverse outcomes than women without endometriosis. While larger studies are needed, these results suggest that endometriosis may not by itself result in adverse outcomes.

Supported by: Author is employed by Optum.
Danazol (D), an anrogenic compound, had been approved for medical treatment of endometriosis whereas D's anrogenic side effects limited its widespread acceptance. Anrogenic compounds have also been implicated in induction of endometrial atrophy yet the mechanisms of anrogen effects on endometrium have not been well studied. We hypothesize that anrogens may promote their endometrial effects via modulation of progesterone receptor (PR) expression. We evaluated the effects of selected anrogens on endometrial PR expression by treating human endometrial explants and Ishikawa cells.

**DESIGN:** We evaluated the effects of selected anrogens on endometrial PR expression by treating human endometrial explants and Ishikawa cells.

**MATERIALS AND METHODS:** Endometrial explants in culture were used to investigate the effects of anrogens on human endometrium. Ishikawa cells were used as a model for endometrial glandular cells and for further functional studies. Both explants and Ishikawa cells were treated with vehicle, 5α-dihydrotestosterone (DHT), testosterone (T) and D for 24h. Ishikawa cells were also co-treated with flutamide. We used estradiol treatment as a positive control. Total PR mRNA expression was measured by qPCR using primers for C-terminal common for all PR isoforms and immunoblotting using PR antibodies.

**RESULTS:** Estradiol induced PR expression in treated cells and explants. Interestingly, both in endometrial explants and Ishikawa cells, DHT treatment resulted in increased PR expression compared with vehicle. In Ishikawa cells, all anrogens (DHT, T, and D) increased PR expression. Expression levels correlated with potency of the anrogenic compound (DHT>T>D). Further, both PR-A and PR-B protein were induced with DHT treatment. Although flutamide treatment alone did not affect PR expression, flutamide diminished androgen-induced upregulation of PR in both endometrial explants and Ishikawa cells.

**CONCLUSION:** As a novel finding, anrogens may mediate endometrial effects through upregulation of PR gene expression. Endometrial PR upregulation by anrogens may be mediated via androgen receptor (AR). Understanding AR pathway and its potential association with PR expression in endometrium may lead to development of new strategies for treatment of endometriosis.

**O-233** Wednesday, October 22, 2014 11:15 AM

**ENDOMETRIAL INFLAMMATORY CELLS IN WOMEN WITH RECURRENT PREGNANCY LOSS AND CHRONIC ENDOMETRITIS.** D. McQueen, a C. Perfetto, b F. Hazard, b R. Lath l b

**OBJECTIVE:** To evaluate the distribution of inflammatory cell subpopulations within the endometrium of women with recurrent pregnancy loss and chronic endometritis (CE).

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** 107 women with a history of 2 or more pregnancy losses of less than 20 weeks were included. As part of a work up for recurrent pregnancy loss, all women routinely underwent an endometrial biopsy. CE was defined as the presence of plasma cells on hematoxylin and eosin (H&E) staining of the endometrium. Subjects with CE were offered treatment with doxycycline and a second endometrial biopsy. Immunohistochemistry was performed for markers of hematolymphoid subpopulations using CD138, CD56, CD163, CD14, CD20 and CD79a antibodies (see table 1). Expression of these markers was scored as follows: 0 = none, <1/hpf; 1 = 1-5/hpf or clusters of less than 20 cells; 2 = 5-20/hpf or clusters of at least 20 cells; 3 = >20/hpf or sheets of cells.

**RESULTS:** CE was found in 13% (14/107) of endometrial biopsies. On initial biopsy, the endometrium of subjects with CE demonstrated significantly greater numbers of plasma cells by CD138 staining compared to subjects without CE (p<0.013). There were no statistically significant differences in the quantity of B cells, NK cells or macrophages in the endometrium of women with and without CE. Seven women with CE were treated with doxycycline and received a second endometrial biopsy. Following treatment, CD138 staining was undetectable and there was a significant decrease in the number of NK cells and B cells within the endometrium (p<0.05).

There was no change in the number of macrophages.

**CONCLUSION:** Immunohistochemical staining for CD138 confirms the morphologic presence of plasma cells seen on H&E staining and the clinical diagnosis of chronic endometritis. Additionally, endometrial biopsies from women with CE treated with doxycycline showed no remaining plasma cells and a marked reduction in B cells.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Cell Type</th>
<th>CE (n = 14)</th>
<th>No CE (n = 93)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD138 (SD)</td>
<td>Plasma Cells</td>
<td>1.1 (0.7)</td>
<td>0.6 (0.7)</td>
<td>0.01*</td>
</tr>
<tr>
<td>CD56 (SD)</td>
<td>Natural killer Cells</td>
<td>2.9 (0.3)</td>
<td>2.9 (0.3)</td>
<td>1</td>
</tr>
<tr>
<td>CD163 (SD)</td>
<td>Macrophages</td>
<td>2.8 (0.4)</td>
<td>2.9 (0.2)</td>
<td>0.18</td>
</tr>
<tr>
<td>CD14 (SD)</td>
<td>Macrophages</td>
<td>2.9 (0.3)</td>
<td>2.8 (0.4)</td>
<td>0.37</td>
</tr>
<tr>
<td>CD20 (SD)</td>
<td>B Cells</td>
<td>2.1 (0.7)</td>
<td>2.0 (0.8)</td>
<td>0.64</td>
</tr>
<tr>
<td>CD79a (SD)</td>
<td>B Cells</td>
<td>2.1 (0.8)</td>
<td>1.8 (1.0)</td>
<td>0.26</td>
</tr>
</tbody>
</table>

**Expression score:** 0 = None, <1/hpf; 1 = 1-5/hpf or clusters of less than 20 cells; 2 = 5-20/hpf or clusters of at least 20 cells; 3 = >20/hpf or sheets of cells

**O-234** Wednesday, October 22, 2014 11:30 AM

**ALTERED THYROID HORMONE LEVEL IN OFFSPRING EXPOSED TO HIGH ESTROGEN LEVEL DURING THE FIRST TRIMESTER OF PREGNANCY.** P. P. Lv, a Y. Meng, a J. Y. Li, a M. L. Lv, b D. Q. Yu, a Y. Shen, a S. Dong, a G. L. Ding, a H. F. Huang, a

**OBJECTIVE:** To investigate whether the maternal high E2 environment in the first trimester increases the risk of thyroid dysfunction in children born after in vitro fertilization.
RESULTS: The overall rate of chromosomal abnormalities was 54%. Of the remaining specimens 34% were normal and 12% had maternal cell contamination (MCC). The aneuploidies were 86% maternal and 14% paternal in origin. ART pregnancies had similar rates of chromosomal abnormalities as spontaneous pregnancies, 51.5% vs. 59.6% respectively ($X^2 = 1.2$, $P = 0.53$). The rates of abnormalities in the four age groups were 42.3% vs. 59.5% vs. 57.1% vs. 84.4%, respectively, with a significant increase above age 40 ($X^2 = 11.5$, $P = 0.009$). The most common abnormalities were trisomy 21 and 22.

CONCLUSION: To our knowledge this is the largest study investigating the rate of chromosomal abnormalities detected by SNP microarrays in early pregnancy loss. Our study found that the rate of chromosomal abnormalities is similar between miscarriages in spontaneous pregnancies vs. those achieved by ART. Furthermore, we noted a significant increase in the rate of chromosomal abnormalities above maternal age of 40.

O-236 Wednesday, October 22, 2014 12:00 PM

ETHNICITY AND MISCARRIAGE: A LARGE PROSPECTIVE OBSERVATIONAL STUDY AND META-ANALYSIS. H. M. Harb, a F. Al-rshoud, b R. Dhillon, a M. Harb, a A. Coomarasamy, a "School of Clinical and Experimental Medicine, University of Birmingham, Birmingham, West Midlands, United Kingdom; 3Department of Obstetrics and Gynaecology, Sandwell and West Birmingham Teaching Hospitals, Birmingham, West Midlands, United Kingdom."

OBJECTIVE: To investigate the association between ethnicity and miscarriage in spontaneous and IVF (In Vitro Fertilisation) pregnancies.

MATERIALS AND METHODS: A prospective observational study including all women undergoing first cycle of IVF or Intra-cytoplasmic Sperm Injection (ICSI) at 5 centres for assisted reproduction in the UK, from 2008 to 2012. Miscarriage per clinical pregnancy was reported for women within the following ethnic groups: White, Asian, Black, Chinese, Mixed and any other. Regression and multiple logistic regression analyses were performed. Variables used within the model were age, body mass index, previous live birth and previous miscarriage. Secondly, a systematic review and meta-analysis of studies comparing miscarriage rate in women of different ethnic groups was performed.

RESULTS: A total of 5110 clinical pregnancies were reported between 2008 and 2012 and in five assisted reproduction clinics in the UK, including; White (3970), Black (48), Asian (409), Chinese (27), Mixed (175), Other (48) and Not stated (591). The crude miscarriage rates were; White 9.5%, Asian 11%, Black 12.5%, Chinese 3.7%, Mixed 10.3%, other 6.3% and not stated 8.3%. Multivariate analysis (adjusting for age, BMI, previous live birth, previous miscarriage) revealed a statistically significant increase in miscarriage rate (OR= 1.63, CI: 1.05 -2.54, p=0.03) in women of Asian ethnicity when compared to White women. There was no difference in miscarriage rate in women of other ethnic backgrounds. The review included 16 studies, comprising 18,288,066 women. Meta-analysis of 6 studies in spontaneously conceived pregnancies found that Black women have a two fold increased risk of miscarriage (OR= 1.95, 95% CI 1.18-3.21, p=0.009) in comparison to women of Caucasian origin. No difference in miscarriage risk was observed in Asian women. In pregnancies achieved by IVF/ICSI (160,165 cycles), women of Black origin were found to have a higher risk of miscarriage (OR= 1.62, 95% CI 1.52 – 1.72, p<0.00001) in comparison to women of white origin. A stastically significant increase in miscarriage was also observed in Asian women (OR=1.87, 95% CI 1.25 - 2.81, p=0.002).

CONCLUSION: There is evidence to suggest that women of Black and Asian ethnicity may be at increased risk of miscarriage when compared to Caucasian women.

O-237 Wednesday, October 22, 2014 12:15 PM

MATERIALS AND METHODS: A total of 150 first-trimester miscarriage samples analyzed by traditional karyotype, are due to maternal cell contamination (Lathi et al. 2014). This error is eliminated by SNP microarray analysis in patients who experienced first trimester miscarriage.

OBJECTIVE: To determine if maternal serum anti-Mullerian hormone (AMH) levels in early pregnancy, both in isolation and in combination...
with other biomarkers of feto-placental health, are associated with preterm birth (PTB).

**DESIGN:** This was a retrospective case-control study of women in Iowa who conceived spontaneously and delivered between January 1, 2009 and December 31, 2010.

**MATERIALS AND METHODS:** PTB was defined as delivery at <37 weeks gestation. Cases and controls were selected from a tissue bank at the University of Iowa that included birth certificate data linked to paired 1st and 2nd trimester maternal serum samples which were used for AMH testing. Multivariable logistic regression models were built with one of two exposure variables: 2nd trimester AMH and the mathematical difference between the 1st and 2nd trimester AMH (AMH difference). All models were adjusted for maternal age, weight change between trimesters, history of preterm birth, and smoking history. Once the association between PTB and AMH was established, biomarkers of feto-placental health (maternal alpha-fetoprotein (MSAFP), estriol, inhibit A, pregnancy-associated plasma protein A (PAPP-A), and human Chorionic Gonadotropin (hCG) levels) were added to the model to determine if they strengthened the association between AMH and PTB.

**RESULTS:** 2nd trimester AMH levels were not associated with PTB in any model. However, AMH difference was significantly associated with PTB both in isolation (p=0.04) and in combination with MSAFP (p=0.006), after controlling for weight change between trimesters. A previous history of PTB did not contribute to model performance and was not included in the final model. Further, after stratified the results by MSAFP (≤ 1 MoM vs. >1 MoM), the highest probability for PTB was found in women with a stable or rising AMH levels along with an MSAFP >1 MoM. This equates to a 45% probability of PTB with an incidence rate of 15%.

**CONCLUSION:** The change in AMH levels between the 1st and 2nd trimesters can be used as an indicator of PTB risk, and should be considered in future PTB risk models. This association does not depend on a previous history of PTB, and therefore can be used for both primiparous and multiparous women. Additional studies are needed to validate these findings and to further define the role of the ovary in early pregnancy.

**Supported by:** The Iowa Women’s Reproductive Health Research Career Development Award and KL-2 grant, University of Iowa, 2009-2011.

---

**O-238** Wednesday, October 22, 2014 12:30 PM

**PERINATAL OUTCOME OF TWIN PREGNANCIES FOLLOWING MULTIFETAL PREGNANCY REDUCTION: WHEN IS THE OPTIMAL TIME TO PERFORM IT?** J. Haas,¹ Y. Yinon,² A. Shulman.³ Department of Obstetrics and Gynecology, Chaim Sheba Medical Centre, Tel-Hashomer, Ramat Gan, Israel; ²Department of Obstetrics and Gynecology, Meir Medical Center, Kfar Saba, Israel.

**OBJECTIVE:** To compare the perinatal outcome of early (7-8 weeks of gestation) transvaginal multifetal pregnancy reduction (MPR) from triplets and higher order multiples to twins with late (11-14 weeks) transabdominal MPR.

**DESIGN:** A cohort historical study.

<table>
<thead>
<tr>
<th>Perinatal outcome of early vs. late multifetal pregnancy reduction to twins</th>
<th>Early MPR (n=83)</th>
<th>Late MPR (n=134)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age at delivery (median, range)</td>
<td>36 (24-41)</td>
<td>36 (24-39)</td>
<td>1</td>
</tr>
<tr>
<td>Birth weight (median, range)</td>
<td>2183.6±628</td>
<td>2148.6±551</td>
<td>0.57</td>
</tr>
<tr>
<td>Early pregnancy loss &lt;24 weeks (%)</td>
<td>4 (4.8)</td>
<td>5 (3.7)</td>
<td>0.74</td>
</tr>
<tr>
<td>Preterm delivery ≤ 34 weeks (%)</td>
<td>20 (25.3)</td>
<td>33 (25.6)</td>
<td>1</td>
</tr>
<tr>
<td>Gestational diabetes (%)</td>
<td>12 (15.2)</td>
<td>15 (11.6)</td>
<td>0.53</td>
</tr>
<tr>
<td>Gestational hypertension (%)</td>
<td>12 (15.2)</td>
<td>24 (18.6)</td>
<td>0.57</td>
</tr>
<tr>
<td>Intrathecal growth restriction</td>
<td>5 (6.0)</td>
<td>24 (17.9)</td>
<td>0.01</td>
</tr>
<tr>
<td>MA (Missed abortion) of one fetus</td>
<td>5 (6.0)</td>
<td>2 (1.5)</td>
<td>0.1</td>
</tr>
</tbody>
</table>

**MATERIALS AND METHODS:** A retrospective cohort study of 83 (58 triplets and 25 higher order) multiple pregnancies who underwent early first trimester transvaginal reduction and 134 (109 triplets and 25 higher order) multiple pregnancies who underwent late transabdominal reduction. The rates of early miscarriage, pregnancy loss before 24 weeks, preterm delivery prior to 32 weeks and 37 weeks of gestation as well as pregnancy complications such as, gestational diabetes, hypertension and small gestational age infants (SGA), defined as birth weight less than 10th centile, were compared between the two groups.

**RESULTS:** The rates of early spontaneous miscarriage of one of the fetuses, pregnancy loss prior to 24 weeks, preterm delivery prior to 32 weeks and 37 weeks of gestation gestational diabetes or hypertensive disease of pregnancy were similar among both groups. However, the rate of SGA, was significantly higher among the late reduced twins (6% vs. 18.6%, P=0.01). Comparison of triplets only reduced to twins revealed that women in the “late” MPR group delivered earlier (36.3 vs. 35.6 weeks, p=0.03) and had smaller babies (2344 gr vs. 2175 gr p<0.01), but the rates of preterm delivery prior to 32 weeks and 37 weeks of gestation remained similar among both groups.

**CONCLUSION:** The perinatal outcome of twin pregnancies following early and late MPR is comparable. However, the perinatal outcome of triplets reduced to twins seems to be more favorable when the procedure is performed early at gestation.

---

**O-239** Wednesday, October 22, 2014 12:45 PM

**LIVE BIRTH RATE AND TIME TO LIVE BIRTH IN WOMEN WITH UNEXPLAINED RECURRENT MISCARRIAGE WITH OR WITHOUT INHERITED THROMBOPHILIA.** P. G. de Jong,¹ R. O. Kool,¹ S. P. Kaandorp,¹ B. A. Hutton,ⁿ S. Middeldorp,ⁿ M. Goddijn,ⁿ ¹Vascular Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands; ²Center for Reproductive Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands; ³Clinical Epidemiology, Biostatistics and Bioinformatics, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands.

**OBJECTIVE:** We aimed to investigate the probability of and time to first live birth after recurrent miscarriage (RM).

**DESIGN:** A cohort study in participants of the ALIFE study¹, including follow-up of up to 10 years after randomization.

**MATERIALS AND METHODS:** Women with unexplained RM (with or without inherited thrombophilia) who participated in the ALIFE study were contacted by phone. Data on all pregnancies, including date and outcome were collected in a predefined CRF. We calculated the proportion of women from the ALIFE study who had at least one live birth since randomization at 12, 24, 36 and 48 months. The relative prognostic significance of female age (<36 vs. ≥ 36 years), the number of preceding miscarriages (2 vs. ≥ 3), a previous live birth and inherited thrombophilia was evaluated by Cox regression for time to live birth. Data were censored when participants quit trying to conceive or when pregnancy was not reached at the age of 43. Data of women who were not reached in follow-up were censored based on ALIFE pregnancy data (i.e. censored at time of pregnancy loss or at end of study in case no pregnancy occurred).

**RESULTS:** Follow-up data of 233 (64%) of 364 women were obtained. Seventeen women were excluded because of drop-out in the ALIFE study. Of the analyzed 347 women, 242 (69.7%) had at least 1 live birth after randomization. 197 of these live births occurred during the ALIFE study; additionally 45 women had a live birth during follow up. A total of 349 babies were born. After censoring, 140 (40.3%), 213 (61.4%), 227 (65.7%) and 233 (67.1%) of 347 women had a first live birth at 12, 24, 36 and 48 months respectively. Cox regression analysis showed a lower probability of live birth in women with ≥3 miscarriages compared to women with 2 miscarriages (HR 0.73 [95% CI 0.55-0.97]), and higher probability in women with inherited thrombophilia (HR 1.65 [95% CI 1.13 – 2.4]). An effect of potential bias due to selective loss to follow-up is uncertain as it could affect the live birth estimate in both a positive of negative manner.

**CONCLUSION:** 69.7% of women with unexplained RM had at least one live birth. The probability of live birth was lower in women with ≥3 miscarriages and higher in women with inherited thrombophilia.

**Supported by:** Netherlands Organization for Health Research and Development.
O-240 Wednesday, October 22, 2014 11:15 AM

CHROMOSOME ERRORS INVOLVING LARGE METACENTRIC AND SUBMETACENTRIC CHROMOSOMES ARE MORE COMMON IN YOUNG INFERTILITY PATIENTS. S. McCormick, a J. Stevens, a A. Schneiderman, a R. Smith, b W. B. Schoolcraft, a,b M. G. Katz-Jaffe, a,b Obstetrics Laboratories of Colorado, Lone Tree, CO; a,b Colorado Center for Reproductive Medicine, Lone Tree, CO.

OBJECTIVE: Advanced maternal age (AMA) is the most significant risk factor associated with oocyte aneuploidy. Women at 40 years of age have a 50% reduction in fecundity compared to women a decade younger, including a significant increase in pregnancy loss. Chromosome analysis of pre-implantation embryos has revealed that all 23 pairs of chromosomes are involved in both chromosome gains and losses. The aim of this study was to evaluate the classification of blastocyst chromosome errors in association with maternal age at the time of infertility treatment.

DESIGN: Research study.

MATERIALS AND METHODS: Infertility patients consented, with IRB approval, to an IVF cycle with comprehensive chromosome screening (CCS). All embryos were cultured to the blastocyst stage with a trophectoderm biopsy performed for CCS using either SNP microarray or quantitative PCR (RMA-NJ). Aneuploid blastocysts from infertility patients were divided among two groups: Group A = maternal age < 35 years (n=1,091 aneuploid blastocysts) and Group B = maternal age ≥ 40 years (n=3,624 aneuploid blastocysts). Chromosome aneuploidy results were analyzed using Chi square test with p value of 0.05 for significance.

RESULTS: Predictably, a significant difference was observed for the incidence of aneuploidy relative to AMA (Group A = 33% vs. Group B =73%; P<0.0001). Interestingly, the classification of these chromosome errors was significantly different between the two maternal age groups. AMA infertility patients (Group B) displayed a significant increase in errors (71.2% vs. 63.3% in Group A; p<0.0001) involving small metacentric and acrocentric chromosomes (13-22) that are observed in clinical pregnancy losses. Conversely, younger infertility patients (Group A) showed a significant increase in errors (16.5% vs. 13.5% in Group B; p=0.01) involving the large metacentric and submetacentric chromosomes (1-5), which are not typically observed in miscarriages but predominantly result in implantation failure.

CONCLUSION: The frequency and classification of chromosome errors in human blastocysts were significantly different between AMA (≥40 years) and younger infertility patients (<35 years), reflecting potential alternative mechanisms associated with aneuploidy generation. These results indicate a clinical advantage of blastocyst aneuploidy screening for younger infertility patients with a history of recurrent implantation failure.

O-241 Wednesday, October 22, 2014 11:30 AM

FROZEN VERSUS FRESH DONOR EGG IVF: SIMILAR EFFICACY AND GREATER EFFICIENCY IN A LARGE DONOR EGG IVF PROGRAM. L. H. Sekhon, a R. Holmes, b J. Bower, b B. Berger, b D. Salkhas, a Obstetrics, Gynecology and Reproductive Medicine, Icahn School of Medicine at Mount Sinai, New York, NY; bBoston IVF, Beth Israel Deaconess, Harvard Medical School, Waltham, MA.

OBJECTIVE: To evaluate the use of fresh donor (FrD) and vitrified donor (VitD) oocytes for in vitro fertilization (IVF) cycles during the same time period and compare the reproductive efficiency of both techniques in treating patients requiring donor oocytes.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: A chart review was conducted to compare the outcomes of donor egg IVF cycles conducted at a single, large IVF practice from 8/1/12 to 12/31/13, using FrD vs. VitD oocytes. The number of embryos transferred, implantation rates and ongoing pregnancy rates (defined as a confirmed pregnancy with fetal heart beat (FHB) per transfer) were compared. The number of post-cycle surplus embryos, vitrified for cryostorage, was noted for each group. The final expected FHB per oocyte used was calculated by addition of the number of fetuses with FHB from the fresh transfer and those expected utilizing the remaining vitrified blastocyst embryos; assuming 100% survival of vitrified blastocysts and a 35% implantation rate after transfer. A chi square analysis was used to compare IVF outcomes.

RESULTS: A total of 2812 FrD and 859 VitD oocytes were used. No significant differences were observed between FrD and VitD implantation rate (36.4% vs. 31.1%, P=0.05) and ongoing pregnancy per transfer rate (48.4% vs. 39.0%, P>0.05). Cycle cancellation rate was similar in both groups (3.8% vs. 2.8%). The mean number of embryos transferred was similar in both groups (1.53 ± 0.93 vs. 1.52 ± 0.66). The number of embryos transferred was FrD: N=228 (28.9% blastocysts; 71.1% Day 3) versus VitD: N=212 (31.3% blastocysts; 68.9% Day 3). A significantly greater number of embryos were frozen in the FrD group (N=904 vs. N=96). The final expected FHB per oocyte used was similar for both groups (FrD: 13.6% versus VitD: 11.4%), respectively.

CONCLUSION: VitD oocytes can provide high ongoing pregnancy and implantation rates, equivalent to the outcomes seen with FrD oocytes. Expected FHB per FrD oocyte vs. VitD oocyte was 13.6% vs. 11.4%, respectively. VitD cycles led to a drastically lower number of cryopreserved surplus embryos which may spare patients from the psychological distress often provoked by the dilemma of deciding what to do with surplus embryos in the future. VitD oocytes provide comparable final ongoing pregnancy rates per mature oocyte and are a beneficial and efficient treatment option.

O-242 Wednesday, October 22, 2014 11:45 AM

INVESTIGATION INTO METHYLATION OF IMPRINTED GENES IN CHILDREN CONCEIVED VIA ASSISTED REPRODUCTIVE TECHNOLOGIES (ART) COMPARED TO NATURALLY CONCEIVED (NC) CONTROLS. R. N. Vincent, K. B. Dong, E. Chan Wong, L. P. Cao, S. Ma. Obstetrics and Gynecology, University of British Columbia, Vancouver, BC, Canada.

OBJECTIVE: To investigate DNA methylation at the KvDMR1, PLAGL1 and PEG10 differentially methylated regions (DMRs) and the repetitive LINE-1 element in cord blood (CB) and placental chorionic villi (PCV) from babies conceived via ARTs compared to NC controls.

DESIGN: A case-control study was completed to determine DNA methylation levels at genes associated with known imprinting disorders, and at LINE-1 elements (gauge of global methylation) in ART and NC babies.

MATERIALS AND METHODS: DNA from babies born by in vitro fertilization (IVF) (N=59 CB, 66 PCV), intracytoplasmic sperm injection (ICSI) (N=52 CB, 65 PCV) and NC (N=59 CB, 69 PCV) was extracted and bisulfite-converted. The desired region was then amplified by PCR and methylation analysis at specific CpG sites was quantified by pyrosequencing. For the KvDMR, PLAGL1, PEG10 and LINE-1 regions, five samples were randomly chosen from each of the above cohorts and the results were calculated and significantly compared.

RESULTS: A total of 2812 FrD and 859 VitD oocytes were used. No significant differences were observed in DNA methylation between FrD and VitD oocytes. No significant difference in KvDMR methylation was observed in IVF (P=0.62) or ICSI (P=0.87) pregnancies compared to NC controls. No differences were observed in the PCV at PLAGL1 for IVF (P≤0.72) or ICSI (P≤0.54) infants. We observed lower than expected methylation at the PEG10 region in CB from NC, IVF and ICSI infants with mean values of 5.64%, 6.23% and 4.24%, respectively. No significant difference in PEG10 methylation was seen in IVF (P=0.62) or ICSI (P=0.95) CB samples or IVF (P=0.70) or ICSI (P=0.28) PCV samples compared to controls. At KvDMR1, mean methylation levels for IVF and ICSI were not significantly different from NCs in CB (P=0.56 and P=0.34) or in PCV (P=0.54 and P=0.78), respectively. No differences were detected between methylation levels of LINE-1 in CB of IVF (P≤0.19) or ICSI (P≤0.55) or in PCV of IVF (P=0.069) or ICSI (P≤0.48).

CONCLUSION: PLAGL1 is a known tumor suppressor (1) and changes in PLAGL1 have been implicated in Transient Neonatal Diabetes (2). Therefore, our findings indicate that children born via ART may be at an increased risk of imprinting disorders and certain types of cancers. Also, the lower than expected levels of methylation at the PEG10 region in the cord blood of babies of all conception modes may indicate a loss of imprinting in normal development and is an area requiring further research.

Supported by: This study was supported by the Canadian Institutes of Health Research (CIHR, grant to Sai Ma).
O-243 Wednesday, October 22, 2014 12:00 PM

MOST PATIENTS WOULD BENEFIT FROM MOVING MORE QUICKLY TO IVF: RESULTS FROM A COST-EFFECTIVENESS ANALYSIS OF A LARGE RETROSPECTIVE COHORT. H. V. Karvir, a M. Elashoff, a D.-E. Parfitt, a A. B. Copperman, b P. Yurttas Beim. a, b Celmatrix, Inc, New York, NY; b Reproductive Medicine Associates of New York, New York, NY.

OBJECTIVE: Given limited insurance coverage for many infertility treatments, cost is often a major driver of patient decision-making and, therefore, outcomes. Patients understandably tend to gravitate to less expensive options first, which in turn, these options often convey lower likelihoods of success. In this study, we aimed to generate personalized models to accurately predict the most cost effective strategy to achieving a live birth (LB) outcome on a patient-level basis.

DESIGN: De-identified retrospective data from a large academic reproductive medical center (38,036 cycles; 13,086 patients).

MATERIALS AND METHODS: Using clinical metrics available before treatment initiation, predictive models were developed to assess a patient’s personal chances of achieving LB over multiple cycles of self fresh/frozen embryo transfer (FET) in-vitro fertilization (IVF), donor-egg recipient (ER) fresh/FET IVF, and non-IVF fertility treatments (n-IVF). Predicted probabilities from the three treatment options were then propagated through a decision model that simulated all possible treatment permutations over six cycles. Inverse probability weighted cumulative cost was then used to determine the most cost effective treatment plan for each patient.

RESULTS: The majority of patients in our study (72%) were treated first with hormonal treatments and/or inseminations prior to IVF (mean 4.6 ± 2.5 cycles). Among these patients, 35% discontinued treatment without progressing to IVF. Only 28% of patients initiated with IVF first. In contrast, our cost effectiveness analysis demonstrated that a majority of these patients (73%) would have benefited most from initiating IVF upfront. The remaining patients would have benefited most from undergoing two cycles of n-IVF followed by IVF.

CONCLUSION: The most cost effective treatment strategy for achieving a LB outcome for the majority of patients is to pursue IVF treatment upfront. Third party payers have the opportunity to use these observations to revise “less before more” treatment strategies and consider “direct-to-IVF” as a more efficient paradigm. These findings have significant implications given our observation that, all too commonly, infertility treatment does not employ a most cost effective strategy.

Supported by: Celmatrix, Inc.

O-244 Wednesday, October 22, 2014 12:15 PM

IMPACT OF INSURANCE COVERAGE AND FINANCIAL INCENTIVES ON ESET ACCEPTANCE IN A NON-MANDATED STATE. F. I. Sharara, a, b Virginia Center for Reproductive Medicine, Reston, VA; OB/Gyn, George Washington University, Washington, DC.

OBJECTIVE: The use of elective single embryo transfers (eSET) in ART was only 15% in 2012, and significantly lags behind Western European countries where eSET is the norm. The reasons for this are many, including the absence of universal coverage for ART, lack of patient education, desire for twin pregnancy, financial reasons, inadequate counseling by providers, and fear of a lower success rates compared to double embryo transfers (DET). Even in mandated states, eSET utilization is suboptimal. We have designed an innovative trial to encourage couples undergoing ART to choose eSET. Patients were offered free HMG (Menopur), free freezing of all extra embryos, and free storage for one year (> $5,000 savings) if they agreed to have an eSET.

DESIGN: Prospective, pilot study.

MATERIALS AND METHODS: We included couples with age up to 38 yo, couples with prior failed cycles, women with uterine fibroids or prior uterine surgery, and women with diminished ovarian reserve. Only couples with very severe male factor (testicular sperm or counts < 1 million/cc) were excluded. To date, 68 women (< 38 yo) have participated in the study. All couples had an extensive consultation stressing the perinatal, neonatal, and maternal morbidities associated with twin gestation before starting ART, and again at the time of ET. All patients received daily injections of HP-hMG (Menopur) in GnRH-a or GnRH-ant cycles, and all cycles were blastocyst transfers. Only one cycle/patient was included. P<0.05 was considered significant.

RESULTS: Of the 68 couples, 41 (60.3%) agreed to have an eSET after counseling. Of the 68 couples, 30/68 (44.1%) had insurance coverage, and of these, 21 (70.0%) agreed to eSET. In contrast, 36 couples were self-pay (63.9%), and 20 (52.6%) agreed to undergo eSET (P = NS). Of the 30 couples with insurance coverage, 26 cycles were completed to date, the clinical PR was 73.1% (19/26), and ongoing/delivery PR was 57.7% (15/26). Of those without insurance coverage, 33 completed a cycle and the clinical PR and ongoing/delivery PR were 72.7% (24/33) and 54.3% (18/33) (P = NS compared to those with insurance).

CONCLUSION: By using innovative incentives and extensive counseling we were able to increase our eSET rate in this pilot study to over 60% in a non-mandated state using “real-world” patients. While statistically not different, couples who have insurance coverage for ART were more accepting of eSET for their initial cycle. Insurance companies need to not just strongly encourage but to incentivize couples to proceed with eSET. The study is ongoing.

Supported by: Ferring.

O-245 Wednesday, October 22, 2014 12:30 PM

SELECTION OF CHROMOSOMALLY NORMAL EMBRYOS IMPROVES EGG DONOR FROZEN TRANSFER PREGNANCY RATES. C Wagner Couglin, a B. Kaplan, a A. Beltsos, a S. Munne, b Global Genetics Institute; In contrast, 36 couples were self-pay (63.9%), and 20 (52.6%) agreed to undergo eSET (P = NS). Of the 30 couples with insurance coverage, 26 cycles were completed to date, the clinical PR was 73.1% (19/26), and ongoing/delivery PR was 57.7% (15/26). Of those without insurance coverage, 33 completed a cycle and the clinical PR and ongoing/delivery PR were 72.7% (24/33) and 54.3% (18/33) (P = NS compared to those with insurance).

CONCLUSION: By using innovative incentives and extensive counseling we were able to increase our eSET rate in this pilot study to over 60% in a non-mandated state using “real-world” patients. While statistically not different, couples who have insurance coverage for ART were more accepting of eSET for their initial cycle. Insurance companies need to not just strongly encourage but to incentivize couples to proceed with eSET. The study is ongoing.

Supported by: Ferring.

Donor Egg Blastocyst Transfers

<table>
<thead>
<tr>
<th>Fresh Transfer</th>
<th>Frozen Transfer</th>
<th>Frozen PGS Transfer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transfers (N=)</td>
<td>55</td>
<td>35</td>
</tr>
<tr>
<td>Ave # embryos transferred</td>
<td>1.8</td>
<td>1.7</td>
</tr>
<tr>
<td>Clinical Pregnancy Rate</td>
<td>67.3% (37/55)</td>
<td>57.1% (20/35)</td>
</tr>
<tr>
<td>SAB Rate</td>
<td>10.8% (4/37)</td>
<td>15.0% (3/20)</td>
</tr>
<tr>
<td>Ongoing/Delivered Rate</td>
<td>58.2% (32/55)</td>
<td>48.6% (17/35)</td>
</tr>
<tr>
<td>Implantation Rate</td>
<td>53.9% (55/102)</td>
<td>44.3% (27/61)</td>
</tr>
</tbody>
</table>

CONCLUSION: There was no difference in outcomes comparing fresh transfers and frozen transfers. Pregnancy outcomes were significantly higher in the PGS FET group compared to FET only for Pregnancy rates (p<0.05), ongoing pregnancy rates (p<0.05) and Implantation rates (p<0.025). Donor egg recipient transfers are an ideal paradigm to assess the effect of PGS due to favorable prognosis and controlled uterine environment. In this ideal patient population, PGS of donor egg embryos showed high implantation rates and low miscarriage rates suggesting PGS may be considered for recipient patients requesting PGS of donor egg embryos.
### O-246 Wednesday, October 22, 2014 12:45 PM

**PREDICTORS OF LIVE BIRTH RATE (LBR) AFTER INTRAUTERINE INSEMINATION (IUI) CYCLES.** Z. Khan, E. P. Barnard, D. E. Morbeck, J. R. Jensen. Obstetrics & Gynecology, Mayo Clinic, Rochester, MN.

**OBJECTIVE:** For patients undergoing fertility treatments, LBR is a more clinically relevant measure of successful outcome than clinical pregnancy rates (CPR). We report prognostic variables related to LBR after IUI cycles.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** All women undergoing IUI from 1/1/2005-12/31/2013 at Mayo Clinic, Rochester who gave IUI consent were included in the study. Information regarding ovulation induction (OI) protocols and semen parameters was collected. Regression models were utilized to identify independent factors associated with LBR.

Comparison of Successful vs. Unsuccessful IUI Outcome

<table>
<thead>
<tr>
<th></th>
<th>Live Birth (n=248)</th>
<th>No Live Birth (n=2402)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>31.63±4.2</td>
<td>32.73±4.8</td>
<td>0.0002</td>
</tr>
<tr>
<td>Ovulation Induction with Oral Agents</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>73(7.3)</td>
<td>927(92.7)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>175(10.9)</td>
<td>1430(89.1)</td>
<td></td>
</tr>
<tr>
<td>Ovulation Induction with Gonadotropins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>89(13.2)</td>
<td>588(86.9)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>159(8.3)</td>
<td>1769(91.8)</td>
<td></td>
</tr>
<tr>
<td>hCG Trigger</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>204(10.5)</td>
<td>1748(89.6)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>44(6.7)</td>
<td>609(93.3)</td>
<td></td>
</tr>
<tr>
<td>Semen Source</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>246(9.8)</td>
<td>2259(90.2)</td>
<td></td>
</tr>
<tr>
<td>Frozen</td>
<td>2(2)</td>
<td>98(98)</td>
<td>0.009</td>
</tr>
<tr>
<td>Total Motile Sperm in Ejaculate</td>
<td>124.7±11.3</td>
<td>120.7±12.9</td>
<td>0.51</td>
</tr>
<tr>
<td>Motility of Sperm in Ejaculate</td>
<td>63.1±13.3</td>
<td>59.6±15.9</td>
<td>0.0001</td>
</tr>
<tr>
<td>Total Motile Sperm in Inseminate</td>
<td>53±5</td>
<td>52.1±60.3</td>
<td>0.81</td>
</tr>
<tr>
<td>Motility of Sperm in Inseminate</td>
<td>86.1±10.4</td>
<td>82.7±16.1</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Numbers shown as: Mean±Standard Deviation or Count(Percentage)

**RESULTS:** A total of 959 women who underwent 2605 IUI cycles were included. Overall 13.6% women had a positive pregnancy test. CPR and LBRs were 12.7% (n=332) and 9.5% (n=248). Mean gestation age at delivery was 39.8±10.2 weeks. Women with successful live birth (LB) were younger; more of them used gonadotropins for OI and had hCG trigger compared to women without LB. They also used fresh semen sample for IUI and the sperm motility in the ejaculate (SME) as well as the inseminate (post wash) (SMI) was higher in this group compared to women without LBs. In the final multivariable model maternal age ≥ 38 years, stimulation protocol (oral agents vs. gonadotropins), SME >40% and SMI >70% were independently associated with LB with adjusted odds ratios (95%CI) of: 0.43 (0.27-0.67), 0.74 (0.46-0.84) vs. 1.51 (1.10-2.09), 1.87 (1.04-3.69) and 1.92 (1.13-3.59) respectively. No LBs were reported in women ≥43 years, where total motile sperm in the inseminate was <1 million, SME was <20% or SMI was <30%.

**CONCLUSION:** Although clinically relevant to patients, IUI success as LBR is rarely reported. This large cohort reports maternal age, mode of stimulation for OI and sperm motility as important prognostic markers for LB after IUI cycles.

### O-247 Wednesday, October 22, 2014 11:15 AM

**EPIGENETICS: ARE BABIES HEALTHIER CONCEIVED THROUGH IUI COMPARED WITH FRESH OR FROZEN IVF CYCLES?** J. M. Bolnick, A. D. Bolnick, M. S. Estill, M. P. Diamond. "Department of Obstetrics and Gynecology, Wayne State University, Detroit, MI; *Center for Molecular Medicine and Genetics, Wayne State University, Detroit, MI; †Department of Obstetrics and Gynecology, Georgia Regents University, Augusta, GA.

**OBJECTIVE:** Assisted reproductive technologies have been implicated in adverse pregnancy outcomes. The available data lacks significance with potential risks of methylation alterations and genomic imprinting. Our objective was to compare epigenetic alterations in newborns conceived from infertile patients who underwent either in vitro fertilization (IVF) or intrauterine inseminations (IUI) treatments.

**DESIGN:** Laboratory investigation.

**MATERIALS AND METHODS:** Three groups of infertile patients that underwent either IVF or IUI and delivered a viable infant were randomly chosen, Michigan Neonatal Biobank Bloodspots were obtained and identified, then placed into the appropriate intervention groups: IUI (N=17), Fresh IVF embryo transfer(N=38), and Frozen IVF embryo transfer(N=38). DNA methylation profiles were obtained using Illumina Infinium Human Methylation 450 BeadChip system.

**RESULTS:** Genders were identified utilizing Y chromosome probes. There were differences in methylation in male compared with female newborns: IUI vs. Fresh (1378 to 2082), IUI vs. Frozen (1860 to 3661) and Fresh vs. Frozen (1613 to 2165). A number of genes were present in both the male and the female comparisons: IUI vs. Fresh (638 genes), IUI vs. Frozen (1252 genes) and Fresh vs. Frozen (720 genes). Of these genes, a subset showed the same pattern of methylation changes (hypo- or hypermethylation) in both male and female comparison groups: IUI vs. Fresh (498 genes), IUI vs. Frozen (1052 genes), and Fresh vs. Frozen (559 genes). Within these groups of genes that exhibited similar patterns, 90% were hypermethylated in the IUI vs. IVF and the IUI vs. the IVF Frozen groups, while 81% of the genes in the Fresh IVF vs. Frozen comparison were hypomethylated. Disease association analysis, using Genomatix software GEPIs, identified both Autism and Schizophrenia may be associated with either hypomethylation or hypermethylation changes found in IUI and IVF comparisons.

**CONCLUSION:** Our data is consistent with an alteration of DNA methylation profiles in babies conceived through IVF treatments compared with IUI. Furthermore the data suggests that embryo freezing may be associated with other changes in methylation status. Additional studies are required to ascertain the spectrum of effects and pregnancy outcomes on newborns conceived through ART.

**Supported by:** This work was supported in part by the WSU Ob/Gyn Research fund to MPD

### O-248 Wednesday, October 22, 2014 11:30 AM

**THREE DAY PROGESTERONE AREA UNDER THE CURVE: A BETTER TEST THAN PROGESTERONE ON THE DAY OF HCG?** G. D. Royster, IV, M. J. Hill, S. M. Zarek, A. H. DeCherney, E. Levins, E. Wida, M. J. Levy. "Program on Reproductive and Adult Endocrinology, National Institutes of Health, Bethesda, MD; †Reproductive Endocrinology and Infertility, Walter Reed National Military Medical Center, Bethesda, MD; ‡Shady Grove Fertility, Rockville, MD.

**OBJECTIVE:** Elevated progesterone (P) on day of hCG trigger (P-hCG) has been associated with reduced ART pregnancy rates. However, less is known about the effect of persistently elevated P days before trigger. The

---

**FERTILITY & STERILITY®**

e85
OBJECTIVE was to compare the effect of P area under the curve (P–AUC) over multiple days to P–hCG, the primary outcome of a retrospective cohort study.

MATERIALS AND METHODS: All fresh autologous ART cycles from Aug 2013–Jan 2014 were included. Primary analyses performed with generalized estimating equations (GEE) with nesting for patients with multiple cycles. Univariate GEE assessed variables associated with P–levels and clinical pregnancy with a multivariate GEE model accounting for all significant confounders. The primary outcome was clinical pregnancy rate.

RESULTS: 1448 consecutive patients undergoing 1620 fresh autologous ART cycles met inclusion criteria (84 cycles were excluded because of an elevated P–hCG with no embryo transfer). Serum P–hCG, P–levels for each of the 3 days prior to hCG trigger and P–AUC for 1, 2, and 3 days were all negatively associated with pregnancy. The 3 day P–AUC had the strongest negative correlation and persisted in multivariate analysis. In multivariate GEE controlling for age, embryo quality, and day of embryo transfer, 3 day P–AUC remained significantly associated with pregnancy [p = 0.0003, OR 0.83 (0.75 – 0.92)]. Threshold analysis suggested cutoffs of ≥ 2 ng/mL for P–hCG and ≥ 4.5 ng/mL for 3 day P–AUC to predict clinical pregnancy failure. The PPV for pregnancy failure was 69% for elevated P–hCG versus 76% for 3 day P–AUC. Additional analyses demonstrated that 3 day P–AUC was negatively associated with clinical pregnancy rate regardless of day of transfer or embryo grade.

CONCLUSION: 3 day P–AUC had a stronger negative association with clinical pregnancy than P–levels on day of hCG, suggesting that chronic elevations in progesterone may be a more useful clinical marker to predict pregnancy failure. Clinicians should consider measuring P when lead follicles are ≥ 14 mm in order to identify cycles at risk of pregnancy failure.

Supported by: This work was supported, in part, by the Program in Reproductive and Adult Endocrinology (PRAE), Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), National Institutes of Health (NIH), Bethesda, MD.

O-294 Wednesday, October 22, 2014 11:45 AM

OXIDATIVE STRESS BIOMARKERS IN FOLLICULAR FLUID OF WOMEN WITH PCOS AND TUBAL FACTOR INFERTILITY: IS THERE A CORRELATION WITH IN-VITRO-FERTILIZATION OUTCOME? N. Malhotra, a K. Gogadashetty, a R. Duda, b N. Singh, a aART Center, Department of Obstetrics and Gynecology, All India Institute of Medical Sciences, New Delhi, Delhi, India; bLaboratory for Molecular and Genetics, Department of Anatomy, All India Institute of Medical Sciences, New Delhi, Delhi, India.

OBJECTIVE: To compare oxidative stress (OS) biomarkers in follicular fluid of women with PCOS and tubal factor infertility undergoing in-vitro-fertilization (IVF) and correlate them with assisted reproductive technique (ART) outcome including oocyte fertilization, embryo cleavage, and clinical pregnancy and miscarriage rate.

DESIGN: Cross sectional study.

MATERIALS AND METHODS: Follicular fluid collected during oocyte retrieval from 43 women with PCOS (group I) and 57 women with tubal factor infertility (group II) were assessed for OS biomarkers including Reactive oxygen species (ROS), Total anti-oxidant capacity (TAC), and 8-Isoprostane (8-IP) levels. ROS levels were detected by chemiluminescence, TAC and 8-IP by enzyme immunoassay methods. OS biomarkers were compared between both groups using Wilcoxon ranksum test and correlation with ART outcome with Spearman’s rank correlation coefficient. A p value of < 0.05 was considered significant.

RESULTS: Women in both groups were comparable in age, BMI and duration of infertility. There were no significant difference in the median levels of ROS [71.7 cpm vs 54.8 cpm] (p = 0.570) and TAC [44.4 Mm/ul vs 40.7 Mm/ ul of trolox equivalent] (p = 0.17) in the follicular fluid from women in group I when compared to group II. Levels of 8-IP were significantly higher in follicular fluid from women in group I as compared to group II [57.1 pg/ul vs 39.2 pg/ul] (p = 0.04). The levels of TAC were significantly higher in those women who were pregnant compared to those without pregnancy in both groups. OS biomarkers did not correlate with ART outcome including fertilization, cleavage or pregnancy rate in both PCOS and tubal factor infertility. Ongoing clinical pregnancy were no different in both groups (25.4% vs 29.1%, p = 0.34). Follicular fluid Levels of 8-IP in group I were significantly higher in women who had miscarriage when compared to those with ongoing pregnancy (p = 0.04). There was a positive correlation (0.16, p = 0.01) of 8-IP with miscarriage rate.

CONCLUSION: 8-IP an OS biomarker is elevated in follicular fluid from women with PCOS indicating the role of oxidative stress in PCOS, 8-IP levels correlate with miscarriage rate in women with PCOS undergoing IVF.

O-250 Wednesday, October 22, 2014 12:00 PM

MECHANICAL BIOMARKERS OF OOCYTE MATURATION. L. Zarnescu, a J. Han, b B. Behr, b R. Reijo Pera, a D. Camarillo. aDepartment of Bioengineering, Stanford University, Stanford, CA; bDepartment of Obstetrics and Gynecology, Stanford University, Stanford, CA; cDepartment of Chemistry and Biochemistry, Montana State University, Bozeman, MT.

OBJECTIVE: In the in vitro fertilization (IVF) clinic, several oocytes are collected from a patient and assessed for maturation. Oocytes which have visibly completed nuclear maturation (MII stage) and extruded a polar body are deemed mature and ready for fertilization. Those which lack a polar body (GV or MI stage) then undergo in vitro maturation (IVM) until they reach the MII stage and are ready for fertilization. Although nuclear maturation is easy to assess by simple observation, oocytes must also undergo cytoplasmic maturation before acquiring optimal developmental competence. Cytoplasmic maturation is still poorly understood and difficult to detect non-invasively. We have developed an approach to measure the mechanical parameters of oocytes, and our objective is to use these parameters to potentially predict cytoplasmic maturation, fertilizability, and viability.

DESIGN: We measured the mechanical parameters of mouse oocytes at the GV, MI, and MII stages in order to characterize how these parameters change over the course of nuclear maturation and whether mechanical properties could provide more information about fertilization and viability than visual observation alone.

MATERIALS AND METHODS: The mechanical parameters describing the viscous and elastic properties of oocytes were measured by observing their response to micropipette aspiration, and fitting the aspiration depth of each oocyte into the pipette to a 4-parameter bulk mechanical model.

RESULTS: Oocytes exhibited large changes in mechanical properties between the GV, MI, and MII stages. We also found many oocytes which did not appear to have reached the MII stage but had mechanical properties similar to MII oocytes, and were capable of fertilization and blastocyst formation. This leads us to believe that mechanical properties of oocytes could provide more information about developmental competence when compared to visual assessment alone, and may be related to cytoplasmic maturation.

CONCLUSION: We present a novel technique for demonstrating that the mechanical parameters of oocytes could be used to noninvasively assess both nuclear and cytoplasmic maturation.

Supported by: Bio-X seed grant program at Stanford University.

O-251 Wednesday, October 22, 2014 12:15 PM

A PILOT STUDY EVALUATING PLOIDY PREDICTIVE MODELS VIA TIME LAPSE MICROSCOPY (TLM) MORPHOKINETIC PARAMETERS; EXPOSING UNLIKELY UNIVERSAL PREDICTIVE METHODS. Y. G. Kramer, J. D. Kofinas, K. Melzer, N. Noyes, C. McCaffrey, D. H. McCulloh, J. A. Grifo. NYU Fertility Center, NYU Langone School of Medicine, New York, NY.

OBJECTIVE: To determine if Aneuploidy Risk Classification Model presented in the literature is predictive of embryo ploidy at the NYU Fertility Center (NYUFUC).

DESIGN: Retrospective Cohort Analysis.
RESULTS: Observed aneuploidy frequencies were significantly different from the expectations predicted using the algorithm and not significantly different than expected if there is no discrimination.

NYUFC Application of Aneuploidy Risk Model

<table>
<thead>
<tr>
<th>Category</th>
<th>Observed</th>
<th>Expected (Campbell)</th>
<th>Expected (no discrim)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>54.5% (42 / 77)</td>
<td>37% (28.5 / 77)</td>
<td>56.4% (43.4 / 77)</td>
</tr>
<tr>
<td>Medium</td>
<td>56.8% (25 / 44)</td>
<td>69% (30.4 / 44)</td>
<td>56.4% (24.8 / 44)</td>
</tr>
<tr>
<td>High</td>
<td>66.7% (8 / 12)</td>
<td>97% (11.6 / 12)</td>
<td>56.4% (6.8 / 12)</td>
</tr>
<tr>
<td>Compare</td>
<td>0.01 &lt; P &lt; 0.02</td>
<td>Not Significant</td>
<td></td>
</tr>
</tbody>
</table>

The model failed to segregate euploid embryos from aneuploid embryos cultured at our facility. 33.3% of euploid embryos from the high category with potential for transfer were missed.

CONCLUSION: Non-invasive morphokinetics is unlikely to be universally reliable in discriminating ploidy. Moreover, it cannot approach the accuracy of PGS. Patients not electing PGS or clinics without access to PGS may not improve cycle outcomes utilizing currently available aneuploidy risk models, even when using identical culturing systems. Despite their poor ability to discriminate ploidy, morphokinetics may be useful as an adjunct to PGS in selecting those PGS-screened euploid embryos with the best chances of implantation and live birth.

O-253 Wednesday, October 22, 2014 12:45 PM

CLINICAL VALIDATION OF EMBRYO CULTURE AND SELECTION BASED ON MORPHOKINETIC ANALYSIS: A RANDOMIZED CONTROLLED TRIAL BY TIME-LAPSE IMAGING. J. M. de los Santos,1 I. Rubio,2 Z. Larreategui,3 F. Ayerdi,4 J. Remohi,5 M. Meseguer,6 IVF Laboratory, Instituto Universitario IVI, Valencia, Spain;7 IVF Laboratory, IVI Bilbao, Leioa, Vizcaya, Spain.

OBJECTIVE: To determine if incubation in the integrated EmbryoScope(TM) time-lapse monitoring system improves the reproductive outcome.

MATERIALS AND METHODS: Data was analysed in Microsoft Excel using Analysis of Variance package (statistiXL plugin). Successful pregnancy was considered one which resulted in at least one foetal heart. A score of 2 was given for a successful pregnancy and 1 for an unsuccessful pregnancy. A pregnancy score was calculated against each variable (e.g. clinician A, average pregnancy score = 1.2). This then allowed the performance of analysis of variance between the different variables to determine whether the pregnancy score difference was significant or insignificant.

RESULTS: The result of analysing 1346 patients showed that no one catheter was better than another in achieving a pregnancy (P = 0.267). Clinician transferring the embryo had no significant effect on pregnancy outcome (P = 0.421). There was no statistically significant effect of reloading on pregnancy rate (P = 0.322). Some catheters were more likely than others to cause an additional reloading attempt (Long New Wallace is the most frequently associated with an additional loading attempt, P = 0.001). IVF had a higher likelihood of achieving a pregnancy than ICSI (P = 0.018). As expected, blastocyst culture and a later day of transfer was associated with a higher pregnancy rate (P = 0.000). Number of embryos transferred had no significant effect on pregnancy outcome. A larger number of embryos transferred was not associated with a higher rate of pregnancy. Clinicians need to consider this in view of the risk of multiple pregnancy in patients with more than one embryo transferred.

O-254 Wednesday, October 22, 2014 12:30 PM

DOES THE TYPE OF CATHETER USED, THE CLINICIAN LOADING THE EMBRYO OR THE NUMBER OF ATTEMPTS OF LOADING AN EMBRYO AFFECT PREGNANCY OUTCOME? A RETROSPECTIVE STUDY OF 1346 PATIENTS. A Abd El Maksoud. Centre of Reproductive Medicine, University Hospital Coventry Warwickshire, Coventry, the UK (1346 patients).

OBJECTIVE: To determine if certain factors have an effect on pregnancy rate including catheter used, clinician attempting the transfer and number of attempts of loading the embryo.

DESIGN: A retrospective study looking at pregnancy rates in all patients treated with In Vitro Fertilization (IVF) or Intra Cytoplasmic Sperm Injection (ICSI) between January 2010 to January 2012 at the Centre of Reproductive Medicine, University Hospital Coventry Warwickshire, Coventry, the UK (1346 patients).

MATERIALS AND METHODS: Data was analysed in Microsoft Excel using Analysis of Variance package (statistiXL plugin). Successful pregnancy was considered one which resulted in at least one foetal heart. A score of 2 was given for a successful pregnancy and 1 for an unsuccessful pregnancy. A pregnancy score was calculated against each variable (e.g. clinician A, average pregnancy score = 1.2). This then allowed the performance of analysis of variance between the different variables to determine whether the pregnancy score difference was significant or insignificant.

RESULTS: The result of analysing 1346 patients showed that no one catheter was better than another in achieving a pregnancy (P = 0.267). Clinician transferring the embryo had no significant effect on pregnancy outcome (P = 0.421). There was no statistically significant effect of reloading on pregnancy rate (P = 0.322). Some catheters were more likely than others to cause an additional reloading attempt (Long New Wallace is the most frequently associated with an additional loading attempt, P = 0.001). IVF had a higher likelihood of achieving a pregnancy than ICSI (P = 0.018). As expected, blastocyst culture and a later day of transfer was associated with a higher pregnancy rate (P = 0.000). Number of embryos transferred had no significant effect on pregnancy outcome. A larger number of embryos transferred was not associated with a higher rate of pregnancy. Clinicians need to consider this in view of the risk of multiple pregnancy in patients with more than one embryo transferred.
LEIOMYOMA

O-254 Wednesday, October 22, 2014 11:15 AM
EXOME CHIP EVALUATION OF GENETIC VARIANTS FOR ASSOCIATION WITH UTERINE FIBROIDS: T. L. Edwards, K. E. Hartmann, D. R. Velez Edwards, Department of Medicine and Division of Epidemiology, Nashville, TN; Vanderbilt Epidemiology Center, Nashville, TN; Center for Human Genetics Research, Nashville, TN; Institute of Medicine and Public Health, Nashville, TN; Department of Obstetrics and Gynecology, Nashville, TN.

OBJECTIVE: Uterine fibroid(UFs) affect up to 77% of women by menopause with large racial disparities and account for $9.4 billion in yearly healthcare costs. Although UFs are heritable, genetic risk is poorly understood. The first genome-wide association study(GWAS) of UFs was performed in 2011 in a Japanese population, however, to-date few large-scale genetic studies have been performed in US populations.(1) The objective of this study is to conduct a whole exome association study of UF risk in European Americans(EA) and African Americans(AA).

DESIGN: This is case-control genetic association study of UFs defined using pelvic imaging data obtained from an electronic medical record repository (BioVU DNA Repository).

MATERIALS AND METHODS: Logistic regression adjusted for ancestry and age was used in 728 EA and AA DNA samples (EA: 246 cases, 243 controls; AA: 121 cases, 118 controls) to evaluate SNPs for association, stratified by race. Meta-analyses of results with fixed effect models were performed to obtain combined evidence for associations across racial groups.

RESULTS: Our strongest association within EAs was within t-complex 11, testis-specific like 1 TCP11LI1, rs1064005, OR 1.79, 95% CI 1.36-2.32, p=3.94x10-5 and within multiple SNPs within the HLA region (smallest est p=9.28x10-5). It is of note that the exon chip has enhanced coverage of the HLA region compared to GWAS arrays. Among AAs strong associations were observed within glipican 4 (GC4, OR = 0.46, 95% CI 0.31-0.68, p = 8.73x10-5). Meta-analysis across EA and AA further strengthened the associations observed at TCP11LI1 (smallest meta-p=4.09x7, with effect sizes consistent across races).

CONCLUSION: Prior gene expression studies of TCP11LI1 have shown increased expression in tumor tissues compared to normal tissues across several different cancers and variants within HLA genes have been associated with UF risk in prior studies. These pilot data suggest common variants in exonic regions increase risk for UF in both EA and AA populations. However, further validation of our study findings is needed to confirm our results. We are currently genotyping >4,000 samples for GWAS and exome arrays to further evaluate the relationship between gene variants and UF risk.

Supported by: The NICHD supports Dr. Velez Edwards (1R01HD074711-01 and 1R03HD078567-01).

O-255 Wednesday, October 22, 2014 11:30 AM
AN ALTERED PERCEPTION OF NORMAL: A QUALITATIVE ASSESSMENT OF WOMEN’S EXPERIENCES WITH SYMPTOMATIC UTERINE FIBROIDS: M. S. Ghant, K. Sengumba, H. Recht, K. A. Cameron, R. Vogelzang, E. E. Marsh, Obstetrics and Gynecology - REI Division, Northwestern University Feinberg School of Medicine, Chicago, IL; Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL; Radiology, Northwestern University Feinberg School of Medicine, Chicago, IL.

OBJECTIVE: To qualitatively identify and characterize factors that impact women’s experiences with uterine fibroids.

DESIGN: Qualitative semi-structured interviews and demographic survey.

MATERIALS AND METHODS: Forty-eight women diagnosed with symptomatic uterine fibroids were recruited from an urban academic medical center and organizations within the community. Participants completed in-depth, one-on-one interviews, which were transcribed verbatim and uploaded to NVivo version 10 for data management and thematic coding. Participants also completed a demographic survey and a health literacy assessment. Themes were identified by three coders using a grounded theory approach.

RESULTS: A total of 29 hours of interviews were conducted yielding 1,108 transcribed pages. The k across coders was 0.94. Mean age of participants was 42.8 ± 7.4 (mean ± SD). 62.5% of the subjects were African-American, 20.8% were Caucasian, 10.4% were Hispanic and 6.3% were Asian. A significant portion of the thematic content focused on delayed diagnosis and women’s perceptions of their symptoms. Many subjects did not obtain an immediate diagnosis despite experiencing severe heavy menstrual bleeding. The most commonly cited reason for this delay was their perception that what they were going through was normal and something they “must endure as a woman.” When the women recognized that something was wrong, most reported surprise regarding their diagnosis of fibroids, because they either did not perceive themselves at risk or had never heard of fibroids. Over half of the participants expressed that they were now “just dealing” with their fibroids and minimized the severity of their symptoms. Several women also dissociated themselves from the fibroids through referring to them as “alien” and as a problem they compartmentalized.

CONCLUSION: Women with symptomatic fibroids may live with this chronic condition without seeking care because they think their symptoms are normal and something with which they must “just deal.” These findings suggest that there is a significant need for both patient-focused and community-centered education to inform women of fibroids, symptoms and specifically to promote treatment options. These data highlight an opportunity and need for a multidisciplinary approach to this vulnerable population of women.

Supported by: NIH WWHR Program K12HD050121, RWJ Foundation, NHM, and NMH, Evergreen Foundation (EEM); and AHRQ 1P01HS021141-01 (KAC).

O-256 Wednesday, October 22, 2014 11:45 AM
THE MEDIATOR COMPLEX SUBUNIT 12 (MED12) MODULATES THE EXPRESSION OF EXTRACELLULAR MATRIX GENES IN HUMAN UTERINE FIBROID CELLS: S. K. Halder, A. Al-Hendi, Department of Obstetrics and Gynecology, Medical College of Georgia, Georgia Regents University, Augusta, GA.

OBJECTIVE: Uterine fibroids are benign tumors of reproductive age women. Mutations in the MED12 gene have been detected in up to 80% of human fibroid lesions. Using shRNA technology, we have silenced MED12 gene expression in human uterine fibroid cells, and as such, previously demonstrated that MED12 is related with fibroid cell proliferation (1, 2). Since excessive production of extracellular matrix (ECM) is one of the major feature of uterine fibroids, this study is set to evaluate whether MED12 is associated with ECM gene expression in human uterine fibroid cells.

DESIGN: To determine the role of MED12 in ECM production we will use our established MED12-KD (knock-down) clone and non-functional scramble control clone.

MATERIALS AND METHODS: We knocked-down the expression of MED12 gene in human uterine fibroid cell line (HuLM) using MED12-specific shRNA. HuLM cells were infected with lentiviruses carrying plasmid constructs containing MED12 gene-specific short sequences or scramble-control with green fluorescence protein. Stable clones that expressed the lowest levels of MED12 gene was selected for further analyses.

RESULTS: We performed western blot analyses using protein lysates from scramble-control clone#12 and MED12-KD stable clone#16. We noticed that MED12-KD clone exhibited significant reduction of fibronectin (55%) and collagen type 1 (70%) protein expression when compared with scramble-control clone (p<0.05). We also observed that the MED12-KD clone showed reduced expression of transforming growth factor beta receptor type II (TGFβRII) (p<0.05). MED12-KD clone exhibited reduced levels of Smad activation as determined by the reduction of phospho-Smad2 when compared with scramble-control clone. Furthermore, immunofluorescence analyses with rabbit polyclonal anti-fibronectin and anti-collagen type1 antibodies confirmed the reduced expression and localization of fibronectin and collagen type 1 in the MED12-KD clone. These results suggest that MED12 can modulate the expression levels of several ECM-related genes in uterine fibroid cells. Using immunofluorescence and co-immunoprecipitation assays we are currently verifying the nuclear translocation of Smad proteins and their functional complex formation among Smad2, Smad3, and Smad4 in MED12-KD clone in presence or absence of TGF-β3.

CONCLUSION: Loss of MED12 gene expression reduces the levels of fibronectin and collagen type 1 in human uterine fibroid cells. Alteration of MED12 function may play pivotal role in initiation and/or propagation of uterine fibroid pathogenesis.

Supported by: RCMI pilot 2G12R003032-26, and NIH/NICHD R01 HD046228.
O-257 Wednesday, October 22, 2014 12:00 PM

ASSOCIATION OF BODY MASS INDEX (BMI) AND ADIPOKINES WITH THE PREVALENCE OF FIBROIDS IN YOUNG AFRICAN-AMERICAN WOMEN: A CASE-CONTROL STUDY. E. E. Marsh,* M. Steinberg,* L. Bernardi,* P. de Chavez,* M. S. Ghant,* L. M. Neff,* M. Carneith.Obstetrics and Gynecology - REI Division, Northwestern University Feinberg School of Medicine, Chicago, IL; 2Preventive Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL; 3Medicine - Division of Endocrinology and Metabolism, Northwestern University - Feinberg School of Medicine, Chicago, IL.

OBJECTIVE: To determine a relationship between fibroid prevalence and markers of obesity in young African-American women (AAW).

DESIGN: Case-Control.

MATERIALS AND METHODS: Study participants were selected from the Study of Environment, Lifestyle & Fibroids (SELF) (n=1,695). Inclusion criteria for participating in SELF included AA race, age 23-34 at recruitment, no known diagnosis of fibroids, no history of hysterectomy, and no history of cancer, Lupus, Grave’s Disease, Sjogren’s Disease, scleroderma, or multiple sclerosis that required medical or radiation treatment. All of the subjects underwent ultrasound and 364 women were found to have fibroids (cases). 364 age-matched controls were selected from the subjects without fibroids. Serum leptin and total adiponectin were determined with ELISA assays. Conditional logistic regression analysis was performed to obtain odds ratio (OR) and 95% confidence intervals (95% CI).

RESULTS: The prevalence of fibroids was significantly less common among women who reported ever-using hormonal contraception (OR=0.54, 95% CI=0.35-0.82 vs never), prior pregnancy (OR=0.52, 95% CI=0.36-0.74 vs never pregnant) and parity (OR=0.48, 95% CI=0.35-0.62 vs. nulliparous) in univariate models. There was no association of fibroids with age at menarche, current hormonal birth control use, marital status, education level, smoking status, alcohol history, BMI, and serum levels of leptin and total adiponectin. In a multivariable model, both ever-using hormonal contraception and prior pregnancy remained significantly associated with a lower likelihood of having fibroids (OR=0.62, 95% CI=0.40-0.96 vs never; OR=0.54, 95% CI=0.38-0.78 vs never pregnant respectively).

CONCLUSION: While previous reports in the literature have suggested a potential association with fibroid prevalence and obesity, our study of African American women, we found no difference between several markers of adiposity i.e. BMI, leptin, and adiponectin, and the prevalence of fibroids. Further studies are needed to determine whether the incidence (versus the prevalence) of fibroids is impacted by serum and biometric markers of obesity.

Supported by: NICHHD Women’s Reproductive Health Research (WRHR) Scholar; Robert Wood Johnson Foundation; Friends of Prentice; and Evergreen Invitational (EEM).

O-258 Wednesday, October 22, 2014 12:15 PM

COMMON GENETIC VARIANTS MAY IDENTIFY WOMEN WITH LARGE UTERINE FIBROIDS ON ULTRASOUND: THE CARDIA WOMEN’S STUDY (CWS). M. Wellons,a T. M. Edwards,a K. E. Hartmann,a M. Parnage,a D. R. Velez Edwards,a* Vanderbilt University Medical Center, Nashville, TN; 2University of Texas Health Science Center at Houston, Houston, TX.

OBJECTIVE: Uterine fibroids (UFs) affect 77% of women by menopause and account for $9.4 billion in yearly healthcare costs. Although UFs are heritable, genetic risk is poorly understood. We performed a genome-wide association study (GWAS) among women of European descent, a single nucleotide polymorphism (SNP), rs4247357, reached genome-wide significance in association with UF. This SNP is located on chromosome 17 in a large linkage disequilibrium (LD) block that contains three genes, fatty-acid synthase (FASN), coiled-coil-domain-containing 57 (CCDC57), and solute carrier family 16, member 3 (SLC16A3). Levels of FAS, the enzyme responsible for de novo fatty-acid synthesis, were shown to be higher in UL tissue than in matched myometrium. We sought to replicate the association between rs4247357 and UL risk in the Black Women’s Health Study.

DESIGN: Prospective cohort study of African American women.

MATERIALS AND METHODS: We genotyped 2,014 incident UL cases and 1,939 controls for rs4247357 and a panel of validated ancestry informative markers (AIMs). All women were premenopausal, had intact uteri, and were aged 23-50 years in 1997. Associations were assessed using logistic regression with control for age, geographic region of residence, and percent European ancestry.

RESULTS: Overall, rs4247357 was not associated with risk of UL. Relative to the CC genotype (28.7%), the odds ratio was 0.96 (95% confidence interval (CI): 0.83, 1.11) for the AC genotype (52.2%) and 1.01 (CI: 0.84, 1.22) for the AA genotype (19.1%) (P-trend=0.9572). No appreciable associations were found between rs4247357 and UL risk when we stratified the data by age at baseline, surgical treatment, family history of UL, and recency of pelvic ultrasound. In contrast, we found evidence of an association among the women with the highest levels of %European ancestry (≥40%), but numbers were small: relative to the CC genotype (24.0%), the odds ratio was 1.94 (CI: 0.83, 1.11) for the AC genotype (54.0%) and 2.29 (CI: 1.00, 4.52) for the AA genotype (22.0%) (P-trend=0.1248).

CONCLUSION: Our data indicate that rs4247357 is not associated with UL incidence in African American women. This observation is further supported by suggestive evidence of a positive association only among women with ≥40% European ancestry. Fine-mapping may be warranted to determine whether this genomic region is causally related to UL in African American populations and, if so, whether there are other SNPs that are more closely correlated with the causal variant.

Supported by: This work was supported by grant R01HD057966 from the Eunice Kennedy Shriver National Institute of Child Health and Human Development and by grants RO1CA058420 and RO1CA098663 from the National Cancer Institute, Division of Cancer Control and Population Science.

FERTILITY & STERILITY® e89
O-260 Wednesday, October 22, 2014 12:45 PM

TUMOUR-TARGETED ADENOVIRAL VECTORS SHOW ENHANCED EFFICACY AGAINST UTERINE FIBROIDS IN A MU- RINE MODEL. S. A. Mohamed,1,2 A. M. Shalaby,1,2 A. A. Lakkunar,1 S. Nair,1 A. Al-Hendy,1 1Masoura Medical School, Mansoura, Dakahliya, Egypt; 2Tanta Medical School, Tanta, Gharbeyia, Egypt; 3Georgia Regents University, Medical College of Georgia, Augusta, GA.

OBJECTIVE: Fibroids are steroid hormone-dependent uterine tumors with no effective medicinal therapy at the moment; thus hysterectomy is the mainstay of treatment. Our laboratory has been developing selective targeted gene therapy as a potentially viable alternative localized therapy for uterine fibroids. In this study, we are investigating the effect of a modified adenovirus vector, Ad-SSTR-RGD-TK (Adenovirus human somatostatin receptor subtype 2- arginine, glycine and aspartate thymidine kinase), as a suicide gene targeted therapy to fibroids lesions in nude mice is indeed more effective than Ad-TK.

DESIGN: in vivo study.

MATERIALS AND METHODS: Sixteen nude mice implanted with estrogen pellets were subcutaneously injected with rat ELT3 fibroid cells (10 million/mouse) in the right flank. After the tumors were palpable, a single direct intra tumor injection of Ad-SSTR-RGD, untargeted Ad-TK, or Ad-LacZ was given followed by daily intra-peritoneal injection of GCV (5 days). Animals were evaluated regularly and tumors measured weekly. Samples from tumors and other body organs were collected at day 20 and day 40 of treatment to assess the efficacy of the modified vector. Evaluation of tumor reduction on day 20 and day 30, as well as apoptosis and proliferation markers was performed in our laboratory.

RESULTS: Western blot analysis showed that, in comparison with the control Ad-LacZ vector, Ad-TK caused a 44% reduction in the expression of cell proliferation protein PCNA (Proliferating Cell Nuclear Antigen), and Ad-SSTR-RGD-TK/GCV more effectively mediated a 60% reduction in the expression of PCNA. Regarding the activity of the apoptotic protein product of cleavage of PARP1 (Poly ADP Ribosyl Polymerase1) we found that Ad-SSTR-RGD-TK/GCV induced apoptosis 6 folds compared to Ad-TK (P<0.05). Immunohistochemistry using Trichrome staining showed dramatic decrease in cellularity in fibroid lesions treated with Ad-SSTR-RGD group compared to Ad-TK group. Additionally, IHC using anti PCNA antibody showed that Ad-TK & Ad-SSTR-RGD-TK/GCV effectively reduced expression of PCNA by 70% and 90% respectively, compared to Ad-LacZ control.

CONCLUSION: Fibroid-specific targeting strategies for adenoviral vectors enhance their ability to reduce cell proliferation and induce apoptosis in a fibroid mouse model. Adenoviral-based gene therapy may be a viable localized non-surgical option for effective treatment of uterine fibroid.

Supported by: NIH/NICHD R01 HD046228.

ENVIRONMENT AND REPRODUCTION

O-261 Wednesday, October 22, 2014 11:15 AM

URINARY CONCENTRATIONS OF BENZOPHENONE-TYPE UV FILTERS AND COUPLE FECUNDITY. G. M. Buck Louis,4 R. Sundaram,4 K. Supa,4 J. Maisog,4 K. Kuman,4 Division of Intramural Population Health, NICHD, Rockville, MD; 2Division of Intramural Population Health, Glotech, Inc., Rockville, MD; 3Wadsworth Center, NYS Department of Health, Albany, NY.

OBJECTIVE: Recently, concern has arisen about the potential toxicity of benzophenone-type ultra violet (UV) filters, chemicals added to sunscreen and personal care products, that are reported to have estrogenic and antiandrogenic in vivo and in vitro activity.

DESIGN: Prospective cohort with longitudinal followup through one year of trying.

MATERIALS AND METHODS: 501 couples stopping contraception to become pregnant were recruited from 16 counties in Michigan and Texas. Each partner of the couple provided urines following the baseline interview and each completed daily journals. Women utilized home fertility monitors to time intercourse relative to ovulation, and digital home pregnancy tests starting on the day of expected menstruation. Five UV filters were quantified (ng/ml): using triple-quadrupole mass spectrometry with ongoing quality control procedures: BP-1 (2,4-dihydroxybenzophenone), BP-2 (2,2‘,4,4’-tetrabromobenzophenone), BP-3 (2-hydroxy-4-methoxybenzophenone), BP-5 (2,2‘,4,4‘,5-pentamethylbenzophenone), BP-8 (2,2‘-dihydroxy-4-methoxybenzophenone), and 4-OH-BP (4-hydroxybenzophenone). Adjusted fecundability odds ratio (FORS) and 95% confidence intervals (CIs) were estimated for each filter dichotomized at the 75th percentile and adjusting for age, creatinine, body mass index, serum cotinine, and site while accounting for time off contraception. Separate models were run for each UV filter and partner, but final models included both partners’ concentrations given their low correlations (r = 0.017).

RESULTS: For male partners, BP-2 was significantly associated with reduced fecundity (FOR = 0.95 95% CI = 0.85-0.95). In models adjusting for both partners’ concentrations, BP-2 remained significantly associated with reduced fecundity for males (FOR = 0.70. 95% CI = 0.50, 0.97). When modeled as continuous concentrations however, the directionality for BP-2 remained but no longer retained significance. No UV-filters were significantly associated with fecundity when assessing females’ urinary concentrations.

CONCLUSION: To our knowledge, these are the first human evidence suggesting that male partners’ exposures to select UV filters may diminish fecundity resulting in a longer TTP. If the findings are corroborated, reducing exposure to these estrogenic compounds may help improve fecundity. Supported by: NICHD Intramural funding (contracts #N01-HD-3-3355; N01-HD-3-3356; NOH-HD-3-3356; HHSN2752000001).
STATISTICALLY SIGNIFICANT IMPROVEMENTS IN CLINICAL OUTCOMES USING ENGINEERED MOLECULAR MEDIA AND GENOMICALLY MODELED ULTRAVIOLET LIGHT FOR COMPREHENSIVE CONTROL OF AMBIENT AIR (AA) QUALITY. M. Forman, A. E. T. Sparks, S. Degelos, G. Koulilanos, K. C. Worthing, A. Ob Gym, University of Iowa Hospitals and Clinics, Iowa City, IA; Center for Reproductive Medicine, Mobile, AL; LifeAire Systems, LLC, Allentown, PA.

OBJECTIVE: Successful preimplantation embryogenesis is critically dependent upon the culture environment and changing organic chemistry of the AA within the IVF laboratory. AA contains dynamic levels of embrittoxic factors and volatile organic compounds that play a critical role in preimplantation toxicity. Our retrospective analysis examined the impact of comprehensive control of airborne pathogens on measures of embryogenesis in multiple IVF programs using a proprietary air purification system (Aire). Aire® was designed specifically to protect the human embryo.

DESIGN: Retrospective analysis of AA and clinical outcome data.

MATERIALS AND METHODS: Clinical outcome data from all non-donor IVF patients (n = 1245) cycling through 3 independent IVF programs were evaluated over a 24-month period. Data were collected for 692 patients cycling in an environment protected by existing means of air filtration and 553 patients after the installation of Aire® (LifeAire Systems). Blastocyst conversion rate (BCR) was defined by zygotes reaching the blastocyst stage by Day 5, clinical pregnancy rate (CPR) by an intruterine gestational sac, ongoing pregnancy (OP) by positive fetal cardiac activity and loss rate (LR) as positive CPR without subsequent fetal cardiac activity. Statistical analyses included odds ratios calculated with 95% confidence intervals and α = 0.05 using MedCalc Software 13.1.2, Ostend, Belgium.

RESULTS: Patients whose embryos were cultured under installation of the Aire®-IVF system demonstrated a significant increase in BCR (27.6% vs. 46.3% [p = 0.001]), implantation rate (IR) (26.4% vs. 40.6%, [p = 0.001]), CPR (57.1% vs. 67.6% [p = 0.001]), and OP (36.4% vs. 51.2% [p = 0.001]) for pre-Aire®-IVF and post-Aire®-IVF rates, respectively. The mean number of embryos transferred decreased from 2.39 to 2.08 post-Aire®-IVF installation (p = 0.053). Patients whose embryos were cultured in an Aire®-IVF environment (n = 769) demonstrated a significant decrease in LR (36.2% vs. 24.3%, [p = 0.001]).

CONCLUSION: Concomitant with an in vitro culture environment protected by the Aire®-IVF system was a statistically significant increase in BCR, IR, CPR, OP and a decrease in LR. Removal of airborne pathogens and comprehensive control of the AA serving the in vitro culture environment is critical to successful preimplantation embryogenesis and clinical outcomes.


OBJECTIVE: Few studies have looked at substance use among infertile men and their relationship to sexual satisfaction, and erectile function. This study sought to evaluate whether there was a correlation between alcohol and tobacco use with subjective parameters of sexual health and satisfaction among infertile men.

DESIGN: After IRB approval, we retrospectively reviewed 753 surveys completed by men who presented to an infertility clinic between 2003-2011.

MATERIALS AND METHODS: We evaluated patient’s International Index of Erectile Function (IIEF) Domain scores according to reported alcohol and tobacco use.

RESULTS: The mean age of the cohort was 34.7 years (SD 5.9 yrs). Of the men surveyed, 16% were tobacco users and 73% consumed alcohol. As compared to non smokers, men who smoked were more likely to have low confidence in their ability to get and keep an erection, (9.9% vs 5%, p = 0.04), feel that their erections were not hard enough for penetration, (8.2% vs 2.8%, p=0.03), that it was more difficult to maintain their erections to completion of intercourse, (8.3% vs 2.6%, p=0.02) and they were more likely to feel unsatisfied with sexual intercourse (7% vs 2%, p = 0.02). Among smokers, 3.4% had severe erectile dysfunction and 5% had moderate erectile dysfunction based on their IIEF Domain Scores. As compared to men who drink alcohol, men who did not drink any alcohol were more likely to report that their erections were not hard enough for penetration (7% vs 2%, p = 0.002) and that they would be unable to maintain erections after penetration (6.2% vs 1.6%, p = 0.0007). Non-alcohol drinkers were also more likely to report that they could not maintain an erection to completion of intercourse (5% vs 2%, p = 0.05). Interestingly there was no difference in overall sexual satisfaction between men who drank alcohol and those that did not.

CONCLUSION: Sexual dysfunction/Erectile dysfunction is more prevalent in infertile men who use tobacco and, unpredictably, those who do not consume alcohol. Tobacco use and decreased alcohol consumption may be predictive of men’s assessment of their sexual/erectile function.


OBJECTIVE: To evaluate whether critical windows of exposure to air pollutants early in gestation increase preterm delivery risk.


MATERIALS AND METHODS: Preterm delivery (<37 completed weeks) was based on the best clinical estimate reported in electronic medical records at delivery and modified Community Multiscale Air Quality models calculated peak exposures for four time windows (gestational weeks 1-7, 8-14, 15-21, 22-28) for particulate matter (PM) ≤2.5 microns and ≤10microns, nitric oxides (NOx), sulfur dioxide (SO2), and ozone. Logistic regression with generalized estimating equations calculated the odds ratio (OR) and 95% confidence interval (CI) for each pollutant window peak exposure in relation to preterm delivery after adjustment for study site, maternal age and gestational age at delivery. We also evaluated whether peak exposures were associated with preterm delivery based on gestational age and severity interaction terms.

RESULTS: Preterm delivery occurred in 26,103 pregnancies (11.7%). The average peaks were similar across time windows. In the first seven weeks, the mean (range) of PM2.5 = 17.6 ug/m3 (5.3-54.7); PM10 = 30.9 ug/m3 (11.0-81.0); NOx = 43.4 parts per billion (ppb) (5.8-145.1); CO = 708 ppb (172.9-1679.0); SO2 = 5.5 ppb (0.9-33.7); and ozone = 35.3 ppb (7.8-64.3). A ten-unit change in peak exposure during the first 7 weeks of gestation was significantly associated with preterm delivery for PM2.5 (OR = 1.05; CI: 1.03, 1.08); PM10 (OR = 1.01; CI: 1.02, 1.06) and NOx (OR= 1.01; CI: 1.00, 1.01). Peak exposures during later time windows generally showed reduced risk estimates. No interaction between ART and exposure was observed, suggesting similar effects for ART and spontaneous conceptions.

CONCLUSION: Peak exposure to air pollutants, particularly PM2.5, PM10 and NOx in the first 7 weeks of gestation increased preterm risk while later time points did not.

Supported by: Eunice Kennedy Shriver National Institute of Child Health and Human Development Intramural Research Program.

URINARY CONCENTRATIONS OF BENZOPHENONE-TYPE UV FILTERS AND SEMEN QUALITY. G. M. Buck Louis, Z. Chen, S. Kim, K. Supra, J. Bae, K. Kamman. Division of Intramural Population Health, NICHD, Rockville, MD; Department of Environmental Health, Wadsworth Center, Albany, NY.

OBJECTIVE: Benzophenone-type ultra violet (UV) filters are highly effective chemicals added to sunscreen and personal care products for the protection of skin and hair against harmful UV rays. Despite evidence suggesting estrogenic and anti-androgenic in vivo and in vitro activity, no human studies have assessed reproductive outcomes or semen quality, specifically.

DESIGN: Prospective cohort.

MATERIALS AND METHODS: The study population comprises male partners of 501 couples recruited preconception when trying for pregnancy and followed until pregnant or up to 12 months of trying, 2005-2009. At enrollment, males provided urine and blood samples for the analysis of UV-filters and serum cotinine, respectively. Men provided 1-2 semen samples approximately one month apart using at home collection protocols for the analysis of 35 semen quality endpoints: 5 general, 8 motility, 6 sperm head, 14 morphology, and 2 sperm chromatin stability assay endpoints. Five UV filters were quantified (ng/mL) using triple-quadrupole mass spectrometry: BP-1 (2,4-dihydroxybenzophenone), BP-2 (2,2',4''-tetracydroxybenzophenone), BP-3 (2-hydroxy-4-methoxybenzophenone), BP-8 (2,2',6',4''-tetrahydroxybenzophenone), BP-8 (2,4-dihydroxybenzophenone), BP-2 (2,2',4''-tetracydroxybenzophenone), BP-3 (2-hydroxy-4-methoxybenzophenone), BP-8...
(2,2’-dihydroxy-4-methoxybenzophenone), and 4-OH-BP (4-hydroxybenzophenone). Linear regression models with fixed and random effects were used to estimate beta coefficients (β) and standard errors (SE) for each UV filter and Box-Cox transformed semen quality endpoint, after adjusting for age, body mass index, cotinine, and research site.

RESULTS: 413 (88%) men provided urine and ≥1 semen samples available for analysis. Four UV filters were significantly associated with ≥1 semen parameters; 3 were negatively associated with percent motility (BP-1, BP-3, 4-OH-BP). BP-8 was associated with both high DNA stainability (β = 0.80; SE = 0.23; p = 0.004) and DNA fragmentation (β = 1.04 ±SE = 0.52; p = 0.04).

CONCLUSION: Our findings provide the first evidence suggesting that UV filters may affect semen quality, but corroboration of findings is needed given our exploratory analysis.

Supported by: NICHD Intramural funding (contracts #N01-HD-3-3355; N01-HD-3-3356; NOH-HD-3-3358; HHSN27520001).

O-267 Wednesday, October 22, 2014 14:45 PM

THE ASSOCIATION OF DIETARY FAT INTAKE AND ANTRAL FOLLICLE COUNT (AFC) AMONG WOMEN UNDERGOING INFERTILITY TREATMENTS. I. Souter, Y.-H. Chiu, M. Afeiche, R. Hauser, J. E. Chavarro, S. C. Baumgarten, S. C. Star, C. Stocco, A. M. Zamah, Y.-H. Chiu, 0.05), while P levels were high (20 ng/ml; p < 0.05) during subsequent culture. While exogenous rhFST during wk 0-3 did not alter follicle survival and diameters (88%; 774 μm), rhFST exposure during wk 0-3 reduced follicle survival rate and growth at wk 5 (49%; 652 μm; p < 0.05) compared with controls (52%; 785 μm). Growing follicles formed an antrum at wk 3, and steroid production increased. For follicles cultured with rhFST during wk 0-3, E2 levels were low at wk 3 (97 pg/ml; p < 0.05), while P levels were high at wk 5 (20 ng/ml; p < 0.05), compared with controls (199 pg/ml E2; 10 ng/ml P). For follicles cultured with rhFST during wk 3-5, E2 levels were low (<346 pg/ml; p < 0.05), while P levels were high (21 ng/ml; p < 0.05) at wk 5, compared with controls (5689 pg/ml E2; 10 ng/ml P). Healthy, germinal vesicle oocytes were retrieved from all groups after 34-hr human chorionic gonadotropin exposure, but only the control group yielded 2 metaphase II oocytes which were successfully fertilized.

CONCLUSION: Macaque follicles produce FST in vitro which may have local effects on follicle growth and function. FST suppressed the survival and growth of preantral follicles, as well as E2 production by antral follicles in vitro.

Supported by: NIH P51OD011092, NIH 2K12HD043488, American Society for Reproductive Medicine.

O-269 Wednesday, October 22, 2014 11:30 AM

FSH RECEPTOR (FSHR) AND IGF-1 Receptor (IGF-1R) COORDINATED ACTIVATION OF AKT ESSENTIAL FOR HUMAN GRANULOSA CELL DIFFERENTIATION. A. M. Zamah, S. C. Baumgarten, M. A. Fiero, N. J. Winston, C. Stocco, B. Scocchia, Obstetrics and Gynecology, University of Illinois, Chicago, IL; Physiology and Biophysics, University of Illinois, Chicago, IL.

OBJECTIVE: FSH is used in IVF to induce follicle maturation, which results in granulosa cell differentiation and coordination of oocyte maturation. Insulin-like growth factors (IGFs) in conjunction with FSH play an important role in folliculogenesis and steroidogenesis. Our objective was to study the molecular crosstalk between FSH and IGF-1 signaling and the regulation of human granulosa cell differentiation.

DESIGN: Prospective in vitro studies using human primary granulosa cultures.

MATERIALS AND METHODS: Human cumulus and mural granulosa cells were isolated from follicular aspirates of IVF patients and analyzed immediately or cultured in serum-free media in the presence of FSH, IGF-1, or an inhibitor of type I IGF receptor (IGF1R) activity. We quantified messenger RNA and protein levels of steroidogenic enzymes, components of the IGF system, and gonadotropin receptors, measured 17β-estradiol levels, and examined the activation of intracellular signaling pathways to assess granulosa cell differentiation as well as FSH and IGF actions in both cumulus and mural cells. Experiments were run in triplicate and between group statistical comparisons were performed with t-tests and multiple comparisons were performed with one-way ANOVA. P < 0.05 was considered significant.

RESULTS: In freshly isolated human granulosa cells, luteinizing hormone receptor (Lhhr) and steroidogenic acute regulator (Star) were expressed at lower levels in cumulus than in mural cells (p < 0.05), whereas FSH receptor (Fsfrh) and anti-Müllerian hormone (Amhn) were expressed at higher levels in cumulus than in mural cells (p < 0.05). In vivo, the expression of Igfl2, the differentiation markers Lhhr, Star, and Cypl9a1 (aromatase) as well as 17β-estradiol production remained low in untreated cumulus cells, but increased significantly after FSH treatment (p < 0.05). Strikingly, this stimulatory effect of FSH was abolished by the inhibition of IGF1R activity. FSH-induced activation of AKT required IGF1R activity, and overexpression of constitutively active AKT rescued the induction of differentiation markers and 17β-estradiol production by FSH in the presence of the IGF1R inhibitor.

CONCLUSION: Our results indicate for the first time that the human cumulus cell response to FSH resembles the differentiation of preantral to
O-270 Wednesday, October 22, 2014 11:45 AM


OBJECTIVE: Conflicting reports have emerged regarding the impact of obesity on in-vitro fertilization (IVF) outcomes. Some of this variability may result from analyses that aggregate patients across diagnoses. In this study, we investigated the possibility that obesity may have differential impacts on success rates in patients with different diagnoses.

DESIGN: Retrospective analysis using de-identified fresh and frozen, non-donor IVF cycles (N=5208, 2738 patients) from a large reproductive medical center.

MATERIALS AND METHODS: Patients with a body mass index (BMI) ≥ 30 kg/m² were classified as obese. We investigated the effect of obesity on outcomes including: number of oocytes retrieved (Ret), fertilization rates (Fert), number of viable embryos on Day 3 and 5 (NumEm), clinical pregnancy, and live birth in patients diagnosed with polycystic ovarian syndrome (PCOS), male factor, endometriosis, tubal factor, diminished ovarian reserve, and idiopathic infertility. Analysis was performed using generalized estimating equations (GEE), while controlling for possible confounding factors (age, day 3 follicle stimulating hormone (FSH) levels, peak estradiol levels, number of oocytes retrieved, number of embryos transferred, and intra-cytoplasmic sperm injection (ICSI)) for the respective outcomes.

RESULTS: For the aggregate data across diagnoses, obesity had no significant effect on any of the outcomes analyzed. Among patients diagnosed with PCOS (N=439 cycles), however, obesity had significant negative effects on implantation rate (< 50%, OR=0.55, p=0.02), clinical pregnancy (OR=0.57, p=0.03) and live birth (OR=0.44, p=0.02) outcomes. No adverse effects were observed on Ret, Fert, and NumEm. Among the remaining diagnostic groups, there were no significant adverse effects from obesity on any of the analyzed outcomes.

CONCLUSION: For PCOS patients, we find that obesity increases the risk of IVF treatment failure over two-fold. Among these women, the adverse effects were observed on implantation rate, clinical pregnancy and live birth outcomes. When controlling for implantation rate (<50%), the effect of obesity on live birth was no longer significant (p=0.12), indicating the potential negative influence is isolated at the embryo implantation stage.

Supported by: Celmatix Inc.

O-271 Wednesday, October 22, 2014 12:00 PM

SKELETAL MUSCLE PHENOTYPE AND RECEPTOR GENE EXPRESSION IN POLYCYSTIC OVARY SYNDROME. J. J. Berger, M. M. Bannman, B. A. Gower, G. W. Bates, Jr OBGYN- Division of Reproductive Endocrinology and Infertility, University of Alabama at Birmingham, Birmingham, AL.

OBJECTIVE: Polycystic ovarian syndrome (PCOS) is associated with androgen excess and the metabolic syndrome. Despite the widespread nature of PCOS, a precise link with metabolic dysfunction has remained elusive. Alterations in skeletal muscle phenotype have been described in men and women with obesity and metabolic syndrome and may play a role in the pathophysiology of PCOS.

DESIGN: Characterize skeletal muscle fiber phenotype and receptor gene expression for estrogen, androgen and insulin in a cohort with PCOS.

MATERIALS AND METHODS: Muscle biopsies were obtained from the vastus lateralis (n=10, age = 40±5.8y) of women who met the NIH criteria for PCOS and compared to age and race matched controls (n=10). Myofiber size and type distribution (I, IIA, IIX) were quantified using immunohistological techniques, and gene expression was analyzed using real time polymerase chain reaction.

RESULTS: In PCOS vs control, Myofiber size was greater for type IIX (3377 vs 2561 μm², p<0.05), type IIA percent distribution was higher (56.7% vs 45.7%, p<0.01), and type I percent distribution was low (29.3% vs 37.8%, p<0.05) with no group differences in type IIX distribution. There was substantial down-regulation of gene expression in the PCOS group compared to controls for estrogen receptor (relative mRNA expression 0.66, p<0.01) and insulin receptor (relative mRNA expression 0.56, p<0.01). Although not significant, there was also a trend for lower androgen receptor expression in PCOS (relative mRNA expression 0.83, p=0.1).

CONCLUSION: We report for the first time that women with PCOS have an inherently low distribution of oxidative, fatigue-resistant type I myofibers and blunted expression of key receptors in skeletal muscle. These findings suggest that the heightened risk of metabolic disease among women with PCOS may in part result from low type I muscle fiber distribution leading to reduced exercise tolerance or activity participation; further, low levels of insulin receptor expression in skeletal muscle may exacerbate this risk.

O-272 Wednesday, October 22, 2014 12:15 PM


OBJECTIVE: To examine the relationship between weight change since age 18 and time to pregnancy at ages 22 to 49.

DESIGN: Prospective cohort study.

MATERIALS AND METHODS: Our study included 2,880 women trying to become pregnant or with a recent planned pregnancy in the Nurses’ Health Study 3 cohort (2010-present). Every 3 to 6 months women report the duration of their pregnancy attempt. Women were considered at risk for pregnancy for the duration of their pregnancy attempt until they became pregnant, stopped trying to become pregnant, or were lost to follow-up. Height, current weight, and weight at age 18 were self-reported on the baseline questionnaire. Multivariable Cox proportional hazards models for discrete survival time were used to estimate the fecundability odds ratios (FOR) and 95% confidence intervals (CI). FORs < 1 indicate longer TTP and FORs > 1 indicate shorter TTP.

RESULTS: Women in this cohort were 34 years old on average. 94% Caucasian, 77% never smokers, with a mean BMI of 25.7 kg/m². After adjusting for age, smoking, marital status, race, and BMI at age 18, weight change since age 18 was significantly associated with TTP (p-value<0.001). The adjusted FOR for a 5 kg increase in body weight from age 18 was 0.91 (95% CI 0.88, 0.92). This association was similar in women who were <25 kg/m² and ≥25 kg/m² at age 18 (p-interaction=0.66). Current BMI and BMI at age 18 were also independently associated with TTP. The adjusted FOR (95% CI) for a 5 kg/m² increase in current BMI and age 18 BMI were 0.82 (0.78, 0.85) and 0.88 (0.82, 0.93), respectively.

CONCLUSION: Gaining weight in adulthood is associated with reduced fertility, independent of BMI at age 18.

Supported by: Supported by a grant from the Breast Cancer Research Foundation and Nutricia Early Life Nutrition US and NIH grants T32DK007703-16 and T32HD060454.

O-273 Wednesday, October 22, 2014 12:30 PM

SERUM PROGESTERONE CONCENTRATION AT DAY OF BLASTOCYST TRANSFER IS A REFLECTION OF OVARIAN RESPONSE TO CONTROLLED OVARIAN STIMULATION AND DOES NOT IMPACT LIVE BIRTH. A. Nyboe Andersen, J.-C. Arce. Fertility Clinic, Copenhagen University Hospital, Copenhagen, Denmark; 2Global Clinical R&D (Reproductive Health), Ferring Pharmaceuticals, Copenhagen, Denmark.

OBJECTIVE: To explore the relationship of ovarian response and serum progesterone levels at the day of blastocyst transfer and 13-15 days after transfer and to investigate the predictive ability of progesterone at transfer on live birth.

DESIGN: Retrospective analysis of a large, assessor-blind, multicenter trial (MEGASET®; N=749).

MATERIALS AND METHODS: Patients underwent stimulation with a starting dose of 150 IU/day of a GnRH antagonist cycle with single blastocyst transfer. Blood samples were analyzed centrally. Luteal phase support (3x 200 mg daily, vaginal progesterone capsules) was used from the day after oocyte retrieval till the β-hCG visit (13-15 days after transfer). The association between progesterone at transfer and parameters of interest was evaluated using Spearman correlation coefficient (r). The predictive value of progesterone on live birth was evaluated using logistic regression.

RESULTS: The distribution in serum progesterone at the blastocyst transfer visit was similar between patients with and without live birth, with a median (25th, 75th percentile) of 105 (70; 147) ng/mL and 99 (67; 145) ng/mL, respectively. All patients except one had progesterone above 10 ng/mL. The 5th percentile was 43 and 44 ng/mL among patients with and without live birth, respectively. The live birth rate in the lowest (Q1) and highest (Q4)
progesterone quartiles was 29% and 32%, respectively, and similar to the 33% in the middle group (Q2-Q3). The AUC of the ROC-curve for live birth was 0.51, indicating no predictive value of progesterone concentration at transfer. The progesterone concentration at transfer was significantly (p<0.001) positively correlated with number of follicles at end of stimulation (r=0.44; 95% CI 0.38-0.50), estradiol at end of stimulation (r=0.50; 95% CI 0.44-0.55), and number of oocytes retrieved (r=0.56; 95% CI 0.51-0.61). Estradiol at transfer (r=0.58; 95% CI 0.52-0.63) was also significantly (p<0.001) positively correlated with progesterone at transfer. The progesterone concentration at end of stimulation was significantly (p<0.001) positively correlated with the serum βhCG concentration, with no correlation to any follicular or endocrine parameters at end of stimulation.

CONCLUSION: Serum progesterone at time of blastocyst transfer is mainly a reflection of the number of corpora lutea subsequent to ovarian stimulation, and is not predictive of live birth. Serum progesterone at time of βhCG test is independent of earlier ovarian response.

Supported by: Ferring Pharmaceuticals.

O-274 Wednesday, October 22, 2014 12:45 PM

PARENTAL X CHROMOSOME INHERITANCE AND VARIABLE GENE EXPRESSION IN TURNER SYNDROME. N. K. Banks, a P.S. Kruszka, a C. Cheng, a A. Elkahlon, a C. A. Bondy, b M. Muenke. a, bNH-GRI, Bethesda, MD. a, bNICHID, Bethesda, MD.

OBJECTIVE: Turner syndrome (TS) occurs in 1 in 2500 live female births and includes a spectrum of phenotypic findings, from more severely affected infants with congenital heart disease and lymphedema to adults with short stature and gonadal dysgenesis. In classic 45,X TS, two-thirds of patients are missing the paternal sex chromosome and carry only the maternal X (Xm), while the other one-third carry a paternal X (Xp). This is an important distinction as some aspects of the TS phenotype are affected by which parental X is inherited. X imprinting may explain some sexually dimorphic traits, and these traits would be predicted to vary in TS based on which X is inherited. Gene expression profiling studies may highlight which genes on the X are potentially imprinted or what genes throughout the genome are differentially expressed, helping to explain phenotypic differences.

DESIGN: Gene expression profiling analysis.

MATERIALS AND METHODS: Twenty-six women with 45,X detected on peripheral karyotype in 50 cells underwent DNA expression array profiling of peripheral blood lymphocytes using Affymetrix GeneChip Human Genome U133 Plus 2.0 Array as part of the IRB approved NICHD protocol “Turner Syndrome: Genotype and Phenotype.” Ten women inherited their X chromosome from their father (Xp) and 16 women inherited their X chromosome from their mother (Xm). Analysis comparing TS Xp and TS Xm gene expression was performed with Partek Genomics Suite, using a 1-way ANOVA test.

RESULTS: Comparing inheritance of the Xm versus Xp in TS, 3443 genes show differences in expression, with p-value less than 0.05, and 63 genes show a highly significant difference (p<0.001). One transcript for a non-coding (nc) RNA (probe ID 242299_at) on the X chromosome was expressed 1.53 fold greater in TS Xp versus Xm (p=0.028).

CONCLUSION: Significant differences in gene expression between Xm and Xp groups do exist. NcRNA 242299_at is located within the X-inactivation center (XIC), suggesting a possible regulatory role in expression of XIST, the primary ncRNA responsible for X inactivation. NcRNAs generally play a major role in transcription regulation throughout the genome and therefore, are important candidates for explaining phenotypic differences in TS. These findings require confirmation using a different expression profiling modality. Further study is needed to understand the molecular actions of ncRNA 242299_at and whether it may play a role in the XIC.

Supported by: NICHD, NHGRI Intramural Research Programs.

PREIMPLANTATION GENETIC DIAGNOSIS II

O-275 Wednesday, October 22, 2014 11:15 AM

ARE PARTIAL ANEUPLOIDIES LINKED WITH CHROMOSOMAL STRUCTURAL ABNORMALITIES? T. Escudero, a L. Ribustello, a A. Suhottova, a J. Skorupski, b J. Garril, a K. Wiemer, a M. Ophal, a S. Munne. a Reprogenetics, Livingston, NJ. bHouston Fert Institute, Tomball, TX. aSBMC - IRMS, Livingston, NJ. aNorthwest Center for Reproductive Sciences, Kirkland, WA.

OBJECTIVE: To determine if partial aneuploidies share the same frequent breakpoints as other common structural abnormalities such as reciprocal translocations.

DESIGN: Retrospective Analysis.

MATERIALS AND METHODS: From 11/4/2013 to 4/7/2014, a total of 1409 PGS cycles (13,347 embryos) from 132 centers were processed at the same reference lab by array CGH. 384 cycles had at least one embryo with partial aneuploidies. A chromosome map was designed locating where the breakpoint of partial aneuploidy occurs and with what frequency. For comparison, a similar map was designed locating the breakpoints from PGD cycles in which one or both partners are carriers of a structural chromosome abnormality, such as reciprocal translocations.

RESULTS: 384 PGS cycles (27.25% of the cases) and 430 embryos (3.4%) showed only a partial aneuploidy in at least one embryo. The breakpoint map of these cases was compared to that of 94 PGD cycles consisting of a carrier with at least one inherited structural abnormality. Table I shows the location of the most frequent hotspots which occurred in partial aneuploidies compared with the most frequent breakpoints in cycles with couples carrying structural abnormalities:

<table>
<thead>
<tr>
<th>hotspots in partial aneuploidies</th>
<th>occurrences</th>
<th>breakpoints in translocations</th>
<th>occurrences</th>
</tr>
</thead>
<tbody>
<tr>
<td>1q21.1</td>
<td>6</td>
<td>6q21</td>
<td>7</td>
</tr>
<tr>
<td>9q21.11</td>
<td>6</td>
<td>22q11.21</td>
<td>6</td>
</tr>
<tr>
<td>15q21.3</td>
<td>6</td>
<td>14q11.2</td>
<td>5</td>
</tr>
<tr>
<td>2q33.1</td>
<td>5</td>
<td>12p11.21</td>
<td>4</td>
</tr>
<tr>
<td>4q28.3</td>
<td>5</td>
<td>14q32.11</td>
<td>4</td>
</tr>
<tr>
<td>7p11.2</td>
<td>5</td>
<td>3p21.1</td>
<td>3</td>
</tr>
<tr>
<td>8p12</td>
<td>5</td>
<td>4q33</td>
<td>3</td>
</tr>
<tr>
<td>8q24.3</td>
<td>5</td>
<td>9p24.1</td>
<td>3</td>
</tr>
<tr>
<td>14q24.3</td>
<td>5</td>
<td>10q26.11</td>
<td>3</td>
</tr>
<tr>
<td>16p11.2</td>
<td>5</td>
<td>10q26.3</td>
<td>3</td>
</tr>
</tbody>
</table>

CONCLUSION: The distribution of breakpoints between partial aneuploidies does not occur randomly along the genome and several hotspots had been observed. The hotspots observed de novo do not correspond with those from translocation carriers. The fact that inherited translocations have different breakpoints than de novo structural abnormalities suggests that the first are more stable and benign than the second group. This also suggests that de novo partial abnormalities should be avoided for transfer.

O-276 Wednesday, October 22, 2014 11:30 AM

NEXT GENERATION SEQUENCING-BASED ANEUPLOIDY SCREENING IMPROVES DETECTION OF LOW-LEVEL MOSAICISM IN HUMAN EMBRYOS. F. Spinella, a A. Birick, b S. Bono, a A. Nuccitelli, a E. Cottone, a G. Cottone, a F. Kokocinski, a C. E. Michel, a F. Fiorentino. a GENOMA, Rome, Italy; aIlluminia, Cambridge, United Kingdom.

OBJECTIVE: Aneuploidy screening of embryos at blastocyst stage can be jeopardized by the presence of chromosomal mosaicism, which is a phenom- enon characterized by the presence a mixture of diploid and aneuploid cell lines in embryos. Although its significance for implantation and the develop- ment of babies is still unclear, it is reasonable to assume that mosaicism is likely to influence IVF success rates. In this study we investi- gated whether next generation sequencing (NGS) technologies applied for preimplantation genetic screening (PGS) purposes have the potential to improve mosaicism detection in human embryos over conventional methods.

DESIGN: This study was organized into three steps. The first involved mixing experiments with different ratios of euploid and aneuploid single cells, to mimic chromosomal mosaics. The aim was to determine the maxi- mum ratio of aneuploid to euploid cells that is needed to detect a copy num- ber variation (CNV) by NGS. The second step was a retrospective blinded assessment of whole genome amplification (WGA) products, selected from previously performed clinical PGS cycles, including embryos with mosaic aneuploidy results. The third consisted in a prospective trial involving a blind parallel evaluation, with both NGS and array comparative genomic hybridiza- tion (aCGH) techniques.

MATERIALS AND METHODS: Reconstruction experiments were performed using different ratio of euploid and aneuploid single cells and
INTENT TO TREAT ANALYSIS OF PREIMPLANTATION GENETIC SCREENING (PGS) AND IN VITRO FERTILIZATION (IVF) VERSUS EXPECTANT MANAGEMENT (EM) IN PATIENTS WITH RECURRENT PREGNANCY LOSS (RPL).


OBJECTIVE: The addition of the Embryoscope (Fertilitech, Rockland MA), with the capability to continuously monitor embryo growth, is a powerful new tool in the IVF laboratory. The objective of this study was to compare morphokinetic parameters of euploid and aneuploid embryos.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: A total of 32 IVF patients with embryos cultured in the EmbryoScope were analyzed. Patients either had PGS with day five trophoderm biopsy (n = 20) or transfer of one embryo resulting in a normal singleton birth (n = 12). Morphokinetic parameters evaluated include: timing of cell divisions (time to two cells (c) t2), (3c (t3), 4c (t4), 5c (t5), 8c (t8)), time to morula (tMor), start of blastulation (tSB), blastocyst (tBL), expanded blastocyst (tEBL). Durations of the second cycle (c2; t2), third cell cycle (c3; t3), time to complete synchronous divisions s2 (s4-t3) and s3 (t8-t5) and interval between 2 and 5 cells (t5-t2) were calculated. All values are reported as mean time in hours +/- standard deviation.

RESULTS: The kinetics of 52 euploid embryos were compared to 47 with known aneuploidy. There were no significant differences in kinetic parameters between groups.

CONCLUSION: This study found no significant difference in the timing of any specific morphological event in embryo development for euploid vs. aneuploid embryos. Further investigation of larger samples sizes is needed to define the impact of continuous time-lapse monitoring on determining genetic competence.

O-279 Wednesday, October 22, 2014 12:15 PM

SINGLE CENTER VALIDATION OF TROPHECTODERM BIOPSY USING PREIMPLANTATION GENETIC SCREENING (PGS)/VITRIFICATION COMPARED TO NON-PGS EMBRYO TRANSFER (ET).


OBJECTIVE: This study aims to validate the performance of current PGS technologies to past treatment protocols.

Does the use of trophoderm biopsy-PGS/vitrification (VTB)-ALL cycles increase pregnancy rates compared to untested ET alone? Do these applied technologies increase live-birth rates on a per cycle first transfer comparison?

DESIGN: From 2011 to 2013, patients were placed into two groups and compared on a first transfer basis. Group 1 included PGS/VTB-ALL cycles (n = 172). These patients elected PGS for aneuploidy screening only using array-CGH. Group 2 included all first ET for non-PGS fresh cycles (n = 160). If enrolled in Group 2 as a VTB-ALL cycle, their first transfer was included. All results were stratified by age, with donor cycles excluded. To evaluate overall PGS performance and eliminate bias, the same groups were then compared on a first transfer basis. Chi-square analysis was used to assess differences.

MATERIALS AND METHODS: Group 1 patients had all embryos laser hatched on Day 3, continued to Day 5/6 for trophoderm biopsy and vitrified. Group 2 patients had embryos transferred on Day 3 or Day 5 of their fresh or Day 5 of their frozen-VTF cycle. All blastocysts were vitrified using the microSecure™ method.
PER TRANSFER COMPARISON

<table>
<thead>
<tr>
<th>Age</th>
<th>Group 1 (PGS) Ongoing/Live Birth(LB) % per ET</th>
<th>Group 2 (no-PGS) Ongoing/Live Birth(LB) % per ET</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 34</td>
<td>48%</td>
<td>48%</td>
<td>*p ≤ 0.01</td>
</tr>
<tr>
<td>35-37</td>
<td>53%</td>
<td>53%</td>
<td>*p ≤ 0.01</td>
</tr>
<tr>
<td>38-40</td>
<td>29%</td>
<td>29%</td>
<td>*p ≤ 0.01</td>
</tr>
<tr>
<td>41-42</td>
<td>7%</td>
<td>7%</td>
<td>*p ≤ 0.01</td>
</tr>
<tr>
<td>43+</td>
<td>0%</td>
<td>0%</td>
<td>p = 0.11</td>
</tr>
</tbody>
</table>

VALIDATION: PER CYCLE COMPARISON

<table>
<thead>
<tr>
<th>Age</th>
<th>Group 1 (PGS) #Cycles / #ET</th>
<th>Group 2 (no-PGS) #Cycles / #ET</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 34</td>
<td>46 / 43</td>
<td>46 / 43</td>
<td>*p ≤ 0.01</td>
</tr>
<tr>
<td>35-37</td>
<td>43 / 41</td>
<td>43 / 41</td>
<td>*p ≤ 0.05</td>
</tr>
<tr>
<td>38-40</td>
<td>47 / 37</td>
<td>47 / 37</td>
<td>*p ≤ 0.01</td>
</tr>
<tr>
<td>41-42</td>
<td>28 / 12</td>
<td>28 / 12</td>
<td>*p ≤ 0.01</td>
</tr>
<tr>
<td>43+</td>
<td>8 / 1</td>
<td>8 / 1</td>
<td>p = 1.0</td>
</tr>
</tbody>
</table>

* % values within rows are different

RESULTS: When utilizing PGS/VTF-ALL cycles, patients under 43 exhibit higher implantation and ongoing/live birth rates per first transfer compared to untested embryo transfer alone. Additionally, for patients 40 and under, PGS/VTF-ALL cycles result in higher take home baby rates per egg retrieval.

CONCLUSION: Validating PGS on a per cycle basis eliminates the bias of non-normal and failure to grow outcomes. Clearly, PGS uses BL more efficiently to achieve success. In women over 40, the cost-to-live birth benefit may be lacking, but the nonparametric significance of understanding why failure occurs and a shortened turnaround time does benefit the patients overall well-being.

Supported by: SCCRM

O-281 Wednesday, October 22, 2014 14:25 PM

PGD FOR MICRO-DELETIONS AND -DUPLICATIONS COMBINED WITH 24-CHROMOSOME ANEUPLOIDY SCREENING. N. R. Treff, a,b X. Tao, a C. Jalas, b L. Levy, c R. T. Scott, Jr., c RMA of New Jersey, Basking Ridge, NJ; c RWJ Medical School-Rutgers University, New Brunswick, NJ.

OBJECTIVE: New data indicates that the prevalence of insertions and deletions (indels) which may produce children with an abnormal phenotype may exceed 1% of viable conceptions. Studies regarding PGD for these indels have been limited by the fact that many are too small for existing methods of comprehensive chromosome screening (CCS) to detect. This study demonstrates the ability to perform testing of indels together with CCS from the same biopsy using qPCR.

DESIGN: Observational.

MATERIALS AND METHODS: Workups included identifying informative SNPs in the parents using SNP arrays and phasing the markers using qPCR on family members. For insertions, a linkage only approach was used. For deletions, multiple informative markers within the deletion (direct testing) were used along with linked markers outside the deletion when possible. Blastocyst biopsies underwent targeted multiplex amplification of several informative SNP-, direct deletion-, and copy number-loci followed by TaqMan-based qPCR for the indels and CCS analyses. Clinical outcomes were monitored and genetic testing of newborns was performed when possible.

RESULTS: The typical workup involved approximately 4 weeks to complete. 14 cases were processed including 1q21.1, 15q11.2, and 16p13.11 duplications, and 2p16.3, 4q35.1, 5q14.3, 10p11.2, 16p12.2, 20p12.2, 22q11, and Xp22.3 deletions. A total of 137 embryos were evaluated all of which were successfully typed and the indel diagnosis was correct. In 7 patients patients have had embryo transfers from which 5 have delivered, 1 has an ongoing pregnancy, and 1 did not become pregnant. In 2 cases, DNA from the newborns was available for analysis and confirmed the PGD and CCS diagnoses.

CONCLUSION: This new approach to combined CCS and indel PGD involves a short and inexpensive workup and the ability to evaluate indels in parallel with CCS from the same biopsy. In cases where deletion screening was needed but family members were not available to develop informative markers, direct testing of multiple markers within the mutation was possible.

e96 ASRM Abstracts Vol. 102, No. 3, Supplement, September 2014
FERTILITY & STERILITY®
e97

MALE FACTOR II

O-282 Wednesday, October 22, 2014 11:15 AM


OBJECTIVE: Some authors describe similar and high fertilization and pregnancy rates in non-obstructive azoospermia (NOA) patients whereas others report lower outcomes in NOA than in obstructive azoospermia (OA) patients. Sperm DNA damage is associated with reduced fertilization rates, embryo quality and pregnancy rates and higher rates of spontaneous miscarriage and childhood disease. Meanwhile, mouse zygotes respond to sperm DNA damage through a non-automatope mechanism that acts by slowing paternal DNA replication and embryonic development. In this study, we compared the early development of embryos fertilized with NOA and OA spermatozoa by using time-lapse monitoring system.

DESIGN: This is a retrospective study.

MATERIALS AND METHODS: Six oocytes with ICSI using testicular spermatozoa from NOA and 35 oocytes with OA from 2013 to 2014 were used. The exact times for each embryo division and developmental parameters were calculated in hours after ICSI. Time-lapse images of each embryo were retrospectively analyzed by PrimoVision software. The developmental events observed in each embryo were at the time of the visibility of two pronuclei (2PN), syngamy, two cells and four cells. These parameters were compared between the NOA group and the OA group.

RESULTS: The time of the visibility of 2PN in the NOA group (9.5 ± 1.4 hours) was significantly earlier than that in the OA group (12.1 ± 2.3 hours). However, the time from the visibility of 2PN to the syngamy in the NOA group (13.2 ± 3.4 hours) was significantly longer than that in the OA group (10.6 ± 2.7 hours). The time between the syngamy and the two cells in the NOA group (12.0 ± 1.3 hours) was comparable with that in the OA group (16.6 ± 10.2 hours). The time from the two cells to the four cells in the NOA group (13.5 ± 3.5 hours) was also comparable with that in the OA group (10.0 ± 4.2 hours).

CONCLUSION: The time from the visibility of 2PN to the pronuclear-fading (syngamy) after ICSI with testicular spermatozoa from NOA was prolonged compared to that after ICSI with testicular spermatozoa from OA.

O-283 Wednesday, October 22, 2014 11:30 AM

RELATIONSHIPS BETWEEN SPERM DNA FRAGMENTATION, AGE OF DONORS AND PATIENTS AND CHILDREN WITH PSYCHIC DISORDERS. D. P. Evenson, a J. Brannian, b D. P. Evenson, c J. Christianson. d Dermatology and Andrology, Mansoura Faculty of Medicine, Mansoura University, Mansoura, Dakahlia, Egypt; e Andrology, Center for Advanced Research in Human Reproduction, Cleveland Clinic Foundation, USA, Cleveland, OH.

OBJECTIVE: To assess the occurrence of spermatogenetic meiotic errors in relation to advancing paternal age and their role in contributing to proper embryo development.

DESIGN: We analyzed the proportion and type of spermatogenetic meiotic errors in relation advancing men age. The age related aneuploid sperm proportion was correlated to the ability to establish a term pregnancy.

MATERIALS AND METHODS: Consenting men seen at our clinic for male infertility screening or with a history of recurrent IVF failure were included. Cytogenetic analysis for chromosomes X,Y,13,15,16,17,18,21, and 22 was performed. Men were categorized into one of 7 age groups in relation to the occurrence of nullisomy, disomy, and diplody. Clinical outcomes were compared.

RESULTS: FISH was carried out on 67 semen samples with concentration of 46.3 ± 4.3 and motility of 52.8 ± 7%, and normal morphology of 2.6 ± 2%. Paternal ages clusters spanned 25-30, 31-35, 36-40, 41-45, 46-50, 51-55, and >55. The overall aneuploidy rate progressively and consistently increased with advancing age to reach 10.4% in >55yo. A positive correlation with age was consistent when the autosomal or gonosomal anomalies were observed separately. Disomy of Chx15 and 17 progressively increased with age, most remarkably in the oldest group of men reaching 2% and 3%, respectively. Interestingly, Chx21 non-disjunction was most frequent in the youngest cluster 20-30yo at 1.25%. The remainder autosomes analyzed did not seem to relate to a particular age. Specific gonosomal non-disjunction involving ChxY maintained a progressive occurrence with age, reaching 2% in the oldest men. A total of 113 ICSI cycles were assessed and grouped according to paternal age. Fertilization progressively decreased from 87.7% in men 20-30yo to 52% in the oldest group, while embryo cleavage and morphological embryo quality appeared unrelated. A +ßhCG at 80% resulted in a clinical pregnancy of 60% in the 20-30yo and progressively decreased when the man became 50yo. Accordingly, pregnancy loss steadily increased with the advancing male age to reach 50.0% in men >50yo due gonosomal and autosomal defects (P<0.0001).

CONCLUSION: As it may be expected but not undeniably documented, advancing paternal age affected spermatogenetic meiosis almost exclusively non-disjunction. The exceptional was with Chx21 non-disjunction, mostly reported in the youngest age group. While we could not fully control for an eventual female factor, increasing spermatogenetic meiotic errors of aging men adversely affect their ability to fertilize and achieve a successful implantation.

Supported by: Reproductive Medicine, Weill Cornell Medical College.

O-285 Wednesday, October 22, 2014 12:00 PM

FOLLICLE STIMULATING HORMONE RECEPTOR POLYMORPHISM AND ANTI MullERIAN HORMONE IN FERTILE AND INFERTILE MEN. A. H. Hassan, a A. A. Zalata, b F. Bragais, c A. Agarwal, d T. Mostafa, d Dermatology and Andrology, Mansoura Faculty of Medicine, Mansoura University, Mansoura, Dakahlia, Egypt; cBiochemistry, Mansoura Faculty of Medicine, Mansoura University, Mansoura, Dakahlia, Egypt; d Andrology, Center for Advanced Research in Human Reproduction, Cleveland Clinic Foundation, USA, Cleveland, OH; dAndrology, Cairo Faculty of Medicine, Cairo, Egypt.

OBJECTIVE: To investigate the occurrence of Asn and Ser FSHR gene variants and its relationship with seminal anti-Mullerian hormone (AMH) among normozoospermic and fertile oligoasthenozoospermic (OAT) males.

MATERIALS AND METHODS: Eighty-two Caucasian males grouped into normozoospermic healthy controls (n = 30) and infertile OAT males (n = 52). FSHR gene variants were determined by DNA from anti-coagulated blood and underwent polymerase chain reaction (PCR) amplification and
 electrophoresis in detecting amplification products. AMH in seminal plasma was determined by ELISA.

RESULTS: The frequency of FSHR gene variants among fertile men was 46.7% Asn/Asn (N680S), 33.3% Asn/Ser, and 20% Ser/Ser, whereas among OAT men were 34.6%, 38.5% and 26% respectively with nonsignificant differences. Seminal AMH was significantly higher in fertile than infertile OAT men. There was significant increase in seminal AMH with Asn/Asn variant of FSHR gene than those with Asn/Ser or Ser/Ser.

CONCLUSION: FSH gene variants showed no difference in distribution between fertile or infertile OAT men. However, when correlated with seminal AMH values, there was an increase in Asn/Asn in men with high seminal AMH.

Supported by: Mansourea Faculty of Medicine.

O-286 Wednesday, October 22, 2014 12:15 PM
SPERM SURFACE GLYCAN MODIFICATION BY CHEMICAL REPORTER CONJUGATION. J. A. Frezzo, a J. K. Montclare, b J. P. Alukal, a V. Kapita. c Chemical and Biomolecular Engineering, New York University Polytechnic School of Engineering, Brooklyn, NY; aObstetrics and Gynecology and Urology, New York University Langone Medical Center, New York, NY; bMechanical Engineering, New York University Polytechnic School of Engineering, Brooklyn, NY.

OBJECTIVE: The sperm surface glycocalyx has long been known to serve an instrumental role in sperm capacitation and zona pellucida penetration; however, utilization of this aspect of sperm for assisted reproduction or diagnostic purposes has been lacking. We describe novel orthogonal chemistry methodologies to characterize the network of sperm sialic acids; this is with the purpose of developing a quantitative measurement of sperm fertility that can be used as a novel sperm separation tool.

DESIGN: In this research project, we investigated the terminal sialic acids of surface-exposed glycoproteins, which are well-established as biomarkers for fertility, and are also amenable to periodate oxidation, a chemical modification method that enables reporter labeling.

MATERIALS AND METHODS: IRB approval was obtained; donated, de-identified sperm that resulted in a positive live-birth pregnancy were isolated by density gradient and subjected to periodate oxidative treatments (0.25 mM, 0.5 mM, 0.75 mM and 1.0 mM) followed by fluorescent tagging with 100 μM amino-oxa AlexaFluor 488, all while retaining the viability of the cells. Fluorescence of labeled cells was measured via excitation at 488 nanometers after repeated washes of unconjugated label.

RESULTS: Isolated sperm cells subjected to periodate oxidation and labeling demonstrates insignificant cytotoxicity across three concentrations of the sodium periodate (p<0.001). While fluorescence labeling did occur with the negative control group, there were substantial increases of 67% and 62% in labeling with two separate periodate concentrations emphasizing the utility of this method.

CONCLUSION: Effective isolation of fertile sperm for assisted reproduction technologies is limited by sperm characterization methods which sacrifice the viability of sperm for further usage. Herein we describe an orthogonal chemistry method for live sperm cell labeling that retains pertinent viability characteristics while yielding quantitative measures useful in fertility diagnosis and assisted reproduction.

Supported by: This work was supported by ARO W911NF-10-1-0228 and W911NF-11-1-0449 (J.K.M.), AFOSR FA-9550-08-1-0266 (J.K.M.) and in part by the NSF MRSEC Program under Award Number DMR-0820341, NSF GK-12 fellow grant DGE-0741714 (J.A.F.) and NYU CTSA grant UL1TR000038 from the National Center for Advancing Translational Sciences (NCATS), NIH (J.K.M.).

O-288 Wednesday, October 22, 2014 12:45 PM
PROGNOSTIC VALUE OF PREIMPLANTATION GENETIC SCREENING (PGS) FOR PATIENTS WITH ALTERED RESULTS IN SPERM FISH (FLUORESCENT IN SITU HYBRIDISATION) ANALYSIS. P. Collins, a E. Garcia-Guixé, b S. Munne, c C. Gíménez, b M. Sandalinas. aReprogenetics, Livingston, NJ; bReprogenetics Spain, Barcelona, Spain.

OBJECTIVE: The aim of this study is to find out whether an altered FISH analysis in sperm (AFAS) (increased aneuploidy rate) has a direct influence on embryo chromosomal abnormalities and if these individuals are good candidates for PGS.

DESIGN: A retrospective study was performed in couples with just AFAS as indication which underwent a PGS by means of arrays of aCGH (Comparative Genome Hybridization). Results were compared with a control group of couples with oocyte donation and without male factor who underwent PGS for SET (Single Embryo Transfer) or to avoid transfer of aneuploid embryos.

MATERIALS AND METHODS: The study group is formed by 36 couples with AFAS that underwent a total of 39 PGS cases (289 biopsied embryos). The control group is formed by 13 patients that underwent 16 PGS cases (100 biopsied embryos). Maternal age mean for each group was 30.4 and 29.1 respectively (p=0.2108).

One blastomere of each embryo was biopsied on Day +3 and processed for WGA (Whole Genome Amplification). The amplification product was analyzed by means of arrays of aCGH according to manufacturer’s protocol (24Sure, BlueGnome®). Statistical analysis was performed using Fisher test (GraphPad Instat 3).

RESULTS: Results were obtained in 93% of the analyzed embryos. In the study group, 44.2% of the analyzed embryos were found to be euploid, 47.6% aneuploid and 8.2% were classified as complex abnormal. In the control group, 57.0% of the analyzed embryos were found to be euploid, 38.7% aneuploid and 4.3% were classified as complex abnormal. There are statistically significant differences between the euploid embryos between the 2 groups (p<0.05). Patients with altered sperm FISH results have 1.3 times more risk of producing aneuploid embryos than the control group. For the study group, pregnancy rate was 39.4% per cycle and 46.4% per transfer (ongoing pregnancy: 33.3% per cycle and 39.3% per transfer). For the control group, pregnancy rate was 37.5% per cycle and 46.2% per transfer (ongoing pregnancy: 31.3% per cycle and 38.5% per transfer). No statistically significant differences were observed in the pregnancy rates between both groups.

CONCLUSION: This study shows that individuals with AFAS are at risk of producing chromosomally abnormal embryos and PGS can be recommended in order to increase their IVF success. Sperm FISH analysis is a useful tool in the genetic counselling of infertile couples.
SPERM BIOLOGY

O-289 Wednesday, October 22, 2014 11:15 AM


OBJECTIVE: To characterize the origin and meaning of round cells (RC) in human ejaculates and their relationship to spermato genesis.

DESIGN: In a prospective fashion, a total of 3,660 men undergoing male infertility screening in a period of 28 months were included in the study. Semen parameters were assessed according to the WHO criteria 2010. Cases displaying ≥ 2 million RC were screened for bacteria presence. The role of RC on clinical outcome was assessed in specimens used for ART.

MATERIALS AND METHODS: Raw specimen containing RC were processed by peroxidase and identification of transmembrane glycoproteins. To identify and characterize eventual immature germ cells (IGC) specific stains for Sertoli-cell cytoplasmic remnants and prostates to assess their spermato genic stage were used. DNA fragmentation (TUNEL) and aneuploidy (9 chromosome FISH) were carried out on IGC and spermatozoa.

RESULTS: Of a total of 3660 men screened, 1.3% presented with RC. Men (n=47) consented to participate were 43.0±9.9 yrs had a concentration of 32.3±27.5x10^6/mL, motility of 34.7±19.6%, and morphology of 2.0±1.7%. The actual proportion of WBC within those specimen was 30.0% with the remainder being IGC (range 0.79–24.90 million). All specimens screened were negative for uropathogens. The IGC were characterized by single/multiple nuclei as proven by their ploidy content. Ejaculates with IGC had higher incidence of aneuploidy in their spermatozoa (P=0.0001). There was also a concordant DNA fragmentation rate between IGC and spermatozoa. Interestingly, IGC stained for vimentin indicating their encasement in Sertoli-cell. A subgroup of men (n=14) underwent 33 cycles whose female partners were 37.4±4yrs. The fertilization was 59.4% resulting in a clinical pregnancy of 33.3% and a pregnancy loss of 9.1%. The presence of RC is clearly associated with impaired fertilization (P=0.0001) and an apparent deleterious effect on pregnancy outcome when compared to a matched control void of RCs.

CONCLUSION: The traditional perception of the seminal round cells has ranged from an infection marker to a “good Samaritan” cell. However, genetic and epigenetic assessments of these cells carried out in this study evidenced that they were largely byproducts of abnormal spermatogenesis proven by the observation of compacted chromatin nuclei engulfed in Sertoli-cell cytoplasm. This suggests that the presence of round cells in the ejaculate may serve as an indicator of an acute spermatogenic insult and may yield important information on compromised gamete competence.

Supported by: Reproductive Medicine, Weill Cornell Medical College.

O-290 Wednesday, October 22, 2014 11:30 AM


OBJECTIVE: Mature sperm that bind hyaluronan, a major constituent of the cumulus matrix surrounding the oocyte, have been reported to have lower DNA fragmentation and potentially lower aneuploidy. The present study evaluated the use of selected hyaluronan-bound (HB) sperm in IVF-ICSI cycles.

DESIGN: Retrospective study.

MATERIALS AND METHODS: An IRB-approved review of 10,834 cycles receiving assisted reproductive therapy during 2010-2013 at a large private IVF center. HB sperm injection was used for patients who had previous poor treatment outcomes using conventional ICSI. HB sperm were selected by their ability to bind hyaluronan microdots while remaining motile for subsequent injection of oocytes. Fertilization rate and implantation rate (IR) were compared by t-test. Clinical pregnancy rate per transfer (CPR) and spontaneous abortion (S) rate were compared by chi-square and Fisher’s exact test. Statistical significance was defined as P < 0.05 for comparisons between the two groups (conventional ICSI vs HB-ICSI).

RESULTS: There were 10,511 cycles undergoing conventional ICSI and 304 cycles undergoing HB-ICSI. Fertilization rates were similar (73.4% vs 72.3%) and clinical pregnancy rates were similar (4.1% vs 3.9%). CPR (46.95% vs 44.2%) and spontaneous abortion (3.7% vs 3.6%) were not different between groups.

CONCLUSION: Sperm DNA methylation patterns differ significantly and consistently between fertile men and fertile, normozoospermic controls. Methylation patterns may also be predictive of embryo quality following IVF.

O-291 Wednesday, October 22, 2014 11:45 AM

SPERM DNA METHYLATION PATTERNS ARE HIGHLY PREDICTIVE OF FERTILITY STATUS AND MAY BE PROGNOSTIC FOR IVF EMBRYO QUALITY. A. Smith, a K. I. Aston, b P. J. Uren, a T. G. Jenkins, b D. T. Carrell, b Molecular and Computational Biology, University of Southern California, Los Angeles, CA; 2Surgery (Urology), University of Utah Andrology and IVF Laboratories, Salt Lake City, UT.

OBJECTIVE: To determine the relationship between genome-wide sperm DNA methylation patterns and fertility status, IVF embryo quality, and IVF outcome.

DESIGN: Genome-wide sperm DNA methylation patterns were compared between 127 IVF patients and 36 normozoospermic fertile men. IVF patients were stratified into three groups: patients with generally high quality embryos and positive pregnancy (n = 53), patients with generally poor quality embryos and positive pregnancy (n = 42), and patients with generally poor quality embryos and negative pregnancy (n = 31) following IVF.

MATERIALS AND METHODS: Following somatic cell lysis and confirmation of the absence of potentially contaminating cells, sperm DNA was extracted, bisulfite converted, and hybridized to Infinium 450HumanMethylation Bead-Chips to measure methylation at > 485,000 sites across the genome. Statistical comparisons were made between patients with good embryogenesis versus patients with poor embryogenesis and between all patients versus controls. A combination of feature-selection, via support-vector machine, and logistic regression under cross-validation was applied to construct a predictive model for infertility.

Hierarchical clustering was applied to good and poor embryogenesis samples, using Euclidean distance between methylation profiles.

RESULTS: Hierarchical clustering of samples based on DNA methylation levels identified two out-groups that accounted for close to half of the poor embryogenesis patients. These out-groups had high purity, consisting of more than 83% poor-embryogenesis samples, pointing to a clear link between sperm DNA methylation status and embryo quality. Further, predictive models constructed based on the current sperm DNA methylation dataset proved highly accurate in classifying samples as fertile or infertile with a sensitivity of 84.3%, a specificity of 92.1%, a positive predictive value of 97.3%, and a negative predictive value of 63.6%.

CONCLUSION: Sperm DNA methylation patterns differ significantly and consistently between infertile men and fertile, normozoospermic controls. Methylation patterns may also be predictive of embryo quality following IVF.

A PROPOSED 360° SCORING METHOD TO REPLACE CONVENTIONAL BIDIMENSIONAL MORPHOLOGICAL ANALYSIS. B. A. Levine, a J. Feinstein, a Q. V. Neri, a D. Goldschlag, b S. Belongie, a Z. Rosenswaks, a G. D. Palermo. aThe Ronald O. Perelman & Claudia Cohen Molecular and Computational Biology, University College, New York, NY; bDepartment of Computer Science, College of Engineering, Cornell University, New York, NY; cCornell Tech, Cornell University, New York, NY.

OBJECTIVE: In a one-year time span, we noticed that only one-third of our semen analysis reports fit the normal morphology parameters. In this study we
sought to investigate the chances of misjudging sperm morphology due to asymmetrical abnormalities or variable head positioning during fixation.

**DESIGN:** Prospective, randomized, double-blind study.

**MATERIALS AND METHODS:** Standard morphology slides were prepared using TestSimplet™ and scored according to the 2010 WHO criteria. An aliquot of semen was added to polyvinylpyrrolidone, placed on a slide, analyzed at 600x, where a single motile spermatozoon was targeted and a ten-second video was recorded. This was repeated for five spermatozoa and ten subsequent still images were obtained for each sperm at different rotational plane. These 50 images were randomly displayed to a team of andrologists who were asked to assess the presence or absence of sperm head irregularities. Inter-observer reliability was assessed by the intra-class correlation coefficient (ICC) using a two-way random effects model.

**RESULTS:** The semen sample used for the study had a volume of 4.5mL, concentration of 28 million/mL, motility of 46%, and 2% normal morphology. Seven andrologists participated in the study. Despite the inter-observer concordance of the sperm images, there was overt discordance with respect to the angle of observation on whether the spermatozoon was normal or abnormal. This indicated that the various images of the same spermatozoon were scored differently according to the presented position. From this we can extrapolate the poor reliability of the standard bidimensional method particularly when asymmetric abnormalities are present. The concordance of inter-observer agreement (ICC) = 0.53 (95% CI=0.21, 0.91) (P<0.0001) demonstrating poor reliability of the morphological assessments of the same spermatozoon.

**CONCLUSION:** These results underscore the innate limitations and the highly subjective nature of standard 2-D sperm staining and assessment. By keeping a spermatozoon in a fluid environment, our analysis is not subject to staining and air-drying artifacts. Because spermatozoa are not spherical but have flattened surfaces, this observation highlights the chances of false-negative interpretation with conventional staining.

**O-293 Wednesday, October 22, 2014 12:15 PM**

**MULTILAYER DENSITY GRADIENT AS A USEFUL TECHNIQUE FOR SPERM SEX SORTING.** P. Nicotta, H. Urriondo, E. Barrios, S. Papier, G. Fiszbajn, F. Nodar, C. Alvarez Sedo. CEGYR - Genetics and Reproductive Medicine, Capital Federal, Buenos Aires, Argentina.

**OBJECTIVE:** Several studies have been carried out for sperm cell sorting in order to allow gender selection. Most of them were performed using flow citometry considering the differences between sperm DNA staining (X or Y). However, flow citometry technologies are expensive and it can have a cost-effectiveness impact over clinical treatments. Kaneko et al., (1983) have demonstrated that performing a multilayer density gradient could improve sperm gender selection in humans. Thus, the objective of this study was to describe our results on sperm gender selection based on centrifugation density gradient without altering sperm characteristics.

**DESIGN:** Prospective cohort study.

**MATERIALS AND METHODS:** This study included the analysis of 18 semen samples from men who underwent ART procedures. The inclusion criteria were: age <45 y/o, sperm concentration > 5million/ml, and normal hormonal levels. Samples with severe teratozoospermia were excluded. Seminal parameters: volume, concentration, progressive motility and morphology were taken into account for the analysis. All samples were processed by centrifugation density gradient consisting in four layers (90%, 70%, 50%, 20%) and scored according to the 2010 WHO criteria. DNA fragmentation was evaluated by TUNEL assay. Further.

---

**O-294 Wednesday, October 22, 2014 12:30 PM**

**EFFECT OF SPERM MATURITY AND ASSISTED OOCYTE ACTIVATION ON MORPHOGENETICS OF EARLY HUMAN EMBRYOS.** T. Takeuchi,* Y. Mori,* Y. Nakajo,* a,b N. Aono,* T. Okuda,* K. Kyono.* "Kyoto ART Clinic Takeanawa, Minatoku, Tokyo, Japan; "Kyoto ART Clinic, Sendai, Miyagi, Japan.

**OBJECTIVE:** ICSI with testicular sperm extraction (TESE) is a feasible therapeutic option for azoospermic patients, however, testicular sperm has lower fertilizability owing to its immaturity. Assisted oocyte activation (AOA) was often used to improve egg activation after ICSI. The objective of this study was to assess the effect of sperm immaturity and induced oocyte activation on early embryonic events by Time-lapse monitoring (TM).

**DESIGN:** Comparative morphological assessment of ICSI zygotes.

**MATERIALS AND METHODS:** A total of 74 patients comprised of 3 groups, 57 with ejaculated sperm (EJ), 10 with AOA, and 7 with TESE. Number of zygotes in each group was 196, 48, and 65, respectively. For AOA, oocytes were briefly exposed to Ca ionophore after ICSI. Inseminated oocytes were individually cultured in a TM system (Embryoscope®). Images were acquired every 10 min for up to 144 hrs. Zygotes were categorized as either those which developed to blastocysts (BL), or those which did not (non-BL). Time points of each morphokinetic event, such as 2nd polar body extrusion (PBII), pronuclear (PN) appearance/disappearance, embryonic cell divisions, and intervals between cell cleavages were analyzed.

**RESULTS:** Blastulation rates for EJ, AOA, and TESE were 54.1, 41.7, and 44.6%, respectively, and all comparable. After transferring 59 EJ embryos 13 (22.0%) implanted (IMP). In EJ, the time point for the 2-cell stage was shorter in BL than in non-BL (P < 0.01), while the interval between the 2- and 3-cell stage was longer in BL (P < 0.01). Among replaced embryos, IMP zygotes developed to the 8-cell stage faster than their non-IMP (55.0 ± 6 vs. 61.1 ± 8 hrs, P < 0.01). In both AO and TESE, time points for PN disappearance, the 2-, and 4-cell stage were shorter in BL than in non-BL (P < 0.05), while intervals between the 2- and 3-cell stage in AO, and between the 4- and 5-cell stage in TESE were longer in BL than in non-BL (P < 0.05). Overall, in comparison to EJ zygotes, TESE extruded PBII more slowly (P < 0.05), while AOA developed faster to the 4-cell stage (P < 0.05).

**CONCLUSION:** Faster and more synchronous cleavage was associated with higher developmental potential of zygotes irrespective of sperm origin and activation methods. Although the blastocyst formation rate was similar, sperm immaturity appeared responsible for slower first cleavage, and chemically induced activation accelerated early cleavage.

---

**O-295 Wednesday, October 22, 2014 12:45 PM**

**NEXT GENERATION BSULFITE SEQUENCING REVEALS CONSISTENT POPULATION-WIDE REGIONAL SPERM DNA METHYLATION ALTERATIONS WITH AGE.** T. G. Jenkins,* K. I. Aston,* C. Pfluger,* B. R. Cairns,* D. T. Carroll.* "Andrology and IVF Laboratories, University of Utah, Salt Lake City, UT; "Department of Oncological Sciences, University of Utah, Salt Lake City, UT.

**OBJECTIVE:** To confirm, and further describe DNA methylation alterations that are common in sperm as a result of aging. Our objective was to better understand the cell population dynamics of DNA methylation alterations associated with aging.

**DESIGN:** Samples from our general population tissue bank were selected based on age alone for a comparison of “young” and “aged” sperm DNA methylation profiles. A total of 19 aged (>45 years of age) and 47 young (<25 years of age) samples were selected for this analysis.

---

<table>
<thead>
<tr>
<th>Initial sperm parameters</th>
<th>N=18</th>
<th>X±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>38.5±4.5</td>
<td></td>
</tr>
<tr>
<td>Volume</td>
<td>3.1±0.9</td>
<td></td>
</tr>
<tr>
<td>Concentration(millions/mL)</td>
<td>63.9±31.2</td>
<td></td>
</tr>
<tr>
<td>Progressive motility</td>
<td>47.5±13.4</td>
<td></td>
</tr>
<tr>
<td>Morphology</td>
<td>10.1±5.9</td>
<td></td>
</tr>
<tr>
<td>Vitality</td>
<td>74.5±8.5</td>
<td></td>
</tr>
<tr>
<td>DNA fragmentation</td>
<td>27.6±8.5</td>
<td></td>
</tr>
<tr>
<td>Acrosome reaction</td>
<td>14.5±3.8</td>
<td></td>
</tr>
<tr>
<td>X rate</td>
<td>50.5%±1.2%</td>
<td></td>
</tr>
<tr>
<td>Y rate</td>
<td>49.5%±1.5%</td>
<td></td>
</tr>
</tbody>
</table>

**Note:** The table above represents the initial sperm parameters with their respective means and standard deviations.
FERTILITY & STERILITY®

O-296 Wednesday, October 22, 2014 11:15 AM
NEONATAL OUTCOMES OF TRANSFER OF BLASTOCYSTS VITRIFIED AND WARMED IN DEFINED SOLUTIONS CONTAINING RECOMBINANT HUMAN ALBUMIN: 354 BABIES BORN FOLLOWING 851 EMBRYO TRANSFERS.


OBJECTIVE: To determine the incidence of placenta accreta in our IVF/ICSI population and to test the hypothesis that CET is an independent risk factor for placenta accreta.

MATERIALS AND METHODS: Among consecutive patients delivering at least one viable infant ≥24 weeks’ gestational age at our hospital from 2005 to 2011 (n=54,947), we identified all patients who underwent a day 3 or day 5 transfer at our IVF center (n=1,569). From this cohort, we confirmed 52 cases of placenta accreta (defined by histology or the clinical finding of an adherent placenta with or without morbid complications such as postpartum hemorrhage, hysterectomy or surgery to remove the placenta). Cases were matched 1:3 by maternal age and history of prior cesarean to IVF/ICSI patients without accreta. Adjusted odds ratios (aOR) with 95% confidence intervals (CI) were calculated by multivariate analysis, controlling for age (to account for any residual confounding), placenta previa, and potential confounders was obtained from national registries. For singletons, each ART pregnancy was matched with four spontaneously conceived (SC) pregnancies by year and parity. For twins, all pregnancies in the study period were included.

RESULTS: In general, there are no major outlying populations of sperm that drive the average age-associated methylation alterations. Instead, it appears that most cells in a sperm population have a subtle, uniform, regional age associated methylation perturbations. While these methylation alterations are significant, they tend to be more significant than those that have been identified in somatic cells based on previous work from Day et al. who showed only a single CpG in somatic tissue that had a yearly change of >0.5%. Our data revealed 13 genomic windows with an average fraction methylation change per year of >0.5%. In regions that undergo hypermethylation with age, the average fraction methylation change was 0.30% per year and in regions undergoing hypo-methylation the average fraction methylation change was 0.28% per year.

CONCLUSION: Our data support the idea that all cells in a population of sperm (and not just small subpopulations) undergo similar DNA methylation alterations with age, and thus may represent a similar risk to the offspring. These results confirm data previously reported in longitudinally collected donor samples in our laboratory.

O-297 Wednesday, October 22, 2014 11:30 AM
RISK OF PREGNANCY-INDUCED HYPERTENSIVE COMPLICATIONS FOLLOWING ASSISTED REPRODUCTIVE TECHNOLOGY (ART) – A COHORT STUDY FROM THE COMMITTEE ON NORDIC ART AND SAFETY.


OBJECTIVE: Women who conceive through ART are at increased risk of pregnancy-induced hypertensive disorders. In this large population based study, we studied whether in-vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) as well as fresh or frozen cycles differed with respect to risk of hypertensive complications in pregnancy.

DESIGN: A population based cohort study including all ART pregnancies in Denmark, Finland, Norway and Sweden throughout 2007. Information on pregnancy complications, type of ART procedure, and potential confounders was obtained from national registries.

RESULTS: In general, there are no major outlying populations of sperm that drive the average age-associated methylation alterations. Instead, it appears that most cells in a sperm population have a subtle, uniform, regional age associated methylation perturbations. While these methylation alterations are significant, they tend to be more significant than those that have been identified in somatic cells based on previous work from Day et al. who showed only a single CpG in somatic tissue that had a yearly change of >0.5%. Our data revealed 13 genomic windows with an average fraction methylation change per year of >0.5%. In regions that undergo hypermethylation with age, the average fraction methylation change was 0.30% per year and in regions undergoing hypo-methylation the average fraction methylation change was 0.28% per year.

CONCLUSION: Our data support the idea that all cells in a population of sperm (and not just small subpopulations) undergo similar DNA methylation alterations with age, and thus may represent a similar risk to the offspring. These results confirm data previously reported in longitudinally collected donor samples in our laboratory.

O-298 Wednesday, October 22, 2014 11:45 AM
CRYOPRESERVED EMBRYO TRANSFER (CET) IS AN INDEPENDENT RISK FACTOR FOR PLACENTA ACCRETA IN THE IVF/ICSI POPULATION.

D. J. Kaser, A. Melamed, C. Racowsky, C. L. Bormann, B. W. Walsh, D. A. Carusi.

OBJECTIVE: To determine the incidence of placenta accreta in our IVF/ICSI population and to test the hypothesis that CET is an independent risk factor for placenta accreta.

MATERIALS AND METHODS: Among consecutive patients delivering at least one viable infant ≥24 weeks’ gestational age at our hospital from 2005 to 2011 (n=54,947), we identified all patients who underwent a day 3 or day 5 transfer at our IVF center (n=1,569). From this cohort, we confirmed 52 cases of placenta accreta (defined by histology or the clinical finding of an adherent placenta with or without morbid complications such as postpartum hemorrhage, hysterectomy or surgery to remove the placenta). Cases were matched 1:3 by maternal age and history of prior cesarean to IVF/ICSI patients without accreta. Adjusted odds ratios (aOR) with 95% confidence intervals (CI) were calculated by multivariate analysis, controlling a priori for age (to account for any residual confounding), placenta previa, prior abdominal or laparoscopic myomectomy and uterine factor infertility.

RESULTS: The overall incidence of accreta in our IVF/ICSI cohort was 3.31% (52/1,569); among CET patients, the incidence was 7.24%
Vitrification of cleavage stage day 3 embryos results in significantly higher life birth rates than slow freezing: A randomized controlled trial.


OBJECTIVE: To compare vitrification and slow freezing for cryopreservation of day 3 embryos.

DESIGN: Randomized controlled trial in an academic tertiary setting.

MATERIALS AND METHODS: Patients younger than 40 years using own oocytes in the 1st ART cycle, with embryo transfer (ET) and with available supernumerary embryos on day 3, were randomized at the time of cryopreservation after informed consent. Day 3 embryos with ≥ 6 cells, <25% fragmentation and even to slightly uneven blastomeres were cryopreserved by slow freezing (EmbryoStore, Gynemed®) or by closed vitrification (Vitr Kit Freeze, Irvine Scientific®). Survival was defined as present if ≥ 50% of cells were intact after thawing. Thawed embryos were further cultured overnight. To test the hypothesis that the life birth rate (LBR) per embryo thawed after vitrification was significantly higher (16%) than after slow freezing (6%), power calculation revealed that 184 thawed embryos were needed in each group (β=0.8, α<0.05). Secondary outcome variables were implantation rate (CPR), implantation rate (IR), and embryo quality. Equilibration, vitrification and loading procedures were employed for vitrification. Equilibration, vitrification and loading procedures were employed for vitrification. Donor stimulation and endometrial preparation for recipients were performed at the discretion of the treating physician. The primary end point was the pregnancy rate (PR). Secondary endpoints included CPR, FHB, and implantation rate (IR).

RESULTS: Between September 2011 and March 2013, 307 patients were randomized to slow freezing (155 patients, 480 embryos) or vitrification (152 patients, 495 embryos). In March 2013, 200 embryos were thawed after slow freezing in 95 cycles for 79 patients and 217 embryos were warmed after vitrification in 121 cycles in 90 patients. The LBR per embryo thawed was significantly higher after vitrification (17.1% [37/217] than after slow freezing (5.5% [11/200]; p=0.0002)). Similarly, the IR per embryo transferred was higher after vitrification (27.1% [48/177]) than after slow freezing (16% [16/100]; p=0.0382). The embryo survival rate was significantly higher after vitrification (84.3% [183/217]) than after slow freezing (52.5% [105/200]; p=0.0001). Significantly more embryos were fully intact after vitrification (75.4% [138/183]) than after slow freezing (28.6% [30/105]; p<0.0001). The number of ETs was significantly higher after vitrification with addition of any potential confounder, none were retained in the final model. Vitrificates not retained included gravidity, parity, race, body mass index, use of donor oocytes, history of curettage or operative hysteroscopy, intrauterine synechiae, oral contraceptive lead-in, pre-treatment with leuprolide acetate, endometrial thickness ≤ 7mm, micromanipulation (ICSI), assisted hatching, embryo biopsy, type of luteal phase support, selective reduction and multifetal delivery. The only significant predictor of acrreta in multivariate analysis were CET, prior myomectomy, and placenta previa (Table 1). Restricting analysis to only morbid cases of accreta strengthened the effect of CET on accreta to 2.78 (95% CI 1.11-6.99; P=0.03).

CONCLUSION: CET was a strong independent risk factor for placenta accreta among IVF/ICSI patients, even after controlling for known accreta risk factors.

O-299 Wednesday, October 22, 2014 12:00 PM

Vitrification of cleavage stage day 3 embryos results in significantly higher life birth rates than slow freezing: A randomized controlled trial.


OBJECTIVE: To compare vitrification and slow freezing for cryopreservation of day 3 embryos.

DESIGN: Randomized controlled trial in an academic tertiary setting.

MATERIALS AND METHODS: Patients younger than 40 years using own oocytes in the 1st ART cycle, with embryo transfer (ET) and with available supernumerary embryos on day 3, were randomized at the time of cryopreservation after informed consent. Day 3 embryos with ≥ 6 cells, <25% fragmentation and even to slightly uneven blastomeres were cryopreserved by slow freezing (EmbryoStore, Gynemed®) or by closed vitrification (Vitr Kit Freeze, Irvine Scientific®). Survival was defined as present if ≥ 50% of cells were intact after thawing. Thawed embryos were further cultured overnight. To test the hypothesis that the life birth rate (LBR) per embryo thawed after vitrification was significantly higher (16%) than after slow freezing (6%), power calculation revealed that 184 thawed embryos were needed in each group (β=0.8, α<0.05). Secondary outcome variables were implantation rate (CPR), implantation rate (IR), and embryo quality. Equilibration, vitrification and loading procedures were employed for vitrification. Equilibration, vitrification and loading procedures were employed for vitrification. Donor stimulation and endometrial preparation for recipients were performed at the discretion of the treating physician. The primary end point was the pregnancy rate (PR). Secondary endpoints included CPR, FHB, and implantation rate (IR).

RESULTS: Between September 2011 and March 2013, 307 patients were randomized to slow freezing (155 patients, 480 embryos) or vitrification (152 patients, 495 embryos). In March 2013, 200 embryos were thawed after slow freezing in 95 cycles for 79 patients and 217 embryos were warmed after vitrification in 121 cycles in 90 patients. The LBR per embryo thawed was significantly higher after vitrification (17.1% [37/217] than after slow freezing (5.5% [11/200]; p<0.0001). Secondary outcome variables were implantation rate (CPR), implantation rate (IR), and embryo quality. Equilibration, vitrification and loading procedures were employed for vitrification. Equilibration, vitrification and loading procedures were employed for vitrification. Donor stimulation and endometrial preparation for recipients were performed at the discretion of the treating physician. The primary end point was the pregnancy rate (PR). Secondary endpoints included CPR, FHB, and implantation rate (IR).

CONCLUSION: The hypothesis was confirmed that the LBR per embryo thawed is higher after vitrification than after slow freezing on day 3, based on better embryo survival, quality and availability in the vitrification group.
OBJECTIVE: The aims of this study were i) to compare the effects of different cryopreservation protocols on the functions of mouse epididymal spermatozoa and ii) to examine the effects of vitrification on IVF outcomes and embryo development using epididymal sperm.

DESIGN: An F1 experimental mouse model was used to provide epididymal sperm samples and oocytes for IVF. The effects of different cryopreservation methods on sperm parameters such as sperm motility, viability and DNA fragmentation and on embryo production and development were compared to select the most effective protocol for future studies.

MATERIALS AND METHODS: The cryopreservation methods and device used were selected from a pilot study prior to the experiment. Spermatozoa cryopreserved using a conventional freezing protocol with 18%w/v raffinose (RC) were compared with vitrified sperm samples (3 and 10μL) using cryohooks, three concentrations of sucrose (SV;0.25M,0.5M,0.75M) and one concentration of raffinose (RV;18%w/v). Sperm motility, viability (hypo-osmotic swelling test) and sperm DNA damage (TUNEL assay) in fresh controls (FC) were compared with post-thawed RC samples and post-warming RV and SV samples. Mature mouse eggs (n=267) were randomly assigned into three groups for insemination using RV (n=102), RC (n=86) and FC spermatozoa (n=79). Numbers of 2-cell embryos and the % of these embryos that developed to the blastocyst stage were assessed using standard criteria.

RESULTS: Sperm motility (14% RV and 30% SV vs. 46% RC; p<0.05) and viability (23% RV and 39% SV vs. 57% RC; p<0.05) were lower after RV and SV than RC. However, sperm vitrified with 0.25M sucrose gave the lowest incidence of DNA fragmentation (15% SV [0.25M] vs. 26% RV and 27% RC; p<0.05). The number of two-cell embryos produced by RC, RV and FC spermatozoa was not significantly different (p>0.05). The number of blastocysts, however, was significantly higher in the RV group than FC (p=0.0053), and the number of expanded blastocyst was higher with RV compared to SV (p=0.048).

CONCLUSION: Cryopreservation of mouse epididymal spermatozoa using the vitrification technique described in this study is simple, fast, and cost-effective with similar embryo success rates for vitrification and conventional cryopreservation methods as well as FC samples. However, blastocyst production was more successful using RV spermatozoa than FC.

Supported by: This research was Supported by funding from the Education Program in Reproduction and Development, Department of Obstetrics and Gynecology, Monash University.

STEM CELLS

O-303 Wednesday, October 22, 2014 11:15 AM

PRELIMINARY EVALUATION OF PGC DIFFERENTIATION PROTOCOL IN RHESUS MODEL. S. Park, N. Vahidi, A. H. DeCherney, E. F. Wolff. NICHD, NIH, Bethesda, MD.

OBJECTIVE: Functional oocyte differentiation from pluripotent stem cell through primordial germ cell (PGC) hold promise as a future treatment for female infertility, which has been shown to be successful in mouse models; however there are many biologic and developmental differences between mouse and human before these therapies could be transplanted into clinical care. Therefore, higher order models such as rhesus macaque are needed to test emerging stem cell technologies for their suitability as human treatments. However, published PGC differentiation models in non-human primates are lacking. The objective of this study is to develop rhesus differentiation protocol from pluripotent stem cells to PGCs.

DESIGN: In vitro study of rhesus iPSCs (rhiPSCs) differentiation into PGCs.

MATERIALS AND METHODS: rhiPSC were which were generated from female rhesus macaques using STEMCCA and pluripotency confirmed by teratoma formation. Undifferentiated rhiPSC colonies were dissociated and plated as a monolayer culture and exposed to a two-step differentiation culture protocol. First, cells are cultured in activin A (20 ng/ml) in the presence of bFGF, MEM-NEAA, beta-mercaptoethanol. Second, cultures are incubated with KO-DMEM with BMP4 (0, 20, 50 or 100 ng/ml), BMP5 (0, 20, 50 or 100 ng/ml), SCF (100 ng/ml), EGF (50 ng/ml) and LIF (10 ng/ml) in a low-attachment plate to allow cell aggregation. Cell density concentrations of 5x10^3 and 5x10^4 /well in a 24-well plate were tested. Frequency of media change (2, 3 or 4 days) was tested and viability was assessed. Cellular aggregation was assessed as evidence of differentiation, as well as RT-PCR and immunofluorescence staining were performed for DDx4, STELLA, NANOS3 genes.

RESULTS: The highest cell viability and largest aggregate formation was observed with 5x10^4 /well compared to 5.0 x 10^3/well in a 24-well plate. Optimal media change was determined to be every 2-3 days. RT-PCR revealed that 50 ng/ml of BMP4 and BMP8 led to highest up-regulation of STELLA and DDx4 expression as compared to 20 ng/ml of BMP4 and BMP8 or its absence and no significant difference with 100 ng/ml DDx4 protein expression in immunofluorescence staining was confirmed suggesting that 50 ng/ml of BMP4 and BMP8 are optimal to differentiate rhiPSCs to PGC like cells.

CONCLUSION: Here we have developed the first rhiPSC differentiation protocol for PGCs. Ongoing studies are underway to optimize terminal differentiation protocols for the rhiPSC derived PGCs into oocytes.

Supported by: Intramural NICHD.

SUCCESSFUL TELOMERE LENGTHENING IN INDUCED PLURIPOTENT STEM CELLS (ipSCs) DERIVED FROM HUMAN GRANULOSA CELLS (hGCs) OF WOMEN WITH DIMINISHED OVARIAN RESERVE (DOR). M. Gualtieri, T. Miki, K. Chung, B. Bendikson, M. Francis, R. J. Paulson. Obstetrics and Gynecology, University of Southern California, Los Angeles, CA. Biochemistry and Molecular Biology, University of Southern California, Los Angeles, CA.

OBJECTIVE: To investigate the feasibility of creating patient-specific iPSCs derived from hGCs obtained from patients with DOR and their subsequent re-differentiation to functional rejuvenated hGCs.

DESIGN: In vitro experiment.

MATERIALS AND METHODS: We obtained hGCs from good prognosis patients (n=10) and DOR patients (n=9) undergoing follicle aspiration during IVF. Good prognosis was defined as FSH<10, AMH>2, and <35 years old. Primary hGCs were isolated from follicular aspirates and then passed onto a cell culture dish. Lentiviral transfection with OCT4, MYC, KLF4, and SOX2 was then performed to induce reprogramming. Pluripotency was investigated by comparing immunohistochemistry (IHC), gene expression with human embryonic stem cells. After validation of pluripotency, telomere length of the primary hGCs and iPSCs was determined with Monochrome Multiplex qPCR. The iPSCs were subsequently re-differentiated to hGCs by culturing the iPSCs in media containing WNT-3a, Activin-A, and BMP-4 for 5 days followed by FSH and MIS for another 5 days. Successful differentiation was investigated by comparing relative gene expression of FOXL2, CYP19A1, AHM, and FSH receptors of the re-differentiated hGCs to that of primary hGCs. Additional validation was accomplished with quantification of estradiol and AMH production.

RESULTS: Successful attainment of pluripotency of the iPSCs was confirmed as noted above. Telomere length was significantly shorter in the hGCs of DOR patients compared to that of good prognosis patients (p<0.05). After induced pluripotency, telomere lengths in iPSCs derived from hGCs in the DOR patients were longer than those in the primary hGCs, and were similar to the hGCs of good prognosis patients. After differentiation, relative expression of FOXL2, CYP19A1, AMH, and FSH receptors were elevated compared to undifferentiated iPSCs (p<0.05). Production of Estradiol and AMH was also confirmed in the differentiated cells.

CONCLUSION: Successful induction of iPSCs from hGCs was confirmed. The telomere length of hGCs obtained from DOR patients was significantly shorter than that of hGCs of good prognosis patients, but was successfully rejuvenated after induction of pluripotency. Human iPSCs were successfully re-differentiated to functional hGCs. These data demonstrate the feasibility of this approach to the reprogramming of impaired hGCs to increase telomere lengths and potentially improve function.

Supported by: California Institute for Regenerative Medicine (CIRM) grant no. TR3-05488.
OPTIMAL METHODS FOR RECOVERY OF TRANSPLANTABLE STEM CELLS FROM FROZEN/THAWED HUMAN TESTICULAR TISSUE. H. Valli, M. Sukhwani, K. A. Peters, A. Althouse, K. E. Orwig. Obstetrics, Gynecology and Reproductive Sciences, Magee Womens Research Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA.

OBJECTIVE: Spermatogonial stem cell (SSC)-based technologies may have application for preserving and/or restoring male fertility. We evaluated the recovery of stem and progenitor spermatogonia from frozen/thawed testicular cell suspensions versus testicular tissue fragments frozen at a controlled rate (CR) or by vitrification.

DESIGN: Laboratory study using human tissue.

MATERIALS AND METHODS: Human testicular tissue was obtained through the Center for Organ Recovery and Education with approval from the University of Pittsburgh Institutional review board (#0506140). Testicular tissue was digested with enzymes to produce a cell suspension and frozen at CR or miniced into large (6-10 mm3) or small (3-5 mm3) testicular tissue fragments and frozen at CR or by vitrification. The efficiency of each technique was analyzed by immunocytochemistry (ICC) for the spermatogonial marker UTF1 and human to nude mouse xenotransplantation.

RESULTS: Cryopreservation method had a significant effect on the recovery of UTF1 positive spermatogonia per gram of tissue (p < 0.001). Large testicular tissue fragments frozen at CR had the greatest number of UTF1 cell/g tissue (14.9±2.2), followed by CR small (10.4±1.3), vitrified small (5.2±0.9), vitrified large (4.4±0.5), and frozen/thawed cell suspension (1.5±0.2). The difference in UTF1 positive cell recovery from CR large tissue fragments versus CR cell suspension was statistically significant (14.9 vs. 1.5, p = 0.027). Human to nude mouse xenotransplants confirmed that recovery of transplantable SSCs (colonies/g tissue) from CR small (1045.9±240.6) and CR large (570.0±147.1) testicular tissue fragments was greater than the CR cell suspension (62.6±12.3, p = 0.05). Recovery of transplantable stem cells from vitrified small (562.4±186.0) and vitrified large (75.4±33.2) testicular tissue fragments was not significantly greater than the CR cell suspension (p = 0.05).

CONCLUSION: CR freezing of testicular tissue fragments (large or small) leads to the greatest recovery of UTF1 positive spermatogonia and transplantable SSCs. Recovery of SSCs from vitrified small testicular tissue fragments was also very good. Freezing and thawing of intact testicular tissue is compatible with several downstream applications, including testicular tissue grafting, testicular tissue organ culture or digestion with enzymes to produce a cell suspension for SSC transplantation.

Supported by: This work was Supported by NIH grants HD055475 and HD061289, Magee-Womens Research Institute, Foundation and the Richard King Mellon Foundation and the United States-Israel Binational Science Foundation.

CHARACTERIZATION OF THE PUTATIVE OVARIAN STEM CELL MARKER DDX4 BY MASS SPECTROMETRY AND FUSION PROTEIN ANALYSIS OF C-TERMINUS EXPRESSION. N. Vahid, S. Park, P. Weitzel, L. Tisdale, E. F. Wolff. *NICHHD, National Institutes of Health, Bethesda, MD; †NHLBI, National Institutes of Health, Bethesda, MD.

OBJECTIVE: The existence of adult ovarian stem cells (OSCs) remains contentious in part due to the controversial surface marker, DDX4, which was purported to identify these elusive cells. DDX4 was historically considered an exclusively intracellular protein, but has been described to have a nuclear and/or cytoplasmic staining. Single and double potency and stress enzyme reporters (AMPKAR) enable detection of telomerase activity in living cells. Single and double potency and AMPK activity reporters in the embryonic stem cells. The objective is to validate the effectiveness of using double potency (Oct4, Rex1) reporter in embryonic stem cells (ESC) to detect stress and double potency and stress enzyme (AMPK) reporters to detect kinetic responses to stress.

DESIGN: Experimental.

MATERIALS AND METHODS: Mouse ESCs were infected with lentivirus expressing Oct4 promoter-driven GFP expression vector and a lentivirus containing AMPK reporter (AMPKAR). AMPKAR enables fluorescence resonance energy transfer (FRET) for real-time AMPK activity in live cells. Single and double potency and stress enzyme reporter expression in live stem cells were isolated by Fluorescence Activated Cell


OBJECTIVE: Telomeres are tandem repeated DNA sequences at the ends of chromosomes, which mediate aging by shortening with cell division and from reactive oxygen in non dividing cells. Progressive telomere erosion culminates in apoptosis and cell death. Polar body telomere length predicts IVF outcome and embryo aneuploidy as well as fragmentation. Overexpression of telomerase lengths telomeres, reverses the signs of aging and prolongs the life span of treated animals. Cycloastragenol, a substance from the Astragalus plant (A. propinquus), is a naturally occurring potent telomerase activator. The effect of cycloastragenol on human embryonic stem cells has not yet been studied.

RESULTS: In vitro study of cycloastragenol’s effects on telomerase activity and telomere length in human embryonic stem cells and fibroblasts.

MATERIALS AND METHODS: A novel monoclonal multiplex quantitative polymerase chain reaction (qPCR) (Cawthon 2009) assay measured telomere (T) signals and single copy gene (S) signals in comparison to a reference DNA to yield relative T/S ratios proportional to average telomere length. Single-cell telomere length pre-amplification qPCR (Wang 2013) measured telomere length in individual cells, telomeric repeat amplification protocol (Kim 1994) measured telomerase activity, and universal single telomere elongation length analysis (Bendix 2010) measured the shortest telomeres in NIH approved human ESC lines and fibroblasts as controls.

RESULTS: Cycloastragenol activates telomerase within 24 hours with duration of over one week, doubles telomere length and preferentially lengthens the shortest telomeres in both hESCs and fibroblasts. The greatest effects are seen after 7 days when the relative telomere length in fibroblasts (0.420±0.040 doubles compared to untreated cells (0.204±0.00030) (R2 = 0.994).

CONCLUSION: Telomerase activation using a natural plant product lengthens telomeres in human embryonic stem cells and fibroblasts. Future studies should determine whether telomere lengthening improves reproductive outcomes of women with advanced reproductive age.

Supported by: T.A. Sciences, NYU Medical School-Department of Obst/Gyn.

DEVELOPMENT OF A VAILABLE DOUBLE POTENCY FACTOR REPORTER FOR HIGH THROUGHPUT SCREENS OF STRESS RESPONSES IN EMBRYONIC STEM CELLS. E. E. Puscheck, Q. Li, Y. Yang, D. A. Rappolee. Obstetrics and Gynecology, Wayne State University School of Medicine; Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI.

OBJECTIVE: Stress induces differentiation (or loss of pluripotency) in mouse embryos through AMPK activity. Here we construct and test viable potency and AMPK activity reporters in the embryonic stem cells. The objective is to validate the effectiveness of using double potency (Oct4, Rex1) reporter in embryonic stem cells (ESC) to detect stress and double potency and stress enzyme (AMPK) reporters to detect kinetic responses to stress.

DESIGN: Experimental.

MATERIALS AND METHODS: Mouse ESCs were infected with lentivirus expressing Oct4 promoter-driven GFP expression vector and a lentivirus containing AMPK reporter (AMPKAR). AMPKAR enables fluorescence resonance energy transfer (FRET) for real-time AMPK activity in live cells. Single and double potency and stress enzyme reporter expression in live stem cells were isolated by Fluorescence Activated Cell

e104 ASRM Abstracts Vol. 102, No. 3, Supplement, September 2014
MATOGONIA STEM CELL.

OBJECTIVE: In vitro culture of spermatogonia stem cell would be benefit to the research on spermatogenesis mechanism. For those who suffer from azoospermia with a spermatogenesis arrest, there would be a hope to gain functional gamete via inducing their own spermatogonia stem cells if we could achieve long-term culture of these cells in vitro. However, in spite of that the rodent spermatogonia stem cells had been cultured in vitro over months successfully, there was few report on the long-term culture of human spermatogonia stem cells, which was mainly due to the fragile of human spermatogonia cells and the critical culture condition essential for them. This work aimed to explore a gentle way to isolate human spermatogonia stem cells and achieve a long-term in vitro culture of them.

DESIGN: Exploratory research.

MATERIALS AND METHODS: Human testis tissues of obstructive azoospermia patients were obtained via surgery and digested into cell suspension via two-step enzymatic digestion. Matrix selection was applied to isolate the human spermatogonia stem cells. SG medium was used as the condition medium for human spermatogonia stem cell culture. Immunofluorescence was carried out to characterize the isolated cells.

RESULTS: The isolated cells survived over 4 weeks in vitro with a good morphology that was identical with human spermatogonia stem cells and some of them formed colonies. Immunofluorescence showed that over 90% isolated cells expressed the spermatogonia stem cell markers such as GPR125, UCHL1, PLZF and GFRα-1.

CONCLUSION: Matrix selection would be a good way to isolated human spermatogonia stem cells and SG medium was suitable for the long-term culture of these cells. Improvements should be made for a longer period of culture and a higher purity.

Supported by: The work was supported by China National Key Project (2010CB945200 to ZL), a key grant from National Nature Science Foundation of China (31230048 to ZH), grants from National Science Foundation of China (31171422 to ZH).

SPECIAL RESEARCH PRESENTATIONS

O-412 Wednesday, October 22, 2014 11:15 AM

SPECIAL RESEARCH PRESENTATION: ANTI-MULLERIAN HORMONE LEVELS ARE DIFFERENT AMONG BRCA CARRIERS COMPARED TO LOW-RISK, HEALTHY CONTROLS. L. Johnson, a S. Sambell, a S. Dornhecker, a A. Schanne, b B. Urbani, a C. Gracia, a "Reproductive Endocrinology and Infertility, University of Pennsylvania, Philadelphia, PA; "Biostatistics and Epidemiology, University of Pennsylvania, Philadelphia, PA; "Basser Research Center for BRCA, University of Pennsylvania, Philadelphia, PA.

OBJECTIVE: Retrospective studies suggest that ovarian function may be compromised in BRCA carriers, but these findings have not been confirmed in prospective studies. The purpose of this study is to compare Anti-Mullerian Hormone (AMH) in BRCA carriers and low-risk, healthy controls.

DESIGN: Prospective cohort.

MATERIALS AND METHODS: Reproductive age menstruating women tested for a BRCA mutation without a history of cancer treatment were recruited nationwide through BRCA advocacy groups. Comparable healthy women at low risk for hereditary breast/ovarian cancer were recruited as controls. Subjects completed an online questionnaire and bloodspot home collection kit. Geometric mean AMH levels were compared between groups using multivariable regression models adjusted for confounders, including age, hormonal contraceptive (HC) use, menstrual cycle phase, and geographic region.

RESULTS: 96 women (36 BRCA1, 37 BRCA2, and 23 controls) with mean age of 31.4 years (range 19–45 years) participated. There were no significant differences in baseline characteristics between groups. Serum and bloodspot AMH values were highly correlated (Pearson’s r=0.79, p<0.0001) in 21 subjects with serum values. Age adjusted geometric mean AMH levels were similar between groups. However, after adjusting for confounders, AMH was noted to be significantly lower in BRCA2 carriers compared to healthy controls (2.48 vs. 4.39, p=0.038). There was no significant difference in AMH levels between BRCA1 carriers and controls or between BRCA1 and BRCA2 women.

AMH levels in BRCA1, BRCA2, and Low-Risk Controls

<table>
<thead>
<tr>
<th>BRCA1</th>
<th>BRCA2</th>
<th>Control</th>
<th>BRCA1 vs BRCA2</th>
<th>BRCA1 vs Control</th>
<th>BRCA2 vs Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age- adjusted*</td>
<td>2.19 (1.58-3.02)</td>
<td>1.52 (1.11-2.10)</td>
<td>2.30 (1.53-3.45)</td>
<td>0.85</td>
<td>0.12</td>
</tr>
<tr>
<td>Fully- adjusted*</td>
<td>3.42 (2.23-5.26)</td>
<td>2.48 (1.62-3.80)</td>
<td>4.39 (2.52-7.65)</td>
<td>0.35</td>
<td>0.04</td>
</tr>
</tbody>
</table>

*Adjusted for age, HC use, menstrual cycle phase, and geographic region

CONCLUSION: Compared to healthy low-risk controls, women with BRCA2 mutations were found to have lower AMH after adjustment for confounders. This finding may impact decisions about use of assisted reproductive technologies and fertility preservation in this population.

Supported by: American Society for Reproductive Medicine Fertility Preservation Grant (LJ); NIH T32 HD007440 (LJ); Basser Research Center for BRCA (CG, LJ).

O-413 Wednesday, October 22, 2014 11:30 AM

SPECIAL RESEARCH PRESENTATION: EPIDEMIC PROFILES IN ART PREGNANCIES. M. D. Pisarska, a G. M. Barlow, b N. Xu, c M. O. Goodarzi, d V. Funari, e J. Williams, III, f "Dept. of Obst/Gyn, Division of Reproductive Endocrinology and Infertility, Cedars-Sinai Medical Center, Los Angeles, CA; "Dept. of Medicine, Division of Endocrinology, Cedars-Sinai Medical Center, Los Angeles, CA; "Genomics Core, Cedars-Sinai Medical Center, Los Angeles, CA.

OBJECTIVE: Pregnancies conceived using assisted reproductive technologies (ART) are associated with adverse outcomes. As more than 1% of babies born in the U.S. are conceived using ART, it is essential to determine whether these adverse outcomes specifically result from the in vitro fertilization (IVF) used in ART, or are related to the causes of the underlying infertility. We hypothesize that IVF can induce epigenetic changes independent of genetic predisposition to infertility, and that these changes have lasting effects on placental and ultimately fetal development.

DESIGN: To test our hypothesis, we used first trimester chorionic villous samples (CVS) to compare epigenetic profiles in singleton pregnancies conceived using IVF or non-IVF fertility treatments vs. those conceived spontaneously.

MATERIALS AND METHODS: Genomic DNA was extracted from CVS samples from all three groups which had been preserved in RNAlater using ALLPrep DNA/RNA Minikits (Qiagen). Purified genomic DNA (500 ng) was bisulfite-converted and submitted to genome-wide Infinium HumanMethylation450K BeadChip (Illumina). Detection P values were calculated to
OBJECTIVE: To characterize the pregnancies in volunteers to the Infertility Family Research Registry (IFRR) with regard to number of pregnancy losses and to correlate repeat losses with the presence of infertility diagnoses and health conditions.

DESIGN: Case-control analysis of a volunteer cohort.

MATERIALS AND METHODS: Participants in the IFRR are infertile, fertile, donor and surrogate volunteers who enter self reported information into a secure database. Initial data are entered when the volunteer joins the registry and is updated periodically. Volunteers were reported one or more pregnancies (total 1,311 pregnancies) were compared as to demographic characteristics, pregnancy and delivery outcomes, infertility diagnoses, and health. Two logistic regression models were run: Model 1 compared women with ≥3 losses (118 women, 568 pregnancies) to a referent group with <3 losses (425 women with 743 pregnancies); Model 2 compared women with ≥5 losses (29 women and 1,112 pregnancies) with a referent group ≤-5 losses (514 women, 1,112 pregnancies).

RESULTS: Three hundred eighty three volunteers (70.4%) reported one or more losses. In Model 1 the high loss group had shorter duration of first pregnancy (<12 weeks compared to ≥12 weeks: AOR 8.07, 95% CI 4.62, 14.01) and more pregnancies with short duration (AOR 5.09, 95% CI 3.95, 6.54), but did not have more first pregnancies at ≤20 years. Number of live birth deliveries was lower in the high loss group of Model 1 (1 or more vs. none: AOR 0.35, 95% CI 0.27, 0.45) but longest time to pregnancy did not differ. Of infertility diagnoses, Model 1 had more unexplained (AOR 95%, 95% CI 1.38, 3.51) and less male factor infertility (AOR 0.32, 95% CI 0.185, 0.65). Hypertension (AOR 2.10, 95% CI 1.04, 4.25) and borderline disorders (2.75, 95% CI 1.19, 5.90) were more prevalent in the high loss group Model 1 whereas in Model 2 depression (AOR 3.93, 95% CI 1.08, 5.29), eating disorders (AOR 3.34, 95% CI 1.13, 9.86) and hypothryoidism (AOR 3.20, 95% CI 1.40, 7.32) were more prevalent in the high loss group. Having or more medical indications vs. none increased risk in both models: Model 1 (AOR 2.15, 95% CI 1.12, 4.12) and Model 2 (AOR 6.93, 95% CI 2.10, 22.91).

CONCLUSION: Volunteers to the IFRR exhibit a high rate of pregnancy loss and an increasing number of losses is associated with earlier stage pregnancy loss and medical indications.

Supported by: ASRM.

O-416 Wednesday, October 22, 2014 12:15 PM

“SPECIAL RESEARCH PRESENTATION” ENDOTHELIN REGULATION OF CYR61 IN UTERINE LEIOMYOMAS. K. Wallace,* K. Chatman,* S. K. Spencer,* V. Johnson,* B. LaMarca.* Ob/Gyn and BioInformatics, Dartmouth Medicine, V. Johnson,* B. LaMarca.* Hand of Los Angeles, Inc.

OBJECTIVE: Genetic studies have identified imbalances in angiogenic factors such as caveinte –rich protein 61 (Cyr61) and platelet derived growth factor-C (PDGFC) in women with uterine leiomyomas. Low oxygen conditions, hypoxia, have been identified as a possible mechanism to increase endothelin-1 and potentially lead to aberrant expression of Cyr61 and PDGFC. The objective of this study was to determine if hypoxia induces Cyr61 secretion from fibroid smooth muscle cells (sMBCs) to levels comparable to secretion from myometrial smooth muscle cells (fSMCs) through activation of the Endothelin-A (ETa) receptor.

MATERIALS AND METHODS: We modified a cardiac specific hESC reporter line via lentiviral transduction, creating a dual reporter line in which the Nkx2.5 promoter (cardiac) and the VE-Cadherin promoter (EC) drive respective fluorescent proteins. We also used TALEN targeting to “knock-in” CRE recombinase into the Nkx2.5 locus, allowing for lineage analysis of Nkx derivatives. We defined culture conditions that enrich for double-positive cells (putative CECs), isolated this population via FACS, and analyzed its molecular phenotype via RNA sequencing. We confirmed biologic relevance of our findings with progeny of Nkx2.5-Cre, floxed-stop TgTomato, VEGFR2 GFP, and floxed-beta catenin crosses in mice. We used a mouse model of MI to test engraftment of embryonic CECs.

DESIGN: in vitro cell culture/differentiation/analysis of human embryonic stem cells and in vivo developmental studies in mice.

RESULTS: Lineage tracing in mouse revealed that cardiac crescent ECs ancestrally express Nkx2.5. Cells expressing both reporters were evident day 9 of hESC differentiation. RNA sequencing revealed that WNT signaling was increased in cardiomyocytes and decreased in CECs. Principal component analysis identified a signature similar to CECs from human fetal heart. Subsequent inhibition of WNT during hESC differentiation increased the yield of CECs (42.9% vs 9.8%) at the expense of cardiomyocytes (-32.8% vs 65.6%).

CONCLUSION: Cardiac ECs can be derived from hESCs. Persistent WNT inhibition in progenitors drives CEC identity. This embryonic cell type may have translational benefit as a regenerative or instructive input in the context of disease and wound healing.

Supported by: Starr foundation, internal CRM1 funding, KY Cha ASRM grant.

O-416 Wednesday, October 22, 2014 12:15 PM

SPECIAL RESEARCH PRESENTATION: CHARACTERIZATION OF PREGNANCY LOSS IN A POPULATION WITH PREGNANCIES REPORTED TO THE INFERTILITY FAMILY RESEARCH REGISTRY (IFRR). J. E. Stern,* S. M. Gallagher,* M. B. Goldman,* W. E. Gibbons,* Ob/Gyn and BioInformatics, Dartmouth Medicine, Lebanon, NH, Ob/Gyn, Baylor College of Medicine, Houston, TX.

OBJECTIVE: To report the pregnancy losses in volunteers to the IFRR over a 20 year period.

MATERIALS AND METHODS: Volunteers to the IFRR exhibit a high rate of pregnancy loss and an increasing number of losses is associated with earlier stage pregnancy loss and medical indications.

RESULTS: Three hundred eighty three volunteers (70.4%) reported one or more losses. In Model 1 the high loss group had shorter duration of first pregnancy (<12 weeks compared to ≥12 weeks: AOR 8.07, 95% CI 4.62, 14.01) and more pregnancies with short duration (AOR 5.09, 95% CI 3.95, 6.54), but did not have more first pregnancies at ≤20 years. Number of live birth deliveries was lower in the high loss group of Model 1 (1 or more vs. none: AOR 0.35, 95% CI 0.27, 0.45) but longest time to pregnancy did not differ. Of infertility diagnoses, Model 1 had more unexplained (AOR 95%, 95% CI 1.38, 3.51) and less male factor infertility (AOR 0.32, 95% CI 0.185, 0.65). Hypertension (AOR 2.10, 95% CI 1.04, 4.25) and borderline disorders (2.75, 95% CI 1.19, 5.90) were more prevalent in the high loss group Model 1 whereas in Model 2 depression (AOR 3.93, 95% CI 1.08, 5.29), eating disorders (AOR 3.34, 95% CI 1.13, 9.86) and hypothryoidism (AOR 3.20, 95% CI 1.40, 7.32) were more prevalent in the high loss group. Having or more medical indications vs. none increased risk in both models: Model 1 (AOR 2.15, 95% CI 1.12, 4.12) and Model 2 (AOR 6.93, 95% CI 2.10, 22.91).

CONCLUSION: Volunteers to the IFRR exhibit a high rate of pregnancy loss and an increasing number of losses is associated with earlier stage pregnancy loss and medical indications.

Supported by: ASRM.
NURSING/SexualitY

O-310 Wednesday, October 22, 2014 03:45 PM

OCCUPATIONAL USE OF HIGH LEVEL DISINFECTANTS AND TIME TO PREGNANCY AMONG NURSES. A. J. Gaskins,2 C. C. Lawson,3 J. W. Rich-Edwards,4 S. A. Missmer,5 F. Laden,6 J. E. Chavarro,4 6Harvard School of Public Health, Boston, MA; 5National Institute of Occupational Safety and Health, Cincinnati, OH.

OBJECTIVE: To examine the relationship between occupational use of high level disinfectants among nurses and time to pregnancy (TTP).

DESIGN: Prospective cohort study.

MATERIALS AND METHODS: Our study included 2,581 women trying to become pregnant or with a recent planned pregnancy in the Nurses’ Health Study 3 cohort (2010-present). Every 3 to 6 months women report the duration of their pregnancy attempt. Women were considered at risk for pregnancy for the duration of their pregnancy attempt until they became pregnant, stopped trying to become pregnant, or were lost to follow-up. Occupational exposure to agents used to disinfect medical instruments, devices, or supplies was self-reported on the baseline questionnaire. Multivariable Cox proportional hazards models for discrete survival time were used to estimate the fecundability odds ratios (FOR) and 95% confidence intervals (CI) adjusting for age, race, BMI, smoking, and marital status.

RESULTS: Nurses who reported current exposure to high level disinfectants had a 22% reduction in fecundity (longer TTP) compared to nurses who were never exposed (FOR=0.78 [95% CI 0.67, 0.90]). A longer TTP was associated with current use of glutaraldehyde (FOR=0.77 [95% CI 0.62, 0.96]) but not with ortho-phthalaldehyde (FOR=1.02 [95% CI 0.66, 1.56]), peracetic acid (FOR=1.27 [95% CI 0.82, 1.97]), or hydrogen peroxide (FOR=0.74 [95% CI 0.54, 1.02]). Consistent use of ≥3 types of protective equipment was rare (6.7%) but significantly protected exposed women against longer TTPs. When specific types of equipment were examined, only consistent use of a water resistant gown or respiratory protection (not including surgical mask) significantly protected exposed women against longer TTPs. Use of a disinfection system with dedicated ventilation, eye protection, or protective gloves appeared to be beneficial however they did not reach statistical significance.

CONCLUSION: Occupational use of high level disinfectants, particularly glutaraldehyde, is associated with reduced fertility among women. Nurses using high level disinfectants should be advised to use the recommended protective equipment as these might mitigate the fertility impairments associated with high level disinfectant use.

Supported by: NIH grant T32HD060454 and contract 200-2013-M-54978 from CDC/NIOSH.

O-312 Wednesday, October 22, 2014 04:15PM

THE EFFECT OF LIFESTYLE FACTORS ON ANTI-MULLERIAN HORMONE (AMH) LEVELS IN INFERTILE JAPANESE WOMEN. M. Nakayama,7 E. Kamisawa,8 H. Kawauchi,9 Y. Asada.4 7School of Nursing, Osaka Prefecture University, Habikino, Osaka, Japan; 8School of Nursing, University of Fukui, Yoshida, Fukui, Japan; 9Kitsatsko University Hospital, Sagamihara, Kanagawa, Japan; 4Asada Ladies Clinic, Nagoya, Aichi, Japan.

OBJECTIVE: Anti-Müllerian hormone (AMH) is commonly used in clinical settings to measure fertility as it can provide an indication of ovarian reserve. Only a few studies however have explored how lifestyle factors affect AMH levels. The purpose of this study was to investigate whether lifestyle factors correlate with AMH levels in infertile Japanese women.

DESIGN: Cross-sectional study.

MATERIALS AND METHODS: All 2319 participants were infertile Japanese women undergoing infertility treatment across 6 ART clinics in Japan. AMH levels were measured using the AMH Gen II EUSA kit and lifestyle factors (BMI, smoking habits, alcohol consumption, coffee intake, physical activity, and sleeping patterns) were measured via a questionnaire. We analyzed the association between lifestyle factors and AMH levels by using Student’s t-tests and examined how lifestyle factors affect AMH levels by using multiple linear regression analysis.

RESULTS: The average age of participants was 36.15±4.64 years (range: 20–49) with a mean duration of known infertility of 38.78 months. AMH levels ranged from 0.01 ng/ml to 58.60 ng/ml, with a mean of 3.57 ng/ml. Mean BMI was 20.92 (range: 15.24–38.57) with a mean duration of known infertility of 38.78 months. AMH levels were associated with current use of glutaraldehyde (FOR=0.78 [95% CI 0.67, 0.90]), a longer TTP (FOR=0.77 [95% CI 0.62, 0.96]) but not with ortho-phthalaldehyde (FOR=1.02 [95% CI 0.66, 1.56]), peracetic acid (FOR=1.27 [95% CI 0.82, 1.97]), or hydrogen peroxide (FOR=0.74 [95% CI 0.54, 1.02]). Consistent use of ≥3 types of protective equipment was rare (6.7%) but significantly protected exposed women against longer TTPs. When specific types of equipment were examined, only consistent use of a water resistant gown or respiratory protection (not including surgical mask) significantly protected exposed women against longer TTPs. Use of a disinfection system with dedicated ventilation, eye protection, or protective gloves appeared to be beneficial however they did not reach statistical significance.

CONCLUSION: Occupational use of high level disinfectants, particularly glutaraldehyde, is associated with reduced fertility among women. Nurses using high level disinfectants should be advised to use the recommended protective equipment as these might mitigate the fertility impairments associated with high level disinfectant use.

Supported by: NIH grant T32HD060454 and contract 200-2013-M-54978 from CDC/NIOSH.

Fertility & Sterility®
e107

O-311 Wednesday, October 22, 2014 04:00 PM

GNRH-AGONIST SHORT PROTOCOL VERSUS GNRH-ANTAGONIST FOR OVARIAN STIMULATION IN IVF. J. J. Taylor. City Fertility Centre, Brisbane, Queensland, Australia.

OBJECTIVE: The objective of this study was to compare clinical outcomes of short agonist cycles and antagonist cycles in IVF.

DESIGN: This retrospective analysis compares the clinical outcomes of 5967 cycles between January 2009 and December 2013 across an Australian multi centre clinic. The agonist group contained 1344 patients compared to 4623 patients in the Antagonist group. Statistical analysis used Chi squared test.

MATERIALS AND METHODS: The agonist group contained 1344 patients compared to 4623 patients in the Antagonist group. Statistical analysis used Chi squared test.

RESULTS: The mean maternal age was not statistically significant between the two groups, 37.5% for the agonist group and 36.4% for the antagonist group. Retrospective analysis failed to detect a difference in the duration of ovarian stimulation between the cycles. We found an average of 10 days of stimulation for both cycle types. There was no significant statistical difference (p=0.5) in the number of eggs collected for each group, 7.8 in the agonist group and 9.5 in the antagonist group. The literature does suggest that fewer eggs are collected with antagonist cycles however this has not been our experience. For both stimulation groups, clinical pregnancy rates were not statistically different however there was a trend towards higher pregnancy rates across all ages in the antagonist group. Hospital admissions for moderate OHSS during the study period were not statistically different between the two groups however there was a lower number of admissions for moderate OHSS in the antagonist group. There were no hospital admissions for severe OHSS in either group.

CONCLUSION: This retrospective analysis supported the current body of evidence indicating that GnRH- antagonist cycles reduce the risk of OHSS without negatively affecting pregnancy rates. In fact, although not statistically significant, our study indicated a trend towards increased pregnancy rates with GnRH-antagonist cycles. Slightly lower rates of hospital admission in the antagonist group is consistent with current research.

CONCLUSION: This retrospective analysis supported the current body of evidence indicating that GnRH- antagonist cycles reduce the risk of OHSS without negatively affecting pregnancy rates. In fact, although not statistically significant, our study indicated a trend towards increased pregnancy rates with GnRH-antagonist cycles. Slightly lower rates of hospital admission in the antagonist group is consistent with current research.

O-313 Wednesday, October 22, 2014 04:30 PM

PREVIOUS INFERTILITY TREATMENT ASSOCIATED WITH DIFFERENT LEVELS OF PREGNANCY RELATED ANXIETY DURING IN-VITRO FERTILIZATION PREGNANCIES. E. Stevenson,8 R. Sloane,9 C. Bergh;8 Duke Univ, Durham, NC; 8RMANJ, Basking Ridge, NJ.

OBJECTIVE: In-vitro fertilization (IVF) is stressful; however, little is known if that stress carries into resulting pregnancies. This study examined
whether previous lesser-means treatments for infertility were associated with pregnancy-related anxiety during IVF pregnancies.

DESIGN: Prospective, cross-sectional.

MATERIALS AND METHODS: Subjects were recruited from an infertility center in NJ. Women currently pregnant by IVF, between 25 and 40, singleton or twin, and medically low-risk participated at one time point between 12 and 18 weeks gestation. Subjects completed a survey about their demographic data, previous treatments for infertility including Clomiphene Citrate or gonadotropins/Intercourse (CGI) only, IUI/Intercourse (IUII) only, Clomiphene Citrate or gonadotropins/IUI (CGUI), or no previous treatment (NT) as well as their perception of anxiety specific to pregnancy using the Pregnancy-Related Anxiety Measure (PRAM). In order to explore groups of subjects in the database, Latent Class Analysis (LCA) was used with the group definition variables including stimulated IUII alone, age, number of IVF cycles, number of miscarriages, income, previous children, gestational size, and history of laparoscopy, PGD, or saline sonogram. Groups were evaluated for differences in PRAM levels using ANOVA.

RESULTS: 144 subjects participated and were highly educated, affluent, married, and primarily white. The LCA process yielded two groups that on average had similar levels on most items except for use of IUI and/or ovarian stimulation. This information was used to generate four exhaustive and mutually exclusive groups: CGI, IUII, CGUI, or NT. ANOVA found that those in the CGI group had statistically significantly higher PRAM scores than the CGUII (p = 0.0036) and the NT group (p = 0.0013), and near significance from the IUII group (p = 0.0529).

CONCLUSION: Women who become pregnant via IVF and had a history of lesser-means of treatment with ovarian stimulation (CC or gonadotropins) without IUI, experience more pregnancy-specific anxiety. One possible explanation is that women undergoing treatment without IUI are more likely to have an unexplained infertility diagnosis. Patients experiencing infertility are often relieved at having a “cause.” It gives them a focus and hope for a solution to a specific problem. It is not a stretch to imagine that the ambiguity and uncertainty of “unexplained” infertility causes a higher level of stress, which carries into the pregnancy. This is an important consideration for nurses, as these patients may require additional emotional support.

Supported by: $1000 grant from the Mc Rae Foundation and a $1000 grant from Sigma Theta Tau, Upsilon Chapter.

O-315 Wednesday, October 22, 2014 04:05 PM

PSYCHOLOGICAL STRESS AND SEXUAL FUNCTION OF MALE PARTNER OF INFERTILE COUPLE DURING FERTILE PERIOD, S.-H. Song,① D. S. Kim,① H. J. Kim,① J. W. Kim,① S. W. Ryu,① J. Y. Hong,① D. S. Kim,② Department of Urology, Fertility Center, CHA Gangnam Medical Center, CHA University, Seoul, Korea; ②Department of Obstetrics and Gynecology, Fertility Center, CHA Gangnam Medical Center, CHA University, Seoul, Korea; ③Department of Urology, CHA Bung-dang Medical Center, CHA University, Seongnam, Korea.

OBJECTIVE: Infertility is a major source of life stress and might be associated with sexual dysfunction. We evaluated the sexual function and stress level during timed intercourse (TI) in male partner of infertile couples.

DESIGN: A prospective survey study.

MATERIALS AND METHODS: A total of 236 male partners of couples with more than 1 year of infertility period seeking medical care or an evaluation of couple infertility were included. The participants completed the International Index of Erectile Function (IIEF)-5 questionnaire for evaluation of sexual function. The patients were also asked about the usage of phosphodiesterase type 5 inhibitor (PDE5-I) such as sildenafil or tadalafil for erectile dysfunction (ED) treatment. Stress related to timed intercourse during fertile period was measured using ten-division visual analog scale (VAS) questionnaire. Patients with azoospermia, hypogonadism, underlying chronic medical disease, previous exposure to gonadotoxin were excluded.

RESULTS: All patients had a comprehensive interview, physical examination, semen analysis, and/or hormonal profile study. The VAS score of sexual relationship stress was significantly different between fertile period and non-fertile period. (non-fertile period: 2.1±2.2 vs. fertile period: 3.4±2.6, p < 0.001). The incidence of mild to moderate ED (IIEF-5 score, ≤26.55) in men of infertile couple was 8.9% (21/236) and the incidence of mild ED (IIEF-5 score, 17-21) was 42% (99/236). However, only 5.8% (12/236) have used PDE5-I for ED treatment during fertile period.

CONCLUSION: Male partners of infertile couple experience significantly higher TI related stresses during fertile period. Sexual dysfunction is also common in male partners of infertile couple. Sexual medical dealing with infertile couple should be aware of the male patient’s problem and be able to give better counselling.

O-316 Wednesday, October 22, 2014 04:15 PM

QUANTIFICATION OF SELF-PERCEIVED DELAYED EJACULATION, A. G. Winter, A. Bolyakov, D. A. Paduch. Department of Urology and Reproductive Medicine, New York Presbyterian Hospital- Weill Cornell Medical Center, New York, NY.

OBJECTIVE: Of male sexual dysfunctions, delayed ejaculation (DE), is one of the most poorly defined and least understood phenomena. We collected data on ejaculatory function in a cohort of men presenting with self-perceived DE and a cohort of asymptomatic men to quantify and characterize DE.

DESIGN: Case-control study.

MATERIALS AND METHODS: Male volunteers with no ejaculatory complaints were included as control subjects (Group 1, n = 56). DE cases (Group 2, n = 36) were defined after meeting two criteria: 1) a presenting complaint of ejaculatory dysfunction, 2) responses of “often” or “always” to the survey, “How often has it taken you longer to ejaculate that you would like to?” All men underwent hormonal evaluation, including total testosterone (TT). Sexual function was assessed using validated instruments (Male Sexual Health Questionnaire- MSHQ).
O-317 Wednesday, October 22, 2014 03:50 PM


OBJECTIVE: Evidence supporting best practices for providing fertility counseling for female adolescents is lacking. Adolescent girls desire reproductive information, yet they frequently do not access it. The objective of this study was to review existing literature regarding fertility counseling in the adolescent population and to create a practice guide to assist providers in achieving a successful dialogue.

DESIGN: Systematic review.

MATERIALS AND METHODS: A detailed PubMed search was performed using the terms “adolescent” and “fertility”. Further terms were added to cover specific fertility-related conditions. References of reviewed articles were also used to identify existing literature. Papers were selected if written in English and if deemed relevant by two independent researchers. Professional society guidelines were also reviewed and informed development of our practice guide.

RESULTS: 68 studies and 9 published guidelines were identified. Twenty studies related to sexual education and adolescents’ access to reproductive care, and revealed that though adolescents are interested in learning about sexuality and reproduction, this is overlooked during the standard office visit. As a result, adolescents often turn to less reliable sources, such as peers or the Internet. There was no literature on routinely discussing fertility with healthy teens. The remaining 48 studies addressed specific fertility-related conditions, most frequently menstrual irregularities (11), oncology (8) the polycystic ovary syndrome (7) and sexually-transmitted related conditions, most frequently menstrual irregularities (11), oncology (8) the polycystic ovary syndrome (7) and sexually-transmitted infections (6). Outside of articles on oncology, the literature we reviewed rarely mentioned future fertility, despite acknowledgement that many of these adolescents ultimately desire children and may have enhanced fertility concerns compared to healthy teens. A practice guide was created to assist in provision of critical reproductive health and fertility information to the female adolescent.

CONCLUSION: Providers caring for adolescent girls have the opportunity to provide fertility counseling as part of a larger reproductive health conversation that adolescents desire, and may benefit from. Identifying potential future fertility issues, understanding age-related fertility decline and aiding in health optimization prior to future conception may empower the adolescent to make informed reproductive decisions. Utilization of the suggested guide will enhance providers’ fertility counseling approach and delineate when more detailed counseling is warranted.

O-319 Wednesday, October 22, 2014 04:00 PM

"SPECIAL RESEARCH PRESENTATION" IDENTIFICATION OF ABDERRANT MOLECULAR PATHWAYS IN UNEXPLAINED NON-OBSTRUCTIVE AZOOSPERMIA. P. L. Yang, J. F. Smith, E. Altman, S. Choudhyr, N. D. Tran. Department of Obstetrics, Gynecology and Reproductive Sciences, University of California San Francisco, San Francisco, CA; Department of Urology, University of California San Francisco, San Francisco, CA.

OBJECTIVE: Spermatogenesis is maintained by interactions between spermatogonial stem cells (SSCs) and somatic cells within the testicular niche. However, it is unknown whether defects in either the germ or somatic compartments or a combination of the two are attributed to nonobstructive azoospermia (NOA). The aim of this study is to elucidate the pathophysiology of NOA.

DESIGN: In vitro SSC culture and protein/molecular analyses of SSCs and somatic cells from normal and patients with unexplained NOA.

MATERIALS AND METHODS: SSCs and testicular somatic cells were isolated by fluorescence activated cell sorting (FACS) from testicular biopsies for culture under time-lapse photography and RNA-seq. Differential gene expression was defined by >2 fold change, validated with Fluidigm, and analyzed with Ingenuity Pathway Analysis (IPA).

RESULTS: NOA SSCs exhibited a 50% decrease in the rate of colony formation with smaller colony size in comparison to normal SSCs regardless if they were co-cultured with somatic cells collected from NOA or normal patients. Analyses of culture media from NOA patients demonstrated significantly higher levels of inflammatory chemokines (CCL5-2) and interleukins (IL1b, 6, 8, 12). RNA-seq of SSCs and somatic cells from NOA patients showed 79 (72 upregulated) and 590 (423 upregulated) differentially expressed genes, respectively, in comparison to normal patients. IPA analyses demonstrated activation of the TNF and NFkB pathways in both NOA SSCs and somatic cells. In contrast, genes involved in retinoic acid receptor activation, biosynthesis, and apoptosis signaling were highly expressed specifically in NOA somatic cells. Interestingly, genes involved in reduction of oxidative stress (Vitamin C and pentose phosphate pathways) were highly expressed only in NOA somatic cells.

CONCLUSION: Activation of the inflammatory cascade through the TNF and NFkB pathways in both the germ and somatic compartments play an essential role in the pathogenesis of NOA. While NOA somatic cells were able to activate antioxidative pathways, this was not observed in NOA SSCs.

FERTILITY & STERILITY®

OBJECTIVE: Mounting evidence puts the blame on increased DNA damage as the underlying cause for somatic aging. Recent research showed that age-related decline in DNA double strand break (DSB) repair and as a result, accumulating DSBs may also be responsible for oocyte aging. In this study, we evaluated whether the same mechanism has a role in male gamete aging.

DESIGN: Experimental study.

MATERIALS AND METHODS: First, young (2-3 month old) and productively senescent old (10-14 month) wild type (WT) male mice were studied to determine the impact of age on sperm DNA damage and repair. Then, to determine the vitality of intact DNA DSB repair in maintaining male reproductive health, we inbred a transgenic BRCA1-mutant mouse colony, which has impaired DNA DSB repair function. These were studied at 2-3 months of age. Sperm were isolated from the vas deferens to observe: a) the extent of DNA DSBs as reported by histone H2AX phosphorylation (γH2AX) utilizing laser scanning microscopy; and b) the expression of key DNA DSB repair genes relative to GAPDH by qRT-PCR analysis of extracted sperm RNA. Lastly, we compared the BRCA1-mutant mice to WT for litter size and sperm concentration.

RESULTS: We observed a significant increase in the expression of γH2AX in old vs. young WT mice (16.6±4.1 vs. 5.26±0.706, n=9; p=0.006). This increase was associated with a decline in the relative expression of key DNA repair genes with age, including MRE11 (0.130±0.213 vs. 0.015±0.244, n=5; p=0.019), DMC1 (0.00046±0.127 vs. 0.00032±0.188, n=5; p=0.045), RAD51 (0.009±0.401 vs. 0.0027±0.440, n=3; p=0.046), ATM (0.010±0.168 vs. 0.007±0.207, n=5; p=0.008), and BRCA1 (0.075±0.130 vs. 0.067±0.079, n=5; p=0.024). The BRCA1-mutant mice showed a significant increase in DNA DSBs as reported by γH2AX expression (20.85±5.19 vs. 5.26±0.706, n=9; p=0.02) when compared to similar age WT mice. Furthermore, BRCA1-mutant male mice resulted in smaller litter size when mated with WT females compared to when WT males were mated with the same (3.2±2.3 vs. 6.8±1.27, n=9; p=0.0008) and they were found to have lower sperm concentration (1,621.4 x101±0.074 vs. 2,006.3 x101±0.087, n=6; p=0.013).

CONCLUSION: These data strongly suggest that declining DNA DSB repair results in spermatooza aging. Exploitation of this mechanism may result in future medical interventions to curb reproductive senescence in males.

Supported by: NIH R01 HD053112; R21 HD061259.

O-320 Wednesday, October 22, 2014 04:15 PM

ABNORMAL CLEAVAGE OF FILAMIN A AND CYTOSPLASMIC RETENTION OF ANDROGEN RECEPTOR IN TESTICULAR BIOPSY IN MEN WITH KLINEFELTER SYNDROME (KS): AN EXPLANATION FOR ANDROGEN RESISTANCE IN KS. M. G. Funaro, A. Mielnik, C. Baker, P. N. Schlegel, D. A. Paduch. Dept of Urology and Reproductive Medicine, Weill Cornell Medical College, New York, NY.

OBJECTIVE: Men with KS (47 XXY), frequently demonstrate resistance to exogenous testosterone. Posttranslational splicing of Filamin A (FnHa) into smaller fragments is a critical step for normal trafficking of activated AR and for activation of androgen response elements on DNA (1). The aim of this study was to show that abnormal expression and splicing of FnHa is present in KS and may explain retention of AR within cytoplasm and androgen resistance in KS.

DESIGN: Tissue repository analysis study.

MATERIALS AND METHODS: 10 testicular biopsy samples were obtained from normal male patients (control); 7 testicular biopsy samples were obtained from men with KS. Tissue was prepared with Qiagen AllPrep DNA/RNA/Protein Mini kit. To quantify AR, nuclear and cytoplasmic protein fractions were extracted from snap-frozen testicular tissues. Amounts of AR and HSP-90 proteins were measured with ImageJ and after OD normalization expressed as a ratio of nuclear to cytoplasmic (N/C) AR. Quantitative real-time PCR (qRT-PCR) was performed using Roche Lightcycler 480. Gene expression was normalized to housekeeping gene HPRT. Western blots were run using novex 3-8% tris-acetate gels, Santa Cruz N20 AR, Millipore MAB1680 FnHa, and Sigma A1978 B-Actin antibodies. Expression was quantified using Li-Cor Odyssey.

RESULTS: In patients with KS (N/C=2.1 ± 1.1) there was 3.5 times less AR in nucleus than in cytoplasm as compared to controls (N/C=7.1 ± 1.3) p=0.03, despite normal testosterone levels indicating retention of AR in cytoplasm in KS. qRT-PCR showed 3.4× higher expression of FnHa and elevated AR mRNAs expression in KS vs. normal men. Men with KS had an increase in whole FnHa protein with an 80% decrease in the expression
TABLE 1. Expression of FlmA and AR mRNA and proteins.

<table>
<thead>
<tr>
<th>qRT-PCR Normalized Expression</th>
<th>Normal</th>
<th>KS</th>
</tr>
</thead>
<tbody>
<tr>
<td>AR (Target/Ref)</td>
<td>16.53</td>
<td>30.77</td>
</tr>
<tr>
<td>FlmA (Target/Ref)</td>
<td>11.84</td>
<td>40.33</td>
</tr>
</tbody>
</table>

Western Blot - Normalized Expression ratios

<table>
<thead>
<tr>
<th>AR</th>
<th>FlmA (280)</th>
<th>FlmA (110)</th>
<th>FlmA (90)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KS/Normal</td>
<td>0.88</td>
<td>1.10</td>
<td>0.26</td>
</tr>
<tr>
<td>KS/Normal</td>
<td>0.16</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

of FlmA fragments (required to direct AR toward nucleus) as compared to controls.

CONCLUSION: Men with KS have 3x higher retention of activated AR in cytoplasm, indicating a defect in trafficking of AR toward the nucleus. The 82% decrease in levels of FlmA 90 and 74% reduction in FlmA 110 observed in KS clearly support our hypothesis that abnormal trafficking of AR due to abnormal cleavage of FlmA is responsible for androgen resistance in KS. This study may give rise to new therapeutic avenues to treat men with hypogonadism not responding to treatment.

Supported by: Irena and Howard Laks and the Theresa & Frederick Dow Wallace Fund of NYCT provided funds to support this study. Michael Funaro was Supported by the Eric M Smith scholarship in Andrology and Business.

O-323 Wednesday, October 22, 2014 05:00 PM


OBJECTIVE: To assess the global organization of telomeres within human spermatozoa using both 3D and 2D methodological approaches.

MATERIALS AND METHODS: This study was approved by the local Institutional Review Board and 10 normozoospermic males were recruited. Global telomere organization was assessed using fluorescence in situ hybridization for a pantelomeric probe. Both 3D and 2D imaging was employed to investigate telomere organization in terms of radial (nuclear interior to nuclear periphery) and longitudinal (head to tail). Radial organization of telomeres was assessed using 3D microscopy (n=300). Furthermore, longitudinal telomere organization was measured by both 2D and 3D methods (1,000 and 300 cells respectively). Statistical analysis was performed using the Chi-squared goodness-of-fit test to determine nonrandom organization of telomeres (p<0.05).

RESULTS: All 10 subjects demonstrated reproducible telomere distribution. The organization was nonrandom (p<0.05) by both means of assessment; 3D (radial), 2D and 3D (longitudinal). Specifically, telomeres demonstrated the following radial distribution: 43% nuclear periphery, 43% intermediate localization, and 14% nuclear interior. In terms of longitudinal organization, both 2D and 3D approaches revealed a remarkably similar telomere distribution with 25%, 50% and 25% being found in the head, mid, and tail region of the sperm cells respectively.

CONCLUSION: The main finding of this study is that global telomere chromatin organization is nonrandom within the sperm nucleus and contrary to previous reports NOT all telomeres are situated in the nuclear periphery. This finding suggests that the sperm nucleus is segmented and fits with the hypothesized ordered exodus of chromosomes from the sperm allowing gradual decondensation and preferential remodeling of specific regions of the genome. This sequential exodus and remodeling could impact the differential gene activation patterns observed within the early embryo. Thus telomeres of chromosomes occupying the apical and nuclear periphery are more likely to be exposed and activated first if this preferential organization holds true. We postulate that alterations from this organization may negatively impact fertilization and early embryo-gensis.

Supported by: NM and EJ NIH/NIGMS R25 GM061347.

O-324 Wednesday, October 22, 2014 03:45 PM

IS OOCYTE DONATION AN INDEPENDENT RISK FACTOR FOR PREECLAMPSIA: A PROPENSITY SCORE ANALYSIS. T. Tarlatzi, R. Imbert, I. Demeestere, C. Venetis, Y. Engler, A. Delbaere. Fertility Clinic, Erasmus Hospital, Université Libre de Bruxelles, Brussels, Belgium; Women’s & Children’s Health, St George Hospital, University of New South Wales, Kogarah New South Wales, Australia.

OBJECTIVE: The aim of the present study is to evaluate whether oocyte donation represents an independent risk factor for preeclampsia.

DESIGN: Retrospective analysis of all consecutive oocyte donation IVF/ICSI cycles performed during the period 1990-2013 at the Fertility Clinic of the Erasmus Hospital of the French-speaking Free University of Brussels.

MATERIALS AND METHODS: All resulting singleton pregnancies after oocyte donation and fresh embryo transfer, leading to a delivery at ≥22 weeks of gestation were identified (n=239). The control group was extracted among singleton pregnancies after IVF/ICSI with own oocytes and fresh embryo transfer during the same period with a delivery at the Erasmus Hospital (n=799). Propensity score matching (on maternal age and parity) was performed on a 1:1 basis and 144 pregnancies after oocyte donation (OD group) were compared with 144 pregnancies achieved by using autologous oocytes (AO group). The primary outcome measure was the incidence of preeclampsia and secondary outcome measures were the incidence of pregnancy induced hypertension (PIH), intrauterine growth restriction (IUGR), gestational diabetes, vaginal bleeding during pregnancy, the mode of delivery and all registered neonatal outcomes.

RESULTS: Pregnancies after oocyte donation compared to pregnancies using autologous oocytes were associated with a significantly increased risk for preeclampsia (OR 2.43, 95% CI 1.02-5.8; p=0.046), PIH (OR 5.3, 95% CI 1.1-25.23; p=0.036), and cesarean delivery (OR 2.3, 95% CI 1.43-3.69; p<0.001). Risks for IUGR (OR 3.04; p=0.34), first trimester bleeding (OR: 4.73, 95% CI: 0.95-45.68), and preterm deliveries (OR: 1.38, p=0.29) were increased at the OD group, but not significantly so. Neonatal birth weight and Appgar scores were comparable between the two groups.

CONCLUSION: To the best of our knowledge this is one of the largest cohorts of pregnancies after oocyte donation reported in the literature, where obstetric and neonatal outcome is compared to pregnancies using autologous oocytes matched for age and parity. Based on the results of this study, pregnancies after oocyte donation are at a significantly increased risk for preeclampsia and pregnancy-induced hypertension.

O-325 Wednesday, October 22, 2014 04:00 PM


OBJECTIVE: To compare oocyte to birth efficiency before and after implementation of several changes in embryological procedures.

DESIGN: Retrospective review.

MATERIALS AND METHODS: All oocyte retrievals for autologous IVF from 2004-12 at a single ART center were reviewed. Following fresh transfer, surplus embryos developing into good-quality blastocysts were cryopreserved. Potential children resulting from embryo cryopreservation were estimated using observed outcomes of frozen embryo transfers during the study period and assuming use of all frozen embryos. Retrievals from 2009 through 2012 (Group B), when vitrification replaced slow freeze for cryopreservation and culture enhancements including low oxygen concentration and improved media formulations were incorporated, were compared to earlier retrievals (Group A).

RESULTS: 385,284 oocytes were collected in 29,096 retrieval cycles. Oocyte utilization (% oocytes resulting in transferred or cryopreserved embryos) increased among patients under 41 years (&lt;35: 21.4% vs 22.6%, p&lt;0.0001; 35-37: 22.2% vs 23.2%, p&lt;0.0001; 38-40: 24.4% vs 25.5%, p=0.046), PIH (OR 5.3, 95% CI 1.1-25.23; p=0.036), and cesarean delivery (OR 2.3, 95% CI 1.43-3.69; p&lt;0.001). Risks for IUGR (OR 3.04; p=0.34), first trimester bleeding (OR: 4.73, 95% CI: 0.95-45.68), and preterm deliveries (OR: 1.38, p=0.29) were increased at the OD group, but not significantly so. Neonatal birth weight and Appgar scores were comparable between the two groups.

CONCLUSION: To the best of our knowledge this is one of the largest cohorts of pregnancies after oocyte donation reported in the literature, where obstetric and neonatal outcome is compared to pregnancies using autologous oocytes matched for age and parity. Based on the results of this study, pregnancies after oocyte donation are at a significantly increased risk for preeclampsia and pregnancy-induced hypertension.

OTHER: ART - CLINICAL III
p=0.004). Live born children per thawed embryo increased among all age groups (<35; 14.0% vs 35.0%; 35-37; 11.4% vs 34.1%; 38-40; 9.7% vs 17.4%; 41-42; 7.5% vs 15.5%; 42-43; 6.6% vs 13.3%, p<0.0001 for all). The net result was significant increase in oocyte to baby efficiency in all age groups under 43 years (Table, p<0.0001 for all).

CONCLUSION: Our results demonstrate that the vast majority of oocytes appear to be unable to produce a live birth. Progressive changes in embryological methods are associated with increased efficacy and efficiency of IVF; increased oocyte utilization and higher birth rates per transferred embryo. These findings are potentially confounded by other changes during the study period, however this analysis provides a novel and valuable perspective on factors leading to improved IVF clinical outcomes.

O-326 Wednesday, October 22, 2014 04:15 PM

AN ARTIFICIAL NEURAL NETWORK (ANN) AS A CLINICAL DECISION MAKING TOOL DURING OVARIAN STIMULATION AND IN VITRO FERTILIZATION (IVF). G. S. Letterie J. Borseth. Seattle Reproductive Medicine, Seattle, WA.

OBJECTIVE: To design an ANN to predict gonadotropin dose, hCG trigger and possible cancellation during ovarian stimulation for IVF and to compare accuracy of the ANN to clinical team decision making.

DESIGN: Descriptive and comparative.

MATERIALS AND METHODS: Data from 25 IVF cycles including 5 cancellations were collected and analyzed. Multilayered feed-forward networks were trained using a back propagation algorithm. The networks consisted of 15 inputs, 30 hidden layers and 4 outputs. Input data and training sets included estradiol concentrations (pg/ml) and follicle diameters (measured in mm in two dimensions). The neural output layers included daily gonadotropin dose (same or decreased dose), hCG trigger or cycle cancellation. The ANN was challenged with these four decisions on four cycle days (5, 7, 8 and 10) for 25 patients. After training, ANN output data was compared to decisions by the clinical team. Performance accuracy was defined as the percent agreement of ANN predictions and decisions by the clinical team regarding management. Statistical analysis was performed using Cohen’s kappa as a measure of agreement between ANN and clinical team decisions. Sensitivity and positive predictive values (PPV) were calculated within 95% confidence intervals.

RESULTS: Cohen’s kappa was 0.7632 with 0 as complete disagreement and 1.0 as complete agreement. Accuracy of the ANN was 85.1064%. Table 1 reports the sensitivity, PPV and 95% confidence intervals for four decisions of dose (same or decrease), trigger or cancellation.

CONCLUSION: We designed a very basic ANN and trained with a limited case series for decisions during IVF. Our results suggest that an ANN can predict medication dose, day of hCG trigger and/or decision to cancel with reliability and confidence. Cancellation decisions were less reliable owing in part to a low number of cases for training. These ANN demonstrated a high degree of agreement with clinical team decisions suggesting that an ANN may be a feasible computer based tool to improve the efficiency of day-to-day clinical decision making during ovarian stimulation and IVF and opens the possibility of additional computer based tools for management in IVF.

O-327 Wednesday, October 22, 2014 04:30 PM

A RANDOMIZED DOUBLE BLIND COMPARISON OF ATOSIBAN IN UNSELECTED PATIENTS UNDERGOING IN VITRO FERTILIZATION TREATMENT. E. H. Y. Ng,1 R. H. W. Li,1 L. Chen,2 V. T. N. Lan,3 H. M. Tuong,3 S. Quan,4 Obstetrics & Gynaecology, The University of Hong Kong, Hong Kong, Hong Kong; Center for Reproductive Medicine, Guangdong No.2 Provincial People’s Hospital, Guangzhou, Guangdong, China; Obstetrics & Gynaecology, University of Medicine and Pharmacy of Ho Chi Minh City, Ho Chi Minh, Viet Nam; Obstetrics and Gynecology, Southern Medical University, Guangzhou, Guangdong, China.

OBJECTIVE: To compare the live birth rates of in vitro fertilization (IVF) treatment between women receiving atosiban and placebo prior to embryo transfer (ET).

DESIGN: A randomized double blind multi-centre study.

MATERIALS AND METHODS: Consecutive subfertile women undergoing IVF treatment were recruited if they were aged <43 years and had normal uterine cavity shown on ultrasound scanning. On ET day, patients were randomized into two groups: the atosiban and placebo groups. Patients in the atosiban group received intravenous atosiban 30 min before the transfer with a bolus dose of 6.75 mg and the infusion was continued with an infusion rate of 18 mg/h. After performing ET, the dose of atosiban was reduced to 6 mg/h and the infusion was continued for 2 hours (total administered dose: 37.5 mg). Those in the placebo group received normal saline only. In both groups, patients were medicated by intravenous bags which looked identical. The primary outcome measure was the live birth rate and the secondary outcome measures included biochemical pregnancy, ongoing and miscarriage rates. Assuming 10% increase from 35% in the live birth rate after the use of atosiban, 396 patients in each arm would be required at a power of 80% and a significance level of 5%. A total of 800 patients were recruited in this study.

RESULTS: No significant differences were found in demographic data between the atosiban and placebo groups. Both groups had comparable duration of ovarian stimulation, dose of gonadotrophin used, peak serum estradiol level, endometrial thickness, number of oocytes obtained, number of oocytes fertilized and number of transferable embryos. The live birth rate in the atosiban group (39.9%, 159/398) was similar to that of the placebo group (38.0%, 152/400). Biochemical pregnancy, ongoing pregnancy and miscarriage rates were also similar for both groups. No serious adverse events were reported. Findings remained similar when subgroup analysis was performed for the first cycle vs repeated cycle, fibroids vs no fibroids and those with oocytes <15 vs >15.

CONCLUSION: Use of atosiban prior to ET is not associated with any increase in the live birth rate in unselected women undergoing IVF treatment. Supported by: Centres in Hong Kong and Vietnam received research funding from Ferring.
SUCCESSFUL OUTCOMES OF FRESH AND FROZEN DONOR OVUM CYCLES AMONG RECIPIENTS USING ORAL ESTRADIOL AND VAGINAL PROGESTERONE GELS VS INTRAMUSCULAR AND VAGINAL PROGESTERONE. R. Boostanfar a, J. Frederick b, P. Khanna c, D. H. Barad c.

Center for Human Reproduction, New York, NY; a J. Frederick.

OBJECTIVE: There is a paucity of data evaluating the efficacy of vaginal progestrone replacement in fresh and frozen IVF transfers of recipients undergoing oocyte donation. The purpose of this investigation is to detect any differences in pregnancy rates between vaginal progestrone as compared to Intramuscular (IM) and vaginal progestrone among fresh and frozen donor oocyte cycles among 2 separate practitioners in a large regional ART center.

DESIGN: This is a single center, IRB-approved, retrospective analysis from 1/2009 through 6/2013 of 255 cycles from subjects less than 55 years of age who were oocyte recipients in a anonymous donor program. Recipients in this data base included gestational surrogates and cycles with preimplantation genetic screening.

MATERIALS AND METHODS: Oocyte recipients from fresh and frozen cycles received oral estradiol 2 mg 2–3 times daily in a step-up protocol followed by vaginal progesterone gel (Crinone 8%) 90 mg twice daily for 5 days for blastocyst transfer. The comparative groups of fresh and frozen donor oocyte cycles took oral estradiol in a similar fashion followed by progesterone IM 50 mg once a day for 5 days prior to transfer and subsequently a Progesterone 200 mg vaginal capsule in addition beginning the day of transfer continuing until the tenth week of pregnancy. Subjects were monitored via transvaginal ultrasound, serum estradiol and progesterone levels both on baseline and the week prior to transfer. Serum beta-hCG, estradiol and progesterone were obtained 10 days after blastocyst transfer.

RESULTS: Table 1 demonstrates that the women who prepared the endometrium with vaginal or IM progesterone during fresh and frozen cycles did not significantly differ with regard to number of positive pregnancy tests. \( \chi^2 = 4.41, p = .220 \). Similar outcomes were observed with respect to clinical pregnancy rates, \( \chi^2 = 4.68, p = .196 \).

<table>
<thead>
<tr>
<th>Positive Pregnancy Rate and Clinical Pregnancy Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pregnancy Rate</strong></td>
</tr>
<tr>
<td>Fresh cycles with vaginal progesterone only</td>
</tr>
<tr>
<td>Frozen cycles with vaginal progesterone only</td>
</tr>
<tr>
<td>Fresh cycles with vaginal and IM progesterone</td>
</tr>
<tr>
<td>Frozen cycles with vaginal and IM progesterone</td>
</tr>
</tbody>
</table>

CONCLUSION: Preparing the endometrium with oral estradiol and vaginal progesterone gel among recipients treated in a contemporary donor oocyte program is highly effective and not significantly different from utilizing oral estradiol with IM and vaginal progesterone in fresh and frozen embryo transfers.

Supported by: Research grant provided by Actavis Pharmaceuticals.

O-329 Wednesday, October 22, 2014 05:00 PM


OBJECTIVE: The aim of our study is to compare the effects of two commercially available sequential culture media systems on embryo morphokinetics.

DESIGN: Clinical retrospective study.

MATERIALS AND METHODS: The morphokinetic parameters of a total of 777 transferred cleavage-stage embryos with a known implantation data were retrospectively analyzed in this study. Inclusion criteria were woman age below 40 years old, absence of azospermia and history of any genetic disorders and having less than 3 previous IVF attempts. 413 embryos were cultured in vitrolife G5 series and 364 embryos were cultured in Medicult medium. Morphokinetic variables that were analyzed were the specific cleavage timings such as t2, t3, t4, t5, t6 and t8 (t represents time and the numbers represent the cell number at that specific time point). Statistical analysis was performed by using Student t test.

RESULTS: Patient demographics, all cleavage timings and clinical outcomes of the embryos are given in Table 1. There were no statistically significant differences between both groups in terms of patient demographics, morphokinetic parameters and clinical outcomes (p=0.05).

<table>
<thead>
<tr>
<th>TABLE 1. Comparison of patient demographics, morphokinetic parameters and clinical outcomes of both culture media (data are presented as mean±SD).</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient demographics</strong></td>
</tr>
<tr>
<td><strong>(n=413)</strong></td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>BMI</td>
</tr>
<tr>
<td>Morphokinetic parameters</td>
</tr>
<tr>
<td>t2</td>
</tr>
<tr>
<td>t3</td>
</tr>
<tr>
<td>t4</td>
</tr>
<tr>
<td>t5</td>
</tr>
<tr>
<td>t6</td>
</tr>
<tr>
<td>t7</td>
</tr>
<tr>
<td>t8</td>
</tr>
<tr>
<td>Clinical outcomes</td>
</tr>
<tr>
<td>Positive hCG (%)</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
</tr>
</tbody>
</table>

p<0.05 is considered as statistically significant.

CONCLUSION: Today, many different culture media systems for in vitro fertilization (IVF) are commercially available in the global market. Selection of the culture media that can properly support embryo development and give good clinical outcomes in an IVF laboratory routine is very crucial. Our results demonstrate that both culture systems show a similar effect on embryo morphokinetics and comparable clinical outcomes.

O-330 Wednesday, October 22, 2014 05:15 PM

ASSESSMENT OF PRACTICE IN IVF CLINICS IN WHICH SUCCESS IN AUTOLOGOUS AND DONOR OOCYTE CYCLES DO NOT CORRELATE. V. A. Kushnir a, P. Khanna b, D. H. Barad a, c, N. Gleicher a, b, c, d, e, f, g, h, J. Center for Human Reproduction, New York, NY; a Icahn School of Medicine at Mount Sinai Program, Jamaica, NY; b, f, g, h, i, Foundation for Reproductive Medicine, New York, NY.

OBJECTIVE: We recently demonstrated a significant correlation between national clinic specific live birth rates (LBR) in oocyte donor and autologous oocyte IVF cycles. Here we examine what characterizes outlier clinics where these two outcomes do not correlate.

DESIGN: Retrospective analysis of national U.S. IVF reporting statistics.

MATERIALS AND METHODS: Reviewing 2011 National ART Surveillance System (NASS) data for 451 clinics, only 137 qualified for this study by reporting at least 20 autologous fresh embryo transfers per age group and at least 20 fresh embryo transfers from oocyte donations. This analysis focused on a subgroup of clinics whose autologous LBR for women up to age 40 years did not correlate to LBR in donor oocyte cycles. We further differentiated between those with high donor oocyte LBR yet low autologous IVF LBR (HL clinics) and those with low donor oocyte LBR and high IVF LBR (LH clinics). In the HL clinics we evaluated whether they treated a more adversely selected patient populations based on prevalence of diminished ovarian reserve (DOR). In LH clinics we assessed whether they treated particularly adversely selected recipient/donor populations or if they disproportionately excluded poor prognosis patients from reaching embryo transfer in autologous IVF cycles, thus creating inflated IVF LBRs.

RESULTS: HL outlier clinics revealed only nominally increased prevalence of DOR patients (median= 31%, mode = 44%) in comparison to...
non-outlier clinics (median=29%, mode=52%) (P=0.08). Analysis of cycle exclusion in LH outlier clinics revealed 1.330 excluded cycles out of 25,246 initiated cycles (5.3%) well below the 9.2% average rate among all reporting clinics in 2011.

CONCLUSION: To some degree, LH outlier clinics, indeed, appear to treat a more adversely select patient population with increased prevalence of DOR. LH outlier clinics do not excessively exclude cycles from outcome reporting. They, therefore, either treat more adversely selected recipients (not a likely proposition) or, more likely, select donors poorly. This observation suggests that LH outlier centers may utilize here reported criteria for selection of LH and HL clinics for internal quality control to differentiate between these two possibilities.

Supported by: The Center for Human Reproduction.

O-331 Wednesday, October 22, 2014 04:30 PM
AN EVALUATION OF FACTORS THAT PREDICT LIVE BIRTH AFTER IN VITRO FERTILIZATION (IVF) IN WOMEN AT LEAST 40 YEARS OF AGE.

OBJECTIVE: Parameters which predict live birth in women ≥40 years of age, with IVF have not been evaluated previously in the literature. Therefore, stepwise regression analysis was used to determine predictors of live birth in this age group.

DESIGN: Retrospective analysis.

MATERIALS AND METHODS: We studied the data of 631 women aged 40 to 46 years, who underwent a total of 901 cycles of IVF, with or without intra cytoplasmic sperm injection treatment, who reached the stage of embryo transfer from August 2010 till June 2012. Patients had 1-3 embryos transferred based on quality, provincial and reproductive center guidelines. Guidelines have limited female age at treatment to 43 and 46 birthday at different periods.

The patients were classified based on achieving a live birth. The following data were evaluated: patient & partners’ ages, IVF attempt number, number of follicles less, or at least 14 mm on the day of trigger, number of oocyte(s) collected, viable oocytes, metaphase 2 (MII) oocyte(s), 2 pronuclei (2PN) embryos, number of embryos transferred, number of cells in each embryo(s) transferred. Statistical analysis was done with stepwise logistic regression analysis which controls for confounding effects and multiplicity and was based on cycle outcome. Data is presented as Mean ±SD. IRB #13-053-SDR.

RESULTS: The overall all live birth rate was 7.4% per transfer. Data is presented comparing those with and without a live birth respectively. Statistically significant predictors of live birth were the maternal age (41.±1.0 vs. 42.±1.3 years, p=0.007), paternal age (41.±8 vs. 43.±7 years, p=0.028), number of 2PN embryos (5.7±3.6 vs. 3.4±2.9, p=0.038) and number of cleavage stage embryos (5.7±3.7 vs. 3.4±2.9, p=0.032). Other analyzed factors did not differ significantly when controlling for confounding effects and multiplicity. Male age range was 25 to 73 years with 95% falling bellow 53 years and the median being 41 years.

CONCLUSION: Live birth as expected was related to maternal age and the number of embryos. Unexpectedly, male age was found to be a significant individual predictor of the likelihood of a live birth, at a relatively young mean age. This is some of the first data to support the role of male age in prediction of IVF success. We hypothesize that this is due to the impaired ability of eggs from women > 40 years-old to repair damaged sperm DNA. Studies will be needed to confirm this hypothesis.

OVARIAN RESERVE

O-332 Wednesday, October 22, 2014 03:45 PM
ASSOCIATION BETWEEN ANTI-MULLERIAN HORMONE (AMH) AND TIME-TO-PREGNANCY (TTP) AMONG WOMEN IN THE EFFECTS OF ASPIRIN ON GESTATION AND REPRODUCTION (EAGER) TRIAL.
S. L. Munford, S. M. Zarek, E. M. Mitchell, I. D. Stanley, J. Wactawski-Wende, E. F. Schisterman, NIH, Rockville, MD; University of Utah, Salt Lake City, UT; University at Buffalo, Buffalo, NY.

OBJECTIVE: Although recent studies suggest that lower AMH is associated with reduced fecundity among women seeking fertility treatment, less is known regarding associations with TTP among women with a history of 1-2 prior pregnancy losses and no history of infertility. The objective was to assess a possible association between preconception AMH levels with TTP

DESIGN: Secondary analysis of a multicenter, blocker-randomized, double-blind, placebo-controlled clinical trial.

MATERIALS AND METHODS: 1228 women attempting pregnancy, aged 18-40 years, with one to two prior pregnancy losses and no history of infertility, or other gynecologic disorders were included. Women were random-ized to preconception-initiated daily low-dose aspirin or placebo and fol-lowed for up to 6 cycles while trying to conceive. Preconception AMH levels were measured at baseline using the Gen II ELISA assay (Beckman-Coulter).

AMH was categorized as low (<1.25 ng/mL), normal (1.25 to 4.0 ng/mL), and high (>4.0 ng/mL), based on clinically relevant cut points. Cox proportional hazard regression models were used to compare the time from randomization to a positive urine pregnancy test and corresponding fecundability odds ratio (FOR) between categories of preconception AMH levels adjusting for age.

RESULTS: A total of 776 women had positive urine hCG test over the six menstrual cycles of follow-up. Women were predominately of white race (95.6%) with a mean age of 28.9 years (standard deviation (SD) 4.7) and body mass index (BMI) of 26.1 (SD 6.5), and mean AMH levels were 3.6 (SD 2.6) among women with a confirmed pregnancy compared to 3.4 (SD 2.6) among women without a confirmed pregnancy (p=0.2). Among women with a single recent pregnancy loss, high levels of AMH (>4.0 ng/mL) were associated with a shorter TTP compared to women with low AMH (<1.25 ng/mL) (FOR 1.38, 95% confidence interval 1.10, 1.73).

CONCLUSION: High AMH levels were associated with a shorter TTP among women with a single recent pregnancy loss. These results suggest that AMH is associated with fecundity in women with normal and subfertili-ty.

Supported by: Intramural Research Program, DPHR, PRAE.

CIRCULATING NUCLEIC ACIDS IN SERUM FROM INFERTILE PATIENT AS INNOVATIVE NON-INVASIVE DIAGNOSTIC BIOMARKERS IN IVF/ICSI.

Objective: Parameters which predict live birth in women ≥40 years of age, with IVF have not been evaluated previously in the literature. Therefore, stepwise regression analysis was used to determine predictors of live birth in this age group.

Conclusion: The overall all live birth rate was 7.4% per transfer. Data is presented comparing those with and without a live birth respectively. Statistically significant predictors of live birth were the maternal age (41.±1.0 vs. 42.±1.3 years, p=0.007), paternal age (41.±8 vs. 43.±7 years, p=0.028), number of 2PN embryos (5.7±3.6 vs. 3.4±2.9, p=0.038) and number of cleavage stage embryos (5.7±3.7 vs. 3.4±2.9, p=0.032). Other analyzed factors did not differ significantly when controlling for confounding effects and multiplicity. Male age range was 25 to 73 years with 95% falling bellow 53 years and the median being 41 years.

Conclusion: Live birth as expected was related to maternal age and the number of embryos. Unexpectedly, male age was found to be a significant individual predictor of the likelihood of a live birth, at a relatively young mean age. This is some of the first data to support the role of male age in prediction of IVF success. We hypothesize that this is due to the impaired ability of eggs from women > 40 years-old to repair damaged sperm DNA. Studies will be needed to confirm this hypothesis.

Supported by: The University-Hospital of Montpellier and by a grant from the Ferring Pharmaceutical Company. The authors of the study have no competing interests to report.
**O-334 Wednesday, October 22, 2014 04:15 PM**

**BRCA MUTATION CARRIERS DO NOT SHOW REDUCED OVARIAN RESERVE AS DEMONSTRATED BY IVF TREATMENTS OUTCOME.** D. Meirou,a T. Eldar-Geva,a D. Manela,a M. Brenghausen,a M. Shapiro,a H. Raanani. aFertility Preservation, Sheba Medical Center, Tel Hashomer, Israel; b1IVF Unit, Shaare Zedek Medical Center, Jerusalem, Israel.

**OBJECTIVE:** BRCA1/2 gene mutation which is prevalent in the Ashkenazi Jewish population is associated with an increased risk for developing breast cancer and ovarian cancer. Few recent studies, have established a commonly accepted belief suggesting that BRCA mutations are associated with low ovarian reserve and poor response to ovarian stimulation, as well as early menopause. We aimed to evaluate a possible association between carriage of BRCA1/2 mutations and low ovarian reserve, as demonstrated by ART performance of BRCA mutation carriers.

**DESIGN:** Multicenter retrospective study including BRCA mutation carriers who underwent COH for IVF between 2000 and 2012. Healthy and cancer patients were compared with controls.

**MATERIALS AND METHODS:** The study group was divided into two sub-groups: (1) BRCA positive breast cancer patients, undergoing fertility preservation prior to chemotherapy, were compared with breast cancer patients whose BRCA mutation status was negative or unknown. (2) Healthy BRCA carriers, undergoing fertility treatment or IVF for PGD, were compared with male factor infertility patients and non-BRCA PGD patients. Patient’s age, cancer status, BRCA mutation status, stimulation data and ART outcomes were extracted from patients’ medical records. When more than one IVF cycle was recorded per patient, only the most favorable cycle (maximal oocytes yield) was included in analysis. Low response to COH was defined as retrieval of four or fewer oocytes per cycle.

**RESULTS:** Fifty seven BRCA mutation carriers underwent 124 IVF cycles and 130 controls underwent 233 IVF cycles. A total of 187 best performed cycles were compared. Patients’ age, duration of stimulation and peak E2 levels were comparable between carriers and controls. Number of oocytes retrieved (15.00±8.06 vs. 14±8.24, p=0.44), number of 2PN embryos (9.6±5.91 vs. 8.17±5.55, p=0.077) and low response rate (8.77% versus 8.46%, p=1) were also not significantly different. An age-stratified (<28, 28-37, >37 years) analysis indicated similar oocytes yield in each carriers subgroups and their corresponding age matched control subgroup.

**CONCLUSION:** BRCA mutation carriers constitute a unique population of women who frequently utilize ART treatments, whether for fertility preservation prior to chemotherapy or for PGD. Contrary to previous publications described, our results provide reassuring data and confirm normal IVF performance among BRCA mutation carriers.

**O-335 Wednesday, October 22, 2014 04:30 PM**

**THE TP73 GENE POLYMORPHISM (RS4648551- A/A GENOTYPE) IS RELATED WITH REDUCTION IN THE NUMBER OF ANTRAL FOLLICLES.** L. D. Vagnini,a,b C. G. Petersen,a,b A. Renzi,a,b G. C. Oliveira-Pelegrin,a,b A. L. Mauri,a,b F. C. Massaro,a,b M. Cavagna,a,b J. B. A. Oliveira,a,b R. L. R. Baruffi,a,b J. G. Franco Jr,a,b aPaulista Center for Diagnosis Research and Training, Ribeirao Preto, Sao Paulo, Brazil; bCenter for Human Reproduction Prof. Franco Jr, Ribeirao Preto, Sao Paulo, Brazil; cWomen’s Health Reference Centre, Perola Byington Hospital, Sao Paulo, Brazil.

**OBJECTIVE:** It’s known that members of the TP73 family are involved in the regulation of female reproduction. Studies in mice showed that the TP73 gene (member of this family) plays a role in the size of follicle pool, ovulation rate and maintenance of genomic stability. Therefore, we intended to analyze the association between a polymorphism on the TP73 gene and some characteristics associated with ovarian reserve (age, antral follicles count-AFC, anti-mullerian hormone-AMH levels and the ovarian response prediction index (ORPI) since there is no study in humans.

**DESIGN:** Prospective cohort-study.

**MATERIALS AND METHODS:** A total of 504 infertile female patients were included in the study. The TP73 polymorphism (rs4648551) was genotyped using DNA extracted from peripheral blood and TaqMan SNP genotyping assay. The results were correlated with age, AFC, AMH, ORPI and ORPI (ORPI=(AMH x AFC)/Patient age) using t test.

**RESULTS:** The results showed an association between the number of antral follicles and ORPI with the TP73 polymorphism. Women presenting the AA genotype had fewer follicles (P=0.03) and a lower ORPI (P=0.03) than the group presenting AG + GG genotype. No other correlation was observed. Table 1 summarizes the results.

<table>
<thead>
<tr>
<th>TP73 - WOMEN’S GENOTYPES</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AA</strong></td>
</tr>
<tr>
<td>n (patients)</td>
</tr>
<tr>
<td>Age (years)(Mean±SD)</td>
</tr>
<tr>
<td>AMH (ng/mL)(Mean±SD)</td>
</tr>
<tr>
<td>AFC (n)(Mean±SD)</td>
</tr>
<tr>
<td>ORPI(Mean±SD)</td>
</tr>
</tbody>
</table>

**CONCLUSION:** Our results showed an association between the TP73 gene polymorphism and the antral follicular count and ORPI. The AA genotype was correlated with fewer antral follicles and a lower ovarian response prediction index. To the best of our knowledge, the present study is the first to analyze TP73 gene polymorphism in humans for the assessment of the number of ovarian follicles and, thereafter, the ovarian reserve. Because this was a preliminary study, additional validation is needed to provide more information regarding the potential use of this polymorphism as a follicular count marker.

**O-336 Wednesday, October 22, 2014 04:45 PM**

**NORMAL-RANGE FMR1 REPEAT GENOTYPES AND CORRELATION WITH SERUM ANTI-MÜLLERIAN HORMONE (AMH), CYCLE DAY 3 FSH (D3FSH), ANTRAL FOLLICLE COUNT (AFC) AND INCIDENCE OF DIMINISHED OVARIAN RESERVE (DOR).** S. Davis, B.-S. L. Maskow, J. Nilsen, L. Engmann, C. A. Benadiva. University of Connecticut Health Center/Center for Advanced Reproductive Services, Farmington, CT.

**OBJECTIVE:** FMR1 mutation and premutation carriers are known to be at risk for premature-ovarian failure. Conflicting studies have investigated correlations between particular normal FMR1 genotypes and AMH levels or incidence of DOR. This large study assessed potential associations across all FMR1 genotypes with respect to AMH, D3FSH, AFC and incidence of DOR.

**DESIGN:** Retrospective cohort study.

**MATERIALS AND METHODS:** We reviewed all FMR1 screenings performed at a large fertility center from 1/2011-3/2014 and collected baseline serum AMH, D3FSH, and AFC for all normal results (<55 repeats). Carriers of the full (>200) or premutation (55-200) were excluded. DOR was defined as AMH:<10ng/mL or FSH:>10mIU/mL. We classified normal alleles by previously published ranges (low <26, normal 36-34, high >35) and categorized results into 6 distinct genotypes (low/low, low/normal, low/high, normal/high, high/high, normal/normal). We compared serum AMH, D3FSH, AFC and incidence of DOR (by AMH and FSH) by genotype. Associations were tested overall and stratified by age group (A<35, B 35-37, C 38-40, D 40-41, E=42). Analyses were performed using χ2 and Fisher’s exact test for categorical data and Mann-Whitney U, Kruskall-Wallace for continuous variables. FMR1 testing was performed by Coungs®.

**RESULTS:** 602 women met inclusion criteria. Mean age was 33.7±4.7 years [A=372(61.7%), B=111(18.4%), C=76(12.6%), D=17(2.8%), E=26(4.3%)]. Mean AMH was 3.4±4.4ng/mL, D3FSH 7.6±6.1mIU/mL, and AFC 17±13.3. 53.1% (320/602) of the women were normal/normal genotype, 26.2% (158/602) low/normal, 13.1% (79/602) normal/high, 3.9% (24/602) low/low, 2.8% (17/602) low/high, and 0.7% (4/602) high/high. Overall incidence of DOR was 26.7% (161/602) by AMH and 12.2% (68/558) by FSH. Incidence of DOR stratified by age group was A 18.5% and 7.7%, B 30.6% and 15.2%, C 55.2% and 26.0%, D 52.9% and 21.4%, and D 69.2% and 22.2%, by AMH and FSH respectively. Analysis of mean AMH, D3FSH and incidence of DOR with respect to each of the six genotypes yielded p-values<0.05, both overall and when stratified by age.

**CONCLUSION:** In this large study we failed to find a correlation between any of the FMR1 genotypes and serum AMH, D3FSH or incidence of DOR, even when stratified by age group. These findings agree with prior study lack of correlation between FMR1 genotype and gonadotropin response in oocyte
donors and disagree with smaller studies suggesting FMR1 genotypes may help identify women at high-risk for DOR.

O-337 Wednesday, October 22, 2014 05:00 PM

IMPACT OF METHOTREXATE ON OVARIAN RESERVE. C. E. Boots,* S. Desai,* M. Hill,* E. C. Feinberg,* S. A. Fowler,* E. S. Jungeheim,* OBGYN, Division of Reproductive Endocrinology & Infertility, Washington University, St. Louis, MO; Program in Reproductive and Adult Endocrinology, Eunice Kennedy Shriver NICHD & NIH, Bethesda, MD; Fertility Centers of Illinois, Highland Park, IL.

OBJECTIVE: Methotrexate (MTX) is used commonly to treat ectopic pregnancies resulting from fertility treatment. Previous studies have explored the impact of MTX on ovarian reserve, but these studies have been small and underpowered. Thus, our objective was to estimate the impact of MTX on ovarian function in women treated for ectopic pregnancy during fertility treatment.

DESIGN: Systematic review & Meta-analysis.

MATERIALS AND METHODS: A systematic search was performed in Medline, Embase, Scopus, and CENTRAL for studies comparing markers of ovarian reserve and measures of ovarian responsiveness during IVF before and after receiving methotrexate for an ectopic pregnancy. Authors from the primary studies were contacted for complete data sets, and three authors compiled. With IRB approval, primary data from our center was also included. Meta-analysis of the data was performed using a DerSimonian-Laird random effects model. Results are reported as weighted mean difference and 95% confidence interval.

RESULTS: Seven studies and our center’s primary data included in the meta-analysis, totaling 289 women. There were no differences in FSH, antral follicle count, endometrial thickness, total gonadotropin dose, or fertilization rate between pre- versus post-MTX cycles. There was also no difference in the numbers of oocytes retrieved (WMD: 0.92, 95% CI: -2.1-0.21). A post hoc power analysis revealed that a sample of this size would be able to detect an 8.3% difference in oocytes retrieved (1 oocyte) with 80% power. Post-MTX cycles required significantly more days of stimulation than pre-MTX cycles (WMD: 0.29, 95% CI: 0.06-0.5). Women were also significantly older in post-MTX cycles than in pre-MTX cycles (WMD: 0.9, 95% CI: 0.18-1.7).

CONCLUSION: Few differences were noted in markers of ovarian reserve or responsiveness in pre- versus post-MTX cycles, with the exception of a longer duration of stimulation in the post-MTX cycle. Women were also significantly older in the post-MTX cycles. Whether this significant increase in age is due to time patients are counseled to wait after MTX before proceeding with another cycle or it is influenced by other factors such as cost is unknown. Our findings support the continued use of MTX in the management of ectopic pregnancy without concern for a reduction in ovarian reserve.

Supported by: NIH K12 HD063086 (ESJ).

O-338 Wednesday, October 22, 2014 05:15 PM

A 9-YEAR ANALYSIS OF TRENDS IN OVARIAN RESPONSE TO STIMULATION IN ELECTIVE OOCYTE CRYOPRESERVATION AND IN VITRO FERTILIZATION PATIENTS. L. Schuman, a K. Bergin, a G. Witkin, a,b J. A. Lee, a A. B. Copperman, a,b *Reproductive Medicine Associates of New York, New York, NY; 1Department of OB/GYN and Reproductive Science, Mount Sinai School of Medicine, New York, NY.

OBJECTIVE: Advances in elective oocyte cryopreservation (EOC) have given women the opportunity to preserve their fertility and assist in their future goal of family building. The Society for Reproductive Medicine (SART) data provides infertility patients with realistic expectations for IVF success based on age and number of oocytes retrieved, scant data is available to guide EOC patients. Our study was designed to compare infertility patients to EOC patients over time with regard to ovarian responsiveness to controlled hyperstimulation.

DESIGN: Retrospective analysis.

MATERIALS AND METHODS: EOC and IVF patients from 5/7/2005-3/26/2014 were included. Oocytes retrieved at the time of VOR were evaluated in EOC and IVF patients. Data was segregated by patient ages following the distribution set forth by ASRM guidelines (>35, 35-37, 38-40, >40).

RESULTS: Oocyte count at VOR for EOC (n=722) and IVF (n=12,065) patients were analyzed. No significant difference in the number of oocytes retrieved between groups was observed.

CONCLUSION: Our study answers several key questions regarding trends in EOC patients and IVF patients over nearly a decade. While it had originally been hypothesized that women presenting for EOC often had a “premonition” or “insight” into their need for fertility preservation and were actually patients with diminished ovarian reserve, the data suggest the contrary. EOC patients respond similarly to IVF patients and do not demonstrate a higher incidence of ovarian dysfunction. In addition, the increased awareness and popularity of EOC over the past several years has not resulted in changes in this population’s response to gonadotropins. Within our patient population, IVF patients VOR demographics can be used in setting realistic expectations for EOC patients.

O-339 Wednesday, October 22, 2014 05:30 PM

ABSTRACT WITHDRAWN

OOCYTE BIOLOGY

O-340 Wednesday, October 22, 2014 03:45 PM

MICRONAS REGULATE EXPRESSION OF AGED HUMAN CUMULUS CELLS GENES THAT ARE ESSENTIAL FOR OOCYTE QUALITY. T. Al-Edani, a S. Assou, a F. Ferrière, b C. Brunet, b O. Aït-Ahmed, a S. Hamamah. a Université Montpellier 1, Inserm U1040, IRMB, Montpellier, Hérault, France; b ART-PGD Department, CHRU Montpellier, Montpellier, Hérault, France; c ART-PGD Department, Université Montpellier 1, Inserm U1040, IRMB, Montpellier, Hérault, France.

OBJECTIVE: To evaluate the impact of female aging on the gene expression profile of human cumulus cells (CCs) and to characterize the biological relationships between microRNAs (miRNAs) and impacted CC-genes by aging.

DESIGN: This study includes 47 CCs isolated from mature MII oocytes collected from patients aged <30 years, 31-36 years, and 37-43 years (n=33). All groups of CCs were obtained from patients who underwent COS for male infertility for ICSI.

MATERIALS AND METHODS: CCs from each MII oocyte were analyzed individually using whole genome U133 Plus 2.0 GeneChip Affymetrix microarrays. Significance analysis of microarray was used to analyze the data according to age of patients. Using deep-sequencing technology, we dissected the microRNome of pooled CCs (n=20). The correlation between miRNAs and their corresponding mRNA targets was analyzed using in silico prediction algorithms. Validation was performed by qPCR.

RESULTS: 2,405 genes were differentially expressed among the three groups according to age. In CCs collected from patients >37 years, angiogenic genes including PGF2 (x3.2, FDR=0) were significantly over-expressed. Whereas, genes related to insulin signaling pathway were overexpressed in CCs of patients (31-36 years), like IGFBP3 (x2.0, FDR=0.004). Furthermore, some of the genes whose down regulation in CCs was previously shown to be associated with oocyte aneuploidy such as (TP53 and PSIPB) were down-regulated in older CCs. A bioinformatics analysis was performed to identify the miRNAs that are putative regulators of the differentially expressed genes of the study. It revealed that the pathways impacted by age were potential targets of specific miRNAs identified.
in our CCs small RNAs sequencing. MIR202 is a potential regulator of the hyaluronan synthase-encoding gene HAS2 that is related to aging and angiogenesis. EGFBP3 was target of MIR210, whereas FGF2 was targeted by MIR424.

CONCLUSION: The present study reports for the first time an extensive analysis of gene expression in CCs in relation to female age. Our findings point to aging as a major player in processes and pathways that are of key biological importance for oocyte growth and genome integrity. The characterization of the miRNA regulators of the genes impacted by female age represents a valuable resource for future investigations on the biology of aging and aneuploid oocyte.

Supported by: Ferring and Genevier companies.

O-341 Wednesday, October 22, 2014 04:00 PM

SPECIAL RESEARCH PRESENTATION: BMPR SIGNALING IN THE FEMALE OOCYTE IMPROVES FERTILIZATION AND INFLUENCES EARLY EMBRYO DEVELOPMENT. A. M. Zamah, a A. M. Laeno, b H. Cakmak, b L. Xiong, b Department of Obstetrics and Gynecology, University of Illinois at Chicago, Chicago, IL; b Obstetrics, Gynecology, and Reproductive Sciences, University of California, San Francisco, San Francisco, CA.

OBJECTIVE: Much data supports the concept that there is little to no detectable new transcription during the final stages of oocyte maturation and the initial stages of early embryo development. Therefore most of the critical events at the time of fertilization and very early embryo development rely on an orchestrated program of translation of already existing mRNAs. BMPR (Bone morphogenetic protein receptor) mRNA is increased in poly-some binding during oocyte maturation. Thus, BMP signaling may be important during oocyte maturation and early embryo development, since these events are dependent on the oocyte translational program of pre-formed maternal mRNAs. We sought to assess whether activation or blockade of BMPR would affect in vitro fertilization outcomes.

DESIGN: An in vitro study using murine oocytes.

MATERIALS AND METHODS: Murine oocyte microarray data were previously generated. QPCR was used to validate all microarray data and to assess embryonic genome activation (EGA). BMPR signaling pathways were assessed by Western Blot to phospho-SMAD proteins and immunofluorescence. Murine IVF was performed under standard conditions.

RESULTS: BMPR is significantly increased in translation during oocyte maturation. There is increased signaling to phospho-SMAD when BMPR is activated by exogenous BMP-2 in GV versus MII oocytes (2.1 fold versus 6 fold p <0.05). IVF performed under 4 different conditions [control; BMP-2; BML-275 (a specific competitive antagonist of BMP signaling); and BMP-2 + BML-275] showed improved fertilization rates with BMP-2 (59%; 76%; 57% ±5%, respectively p <0.001) as well as a trend to improved blastulation rates. EGA as assessed by QPCR performed on 2 cell embryos revealed significant differences in early EGA transcripts among the groups.

CONCLUSION: BMPR signaling in the oocyte during the peri-fertilization period improves fertilization rates and affects early embryo development as assessed by embryonic genome activation, but has no significant effect on blastulation. Assessment of oocyte receptors which are increased in translation during oocyte maturation can provide a valuable understanding of signaling pathways important for oocyte competence and may provide a means to improve IVF outcomes.

Supported by: ASRM Research Grant and NIH K12 HD001262 to AMZ.

O-342 Wednesday, October 22, 2014 04:15 PM

INDIVIDUAL CORONA CELL RNA SEQUENCING REVEALED TRANSCRIPTS ASSOCIATED WITH OOCYTE COMPETENCE AND LIVE BIRTH. B. R. McCallie, a A. Strieby, a J. C. Parks, a W. B. Schoolcraft, a,b M. G. Katz-Jaffe, a,b "National Foundation for Fertility Research, Lone Tree, CO; a Colorado Center for Reproductive Medicine, Lone Tree, CO.

OBJECTIVE: Cumulus cells (CC) surround the oocyte and maintain a close relationship via transzonal processes and gap junctions, providing key nutrients and other factors essential for oocyte maturation and developmental competence. Previous studies have shown a potential correlation between the CC transcriptome and implantation outcome. To complement existing data, our study investigated the CC transcriptome of individual euploid oocytes by RNA sequencing to uncover novel biological pathways that may influence implantation outcome.

DESIGN: Research study.

MATERIALS AND METHODS: Under IRB consent, advanced maternal age infertility patients (36-40 years) donated CC from individual cumulus oocyte complexes. Zygotes were individually cultured to the blastocyst stage followed by a single euploid blastocyst frozen embryo transfer. Total RNA was isolated from individual CC samples (n=10) and purified cDNA libraries were constructed, amplified and enriched for sequencing on the ION PI v2 chip (Life Technologies). Trimmed and filtered reads were aligned to human reference genome and transcriptome UCSC hg19 using the Avadis NGS platform (Strand Scientific). Gene expression quantification was performed according to implantation outcome (live birth vs. negative implantation) with DESeq normalization followed by unpaired t-test at significance P<0.05.

RESULTS: RNA sequencing of individual CC samples generated between 11-30 million reads per sample. A total of 343 differentially expressed transcripts were identified in relation to a live birth (P<0.05). The majority (87%) of these differentially expressed transcripts are considered protein-coding, including previously recognized CC genes associated with developmental competence, HDAC2 (chromatin structure), ANG (angiogenesis stimulator) and TNFRSF10A (pro-apoptosis) (P<0.05). Gene ontology analysis identified enriched biochemical signaling pathways involving in downstream processes including: response to hormone stimulus, regulation of cellular protein metabolic processes, transcription and transmembrane transport (P<0.05).

CONCLUSION: RNA transcriptome sequencing generated a library of genes associated with oocyte developmental competence and successful live birth following transfer of a euploid blastocyst. Elucidating the biological pathways involved in acquiring oocyte developmental competence will contribute to the development of a non-invasive viability assay to assist in embryo selection during infertility treatment.

O-343 Wednesday, October 22, 2014 04:30 PM

REGULATION OF FOXO3 SUBCELLULAR LOCALIZATION BY KIT LIGAND IN THE NEONATAL MOUSE OVARY. M. Ezzati, a M. Baker, a,b G. Aloisi, a C. Pena, b Y. Nakada, a I. Cuevas, a,b B. R. Carr, a D. H. Castrillon. a Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, University of Texas Southwestern Medical Center, Dallas, TX; a Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX; a Fort Worth Fertility, Fort Worth, TX.

OBJECTIVE: Foxo3 protein in the oocyte nucleus is required for the maintenance of primordial follicles in a dormant state. PI3K/AKT-dependent phosphorylation of Foxo3 leads to its relocalization to the cytoplasm and subsequent follicular activation. However, the nature of the upstream signals controlling Foxo3 activity and subcellular localization remain unknown. We aimed to study the in vitro effects of Kit ligand (SCF) on the subcellular localization of Foxo3 in primordial follicles within the postnatal mouse ovary.

DESIGN: In vitro study using explants of intact neonatal mouse ovaries. MATERIALS AND METHODS: Neonatal FVB mice ovaries at postnatal day 7 (PD7) were harvested and incubated in culture medium (DMEM) at 37°C and 5% CO2 for 60-90 minutes with (n=3) or without (n=3) Kit ligand at 150 ng/mL (8 nM). Similar experimental conditions were used to establish a dose-response curve for the effects of Kit ligand and assess the effects of imatinib (small molecule inhibitor of the Kit receptor). Immunofluorescence (IF) staining for Foxo3 was performed using a rabbit polyclonal antibody. A microscope equipped with epifluorescence was used to obtain the images. Three sections per ovary were used for the analysis. Relative proportions of nuclear versus cytoplasmic staining were determined using ImageJ software. Western blot was used to measure the relative abundance of phosphorylated versus total Foxo3 in tissue lysates from different experimental groups. A negative binomial regression model, ANOVA and t tests were used to determine statistical significance.
RESULTS: Kit ligand treatment increased the cytoplasmic localization of Foxo3 from 40% in the untreated ovaries to 75% in the treated group (p = 0.007 in paired samples and p = 0.03 in unpaired samples). Furthermore, this effect was reversible with imatinib (p = 0.005). A dose response curve for Kit ligand treatment showed that maximum effect was seen at 150 ng/mL. No significant change in signal intensity was noted for phosphorylated Foxo3 with Western blot.

CONCLUSION: Kit ligand treatment in vitro increases the proportion of cytoplasmic Foxo3 in primordial follicles at PD7, lending support to the idea that Kit receptor/ligand control Foxo3 activity in the context of primordial follicle activation.

Supported by: This work was Supported by NICHD R01 HD048690 grant.

O-344 Wednesday, October 22, 2014 04:45 PM

ANALYSIS OF SLIDING SCALE HCG FOR REDUCTION OF OVARIAN HYPERSTIMULATION SYNDROME (OHSS) IN 10,427 IVF-ICSI CYCLES. V. Gunnala, D. Reichman, G. Schattman, O. Davis, Z. Rosenwaks. The Ronald O. Perelman Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medical College, New York, NY.

OBJECTIVE: To evaluate the incidence of OHSS using sliding-scale intramuscular (IM) hCG to trigger oocyte maturity, determine serum absorption of IM b-hCG according to dose/BMI, and establish a threshold level of serum b-hCG after trigger associated with optimal oocyte maturity.

DESIGN: Retrospective cohort.

MATERIALS AND METHODS: 10,427 ICSI cycles from 9/2004-12/2011 were dosed hCG accordingly: 10,000 IU for E2<150 pg/mL, 5,000 IU for E2 1,501-2,500 pg/mL, 4,000 IU for E2 2,501-3,000 pg/mL, and hCG 3,300 IU vs. leuprolide 2 mg + 1500 IU for E2>3,000 pg/mL. Serum b-hCG was assessed the morning following injection. Spearman correlation, multivariable logistic regression, t-test, and Chi-square were used to evaluate data. Analyses were evaluated for repeated measures bias, with p<0.05 considered significant. Prenatal genetic diagnosis/screening cycles were excluded.

RESULTS: There was a strong inverse linear relationship between BMI and serum hCG for each dose group. Patients with serum hCG 20-30, 30-40, and 40-50 IU/mL all had reduced oocyte maturity as compared to patients with h-b-hCG >50 (67.8% vs. 71.4% vs. 73.8% vs. 78.9%, respectively, p<0.05). Cycles with b-hCG 20-50 IU/mL had a 40.1% reduction in live birth (OR 0.599, 95% CI 0.41-0.87) after controlling for age, BMI, # transferred, and # IVF cycles. B-hCG <50 occurred in 2.3% of cycles, and was more likely in overweight patients receiving <5,000 IU and obese patients receiving <10,000 IU. Incidence of b-hCG <50 in patients with normal BMI receiving <10,000 IU was low, at 0.8% of cycles. Incidence of severe OHSS (requiring paracentesis or hospitalization) was 0.17% (n = 18).

OBJECTIVE: We have previously reported that chromosomes aggregate around nucleoli in human oocytes at the germinal vesicle (GV) stage (Ot-suki and Nagai, 2007) and that this chromosome phase persists after germinal vesicle breakdown (GVBD). However, we have also observed nucleoli without such chromosome aggregation. As GVBD fails to occur in some GV oocytes and there is no explanation for this, we hypothesize that peri-nucleolar chromosome aggregation (PNCA) is necessary for GVBD. In this study we tested this hypothesis.

DESIGN: Prospective and retrospective cohort analyses.

MATERIALS AND METHODS: GV oocytes that were obtained during controlled ovarian stimulation cycles were used in this study. Photographs of GV oocytes were taken using the relief contrast inverted microscope at the Family Medicine Center in Russia or using the differential interference contrast inverted microscope at the Nagai Clinic in Japan. GV oocytes were categorized into two groups (P: positive and N: negative) based on the presence or absence of chromosomes aggregation. Time lapse observations were performed using PrimoVision (VitroLife) with 5-minute intervals. The occurrence of GVBD and polar body (PB) extrusion were recorded and the elapsed time to GVBD and from GVBD to PB extrusion were calculated. In addition, the average of horizontal and vertical diameters of GV oocytes were calculated and compared in the two groups.

Correlation between chromosome accumulation around the nucleoli and GVBD

<table>
<thead>
<tr>
<th>PNCA positive</th>
<th>PNCA negative</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>78</td>
<td>31</td>
</tr>
<tr>
<td>GVBD (%)</td>
<td>87.2 (68/78)</td>
<td>45.2 (14/31)</td>
</tr>
<tr>
<td>Polar body release (%)</td>
<td>74.4 (59/78)</td>
<td>19.4 (6/31)</td>
</tr>
<tr>
<td>Elapsed time (minutes±SD):</td>
<td>358 (±408)</td>
<td>1146 (±939)</td>
</tr>
<tr>
<td>Start-GVBD</td>
<td>997 (±125)</td>
<td>1042 (±144)</td>
</tr>
<tr>
<td>Elapsed time (minutes±SD):</td>
<td>114 (±4.1)</td>
<td>113 (±4.8)</td>
</tr>
</tbody>
</table>

Clinical pregnancy rate per retrieval

<table>
<thead>
<tr>
<th>hCG dose (IU)</th>
<th>35-37</th>
<th>38-40</th>
<th>41-42</th>
<th>&gt;42</th>
</tr>
</thead>
<tbody>
<tr>
<td>10,000</td>
<td>n=1,333; 43.0%</td>
<td>n=1,042; 36.9%</td>
<td>n=1,540; 30.7%</td>
<td>n=1,076; 19.9%</td>
</tr>
<tr>
<td>5,000</td>
<td>n=802; 49.0%</td>
<td>n=559; 40.2%</td>
<td>n=550; 37.5%</td>
<td>n=336; 22.0%</td>
</tr>
<tr>
<td>4,000</td>
<td>n=516; 48.4%</td>
<td>n=291; 47.3%</td>
<td>n=270; 32.2%</td>
<td>n=156; 31.4%</td>
</tr>
<tr>
<td>3,300</td>
<td>n=280; 44.4%</td>
<td>n=150; 37.9%</td>
<td>n=119; 28.6%</td>
<td>n=70; 25.4%</td>
</tr>
</tbody>
</table>

Clinical pregnancy rate per retrieval according to age and hCG dose

CONCLUSION: Conservative stimulation with sliding scale hCG trigger and fresh transfer is associated with very low rates of OHSS and excellent pregnancy rates. Doses as low as 3,300 IU hCG are sufficient for oocyte maturity in patients with normal BMI, however overweight and obese patients likely benefit from higher doses. Programs should consider measuring serum b-hCG 8-10 hours after IM injection and counseling patients with levels <50 mIU/mL regarding potential adverse effects.

RESULTS: In total 109 GV oocytes (57 in the Family Medicine Center and 52 in the Nagai Clinic) were evaluated. The results are shown in Table 1.

CONCLUSION: Our results suggest that the aggregation of chromosomes around nucleoli may be a key factor in germinal vesicle breakdown and its observation could be a useful indicator for evaluating GV oocytes.
O-346 Wednesday, October 22, 2014 05:15 PM

THE PREDICTIVE VALUE OF SERUM CONCENTRATIONS OF ANTI-MULLERIAN HORMONE FOR OOCYTE QUALITY. A. Iaconelli, Jr., D. P. A. F. Braga, A. S. Setti, F. F. Pasqualotto,* R. C. S. Figueira,* E. Borges, Jr., *Fertility - Centro de Fertilização Assistida, São Paulo, SP, Brazil; 3) Instituto Sapientiae, São Paulo, SP, Brazil; Universidade de Caxias do Sul, Caxias do Sul, RS, Brazil.

OBJECTIVE: To identify a possible correlation between serum anti-Mullerian hormone (AMH) concentrations and oocyte quality, embryo developmental competence and pregnancy.

DESIGN: Case-control study.

MATERIALS AND METHODS: A total of 4488 oocytes obtained from 408 patients undergoing ICSI cycles between January 2011 and August 2013, were evaluated. Serum AMH levels were measured prior to the start of each cycle and the concentrations were recorded. The oocyte morphologies were evaluated before ICSI, and the embryos were evaluated on days two, three and five of development. Binary regression analyses, adjusted for maternal age, were performed to evaluate the influence of the AMH level on the oocyte and embryo quality, blastocyst formation and the pregnancy chance.

RESULTS: Significant positive correlations between the serum AMH level and the number of aspirated follicles (CC: 0.626, p < 0.001), number of retrieved oocytes (CC: 0.600, p < 0.001), number of mature oocytes (CC: 0.585, p < 0.001), fertilisation rate (CC: 0.595, p < 0.048), number of obtained embryos (CC: 0.495, p < 0.001), number of high-quality embryos (CC: 0.504, p < 0.001), number of transferred embryos (CC: 0.221, p < 0.001) and implantation rate (CC: 0.116, p = 0.031) were noted. The AMH level did not influence the embryo quality on days two or three, or the chance of blastocyst formation. However, the oocyte quality was influenced by the AMH level (OR: 1.30, CI: 1.13-1.48, p < 0.001). The AMH level did not influence the embryo quality on days two or three, or the chance of blastocyst formation. However, the oocyte quality was influenced by the AMH level (OR: 0.70, CI: 0.52-0.92, p < 0.001). The presence of aggregates of smooth ERC (OR: 0.79, CI: 0.63-0.99, p = 0.014), large PVS (OR: 0.82, CI: 0.75-0.90, p < 0.001), PVS granularity (OR: 0.87, CI: 0.83-0.92, p < 0.001), ZP abnormalities (OR: 0.80, CI: 0.69-0.92, p < 0.001) and shape abnormalities (OR: 0.72, CI: 0.56-0.94, p = 0.003) were affected by the AMH level. The presence of other dysmorphisms, such as cytoplasmic colour, vacuoles in the ooplasm, retractive bodies and fragmented PB were not influenced by the AMH level.

CONCLUSION: Our findings indicate that the serum AMH level is a useful predictor of the oocyte quality and pregnancy. Therefore, not only a decreased follicle reserve, but also a lower oocyte quality may be responsible for the lower pregnancy chance related to low AMH levels.

O-347 Wednesday, October 22, 2014 05:30 PM

THE C-TYPE NATRIURETIC PEPTIDE AND ITS TRANSMEMBRANE GUANYLYL CYCLASE RECEPTOR NATRIURETIC PEPTIDE RECEPTOR B ARE NOT CRITICAL REGULATORS OF MEIOSIS INHIBITION IN RHESUS MACAQUES. C. B. Hanna,* S. Yao,† J. D. Hennebold,‡ J. T. Jensen. †Division of Reproductive & Developmental Sciences, Oregon National Primate Research Center, Beaverton, OR; ‡Department of Ob/Gyn, Oregon Health & Science University, Portland, OR.

OBJECTIVE: C-type natriuretic peptide (CNP) and its transmembrane guanylyl cyclase receptor, natriuretic peptide receptor B (NPR2), have been established as critical regulators of cGMP induced meiosis arrest in mouse oocytes. CNP produced by mural granulosa cells binds NPR2 in cumulus cells to produce cGMP that diffuses into the oocyte to inhibit meiosis. As this pathway represents a potential target for contraceptive development, the study objective was to determine if this mechanism is also functional in primates.

DESIGN: In vitro experiments at a Primate Research Center.

MATERIALS AND METHODS: Rhesus macaques underwent controlled ovarian stimulation (COS) cycles without an ovulatory stimulus. Mural granulosa (MG) cells and cumulus oocyte complexes (COCs) were collected from large antral follicles and quantitative RT-PCR (qRT-PCR) measured the expression of the CNP precursor, natriuretic peptide precursor C (NPPC) and NPR2 mRNA (n = 3). Fluorescent immunoassay measured the concentration of CNP in follicle fluid from large antral follicles of other females (n = 3). Additional stimulation cycles (n = 3) yielded COCs that were cultured in HECM9 only (Control), or supplemented with 100nM estradiol (E2), 50nM CNP, or 100nM E2 + 50nM CNP (E2 maintains NPR2 receptor expression in mice) for 24 hours.

RESULTS: qRT-PCR indicated a 2.5-fold increase in steady state levels of NPPC in COCs compared to MG and a 1.5-fold decrease in NPR2 expression in COCs compared with MG. The concentration of CNP in follicle fluid samples ranged from 57-59 PM. Meiosis resumption rates measured by germinal vesicle breakdown (GVBD) of COCs cultured with CNP alone (46%) or CNP + E2 (32%) were no different from the controls (74%) or those cultured with E2 only (45%, p = 0.17).

CONCLUSION: The small changes in cell specific expression of NPPC and NPR2 suggest the receptor-ligand does not function in a similar capacity as the mouse where NPRPC expression is 10-fold higher in MG than COCs while NPR2 remains only 2-fold greater in COCs [1]. Further, the concentration of CNP secreted into the follicle fluid in macaques was 500-fold less than that reported to maintain meiotic arrest in mouse COCs (30 nM). Although CNP supplementation did not prevent GVBD in COCs, a trend towards reduced GVBD suggests that the NPR2 receptor is active in the primate follicle. Taken together, these data support that CNP and NPR2 are not critical regulators of meiosis arrest in primate follicles.

Supported by: U54HD055744, PS01OD011092.

OVARIAN STIMULATION - ART II

O-348 Wednesday, October 22, 2014 03:45 PM


OBJECTIVE: To assess early determinants of OHSS risk and whether cycle day (CD) 6 Estradiol (E2) level predicts OHSS risk and freeze all decision.

DESIGN: Retrospective data analysis.

MATERIALS AND METHODS: From January 2003 to January 2014 the records of 24552 cycles were retrospectively reviewed from our database. A total of 4536 cycles in which >15 oocytes retrieved were included in this study. According to OHSS development we divided it into three groups. In group 1 there was no OHSS development (3964 cycles). In group 2, all patients developed moderate or severe OHSS (387 cycles), and in group 3 embryo transfer was cancelled due to OHSS risk (185 cycles). Binary multivariate regression analysis was done to determine significant predictors of OHSS risk. Three groups were compared using ANOVA and two group comparisons were done using post hoc analysis. Significance was determined at p < 0.05.

RESULTS: According to regression analysis age, AMH and CD6 E2 levels were significant determinants of OHSS risk in which age was negatively and the other parameters were positively correlated. Comparison of the three groups are presented in Table 1.

Among the three groups, all parameters were significantly different except mean age and BMI. Number of retrieved, MI, fertilized oocytes and E2 levels were significantly different within each group. AMH and E2 levels on day 6 were similar between OHSS and all freeze groups. However these groups were significantly different compared to the group without OHSS.

CONCLUSION: Instead of waiting until HCG day we can give all freeze decision as early as on day 6 according to E2 levels.

Age and AMH level also contribute for early decision from the beginning of the cycle. Caution should be taken in patients with younger age, high AMH and high D6 E2 levels to decrease OHSS development. This would help in counselling patients early in the cycle for freeze all decision.
O-349 Wednesday, October 22, 2014 04:00 PM

CONVENTIONAL IVF STIMULATION CAN BE SAFE AND EFFECTIVE WITHOUT THE REQUIREMENT FOR TRADITIONAL INTENSIVE MONITORING. K. J. Doody, K. M. Doody. Center for Assisted Reproduction, Bedford, TX.

OBJECTIVE: The study aim was to investigate the safety and efficacy of a traditional stimulation protocol in conjunction with minimal monitoring. The studied hypothesis is that gonadotropin dose for the cycle could be determined using AMH and body weight with only one monitoring visit during the stimulation.

DESIGN: Prospective trial (pilot study).

MATERIALS AND METHODS: Inclusion criteria were infertile women age 18-38, AMH 1-3ng/ml, AFC 6-20, BMI < 36 and no prior history of high response to ovarian stimulation. Oral contraceptives were used to program the cycle and overlapped with leuprolide 1mg/day. Leuprolide was continued for 7-14 days and then decreased to 0.5mg/day prior to stimulation. Sonogram was used to confirm absence of follicular cysts prior to starting leuprolide and prior to initiation of human menopausal gonadotropin (HMG). A starting dose of 225 IU per day was 'stepped down' to 150 IU after one or more days based on an algorithm taking into account the AMH and body weight. A single monitoring visit performed on the 10th day of stimulation. Decision to trigger on stimulation day 10, 11 or 12 was based on measurements of follicle size on day 10. Egg retrieval was performed 36 hours following trigger. Up to 10 oocytes were co-incubated with sperm for two to four hours. These oocytes were then cultured in traditional incubators or vaginal culture device (INVO Bioscience, MA, USA) for 5 days.

RESULTS: A total of 40 cycles were planned in the IRB approved trial. An interim analysis of 33 completed cycles has shown the following (ranges are listed in parentheses):

- Mean age: 32.6 (26-38)
- Mean AMH: 1.91 (1.07-2.91)
- Mean body weight: 156.6 pounds (109-207)
- Mean gonadotropin consumption / stimulation: 1.882 IU (1350-2475)
- Mean number of eggs retrieved: 8.1 (1-18)
- Mean number of embryos transferred: 1.7 (1-2)
- Number of interventions to prevent OHSS: 0
- OHSS / cycle (moderate / severe): 0/3
- Ongoing pregnancy rate / cycle: 61%

CONCLUSION: In vitro fertilization is necessary to achieve pregnancy for many patients with infertility. Unfortunately, the process of IVF can be burdensome. Patients are deterred due to the time and effort required for monitoring of ovarian stimulation. Traditionally, the monitoring of the ovarian stimulation requires multiple visits for sonography and hormonal measurements.

This study demonstrates that AMH and body weight can be used in predicted normal responders to conduct a predetermined fixed stimulation for IVF that is effective and safe (low risk of OHSS) and has minimal monitoring requirements. This strategy can be used to improve access to ART services.

O-350 Wednesday, October 22, 2014 04:15 PM

THE FSHR GENE POLYMORPHISM (RS 6165 - ALA/ALA GENOTYPE) IS ASSOCIATED WITH THE USE OF HIGHER DOSES OF RECOMBINANT FSH DURING IVF/ICSI TREATMENT. A. Renzi, a C. G. Petersen, a,b L. D. Vagnini, a G. R. Oliveira-Pelegrin, a L. A. Mauri, a,b F. C. Massaro, a,b M. Cavagna, a,b,c J. A. Oliveira, a,b R. L. Bauru, a,b J. G. Franco, Jr., a,b Paulista Center for Diagnosis Research and Training, Ribeirao Preto, Sao Paulo, Brazil; cCenter for Human Reproduction Prof. Franco Jr, Ribeirao Preto, Sao Paulo, Brazil; dWomen’s Health Reference Centre, Perola Byington Hospital, Sao Paulo, Brazil.

OBJECTIVE: Follicle-stimulating hormone (FSH) is essential to folliculogenesis and acts through the FSH receptor (FSHR) present on the membrane of granulosa cells. Polymorphisms in the FSHR gene may lead to altered pattern of receptor expression on the cell surface or differential affinity towards FSH. The Ala307Thr polymorphism is located in the extracellular domain within the hormone binding region, which can influence the response to endogenous and exogenous FSH stimulation. Herein we investigated the association of this FSHR polymorphism with the ovarian response to exogenous recombinant FSH (r-FSH).

DESIGN: Prospective cohort-study.

MATERIALS AND METHODS: The study included 168 infertile women submitted to GnRH-antagonist protocol for IVF/ICSI. The Ala307Thr FSHR polymorphism (rs6165) was genotyped using DNA extracted from peripheral blood and TaqMan SNP genotyping assay. The results were associated with age, Anti-Mullerian Hormone (AMH) levels, Antral Follicular Count (AFC), mean number of total and MII collected oocytes, blood and TaqMan SNP genotyping assay.

The statistical analyses were made using Kruskal-Wallis test. The results were associated with the following:

- Total dose r-FSH, follicle size and number of total and MII collected oocytes.
- Total dose r-FSH, AFC (n)
- Mean age, AMH (ng/ml)
- Total number of interventions to prevent OHSS.
- Mean number of eggs retrieved.
- Total number of embryos transferred.
- Total number of pregnancies.
- Percentage of live births.

RESULTS: The statistical analysis using Kruskal-Wallis test showed a significant association between the use of higher doses of r-FSH and the following:

- Mean age: 32.6 (26-38)
- Mean AMH: 1.91 (1.07-2.91)
- Mean number of eggs retrieved: 8.1 (1-18)
- Mean number of embryos transferred: 1.7 (1-2)
- Number of interventions to prevent OHSS: 0
- Percentage of live births: 61%

CONCLUSION: These findings suggest that the use of higher doses of r-FSH during IVF/ICSI treatment is associated with the use of higher doses of recombinant FSH (r-FSH).

Ongoing pregnancy rate / cycle: 61%.

OHSS / cycle (moderate / severe): 0/33

Number of interventions to prevent OHSS: 0

Mean gonadotropin consumption / stimulation: 1,882 IU (1350-2475)

Mean AMH: 1.91 (1.07-2.91)

Mean age: 32.6 (26-38)

TABLE 1. Results

<table>
<thead>
<tr>
<th>genotypes</th>
<th>n</th>
<th>Ala/Ala</th>
<th>Ala/Thr</th>
<th>Thr/Thr</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs6165</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ala/Ala</td>
<td>41</td>
<td>24(4%)</td>
<td>79(47%)</td>
<td>48(28%)</td>
<td>0.53</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td>34±1.52</td>
<td>34±3.35</td>
<td>34±3.8</td>
<td>0.53</td>
</tr>
<tr>
<td>AMH (ng/mL)</td>
<td></td>
<td>2.5±2.9</td>
<td>2.7±2.8</td>
<td>3.0±3.5</td>
<td>0.58</td>
</tr>
<tr>
<td>AFC (n)</td>
<td></td>
<td>15.1±11.6</td>
<td>16±8.8</td>
<td>17.6±12.1</td>
<td>0.37</td>
</tr>
<tr>
<td>Total dose r-</td>
<td></td>
<td>2056±828</td>
<td>1675±744</td>
<td>1733±808</td>
<td>0.008, 0.04</td>
</tr>
<tr>
<td>FSH (IU)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oocytes MII</td>
<td></td>
<td>7.1±5.4</td>
<td>6.5±4.3</td>
<td>6.6±5.4</td>
<td>0.85</td>
</tr>
<tr>
<td>Oocytes Total</td>
<td></td>
<td>10.3±6.8</td>
<td>9.1±5.3</td>
<td>9.3±6.6</td>
<td>0.60</td>
</tr>
</tbody>
</table>

Table 1. Results

FSHR (rs6165) - WOMEN’S GENOTYPS
observed. There was no case of ovarian hyperstimulation syndrome. Table 1 summarizes the results.

CONCLUSION: In the population of infertile women, the Ala/Ala genotype seems to be associated with the use of higher doses of r-FSH during IVF/ICSI treatment, suggesting that this allelic variant (Ala) provides lower sensitivity to FSH receptor.

O-351 Wednesday, October 22, 2014 04:30 PM
THE REPRODUCIBILITY OF LUTEINIZING HORMONE SURGES FOLLOWING AGONIST TRIGGER: ANALYSIS OF 1792 AGONIST TRIGGERERS. S. T. Daneshmand, a,b F. C. Garner, a,b S. Shapiro, a,b Reproductive Center of Las Vegas, Las Vegas, NV; aObstetrics and Gynecology, University of Nevada School of Medicine, Las Vegas, NV.

OBJECTIVE: GnRH agonist trigger is frequently used to reduce OHSS risk in high responders on antagonist protocols. It has been reported that oocyte quality is related to the pituitary response of luteinizing hormone (LH) "surge". We examine the reproducibility of LH surges in repeated cycles in the same subjects.

DESIGN: IRB-approved retrospective cohort study.

MATERIALS AND METHODS: Subjects with very high stimulated ovarian response to received GnRH agonist (4mg leuprolide acetate) for oocyte maturation under the antagonist protocol for ovarian stimulation. Serum LH levels were measured the following morning, approximately 12 hours later. LH >50 IU/L was considered "optimal". LH <15 IU/L was considered "inadequate". In patients with repeated cycles, the predictive value of the prior LH value in predicting the next LH value was assessed.

RESULTS: The study included all 1,792 subjects receiving agonist trigger. Mean age was 31.2 ± 5.2 years, mean weight was 68.5 ± 17.2 kg, and the mean oocyte recovery was 21.3 ± 8.9. There were 814 subjects (45.4%) with optimal LH levels (LH >50 IU/L). There were 189 patients with suboptimal LH levels in their first cycle that were again given agonist trigger in a subsequent cycle. A prior optimal LH level was predictive of a subsequent optimal LH level (P < 0.0001). The sensitivity and specificity of an optimal LH level in their first cycle for predicting optimal LH level in a subsequent cycle were 0.682 and 0.626, respectively. The positive predictive value and negative predictive value were 0.560 and 0.738, respectively. There were only 22 patients with inadequate LH levels (<15 IU/L) in their first cycle who were triggered a second time with the agonist. Of these 22 patients, 11 (50%) had a subsequent inadequate LH surge, well above the 4.8% rate of inadequate LH levels in the entire study set. Across the entire set, linear regression revealed a modest but statistically significant decline in LH level with increasing age (P = 0.0018), after controlling for patient weight. The slope of LH level versus age was -0.46 IU/L per year, and the expected LH level in a 70 kg 22-year-old was 56.7 IU/L, while that of a 70kg 42-year old was 47.5 IU/L.

CONCLUSION: The LH serum level following agonist trigger is moderately predictive of levels following subsequent agonist triggers, and appear to decrease with increasing maternal age and weight, suggesting a potential age-related decline in pituitary response to GnRH agonist.

O-352 Wednesday, October 22, 2014 04:45 PM
HCG VERUS AGONIST TRIGGER IN DONOR OOCYTE CYCLES: DOES HCG HAVE A PLACE IN CURRENT PRACTICE? J. M. Cox, a K. Fru, a M. Purcell, b B. Whitcomb, c J. Segars, b E. Levens, b F. Chang, b "PRAE, NICHD, NIH, Bethesda, MD; aShady Grove Fertility, Rockville, MD; bBiostatistics and Epidemiology, University of Massachusetts, Amherst, Amherst, MA.

OBJECTIVE: The use of GnRH agonist trigger has been shown to reduce OHSS. However in autologous ART, previous meta-analysis suggested GnRH agonist trigger might lower clinical outcomes when compared to hCG trigger. Reducing the risk of OHSS without compromising pregnancy outcomes is of paramount importance in donor oocyte ART. Thus we compared clinical outcomes between donors receiving hCG or GnRH agonist triggers.

DESIGN: Retrospective cohort.

MATERIALS AND METHODS: All donor cycles and matched recipients were identified in 2010, the year in which our practice transitioned from hCG to primarily agonist trigger for donor cycles. Groups were compared for baseline characteristics and primary outcome measures: oocyte maturity, OHSS diagnosis, and failed trigger defined as patients who required additional trigger based on lab assessment or no oocytes retrieved at first retrieval. Clinical pregnancy rates (CPR) and live birth rates (LBR) in recipients between the two groups were also compared.

RESULTS: 339 donors were included in the analysis (agonist n = 209, hCG n = 130). There were no significant differences between groups in regard to age (P = 0.8), BMI (P = 0.4), LH (P = 0.3), or FSH (P = 0.3) at baseline. A statistically significant difference in the proportion of mature oocytes was observed favoring GnRH agonist (80.2% vs. 75.2%, p = 0.004), with no difference in oocytes retrieved. OHSS diagnosis was significantly increased in hCG group (3.85% vs. 0.48%, p = 0.033). Failed trigger was only seen in agonist group but was not statistically significant at 1.9% (p = 0.3). CPR and LBR were not different between groups.

O-353 Wednesday, October 22, 2014 05:00 PM
ABSTRACT WITHDRAWN

O-354 Wednesday, October 22, 2014 05:15 PM
EGG DONOR ANEUPLOIDY RATES SIGNIFICANTLY DIFFER BETWEEN FERTILITY CENTERS. S. Munne, a M. Allikani, b J. Barratt, c J. Hesla, c B. Kaplan, c M. Alper, d D. McCulloh, d "Reprogenetics, Livingston, NJ; cThe Center for Human Reproduction, North Shore University Hospital, Manhasset, NY; aART Reproductive Center and Southern California Reproductive Center, Beverly Hills, CA; bOregon Reproductive Medicine, Portland, OR; cFertility Centers of Illinois, Highland Park, IL; dBoston IVF, Waltham, MA; eNew York University Fertility Center, New York, NY.

OBJECTIVE: To investigate if aneuploidy rates differ between fertility centers. Aneuploidy rates could differ due to differences in population age and infertility indication. However, any differences in aneuploidy rates between egg donor cycles are probably induced by differences in center-specific treatments.

DESIGN: Aneuploidy rates in blastocysts from egg donation cycles from different fertility centers were compared.

MATERIALS AND METHODS: 616 Egg donor cycles (average age 25.8 years) underwent blastocyst biopsy for PGS at 20 fertility centers. Biopsied samples were analyzed at the same reference laboratory by array CGH. Ploidy data were examined using multiple logistic regression considering the mean incidence of euploidy per patient to see whether euploidy rates differed among centers. Each facility was compared against the mean incidence of euploidy for all facilities.

RESULTS: Different centers had significantly different incidences of euploidy and these were independent of the number of biopsied embryos and donor age. Euploidy rates significantly deviated from the average of 65% (0.65 ± 0.01) in 7 of the 20 centers included, ranging from 42% to 80%. Four centers had significantly lower euploidy rates (0.42 ± 0.10, 0.47 ± 0.07, 0.51 ± 0.03, 0.51 ± 0.07) and 3 significantly higher (0.77 ± 0.02, 0.79 ± 0.03, 0.80 ± 0.02).

CONCLUSION: Agonist trigger for donor cycles significantly decreased the diagnosis of OHSS without affecting oocyte maturity or other cycle outcomes. These data support the use of a GnRH agonist trigger as the preferred trigger type in this high responding population.

Supported by: In part by the intramural research program of Reproductive and Adult Endocrinology, NICHD, NIH.

O-356 Wednesday, October 22, 2014 05:45 PM
FERTILITY & STERILITY®
e121
CONCLUSION: Aneuploidy rates in egg donors vary significantly per center. The differences were not attributable to cohort size (blastocysts biopsied). This is the first study to investigate the question of center-dependent aneuploidy controlling for indication and patient population by taking into account only egg donor cycles. The reasons for the observed differences between centers can only be speculated but include any of the factors that are known to affect oocyte and embryo development, such as hormonal stimulation regimes, laboratory conditions, gamete and embryo manipulation methods, or environmental differences. Age is not considered to play a role here since young age is a pre-requisite to anonymous egg donation.

O-355 Wednesday, October 22, 2014 05:30 PM

OBJECTIVE: The objective of this study was to identify potential genetic biomarkers for ovarian hyperstimulation syndrome (OHSS) using targeted single molecule sequencing (T-SMS).

DESIGN: This was a retrospective, observational study.

MATERIALS AND METHODS: DNA was isolated from patient blood samples. A T-SMS panel for genes implicated in ovarian response to controlled ovarian hyperstimulation was developed. SMS was carried out using the Pacific Biosciences single molecule molecule, real-time, DNA Sequencing System. Primary data analyses, alignment and filtering utilized the Pacific Biosciences SMRT portal. Secondary analyses was conducted using the Genome Analysis Toolkit for SNP discovery and wANNOVAR for functional analysis of exonic variants. Filtered functional variants were further validated using conventional Sanger DNA sequencing.

RESULTS: Target enrichment using droplet-based, multiplex polymerase chain reaction generated amplicons averaging 1 kb fragment size from 44 target loci (99.8% unique base-pair coverage, 3.18 Mb per sample). SMS produced an average raw read length of 1178 nucleotides (nt) with 5% of the amplicons >6000 nt. After filtering with circular consensus (CCS) reads, the mean read length was 3200 nt (97% CCS accuracy). A total of 46 exonic variants were initially identified with 6 observed in OHSS, 24 in non-OHSS and 16 found in both. All variants were validated by Sanger DNA sequencing.

CONCLUSION: These results offer promise of identifying genetic biomarkers for OHSS risk in controlled ovarian hyperstimulation patients. To the best of our knowledge, this is the first report utilizing emulsion PCR and T-SMS for long reads using human DNA samples.

Supported by: This study was funded by award Number U1HR031988 from the National Center for Research Resources. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center for Research Resources or the National Institutes of Health.

CRYOPRESERVATION AND FROZEN EMBRYO TRANSFER II

O-356 Wednesday, October 22, 2014 03:45 PM
EFFICACY AND EFFICIENCY OF THE “UNIVERSAL WARMING PROTOCOL”: MULTICENTER RANDOMIZED CONTROLLED STUDY ON HUMAN SLOW FROZEN OOCYTES. L. Parmegiani,* C. Garelo,* A. Revelli,* L. Criscioli,* S. Dubizzi,* R. Gualtieri,* R. Tavelli,* M. Filicori.* Reproductive Medicine Unit, GynePro Medical Centers, Bologna, BO, Italy; †Livit Clinic, Turin, TO, Italy; ‡Centre of Reproductive Medicine, Careggi University Hospital, Florence, FI, Italy; §Dipartimento di Biologia, Università di Napoli “Federico II”, Naples, Italy.

OBJECTIVE: Human reproductive cells are cryopreserved via slow freezing (SF) or vitrification (VIT). In a previously published pilot-study, it has already been demonstrated that it is possible to warm SF oocytes by using warming solution for VIT and also to increase their survival rates (1). The aim of the present study was to confirm the efficacy of the “rapid warming protocol” for SF oocytes in a multicenter trial and to compare its efficiency with the standard rapid thawing protocols.

DESIGN: Three Assisted Reproductive (AR) centers in Italy were involved in this study. This was a prospective study on 414 oocytes randomized for conventional rapid thawing (RT) via two ready-to-use thawing media (Oocyte Thawing-KIT Origio–group A; CSC Thawing Medium Kit – group B), or rapid warming (RW) via VIT warming solutions (Vitrification Warming-Kit Sage – group C).

MATERIALS AND METHODS: Primary endpoint was the morphological assessment of survival at 2 hours. Some of the surviving oocytes were divided into 3 sub-groups: i) parthenogenetically activated and observed by time-lapse microscopy ii) fixed and observed by confocal microscopy, iii) evaluated by polarized light for the presence of the meiotic spindle. Secondary endpoints were parthenogenetic activation and development, the assessment of the markers of oocyte preservation by confocal microscopy and the spindle presence by polarized light.

RESULTS: Survival rate was 80.7% (71/88) in group A, 60.4% (125/207) in group B and 91.6 (109/119) in group C. Survival rate was significantly higher in group C (P<0.036 vs group A and P<0.001 vs group B), in which the SF oocytes were warmed via VIT warming solutions. Survival rate was also significantly higher in group A vs group B (P<0.001).

Parthenogenetic activation and meiotic spindle presence were not significantly different in the three groups. At the time of writing this abstract the evaluation by confocal microscopy is still in progress.

CONCLUSION: The findings of this study seem to confirm that is possible to optimize costs and increase the survival rate by using the same warming protocol for both SF and VIT oocytes. This “universal warming protocol” is potentially applicable to all kind of slow frozen reproductive cells, such as zygotes, cleavage stage embryos and blastocysts.

Supported by: Thawing and warming solution were kindly supplied by Origio.

O-357 Wednesday, October 22, 2014 04:00 PM
COMPARISON OF VITRIFICATION DEVICES FOR HUMAN EMBRYOS BETWEEN OPEN AND CLOSED SYSTEM. S. Mizuno,* A. Ohgaki,* H. Matsumoto,* A. Fukuda,* Y. Morimoto.*#IVF Osaka Clinic, Higashi-Osaka, Osaka, Japan; #IVF Namba Clinic, Nishi-ku Osaka, Osaka, Japan.

OBJECTIVE: Vitrification is a freezing method that is applied in almost every IVF clinic in Japan. In our clinic, human embryos are vitrified by Cryotop method in which the solution containing embryos is directly exposed to liquid nitrogen. This method does not completely eliminate the risk of cross-contamination during their storage. Therefore, the closed vitrification device, Rapid-i, has been developed to solve this problem. In the present study, survival rate and subsequent development of warmed embryos were compared between Cryotop and Rapid-i, to evaluate if embryos can be safely cryopreserved by Rapid-i without sacrificing their potential after warming.

DESIGN: Prospective study.

MATERIALS AND METHODS: The preliminary study was performed, using zygotes previously vitrified at pronuclear stage (n=78) and day 3 (n=36). They were warmed once, randomly allocated to either of the two vitrification methods, Cryotop or Rapid-i and then revitalized. Post-warming survival rate and subsequent development at day 5 and 6 were compared between the two vitrification methods. The present study was clinically performed, using 962 blastocysts. They were randomly allocated either Cryotop (n=768) or Rapid-i (n=194) and vitrified-warmed. Survival rates and pregnancy rates of single blastocyst transfer were compared. In evaluation of pregnancy rate, over 39-year old patients were excluded.

RESULTS: The preliminary study showed all 2PNs and day 3 embryos survived after warming in both methods. Blastocyst rate from 2PN after warming was not significantly different between Cryotop and Rapid-i (day 5: 6/35, 17.1% vs. 6/43, 14.0%, day 6: 9/35, 25.7% vs. 11/43, 25.6%, respectively). The evaluation of development to blastocyst from
day 3 embryos after warming also showed similar results between Cryo-
top and Rapid-i (day 5: 5/18, 27.0% vs. 9/18, 50.0%, day 6: 10/18, 55.6% vs. 12/18, 66.7%, respectively). In the present study, there were no differences in survival rates (752/768, 97.9% vs. 190/194, 97.9%) and pregnancy rates (268/488, 54.9% vs. 57/109, 52.3%) between Cryo-
top and Rapid-i.

CONCLUSION: The present study demonstrates that a newly developed
device, Rapid-i, dose not impair not only developmental potential of 2PN,
day 3 embryo and blastocyst after warming, but also subsequent pregnancy
rate compared to a conventional Cryotop method. Therefore, it is concluded
that Rapid-i which does not expose human embryos directly to liquid
nitrogen is a favorable device for storage without the risk of cross-

---

**O-358 Wednesday, October 22, 2014 04:15 PM**

**BANKED FROZEN DONOR OOCYTES DEMONSTRATE HIGH EF-
FICIENCY IN CONVERSION TO LIVE BORN INFANTS: A
COLLABORATIVE MULTI-SITE EXPERIENCE.**

K. Fru, a J. Cox, a R. Dunn, C. Vanijgul, b
J. Lim, b B. M. Berger, b R. Mangal, S. Chauhan, L. Schenk, W.-S. Wu, G. Grunert. Fertility Spe-
cialists of Houston, Houston, TX.

OBJECTIVE: Previous studies have shown a <7% oocyte to live born
infant rate in fresh donor cycles. The purpose of this study was to evaluate the
efficiency of cryopreserved donor oocytes compared to those from fresh
donor cycles.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: Outcomes of all IVF cycles using
previously cryopreserved donor oocytes from a single donor oocyte bank and performed at 19 partner ART institutions(1/2012-12/2013)
were reviewed. These were compared to fresh donor IVF cycles per-
formed at a large participant ART institution in 2012. Outcomes included cycle cancellation, number of oocytes thawed, number of cry-
opreserved supernumerary embryos, clinical pregnancy (CP: intrauterine gestational sac), ongoing pregnancy rates (OPR) and number of infants born.

RESULTS: 626 cycles using thawed oocytes were initiated with a 93%
continuation rate; 41% of embryo transfer (ET) cycles had supernumer-
ary embryos for cryopreservation (n

---

**O-359 Wednesday, October 22, 2014 04:30 PM**

**PRE-IMPLANTATION GENETIC SCREENED (PGS)ABNORMAL
BLASTOCYSTS GIVE SIGNIFICANT LESS SURVIVAL AFTER
VITRIFICATION AND WARMING.**

R. Dunn, C. Vanijgul, R. Mangal, S. Chauhan, L. Schenk, W.-S. Wu, G. Grunert. Fertility Spe-
cialists of Houston, Houston, TX.

OBJECTIVE: Aneuploid embryos are generally considered not fruitful,
i.e. arrested, degenerated. For embryos develop to blastocyst stage,
these embryos have passed through maternal genomic activity to em-
byronic genomic activity and keep developing. Do these PGS abnormal
blastocysts survive vitrification and warming as good as normal blasto-
cysts do? In addition, general hypothesis is that developmental speed
reflects the healthy status of embryos (1). Does day-5 biopsied blasto-
cyst survive better than day-6 biopsied blastocyst does? This study
examines the survival of vitrified blastocysts between PGS normal and
abnormal embryos and the survival between day 5 and day 6 biop-
sied blastocysts.

DESIGN: A retrospective study.

MATERIALS AND METHODS: For PGS normal blastocysts, the frozen
embryo transfer is usually 2 hours after warming. For PGS abnormal blasto-
cysts, the examination of survival is 2 hours after warming then dis-
carded. The definition of survival after 2-hours warming is that at least
half numbers of blastomeres are clear and shining. These PGS cycles with
frozen embryo transfer during period 1/1/2013 – 3/31/2014 are
included. The age distribution is 22-45 with mean of 37.3. Totally 278
PGS normal embryos and 112 abnormal embryos from 416 cycles are
included in the study. For trophectoderm biopsy, only those embryos
develop to full or further advance blastocyst stage are biopsied on day 5.
Other slow development blastocysts are biopsied on day 6. Fisher’s Exact
Tests are used for analysis.

---

**Results**

**Comparison between normal vs. abnormal**

<table>
<thead>
<tr>
<th>Biopsy date</th>
<th>PGS normal # survival/total (%)</th>
<th>PGS abnormal # survival/total (%)</th>
<th>P &lt; 0.0001</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 5</td>
<td>147/148 (99%)</td>
<td>162/20 (80%)</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>Day 6</td>
<td>120/130 (92%)</td>
<td>66/92 (71%)</td>
<td>p &lt; 0.0001</td>
</tr>
</tbody>
</table>

n.s.: not significant

RESULTS: The summary of results is in following table.

CONCLUSION: 1. The survival of PGS abnormal blastocysts is signifi-
cantly less than the PGS normal blastocysts does on both day 5 and day 6 biop-
sied blastocysts.

2. By comparison of PGS normal blastocysts, the survival of day 6 bio-
sied blastocysts is significantly less than day 6 biopsied blastocysts does.

3. By comparison of PGS abnormal blastocysts, there is no significant dif-
fERENCE on the survival between day 5 and day 6 biopsied blastocysts. It sug-
gests PGS abnormality is more detrimental than slow blastocyst de-
velopment.

The results support that embryo growth rate affect the outcome of
warmed blastocysts (Hashimoto et al, 2013). It also indicates there is a nat-
ural selection for normal and healthy blastocyst to avoid un-fruitful preg-
nancy.
**THE PROXIMITY OF WARMED EMBRYO TRANSFER OF “INTENTIONAL FREEZE” EMBRYOS FROM VITRIFICATION IMPACTS IMPLANTATION RATES.** D. L. Hill, a, b J. Crofoot, a, b M. Surrey, a, b H. Danzer, a, b G. E. Kelinger, a, b W. Chang, a, b C. Wambach, a, b J. Barritt, a, b ART Reproductive Center, Beverly Hills, CA; a, bSouthern California Reproductive Center, Beverly Hills, CA.

OBJECTIVE: There is mounting evidence that performing blastocyst transfer at least one menstrual cycle from that in which the patient’s eggs were retrieved improves implantation, presumably due to a more receptive uterine environment. Doing this requires vitrification of blastocysts, warming and transferring them in a subsequent cycle that is 1, 2 or more menstrual cycles from that in which the embryos were created. We wanted to examine if implantation rates were affected by this proximity.

DESIGN: Retrospective data analysis from a private laboratory for assisted reproductive technology.

MATERIALS AND METHODS: From 2011 to 2013, 216 transfers (excluding donors and surrogates) did not have a fresh blastocyst transfer, electing to have all embryos intentionally vitrified. In 104 of these transfers the patient also had PGD by array comparative genomic hybridization (aCGH). Transfers of warmed blastocysts were performed anywhere from within 40 days of the vitrification cycle, 41-70 days or >70 days and beyond, representing endometrial lining recreation from 1–3 cycles. Based on the idea that it would take somewhere <40 days to build a single new endometrial lining, we created three “proximity groups” between vitrification and the warmed ET: <40 days, 41-70 days and >70 days post-vitrification.

RESULTS: Non-PGD embryo transfers occurring <40 days from vitrification had significantly lower pregnancy rates than did those performed 41–70 day group (P<0.0001), but not significantly compared to the >70 day group (P<0.12). In the PGD-defined embryo transfers, the <40 days to ET cases were significantly less likely to conceive (P=0.0001) than the 41–70 day group and the >70 day group. Notably 11/112 (10%) of the cases in the “no PGD” group were >37, whereas 61/104 (59%) of the “PGD” group were >37. This outcome is therefore reflective of a elimination of the “age effect” in patients 37–41 years old when aCGH-defined blastocysts are used for transfer.

CONCLUSION: Our data demonstrates that performing a warmed embryo transfer soon after the vitrification cycle does not allow the most optimal uterine receptivity. Most dramatically demonstrated in warmed cycles after PGD in which at least 2 endometrial lining recreations increased pregnancy rates so significantly that the “age effect” in older patients was overcome by doing intentional freezes with PGD. We strongly recommend not performing warmed ETs of blastocysts <40 days from vitrification.

**MATERIALS AND METHODS:** Ovarian stimulation and oocyte retrievals were performed using standard protocols. Oocyte vitrification was performed on cryoprips (Kitazato, Japan), blastocyst vitrification was performed using Cryotips (Irvine Scientific, USA). Both oocytes and embryos were vitrified using ethylene glycol and DMSO as cryoprotectants in gradually increasing amounts. Blastocysts were collapsed prior to vitrification using a single laser pulse (Xylos). Warming protocols were accomplished using rapid warming first at 37 C, then a gradual return to isotonic media at room temperature.

RESULTS: The results of our analysis can be seen in Table 1. There were no significant differences across any of the parameters studied.

CONCLUSION: Vitrifying and warming oocytes has now become common and is gaining in popularity as an alternative and effective method of delivering donor egg services to patients. Results across the globe are similar to results in fresh donor egg cycles. This has led to the establishment of commercial egg banks. One of the remaining questions is whether or not these previously frozen oocytes, once warmed, inseminated and cultured to later embryonic stages can be again re-vitrified and warmed with similar results as in fresh donor egg cycles. This data set suggests that the laboratory and clinical results are comparable and quite good in both groups.

**TABLE 1. Comparison of Group A and Group B**

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td># Cycles</td>
<td>236</td>
<td>21</td>
</tr>
<tr>
<td># Embryos Vitrified</td>
<td>404</td>
<td>38</td>
</tr>
<tr>
<td>Embryos Survived</td>
<td>388 (96%)</td>
<td>37 (97%)</td>
</tr>
</tbody>
</table>

* Frozen Oocytes from Fairfax Egg Bank

---

**A RANDOMIZED PROSPECTIVE CONTROLLED TRIAL CONFIRMS THE SAFETY AND EFFICACY OF EXTENDED INTRAVAGINAL CULTURE OF EMBRYOS WITH INVOCELL COMPARED TO LABORATORY INCUBATORS.** K. Doody, a J. Broome, a K. Doody, b aCenter for Assisted Reproduction, Bedford, TX; bKelowna Regional Fertility Centre, Kelowna, BC, Canada.

OBJECTIVE: The purpose of this IRB approved trial is to compare the safety and efficacy of intravaginal culture of embryos in INVOCell (INVO Bioscience, MA, USA) to traditional incubator laboratory systems for the purpose of IVF.

DESIGN: Randomized prospective controlled trial.

MATERIALS AND METHODS: Inclusion criteria were infertile women age 18-38, AMH 1-3ng/ml, AFC 6-20, BMI < 36. Patients undergoing stimulation for IVF were randomized on the day of trigger to traditional incubator or the INVOCell vaginal culture device. After a 2-4 hour co-incubation with sperm, up to 10 eggs were placed into the INVOCell device or into traditional incubators. After 5 days, one or two embryos were transferred into the uterus.

RESULTS: 33 enrolled patients underwent ovarian stimulation and were randomized on the day of trigger. Outcome data are reported in the table below (ranges are listed in parentheses).

**TABLE 1.**

<table>
<thead>
<tr>
<th>Number of cycles (patients)</th>
<th>16</th>
<th>17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age</td>
<td>32.3 (26-38)</td>
<td>32.8 (26-38)</td>
</tr>
<tr>
<td>Mean body weight (pounds)</td>
<td>162.0 (121-207)</td>
<td>151.5 (109-184)</td>
</tr>
<tr>
<td>Mean number of oocytes</td>
<td>7.6 (1-10)</td>
<td>6.7 (3-10)</td>
</tr>
<tr>
<td>Mean number of embryos</td>
<td>1.8</td>
<td>1.65</td>
</tr>
<tr>
<td>Mean blastocyst quality score (BQG)</td>
<td>27.24</td>
<td>26.14</td>
</tr>
<tr>
<td>Ongoing pregnancy rate (%)</td>
<td>62.5%</td>
<td>58.8%</td>
</tr>
</tbody>
</table>

CONCLUSION: In vitro fertilization is necessary to achieve pregnancy for many patients with infertility. Unfortunately, the expense of IVF can significantly restrict access to ART services. Many factors contribute to the expense of ART. The modern IVF physical laboratory requires expensive HVAC systems to provide clean air. The embryo has no lung, kidney or liver to filter air contaminants including volatile organic compounds. Incubators
require alarm systems as well as daily quality control checks. Embryologists are highly trained with specialized skills enabling micromanipulation procedures such as ICSI, assisted hatching and embryo biopsy.

Intravaginal culture (INVO) can lower the real cost of conducting IVF. Good blastocyst development and pregnancy outcomes can be achieved with an intravaginal culture system. Implementation of this or similar simple culture systems will likely improve affordability and access to ART services.

O-363 Wednesday, October 22, 2014 05:30 PM

OBJECTIVE: Some state mandates do not distinguish between IVF and FET cycles. This may unintentionally encourage patients to proceed with IVF over FET. Our study aimed to determine which states differentiate IVF and FET cycles and whether state-related differences exist in utilization of FET compared to IVF.

DESIGN: Descriptive.

MATERIALS AND METHODS: Public copies of state legislation mandating ART coverage were reviewed for language distinguishing FET from IVF cycles. We also interviewed financial specialists at large ART centers in each of the relevant states. As a marker of statewide FET utilization, we calculated the proportion of “fresh embryos from non-donor oocytes” cycles to “frozen embryos from non-donor oocytes” transfers from the SART 2012 Data at all centers in each mandated state. Comparisons were performed using χ² test.

RESULTS: 8 states have mandated IVF coverage (AR, CT, HI, IL, MA, MD, NJ, RI). 3 states have either unlimited coverage (MA) or a monetary cap (AR $15,000 and RI $100,000). 2 states (NJ, IL) benefits are limited by number of oocyte retrievals and mandate coverage of associated procedures (including FET). 3 states limit their benefits by number of cycles (HI-1, CT-2, MD-3). HI’s mandate does not cover FET. CT and MD’s mandates do not specify cycle type, leaving the benefit to be exhausted with either IVF or FET. Only the 3 states without cycle limitations (AR, MA, RI) mandate coverage of embryo cryopreservation. States with retrieval-limited mandates had a statistically higher FET/IVF ratio compared to cycle-limited states (3536/10654 vs. 2417/9728, p=0.002). However, the states with the most robust mandate (MA, RI) had the lowest FET/IVF ratios compared to cycle-limited states (p=0.001) and retrieval-limited states (p=0.001).

CONCLUSION: Benefits of FET include decreased morbidity, increased pregnancy rates, and possible improved neonatal outcomes. Cost analyses have demonstrated that an FET cycle costs roughly half as much as IVF. ART centers in cycle-limited states reported cases of mandated patients declining FET in favor of a “covered” IVF cycle. Mandates that limit benefits by cycle number, without respect to type, may financially incentivize patients to undergo the more expensive and morbid IVF cycle. The higher FET/IVF ratio in retrieval-limited states may support this observation. Interestingly, states with the most generous mandates had the lowest proportional utilization of FET, possible owing to the unrestricted nature of coverage.

FERTILITY PRESERVATION II

O-364 Wednesday, October 22, 2014 03:45 PM
NATURAL OVARIAN STIMULATION (NATOS) SUCCESSFULLY COMBINES LOW ESTRADIOL LEVELS AND MULTIPLE FOLLI- CLE DEVELOPMENT AND MAY BE SUITABLE FOR FERTILITY PRESERVATION IN WOMEN WITH ESTROGEN-DEPENDENT CANCERS. E. Adda-Herzog, V. Gallott, A. Le Bras, S. Sebug, N. Frydman, R. Fanchin. Reproductive Medicine, Antoine Beclere Hospital, Clamart, France; Reproductive Biology, Antoine Beclere Hospital, Clamart, France.

OBJECTIVE: Supraphysiologic E2 levels are unwelcome in fertility preservation candidates with breast cancer. Aromatase inhibitors have been used during controlled ovarian hyperstimulation (COH) to avoid hyper-estrogenism but serum oestrogens levels can exceed 600pg/mL when numerous oo-
TOWARD UNDERSTANDING YOUNG WOMEN'S REASONS FOR ACCEPTING OR DECLINING FERTILITY CRYOPRESERVATION TREATMENT FOLLOWING CANCER DIAGNOSIS. P. E. Hershberger,1 H. Sigsgaard,2 L. L. Finnegan,3 J. Hirshfeld-Cytron.1 1University of Illinois at Chicago, Chicago, IL; 2Fertility Centers of Illinois, Chicago, IL.

OBJECTIVE: Effective counseling about fertility cryopreservation treatment for young women with cancer is a critical concern for many clinicians. Yet little is known about young women’s underlying decision processes, including their perspectives on whether to undergo treatment. Therefore, this paper aims to provide insight into young women’s reasons for deciding to accept or decline fertility cryopreservation treatment following a cancer diagnosis.

DESIGN: This descriptive, qualitative study incorporated a mixed-methods approach to corroborate and expand qualitative findings with demographic characteristics.

MATERIALS AND METHODS: Young women (N = 27, mean age = 29 years) were recruited from two clinics in the Midwest and the Internet. Inclusion criteria included women who: were diagnosed with cancer, were eligible for fertility cryopreservation (i.e., egg, embryo freezing), and had recently made a decision about whether to undergo fertility cryopreservation. Each participant completed an in-depth, semi-structured interview and a demographic questionnaire.

RESULTS: Women were diagnosed with 6 cancer types, including breast (n = 14), Hodgkin’s lymphoma (n = 5), ovarian (n = 4), leukemia (n = 3), kidney (n = 1), and non-Hodgkin’s lymphoma (n = 1); one woman was diagnosed with two cancer types. Half (n = 14; 52%) of the women declined fertility cryopreservation treatment, and half (n = 13; 48%) of the women accepted treatment. Women who accepted treatment were more likely to be younger, single, nulliparous, less educated, and at lower income levels compared to women who declined treatment. The results of the qualitative analysis revealed that despite lower income levels, the women who accepted fertility cryopreservation received more financial support from their partners, family, and other sources.

CONCLUSION: Clinicians who are aware of the reasons why women with cancer accept or decline fertility preservation can facilitate decision support in the clinical setting. These findings can also be used as a foundation for the development of survey instruments or decision support tools.


OBJECTIVE: To examine the efficiency and limitations of vitrified oocyte donation (VitOD) vs. fresh oocyte donation (FreOD) when both treatment options are available.

The patient was taken off oral contraceptive pills eight days prior to her exploratory laparotomy. Attempts at cysterectomy were abandoned secondary to intraperitoneal findings and a left salpingo-oophorectomy, lysis of adhesions and appendectomy were performed. Final pathology revealed a benign mucinous cystadenoma. Immediately following the removal of the whole ovary specimen, healthy ovarian tissue was identified, isolated and transferred to our embryology laboratory for aspiration. Ten prophase I oocytes were retrieved. After 24 hours of culture, 4 metaphase II (MII) oocytes were identified and fertilized by intracytoplasmic sperm injection (ICSI). Three two-pronuclear (2PN) zygotes were cryopreserved for future use.

RESULTS: In March 2013, the patient returned for a frozen embryo transfer. She was prepared with a standard protocol of exogenous estrogen followed by timed progesterone. Three zygotes were thawed and she underwent an embryo transfer of 2 cleavage-stage embryos (grade B8 and B7) which resulted in a live birth. In December 2013, the patient delivered a healthy male infant at term.

CONCLUSION: To our knowledge, this is the first IVM of oocytes retrieved from extracorporeal ovarian tissue aspiration leading to a live birth. This technique should be considered as a viable fertility preservation option for any reproductive age woman undergoing oophorectomy or resection of a large segment of viable ovarian tissue.

Supported by: Woodson Foundation.
DESIGN: Patients undergoing VitOD or FreOD cycles from January 2012-April 2014 were included. Laboratory and clinical outcomes were assessed for each group and compared. Comparisons were done using a Students t-test, assuming equal variances on data of a continuous nature, or a Chi-squared test for categorical data.

MATERIALS AND METHODS: Eighty-eight oocyte recipients underwent 90 cycles of VitOD (n=50) or FreOD (n=40), the former using oocytes purchased from an egg bank. The choice of fresh or vitrified oocytes was left to the patient and was based on financial and time considerations and donor availability. Oocytes were warmed according to egg bank protocols.

RESULTS: Results are shown in Table 1.

Cycle Characteristics and Clinical Outcomes of VitOD and FreOD Cycles

<table>
<thead>
<tr>
<th></th>
<th>Vitrified Oocyte Donation</th>
<th>Fresh Oocyte Donation</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td># Cycles (Fresh Transfers)</td>
<td>50</td>
<td>40</td>
<td>.</td>
</tr>
<tr>
<td>Mean Age (±SD)</td>
<td>42.6 (4.11)</td>
<td>41.9 (8.78)</td>
<td>.4903</td>
</tr>
<tr>
<td>Average # MII Eggs (±SD)</td>
<td>6.0 (1.96)</td>
<td>17.0 (7.72)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Normal Fertilization Rate ICSI % (±SD)</td>
<td>82.9 (15.05)</td>
<td>81.4 (21.14)</td>
<td>.6538</td>
</tr>
<tr>
<td>ICSI Damage Rate % (±SD)</td>
<td>4.7 (10.12)</td>
<td>4.2 (6.80)</td>
<td>.7943</td>
</tr>
<tr>
<td>Blastocyst Formation Rate % (±SD)</td>
<td>33.2 (30.25)</td>
<td>64.1 (27.78)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Embryo Cryopreservation/2PN % (±SD)</td>
<td>27.6 (22.98)</td>
<td>47.4 (18.72)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Patients with Cryopreservation/embryo %</td>
<td>64.0%</td>
<td>100.0%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Elective Single Embryo Transfer %</td>
<td>16%</td>
<td>25.0%</td>
<td>0.2888</td>
</tr>
<tr>
<td>Ongoing/Delivered Pregnancy %</td>
<td>42%</td>
<td>52.5%</td>
<td>0.3211</td>
</tr>
<tr>
<td>Implantation Rate (FHE/T) %</td>
<td>33.3%</td>
<td>43.1%</td>
<td>0.2502</td>
</tr>
<tr>
<td>Cumulative Ongoing/Delivered Pregnancy Rate %</td>
<td>58.0%</td>
<td>72.5%</td>
<td>0.1533</td>
</tr>
</tbody>
</table>

CONCLUSION: Vitrified oocytes obtained from commercial egg banks offer an attractive alternative to FreOD for many reasons including convenience and reduced cost. In this study, oocyte survival rates post warming were extraordinarily high and fertilization rates were equivalent to fresh oocytes. Oocytes were warmed according to egg bank protocols.

O-371 Wednesday, October 22, 2014 05:30 PM

DYSREGULATION IN GENE EXPRESSION PROFILE OF CUMULUS CELLS FROM WOMEN WITH OVARIAN AND LUNG CANCERS, S. Assou, A. Ferrières, C. Vincens, A. Gala, D.-B. Sophie, S. Hamamah.

OBJECTIVE: To compare the gene expression patterns of cumulus cells (CCs) from isolated from healthy women and patients with ovarian and lung cancers.

DESIGN: This study includes 16 CCs from healthy women and 16 CCs isolated from mature MII oocytes collected from patients with lung (n=10) and ovarian (n=6) cancers for fertility preservation before chemotherapy treatment.

MATERIALS AND METHODS: CCs from each MII oocyte were analyzed individually using whole genome U133 Plus 2.0 GeneChip Affymetrix microarrays. Analysis of gene expression had been used to identify genes that differentiate CCs from patients with ovarian or lung cancers.

RESULTS: Nine eligible trials (774 women) were included. At the longest follow-up, women receiving GnRH analogues co-treatment didn’t achieve a significant increase in resumption of ovarian function [Risk Ratio (RR) = 1.12, 95% CI: 0.96 to 1.30]. GnRH analogues administration failed to show statistically significant protective effects after multiple subgroup analyses, according to type of malignancy (P=0.13), age (P=0.14), and GnRH analog type (P=0.45). Analogues co-treatment didn’t have a protective effect on ovarian reserve parameters, whether FSH [Mean Difference (MD) = 2.63, 95% CI: -7.33 to 2.07], AFC (MD = 1.6695 CI: -0.69 to 4.01) or AMH (MD = 0.3195 CI: -0.41 to 1.03). The occurrence of spontaneous pregnancy was comparable between GnRH analogues co-treated women and their control (RR of 1.01, 95% CI: 0.36 to 2.81).

CONCLUSION: GnRH analogues administration during chemotherapy does not protect ovaries from gonadal toxicity.

O-370 Wednesday, October 22, 2014 05:15 PM

ADJUVANT GONADOTROPIN-RELEASING HORMONE ANALOGUES FOR THE PREVENTION OF CHEMOTHERAPY-INDUCED GONADAL TOXICITY: A SYSTEMATIC REVIEW AND META-ANALYSIS, E. A. Elgindy, M. I. Mostafa, H. Sibai, A. M. AbdelGhany, Obstetrics and Gynaecology, Faculty of Medicine, Zagazig, Sharkia, Egypt; Obstetrics and Gynaecology, Faculty of Medicine, Cairo, Egypt; Obstetrics and Gynaecology, Faculty of Medicine, Zagazig, Sharkia, Egypt; Obstetrics and Gynaecology, Faculty of Medicine, Zagazig, Sharkia, Egypt.

OBJECTIVE: To study whether the adjuvant administration of gonadotropin-releasing hormone analogues during chemotherapy can protect the ovaries from subsequent development of gonadal toxicity.

DESIGN: Systematic review and meta-analysis.
OBJECTIVE: A preliminary study to determine if the blastocyst stage and grades of the ICM and TE predict implantation for euploid blastocysts transferred in FETs.

DESIGN: Retrospective Analysis of Euploid Blastocyst Implantation.

MATERIALS AND METHODS: Stages and grades of euploid blastocysts transferred in FET cycles were analyzed using multiple logistic regression (MLR) to determine if they indicate any differences in implantation. 254 embryos transferred in 236 FET cycles were considered. All transferred embryos had been biopsied then vitrified and the biopsies underwent array Comparative Genomic Hybridization screening for chromosomal ploidy. All embryos were determined to be euploid (either 46XX or 46XY). MLR was performed using an iterative program that adjusted the fitting parameters using maximum likelihood (in GWBASIC). Significance of parameters was determined using the Akaike Information Criterion. Ability to predict implantation was assessed using area under the curve (AUC) for the receiver operator characteristic (ROC) curves.

RESULTS: 129 euploid embryos resulted in fetal sacs with a heartbeat (FSHB) on ultrasound in which an average of 1.08 euploid embryos were transferred per patient. Age of the oocyte source, day of blastocyst cryopreservation (day 5 or day 6) and Blastocyst scores prior to biopsy (Stage, ICM grade and TE grade) were assessed for their ability to predict FSHBs in FET cycles. ICM and TE grades were coded A = 1, B = 2, C = 3. Day of biopsy/cryopreservation, stage and grade of the ICM were significant predictors of implantation. In(OR) = -0.458 X day – 0.467 X stage – 0.905 X ICM + 6.12. Patient age and TE grade were not associated with implantation. The AUC for the ROC curve constructed using the best fit MLR equation was .65 indicating that this is a marginal predictor of implantation. Blastocysts were segregated using a threshold of In(OR) = 0.298. Above the threshold, 59.4% of the embryos implanted whereas below the threshold 31.4% implanted (p < 0.001).

CONCLUSION: Earlier attainment of expanded blastocyst stage (day of biopsy), less advanced blastocyst Stage and better ICM grade were positively associated with FSHBs arising from euploid blastocysts. While this preliminary analysis provides significant predictability for FSHBs, more data will be required to ascertain whether embryo grades can be predictive of live birth or miscarriage (loss of a fetal sac) for euploid embryos.

O-374 Wednesday, October 22, 2014 04:15 PM

THE ROLE OF PROGESTERONE RECEPTOR ISOFORMS IN THE PREGNANT MYOMETRIUM. M. Peavey,1,2* X. Li,1 M. Wetendorf,1,2 C. Yallampalli,1 J. Lydon,1 F. DeMayo,1,2* Molecular and Cellular Biology, University of Michigan, Ann Arbor, MI, OB/GYN, University of Texas, Galveston, TX, Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX.

OBJECTIVE: Preterm birth complicates 10-12% of pregnancies worldwide with significant economic and healthcare burdens. Progesterone is necessary to establish and maintain pregnancy. Humans undergo a functional progesterone withdrawal for initiation of labor, possibly due to changes in the ratio progesterone receptor isoforms PRA and PRB, as there is an increase in the PRA/PRB ratio at the time of labor. Our goal is to determine the role of these PR isoforms in the maintenance of pregnancy by altering the isoform ratio in the mouse myometrium.

DESIGN: We generated a mouse model in which either the PRA or PRB isoform is specifically overexpressed in the myometrium. The impact of altering myometrial PR isoforms was investigated by assessing fertility, gestational length, pregnancy outcomes, uterine contractility, and myometrial gene expression patterns.

MATERIALS AND METHODS: For overexpression of PR isoforms, the cDNA for the coding region of each isoform was inserted into the CAAG-LSL-NR vector and targeted to the ROSA26 locus. These mice were then crossed to the myosin 11 heavy chain (Myh11) cre mice for tissue-specific expression. The gestational length was unchanged. These mice have a 2-fold increase in uterine contractility in response to uterotonic agents, and increased expression of GJa and decreased ZEB1. Mice with PRB overexpression had an average of 2.0 pups/litter vs 8.3 pups/litter in control mice due to a 50% early pregnancy failure rate. The gestational length was unchanged. These mice have a 2-fold increase in uterine contractility in response to uterotonic agents, and increased expression of GJa and decreased ZEB1. Mice with PRB overexpression had an average of 2.0 pups/litter vs 8.3 pups/litter in controls due to an 80% early pregnancy failure rate. These mice also had an increased gestational length of 21.5 days vs 19.1 days in controls, with a 50% rate of peripartum fetal demise.

CONCLUSION: Mice with overexpression of PRA have reduced litter sizes due to early pregnancy failure, increased uterine contractility in response to stimulants, and increased expression of contraction-associated proteins (CAPs). Overexpression of PRB is associated with significantly decreased litter size, and early pregnancy non-survival in non-running groups. PRB overexpression led to a 50% decrease in implantation compared to control in late pregnancy, in addition to decreased expression of CAPs.

Supported by: NIH U54 HD28934.

O-375 Wednesday, October 22, 2014 04:00 PM

VOLUNTARY EXERCISE IMPROVES ESTROUS CYCLICITY IN PRENATALLY ANDROGENIZED MICE INDEPENDENT OF METABOLIC CHANGES: IMPLICATIONS FOR POLYCYSTIC OVARY SYNDROME (PCOS). L. D. Homa1 S. Moenter1* Ob-Gyn, University of Michigan, Ann Arbor, MI, M.I.Physiol, OB/GYN, Int Med, University of Michigan, Ann Arbor, MI.

OBJECTIVE: To test if exercise improves estrous cyclicity in a mouse model resembling lean PCOS.

MATERIALS AND METHODS: Prenatal androgen (PNA) exposure in mice produces a phenotype resembling lean PCOS. PNA mice were generated by injecting dams with dihydrotestosterone (sc 225 mg/d) on day 16-18 of gestation; control dams were injected with sesame oil vehicle. Four groups of 9 mice each — PNA, PNA running (PNA-R), control (CON), control running (CON-R) — were housed individually at 8 weeks of age, and estrous cycles were monitored daily. Beginning at 10 weeks of age, mice in the running groups were given wheels to allow voluntary exercise. Running wheel activity and estrous cycles were monitored daily; food intake and body mass were monitored weekly. After 15 weeks of running, glucose tolerance and body composition testing by nuclear magnetic resonance were done. Estrous cycles were analyzed by total days spent in diestrus, proestrus (days of luteinizing hormone surge), and estrus. Two 6-week time periods (weeks 1-6 and 10-15 of running) were compared after analysis by week showed changes in estrous cycle parameters after 10 weeks. Data were analyzed by 1-way or 2-way ANOVA with post-hoc testing as appropriate.

RESULTS: Baseline cycles for 2 weeks before running showed PNA treatment disrupted cyclicity with most days in diestrus and few days in proestrus or estrus (p<0.001). There was no effect of running on estrous cycles during weeks 1-6 of running. In contrast, from weeks 10-15 of running, cycles in PNA mice improved with fewer days in diestrus (PNA vs PNA-R d/f=6/0, 37.5±1.9 vs 27.1±2.8 p<0.001), more days in proestrus (2.0±0.9 vs 6.0±1.1 p=0.002), and more days in estrus (2.5±1.0 vs 8.9±1.7 p=0.002). Despite improved cyclicity, PNA-R cycles remained different from CON cycles with more days in diestrus and fewer days in proestrus and estrus. Cycles in CON mice were unchanged by running throughout the study. Food intake was similar in running vs non-running groups.

CONCLUSION: Estrous cyclicity improved after 10 weeks of exercise in PNA mice without change in body mass, body composition, or glucose tolerance due to controls. If this can be translated clinically, the benefit of exercise without the need for weight loss would be important for counseling lean women with PCOS.

Supported by: NIH U54 HD28934.
CONCLUSION: Circulating biomarker levels, as early as 7 and 11 days post embryo transfer (before a positive pregnancy test) are highly predictive of SAB of a normal karyotypic fetus.

Supported by: Institutional.

O-376 Wednesday, October 22, 2014 04:45 PM

MALE FACTOR INFERTILITY IN HETEROGENEOUS PAKISTANI POPULATION. G. Ahmad, a H. L. Khan. a Physiology and Cell Biology, University of Health Sciences, Lahore, Pakistan; Lahore, Punjab, Pakistan; aLahore Institute of Fertility and Endocrinology, Lahore, Punjab, Pakistan.

OBJECTIVE: To analyze semen characteristics in couples consulting for primary infertility.

DESIGN: Cross sectional descriptive study.

MATERIALS AND METHODS: Semen samples from men of 1667 couples consulting for primary infertility of more than year were collected between July 2012 to March 2013. The classic semen parameters i.e. volume, pH, sperm motility, concentration and morphology were analyzed according to the guide lines of WHO.

RESULTS: Among 1667 men 61% of the population appeared with normal semen characteristics and rest 39% had abnormal sperm parameters. Abnormal sperm parameters were further classified into oligospermia, asthenospermia, teratospermia, asthenoteratospermia, oligoteratospermia and azoospermia as displayed in below table:

<table>
<thead>
<tr>
<th>Semen characteristics of study population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td>Normal</td>
</tr>
<tr>
<td>Oligospermia</td>
</tr>
<tr>
<td>Asthenospermia</td>
</tr>
<tr>
<td>Teratospermia</td>
</tr>
<tr>
<td>Asthenoteratospermia</td>
</tr>
<tr>
<td>Oligoteratospermia</td>
</tr>
<tr>
<td>Azoospermia</td>
</tr>
</tbody>
</table>

CONCLUSION: This is the first study conducted on Pakistani men on a larger sample size at one infertility center. We report 39% male factor infertility in Pakistan which is higher than published reports.

O-377 Wednesday, October 22, 2014 05:00 PM

CORRELATION BETWEEN COMPUTER-AUTOMATED TIME-LAPSE ANALYSIS RESULTS AND IMPLANTATION SUCCESS IN PATIENTS OF DIFFERENT AGE GROUPS: A BLINDED, MULTICENTER STUDY. M. D. VerMilyea,a K. Ivani,b G. Gvakharia,a R. Boostanfar,b V. L. Baker,b J. Conaghan.a Penn Fertility Care, Philadelphia, PA; aReproductive Science Center of the Bay Area, San Roman, CA; aPalo Alto Medical Foundation Fertility Physicians of Northern California, San Jose, CA; aHRC Fertility, Encino, CA; aStanford Fertility and Reproductive Medicine Center, Palo Alto, CA; aPacific Fertility Center, San Francisco, CA.

OBJECTIVE: It has been demonstrated that computer-automated measurements of key time-lapse parameters can aid in the selection of embryos with the highest potential to develop to the blastocyst stage (Conaghan et al. 2013). However, the impact of this novel test on clinical outcomes for patients in different age groups remains unclear. The objective of this study was to examine the correlation between computer-automated time-lapse analysis results and embryo implantation for patients in different age groups.

DESIGN: Blinded, multi-center study.

MATERIALS AND METHODS: 205 patients from 6 clinics consented to have embryos imaged using the Eeva® System, a platform technology that automates the analysis of P2 (time between first and second mitosis) and P3 (time between second and third mitosis) and generates a test score of High or Low regarding developmental potential. For this non-selection study, High/Low scores were blinded, and embryos were selected for transfer using only morphology evaluation. Two age groups were analyzed: egg age <35 years and egg age ≥35 years. Implantation was defined by fetal heartbeat at the 6-7 week ultrasound. χ2-test was used for statistical analysis.

RESULTS: In the age group <35 years old, the implantation rates (IR) of High vs. Low embryos were 52% (31/60) vs. 35% (34/97). The difference was statistically significant (p=0.02).

<table>
<thead>
<tr>
<th>IR</th>
<th>&lt;35 years</th>
<th>≥35 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eeva High</td>
<td>52% (31/60)</td>
<td>20% (10/51)</td>
</tr>
<tr>
<td>Eeva Low</td>
<td>35% (34/97)</td>
<td>13% (16/123)</td>
</tr>
<tr>
<td>p-value</td>
<td>0.02</td>
<td>NS</td>
</tr>
</tbody>
</table>

CONCLUSION: It is well known that egg age highly correlates to implantation rates. In this blinded, non-selection study, we have demonstrated that in younger patients Eeva High/Low scores generated from automated cell division timings, correlate well with embryo implantation. We postulate that Eeva Test scores may reflect embryo quality independent of age and that the use of Eeva Test scores to aid embryo selection may improve overall implantation rates. The study is currently ongoing and with increased sample size, will allow us to further evaluate such phenomenon in older patients.

Supported by: Auxogyn, Inc.

O-378 Wednesday, October 22, 2014 05:15 PM

SEQUENTIAL (S) VS. MONOPHASIC (M) MEDIA IMPACT TRIAL (SUMMIT): A PAIRED RANDOMIZED CONTROLLED TRIAL COMPARING BLASTULATION RATES OR ANEUPLOIDY RISK AMONGST SIBLING ZYGOTES. M. D. Werner, a K. H. Hong,a J. M. Fransasiak,a C. V. Reda,b K. Upham,b R. T. Scott, Jr.,b,a REI, RWJ, Rutgers, Basking Ridge, NJ; bRMA NJ, Basking Ridge, NJ.

OBJECTIVE: Optimal media for embryo culture continues to be an area of investigation without consensus. S media claims to be most physiologic and was developed to mimic the in vivo conditions, and consists of two phases. M media seeks to integrate the metabolic requirements of pre- and post-compaction culture into a single formulation for all phases of embryonic growth. The advantage of M formulations is that they require fewer media changes, less manipulation and stress on the embryo, and lower costs. Both culture systems have attained good outcomes, but an adequately
powered controlled assessment of embryo development including usable blastulation rates and aneuploidy risk (where mosaicism may play a role) is lacking.

DESIGN: Paired Randomized Controlled Trial.

MATERIALS AND METHODS: A paired design allowed each patient to serve as their own control, eliminating variables that have confounded prior studies. Due to the paired study design, zygotes were randomized after confirming fertilization and half of each patient’s zygotes were cultured in M medium (Quinn’s Advantage Cleavage Medium; Sage; Blast Assist, Origin) and half in S medium (Continuous Single Culture; Irvine Scientific). Each culture system used separate incubators and went through quality assurance testing and was calibrated per manufacturers’ recommendation. Main outcome measures included blastulation rate, usable blastocyst rate and euploid rate. Usable blastocyst rate included all blastocysts that were suitable for biopsy. Statistical analysis performed via Wilcoxon sum rank tests.

RESULTS: 103 patients (1399 zygotes) had half of their 2 PN embryos randomly assigned to each culture system. All were cultured to the blastocyst stage. No difference was found in overall blastulation rates between M and S culture (p=0.53). A significant difference existed in the proportion of 2 PN embryos which progressed to become usable blastocysts (51.2% sequential vs. 43.9% monophasic, p=0.03). Combining the data from all pairs, the overall odds ratio was 1.34, (95% C.I.1.08-1.65). No difference was noted in the euploid rate amongst usable blastocysts (p=0.50), or for the cohort as a whole (p=0.84).

CONCLUSION: Sequential media yielded a higher usable blast rate for all patients. No difference was noted in the other parameters tested. To determine the ultimate impact of this question paired euploid transfers are now being performed to assess if there is any difference in pregnancy outcome in relation to the type of media utilized.

Supported by: Continuous Single Culture Media was provided by Irvine Scientific.

O-379 Wednesday, October 22, 2014 05:30 PM

SUCCESSFUL OPEN SYSTEM VITRIFICATION OF BOVINE AND HUMAN OOCYTES USING CLEAN LIQUID AIR INSTEAD OF COMMERCIAL LIQUID NITROGEN

P. Patrizi,1 P. Levi Setti,1 F. Menduni,1 M. Leong,1 A. Arau,2 Yale Fertility Center, Yale University, New Haven, CT; 1Gynaecology and Reproductive Medicine, Humanitas Fertility Center, Rozzano, Milano, Italy; 2The Women’s Clinic, Hong Kong, Hong Kong; 1TVF Research Lab, FertileSafe, Ltd, Ness Ziona, Israel.

OBJECTIVE: Oocyte vitrification using open systems has shown superior results over closed systems; however direct exposure of oocytes to commercial liquid nitrogen (LN) carries the risk of contamination (by viruses, bacteria, fungi and spores). To report open system cryopreservation and survival of bovine and human oocytes using clean liquid air produced by a new bench top liquid air system.

DESIGN: Experimental comparative research using bovine and human oocytes.

MATERIALS AND METHODS: Clean liquid air was produced with two size devices (ClAir and ClAir XL, registered trademark FertileSafe, Israel) composed of a sterilized metal container and a cooled inner sink in contact with filtered air, able to produce 70ml and 350 ml of clean liquid air every 10 min, respectively. Bovine GV and MII oocytes and human oocytes were vitrified by direct immersion into sterile styrofoam cups containing liquid air and assessed for survival after warming. Control oocytes were vitrified with standard commercial LN. Human MII oocytes were donated for research (with IRB-protocol) and assessed for survival after vitrification/warming by examining re-expansion and morphology. Vitrification was performed using Cryoleaf (Origio) for the bovine oocytes and Cryotop (Kitazato) for the human oocytes.

RESULTS: Cooling rates between liquefied air and liquid nitrogen were above 20,000 C/minutes for each volume of cryogenic liquid used (50 ml, 25 ml or 10 ml). Bio-burden test showed no contamination in liquid air as opposed to commercial LN. In bovine experiments, 20 GV and 22 MII oocytes were vitrified using clean liquid air while 10 GV’s and 10 MII were controls cryopreserved in LN. The recovery rate was 90% for GV and 91% for MII. The survival rate was 88% (16/18) for GV’s and 95% (19/ 20) for the MII. 88% (16/18) of GV’s matured and the MII cleavage rate was 78% (15/19) (no differences with rates of controls). Of the 7 human MII, 4 were vitrified with liquid air and 3 with LN with experiments repeated three times. Upon warming, 100% survival rate (12/12 for liquid air and 9/9 for LN) was obtained in both groups.

CONCLUSION: The use of clean liquid air instead of LN is a breakthrough methodology for cryopreservation and most importantly eliminates the potential risk of contamination from commercial liquid nitrogen during vitrification with open systems.

CLINICAL FEMALE INFERTILITY II

O-380 Wednesday, October 22, 2014 03:45 PM

A NOVEL PRECISE NON INVASIVE TECHNIQUE TO EVALUATE THE INFERTILE WOMEN: VIRTUAL HISTEROSALPINGOGRAPHY IN 11000 CASES.

P. Carrascosa,1 M. Baronio,1 C. Capunay,2 J. Vallejos,3 C. Suelo,4 S. Papier,5 CT, Diagnosticos Maipu, Vicente Lopez, Buenos Aires, Argentina; 1Cegyr, Capital Federal, Buenos Aires, Argentina.

OBJECTIVE: To illustrate the typical findings of virtual hysterosalpingography (VHSG) by MDCT in daily practice and the differential diagnosis with other pathologies.

DESIGN: We retrospectively evaluated the VHSG studies performed to 11000 patients with infertility (mean age 35.3 ± 3.6 years) derived to our institution between 2006 and 2014.

MATERIALS AND METHODS: We evaluated the V-HSG studies of 11000 patients derived from our institution. Studies were performed using 64,128 and 256 multislice CT scanners. Scanning parameters were: On 64-row CT: slice thickness of 9 mm and a reconstruction interval of 0.45 mm, 120 kV and 100-250 mAs, with an average duration of each scan of 3.6 seconds. On 128 and 256-slice CT: slice thickness of 6 mm and a reconstruction interval of 3 mm, 80 kV and 100-150 mAs, with an average duration of each scan of 1.3 seconds. For visualization of the internal genital organs 10-20 ml of a dilution of low-osmolality iodinated contrast was instilled into the uterine cavity. Images were analyzed using multiplanar reconstructions, 3D and virtual endoscopy. The duration of the CT scan, the radiation exposure and the degree of discomfort of the patients were documented.

RESULTS: The scan time was 3.5 and 1.3 seconds using 64-slice or 128-256-slice CT scanners respectively. The mean radiation dose was 0.9 and 0.3 mSv using 64-slice or 128-256-slice CT scanners respectively. The mean radiation dose was 0.9 and 0.3 mSv using 64-slice or 128-256-slice CT scanners respectively. In the cervical region were identified parietal irregularities (26%), thickening of folds (10%), polyps (8%), diverticula (6%), strictures (7%) and adhesions (1%). In the uterine cavity were visualized polyps (34%), submucosal myomas (9%) and adhesions (4%). In addition changes were observed in the wall of the uterus: myomas (15%), malformations (3.5%), adenomyosis (6%) and cesarean section (11%). Only 4% of the uterine tubes were not visualized completely. Unilateral (8%) and bilateral (1.6%) hydrosalpinx were visualized. Patients reported no or mild discomfort in 85% of the cases.

CONCLUSION: The VHSG allowed a proper assessment of the internal genital organs, providing useful diagnostic information on infertility and other gynecological disorders. The technique is painless, well tolerated by patients with low doses of radiation. These advantages place this modality as a valid alternative algorithm study in patients with infertility.

O-381 Wednesday, October 22, 2014 04:00 PM

TAMOXIFEN IS BETTER THAN LOW DOSE CLOMIPHENE OR GONADOTROPINS IN WOMEN WITH THIN ENDOMETRIUM (<6MM) AFTER CLOMIPHENE IN IUI CYCLES: A PROSPECTIVE STUDY.

S. Sharma, B. R. Geetha, S. Ghosh, I. Saha, A. Sarkar, B. Chakravarty, Reproductive Medicine, Institute of Reproductive Medicine, Kolkata, West Bengal, India.

OBJECTIVE: Second line of treatment in patients with thin endometrium following clomiphene (CC) is gonadotropin stimulation, which is associated with higher cost, multiple births and ovarian hyperstimulation syndrome. Tamoxifen, a selective estrogen receptor modulator acts as an estrogen agonist on endometrium. The objective of the present study was to compare the efficacy of low dose CC, tamoxifen, and gonadotropins in women with thin endometrium (<6mm) following CC in IUI cycle.


MATERIALS AND METHODS: Women (n=502) undergoing IUI with endometrium <6mm after CC 100mg were divided into 3 treatment groups.

ASRM Abstracts Vol. 102, No. 3, Supplement, September 2014
Women in Gr A (n=182, cycles=364) received CC (50mg/day from day3-7),
Gr B (n=179, cycles=221) received Tamoxifen (40mg/day from day3-7) &
Gr C(n=141, cycles=226) received continuous u-FSH 75IU-150IU from day
3 onwards until hCG injection. The number of follicles, size of follicle on day
of hCG , day of hCG, endometrial thickness (ET), pregnancy rate, cancella-
tion rates, multiple pregnancy, miscarriage and live birth were compared.

Comparison of outcomes between different groups

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Gr A (n=182)</th>
<th>Gr B (n=179)</th>
<th>Gr C (n=141)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy rate</td>
<td>4.94%</td>
<td>14.52%</td>
<td>14.89%</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>Miscarriage rate</td>
<td>1.64%</td>
<td>2.2%</td>
<td>2.1%</td>
<td>NS</td>
</tr>
<tr>
<td>Live birth rate</td>
<td>3.2%</td>
<td>12.2%</td>
<td>12.7%</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>No of follicles</td>
<td>2.2±0.58</td>
<td>1.3±0.49</td>
<td>2.3±0.49</td>
<td>ab, bc</td>
</tr>
<tr>
<td>Size of follicles</td>
<td>21.08±0.01</td>
<td>17.61±0.01</td>
<td>11.84±0.01</td>
<td>ab, c</td>
</tr>
<tr>
<td>on day of hCG</td>
<td>7.5±0.46</td>
<td>8.6±0.96</td>
<td>10.07±0.69</td>
<td>ab, ac</td>
</tr>
</tbody>
</table>

RESULTS: Pregnancy and live birth rate were statistically significant in
tamoxifen and gonadotropin gr compared to CC. Tamoxifen results in
cancellation rates due to non response while cancellations in CC were due
to luteinised unruptured follicle and thin endometrium. PCOS women with
tamoxifen had longer follicular phase than CC and gondotropin. In gonad-
otropin group cancellations were mainly due to follicular development
(>4 follicles). There were no multiple pregnancies in tamoxifen gr.

CONCLUSION: Tamoxifen can improve endometrial thickness and live birth rate in patients with thin endometrium after CC. Though its outcome is comparable with gonadotropin, it is devoid of complications.

O-382 Wednesday, October 22, 2014 04:15 PM

ENDOMETRIAL THICKNESS HAS NO IMPACT ON IMPLANTA-
TION RATES OR CLINICAL OUTCOMES IN EUPLOID EMBRYO TRANSFERS.
J. Ginting, a,b M. Whitehouse, a J. Lee, a A. B. Copperman,a,b Reproductive Medicine Associates of New York, New York, NY; Obstetrics, Gynecology and Reproductive Science, Mount Sinai School of Medicine, New York, NY.

OBJECTIVE: Past attempts to correlate endometrial thickness (EnT) with implantation rates (IR) and pregnancy rates (PR) have been biased due to variability in embryo quality and the unidentified genetic composition at em-
byo transfer (ET). With the utility of trophoderm biopsy (TB) and comprehensive chromosome screening (CCS); a more precise analysis can now be performed. To evaluate our hypothesis that a thicker endometrium is more associated with a desired clinical outcome, we assessed whether EnT impacts implantation rate.

DESIGN: Retrospective analysis.

MATERIALS AND METHODS: Patients (n=300) whose embryos undergo-
ted TB and CCS using qPCR technology during IVF cycles (n=399) were included. EnT was measured by transvaginal ultrasound (TV) on the day of hu-
man chorionic gonadotropin hCG trigger and transabdominally at ET. Cohorts were classified by clinical outcomes (chemical pregnancies (CP), TV confirm-
ation of a gestational sac (GS) and ongoing clinical pregnancies (CLP)). Monozygotic twins were considered as one sac in this analysis. EnT ranged from 4.2 - 17.9 mm (median = 8.6 mm). Logistic regression of the EnT (at the time of hCG trigger and ET) against the ratio of the number of GS to the number of euploid ET, along with CP and OCP was evaluated. Statistical analysis was
conducted by chi-square for residual deviance with significance at p<0.05.

RESULTS: CPs (n=280), GS (n=267) and CLPs (n=225) resulted from 538 euploid ET assessed in the study. EnT detected at day of hCG trigger (n=269) was not correlated with CP (p=0.85), GS (p=0.55) or CLP (p=0.72). EnT at the time of embryo trigger (n=399) was not correlated with CP (p=0.83), GS (p=0.88) or CLP (p=0.91). No statistical difference in implantation rates in patients whose endometria were > or <7mm was observed. No correlation between increased thickness and implantation rate was experienced.

CONCLUSION: There has been an extensive debate in the literature regarding the effect EnT on IR. Our study displayed EnT, at the time of hCG trigger or at ET, has no significant correlation with IR, CP or CLP of euploid ETs. Our study demonstrated that within the range of thicknesses studied, EnT did not affect a patient’s chance of achieving a pregnancy. Addi-
tional studies discerning the variability of IR in euploid ET, specifically em-
byo morphology, hormonal factors and abnormal endometrial pathologies, would enhance our current knowledge base on the optimal conditions for suc-
cessful embryo implantation.

Supported by: Medical Scientist Training Program.

O-383 Wednesday, October 22, 2014 04:30 PM

FOLLICLE STIMULATING HORMONE (FSH) DIFFERENTIALLY REGULATES GENE EXPRESSION THROUGH AKT AND ERK1/2 ACTIVATION IN HUMAN GRANULOSA CELLS. S. C. Baungarten,a M. S. Convissar,a M. A. Fierro,b N. J. Winston,b A. M. Zannah,b B. Scoccia,b C. Stocco,b S. Physiology and Biophysics, University of Illinois at Chicago, College of Medicine, Chicago, IL; bDepartment of Obstetrics and Gynecology, University of Illinois at Chicago, College of Medicine, Chi-
cao, IL.

OBJECTIVE: FSH administration is routine for in vitro fertilization (IVF) patients to promote ovarian follicle development; however, the mechanisms by which FSH promotes follicle development, including granulosa cell differ-
entiation, are not clearly defined. The objective of the study was to gain an understanding of signaling pathways activated by FSH to elicit human granulosa cell differentiation.

DESIGN: In vitro studies using primary cultures of cumulus granulosa cells collected from women undergoing IVF at the University of Illinois Hos-
pital.

MATERIALS AND METHODS: Cultured cumulus cells, which respond to FSH similarly to pre-antral granulosa cells, were grown in serum-free and phenol red-free media and treated with FSH for 1 hour. Total protein was isolated and the phosphorylation state of 43 kinases was detected using a phospho-protein array. Results from this array were verified in at least 5 additional patients by Western blot. Additionally, cumulus cells were treated with FSH for 48h in the presence of inhibitors of various intracellular signaling pathways, including MK2206, an AKT inhibitor, and U0126, an ERK1/2 inhibitor. RNA was isolated from these cells and gene expression
measured by quantitative RT-PCR. Differences between the means of different groups were analyzed by t-test and considered statistically signifi-
cant at p<0.05.

RESULTS: After treatment with FSH for 1h, the phosphorylation of AKT as well as PRAS40, a downstream target of AKT signaling, increased nearly 3-fold. This FSH-stimulated AKT phosphorylation was specific for serine 473, and no changes were detected in Akt threonine 308 phosphorylation. When cells were treated with MK2206, the FSH-induced expression of ste-
roidogenic genes, such as Cyp19a1 (aromatase), and growth factors, such as
Igf2 , was abolished. This is the first indication that the AKT/PRAS40 signaling pathway is essential for FSH-induced granulosa cell differentia-
tion. Additionally, ERK1/2 phosphorylation was increased nearly 6-fold after treatment with FSH for 1h. In the presence of U0126, FSH was not able to stimulate Cyp19a1 expression but stimulated Igf2 expression.

CONCLUSION: In human cumulus cells, FSH activates both the ERK1/2 and the AKT/PRAS40 pathways, which differentially regulate the expression of steroidogenic enzymes and growth factors required for granulosa cell differ-
entiation.

Supported by: This work was supported by NIH grant number R01HD057110 (COS); SCB and SMC are supported by NIH training grant number T32HL07692.

O-384 Wednesday, October 22, 2014 04:45 PM

PROGESTERONE CONCENTRATION AT OOCYTE RETRIEVAL DOES NOT PREDICT IVF SUCCESS. S. Chantilis,a,b G. Navarrete,a B. Tilley,a K. Lee,a,b M. Thomas,a,b R. Gada,a,b S. Purcell,a M. Mcintjes,a,e Dallas Fertility Center, Dallas, TX; eDallas Ft.Worth Fertility Associates, Dallas, TX; Frisco Institute for Reproductive Medicine, Frisco, TX.

OBJECTIVE: Elevated progesterone (P) at the time of hCG trigger is re-
ported to negatively impact IVF success. Few studies have investigated the impact of elevated P at the time of oocyte retrieval (TVOR). The objective of this study was to determine if P concentration at the time of oocyte retrieval predicts IVF success.

DESIGN: Prospective, non-randomized observational study.

MATERIALS AND METHODS: Serum P concentrations were measured on a Tosoh AIA 900 at TVOR (36 h post hCG trigger) in IVF patients with a
fresh ET undergoing COH with FSH/hMG mixed regiment and either an agonist (n = 189) or antagonist protocol (n = 124). Donor oocyte and gesta-
tional carrier cycles were excluded. Implantation and ongoing pregnancy were defined as a heartbeat were measured. Patients age groups were: <35 (n = 165), 35-37 (n = 61), 38-40 (n = 56) or >40 (n = 31). Differences between P concentrations in pregnant and non-pregnant patients were compared by paired-t-test. The effect P level at TVOR, age group, and their interaction on implantation and ongoing pregnancy were compared using ANOVA and Chi-square. Cycle type did not have an effect on P at TVOR; therefore, all cycles were analyzed together.

RESULTS: Overall progesterone concentration and number of oocytes retrieved did not differ between pregnant and non-pregnant patients (12.1 vs 11.9 and 16.0 vs 14.8; respectively); however, age was significantly greater in the non-pregnant group (P < 0.05). There was no significant differ-
cence in P concentration between pregnant and non-pregnant patients within any age group. There was no interaction between age group and P level on implantation or ongoing pregnancy, and transfer outcomes based on a range of P values for all patients are shown in the table below (mean ± SE).

<table>
<thead>
<tr>
<th>P at retrieval (ng/mL)</th>
<th>1 - 5.0</th>
<th>5.1 - 10.0</th>
<th>10.1 - 15.0</th>
<th>&gt;15</th>
</tr>
</thead>
<tbody>
<tr>
<td>N patients</td>
<td>41</td>
<td>116</td>
<td>76</td>
<td>80</td>
</tr>
<tr>
<td>Age</td>
<td>37.2±0.8</td>
<td>34.4±0.4</td>
<td>33.1±0.5</td>
<td>34.6±0.4</td>
</tr>
<tr>
<td>Oocytes retrieved</td>
<td>5.9±0.5</td>
<td>12.3±1.0</td>
<td>18.3±0.9</td>
<td>21.8±1.1</td>
</tr>
<tr>
<td>Implantation</td>
<td>24.6±5.7</td>
<td>37.1±3.9</td>
<td>29.2±4.7</td>
<td>36.0±4.7</td>
</tr>
<tr>
<td>Ongoing pregnancy</td>
<td>34.1±5.1</td>
<td>44.0±4.0</td>
<td>36.8±4.2</td>
<td>43.8±4.9</td>
</tr>
</tbody>
</table>

a,b,c,d Means in the same row with different superscripts are different (P < 0.05).

CONCLUSION: Progesterone at the time of TVOR is not a predictor of IVF success, but does correlate with patient age and oocytes retrieved. Caution should be exercised in determining when to cryopreserve all embryos from a cycle based on a single measurement of progesterone.

O-385 Wednesday, October 22, 2014 05:00 PM

SUPEROVULATION ALTERS THE EXPRESSION OF ENDOMETRIAL GENES CRITICAL TO ANGIOGENESIS, TISSUE REMODELING AND PLACENTATION. M. A. Mainigi,* S. Schon,* F. Wang,* T. Ord,* R. Feng,* C. Coutifaris.* *Department of Obstetrics and Gynecology, University of Pennsylvania, Philadelphia, PA; †Department of Biostatistics and Epidemiology, University of Pennsylvania, Philadelphia, PA.

OBJECTIVE: To examine the effect of ovarian stimulation on endometrial gene expression and DNA methylation during the earliest stages of embryo invasion.

DESIGN: Prospective cohort study.

MATERIALS AND METHODS: Endometrial biopsies were obtained from 12 oocyte donors 11 days following hCG trigger and 12 naturally cycling women 11 days following LH surge. Gene expression in the biopsy specimens were analyzed using the Affymetrix Human Gene 1.1 ST array and the Infinium DNA methylation 450K BeadChip. Pathway analysis was performed to determine functional gene groups represented in the dataset and quantitative real time PCR (qRT-PCR) was performed to validate a subset of genes from the microarray results.

RESULTS: 207 genes were differentially expressed in the endometrium following superovulation when compared to controls. None of these genes with altered expression showed significant changes in DNA methylation. Analysis of this gene set demonstrated enrichment for multiple genes involved in endometrial remodeling, including PLAT, HSPSE2, MMP2 and TIMP1. Validation studies utilizing qRT-PCR found a 75% reduction in expression of heparanase 2 (HSPE2) and another associated with both angiogenesis and cell invasion (P < 0.0007). Conversely, superovulated endo-
methym presented a 2.3 fold increase in tissue-type plasminogen activator (PLAT), a serine protease participating in matrix degradation (P = 0.01).

CONCLUSION: Fresh IVF cycles have been associated with an increased risk of abnormal placentation leading to higher rates of low birth weight and preeclampsia when compared to pregnancies occurring after either natural conceptions or frozen embryo transfer. In this study we demonstrate that superovulation alters the expression of genes (such as PLAT and HSPE2) critical to endometrial remodeling during the earliest stages of placentation. Such gene expression changes could lead to altered trophoblast migration and impaired endovascular invasion and may suggest a potential mechanism for the adverse perinatal outcomes observed following embryo transfer during fresh IVF cycles.

Supported by: This work was supported by the Penn Presbyterian George L. and Emily McMichael Harrison Fund for Research in Obstetrics and Gy-
necology and the Reproductive Scientist Development Program (2K12HD00849-26).

O-386 Wednesday, October 22, 2014 05:15 PM

OPTIMIZING PATIENT ANALGESIC EXPERIENCE DURING IN VITRO FERTILIZATION. T. Gotz,* D. A. Kiddoo,* T. Motan;* Obstetrics & Gynecology, University of Alberta, Edmonton, AB, Canada; ¹Urology, University of Alberta, Edmonton, AB, Canada.

OBJECTIVE: To determine if lidocaine paracervical block (PCB) is effective in reducing pain during oocyte retrieval when combined with modern conscious sedation.

DESIGN: Double-blind, placebo-controlled, randomized control trial.

MATERIALS AND METHODS: Women receiving IVF/ICSI at an academic fertility centre were randomized to either lidocaine PCB (study) or placebo with saline (control) during egg retrieval. All patients received conscious sedation with fentanyl & midazolam based on weight. Patients could request additional narcotics for excess pain. Prior to discharge, patients completed the Short Form McGill Pain Questionnaire (SFMPQ), a validated tool for measuring gynecological pain. Primary outcome was pain score measured by SFMPQ. Secondary out-
comes were amount of conscious sedation required & number of good quality embryos cultured.

RESULTS: 200 women were enrolled & analysed. There was no differ-
ence in baseline demographics or stimulation parameters between study & control groups. Means for age [34.1], BMI [25.2], FSH [7.3], total gonadotropin dose [3666 ug], stimulation days [9.7] & peak E2 [4436 pg/ml]. The study group had significantly less pain than controls: total SFMPQ score [7.4 vs 10.5; P = 0.002] & visual analog scale (VAS) [27.2 vs 42.3; P < 0.005]. The Short Form McGill Pain Questionnaire (SFMPQ) consists of a sensory component & an affective component that combines to give a total score. Pain was scored on a 100 mm line, the Visual Analog Scale (VAS). Patients also rated their Present Pain Intensity (PPI) on a scale of 0-5. The study group required significantly less IV fentanyl during conscious sedation [112 vs 121 mcg; P = 0.05]. There was no difference in stimulation or embryo outcomes between groups. Means for oocytes retrieved [12.8], fertilization rate [61.7%] & good quality embryos [4.2]. No adverse reactions were reported in either group.

CONCLUSION: Patients receiving lidocaine PCB have less pain & require less narcotic than controls. Lidocaine PCB is safe & does not impact preimplantation embryo development.

Mean Pain Scores & conscious sedation used during egg retrieval

<table>
<thead>
<tr>
<th></th>
<th>Study-Lidocaine (SD)</th>
<th>Control-Saline (SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFMPQ (sensory)</td>
<td>6.0 (5.1)</td>
<td>8.9 (5.7)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>SFMPQ (affective)</td>
<td>1.4 (1.5)</td>
<td>1.6 (1.9)</td>
<td>0.42</td>
</tr>
<tr>
<td>SFMPQ (total)</td>
<td>7.4 (6.0)</td>
<td>10.5 (6.9)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>VAS (mm)</td>
<td>27.2 (23.2)</td>
<td>42.6 (22.1)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>PPI</td>
<td>1.2 (0.8)</td>
<td>1.3 (1.0)</td>
<td>0.98</td>
</tr>
<tr>
<td>Fentanyl (mcg)</td>
<td>112.2 (34.7)</td>
<td>122.7 (37.6)</td>
<td>0.05</td>
</tr>
<tr>
<td>Midazolam (mcg)</td>
<td>1.9 (0.3)</td>
<td>1.9 (0.3)</td>
<td>0.57</td>
</tr>
</tbody>
</table>

O-387 Wednesday, October 22, 2014 05:30 PM

THE UTILITY OF THE SHOCK INDEX TO PREDICT HEMOPERI-
TONEUML OF ECTOPIC PREGNANCY. M. Morita, Y. Katagiri, T. Tsuchiya, M. Kitamura. Department of Obstetrics and Gynecology, Toho University School of Medicine, Ota-ku, Tokyo, Japan.

OBJECTIVE: To assess the clinical utility of the Shock Index (SI: ratio of the heart rate to systolic blood pressure) to predict hemoperitoneum in cases of ectopic pregnancy.
O-388 Wednesday, October 22, 2014 04:35 PM

MATURATION RATE AND SUBSEQUENT BLASTOCYST DEVELOPMENT OF HUMAN OOCYTES DERIVED FROM VARIOUS FOLLICLE SIZE AT THE TIME OF OOCYTE PICK UP: T. Wada,a M. Ikegami,b Y. Nagase,b Y. Yamamoto,b Y. Matsuda,b J. Yonezawa,b M. Ikegami,b H. Yonezawa,a T. Matsuura.a aACT Tower Clinic, Hamamatsu, Sizuoka, Japan; bCenter for Reproductive & Developmental Medicine, Hamamatsu, Sizuoka, Japan.

OBJECTIVE: Mature oocytes were often obtained from various follicle diameter by ultrasound diagnosis. We have estimated follicle size based on amount of follicle fluid at the time of oocyte pick up (opu) in conventional IVF and ICSI since 2013. The objective of this study was to make it appear that the relationship between follicle size at the time of opu, maturation rate of oocyte and subsequent development.

DESIGN: This study was a retrospective analysis of maturation rate of oocytes derived from large (≥ 18 mm; LF), medium (16-17 mm; MF) and small (≤ 15 mm; SF) follicle at the time of opu, and subsequent cleavage of the oocytes and blastocyst development were assessed on day 2 and day 5 after ICSI between 2013 and 2014.

MATERIALS AND METHODS: From August 2013 to March 2014, we studied maturation rate of oocytes and developmental rate of embryos to the blastocyst stage derived from large (≥ 18 mm; LF), medium (16-17 mm; MF) and small (≤ 15 mm; SF) sized follicles for 306 cycles induced by clomiphene or letrozole-RFSH-GnRH administration. Follicle fluid was measured individually follicles after oocyte picked up. Then, the follicle size was calculated with volume of the fluid. The oocytes were examined for maturation and fertilization. Cleavage of the oocytes and blastocyst development were assessed on day 2 and day 5. All blastocysts were vitrified and stored for a single blastocyst transfer in the next cycle.

RESULTS: Maturation rate of oocytes derived from large follicle 87.5% (239/273) were significantly higher than medium and small follicle (69.5% (41/59) and 36.8% (53/144) P<0.05, respectively). The percentages of normal fertilization did not differ between large 62.8% (150/239), medium 78.0% (32/41) and small 64.2% (34/53) follicle size. Although there was not significantly different in the blastocyst development rates, the oocytes derived from medium 51.2% (20/41) follicle were higher blastocyst development rate than large 39.3% (145/239) and small 30.2% (7/53) follicle size.

CONCLUSION: These results suggest that follicle size affected maturation rate at the time of opu. Although follicle size did not affected fertilization, the blastocyst development could be higher with oocytes derived from medium follicle. Follicle size based on amount of follicle fluid at the time of opu could be one of criteria for selecting to transfer embryo.

O-389 Wednesday, October 22, 2014 04:00 PM

DELAYED AND IMPAIRED DEVELOPMENT IN EARLY MOUSE EMBRYO WAS INDUCED BY SPERM DNA DAMAGE. M. Kobayashi, A. Yoshida. Kiba Park Clinic, Koto, Tokyo, Japan.

OBJECTIVE: Some studies have indicated that DNA damage in spermatozoa could induce some developmental defects such as low fertilization rates, poor embryo quality, and developmental arrest. Further, it is suggested that spontaneous miscarriage and childhood disease may be related to sperm DNA damage. However, the mechanisms of these defects on embryos with damaged sperm DNA have not yet been cleared. In this study, we created embryos using spermatozoa with DNA damage and evaluated the influence of the damage on some developmental events of early embryos such as pronuclear formation, DNA replication, and cleavage.

DESIGN: The timing of pronuclear formation, DNA replication, and cleavage were compared between embryos with DNA damage-induced spermatozoa and those with normal spermatozoa.

MATERIALS AND METHODS: Spermatozoa derived from C57BL/6J mice were exposed to 1mM H2O2 for 30 min to induce DNA damage (oxidatively damaged spermatozoa: ODS). ODS and normal spermatozoa (control) were injected into ovulated MII oocytes, and both embryos were cultured in KSOM. After 5.5 hr from ICSI, DNA replication in pronuclei was estimated by using the DNA replication assay kit with EdU. Time-lapse monitoring has been used to examine the development of each embryo. Further, the damages in DNA in male pronucleus and developing embryos were detected by immunohistochemical staining with γ-H2AX antibody.

RESULTS: There was no significantly difference in the rate of male and female pronuclear formation between ODS and control groups. However, DNA replication assay at 5.5 hr after ICSI revealed that the rate of EdU-positive pronuclei in ODS embryos was significantly lower than that of control (58.8% vs 81.3%, P<0.05). The time-lapse analysis revealed that the average time points of pronuclear, 1st cleavage, and 3rd cleavage of embryos with ODS were delayed remarkably compared with control (16.9 vs 14.7hr, 20.1 vs 17.0, 43.5 vs 40.1, respectively, P<0.01). By immunohistochemical staining, male pronucleus and nuclei in developing embryos with ODS were positive for γ-H2AX, and extranuclear DNA fragments were frequently observed in ODS embryos. In addition, the blastocyst rate of ODS was significantly lower than control (15.4% vs 76.7%, P<0.05).

CONCLUSION: In this study, we demonstrated that DNA damage in spermatozoa induced slowing DNA replication at the pronuclear stage and delayed time points of early developmental events. In addition, it was suggested that sperm DNA damage caused abnormal DNA fragmentation in embryos and finally led to impaired embryonic development.

O-390 Wednesday, October 22, 2014 04:15 PM


OBJECTIVE: Validating the temperature and humidity of equipment accurately and continuously is extremely difficult. In particular smaller incubators, such as Benchtop incubators used in IVF laboratories, are difficult to continually access and it is not acceptable to rely solely on the external display readings. The aim of this study was to assess a novel small calibrated incubator 24h a day.

MATERIALS AND METHODS: Different commercial incubators were assessed over time using a Thermochron temperature and humidity monitoring device (Maxim Products), along with a data collection system developed by Thermodata Corporation. The Thermochron devices are calibrated by Thermodata using a system accredited to ISO/IEC 17025 and traceable to National Institute of Standards and Technology (NIST) standards. The monitoring device was adapted for use in an incubator to continually record temperature and humidity in small areas. The Thermochron button is approximately 16 mm in diameter and 6 mm thick, does not take up valuable dish space, and enables the user to take readings from 1 to 60 minute intervals to examine the accuracy of an incubator 24h a day.
RESULTS: The Thermochron buttons have been validated in a large IVF laboratory for >18 months. Tracking of Benchtop incubators has shown them to be extremely reliable in maintaining any±0.1 deg. C and a humidity reading >80%. Temperature measurements in media and in a dry petri dish were the same. The continual monitoring allowed us to: (i) validate external display readings; (ii) assess incubator usage during the daytime so as to distribute usage more evenly; (iii) gauge recovery times of different types of incubators and, (iv) challenge incubators with alterations in temperature with the same device detector instance. One side of a Benchtop incubator read normal (37.0) on the display however the Thermochron button recorded a continual overnight reading of 37.4. The ability to monitor this deviation and validate it with other Thermochron buttons allowed a rapid detection and correction, pre-empting any disturbance in embryo culture conditions.

CONCLUSION: Real time temperature and humidity monitoring allows a strict Quality Control of routine IVF instrumentation and in particular Benchtop incubators. The monitoring of temperature sensitive areas to maintain a constant, regulated state of control for products affected by temperature changes is critical for proper medical laboratory operation from a quality perspective.

O-391 Wednesday, October 22, 2014 04:30 PM
EXTENDED CULTURE TO DAY 7 INCREASES EUFLOID EMBRYO YIELD PER IVF CYCLE. S. Vaccari, J. Conaghan, P. Chenette. Pacific Fertility Center, San Francisco, CA.

OBJECTIVE: Improved culture conditions allow extended culture of blastocysts to day 7 (D7), generating additional clinically useful embryos (Kovalskiy et al., 2013, Fertil Steril, 100:1008-12). The objective of this study is to compare euploidy rates in D7 embryos to those found in sibling D5 and D6 embryos across patients in all age groups.

DESIGN: The study included IVF cycles in which trophectoderm biopsy was performed without fresh embryo transfer. All embryos were vitrified immediately after biopsy and frozen embryo transfers were scheduled for a subsequent cycle. Comprehensive chromosome screening (CCS) for 24 chromosomes was performed for each embryo using SNP microarray (Natera Inc., San Carlos, CA).

MATERIALS AND METHODS: D5, D6 or D7 embryos were biopsied once embryos developed beyond the early blastocyst stage and were of reasonable quality. The zona was breached with laser assist on day 3. After hatching, 3-6 trophoderm cells were removed using a laser (Research Instruments, UK) in a region where the ICM would not be harmed.

RESULTS: Over the span of 3 months following the introduction of D7 embryo biopsy, 56% of cycles (79/142) were scheduled for CCS. One or more blastocysts were biopsied in 66 cycles (84%), and in 27 cycles (40%) at least one D7 embryo was biopsied. Out of 157 embryos, 43 (27%) were biopsied on D7. Of blastocysts biopsied on D5, D6, and D7, euploid embryos comprised 78% (21/27), 70% (60/86) and 50% (21/42) of the blastocysts respectively. D5 and D7 contributed equally to the pool of euploid embryos. The number of embryos available for biopsy on D7 increased with maternal age as did the likelihood of finding a euploid embryo.

CONCLUSION: This is the first study that focuses on the ploidy of human embryos cultured to D7 after oocyte pickup. Most euploid embryos were found on D6. D5 and D7 contributed equal numbers of euploid embryos. Maternal age is correlated with slower blastocyst development, and as a result, more euploid embryos were found at D7 in older patients. Culture to D7 resulted in 27% more embryos to biopsy, and 50% of biopsied D7 embryos were euploid, suggesting that D7 embryos could be clinically important. Implantation and live birth rates will be determined when these embryos are warmed for transfer.

O-392 Wednesday, October 22, 2014 04:45 PM
EFFECT OF LASER ASSISTED HATCHING (LAH) ON FRESH BLASTOCYSTS AND CLINICAL OUTCOMES. S. Henderson, E. Garcia-Cerrudo, A. Mahfoudh, H. Holzer, T. Tulandi, W.-Y. Son. MUHC Reproductive Centre, Montreal, QC, Canada.

OBJECTIVE: The purpose of this study was to evaluate the clinical outcomes of LAH performed on fresh-day 5 blastocysts prior to transfer.

DESIGN: This was a retrospective matched case-control study which included patients who underwent a fresh-day 5 blastocyst transfer between May 2013 and April 2014 at the MUHC Reproductive Centre.

MATERIALS AND METHODS: Patients undergoing fresh stimulated cycles as well as in-vitro maturation cycles were included in this study. Blastocysts were collapsed first using the laser then had LAH performed on the same side as the inner cell mass. Approximately 5-8 laser pulses were delivered in order to completely rupture the zona pellucida (ZP) along roughly ¼ of its diameter. Early stage blastocysts did not undergo shrinking prior to LAH but an opening in the zona was created via 2-3 laser pulses ensuring that the inner ZP layer had been breached. A control group was generated from fresh day 5 blastocyst transfer cycles that did not undergo LAH. Characteristic differences among the groups were analyzed using Z-test or Student’s t-test and outcomes were compared using chi-square test and Fisher’s exact test when appropriate. A P-value <0.05 was considered significant.

RESULTS: A total of 208 cycles were included in the study, 104 in each group. The two groups were comparable in that there were no significant differences in age, serum FSH level, antral follicle count, stimulation protocol or cause of infertility. Additionally, the number of oocytes collected and fertilized as well as the number of embryos transferred and implanted did not differ. The overall pregnancy rate and clinical pregnancy with fetal cardiac activity in the LAH group were 56.73% and 40.38% and in the control group were 49.04% and 36.54% respectively. Among women over 40 years old, the implantation rate was 28.57% in the LAH group compared to 8.33% in the control group (p<0.05). Similarly, the rate of pregnancy with fetal cardiac activity was higher in the study group than in the control group (32.14% vs. 8.33%, p<0.05).

CONCLUSION: Women over 40 years old have a decreased pregnancy rate. Assisted hatching of fresh blastocysts can benefit older patients who traditionally have lower chances of success.

O-393 Wednesday, October 22, 2014 05:00 PM
NON-INVASIVE OMICS ANALYSIS OF ENDOMETRIAL SECRETIONS 24 HOURS PRIOR TO FROZEN EMBRYO TRANSFER IS PREDICTIVE OF IMPLANTATION OUTCOME. J. C. Parks, B. R. McCallie, A. Strieby, S. McReynolds, W. B. Schoolcraft, M. G. Katz-Jaffe, a,b "National Foundation for Fertility Research, Lone Tree, CO; "Fertility Laboratories of Colorado, Lone Tree, CO.

OBJECTIVE: Management of unexplained repeated implantation failure (RIF) patients is a major challenge in ART clinic. It is well known that successful implantation is dependent on the intimate dialogue between a viable embryo and a receptive endometrium. On the maternal side, specific property changes in adhesion need to occur for blastocyst attachment, as well as tight regulation of signaling pathways in the microenvironment of the invading embryo. The aim of this study was to examine ahead of embryo transfer the uterine fluid milieu, including the microRNAome and proteome, in association with implantation outcome.

DESIGN: Research study.

MATERIALS AND METHODS: Infertile patients (n=30) were recruited with IRB consent prior to an estradial/progesterone replacement frozen embryo transfer (FET) with euploid blastocyst. Uterine secretions were collected by gentle aspiration, either 24h ahead or at FET. Uterine secretome analysis was performed blinded of implantation outcome using TaqMan® Low Density human miRNA Array (Thermo Fisher) and tandem liquid chromatography mass spectrometry (LC-MS/MS) (ThermoFinnigan). Analysis of miRNA profiles was performed with REST® statistical software. MS/MS spectra was analyzed using MascotTM (version 2.2) and Scaffold (version 2.06) software. P value of <0.05 was considered significant.

RESULTS: The maternal expression of 29 miRNAs was associated with positive implantation 24h ahead of FET (P<0.05). Notably, 3 miRNAs, miR-891a, miR-522, miR-198, were completely absent from the aspirated uterine fluid of failed implantation. Using the LC-MS/MS proteomic strategy, 469 uterine fluid proteins were reliably identified. Relative quantification revealed significantly reduced expression with positive implantation for the MUC protein family (P<0.05). MUC1 proteins are epithelial cell surface proteins that have considerable effect on endometrial function creating a barrier to implantation. Specifically, MUC1 removal is essential for successful implantation, and was observed at increased protein levels with implantation failure.

CONCLUSION: Embryo-endometrial dialogue is a crucial contributor to implantation success. Aberrant uterine miRNA and protein secretions have

OBJECTIVE: To investigate if nuclear status at 2 cell stage and reverse cleavage between 2–4-cell stage of transferred embryo, are related to implantation outcome.

RESULTS: Retrospective study.

MATERIALS AND METHODS: After Intracytoplasmic sperm injection, the metaphase II oocytes were cultured in EmbryoScope (Unisense) time-lapse system. Transferred embryos were retrospectively analyzed in EmbryoViewer database. Ninety-one transferred embryos from 52 women were analyzed. Forty-one implanted transferred embryos were from 25 patients and 50 non-implanted from 27 patients. The presence of multinucleation at 2-cell stage and reverse cleavage were compared between positive and negative implanted groups. Reverse cleavage (RC) was defined as a decrease in the number of cells during cytokinesis. Namely, cells in blastomere fused together to form a blastomere with lesser cells, and they cleaved again after that. This phenomenon was specifically observed with time-lapse imaging system, and noticed previously [1]. Implantation was assessed by ultrasound in 4 weeks after embryo transfer. Double embryo transfers with only one heart beat were excluded. Data were analyzed using χ²-test and P-value < 0.05 was considered significant.

RESULTS: In the implanted group, 21(51.2%) embryos were observed with mononucleation at 2-cell stage, while 15 (36.6%) presented 1-cell multinucleation (C1), and 5 (12.2%) presented both cells multinucleation (C2), and 5 (12.2%) presented RC. In the non-implanted group, 25(50%) embryos were observed with mononucleation at 2-cell stage, while 14 (28%) presented C1, 11 (22%) presented C2, and 6 (12%) presented RC. Comparisons of 2-cell stage mononucleation and multinucleation rates between implanted and non-implanted were not significant.

The implantation rates of mononucleation, C1, and C2 were 45.7%, 51.7%, and 31.3% respectively. The implantation rates of RC and non-RC groups were 45.5 % and 45%. The differences between these implantation rates were not significant either.

CONCLUSION: Blastocystcs from mono and multinucleated 2-cell embryos have equal chances of implantation success. Reverse cleavage is not a significant factor in unsuccessful implantation.

O-395 Wednesday, October 22, 2014 05:30 PM

INJECTION DEVICE BUILT ON A 3D PRINTER FOR A LAB-ON-A-CHIP. S. H. Cheong, Q. V. Neri, Z. Rosenwaks, G. D. Palermo, Reproductive Medicine, Weill Cornell Medical College, New York, NY; Clinical Sciences, College of Veterinary Medicine, Ithaca, NY.

OBJECTIVE: To produce an inexpensive and reliable needle guide for a microfluidic cartridge to carry out ICSI-on-a-chip and obviate the need for standard micromanipulators.

DESIGN: An integrated ICSI microfluidic chip consisting of sections such as oocyte loading, cumulus removal, injection chamber, and embryo culture was placed within a 3D printed cartridge. A separate 3D printed needle holder was produced which incorporates a tongue-in-groove feature to fit the microfluidic cartridge.

MATERIALS AND METHODS: Microfluidic chips were produced by casting PDMS on SU-8 molds patterned by photolithography, and oxygen plasma bonded to a quartz base. The microinjection device as was 3D printed using a Rocket DVI UII catheter (Hingham, MA) utilizing the outer sheath to limit contamination from the vagina and cervix. Aspirates were placed in DNA free PCR tubes and DNA sequencing performed along with negative controls. The sequence was compared to the SILVA ribosomal database allowing for identification and relative quantification of bacteria present in the sample.

RESULTS: 25 unique samples were obtained for analysis. The bacterial genus depth count over all the samples was ranked yielding the most common bacterial genus across all samples pooled together. Additionally, the average ratio of counts within each sample was ranked yielding the most common genus when taking into account the rank order from each individual specimen.

CONCLUSION: The popularity of time-lapse culture chambers has revamped the concept of microfluidic chips that would envision a micro-embryology lab environment. The possibility to add a module to the microfluidic chamber capable of insemiinating oocyte(s) by ICSI would bring this vision to fruition. A needle guide on a microchip holder produced on a 3D printer would serve this purpose. While the device requires further refinement, the next challenge is to deal with the back loading of individual spermatozoa.

Supported by: Reproductive Medicine, Weill Cornell Medical College.

REPRODUCTIVE BIOLOGY - HUMAN

O-396 Wednesday, October 22, 2014 03:45 PM


OBJECTIVE: There is growing interest in the microbiome (MB) of the reproductive tract. The vaginal and placental MB have been partially characterized and shown to be related to obstetric outcomes. Given the large number of unexplained IVF failures, it is plausible that the MB of the uterus is impactful. However, to date the uterine MB is uncharacterized and difficult given the small fraction of microbes that can be cultured successfully. This has led to novel culture free approaches to bacterial genus detection which focus on sequencing of 16S ribosomal DNA. This study utilizes a highly sensitive and specific molecular genetic technology to characterize what, if any, bacteria are present in it in the uterus.

DESIGN: Laboratory.

MATERIALS AND METHODS: An extensive validation of 16S ribosomal gene sequencing, focusing on the V4 region (a 250-270 nucleotide subset), using Next Generation Sequencing was performed to enable analysis. Aspiration of uterine fluid was performed using a Rocket DVI UII catheter (Hingham, MA) utilizing the outer sheath to limit contamination from the vagina and cervix. Aspirates were placed in DNA free PCR tubes and DNA sequencing performed along with negative controls. The sequence was compared to the SILVA ribosomal database allowing for identification and relative quantification of bacteria present in the sample.

RESULTS: 25 unique samples were obtained for analysis. The bacterial genus depth count over all the samples was ranked yielding the most common bacterial genus across all samples pooled together. Subsequently, the average ratio of counts within each sample was ranked yielding the most common genus when taking into account the rank order from each individual specimen.

Most common bacterium in the uterine microbiome by genus

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Average of Ratios from Individual Samples</th>
<th>Overall Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gardnerella</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bifidobacterium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eremococcus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactobacillus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Veillonella</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptococcus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acinetobacter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aerococcus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corynebacterium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactobacillus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bifidobacterium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aerococcus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clostridium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Veillonella</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptococcus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sphingomonas</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FERTILITY & STERILITY®
e135
CONCLUSION: More difficult embryo transfers were strongly associated with a lower likelihood of ongoing pregnancy. After controlling for confounding variables, the presence of microscopic blood in the transfer catheter was not associated with the likelihood of pregnancy and thus was not an independent predictor of cycle outcome. This indicates that the difficulty of the transfer itself was a much stronger negative predictor of pregnancy.

Supported by: This work was supported, in part, by the Program in Reproductive and Adult Endocrinology, NICHD, NIH, Bethesda, MD.

O-398 Wednesday, October 22, 2014 04:15 PM


OBJECTIVE: Recurrent pregnancy loss (RPL) is defined by two or more failed pregnancies [1]. This is an etiologically diverse group, but for substantial part of the group no explanation for the cause is found (40-50% cases). Autoimmune abnormalities are considered as etiological factors in up to 20% of cases [2]. The recognition of HLA-C expressing trophoblasts by maternal KIR positive NK cells populating uterine mucosa during pregnancy is important for successful placentation [3]. Previous studies on HLA-C and KIR genes suggest that certain combinations of these polymorphic genes might be associated with pregnancy disorders and the presence of activating KIR2DS1 might be beneficial for placentation.

METHODS: KIR genes and haplotype frequencies were analyzed in a N. American cohort of women with RPL to evaluate whether there is a genetic susceptibility to RPL based on KIR repertoire along with cognate HLA-C ligand co-expression and etiological differences depending on presence of KIR2DS1.

MATERIALS AND METHODS: 117 women with 2 or more RPL and without uterine/hormonal abnormalities, inflammatory/neoplastic disorders were genotyped for KIR and HLA-C. Gene frequencies were compared with data for corresponding populations obtained from Allele Frequency Net. Also 68 of the RPL women were tested for various auto-antibodies.

RESULTS: Analysis of the study population based on presence/absence of KIR2DS1 revealed that the distribution of HLA-C1 and C2 alleles differs significantly between two groups. KIR2DS1 was detected in 40.2% of women and was associated with increased frequency of its cognate ligand HLA-C2 compared to KIR2DS1neg population (0.5213 versus 0.3786, p=0.0274) or compared to frequencies for reference populations. Also, KIR2DS1 presence was accompanied with increased frequency of a non-functional deletion form of KIR2D4. The functional KIR2DS4 allele was genotyped only in 17% KIR2DS1pos women in contrast to 54.3% among KIR2DS1neg women (p=0.005). Abnormal laboratory tests for autoantibodies including antinuclear Abs, anti-DNA, Sc70, anti-histone and anti-thyroid Abs were more often detected in KIR2DS1neg women. IgG anti-phospholipid antibodies against 6 phospholipids also were found at significantly higher levels in KIR2DS1neg women than in the KIR2DS1pos group.

CONCLUSION: Our results suggest that KIR and HLA-C genotyping can be useful tests for predicting immune related problems in women with RPL. Supported by: Clinical Immunology Laboratory at Rosalind Franklin University of Medicine and Science.

O-399 Wednesday, October 22, 2014 04:30 PM

SALPINGECTOMY VERSUS PROXIMAL TUBAL OCCLUSION FOR HYDROSALPINGES PRIOR TO IN-VITRO-FERTILIZATION (IVF) CYCLE; IS THERE A DIFFERENCE IN OVARIAN RESERVE OR RESPONSE TO GONADOTROPINS? N. Malhotra, C. P. Vignarajan, N. Singh. ART Centre, Department of Obstetrics and Gynecology, All India Institute of Medical Sciences, New Delhi, Delhi, India.

OBJECTIVE: To compare the effects of prophylactic salpingectomy or proximal tubal occlusion on ovarian reserve and response to controlled ovarian hyperstimulation (COH) during IVF cycles.

DESIGN: Prospective randomized trial.

MATERIALS AND METHODS: Seventy two women with bilateral hydrosalpinges were randomized to laparoscopic salpingectomy (Group I, n=35) or proximal tubal occlusion (Group II, n=37) prior to IVF. Ovarian reserve parameters including day 2 levels of FSH, estradiol (E2), AMH and antral follicle count (AFC) were assessed before and 12 weeks after surgery. Primary outcome was effects on ovarian reserve tests after surgery, implantation rate, clinical and ongoing pregnancy rate between the two groups. Secondary outcome was response to gonadotropins during the IVF cycle.

RESULTS: There was a significant rise post compared to pre-surgery levels of Day 2 FSH [7.3 vs 6.0 IU/L (p=0.007)] and a significant fall in AMH [3.3 vs 2.3 ng/ml (p=0.001)] and AFC [10.8 vs 9.0 (p=0.01)] in group I. There was no significant difference in day 2 FSH, E2, AMH and AFC before and after surgery in group II. Post-surgery AMH and AFC were significantly lower in group I (salpingectomy) compared to group II (tubal occlusion). There was no significant difference in the ovarian response including total dose, days of gonadotropins, number of follicles, E2 levels on day of hCG or number of oocytes retrieved between two groups.
Post surgery ovarian reserve and response to gonadotropins

<table>
<thead>
<tr>
<th>values (SD)</th>
<th>Group I</th>
<th>Group II</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH (IU/L)*</td>
<td>7.3 (1.8)</td>
<td>6.5 (2.6)</td>
<td>0.26</td>
</tr>
<tr>
<td>E2 (pg/ml)*</td>
<td>51.5 (12.2)</td>
<td>44.6 (16.1)</td>
<td>0.14</td>
</tr>
<tr>
<td>AMH (ng/ml)*</td>
<td>2.3 (1.2)</td>
<td>3.1 (0.9)</td>
<td>0.01</td>
</tr>
<tr>
<td>AFC *</td>
<td>9.0 (2.7)</td>
<td>10.5 (3.6)</td>
<td>0.02</td>
</tr>
<tr>
<td>Days of stimulation</td>
<td>11.1 (1.6)</td>
<td>10.4 (1.5)</td>
<td>0.13</td>
</tr>
<tr>
<td>Total dose of gonadotropins (IU)</td>
<td>3607.0 (1219.7)</td>
<td>3314.7 (664.7)</td>
<td>0.28</td>
</tr>
<tr>
<td>E2 on day of hCG(pg/ml)</td>
<td>3040 (1822.1)</td>
<td>3210.4 (1908.2)</td>
<td>0.72</td>
</tr>
<tr>
<td>Number of follicles (15-20mm)</td>
<td>10.3 (3.6)</td>
<td>10.8 (4.8)</td>
<td>0.49</td>
</tr>
<tr>
<td>Number of oocytes retrieved</td>
<td>8.4 (4.1)</td>
<td>8.6 (4.8)</td>
<td>0.97</td>
</tr>
<tr>
<td>pregnancy rate per cycle (%)</td>
<td>17.1</td>
<td>24.3</td>
<td>0.25</td>
</tr>
</tbody>
</table>

* indicate values 3 months post surgery

There was no significant difference in the implantation, clinical and ongoing pregnancy and miscarriage rate between the two groups.

CONCLUSION: Prophylactic salpingectomy prior to IVF lowers AMH levels and AFC compared to tubal occlusion without significantly affecting ovarian response. With comparable pregnancy rate salpingectomy or tubal occlusion may be opted depending on technical difficulty or surgical skill.

O-400 Wednesday, October 22, 2014 04:45 PM

**DENDRITIC CELLS AND IMPAIRED IMMUNE TOLERANCE IN INFERTILITY.** C. Wong,* A. Riaz,* M. Berbic,* D. Hart,* P. D. Fromm,** F. Kupresanin,* R. P. S. Jansen,* R. Markham,* I. S. Fraser,* A. J. Hey-Cunningham,* Obstet., Gynaec. and Neonat., QEII Research Institute for Mothers and Infants, SYD, NSW, Australia; *Dendritic Cell Biology, ANZAC Research Institute, SYD, NSW, Australia; *Genea Limited, SYD, NSW, Australia.

OBJECTIVE: Characterise dendritic cell (DC) subpopulations in the blood and endometrium of fertile and infertile women. It has been shown that DCs contribute to local tolerance during a normal pregnancy (1). Increased number of mature DC appears to be associated with recurrent miscarriage (2). However detailed knowledge is lacking in DC and infertility.

DESIGN: Fertile and unexplained endometriosis infertile participants of reproductive age were recruited from gynaecology theatre lists at Royal Prince Alfred Hospital. Blood and endometrial curettings were collected to study DC populations.

MATERIALS AND METHODS: Blood samples (n=31) were prepared as single cell suspensions for multi-colour flow cytometry. Live and dead populations were discriminated and DCs identified by their lack of expression of HLA-DR. Three CD11c+ myeloid DC (mDC; CD1c+,141+ and 16+) and single cell suspensions for multi-colour flow cytometry. Live and dead populations were collected to study DC populations.

RESULTS: Circulating CD2-DC proportions are significantly reduced in infertile women (p=0.031) compared to fertile women during the secretory phase. Density of endometrial IRF-8+ pDCs was also significantly decreased in infertile women (p=0.023) compared to fertile women during the secretory phase. A trend was also observed for infertile women to have a lower density of IRF-8+ pDCs compared to fertile women during menstruation (p=0.072).

CONCLUSION: This study has shown that circulating and endometrial DCs are disturbed in infertile women. CD2-DCs have tolerogenic functions and decreased tolerogenic properties of circulating DCs are linked with increased inflammatory immune responses in infertility and miscarriage (3). Reduced density of endometrial IRF-8+ during the secretory phase may indicate reduced tolerance of possible implantation. IRF-8+ pDCs promote IDO expression which creates tolerance by inducing proliferation and expansion of regulatory T cells (4). Regulatory T cells are decreased in the endometrium of infertile women (5). Dysregulation of DC might reduce immune tolerance to embryo implantation and thus, lead to infertility.

Supported by: This study was funded by the QE II Research Group for Mothers and Infants and Genea Limited.

O-404 Wednesday, October 22, 2014 05:00 PM


OBJECTIVE: In recent years the role of the adipose tissue as an endocrine gland, independently producing a number of cytokines and hormones, has been actively studied. It is known that obesity leads to pimelitis, which affects metabolic and secretory functions of the adipose tissue and plays a key role in the development of pathologic processes that accompany obesity. A significant role in the development of metabolic syndrome is performed by the system of the innate immunity.

OBJECTIVE: To study expression of TLR-2 and TLR-4 on monocytes of the peripheral blood, content of IL-1β and TNF-α in teenage girls, who are overweight and have menstrual disorders.

DESIGN: 22 patients with obesity and menstrual disorders (group I) and 25 healthy teenage girls with a normal body mass index (BMI) and regular menstrual cycle (control group) were included in the research.

MATERIALS AND METHODS: We performed a retrospective analysis of eating disorders that preceded manifestation of a clinical picture, studied clinical features of the disease course – dynamics of BMI, time of amenorrhea onset, determined the BMI and also performed gynaecological counselling to confirm the diagnosis of amenorrhea and oligomenorrhea. The determination of TLR-2 (CD14+CD282+) and TLR-4 (CD14+CD284+) expression on monocytes of the peripheral blood was performed by the method of two-color flow cytometry using diagnostic kits Caltag, HyCultbiotechnology. The content of IL-1β and TNF-α in the blood serum was defined by immune-enzyme analysis (Bender Medsistic).

RESULTS: It was defined that in the patients with obesity and menstrual disorders as compared with the control group there were apparent changes in the indices of the innate immunity in the form of statistically significant increase of TLR-4 expression on monocytes (CD14+CD284+) (84.1±6.4% compared to 51.7±8.3%), (p<0.05). Besides, we registered the increase of the production of IL-1β (80±8.1) compared to 25±6.9 (p<0.05) and TNF-α (71±8.9 pg/ml compared to 31±7.3 pg/ml), (p<0.05).

CONCLUSION: The activation of the innate immunity receptors may result in the increase of the cytokine formation and pimelitis that promote the development of the ovarian dysfunction. The revealed peculiarities of the immune response in teenage girls with obesity will contribute to the prevention of possible reproductive complications; they can help to broaden the scheme of treatment to restore the reproductive function in teenage girls with the given pathology.

O-402 Wednesday, October 22, 2014 05:15 PM

**THE ROLE OF CLASSICAL AND NON-CLASSICAL CONGENITAL ADRENAL HYPERPLASIA (CAH) IN INFERTILITY: AN ANALYSIS OF 2000 CLINICAL SAMPLES.** S. L. Bristow,* N. Kumar,* S. Rodriguez,* A. Bisignano,* D. Hoffman.* Recombine, New York, NY; *IVF Florida, Margate, FL.

OBJECTIVE: Several genetic variants in the CYP21A2 gene eliminate or reduce P450c21 activity, which is involved in cortisol biosynthesis. These variants can cause 21-hydroxylase deficient classical or nonclassical CAH, which are associated with several clinical symptoms including decreased fertility in males and females. The purpose of this study was to measure the carrier rates of genetic variants in CYP21A2 in fertility patients to determine if testing for these variants should be considered in this population.

DESIGN: Retrospective.

MATERIALS AND METHODS: The Illumina Infinium HD Custom Genotyping platform was used to test for variants in the CYP21A2 gene, including c.293-13C>G (rs6467) and p.G425S (rs72552758) associated with classical CAH and p.H63L (rs9378252) and p.P454S (rs6445) associated with nonclassical CAH. Genotype frequencies were calculated based on data obtained from 2,188 clinical referrals from fertility centers. Documented informed consent to utilize clinical data in a de-identified manner was obtained.

RESULTS: We found that 1,908 individuals (87.2%) do not carry any of the tested CYP21A2 variants. A total of 237 (10.83%) and 22 (1.01%) of the tested individuals were carriers or homozygous for the p.H63L nonclassical variant, respectively. Only one individual (0.05%) was homozygous for the p.P454S nonclassical variant. We identified 18 individuals (0.82%) that were carriers of the p.H63L nonclassical variant, respectively. Only one individual (0.05%) was homozygous for the p.P454S nonclassical variant. We identified 18 individuals (0.82%) that were carriers of the p.H63L nonclassical variant, respectively.

FERTILITY & STERILITY®
carried both nonclassical CAH variants. Further, two individuals (0.09%) carried both the classical c.293-13C>G and nonclassical p.H63L variants.

CONCLUSION: Compared to findings from other studies, the nonclassical p.H63L variant is much more common in our population of fertility patients than previously reported. As of 2013, only 31 cases of p.H63L had been identified in individuals presenting with CAH [1]. However, we found that 12.76% of our population carries at least one copy of this variant. The literature suggests that the p.H63L variant reduces FSH levels and is also associated with mild clinical features. The relatively high frequency of this variant in our population of fertility patients suggests the need to investigate the association of this variant with infertility specifically. If an association exists, this could inform hormone-based fertility treatment decisions.

O-403 Wednesday, October 22, 2014 05:30 PM

OBJECTIVE: To determine if chronic vitamin D deficiency (VD-) affects ovarian oxidative stress gene transcription.

DESIGN: Vitamin D3 deficiency (VD-) is hypothesized to adversely affect fertility and pregnancy outcomes. However, the mechanism(s) by which VD-impairs reproductive physiology in females is poorly understood. VD reduces markers of oxidative stress in vascular and nervous tissue. We previously reported VD-results in oocyte chromatid instability. We tested the hypothesis that VD-disruptions gamete health through mechanisms that involve oxidative stress. To test this hypothesis we performed a prospective study with C57Bl/6 female mice exposed to a VD sufficient (VD+) or VD- diet for 6-8 weeks post weaning. Ovarectomy was performed during the follicular stage and quantitative real-time PCR (qRT-PCR) was used to quantify changes in genes affected by oxidative stress. Serum calcium (Ca2+) and VD were quantified.

MATERIALS AND METHODS: Study female mice were weaned onto a VD+ (control) or VD- diet supplemented with Ca2+ for bone health and Ca2+ homeostasis. VD was determined with LC-MS and Ca2+ determined with a colorimetric enzymatic assay. Females were killed between 9-12 wks of age, ovaries collected, and RNA extracted. Fold changes in ovarian mRNA levels of Gclc, Gclm, Sod1, Sod2, Sod3, Cat, Gpx1, Gpx3, Grs, Glrx1, Glrx2, Txn1, Txn2, Txnr1, Txnr2, Gst4, Gstm1, Gstm2, Gstp1, Gstt1, Mgst1, Prdx3, Ccs were determined with qRT-PCR. Data were normalized to the expression of 18S. qRT-PCR values were calculated using ∆∆CT quantification method. Student’s T-test and one-way ANOVA were used to determine statistical significance. N = 5-7, p<0.05 was considered a significant difference.

RESULTS: Females exposed to VD-diet had reduced VD levels compared to control mice (P=0.002) and equivalent serum calcium levels. Ovarian expression of Gclc, Gclm, Sod1, Sod3, Cat, Glrx1, Glrx2, Txn1, Txn2, Mgst1, Prdx3 was significantly increased and Gsr was significantly decreased compared to control females (P<0.05).

CONCLUSION: Increased expression of genes responsible for enzymes involved in cellular antioxidant defense systems in chronically VD-females suggest higher levels of intracellular oxidative stress. A decrease in Gsr expression, which is a sulfur-redox cycle enzyme important in maintaining Ca2+ homeostasis. VD were quantified.

CONCLUSION: Compared to findings from other studies, the nonclassical p.H63L variant is much more common in our population of fertility patients than previously reported. As of 2013, only 31 cases of p.H63L had been identified in individuals presenting with CAH [1]. However, we found that 12.76% of our population carries at least one copy of this variant. The literature suggests that the p.H63L variant reduces FSH levels and is also associated with mild clinical features. The relatively high frequency of this variant in our population of fertility patients suggests the need to investigate the association of this variant with infertility specifically. If an association exists, this could inform hormone-based fertility treatment decisions.

O-405 Wednesday, October 22, 2014 04:00 PM
STRESS-INDUCED ACTIVATION OF OVARIAN HEAT SHOCK PROTEIN 90 IN A RAT MODEL OF POLYCYSTIC OVARY SYNDROME. M. H. Jung, a Y. I. Ji, b Kyung Hee University Hospital, Seoul, Korea; a Inje University Haeundae Paik Hospital, Busan, Korea.

OBJECTIVE: Polycystic ovarian syndrome is the most common endocrine disorder affecting infertile women of reproductive age. This study evaluated the activation of heat shock protein 90 (Hsp 90) during the formation of stress-induced polycystic ovaries.

DESIGN: Animal model.

MATERIALS AND METHODS: Female Sprague-Dawley rats (180-200 g) were subjected to one of two stress-inducing conditions; animals were either treated with adrenocorticotropic hormone daily for 18 days or were exposed to daily cold stress for three weeks. Non-treated rats sampled during proestrus or diestrus served as controls. Blood samples were collected from the left ventricles of anesthetized rats and concentrations of follicle-stimulating hormone, luteinizing hormone, estradiol, testosterone and corticosterone were measured in all rats. The expression of messenger RNA for androgen receptor, estrogen receptor-α and -β, nerve growth factor receptor, and glucocorticoid receptor, and protein expression for Hsp 90 was also assessed in the rat ovaries.

RESULTS: Stress increased glucocorticoid receptor and androgen receptor expression, and decreased estrogen expression. Nerve growth factor receptor expression was greater in treated than diestrus rats and less in treated than proestrus rats. Ovarian Hsp 90 protein expression was increased in rats treated with adrenocorticotropic hormone or cold stress. Serum follicle-stimulating hormone levels were reduced and testosterone and corticosterone levels increased by stress, whilst luteinizing hormone and estradiol levels were similar to levels in diestrus and proestrus control rats respectively.

CONCLUSION: The results indicate that stress, via the activation of ovarian Hsp 90 and changes in steroid hormone receptor expression and serum reproductive hormone levels, may be involved in the induction of polycystic ovaries in rats.
INSULIN SENSITIZING AGENT (METFORMIN) IMPROVES CLINICAL PREGNANCY RATE IN CLOMIPHENE CITRATE RESISTANT POLYCYSTIC OVARIAN SYNDROME PATIENTS WITH ACANTHOSIS NIGRICANS. S. A. Salman,1 A. T. Farghaly,1 D. A. Attallah,1 H. A. Abdel-Hafeez,1 O. M. Shaaban.2 1Women Health Hospital, Assiut University, Assiut, Egypt; 2Andrology Department, Assiut University, Assiut, Egypt; 3Clinical Pathology, Assiut University, Assiut, Egypt.

OBJECTIVE: There are several studies that showed a positive correlation between acanthosis nigricans (AN) with hyperinsulinemia and increased insulin resistance. The present study evaluated the effect of adding insulin sensitizing agent (metformin) to clomiphene citrate (CC) on improving insulin resistance parameters and pregnancy rate (PR) in clomiphene-resistant polycystic ovary (PCOS) patients with AN.

DESIGN: A double blinded randomized control trial.

MATERIALS AND METHODS: Sixty three CC resistant PCO patients (unsatisfactory ovulation after at least 3 month of CC induction) with AN had been recruited in the study. All study participants had proper clinical evaluation to ensure the diagnosis of AN. Additionally, day 3 follicle stimulating hormone level, fasting insulin, fasting glucose and homostatic model assessment used to quantify insulin resistance (HOMA–IR) were done. Randomization was done using serial number closed opaque envelopes. Blinding was done using two identical packages for induction (group I) two 50 mg CC tablets taken from day 3 to day 7 of the cycle + placebo tablets taken twice daily all through the cycle (Group II) received the above CC dose + metformin 500 mg twice daily continuously for three cycles. Insulin resistance parameters as well as clinical pregnancy rate had been evaluated in both groups. The statistical analysis was done using Chi-square and Fischer exact tests.

RESULTS: Our data showed that there was a statistically significant higher cumulative pregnancy rate after three cycles of stimulation between group II (15/33/45.5%) as compared with Group I (7/33/21.1%) (P=0.03). There were significant improvements in the insulin resistance parameters after three months of combined CC and Metformin treatment as compared with CC alone.

CONCLUSION: Addition of metformin to CC in PCOS patients with AN, decreases insulin resistance and improve clinical pregnancy rate in patients with previous unsatisfactory response to CC alone.

O-407 Wednesday, October 22, 2014 04:30 PM

MITOCHONDRIAL DYSFUNCTION INDUCED BY DEHYDROEPIANDROSTERONE IS REVERSED BY METFORMIN TREATMENT IN MOUSE OOCYTE. Y. Huang, Y. Zhao, Y. Yu, R. Li, J. Qiao. Assisted Reproductive Center and Department of Obstetrics and Gynecology, Peking University Third Hospital, Beijing, China.

OBJECTIVE: To explore the influence of hyperandrogenism on oocyte quality in mice and to evaluate the prevention effect of metformin. DESIGN: Female BALB/c mice were treated with a vehicle control or dehydroepiandrosterone (DHEA) for 20 days, with subsequent analyses of changes in oocyte quality. Rescue group treated with metformin was also analyzed.

MATERIALS AND METHODS: The polycystic ovary syndrome (PCOS) model was induced by daily injection of DHEA s.c. to 25 days old female BALB/c mice (6mg /100g body weight), the rescue group was administrated daily with metformin(50mg /100g body weight) by gavage in addition to DHEA. Metaphase II (MII) oocytes were retrieved after PMSG and HCG administration. The mitochondrial function, oxidative stress level and spindle assembly of matured MII oocytes were measured by real-time PCR, chemiluminescence immunofluorescence and immunofluorescence. Germinal vesicle (GV) oocytes from non-primed mice were cultured under time-lapse imaging for in vitro maturation (IVM).

RESULTS: DHEA-induced mice resembled some aspects of human PCOS, such as irregular sexual cycle and polycystic ovary. DHEA-treated mice yielded fewer MII oocytes, which displayed decreased mtDNA copy number, ATP content and inner mitochondrial membrane potential compared with controls. In addition, an excessive oxidative stress of DHEA-treated mice was revealed by increased contents of reactive oxygen species and decreased concentration of glutathione (GSH) and GSH/GSSG ratio per MII oocyte compared with control group. Metformin treatment dramatically attenuated the mitochondrial damage induced by DHEA exposure, as evidenced by increases of ATP content, inner mitochondrial membrane potential, GSH concentration and GSH/GSSG ratio, and a decrease of reactive oxygen species contents. No obvious differences of abnormal spindles and misaligned chromosomes of MII oocytes were observed in DHEA-treated group compared with control and rescue groups. During IVM, the periods of germinal vesicle breakdown (GVBD) and first polar body extrusion were extended and the maturation rate of GVBD oocytes was decreased in DHEA-treated mice compared with controls.

CONCLUSION: Mitochondrial dysfunction and oxidative stress in mouse oocyte caused by DHEA-induced hyperandrogenism can be rescued by metformin treatment.

Supported by: This research was Supported by National Natural Science Foundation of China (No.81170538).

O-408 Wednesday, October 22, 2014 04:45 PM

COMPARATIVE STUDY OF THE THERAPEUTIC EFFECTS OF TWO DIFFERENT DOSAGES OF ETHINYL ESTRADIOL (EE: 20 MCG VERSUS 30 MCG) IN ASSOCIATION WITH DROSPIRONONE (DRSP: 3 MG) IN POLYCYSTIC OVARY SYNDROME (PCOS), S. M. Bhattacharya1, A. Jha.2 1Obstetrics and Gynecology, S.C. Roy Memorial Medical and Research Center, Kolkata, West Bengal, India; 2Obstetrics and Gynecology, KPC Medical College, Kolkata, West Bengal, India; 3Harvard School of Public Health, Boston, MA.

OBJECTIVE: To compare the therapeutic effects of two different dosages of Ethinyl estradiol (EE: 20 mcg versus 30 mcg) in combination with Drospirenone (DRSP: 3 mg) in polycystic ovary syndrome (PCOS) after 6 and 12 months of treatment.

DESIGN: Single blind randomized controlled study.

MATERIALS AND METHODS: 112 women with PCOS (Rotterdam criteria, 2003) with preset inclusion and exclusion criteria were randomized in 1:1 ratio, between May, 2012 and January, 2013 into 2 groups, receiving DRSP (3mg) with either EE (20 mcg, n= 57; cyclically 24+4 regimen) or EE (30 mcg, n= 55; cyclically 21+7 regimen). Prior ethical approval and informed written consent (from all participants) were obtained. The interventions were sealed in sequentially numbered identical opaque containers according to allocation sequence. The primary outcome was comparison of the absolute change in the free androgen index (FAI) between the two groups at 6 and 12 months of treatment. Body mass index (BMI), abdominal circumference (AC), Ferriman Galwey score (FG), presence of acne (%), and acanthosis nigricans (%), (AN), blood pressure (systolic, SBP: diastolic, DBP) were measured. Serum testosterone (T), sex hormone binding globulin (SHBG), glucose (PPG) and insulin (PPI) levels 2 hours after 75 gram oral glucose intake were measured. FAI was calculated as [T (ng/ml) x 100 x 300 / SHBG (nmol/l)]. PPG/PPG less than 1.0 was considered as indicative of insulin resistance.1 From a pilot study, a sample size of 50 patients per group was found to have 80% power and 95% confidence level with 2 sided test of significance to detect a mean difference of 3.67 between the two groups at 6 months of treatment.

RESULTS: Intent to treat analysis showed that after 6 months of treatment, therapeutic effects of the two types of pills were comparable except a significant increase in SHBG with the 30 mcg pill. After 12 months of treatment, identical therapeutic effects were observed. There was no difference in insulin resistance state between the two groups after 6 and 12 months.

CONCLUSION: There were no differences in therapeutic effects between 20 mcg and 30 mcg EE pills (with DRSP: 3 mg) except in SHBG values. Hence for long term treatment in PCOS, a lower dose estrogen containing pill can be recommended.

CITR registration no. - CTRI/2012/04/002571.

O-409 Wednesday, October 22, 2014 05:00 PM

OBJECTIVE: To determine if elevated fasting insulin is associated with metabolic abnormalities in women with and without PCOS.

DESIGN: Cross sectional study.

MATERIALS AND METHODS: Women seen at a multidisciplinary PCOS clinic between 2006 and 2013 and diagnosed with PCOS by Rotterdam 2003 criteria were considered for study inclusion. Controls were healthy, ovulatory women between 25-45y with regular menstrual cycles identified from a community-based longitudinal study. Data were collected on fasting glucose and insulin, fasting lipids, body mass index (BMI), and waist circumference. PCOS patients and controls were subgrouped by fasting insulin level (abnormal fasting insulin defined as ≥12 mU/L) and metabolic parameters were compared after controlling for age. Data were analyzed using SAS, and ANOVA was used for comparisons.

RESULTS: 301 women with PCOS and 1017 controls were included in the analysis. Prevalence of abnormal fasting insulin was 11.4% in the control group and 41.5% in the PCOS group. Globally, both PCOS and controls with abnormal fasting insulin had greater derangement in many metabolic syndrome parameters. When compared to controls, women with PCOS had significantly higher total cholesterol and LDL in both the normal and abnormal fasting insulin groups (see Table). No difference was seen in HDL. PCOS patients and controls were subgrouped by fasting insulin level (abnormal fasting insulin defined as ≥12 mU/L) and metabolic parameters were compared after controlling for age. Data were analyzed using SAS, and ANOVA was used for comparisons.

CONCLUSION: An elevated fasting insulin is associated with abnormal metabolic parameters in women with and without PCOS. Women with PCOS show more pronounced dyslipidemia compared with controls, when stratified by fasting insulin status.

Supported by: NICHD: HD054956 (MIC).

O-410 Wednesday, October 22, 2014 05:15 PM

GENE EXPRESSION PROFILING OF GRANULOSA CELLS FROM WOMEN WITH POLYCYSTIC OVARY SYNDROME SHOWS ASSOCIATION WITH MITOGEN-ACTIVATED PROTEIN KINASE AND EXTRACELLULAR REGULATED KINASE SIGNALING. H.-N. Ho, H.-F. Chen, Y.-S. Yang, C.-W. Lan. Obstetrics and Gynecology, National Taiwan University Hospital, Taipei, Taiwan; Graduate Institute of Clinical Medicine, National Taiwan University College of Medicine, Taipei, Taiwan.

OBJECTIVE: Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women at the reproductive age. Although its etiology and pathogenesis remain unclear, recent studies suggest that granulosa cell dysfunction may partly be responsible.

DESIGN: This study aimed to use cDNA microarray technology to compare granulosa cell gene expression profiles in women with and without PCOS to identify those genes that may be etiologically implicated.

MATERIALS AND METHODS: This study included 12 women undergoing in vitro fertilization (IVF): 6 without PCOS and 6 with PCOS. Granulosa cells were collected for RNA extraction, amplification, and microarray hybridization, and each sample was assessed using microarray chips. Differential expressions were classified using the Kyoto Encyclopedia of Genes and Genomes and post-analyses of microarray data, followed by western blot analysis of selected genes.

RESULTS: In total, 243 genes showed differential expression, including 125 genes that were upregulated and 118 genes that were downregulated in the PCOS granulosa cells compared with the non-PCOS granulosa cells. These genes are involved in reproductive system development, amino acid metabolism, and cellular development and proliferation. Comparative analysis revealed genes involved in the mitogen-activated protein kinase/extra-cellular regulated kinase (MAPK/ERK) signaling pathways. Western blot analyses confirmed that MAPK4 and phospho-ERK1/2 were decreased in the granulosa cells of women with PCOS.

CONCLUSION: This study identified candidate genes that may influence granulosa cell function in PCOS. We conclude that the MAPK/ERK signaling pathways are altered in granulosa cells of women with PCOS.

Supported by: The grant from Taiwan NSC

O-411 Wednesday, October 22, 2014 05:30 PM

SYMPTOM PATTERNS AND PHENOTYPIC SUBGROUPING OF WOMEN WITH POLYCYSTIC OVARY SYNDROME: ASSOCIATION BETWEEN ENDOCRINE CHARACTERISTICS AND METABOLIC aberrations. C.-C. Huang, Y.-J. Tien, M.-J. Chen, C.-H. Chen, H.-N. Ho, Y.-S. Yang. Department of Obstetrics and Gynecology, National Taiwan University Hospital Hsin-Chu Branch, Hsinchu, Taiwan; Institute of Statistical Science, Academia Sinica, Taipei, Taiwan; Department of Obstetrics and Gynecology, National Taiwan University Hospital, Taipei, Taiwan.

OBJECTIVE: The aim of this study was to subtype women with polycystic ovary syndrome (PCOS) according to heterogeneous phenotypes, and to determine the potential endocrine characteristics related to risk and severity of metabolic disturbance.

DESIGN: We conducted a cross-sectional study in a reproductive endocrinology outpatient clinic of a tertiary medical center.

MATERIALS AND METHODS: Four hundred sixty patients with PCOS diagnosed according to the 2003 Rotterdam criteria were studied. Clinical history recorded by questionnaires, anthropometric measurements, biochemistry tests after an overnight fast, and pelvic ultrasonography were collected from all patients. Generalized association plots (GAP) analysis was adopted to determine the symptom patterns and phenotypic clustering of the patients. GAP is a graphical technique that can simultaneously explore the associations of up to thousands of subjects, variables, and their interactions, without first reducing dimensions.

RESULTS: Applying GAP analysis, the patients were divided into five distinct clusters according to the correlation with six endocrine parameters, including follicular stimulating hormone, luteinizing hormone (LH), estradiol, testosterone, sex hormone-binding globulin (SHBG), and dehydroepiandrosterone sulfate. Each cluster exhibited specific endocrine characteristics and the prevalence of metabolic syndrome (MS) was significantly different among the clusters (P<0.0001). The mean prevalence of MS in our study population was 19.3% (89/460). Cluster 5 had a significantly lower prevalence of MS (2.4%), yet significantly higher mean SHBG and LH levels. Although cluster 1 had a lower mean serum testosterone level (0.72±0.26 ng/ml), the risk of MS was still high (34.9%).

CONCLUSION: Women with PCOS could be subtyped into five clusters according to heterogeneous endocrine characteristics and each had significantly different metabolic risks. The major predictive endocrine factors for metabolic aberrations were serum SHBG and LH levels. The serum testosterone level did not appear to be the key determinant of metabolic risk. PCOS is a heterogeneous and complex disease. Stratifying women with PCOS into meaningful subtypes could provide a better understanding of related risk factors and potentially enable the design of more effective screening and treatment intervention.
CONTRACEPTION/FAMILY PLANNING

P-1 Tuesday, October 21, 2014

POLIDOCANOL FOAM INDUCES COLLAGEN DEPOSITION. D. O. Lee, a,b,c C. Hanna, a,b,c S. Yao, a,c Y. Earian, a,c O. Shayden, a,c J. Jensen, a,c b Division of Reproductive Science, Oregon National Primate Research Center, Beaverton, OR; a Department of Obstetrics and Gynecology, Oregon Health & Science University, Portland, OR; b Center for Uterine Cancer, National Cancer Center, Goyang-si, Gyeonggi-do, Korea.

OBJECTIVE: Transcervical therapy with polidocanol foam (PF) causes tubal occlusion in nonhuman primates and is being studied as a method of nonsurgical permanent contraception (PMID:24560476). Tubal obstruction is correlated with an increase of fibrosis surrounding intramural tube where it passes through the uterine cornua. We hypothesized that the fibrosis contains an abnormal deposition of collagen in the extracellular matrix. Our objective in this study was to characterize collagen types in the zone of tubal obstruction.

DESIGN: In vivo treatments in a Primate Research Center setting.

MATERIALS AND METHODS: Adult cycling rhesus macaques underwent treatment with either 5% PF (n=2); or 1% methylcellulose foam (inert control; n=2); during the proliferative phase of the menstrual cycle. One additional cycling animal was assayed as an untreated control. Tissues samples were collected 2 weeks after treatment. Paraffin sections of uterine cornua were subjected to antigen retrieval with citrate buffer and then immunohistochemistry using mouse monoclonal antibodies for collagen I (ICN Biomedicals Clone 1H8S), III (ImmunotechTM Clone I-53) and goat polyclonal antibodies for collagen IV (Novus Biologicals NBP1-26549).

RESULTS: In the untreated controls the tubal epithelium was intact and light staining for collagen I and III was observed in the serosal layer and subepithelial matrix. Small amounts of collagen I and III were observed away from the sub-epithelium. Collagen IV was distributed evenly in extracellular matrix. In the animals treated with methyl cellulose, oviductal epithelium was intact and the distribution of collagen was identical to the untreated control.

However, treatment with PF resulted in a loss of normal epithelium, which was substituted with Collagen I and III. There was a striking buildup of Collagen I and III associated with the zone of obstruction. Collagen IV was also increased but with less density than collagen I and III.

CONCLUSION: Polidocanol induced tubal obstruction involves loss of normal epithelium and accumulation of collagen I and III at the site of obstruction. Therapies that promote the deposition of extracellular matrix may improve the efficiency of polidocanol-induced tubal blockade.

Supported by: Bill and Melinda Gates Foundation OPP1025233, P51OD011092, U54-O55744.

P-2 Tuesday, October 21, 2014

GLOBAL ENDOMETRIAL GENE EXPRESSION WITH CONTINUOUS EXPOSURE TO LEVONORGESTREL (LNG) VIA AN INTRAUTERINE SYSTEM (IUS). T. D. Kimble, a,* C. F. Esteban, a J. A. Horcajadas, a,b N. Yousefieh, a,c S. Yao, a,c Y. Earian, a,c O. Shayden, a,c J. Jensen, a,c b Division of Reproductive Science, Oregon National Primate Research Center, Beaverton, OR; a Department of Obstetrics and Gynecology, Oregon Health & Science University, Portland, OR; b Center for Uterine Cancer, National Cancer Center, Goyang-si, Gyeonggi-do, Korea.

OBJECTIVE: There is limited data on effects of LNG on endometrial gene expression; almost no information is available on effects of a LNG IUS on global endometrial gene expression in regards to genes associated with embryo implantation. 2

DESIGN: Prospective, nonrandomized, open-label study. Baseline served as the control.

MATERIALS AND METHODS: Adult nulliparous women, aged 12-17 years, with regular menstrual cycles (21-35 days), requesting contraception were recruited.

RESULTS: Of the 343 subjects enrolled, 39 were excluded at screening: LNG-IUS13.5mg placement was attempted in the remaining 304 subjects (the full analysis set). The mean age was 16.2 years, mean BMI was 22.1 kg/m2, and 97.7% were nulliparous. At screening, 51.6% of subjects used oral contraceptives, 22.7% used barrier methods, and 22.7% used no contraception. The 1-year Pearl Index (PI) was 0.00. However, only 72% of the total exposure was relevant to the PI calculation because subjects were counseled to use barrier methods to prevent sexually transmitted infections. Placement was successful in 303/304 subjects. Investigators rated 94.4% of successful placements as ‘easy’. Pain on placement was rated as ‘none’, ‘mild’, ‘moderate’, and ‘severe’, respectively, by 20.5%, 34.3%, 34.3%, and 10.9% of subjects. Between the 1st and 13th (final) 28-day reference intervals (RI), the mean number of bleeding/spotting episodes per RI remained relatively constant over time (range: 0.8-1.4). However, the number of subjects contributing data to bleeding analyses decreased from 141 in RI 1 to 36 in RI 13. At 1 year, 83.9% of subjects

FERTILITY & STERILITY® e141

P-3 Tuesday, October 21, 2014

A PHASE III SINGLE-ARM STUDY OF A NEW 13.5 MG LEVONORGESTREL INTRAUTERINE CONTRACEPTIVE SYSTEM IN POSTMENARCHEAL ADOLESCENTS: AN EVALUATION OF EFFICACY, BLEEDING, USER SATISFACTION, AND PLACEMENT. K. Gemzell-Danielsson, a S. Dernout, b E. Lukkari-Lax, a E. Montegriffo, a S. Rybowski, e D. Apter, e Karolinska Institutet, Stockholm, Sweden; b Gynaecological Centre Dernout & Albicher, Alkmaar, Netherlands; c Bayer Oy, Espoo, Finland; d Bayer HealthCare, Newbury, United Kingdom; e Bayer HealthCare, Whippny, NJ; Sexual Health Clinic, Västöliitto, Finland.

OBJECTIVE: To evaluate the efficacy, bleeding profile and user satisfaction associated with the 13.5 mg total content levonorgestrel intrauterine contraceptive system (LNG-IUS13.5mg; Skyla in the US, Jaydess elsewhere) in postmenarcheal adolescents, and to evaluate the placement of LNG-IUS13.5mg in this group. Safety (primary outcome) data are presented in another abstract.

DESIGN: A 1-year Phase III study conducted at 36 centers in Europe (with an option to continue use for 2 more years).

MATERIALS AND METHODS: Nulliparous and parous women, aged 12-17 years, with regular menstrual cycles (21-35 days), requesting contraception were recruited.

RESULTS: Of the 343 subjects enrolled, 39 were excluded at screening: LNG-IUS13.5mg placement was attempted in the remaining 304 subjects (the full analysis set). The mean age was 16.2 years, mean BMI was 22.1 kg/m², and 97.7% were nulliparous. At screening, 51.6% of subjects used oral contraceptives, 22.7% used barrier methods, and 22.7% used no contraception. The 1-year Pearl Index (PI) was 0.00. However, only 72% of the total exposure was relevant to the PI calculation because subjects were counseled to use barrier methods to prevent sexually transmitted infections. Placement was successful in 303/304 subjects. Investigators rated 94.4% of successful placements as ‘easy’. Pain on placement was rated as ‘none’, ‘mild’, ‘moderate’, and ‘severe’, respectively, by 20.5%, 34.3%, 34.3%, and 10.9% of subjects. Between the 1st and 13th (final) 28-day reference intervals (RI), the mean number of bleeding/spotting episodes per RI remained relatively constant over time (range: 0.8-1.4). However, the number of subjects contributing data to bleeding analyses decreased from 141 in RI 1 to 36 in RI 13. At 1 year, 83.9% of subjects

CONCLUSION: The presence of LNG IUS is associated with dys-regulation of >439 genes, of which 21 dys-regulated genes are shared amongst all time points. Of these, there is one up regulated gene associated with endometrial receptivity according to the literature, and one gene that is down regulated. There are three genes associated with implantation and that are up regulated and four genes that are down regulated.

Supported by: Bayer Healthcare, Inc.
reported that they were either ‘very satisfied’ or ‘satisfied’ with LNG-IUS13.5mg.

CONCLUSION: In this adolescent population, LNG-IUS13.5mg was highly effective, associated with high user satisfaction, and had a similar bleeding profile to that observed in the adult population. Most placements were rated as ‘easy’, and most subjects experienced no more than ‘mild’ pain on placement.

Supported by: Study and abstract funded by Bayer HealthCare.

P-4 Tuesday, October 21, 2014

EFFECT OF POLIDOCANOL FOAM CONCENTRATION ON SUCCESS OF TUBAL OCCLUSION FOLLOWING TRANSCERVICAL ADMINISTRATION IN BABOONS. J. T. Jensen,a,b C. Hanna,a S. Yao,a C. Bauer,a C. Hergert,a A. B. Edelman,a,b O. D. Slayden,a Reproductive and Developmental Sciences, Oregon National Primate Research Center, Beaverton, OR; Obstetrics & Gynecology, Oregon Health & Science University, Portland, OR; Southwest National Primate Research Center, San Antonio, TX.

OBJECTIVE: Our goal is to develop a single-treatment nonsurgical method of female permanent contraception. We have shown that a single transcervical administration of 5% polidocanol foam (PF) can block the fallopian tubes of baboons when animals also receive depomedroxyprogesterone acetate (DMPA). The objective of this study was to determine if occlusion would occur with a lower concentration (1%) of PF with co-administration of DMPA.

DESIGN: In vivo treatments in a Primate Research Center.

MATERIALS AND METHODS: Healthy regularly cycling female baboons were sedated and underwent a hysterosalpingogram (HSG) followed by transcervical infusion of polidocanol foam with (+) or without (-) an intramuscular injection of depomedroxyprogesterone acetate (DMPA, 3.5mg/kg). Two concentrations of PF were evaluated: 1% [(+) DMPA, n=5; (-) DMPA, n=3] and 5% [(+) DMPA, n=4; (-) DMPA, n=1]. Control females [n=2] received (+) DMPA only; additional [+] DMPA, n=2) controls did not undergo baseline HSG. PF-treated animals were examined after ≥60 days and the procedure was repeated if unilateral or bilateral patency was noted. The reproductive tracts of all animals were removed for detailed histologic examination at the end of study.

RESULTS: All 4 females that received a single treatment with 5% PF and DMPA developed clinical and histologic evidence of bilateral tubal occlusion; in contrast the one female treated with 5% PF without DMPA did not develop bilateral occlusion after two PF treatments. None of the 1% PF or control females that did or did not receive DMPA developed bilateral tubal occlusion after a single treatment with PF. In conclusion: A single transcervical treatment with 5% PF resulted in bilateral tubal occlusion in baboons that also received DMPA, but 1% PF was not effective. Additional studies to demonstrate feasibility of the approach as a method of nonsurgical permanent female contraception are warranted.

Supported by: Bill and Melinda Gates Foundation OPP1025233, P51OD011092, P51 OD011133, U54-055744.

P-5 Tuesday, October 21, 2014

A 12-MONTH MULTICENTER, RANDOMIZED PHASE III STUDY COMPARING A 13.5 MG LEVONORGESTREL INTRAUTERINE CONTRACEPTIVE SYSTEM WITH THE ETONOGESTREL SUBDERMAL CONTRACEPTIVE IMPLANT IN WOMEN AGED 18–35 YEARS. M. Tuppurainen,a E. Lukkari-laex,b J. Grunert,b aS. Yao,a C. Bauer,a C. Hergert,a A. B. Edelman,a,b O. D. Slayden,a Reproductive and Developmental Sciences, Oregon National Primate Research Center, Beaverton, OR; Obstetrics & Gynecology, Oregon Health & Science University, Portland, OR; aSouthwest National Primate Research Center, San Antonio, TX.

OBJECTIVE: To compare 12-month rates of discontinuation (primary outcome), user satisfaction and adverse events (AEs) (secondary outcomes) associated with the use of a new 13.5 mg (total content) levonorgestrel intrauterine contraceptive system (LNG-IUS 13.5 mg; Jaydess®) and the etonogestrel subdermal contraceptive implant (ENG implant, Nexplanon®).

DESIGN: A randomized, 2-arm, open-label Phase III study conducted in Australia, Finland, France, Norway, Sweden and the UK.

MATERIALS AND METHODS: Healthy nulliparous and parous women aged 18-35 years with regular menstrual cycles (21-35 days), requiring contraception, were randomized to use LNG-IUS 13.5 mg or ENG implant for 12 months.

RESULTS: In total, 766 women were randomized to use either LNG-IUS 13.5 mg (n=385) or the ENG implant (n=381). In the LNG-IUS 13.5 mg and ENG implant groups, respectively, the mean age was 24.8 years and 25.0 years, the mean BMI was 23.6 kg/m2 and 24.3 kg/m2, and 76.2% and 72.2% were nulliparous. Within 12 months, 19.6% of LNG-IUS 13.5 mg users and 26.8% of implant users prematurely discontinued treatment; this difference in favor of LNG-IUS 13.5 mg was statistically significant (95% CI: -13.2%, -1.2%). Premature discontinuation rates owing to AEs were 14.3% (LNG-IUS 13.5 mg) and 21.8% (ENG implant). Premature discontinuation rates owing to altered bleeding pattern were 4.2% (LNG-IUS 13.5 mg) and 11.5% (ENG implant). Depending on time point (month 6, month 12, end of study), overall user satisfaction rates ranged from 80.2% to 86.5% (LNG-IUS 13.5 mg) and from 66.1% to 75.9% (ENG implant). Among LNG-IUS 13.5 mg users, the most frequently reported AEs were dysmenorrhea (33.5%), uterine spasms (16.2%), and procedural pain (13.6%). The most frequent AEs in the ENG implant group were acne (15.5%), headache (12.3%), and dysmenorrhea (12.3%). No unexpected safety events were reported. Three pregnancies were reported, all in the LNG-IUS 13.5 mg group; however the study was not powered to accurately determine true efficacy.

CONCLUSION: The primary outcome was reached; the 12-month discontinuation rate in the LNG-IUS 13.5 mg group was significantly lower than in the ENG implant group. Additionally, compared with the ENG implant, the LNG-IUS 13.5 mg was associated with a lower discontinuation rate owing to altered bleeding pattern. Greater user satisfaction was seen with LNG-IUS 13.5 mg than with the implant. Both the LNG-IUS 13.5 mg and the ENG implant were well tolerated.

Supported by: Study and abstract funded by Bayer HealthCare.

P-6 Tuesday, October 21, 2014

POSTPARTUM CONTRACEPTION EDUCATION: IMPLICATIONS FOR CONTRACEPTIVE CHOICE AND USE OF LONG ACTING REVERSIBLE CONTRACEPTION (LARC). E. S. Constance, C. Satterwhite, D. Bi, M. Duarte, K. Gorman, C. Wienceke. Obstetrics and Gynecology, University of Kansas Medical Center, Kansas City, KS.

OBJECTIVE: To determine whether comprehensive contraception counseling provided in the postpartum period affects contraceptive choice and LARC use.

DESIGN: Prospective observational study.

MATERIALS AND METHODS: Patients admitted in the immediate postpartum period at the University of Kansas Hospital from 2/1/14 to 5/1/14 were eligible for the study. Eligible patients presenting for delivery were approached for enrollment during their postpartum admission. Exclusion criteria included non-English speaking, undergoing a permanent sterilization procedure during the same hospitalization, and medical conditions precluding the use of hormonal birth control methods. All women who consented to participate received a preliminary survey regarding their intended choice of postpartum contraception method. After accessing comprehensive contraception education through an online interactive educational tool (Bedside.org), participants were given a second survey to determine whether their choice of postpartum contraception method changed following education.

RESULTS: Of 103 enrolled patients, 64 patients completed surveys (62.1%). Of these, 11 (17.2%) reported their initial contraception of choice as LARC. Following an average of 5-10 minutes using the education tool, 23.4% selected a LARC method. Overall, 8 (12.5%) patients reported changing their choice of postpartum contraception following use of the education tool; 4 of 7 (57.1%) patients switched from a non-LARC to a LARC, and one patient switched LARC methods. Nine patients (14%) were undecided on postpartum birth control prior to use of the education tool; 55% selected a preferred method after viewing the tool, with 3 (60%) selecting LARC.

CONCLUSION: Self-directed contraceptive education delivered in the postpartum period may be effective at increasing intended LARC use, particularly for those patients who are undecided regarding postpartum contraception. However, most patients in the postpartum period had already chosen a preferred method and were not influenced by the education tool. Counseling and education regarding LARC may be more effective at influencing contraceptive choice when delivered in the prenatal period.
A RANDOMIZED PHASE III STUDY COMPARING A NEW 13.5 MG LEVONORGESTREL INTRAUTERINE CONTRACEPTIVE SYSTEM WITH A COMBINED ORAL CONTRACEPTIVE: ANALYSIS OF EFFICACY AND BLEEDING PROFILES. L. Borghatta, K. Roth, S. Rybowski, K. Rosen. Boston University School of Medicine, Boston, MA; 2Bayer Pharma AG, Berlin, Germany; 3Bayer HealthCare, Whippany, NJ.

OBJECTIVE: To compare contraceptive efficacy and bleeding profiles associated with use of the 13.5 mg (total content) levonorgestrel intrauterine contraceptive system (LNG-IUS13.5mg) and a 30 µg ethinyl estradiol and 3 mg drospirenone combined oral contraceptive (COC) in younger women.

DESIGN: A randomized Phase III study conducted at 42 centers in Europe and the US.

MATERIALS AND METHODS: Nulliparous and parous women aged 18-29 years with regular menstrual cycles (21-35 days), requesting contraception, were randomized to use LNG-IUS13.5mg or COC for 18 months.

RESULTS: The full analysis set included 279 women randomized to LNG-IUS13.5mg and who had a placement attempt (successful in 279) and 281 women randomized to COC who took ≥ 1 pill. For LNG-IUS13.5mg and COC groups, respectively, the mean age was 23.7 years and 23.9 years, and 77.4% and 73.3% were nulliparous. At Month 18/End of study, 70/247 COC users (28.3%) reported that they ‘sometimes missed pills’ and 132/247 (53.4%) reported that they ‘sometimes took a pill late’. There were 6 pregnancies in the COC group; the unadjusted Pearl Index (PI) was approximately twice that of the adjusted PI (1.82 vs 0.91). By contrast, there were 2 pregnancies in the LNG-IUS13.5mg group; the unadjusted and adjusted PIs were identical (0.57). The mean number of combined bleeding and spotting days declined over time in the LNG-IUS13.5mg group, from 31.7 days in the first 90-day reference interval (RI) to 13.5 days in the sixth (final) 90-day RI, but remained relatively constant during COC use (range: 15.6–19.2 per 90-day RI). By the final 90-day RI, LNG-IUS13.5mg and COC users, respectively, 13.6% and 0.5% had amenorrhea; 29.5% and 16.6% had infrequent bleeding; 21.8% and 7.5% had irregular bleeding; 31.4% and 74.3% had normal bleeding; 3.6% and 0.5% had frequent bleeding; and 3.6% and 0.5% had prolonged bleeding (according to WHO criteria). Regardless of bleeding pattern, discontinuations due to bleeding pattern alterations were uncommon: occurring in 7 women and 1 woman in the LNG-IUS13.5mg and COC groups, respectively.

CONCLUSION: Even in the context of a clinical trial, the efficacy of COC was apparently reduced by non-compliance. In contrast, the efficacy of LNG-IUS13.5mg generally experienced shorter, less frequent bleeding over time, and were more likely to experience amenorrhea and infrequent bleeding than COC users.

Supported by: Study and abstract funded by Bayer HealthCare.

FERTILITY & STERILITY®

P-7 Tuesday, October 21, 2014

RESULTS: 184 YCS, mean age 31.4±5.8, were 2.5 [IQR 4.3] years since cancer diagnosis and at risk of unintended pregnancy. 30 (16%) did not use contraception using family planning services in the prior 12 months. No contraception was associated with older age (≥21 vs. <31, RR 2.3, p=0.03) and partnered status (RR 3.6, p=0.01), and inversely associated with using family planning services (RR 0.4, p=0.01). In adjusted analysis, partnered status was associated with higher rate of non-contraception (RR 3.0, p=0.04) and family planning services was attenuated (RR 0.6, p=0.10). Among those contracepting (n=154), 60 (39%) used Tiers 3/4 methods. Compared to users of Tiers 1/2 methods, these participants were older (RR 2.1, p<0.01), more likely to have breast cancer (RR 2.6, p<0.01) and less likely to use family planning services (RR 0.3, p<0.01). In adjusted analysis, family planning services was the only factor significantly associated with Tier 3/4 methods use (RR 0.4, p<0.01).

CONCLUSION: Fewer than 2/3 of YCS in this cohort used family planning services in the prior year. Receipt of family planning services was significantly associated with use of contraception and more effective methods of contraception. Integrating family planning into survivorship care is needed.

Supported by: UL1 RR024926, HD-058799, MIRS-G-08-110-01-CEE.

P-9 Tuesday, October 21, 2014

FERTILITY AND CONTRACEPTIVE KNOWLEDGE IN A REPOROUG-DUCTIVE-AGE HISPANIC POPULATION. L. L. Kroener, J. Chen, M. Pisarska, E. Wang, Obstetrics and Gynecology, Division of REI, University of California, Los Angeles, Los Angeles, CA; 3Obstetrics and Gynecology, Division of REI, Cedars Sinai Medical Center, Los Angeles, CA.

OBJECTIVE: To compare the perception of fertility knowledge to contraception knowledge in a reproductive-age Hispanic population.

DESIGN: Cross-sectional self-administered survey study.

MATERIALS AND METHODS: A total of 71 surveys from reproductive-age women and men were included in the study. Survey participants were a random sample of attendees at the 2014 Telemundo Health Fair in Los Angeles, CA aimed at providing free health services and information to the Hispanic community. Over 90% of individuals who were approached completed the survey. A multiple-choice 12-question survey was administered in both English and Spanish. The survey primarily assessed knowledge of fertility and contraception on a Likert rating scale with 1 being “no” knowledge and 5 being “very” knowledgeable, as well as childbearing intentions and desire for further education regarding reproduction. Comparisons among groups were made using the Wilcoxon sign rank test and the Chi-squared test as appropriate.

RESULTS: Of the 71 participants, 55 (79%) were women and 16 (21%) were men. Overall, participants were young (mean 32.1±7.5 years), had at least 1 child (56.3%), desired or were considering having children in the future (50.7%). Only 12.9% of participants reported a history of infertility. Participants reported being significantly less knowledgeable about fertility compared to contraception (2.8±1.0 vs 3.3±1.2, P=0.004). Only 21% of participants reported that a healthcare provider had ever discussed fertility with them or when they would like to have children. In addition, 33% of women and 0% of men reported they were “fairly” or “very” knowledgeable about fertility (P=0.008), whereas 45.5% of women and 50% of men reported being “fairly” or “very” knowledgeable about contraception (P=0.748). Interestingly, both women and men desired more information about fertility (45% and 81%, P=0.054) and contraception (54% vs 81%, P=0.012).

CONCLUSION: In this young, reproductive-age Hispanic population, there is an unmet need for further education on fertility, as well as contraception. Raising awareness among providers may lead to changes in practice. Additional studies are needed to compare whether this gap in knowledge is comparable to other ethnic/racial groups.

Supported by: NIH/NCATS Grant# UL1TR000124.

P-10 Tuesday, October 21, 2014


OBJECTIVE: Fertility awareness methods (FAM): cervical mucus monitoring (CMM), ovulation predictor kits (OPK), and basal body temperature
chaining (BBT) are effective methods to predict ovulation. We sought to compare the efficacy of these methods as tools to improve natural fertility.

MATERIALS AND METHODS: Women, 30-44 years old, with no history of infertility, who were trying to conceive for less than 3 months, were enrolled and then followed until pregnancy. Women were not required to use a FAM. Each day women recorded bleeding, use and result of any FAM, intercourse, and pregnancy test results for up to 4 months while attempting to conceive. Each cycle was classified by presence or absence of type 4 cervical mucus (CMM), a positive ovulation predictor kit result (OPK), or basal body temperature charting for at least 10 consecutive days (BBT). Discrete Time Event History Analysis models were used to calculate fecundability ratios (FR) to compare FAMs adjusting for maternal age.

RESULTS: 1533 cycles from 571 women were included in this analysis. CMM, OPK, BBT were used in 32%, 17%, 16% of cycles, respectively. Choice of FAM differed by age, race, education level, and parity. After adjusting for age, fecundability was similar when CMM was compared to OPK (FR: 0.87, 95% CI: 0.52-1.44) and BBT (FR: 0.82, 95% CI: 0.48-1.42). Cycles in which a woman achieved a positive OPK were as likely to result in a pregnancy as cycles in which BBT was used (FR: 0.95, 95% CI: 0.53-1.70). Results did not change when the analysis was restricted to cycles 25-35 days in duration.

CONCLUSION: While FAMs improve natural fertility by increasing fecundability and reducing time to pregnancy, these results suggest that no single method is superior. A randomized trial is needed to support or refute these findings.

Supported by: This work was funded by the NIH/NICHD grant RO1067683.

NURSING

P-11 Tuesday, October 21, 2014


OBJECTIVE: To examine practice patterns of assisted-reproduction programs regarding required or recommended genetic screening of anonymous and known oocyte donors and known sperm donors.

DESIGN: Cross-sectional survey.

MATERIALS AND METHODS: A one-page questionnaire was offered to attendees at a 2014 national symposium on third-party reproduction. Descriptive statistics were compiled and analyzed by Fisher’s exact test.

RESULTS: There were 46 responses from practices in 22 states; 6 (13%) were academic programs and the remainder were from private practices. The majority of respondents (65%) were nurses. Practices did a median of 35 (range 4-1093) donor egg cycles yearly. Of the 46 programs responding, only 8 (17%) have an onsite genetic counselor. All programs obtain genetic carrier screening (GCS) of anonymous oocyte donors, but its scope (Table) varies; multiple screening guidelines are endorsed by 22 programs (48%).

One of 2 specialty labs is routinely used by 3 programs (83%). Only 10 programs (22%) routinely require genetic screening of the recipient’s male partner. When oocyte donors are known to intended parents, 34 programs (74%) require the same genetic screening as for an anonymous donor, while 11 (24%) allow the recipient to decide its scope. For the 30 programs accepting known sperm donors, endorsed genetic screening guidelines are shown in the Table. The presence of an onsite genetic counselor was not associated with use of comprehensive GCS for either egg donors or known sperm donors, or with the use of one of the 2 specialty labs.

CONCLUSION: Genetic screening of oocyte donors is obtained universally but inconsistently follows published guidelines. Genetic screening of known gamete donors tends to track that used for anonymous oocyte donors, but use of comprehensive or history-targeted GCS of known sperm donors is less frequent than for anonymous egg donors.

P-12 Tuesday, October 21, 2014


OBJECTIVE: Guidelines for treatment of mild hypothyroidism prior to conception have recently become more inclusive. The objective of this study was to examine practice patterns among donor oocyte programs in regard to screening and treatment of donor-oocyte recipients for hypothyroidism.

DESIGN: Cross-sectional survey.

MATERIALS AND METHODS: A one-page questionnaire was offered to attendees at a 2014 national symposium on third-party reproduction.

RESULTS: There were 46 responses from practices in 22 states; 6 (13%) were academic programs and the remainder were from private practices. The majority of respondents (65%) were nurses. Programs performed 35 donor egg cycles (median; range 4-1093) per year. Only four programs (9%) denied routinely screening prospective recipients for hypothyroidism; the majority of programs (57%) screen with TSH level only at the initial visit, while 35% screen annually. In early pregnancy, 29 programs (71%) screen recipients again: at the time of the first or second beta-hCG in 17 of programs (37%), and with the first ultrasound scan in 8 programs (17%). The TSH level at or above which programs treat with levothyroxine is 2.5 mIU/L in 26 programs (56%), 2.6-3.0 mIU/L in 9 programs (20%), and 3.1-4.5 mIU/L in 4 programs (9%). The frequency of TSH testing is every 4 weeks in 15 programs (33%), every 4.5-5 weeks in 9 programs (20%) and every 6 weeks in 13 programs (28%). The choice of levothyroxine was brand-name in 8 programs (17%) and generic in 38 (85%). Of the latter, 17 (45%) stated they require the same generic to be dispensed with refills, while 16 (42%) said they do not.

CONCLUSION: Screening of planned donor-egg recipients for hypothyroidism, treatment with levothyroxine, and TSH re-testing generally follow recommended guidelines, with early-pregnancy TSH measurement often deferred to the time of first ultrasound. The majority of programs seek consistent daily levothyroxine dosing through either brand name or same-generic prescription.

OVARIAN RESERVE

P-13 Tuesday, October 21, 2014

OVARIAN RESERVE AFTER FIXED VERSUS ADJUSTED LAPAROSCOPIC OVARIAN DRILLING IN CLOMIPHENE RESISTANT PCOS PATIENTS. M. S. Zakherah. Obstetrics and Gynecology, Assuit Medical School, Assuit, Egypt.

OBJECTIVE: To evaluate the impact of fixed versus adjusted laparoscopic ovarian drilling (LOD) on ovarian reserve assessed by serum anti-Mullerian hormone (AMH), basal follicle stimulating hormone (FSH), antral follicle count (AFC) and ovarian volume in women with clomiphene citrate (CC)-resistant polycystic ovary syndrome (PCOS).

DESIGN: A randomized controlled clinical trial.

MATERIALS AND METHODS: 120 women with CC-resistant PCOS were recruited for this study. They were randomly allocated into two groups: group A [60 women underwent fixed LOD (600J/each ovary) and group B [60 women underwent adjusted LOD (based on ovarian volume with use of a new) model for dose calculation (60 J/cm3 of ovarian tissue)] Basal levels of serum AMH, FSH, AFC and ovarian volume were measured in the 2 groups before and after ovarian drilling (one and six months). Changes in serum AMH levels and AFC were taken as the primary outcome measure. Assuming a 20% difference between the groups, with an α of 5% and a β of 20%, it was calculated that 60 women are required in each arm of the study to detect a true difference at the 95% confidence level with 80% power. Statistical
A NEW ALGORITHM TO PREDICT OVARIAN AGE COMBINING CLINICAL, BIOCHEMICAL AND 3D-ULTRASONOGRAPHIC PARAMETERS. R. Venturella, D. Lico, A. Sarica, M. P. Falbo, E. Guidera, M. Cannataro, F. Zullo, Obstetrics and Gynecology, Magna Graecia University of Catanzaro, Catanzaro, Italy; School of Informatics and Biomedical Engineering-Bioinformatics Laboratory, Magna Graecia University of Catanzaro, Catanzaro, Italy; Chair of Clinical Pathology, Magna Graecia University of Catanzaro, Catanzaro, Italy.

OBJECTIVE: Ovarian reserve is a crucial parameter for guiding women's and gynecologist's choices but it is not yet assessable. A reliable test is urgently needed. We aim to create a multimodal evaluation of ovarian reserve and design a new algorithm able to predict ovarian age (OvAge) with an intuitive, universally accepted and reproducible output.

DESIGN: Prospective observational study.

MATERIALS AND METHODS: 652 healthy women, 18 to 55 years age; 29 women with clinical suspect of premature ovarian failure (POF) and 29 women with PCOS were prospectively enrolled. In all women data on AMH, FSH, Estradiol, 3D Antral Follicle Count, ovarian volume, Vascularization Index, Flow Index and Vascularization Flow Index between day 1 and day 4 of menstrual cycle were collected. A Generalized Linear Model (GLM) was realized. The predictors variables were assessed by backward stepwise multiple regression. Backward selection of parameters was applied. The variables reaching the statistical significance in multivariate regression analysis were then used in the calculation for the final optimal model chosen using the Akaike information criterion.

RESULTS: Among the 10 models studied, the best-fitting is GLM#8** and the best equation is: OvAge = 48.05-3.14*AMH+0.07*FSH-1.55*AF-0.11*FI+0.23*VI+0.2*AMH+0.04*FSH+AF. With this model, women enrolled as healthy controls a high level of fit between chronological age and OvAge was shown whereas in POFs and PCOS patients a significant difference between these two parameters was shown, indicating that the our algorithm is able to recognize pathological deviation from normal ovarian function.

CONCLUSION: The innovation introduced by our algorithm is that the final output is not a generic definition of good or poor ovarian reserve, like others single tests already do. Our test answers with a number. It will be useful for the gynecologist to guide patients' reproductive attempts and to reduce the rate of unnecessary surgeries and for women for guiding their reproductive and working planning.

Comparing IVF outcome in endometrioma and in the contralateral intact ovary

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Endometrioma</th>
<th>Contralateral</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antral follicle count</td>
<td>1.2±0.5</td>
<td>2.0±0.4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Number of follicle flushings</td>
<td>1.1±0.3</td>
<td>1.6±0.7</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Number of oocytes in metaphase II retrieved</td>
<td>0.7±0.3</td>
<td>1.3±0.3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Number of embryo obtained</td>
<td>0.4±0.2</td>
<td>0.8±0.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>High-quality blastocyst obtained(%)</td>
<td>33.3</td>
<td>25.0</td>
<td>NS</td>
</tr>
<tr>
<td>Clinical pregnancy rate (%)</td>
<td>11.1</td>
<td>14.2</td>
<td>NS</td>
</tr>
</tbody>
</table>

Note: Values are mean±SD. NS=not significant.

CONCLUSION: Small endometrioma appear to cause a damage in ovarian reserve, but not qualitative injury through IVF/ET.
was not different between groups. FSH, AMH, tAFC, and AFC-LD were significantly different between the three groups. AFC-LD was highly correlated to tAFC (r = 0.83) and AMH (r = 0.80). The optimal cut point for AFC-LD was >96.26% and specificity of 74.20% for normal ovarian reserve.

CONCLUSION: Measuring the AFC-LD correlates well to established measures of ovarian reserve and a cut-off of at least 6 indicates good ovarian reserve. AFC-LD is a novel measure that shows promise for assessing ovarian reserve in cancer survivors and naturally aging women.

Supported by: RO1HD062797

P-17 Tuesday, October 21, 2014

PROTECTIVE EFFECTS OF SILDENAFIL CITRATE ADMINISTRATION ON CISPLATIN-INDUCED OVARIAN DAMAGE IN RATS, M Isliyeme Taskin, E. Ayay, E. Adali, E. Balciglug, U. Inceoguz.* "Obstetrics and Gynecology, Balikesir University School of Medicine, Balikesir, Bigadik, Turkey; Histology and Embryology, Erciyes University School of Medicine, Kayseri, Turkey.

OBJECTIVE: The aim of this study is to evaluate the effects of sildenafil citrate on cisplatin-induced ovarian toxicity.

DESIGN: This study was designed as an experimental study and was held in university-based research laboratory. A total of 32 Wistar albino female rats of cycling reproductive age were used in the experiments. Rats were utilized to create four groups. Group 1: Saline control (n=8); Group 2: Cisplatin (n=8); Group 3: Sildenafil citrate (n=8); Group 4: Cisplatin plus sildenafil citrate group (n=8).

MATERIALS AND METHODS: The rats in group 2 were injected with cisplatin at a dose of 5 mg/kg intraperitoneally (i.p.). The rats in group 4 were given sildenafil citrate i.p. at a dose of 1.4 mg/kg. Subsequently, 30 min later, cisplatin was administrated i.p. at a dose of 5 mg/kg. The rats in group 3 were injected with sildenafil citrate at a dose of 1.4 mg/kg i.p. The rats in group 1 were given saline i.p. in equal volumes. These injections were repeated weekly twice in total. Ovaries were removed two weeks later in all groups. Histopathologic examination, follicle counting and classification was performed. The expression of anti-Mullerian hormone (AMH) was detected immunohistochemically in the ovarian tissues. Primordial, primary, preantral, secondary, tertiary follicle counts and immunoreactivity intensity of AMH were evaluated statistically among groups.

RESULTS: Sildenafil alleviated cisplatin-induced histopathological changes in the ovarian tissue. Primordial, secondary and tertiary follicles were diminished in group 2 compared with the group 1 (p<0.05). Pre-treatment with sildenafil citrate was increased primordial follicle count in group 4 according to group 2 and this increase was statistically significant (p<0.05). Secondary and tertiary follicle counts were not ameliorated in group 4 according to group 2. Primary and preantral follicle counts were not differed among the groups. According to our results, immunoreactivity intensity of AMH was decreased in group 2 (92.4±3.97 vs. 88.8±1.77) with no statistically significant difference. Whereas, immunoreactivity intensity of AMH was increased in group 4 according to group 2 (88.8±1.77 vs. 94.1±2.36) (p<0.05).

CONCLUSION: Our pilot study shows that AMH values can vary significantly depending on storage conditions, storage time, and type and lot of assay used. Whether this is due to assay interference from other factors such as complement (BC field notice) will continue to be investigated. Several AMH assays exist and clinicians must be diligent with regard to understanding where and how samples are tested for both clinical and research purposes. Given the increasing importance placed on AMH values in clinical decision making for patients, further research in this area is of utmost importance.

Supported by: 1K12HD063086-01 (ARC), Beckman Coulter Inc., support (ARC) and grant funding (GLM), ST32HD04135-12 (JSR).

P-19 Tuesday, October 21, 2014

DIMINISHED FUNCTIONAL OVARIAN RESERVE IS ASSOCIATED WITH DYSBOLISM OF AMYLOID PRECURSOR PROTEIN IN GRANULOSA CELLS. J. Niu, S.-L. Chen, F.-H. Duan, X. Chen, P. Li. Nanfang Hospital, Southern Medical University, Guangzhou, Guangdong, China.

OBJECTIVE: Diminished ovarian reserve is a primary cause of age-related decline in female fertility. However, its underlying mechanism remains unknown. Overexpression of amyloid precursor protein (APP) in granulose cells (GCs) is related with ovarian aging have been demonstrated. This study detected APP, β-site APP-converting enzyme(BACE1),amyloid-β42(AD42) and apoptosis family members to further explore the impact of APP and its metabolites on ovarian.

DESIGN: The study involved in 19 women with premature ovarian aging/occult primary ovarian insufficiency(POA/OPO),defined as age<38years and abnormally low functional ovarian reserve (FOR) by age-specific FSH and/or anti-Mullerian hormone(AMH);29 women with physiologic diminished ovarian reserve(DOR),defined as age>40 years;32 control patient-s<38years demonstrated normal ovarian reserve by FSH and/or AMH.

MATERIALS AND METHODS: Total RNA was extracted from each GC sample and expression of APP,BACE1,Bcl-2,Bax was performed by qRT-PCR. Follicular fluid of all patients were collected and ELISA was applied to detect the expression of AD42. Comparison among the three groups was performed by significance analysis of one-way ANOVA.

CONCLUSION: Our pilot study shows that AMH values can vary significantly depending on storage conditions, storage time, and type and lot of assay used. Whether this is due to assay interference from other factors such as complement (BC field notice) will continue to be investigated. Several AMH assays exist and clinicians must be diligent with regard to understanding where and how samples are tested for both clinical and research purposes. Given the increasing importance placed on AMH values in clinical decision making for patients, further research in this area is of utmost importance.

Supported by: 1K12HD063086-01 (ARC), Beckman Coulter Inc., support (ARC) and grant funding (GLM), ST32HD04135-12 (JSR).
RESULTS: APP significantly varied between the three groups (P<0.001), with older women with DOR actually demonstrating higher levels than controls (P<0.001). BACE1, Bax, and Aβ42 differed between the three groups more profoundly (all P<0.001), with women with POA/OPOI showing significantly higher levels than controls (all P<0.001). There was a lower level of Bcl-2 in DOR and POA/OPOI groups (P=0.001). The correlation analysis showed that APP mRNA and Aβ42 were positively correlated with BACE1 and Bax, and negatively correlated with the expression of Bcl-2 (P<0.05). Aβ42 was negatively related with number of oocytes (P<0.05).

CONCLUSION: The increased expression of APP, BACE1, Aβ42 may be associated with diminished ovarian reserve. High expression of APP and Aβ42 may reduce Bcl-2 levels in ovarian granulosa cells, which may play a role in the development of follicles, the growth and apoptosis of granulosa cells and maturation of oocytes, thus affect functional ovarian reserve.

Supported by: Supported by National Natural Science Foundation of China (81170574, 31371517) and Science Foundation of Nanfang Hospital (GT201206).
contraception decreases the AMH produced by granulosa cells of the early growing follicles through inhibition of follicular development. We investigated the effect of combined hormonal contraceptives on AMH levels in an oocyte donor population.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: Per protocol, AMH levels and contraceptive history were routinely obtained from potential oocyte donors at the initial office visit. All anonymous oocyte donors who had pretreatment AMH levels (AMH II assay) were included. Donors were split into user and non-user groups. Charts were reviewed to assess for history of contraceptive use. The non-user group included all donors not taking hormonal contraceptives for three or more months. T-test was performed. P<0.05 was considered significant. Data presented as mean±SEM.

RESULTS: 82 non-users and 52 users were identified.

<table>
<thead>
<tr>
<th></th>
<th>Non-user (n=82)</th>
<th>User (n=52)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMH(ng/ml)</td>
<td>3.31±0.33</td>
<td>2.36±0.29</td>
</tr>
<tr>
<td>Age</td>
<td>26.47±0.35</td>
<td>26.31±0.38</td>
</tr>
<tr>
<td>BMI</td>
<td>22.54±0.29</td>
<td>23.17±0.47</td>
</tr>
<tr>
<td>Total start</td>
<td>201.73±4.5</td>
<td>218.75±7.82</td>
</tr>
<tr>
<td>gonadotropins (iu)</td>
<td>2095.98±78.58</td>
<td>2754.17±132.12</td>
</tr>
<tr>
<td>Total amount of gonadotropins (iu)</td>
<td>10.04±0.15</td>
<td>10.96±0.14</td>
</tr>
<tr>
<td>Days of GnRH antagonist used</td>
<td>4.57±0.11</td>
<td>4.73±0.13</td>
</tr>
<tr>
<td>Peak E2 levels (pg/ml)</td>
<td>2141.44±100.8</td>
<td>2082.9±119.7</td>
</tr>
<tr>
<td>Oocytes harvested</td>
<td>18.95±1.12</td>
<td>16.94±0.94</td>
</tr>
<tr>
<td>Mature oocytes</td>
<td>15.38±0.87</td>
<td>14.38±0.82</td>
</tr>
</tbody>
</table>

NS= Not significant.

CONCLUSION: Use of combined contraception is associated with lower AMH levels. Oocyte donors on combined contraception required more gonadotropins for a longer period of time showing a higher degree of ovarian suppression. In both groups peak E2 and number of oocytes harvested were similar.

P-24 Tuesday, October 21, 2014

ANTII-MULLERIAN HORMONE OR FOLLICLE STIMULATING HORMONE: WHICH HORMONE BETTER PREDICTS STIMULATING RESPONSE AND PREGNANCY OUTCOME?.

I. M. Abdullahi, M. A. Damaro, N. L. Bossert, R. Isaksson. Obstetrics and Gynecology, University of Minnesota, Minneapolis, MN.

OBJECTIVE: Is there a difference between serum AMH and FSH in predicting stimulation response and pregnancy outcomes during in vitro fertilization?

DESIGN: Retrospective study.

MATERIALS AND METHODS: PATIENTS: A total of 341 eligible patients met criteria for first cycle of autologous IVF with complete AMH and FSH data. Patients utilizing donor oocytes were excluded from the study.

MAIN OUTCOME MEASURES: Primary outcomes were divided into stimulation response and pregnancy outcomes. Stimulation response included oocyte yield and peak estradiol concentration on the day of hCG administration. Pregnancy outcome included positive pregnancy test as well as presence or absence of live birth.

STATISTICAL METHODS: The correlation between AMH and FSH was calculated using Spearman’s correlation coefficient. AMH and FSH levels were additionally categorized based on clinical and published cut-offs. In particular, we considered FSH (IU/L) as either a dichotomous variable (<10,>10) or as multiple categories (<6, 6-7.9, 8-9.9, 10-11.9, 12+) and AMH (ng/dl) as either a dichotomous variable (<8.0,>8.0) or as quintiles (8.0-1.39, 1.40-2.39, 2.40-4.19, >4.2). Using univariate and age-adjusted logistic regression models, associations were made between patient demographic and clinical characteristics to pregnancy (yes/no) and live birth (yes/no). Odds ratios (OR) and 95% confidence intervals (CI) are presented. P-values less than 0.05 were considered statistically significant.

RESULTS: Data demonstrated that oocyte yield and peak estradiol levels were all statistically associated with both FSH and AMH. AMH demonstrated statistically significant in predicting pregnancy rate and live birth rate with regards to both cut-off and quintile variables (P-value: 0.0003 and 0.006, 0.001 and 0.016, respectively). When adjusted for age, AMH remained statistically significant for cut-off (P-value: 0.001, .033, respectively) but not for quintiles. FSH was not able to predict pregnancy rate or live birth rate with statistical significance for both cut-offs and quintiles even when it was adjusted for age.

CONCLUSION: When comparing AMH and FSH, they both were able to predict stimulation response, which included oocyte yield and peak estradiol levels. However, AMH better predicts pregnancy outcomes, which included pregnancy rate and live birth rate compared to FSH. When adjusted for age, AMH cut-offs were able to predict pregnancy and live birth rates.

P-25 Tuesday, October 21, 2014

EXTENDING OVARIAN STIMULATION IN PATIENTS WITH DIMINISHED OVARIAN RESERVE BEYOND NORMAL TRIGGER CRITERIA OF TWO FOLLICLES ≥18MM INCREASES OOCYTE YIELD AND MATURITY BUT NOT FERTILIZATION RATES.

J. E. Swat, M. G. Katz-Jaffe, J. S. Stevens, W. B. Scholcraft. a b *Fertility Laboratories of Colorado, Lone Tree, CO; bColorado Center for Reproductive Medicine, Lone Tree, CO.

OBJECTIVE: Obtaining mature and competent oocytes is critical for successful IVF. Ovarian stimulation protocols are developed to achieve a balance of high oocyte yield and resulting quality. Competent oocytes are dependent upon proper follicular growth during stimulation, with larger follicles often yielding higher quality eggs. However, in patients with diminished ovarian reserve (DOR), there is a tendency to extend ovarian stimulation prior to ovulation trigger in order to improve follicular development and increase oocyte yield. The aim of this study was to examine a patient population with DOR and assess the impact of extending ovarian stimulation prior to hCG trigger.

DESIGN: Retrospective analysis.

MATERIALS AND METHODS: DOR patients with AMH levels <0.5ng/ml undergoing embryo banking (n=206) were analyzed to determine number of days ovarian stimulation was extended beyond normal hCG trigger criteria of 2 follicles ≥18mm. Patients were stratified into groups of 0, 1, or 2 days of extended stimulation. Endpoints were compared, including number of oocytes retrieved, number of mature oocytes and fertilization rates following ICSI. Data were analyzed using ANOVA and differences determined using Tukey, with significance at p<0.05.

RESULTS: No significant difference in maternal age at time of oocyte retrieval was apparent among the groups (Group 0=40.57±0.3 years, n=39; Group 1=40.2±0.3 years, n=40; Group 2=40.0±0.4 years, n=77). Significantly more oocytes were retrieved from patients with extended stimulation for 1 (8.5±0.4 oocytes) or 2 days (8.9±0.6 oocytes) compared to an “on-time” day 0 trigger (6.0±0.4 oocytes), p<0.001. Similarly, more mature oocytes were obtained when extending stimulation for 1 (6.2±0.4 M2 oocytes) or 2 days (6.2±0.5 M2 oocytes) compared to “on-time” trigger (4.1±0.4 M2 oocytes), p=0.012. Nevertheless, a trend towards decreased fertilization rates was observed when extending stimulation for 1 and 2 days (Group 0=71.4%±0.3, Group 1=68.2%±0.03, Group 2=63.3%±0.04, ns).

CONCLUSION: Extending ovarian stimulation in DOR patients increases oocyte yield and maturity, but does not improve fertilization rates. Thus, the additional oocytes retrieved may be post-mature or of suboptimal quality. Future studies, following frozen embryo transfers, will examine additional outcome measures, such as blastocyst conversion and implantation, to determine if extending ovarian stimulation in DOR patients has any additional benefit or detriment.

P-26 Tuesday, October 21, 2014

DISCONTINUATION OF LONG TERM USE OF HORMONAL CONTRACEPTIVES LEADS TO A SIGNIFICANT INCREASE IN AFC AND OOCYTE YIELD IN WOMEN UNDERGOING, ELECTIVE FERTILITY PRESERVATION OR DONOR CYCLES.


OBJECTIVE: Antral follicle count (AFC) is used by clinicians to counsel their patients about their ovarian reserve and approximates the oocyte

Vol. 102, No. 3, Supplement, September 2014
yield after ovarian stimulation. Hormonal contraceptives suppress the hypothalamic–pituitary–ovarian axis and may potentially influence follicle recruitment and response to controlled ovarian stimulation (COS). In this study, we aimed to determine whether stopping hormonal contraception for at least two months improves AFC and egg yield in women undergoing elective fertility preservation or donor cycles.

**DESIGN:** Retrospective cohort.

**MATERIALS AND METHODS:** Between 2012 and 2014, total of 228 (oocyte donors, n=156; elective fertility preservation, n=72) women were included in the study. The inclusion criteria for the study required being on hormonal contraceptives at the initial evaluation for greater than 1 year, or no history of long term hormonal contraceptives within the last 6 months. Each patient had AFC measurement at the initial visit (AFC1) and repeat evaluations of AFC at least 2 months later (AFC2) on the first day of their ovarian stimulation. Every patient underwent antagonism IVF cycle and egg retrieval. Change in AFC and egg yield after COS were assessed with paired t-test and p<0.05 was accepted as significant.

**RESULTS:** 158 patients (Group A) had no recent history of hormonal contraceptive use and 70 patients had been on birth control pill more than one year. The average length of OCP use was 5.2±2.3 years. 39 of these 70 patients had been on birth control pill more than one year. The rest of OCP users (Group B) continued hormonal contraception until COS. The rest of OCP users (Group C, n=31) stopped hormonal contraception after this initial visit for average of 3.9±1.4 months (range: 2-8 months) before COS. No change in AFC was observed in Groups A and B (Table). However, in Group C, AFC significantly increased from 13.2±6.2 to 23.3±6.2 (p<0.001). In groups A and B, both AFC1 and AFC2 were similar to number of oocytes retrieved and, therefore, were able to approximate the oocyte yield after COS. But, in Group C, only AFC2, but not AFC1, was similar to the number of oocytes and was able to predict the oocyte yield (Table).

<table>
<thead>
<tr>
<th>AFC1</th>
<th>AFC2</th>
<th>Oocytes Retrieved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>19.7±9.0</td>
<td>19.4±9.9</td>
</tr>
<tr>
<td>Group B</td>
<td>17.9±4.72</td>
<td>16.6±4.1</td>
</tr>
<tr>
<td>Group C</td>
<td>13.2±6.2*</td>
<td>23.3±6.2*</td>
</tr>
</tbody>
</table>

*p<0.001, *p<0.001

**CONCLUSION:** AFC does not retain its accuracy as predictor of the ovarian reserve in women using hormonal contraception for longer than a year. Therefore, stopping OCP at least 2 months before ovarian stimulation may reveal the patient’s true ovarian potential and increase the mature oocyte yield.

**P-28 Tuesday, October 21, 2014**

**PLATINUM AND TAXANE-INDUCED OVARIAN DAMAGE IN A MOUSE MODEL: MECHANISMS AND IMPLICATIONS FOR GONADOPROTECTION**

**OBJECTIVE:** Determine the mechanisms by which carboplatin and paclitaxel combination chemotherapy (CT) damage the ovary.

**DESIGN:** Experimental laboratory animal study.

**MATERIALS AND METHODS:** C57Bl/6 mice were divided into groups and treated with vehicle (control) or CT (carboplatin 80mg/kg and paclitaxel 20mg/Kg). Mice were sacrificed at 48 hours (n=3 per group) or 21 days (n=10 per group). Outcomes at the 21 day time-point included differential follicle counts, serum antimullerian hormone (AMH) levels, and microvessel density (MVD) quantified by immunohistochemistry (IHC) for CD31. Outcomes for the 48 hour time-point included quantifying DNA damage and apoptosis by IHC for γ-H2AX and caspase-3, respectively. Groups were compared with a student’s t-test using a one-tailed p-value of 0.05.

**RESULTS:** Primordial follicles (PMF) per ovary were reduced by over 90% in the CT group (68±51.7 compared with controls (846±122; p<0.001); and growing follicles (GF) were reduced by 80%, from 432±43.4 to 85±28, respectively (p<0.001). AMH levels were significantly reduced in the CT group (64±6.3ng/ml compared with controls (96±8.15ng/ml; p=0.016). MVD was not different between control (1.33±0.2mm²) and CT (1.65±0.2mm²; p=0.09). Caspase-3 staining oocytes were absent in control mice, but 65±4.2% of PMF oocytes (p<0.001) and 6.7% of GF oocytes (p=0.26) were apoptotic in the CT group. Likewise, 67.6±8.8% of PMF oocytes were γ-H2AX positive in CT mice compared to 1.4% in controls (p<0.001). The GF pool oocytes did not exhibit γ-H2AX staining in either group. Less than 5% of granulosa cells (GC) stained for caspase-3 or γ-H2AX, and results were not different between groups.

**CONCLUSION:** Paclitaxel and carboplatin combination chemotherapy reduces PMFs and ovarian reserve by inducing DNA damage and apoptosis of the PMF oocytes. Future study will evaluate breeding outcomes and gonadoprotective agents targeting this mechanism.

**Supported by:** Supported by: NIH grant HD055475, Magee-Womens Research Institute and Foundation and gifts from Julie and Michael McMullen and Sylvia Bernassoli.

**P-29 Tuesday, October 21, 2014**

**ANTI-MULLERIAN HORMONE (AMH) AS A PREDICTIVE MARKER IN COLLEGE AGE WOMEN AND THEIR SISTERS**

**OBJECTIVE:** Anti-Müllerian hormone (AMH) is a novel marker of ovarian reserve. It reflects the number of ovarian follicles, it functions as a marker of ovarian aging.
AMH has gained widespread use in fertility clinics because low AMH is believed to predict impaired fertility and imminent menopause. Most of the studies have involved evaluation of AMH levels in individuals, but variability in AMH levels within families has not been explored. The aim of this study is to investigate the range of AMH levels in college aged healthy women and their sisters.

**DESIGN:** Cross sectional study.

**MATERIALS AND METHODS:** Blood samples were collected from 738 female college students and their sister’s at the University of Michigan between June 2006 and January 2009 (1). Participants were between the age of 14 to 36 who completed a phenotyping survey, including lifestyle questionnaire and menstrual history. Hormone concentrations were determined by two-site enzyme-immunoenzymetric assay for FSH (detection range: 1- 200mIU/mL; CV:1.2-3.1%) and E2 (25-3000pg/mL; CV:3.9%) and enzyme-linked immunosorbent assay (ELISA) for AMH (detection range: 0.16 - 25 ng/mL; CV: 5.1% - 6.7%). Correlation between AMH, smoking, birth control use, menstrual characteristics, ethnicity, BMI, age of menarche, FSH and E2 were analyzed by Pearson Correlation and ANOVA where appropriate. AMH correlation between sisters was evaluated by using mixed effect model to log (AMH) values. $P < 0.05$ was used for statistical significance.

**RESULTS:** There was a statistically significant correlation between birth control use and AMH level ($P = 0.0062$; CI: -0.89 to -0.13). Birth control use is associated with a 0.5 ng/mL decrease in AMH level. Our mixed effect model revealed a statistically significant correlation of AMH levels between sisters ($P = 10-8$; CI: 2.4-5.3). There was no correlation between AMH levels and the variables mentioned above.

**CONCLUSION:** Use of hormonal contraception is associated with a minimal, but statistically significant, reduction of AMH values in young women. AMH levels were positively correlated between sisters, suggesting that ovarian reserve may have a significant genetic contribution and AMH may be a marker of future fertility within families. To our knowledge, this is the first study evaluating the possible hereditability of AMH between sisters.

**Supported by:** 1. K12HD065257; National Institute of Child Health and Human Development funding; Women’s Reproductive Health Research Scholar Award (NF). 2. IRWG PG U039134; Institute for Research on Women and Gender, University of Michigan (NF).

**P-30 Tuesday, October 21, 2014**

**ETHANOL USE AND ITS ASSOCIATION WITH OVARIAN NON-GROWING FOLLICULAR COUNT.** J. D. Peck, a A. M. Quass, b L. B. Craig, c M. R. Soules, d N. A. Klein, e K. R. Hansen, f Dept. of Biostatistics and Epidemiology, University of Oklahoma Health Sciences Center, Oklahoma City, OK; f Dept. of Obstetrics and Gynecology, Section of Reproductive Endocrinology and Infertility, University of Oklahoma Health Sciences Center, Oklahoma City, OK; e Seattle Reproductive Medicine, Seattle, WA.

**OBJECTIVE:** Multiple studies have investigated the impact of lifestyle choices (tobacco use, oral contraceptives [OCPs], BMI, and ethanol use) on reproductive lifespan by correlating the impact of these exposures with the age of spontaneous menopause. Although occasionally in agreement, many of these studies have reported contradictory findings. The purpose of this investigation was to determine the relationship between lifestyle factors and the true ovarian reserve, the ovarian non-growing follicle (NGF) number, in women undergoing oophorectomy for benign indications.

**DESIGN:** Cross-sectional study, university setting.

**MATERIALS AND METHODS:** Normal ovaries were collected from 113 women (age 21-52 years) undergoing oophorectomy for benign indications at two clinical sites. BMI, tobacco, ethanol and OCP use were determined prior to surgery with a detailed questionnaire. Using systematic random sampling and a validated fractionator/optical disector technique, ovarian NGF counts were determined. The relationship between log-transformed ovarian NGF count and lifestyle factors was determined by multivariable linear regression. A $P$-value of $< 0.05$ was considered statistically significant.

**RESULTS:** After controlling for age, BMI, OCPs, tobacco use and site of collection, cumulative ethanol use (measured in alcoholic drinks per day multiplied by years of drinking) was associated with ovarian NGF count. Women reporting light (< 1 drink-years) and moderate (1-3 drink-years) ethanol use had greater NGF counts ($\beta = 1.10$, $p = 0.03$, and $\beta = 1.79$, $p = 0.009$, light and moderate use, respectively) compared to non-users. Neither heavier ethanol use (>3 drink-years), BMI, OCPs, nor tobacco use were significantly associated with ovarian NGF count.

**CONCLUSION:** In this investigation, light to moderate ethanol use was associated with a greater ovarian NGF count. This finding is consistent with investigations that have suggested that light to moderate ethanol use is associated with a delayed age of spontaneous menopause.

**Supported by:** HR04-115 (OCAST, K.R.H.).

**P-31 Tuesday, October 21, 2014**

**AMH REGULATES SCF VIA THE CAMP/PKA PATHWAY IN HUMAN GRANULOSA CELLS.** R. Hu, a,b F. Wang, c L. Yu, c S. Oehninger, c S. Bocca. c Reproductive Medicine Center, General Hospital of Beijing Medical University, YingChuan, NingXia, China; c Key Laboratory of Fertility Preservation and Maintenance of Ministry of Education, Ningxia Medical University, Yingchuan, Ningxia, China; The Jones Institute for Reproductive Medicine, Department of Obstetrics and Gynecology, Eastern Virginia Medical School, Norfolk, VA.

**OBJECTIVE:** Anti-Mullerian hormone (AMH) and stem cell factor (SCF) are cytokines secreted by granulosa cells (GCs), playing different roles in ovarian physiology. We have demonstrated that recombinant human (rh) AMH inhibits the expression of SCF in cultured human (h)GCs. Little is known about the molecular mechanisms by which AMH regulates SCF. Here we investigated if there is a correlation between serum and follicular fluid (FF) AMH and SCF concentrations, and we evaluated if AMH has a regulatory effect on SCF at the transcription and translational levels.

**DESIGN:** Prospective clinical and experimental study.

**MATERIALS AND METHODS:** AMH and SCF were analyzed in 163 patients who underwent IVF cycles in their serum, follicular fluid, and hGCs, using ELISA, RT-PCR and immunoblotting.

**RESULTS:** There was a significant negative correlation between AMH and SCF in FF, and in the mRNA expression of AMH and SCF in lGC. Conversely, there was no correlation between AMH and SCF in serum. In primary cultures of hGCs, SCF mRNA and protein expression were evaluated after pretreatment with AMH and CAMP (alone or in combination) in the presence or absence of a PKA inhibitor (H89). SCF was down-regulated in AMH groups and was increased in the cAMP groups. The effect of rhAMH on SCF expression showed a dose-dependent response. H89 had no effect on SCF expression.

**CONCLUSION:** This is the first report on a modulatory role for AMH as an ovarian/follicular autocrine/paracrine factor controlling SCF expression. We demonstrated that AMH regulates SCF via the cAMP/PKA pathway.

**Supported by:** This work was supported by the National Natural Science Foundation of China (No. 81260109/80425), the Natural Science Foundation of Ningxia province (No. NZ10115), and the State Key Foundation of Science and Technology of Ningxia, China (No.2010297).

**P-32 Tuesday, October 21, 2014**

**LARGE PROSPECTIVE CLINICAL STUDY MEASURING OVARIAN RESERVE IN FEMALES 4-50 YEARS OF AGE WITH AUTOIMMUNE DISEASE FROM 2008-2014: ARE “BENIGN” THERAPIES REALLY SAFE?** A. R. Cooper, a,b G. Lambert-Messner, a R. E. Eklund, b J. D. Peck, e K. R. Hansen, e A. French, f A. White, f V. S. Ratts, a Ob/Gyn, Washington Univ., St Louis, MO; f Pathology/Lab Medicine, Brown Univ., Providence, RI; Pediatrics, Washington Univ., St Louis, MO.

**OBJECTIVE:** Females are prone to autoimmune diseases requiring years of potentially cytotoxic & biologic therapies. Such therapies, in high doses, can be toxic to the ovaries. Little is known about the impact of therapies used daily in lower doses for more “benign” autoimmune conditions on ovarian reserve.

**DESIGN:** 5 year IRB approved prospective clinical study. The Jones Institute for Reproductive Medicine, Las Vegas, NV; with support from the State Key Foundation of Science and Technology of Ningxia, China (No.2010297).

**RESULTS:** After controlling for age, BMI, OCPs, tobacco use and site of collection, cumulative ethanol use (measured in alcoholic drinks per day multiplied by years of drinking) was associated with ovarian NGF count. Women reporting light (> 0.1 drink-years) and moderate (0.2-1.9 drink-years) ethanol use had greater NGF counts ($\beta = 1.10$, $p = 0.03$, and $\beta = 1.79$, $p = 0.009$, light and moderate use, respectively) compared to non-users. Neither heavier ethanol use (>3 drink-years), BMI, OCPs, nor tobacco use were significantly associated with ovarian NGF count.
RESULTS: 86% of patients approached participated. Of 300 enrolled, 270 were followed to completion. 252 females, mean age 27±14 with RA (45%), IRA (44%), SpA (11%) on MTX (33%), TNFα (11%), combo (36%), other (5%) or none (16%) were 6.8±8 years from diagnosis. 18% of girls were premenarchal. Mean time between serum draws was 133±147 days. At varying time points none of the therapies significantly impacted AMH values in the cohort, subgroups, or based on treatment duration in the multivariate analysis (MTX p=0.09-0.38, TNFα p=0.09-0.66, other p=0.25-0.73). In the younger cohort (12±8 years) we saw a nonsignificant decrease in AMH during which time AMH in healthy controls should be increasing.

CONCLUSION: Young females with debilitating chronic autoimmune diseases are on potentially cytotoxic therapies for years. We present an exciting large clinical study with a tremendous amount of data on therapies & outcomes, including some reassuring data that at least short term impacts on the oocyte pool may not be significant. The lack of an increase in the young children warrants further research and based on our additional data may improve when medications are stopped.

Supported by: 1K12HD063086-01, UL1TR000448 and 2T32HD040135-07 (ARC), Beckman Coulter Inc. support (ARC) and grant funding (GLM).

P-33 Tuesday, October 21, 2014

EFFECT OF ABO BLOOD TYPE ON OVARIAN RESERVE. S. Lin, P. Liu, J. Qiao, Department of Obstetrics and Gynecology, Reproductive Medical Center, Peking University Third Hospital, Beijing, China.

OBJECTIVE: Over the years, many ovarian reserve tests have been performed to evaluate the oocyte reserve quality, and predict the outcomes of assisted reproductive technology (ART). Some studies have shown that there maybe a nonlinear relationship between ABO blood type and ovarian reserve. The present study was conducted to explore the underlying mechanism of ABO blood type on DOR.

METHODS: 35,479 women were included. The day 3 serum FSH level was determined using commercially available immunoassays. AMH levels were measured in frozen sera with an in-house ultrasensitive ELISA assay. and were log transformed due to non-normal distribution. The age, body mass index (BMI), blood type, duration of subfertility, sub-fertility type, history of endometriosis, history of ovarian surgery, and antral follicle count (AFC) were collected from each patient. The day 3 serum FSH level was determined using commercially available immunoassays.

RESULTS: Among the 35,479 women, 11,395 (32.12%) were blood type B, 10,583 (29.83%) were blood type O, 9861 (27.79%) were blood type A, and 3640 (10.26%) were blood type AB. There was a significantly higher percentage of blood type O in patients with a FSH ≤ 10 IU/L compared with the FSH > 10 IU/L group. Conversely, a significantly higher percentage of blood types B and AB existed in patients with DOR. No significant difference was observed between blood type A and DOR. Multivariate logistic regression analysis showed that blood type O was significantly less associated with DOR (OR = 0.656; 95% CI = 0.598-0.719; P < 0.001) and B antigen (blood type B or AB) significantly increased the relationship with DOR (OR = 1.525; 95% CI = 1.392-1.672; P < 0.001).

CONCLUSION: In conclusion, the present study showed that there is an association between ABO blood types and DOR. More research needs to be conducted to explore the underlying mechanism of ABO blood type on DOR.

Supported by: National Natural Science Foundation of China for Young Scholars (81200437).

P-34 Tuesday, October 21, 2014

A PROSPECTIVE LONGITUDINAL STUDY OF THE IMPACT OF BREAST CANCER CHEMOTHERAPY ON OVARIAN RESERVE: DO INDIVIDUALS HAVE DIFFERING SUSCEPTIBILITIES? K. Oktay, a,b S. Goldfarb, c G. Bedoschi, c K. Oktay, a,b J. Quistoff, c E. Grunblatt, c T. Cigler, b F. Moy, a S. Patil, a S. Goswami, d M. Dickler, c "New York Medical College, Valhalla, NY; "Innovation Institute for Fertility Preservation and in Vitro Fertilization, New York, NY; "Memorial Sloan-Kettering Cancer Center, New York, NY; "Yeshiva University, New York, NY; "Weill Cornell Medical College, New York, NY.

OBJECTIVE: While the knowledge is accumulating on the adverse impact of chemotherapy (CtH) on fertility, individual longitudinal data on the sensitivity of ovarian reserve to CtH is lacking. Here we performed the largest longitudinal study of the impact of CtH on ovarian reserve in women with breast cancer. We hypothesized that while CtH reduces ovarian reserve, individuals may have differing susceptibilities to this CtH-induced ovarian damage.

DESIGN: Prospective-longitudinal. 207 subjects enrolled into the study. After the exclusions (failure to follow up, tamoxifen-only treatment, treatment sample inadequacy, possible PCOS and consent withdrawal), 103 were available for final analysis.

MATERIALS AND METHODS: 103 women aged 26-46 (mean age 37.4 ± 4.6) with newly-diagnosed stage 0-3 breast cancer had blood sampling prior to and 1 year post completion of 4-6 month CtH with anthracycline-based (AC/TEC-T) and non-anthracycline based (CMF and TC) protocols. AMH levels were measured in frozen sera with an in-house ultrasensitive ELISA assay and were log transformed due to non-normal distribution. Results were analyzed with Wilcoxon rank sum test and repeated measures ANOVA to adjust for age, adjuvant tamoxifen use, and tumor stage.

RESULTS: Compared to baseline (median 0.21, range 0.001-3.9 ng/ml), AMH levels declined significantly (median 0.11, range 0.001-4.47 ng/ml; p<0.0001) 12 months post-ChT. Type of ChT, tumor stage, and adjuvant tamoxifen use did not significantly affect the results. However, AMH levels did not decline in a subset of patients (N=31; those declining within the intra-say variability or showing increase) despite receiving gonadotoxic CtH. The non-declining group did not show difference in age, receptor status, ChT regimen, and tamoxifen use when compared to those who showed significant decline.

CONCLUSION: This prospective longitudinal study shows that breast cancer CtH is detrimental to ovarian reserve. Of interest, there seems to be an individual variability in the sensitivity of ovarian reserve to CtH-induced damage. Further research is needed to explore the genetic factors that confer resistance to CtH-induced ovarian damage.

Supported by: NIH RO1 HD053112 and R21 HD061259, and Jodi Spiegel Fisher Cancer Foundation.

P-35 Tuesday, October 21, 2014

NORMATIVE ANTIMITOGEN HORMONE (AMH) LEVELS AMONGST YOUNG AFRICAN-AMERICAN WOMEN (AGES 23-35 YEARS OLD). E. E. Marsh, a L. Bernardi, a P. de Chavez, b M. S. Ghant, a J. C. Robins, a D. D. Baird, b M. Carnethon. a Obstetrics and Gynecology - REI Division, Northwestern University-Feinberg School of Medicine, Chicago, IL., b Preventive Medicine, Northwestern Univ., Chicago, IL. c Epidemiology, NIEHS, Research Triangle Park, NC.

OBJECTIVE: To characterize the normative AMH levels amongst young African American women (AAW).

DESIGN: Cross-sectional Study.

MATERIALS AND METHODS: 1,654 AAW participating in the Study of Environment, Lifestyle & Fibroids (SELF) were included in this study. Inclusion criteria for participation in SELF were AA race, age 23-34 years at recruitment, no known diagnosis of fibroids, no history of hysterectomy, and no history of cancer, Lupus, Grave’s disease, Sjogren’s disease, scleroderma, or multiple sclerosis that required medical or radiation treatment. AMH was run using an ultrasensitive ELISA assay.

RESULTS: The mean age of the subjects was 28.7 ± 3.5 years (mean±SD). The mean AMH level was 3.99±3.485 with a range of (<.002 to 39.4 ng/ml). 20.6% of the subjects were currently using some form of hormonal birth control. 12.4% of the subjects had decreased ovarian reserve (≤ 1.0 ng/ml) and 0.6% of the subjects had excessively high AMH levels (> 20 ng/ml). There is a nonlinear relationship between age and AMH with peak AMH levels seen at age 26. Using linear regression analysis, a significant association was found between AMH and age, body mass index (BMI), the current use of hormonal contraception, history of abnormal uterine bleeding, ever having been pregnant, and ever had any pregnancies that ended in a live birth. There was no significant association between AMH and age of menarche, a diagnosis of polycystic appearing ovaries (PCO) or PCOS, past use of hormonal contraception, amenorrhea, having sought medical care for difficulty becoming pregnant, currently smoking or a smoking history. Older Age (β= -0.007; SE=.002; P<.01), higher BMI (β= -.013; SE=.003; P=.01), and current contraceptive use (β= -.357; SE=.067; P<.01) remained significantly associated with lower AMH in the multivariable model.

CONCLUSION: This cross-sectional study suggests that ovarian aging in young AAW peaks at a later age than previous studies have reported in other races. While age is correlated with AMH, it only accounts for a relatively small amount of the variation seen in AMH levels amongst young AAW. Longitudinal studies are needed to better characterize ovarian aging in this understudied segment of the population so that appropriate counseling can be provided in terms of fertility and menopausal health implications.
Supported by: NIH Women’s Reproductive Health Research Scholar Award; Robert Wood Johnson Foundation, Friends of Prentice and the Evergreen Invitational.

P-36 Tuesday, October 21, 2014

ABSTRACT MOVED TO OR-412

P-37 Tuesday, October 21, 2014

aDepartment of Obstetrics & Gynecology, Juntendo University, Faculty of Medicine, Tokyo, Japan; bMetabolism & Endocrinology, Juntendo University, Faculty of Medicine, Tokyo, Japan; cObstetrics & Gynecology, Tatedebari Sato Hospital, Takasaki, Gunma, Japan.

OBJECTIVE: Thyroid dysfunction and autoimmunity are related to adverse impact on fertility in reproductive aged women. Anti-Mullerian hormone (AMH), biomarker of ‘ovarian age’ may be affected by impaired thyroid function, but it is unclear the relationship between AMH and thyroid hormone. The aim of this study was to determine the impact of thyroid hormone on AMH levels.

DESIGN: Levels of thyroid-related hormones and serum AMH were measured in women with infertility and normal fertility.

MATERIALS AND METHODS: Between December 2012 and December 2013, consecutive 251 Japanese infertile patients visited to fertility outpatient clinic at the department of Obstetrics and Gynaecology of Juntendo University Hospital. Twenty-seven subjects, who had recent normal delivery and no history of treatment for infertility and thyroid disorder at the age of 30-39 years old, were also recruited. We excluded 184 patients with adverse factors on thyroid hormone and AMH; polycystic ovary syndrome, primary ovarian insufficiency and premature ovarian failure, treated thyroid dysfunction, endometriosis, ovarian tumor, post-surgical ovarian, smoking and aged 40 or over. And we compared thyroid hormones and AMH levels in between 67 infertile patients and 27 healthy fertile women.

RESULTS: The statistical analysis in post-matching between infertile and healthy women showed that both thyroid stimulating hormone (TSH) level and the patient age were impact factors on AMH in infertile patients (patient age, TSH: partial regression coefficient: -0.291, -0.615, p=0.036, 0.003, respectively). This result was also comparable with the statistical result in pre-matched subject.

Baseline characteristics of women with normal fertility and infertility

<table>
<thead>
<tr>
<th>Fertility</th>
<th>Infertility</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients number</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>33.7±3.5</td>
<td>33.7±3.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>20.4±2.0</td>
<td>20.5±2.0</td>
</tr>
<tr>
<td>AMH (ng/ml)</td>
<td>4.36±2.07</td>
<td>2.55±1.86</td>
</tr>
<tr>
<td>TSH (U/ml)</td>
<td>1.52±0.86</td>
<td>2.01±1.09</td>
</tr>
<tr>
<td>FT4 (ng/dl)</td>
<td>1.20±0.18</td>
<td>1.18±0.12</td>
</tr>
<tr>
<td>Prolactin (ng/ml)</td>
<td>10.6±5.2</td>
<td>14.5±3.1</td>
</tr>
</tbody>
</table>

The data are expressed as mean ± SD. BMI, body mass index; AMH, anti-Mullerian hormone; TSH, thyroid stimulating hormone; FT4, free thyroxine.

CONCLUSION: AMH level correlated inversely with TSH concentration in infertile reproductive-aged patients.

P-38 Tuesday, October 21, 2014

OVARIAN RESERVE AND THE AVAILABILITY OF AT LEAST ONE EUPLOID EMBRYO AFTER PREIMPLANTATION GENETIC SCREENING. L. Londra,a K. Tobler,a L. Fahny,a R. Kaufmann,a R. Ross,a W. G. Kearns.a Johns Hopkins Medical Institutions, Baltimore, MD; bFort Worth Fertility, Fort Worth, TX; cCenter for Preimplantation Genetics, LabCorp, Rockville, MD.

OBJECTIVE: To assess to what extent parameters of ovarian reserve affect the chances of having at least one euploid blastocyst after vitro fertilization (IVF) cycles with preimplantational genetic screening (PGS).

DESIGN: Retrospective cohort review.

MATERIALS AND METHODS: Sixty eight infertile patients underwent ovarian reserve testing using AMH and ovarian stimulation with a standard antagonist protocol. All resulting embryos underwent PGS with comparative genomic hybridization. Logistic regression (LR) analysis was used to assess the influence of age, AMH and total gonadotropin requirement on the odds of having at least one euploid embryo available for embryo transfer. Odds ratios (OR) were based on increments of 1 year for age, 0.5 unit of value for AMH and 100 IU of increments of gonadotropin dose.

RESULTS: Mean maternal age was 36.2 ± 4.5 years (range 26-46), mean AMH 1.97 ±1.89ng/mL (range 0.12-8), and mean total gonadotropin dose was 3611 ±190 IU (range 825-5,400). There were 30 (44%) patients older than 37 years and 24 (35%) patients with an AMH <1 ng/mL. A mean of 4.9 ± 2.2 embryos per patient were available for PGS testing, of which a mean of 1.9 ± 2 embryos (18%) had one normal embryo and 34 (50%) had more than one normal embryo after PGS. On univariate analysis, age, AMH and gonadotropin requirement were significantly associated with the availability of at least one normal embryo per cycle (OR 0.75, 95% confidence interval [CI] 0.65-0.88, p<0.001, OR 1.74, 95% CI 1.24-2.43, p=0.001, and OR 0.82, 95% CI 0.71-0.94, p=0.004, respectively). Gonadotropin requirement was not associated with the main outcome after adjusting for AMH and age. However, for every one unit increase in AMH, there was a 33.7 IU decrease in gonadotropin used. Adjusted analysis with age and AMH indicated that for every 0.5 unit increase in AMH and for every 1 year of age increase there was a significant increase and decrease, respectively, in the odds of having at least one euploid embryo (OR 1.83, 95% CI 1.22-2.74, p=0.003 for AMH and OR 0.72, 95% CI 0.59-0.88, p=0.004 for older age).

CONCLUSION: AMH appears to be useful to predict not only ovarian reserve but euploidy in the resulting embryos after IVF-PGS cycles. Supported by: Biostatistics Dept, Bloomberg School of Public Health, Grant Number: 1UL1TR001079 NIH/NICATS.

P-39 Tuesday, October 21, 2014

VITAMIN D STATUS IN FOLLICULAR FLUID OF YOUNG WOMEN WITH DIMINISHED OVARIAN RESERVE AND THE ROLE IN REGULATING ANTI-MULLERIAN HORMONE EXPRESSION. Y. Li, X. Liang, X. Zhang, Y. Deng, J. Chen, C. Di, P. Sun, Y. Xu. Reproductive Medicine Research Center, Sixth Affiliated Hospital, Sun Yat-sen University, Guangzhou, Guangdong, China.

OBJECTIVE: Vitamin D deficiency is common among reproductive-aged women and the seasonal change in anti-mullerian hormone (AMH) levels correlates with the magnitude of change in vitamin D levels. But little known about the vitamin D status in young patients with diminished ovarian reserve (DOR) and how vitamin D affects AMH. This study aims to evaluate the 25-hydroxyvitamin D (25-OHD) and AMH levels in young women with DOR and to study the effect of 1α,25-dihydroxyvitamin D (1α,25(OH)2D3) on the expression of AMH in cultured human luteinized granulosa cells (GCs).

DESIGN: Experimental study.

MATERIALS AND METHODS: From December,2013 to April 2014, 87 infertile women ages 24-36, including 46 women with DOR and 41 women with normal ovarian reserve (NOR) undergoing in vitro fertilization (IVF) were enrolled. 25-OHD and AMH levels in follicular fluid (FF) from large follicles (LF ≥ 18mm) were measured by electrochemiluminescence immunoassay and enzyme linked immunosorbent assay, respectively. In separate experiments, mural GCs were isolated from aspirated fluid of 12 patients and primary murial GCs were cultured in DMEM/F12 culture medium supplemented with or without 1nM, 10nM or 100nM of 1α,25(OH)2D3 (1nM-8 for each group) followed by real time polymerase chain reaction for mRNA expression levels. All statistical analyses were performed with SPSS 13.0 software. The FF AMH levels, AMH mRNA expressions were analyzed by the Mann-Whitney U test and the 25-OHD statuses were evaluated by the Chi-square test.

RESULTS: AMH concentration in FF from patients with DOR was significantly lower than which in patients with NOR [1.50 ng/ml (range 0.31-4.60) vs 4.39 ng/ml (range 0.74-18.70), P<0.001]. Vitamin D insufficiency/deficiency presented in 93.5% of patients with DOR and 78% of patients with NOR (P=0.037). 10nM and 100nM of 1α,25(OH)2D3 (n=8 for each group) followed by real time polymerase chain reaction for mRNA expression levels. All statistical analyses were performed with SPSS 13.0 software. The FF AMH levels, AMH mRNA expressions were analyzed by the Mann-Whitney U test and the 25-OHD statuses were evaluated by the Chi-square test.

CONCLUSION: Vitamin D insufficiency/deficiency is more prevalent in infertile women with DOR who undergo IVF. 1α,25(OH)2D3 regulates AMH expression in cultured human luteinized GCs. Vitamin D is needed for the young infertile women and supplement 1α,25(OH)2D3 may benefit patients with DOR by promoting AMH levels.
FEMALE CANCER SURVIVORS WITHOUT GONADOTOXIC TREATMENTS HAVE FEWER CHILDREN THAN DESIRED.

OBJECTIVE: To evaluate whether female cancer survivors not treated with chemotherapy or radiation meet their reproductive goals.

RESULTS: Both cancer survivors and cancer-free women desired a median of 2 children. Cancer-free women were more likely to report that it was important to have a biologic child (80% vs. melanoma 74%, thyroid 69%, other 75%) but were less likely to report that they would be disappointed if they could not get pregnant (34% vs. melanoma 45%, thyroid 38%, other 38%). Thyroid cancer survivors were told that they had a medical condition that could prevent pregnancy more frequently than cancer-free women (20% vs. 16%). Endometriosis was more common among survivors of melanoma (14%) and other cancers (17%) than among cancer-free women (9%), but polycystic ovary syndrome was similar across groups. Survivors were less likely to have been pregnant (melanoma 71%, thyroid 70%, other 79%) than cancer-free women (85%). They were also less likely to have had a live birth (melanoma 64%, thyroid 66%, other 73% vs. cancer-free women 82%). Pregnancy loss was more common among survivors of thyroid (37%) and other cancers (39%) than among cancer-free women (32%). Induced abortion was more common among survivors of other cancers than among cancer-free women (22% vs. 17%). After adjusting for age, race, education, income, and marital status, survivors were more likely to have fewer children than desired compared with cancer-free women (odds ratio, 95% confidence interval; melanoma 1.6, 1.0-2.5; thyroid 1.9, 1.2-2.8; other 1.4, 1.0-2.1). Fertility counseling at cancer diagnosis was uncommon (melanoma 7%, thyroid 37%, other 21%).

CONCLUSION: Cancer survivors not treated with gonadotoxic therapies are not meeting their reproductive goals. The reason is unclear, but greater efforts to provide fertility counseling may be warranted.

Supported by: NICHD SR01HD060609.

COMPARING IVF OUTCOMES FOR CANCER PATIENTS FOLLOWING EMBRYO AND OOCYTE CRYOPRESERVATION WITH AGE-MATCHED CONTROLS.

OBJECTIVE: To compare the IVF outcomes of patients with cancer who underwent cryopreservation of embryos and oocytes prior to gonadotoxic therapy with IVF outcomes of age-matched controls.

RESULTS: There was no statistically significant difference between the IVF outcomes of patients with cancer who underwent embryo and oocyte cryopreservation at the Massachusetts General Hospital Fertility Center from 1997-2014 and age-matched controls. Their cycles were compared to the IVF cycles (with cryothaw) of 122 age-matched controls with tubal-factor infertility who underwent fresh embryo transfer within 3 years of the comparison cancer case. Wilcoxon rank sum test for equality of means and Pearson chi-square test were used to assess differences in IVF treatment outcomes between the groups. A p-value of <0.05 was used to determine significance.

RESULTS: There was no statistically significant difference between cancer patients (n=63) and controls (n=122) with respect to age (33.7±4.1 vs. 34.5±3.5 years; p=0.08), BMI (24.0±4.8 vs. 24.8±4.4 kg/m2; p=0.11), Day 3 estradiol levels (49.8±25.7 vs. 45.1±16.3 pg/ml; p=0.91), follicle stimulating hormone (38.0±2.0 vs. 35.6±1.5; p=0.28), day of hCG administration (11.7±2.0 vs. 11.9±2.0; p=0.15), peak estradiol level (2049.9±1335.2 vs. 2162.2±713.7 pg/ml; p=0.21), embryos retrieved (12.4±7.8 vs. 10.9±5.2; p=0.36), fertilization rate (67.7%±29.3% vs. 74.4%±18.3%; p=0.34), number of pronuclear embryos (6.6±0.7 vs. 7.1±0.4; p=0.11), average number of embryos transferred (2.0±0.1 vs. 2.0±0.1; p=0.79), implantation rate (27.8%±7.3% vs. 29.9±3.4% vs. 29.9±3.4%; p=0.79), pregnancy rate per transfer (36.7% vs. 43.4%; p=0.49), and live birth rate per transfer (30% vs. 31.5%; p=0.87). There was a statistically significant difference in day 3 FSH levels between cancer patients and controls (6.4±2.2 vs. 7.3±2.1, respectively; p=0.01), and percentage of twin live births (44%±17.6% vs. 14%±6.0%, respectively; p=0.035). Six cancer patients cryopreserved oocytes but have not returned.

CONCLUSION: Our results indicate no difference in most IVF outcomes between cancer patients who underwent embryo cryopreservation and age-matched controls. Higher twin pregnancy rates in cancer patients may reflect lack of underlying infertility or need for cancer-specific transfer guidelines. Further research in this population is indicated.

THE ROLE OF DEMOGRAPHICS AND DIAGNOSIS ON FERTILITY PRESERVATION DECISION MAKING AMONG FEMALE CANCER PATIENTS OF REPRODUCTIVE AGE.

OBJECTIVE: To understand if cancer patient demographics or diagnosis is correlated with the decision to pursue or not to pursue fertility preservation.

RESULTS: The mean age of women was 31.6 (±5.7) years and ranged from 19-44 years. 45.9% of all patients referred elected to pursue a fertility preservation option, and 46.8% of women referred had a diagnosis of breast cancer. Among all patients referred (n=109), there was no significant association between age (p=0.72), race (p=0.44), parity (p=0.54), partner status (p=0.26), type of cancer (solid vs. hematologic, p=0.92), breast vs. non-breast, p=0.72; gynecologic vs. non-gynecologic, (p=0.47), having systemic treatment prior to consultation (p=0.28), education level (p=0.12), or insurance status (p=0.97) and decision to pursue or not to pursue fertility preservation. Among breast cancer patients (n=51), there was no significant association between age (p=0.95), race (p=0.72), having systemic treatment prior to consultation (p=0.81), education level (p=0.12), insurance status (p=0.35), partner status (p=0.33), parity (p=0.54), cancer stage (p=0.82), cancer grade (p=0.47), cancer hormone receptor status (p=0.51), HER2 status (p=0.28), use of tamoxifen (p=0.24), or BRCA carrier status (p=0.93) and the decision to pursue or not to pursue fertility preservation.

CONCLUSION: Our results indicate no association between demographic characteristics related variables and female oncology patient’s decision to pursue or not to pursue fertility preservation. This suggests that providers are unlikely to be able to predict which patients will wish to pursue fertility preservation, thus it is important that fertility preservation consultation be offered to all female oncology patients of reproductive age. Future research in larger populations is warranted.

THE ROLE OF DEMOGRAPHICS AND DIAGNOSIS ON FERTILITY PRESERVATION DECISION MAKING AMONG FEMALE CANCER PATIENTS OF REPRODUCTIVE AGE.

OBJECTIVE: To understand if cancer patient demographics or diagnosis is correlated with the decision to pursue or not to pursue fertility preservation.

RESULTS: The mean age of women was 31.6 (±5.7) years and ranged from 19-44 years. 45.9% of all patients referred elected to pursue a fertility preservation option, and 46.8% of women referred had a diagnosis of breast cancer. Among all patients referred (n=109), there was no significant association between age (p=0.72), race (p=0.44), parity (p=0.54), partner status (p=0.26), type of cancer (solid vs. hematologic, p=0.92), breast vs. non-breast, p=0.72; gynecologic vs. non-gynecologic, (p=0.47), having systemic treatment prior to consultation (p=0.28), education level (p=0.12), or insurance status (p=0.97) and decision to pursue or not to pursue fertility preservation. Among breast cancer patients (n=51), there was no significant association between age (p=0.95), race (p=0.72), having systemic treatment prior to consultation (p=0.81), education level (p=0.12), insurance status (p=0.35), partner status (p=0.33), parity (p=0.54), cancer stage (p=0.82), cancer grade (p=0.47), cancer hormone receptor status (p=0.51), HER2 status (p=0.28), use of tamoxifen (p=0.24), or BRCA carrier status (p=0.93) and the decision to pursue or not to pursue fertility preservation.

CONCLUSION: Our results indicate no association between demographic characteristics related variables and female oncology patient’s decision to pursue or not to pursue fertility preservation. This suggests that providers are unlikely to be able to predict which patients will wish to pursue fertility preservation, thus it is important that fertility preservation consultation be offered to all female oncology patients of reproductive age. Future research in larger populations is warranted.
OBJECTIVE: The rate of type I endometrial cancer (EC) in reproductive-age women continues to rise due to the national obesity epidemic. In addition, women with anovulatory cycles are at increased risk of EC. Fertility preservation in EC patients was not encouraged previously; now oncofertility consultation is recommended and patients may be given a time-limited period to try to conceive. We performed a cost-effectiveness (C/E) analysis to examine reproductive options in premenopausal women with EC.

DESIGN: Cost-effectiveness analysis.

MATERIALS AND METHODS: A decision analysis model compared 4 strategies for achieving pregnancy in premenopausal women with stage I EC after successful regression of EC with progestin treatment. Strategies included: 1) unassisted pregnancy attempts (UAP) x 12 months; ovulation induction with either 2) clomiphene (CC) x 6 cycles or 3) gonadotropins (GND) x 3 cycles; and 4) in vitro fertilization (IVF) x 3 cycles. Effectiveness was defined as pregnancy rate at 12 months. Medication cost estimates were obtained from a national mail-order fertility pharmacy; procedural cost information was estimated from institutional charges. Pregnancy outcomes were derived from published data in anovulatory patients. The C/E ratio was defined as cost per pregnancy. Incremental cost-effectiveness ratios (ICER) were calculated and sensitivity analyses were performed on pertinent uncertainties.

RESULTS: An estimated 1200 reproductive-aged women are diagnosed annually with stage I EC amenable to fertility preservation. If all pursued UAP, 468 pregnancies would result at a total cost of $216,000 and cost per pregnancy of $462. CC therapy would result in 612 pregnancies at a total cost of $26,988 and cost per pregnancy of $5582, and an ICER of $23,500. GND would result in fewer pregnancies and greater cost than CC. IVF would result in 1,008 pregnancies at a total cost of $43,2M, cost per pregnancy of $42,857, and an ICER of $100,000 per each additional pregnancy.

CONCLUSION: Several assisted reproduction options are available to young women with stage I EC. Our data demonstrate that CC is a cost-effective strategy while GND should not be pursued unless the patient fails CC. Although IVF is the most effective strategy, its costs are prohibitive and should not be considered first-line therapy. Consultation with a physician experienced in oncofertility is paramount to optimize delivery and timing of care.

P-44 Tuesday, October 21, 2014

CURRENT AND FUTURE TRENDS IN FERTILITY-PRESERVING SURGERY: A SURVEY OF THE SOCIETY OF GYNECOLOGIC ONCOLOGY (SGO) ON THE USE OF RADICAL TRACHELECTOMY FOR EARLY-STAGE CERVICAL CANCER.

S. J. Churchill,1 S. Armbruster,1 K. M. Schmeler,1 M. Frumovitz1,2 M. Greer,1 J. Garcia,1 G. Radworth,1 P. T. Ramirez,2 1Department of Obstetrics and Gynecology, Cedars-Sinai Medical Center, Los Angeles, CA; 2Department of Gynecologic Oncology and Reproductive Medicine, Division of Surgery, The University of Texas MD Anderson Cancer Center, Houston, TX; 3Department of Institutional Research, The University of Texas MD Anderson Cancer Center, Houston, TX.

OBJECTIVE: To survey gynecologic oncologists and fellows-in-training regarding the current and future role of radical trachelectomy (RT) for fertility preservation in patients with early-stage cervical cancer.

DESIGN: Cross sectional survey.

MATERIALS AND METHODS: From 06/2012 to 09/2012 surveys were mailed to all SGO member practitioners (N=1,353) and gynecologic oncology fellows (N=156). Practitioners were asked 37 questions and fellows were asked 19 questions to assess opinions on the current practice and training, and future role of radical trachelectomy in the treatment of early-stage cervical cancer.

RESULTS: A total of 303 (22.4%) practitioners and 38 (24.4%) fellows completed the survey. Several important trends were noted. Approximately half (49.8%) of respondents reported that they perform RT. The most common reason provided for not performing the procedure was lack of training (53.4%). Of those, the majority (70%) performs one case annually however, the majority of practitioners (90%) and fellows (97%) propose that at least 2-5 annual cases should be performed to maintain expertise. Currently abdominal (40%) and robotic (47%) RT approaches are the most commonly performed. Most practitioners (75%) and fellows (64%) project that in the next 5 years there will be even less radical procedures to manage early stage cervical cancer; however, most (77%, 92%) still believe RT will continue to have a lasting role.

CONCLUSION: RT has an important role in the management of patients with early-stage cervical cancer who wish to preserve future fertility. The most commonly used approach for RT is the robotic approach. Increased awareness is needed so that potential candidates for this procedure may benefit from this approach. Given that few SGO practitioners perform a low number of annual cases, consideration should be given to strong emphasis for referral to specialized centers.

Supported by: This study was supported through funding provided by the Innovative Surgery Fund in the Department of Gynecologic Oncology and Reproductive Medicine at MD Anderson Cancer Center, Houston, Texas.

P-45 Tuesday, October 21, 2014

ABSTRACT WITHDRAWN

P-46 Tuesday, October 21, 2014

AN APPROACH TO ESTABLISH BEHAVIORAL DETERMINANTS FOR PREDICTING PARTICIPATION IN CERVICAL CANCER SCREENING OF HAITIAN WOMEN.

L. F. Conover,1 L. F. Conover.2 1OB/Gyn & Reproductive Sciences, University of California San Francisco, San Francisco, CA; 2OB/Gyn & Reproductive Sciences, Maine Medical Center, Portland, ME.

OBJECTIVE: Disparities in health outcomes exist where there is limited utilization of primary and secondary prevention; cervical cancer is no exception. We sought to establish a stepwise approach for defining the behavioral determinants associated with predicting a woman’s intention to participate in a screening program in Haiti.

DESIGN: We used the Theory of Planned Behavior (TPB)1 to design a survey to investigate the behavioral determinants associated with predicting a woman’s intention to present for cervical cancer screening, the desired behavior, and ultimately craft a social marketing campaign encouraging participation. Assumed by this model is that intention is based on: (1) behavioral beliefs and attitudes towards the screening test, (2) subjective norms, (3) perceived control, and (4) actual control.

MATERIALS AND METHODS: During a four-week period in 2013, the research team performed a cross-sectional study by administering 61 surveys to women in Milot, Haiti. Factor analysis was used to define each model component in predicting the behavior. We analyzed the likelihood that a woman would be screened using Wilcoxon Rank Sum test, Chi Square tests and factor analysis. A logistic regression model was created from the most significant factors to predict the odds of the behavior.

RESULTS: Every participant had at least heard of cervical cancer, and 37.7% thought the disease was preventable. One third of women surveyed (34.4%) had ever had a screening test. Neither age nor years of education were significantly associated with ever having a screening test, but knowledge that sexual intercourse is a risk factor was. Factors significantly associated with performing the intended behavior were immediate personal awareness (p value 0.01), core social support (0.01) and perceived power (0.04). Sexual position was most commonly listed as a qualitative response for causes of cervical cancer.

CONCLUSION: Exploring the extent to which the population has the intention to engage in the desired behavior enabled us to perform a meaningful factor analysis in which we could assess which components of the TPB model contributed to the outcome. We then targeted future interventions through a tailored social marketing campaign. This theory based approach to determining predictors of behavior may be replicated in methodology to craft targeted interventions in the field of gynecologic and reproductive health sciences.

Supported by: Thank you to the Tufts University School of Medicine for travel support.
**FERTILITY & STERILITY®**

**P-47 Tuesday, October 21, 2014**


**OBJECTIVE:** Thyroid cancer is the fifth-most commonly diagnosed female malignancy. Treatment includes thyroidectomy followed by radioactive iodine (RAI) ablation therapy. There is insufficient evidence regarding the impact of RAI therapy on reproductive function. Our study aim was to investigate whether administration of RAI therapy to premenopausal women with thyroid cancer impacts fertility and menstrual function.

**DESIGN:** We conducted a retrospective survey study.

**MATERIALS AND METHODS:** We contacted women from the UCSF Cancer Registry with a history of thyroid cancer diagnosed from 1982-2012 who underwent a thyroidectomy followed by RAI between the ages of 18-45 (with no prior history of chemotherapy, radiation exposure, or fertility compromising surgery). Controls with the same exclusion criteria included women with benign thyroid disease who underwent a thyroidectomy without administration of RAI. All women were contacted by phone and then sent an electronic survey to collect information on menstrual and pregnancy history pre- and post-treatment.

**RESULTS:** Preliminary data were available for 104 patients (67 cases, 37 controls), the remainder (n=323) will be collected over the next two months. 88% of eligible participants contacted completed the survey. The average age for women that received RAI was 31.8 and for the controls was 36.4. Menstrual irregularity within the first 6 months of treatment were similar among groups (14% of cases vs. 26% of controls, p=0.44). Infertility rates were the same within each group (11%). The miscarriage rate among women who underwent RAI was 26% vs. 37.5% among controls, which was not statistically different when age-adjusted (p=0.54). 32% of cases were counseled about the reproductive effects of their treatment compared to 25% of controls.

**CONCLUSION:** Preliminary data suggests that RAI does not increase a women’s risk of infertility or early pregnancy failure. Furthermore, menstrual function does not appear to be affected by the addition of RAI. These data can be used to counsel women undergoing treatment for thyroid cancer on the safety of treatment on reproductive function.

**Supported by:** NIH/NCCR/OD UCSF-CTSI TL1 RR024129.

**P-48 Tuesday, October 21, 2014**

**INTRACA VITARY VAGINAL BRACHYTHERAPY IN EARLY STAGE ENDOMETRIAL CARCINOMA - IMPACT ON SEXUAL FUNCTION.** C. Singh. Department of Radiotherapy and Clinical Oncology, S.M.S Medical College and Hospital, Jaipur, Rajasthan, India.

**OBJECTIVE:** To describe the effects of intracavitary brachytherapy (IVB) on sexual function and quality of life of women with early-stage endometrial carcinoma.

**DESIGN:** This was a cross-sectional study conducted from December 2011 to December 2013 using Questionnaires given to follow-up patients of early stage endometrial carcinoma. Informed consent was taken from the patients before enrollment into the study.

**MATERIALS AND METHODS:** 60 Women with International Federation of Gynecology and Obstetrics stage I to II endometrial cancer treated in our institution surgically with (n=30) or without IVB (n=30) were selected for study. Quality of life and sexual function were measured using the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core 30 and the cervical cancer disease-specific module. Pertinent data from prior surgery and radiation treatments were abstracted retrospectively.

**RESULTS:** 30% of the IVB patients and 46.66% of the surgery-alone patients felt their vagina was dry during sexual activity and 16.66% versus 20% felt their vagina was short. 20% of patients in the IVB group felt their vagina was tight compared to 30% in the surgery-alone group and 0% versus 16.66% of patients reported pain during intercourse. There was no statistically significant difference in sexual/vaginal functioning, sexual worry, or sexual enjoyment between the 2 groups.

**CONCLUSION:** IVB reported similar outcomes on a sexual function questionnaire compared to patients treated with surgery alone though both groups had vaginal changes affecting sexual function.

**FERTILITY PRESERVATION**

**P-49 Tuesday, October 21, 2014**

**CISPLATIN INDUCE OVER ACTIVATION OF THE DORMANT PRIMORDIAL FOLLICLE POOL THROUGH PTEN/PI3K SIGNALING, RESULTING LOSS OF OVARIAN RESEARVE.** E. Chang, W. Lee, Y. Choi, E.-J. Lim, T. Yoon, S. Ryu, M. Park. 1Department of Obstetrics and Gynecology, Seoul National University College of Medicine, Seoul, Korea; 2Department of Obstetrics and Gynecology, Inje University Sanggyepaik Hospital, Seoul, Korea.

**OBJECTIVE:** While fate of ovarian failure after chemotherapy has been well established, the precise mechanism by which this occurs is less clear. Recent study has revealed that PTEN/PI3K/Akt signaling in oocytes is critically important for maintenance of the primordial follicle pool. Current study was designed to investigate role of this pathway in cisplatin induced primordial follicle depletion.

**DESIGN:** This was an experimental study using animal model.

**MATERIALS AND METHODS:** Mouse was given daily intraperitoneal injection of 2mg/kg (Dong A Pharmaceutical, Korea) for 15days. Ovaries were collected at time point 0, 5, 10, 12, 15 days after the start of daily Cisplatin injection. Ovaries were fixed in PFA and paraffin embedded. Serially sectioned (10μm section) ovarian tissues were H&E stained for differential follicle count. Early stage follicle were counted according to previous definitions after staining with early stage ovarian follicle marker Lhx8. Ovary samples were used for immunohistochemistry and evaluated for apoptosis using Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. Antibodies used in western blotting were PTEN, Akt, phospho-Akt, ERK, phospho-ERK, GSK3-β, phospho-GSK3-β and FOXO3α with visualization by enzymatic chemiluminescence.

**RESULTS:** Quantification of the different follicle populations in each time point after Cisplatin injection showed that as cumulative dose of Cisplatin increase, there is significant increase in ratio of growing follicle versus dormant follicles. As with the TUNEL staining, apoptosis was only observed in early stage cells of growing or mature follicle until day 10 and relatively few primordial follicle was stained compared to growing and mature follicle at day15. Analysis of the PI3K/PTEN/Akt/Foxo3 pathway demonstrated decreased PTEN as cumulative dose of cisplatin increases and activated pathway cascade resulting increase in cytoplasmic translocation of Foxo3 in cisplatin treated follicles.

**CONCLUSION:** Cisplatin over activates the dormant primordial follicle in mice resulting loss of resting pool of ovarian follicle. Effect of the treatment showed activation of PTEN/Akt/PI3K/Foxo3 pathway thus increase in growing follicle pool while dormant follicles exhibit rapid depletion. Our finding suggests promising therapeutic target for fertility preservation during chemotherapy.

**Supported by:** The present study was Supported by a grant (A120080) from the Korean Healthcare Technology R&D project, Ministry for Health, Welfare and Family Affairs, Republic of Korea.

**P-50 Tuesday, October 21, 2014**

**EFFECT OF THREE DIFFERENT TYPES OF ANTIFREEZE PROTEINS SUPPLEMENTATION ON MOUSE OVARIAN TISSUE VITRIFICATION AND TRANSPLANTATION.** J. Lee, J. Lee, H. W. Youn, J. R. Lee, B. C. Jee, C. S. Suh, H. Choi, S. H. Kim. 1Department of Obstetrics and Gynecology, Seoul National University Bundang Hospital, Seongnam, Gyeonggi-do, Korea; 2Department of Obstetrics and Gynecology, Seoul National University College of Medicine, Seoul, Korea; 3Department of Obstetrics and Gynecology, Inje University Sanggyepaik Hospital, Seoul, Korea.
OBJECTIVE: Intracellular ice crystal formation is one of the obstacles for the ovarian tissue (OT) cryopreservation. To alleviate this issue, several studies were performed using antifreeze proteins (AFPs). The aim of this study was to evaluate and compare the effect of 3 different types of AFPs in OT vitrification followed by autotransplantation.

DESIGN: Experimental mouse model.

MATERIALS AND METHODS: Ovaries from B6D2F1 mice were randomly divided into 4 groups: a control and 3 AFP groups (FfIBP, LeIBP and Type III AFP). The OTs were vitrified and 3 different types of AFPs (10 mg/mL) were added into each vitrification-warming media for the AFP groups. After warming, the OTs were analyzed by hematoxylin-eosin staining for histological examination, TUNEL assay for apoptosis, and phospho-histone H2AX staining for DNA double-strand breaks. In addition, the OTs vitrified using the most effective AFP among the 3 different AFPs were autotransplanted. After 7 days of grafting, the OTs were retrieved and evaluated for their revascularization and hormone recovery by CD31 immunohistochemistry and serum follicle stimulating hormone (FSH) levels respectively.

RESULTS: The LeIBP group showed significantly higher ratio of good quality (G1) follicles and lower ratio of apoptotic follicles than the control group. Especially for the primordial follicle, the G1 ratio were significantly increased in all AFP groups, while the ratio of DNA damage (H2AX(+)) follicles were decreased in all AFP groups when compared to the control group. After autotransplantation using the OT vitrified with LeIBP, there were significant increases of the total G1 follicle ratio, primordial G1 follicle ratio and CD31(+) area. Also, significant decrease of serum FSH level and TUNEL(+) follicle ratio were observed when compared to the control group.

CONCLUSION: Our study showed that the AFPs (FfIBP, LeIBP and Type III AFP), especially LeIBP, had beneficial effects on mouse OT vitrification, and such well vitrified OTs could improve the follicle quality, vessels quantity and OT functions after grafting. However, further studies for exact mechanisms of AFPs are required.

Supported by: This study was Supported by a grant of the Korea Health & Care technology R&D Project, Ministry of Health & Welfare, Republic of Korea. (HI12C0055).

P-51 Tuesday, October 21, 2014

AMH VALUES IN REPRODUCTIVE AGED WOMEN WITH AND WITHOUT THE BRCA1 MUTATION. M. E. Pavone, N. Mittal, K. Smith, S. Barnato Giordano. Obstetrics and Gynecology, Northwestern, Chicago, IL. Hematology/Oncology, Medical University of South Carolina, Charleston, SC.

OBJECTIVE: To examine Anti-mullerian hormone (AMH) levels in women with and without the BRCA1 mutation.

DESIGN: Subset analysis of serum samples taken from Northwestern Ovarian Cancer Early Detection Program (NOCEDPP) of reproductive aged women who had testing for the BRCA mutation.

MATERIALS AND METHODS: Using an IRB approved protocol, the NOCEDPP database was searched for women 18-45 years old who had previous BRCA testing. These women must have also provided consent for their stored serum to be used for research purposes. We excluded those with a BRCA2 mutation, a previous history of cancer and/or cancer treatment, and those with a previous unilateral or bilateral oophorectomy or other ovarian surgery. Statistical analysis was done using parametric and nonparametric testing.

RESULTS: A total of 125 met the criteria for our study. 66 women were BRCA1 positive and 59 were BRCA1 negative. The median age for BRCA1 positive was 33.5 while for BRCA1 negative patients was 37 (p<0.05). Body mass index, gravidity, parity, and duration of birth control were similar between the groups. Overall median AMH values were 2.6ng/mL in both groups. However, when broken into age groups, BRCA1 positive women aged 35-39 had a significantly lower AMH level than BRCA1 negative women (median 3.6ng/mL vs 1.3ng/mL; p<0.05).

CONCLUSION: AMH values were significantly lower in BRCA1 positive women aged 35-39. Women with the BRCA1 mutation should be counseled regarding this potential decrease in ovarian reserve.

Supported by: NIST/NIH/Evergreen National Women’s Health Grants.

P-52 Tuesday, October 21, 2014

EFFECT OF OVARIAN TISSUE CRYOPRESERVATION ON GENE EXPRESSION AND GROWTH OF HUMAN INDIVIDUAL FOLLICLE IN VITRO. T. Wang, J. Yan, L. Yan, C. Lu, X. Xia, T. Yin, J. Qiao. “Center for Reproductive Medicine, Department of Obstetrics and Gynecology, Peking University Third Hospital, Beijing, China; Center for Reproductive Medicine, Department of Obstetrics and Gynecology, Shengjing Hospital of China Medical University, Shenyang, Liaoning, China.

OBJECTIVE: To investigate the effect of either slow freezing or vitrification for ovarian tissue cryopreservation on individual human early follicle gene expression and growth in three-dimensional (3D) culture system.

DESIGN: Experimental study.

MATERIALS AND METHODS: Ovarian cortex tissue was collected from 11 patients (31.8±8.36 years of age) with obtaining written informed consent and cryopreserved using slow-rate freezing or vitrification. Tissues were fixed for histology analysis or used for early follicles isolation. Follicles were individually embedded in alginate(1% w/v) and cultured in vitro for 8 days. Follicle survival and growth were assessed by microscopy. Follicle viability was observed under confocal laser scanning microscope following Calcein-AM and Ethidium homodimer-I (Ca-AM/EthD-I) staining. Follicle gene expression was evaluated by single-cell mRNA analysis.

RESULTS: After thawing, the percentage of damaged oocytes and granulosa cells was significantly higher in both slow freezing and vitrification group, as compared with fresh control (p<0.05). The growth of follicles in vitro was significantly delayed in vitrification group than fresh group (p<0.05), while the survival rate and viability appeared similar after both slow freezing and vitrification compared with the fresh control. Both of the slow freezing and vitrification down-regulated the gene expression of oct4, stella, zp3 and cyp11a (p<0.05), while there is no significant difference between the two groups.

CONCLUSION: Although cryopreservation affect the morphology of human follicles and down-regulated gene expression of oct4, stella, zp3 and cyp11a, slow freezing offers similar conditions to fresh tissue for follicle growth, survival rate and viability than vitrification after in vitro culture. Compared to vitrification, slow freezing is considered to be the first choice for human ovarian cryopreservation.

Supported by: This work was Supported by National Basic Research Program of China (2011CB944504) and National Natural Science Foundation of China (81200470, 81170538).

P-53 Tuesday, October 21, 2014


OBJECTIVE: The safety of autotransplantation of frozen-thawed ovarian tissue cannot yet be secured in oncology patients, as it remains conjectural whether the cortical ovarian transplants harbor metastases. This is due to the fact that the current tumor detection methods undermine the validity of ovarian tissue. Near-infrared fluorescence (NIRF) imaging bears considerable potential as a solution to this problem, as it discriminates malignant from healthy tissues while leaving the examined tissues unaffected. A NIRF probe consists of a fluorophore that emits light in the near-infrared range (700-900 nm) and an antibody binding to a protein overexpressed on the membrane of tumor cells. Our study was designed to investigate which tumor proteins can be used in order to detect breast cancer cells within an ovary environment using NIRF imaging.

DESIGN: A comprehensive immunohistochemical analysis.

MATERIALS AND METHODS: Immunohistochemistry was performed on paraffin-embedded specimens of ten normal ovaries and tissue microarrays (TMAs) containing breast tumor cores from 24 premenopausal patients. A panel of established membrane (breast) tumor markers was
selected comprising αvβ6 integrin, CEA (carcinoembryonic antigen), E-cadherin, EpCAM (epithelial cell adhesion molecule), EMA (epithelial membrane antigen), Her/2neu, FR-α (folate receptor) and uPAR (urokinase type plasminogen activator receptor). The suitability thresholds were set on 80, 90 and 100% expression.

RESULTS: None of the normal ovaries demonstrated staining for each of the investigated markers in the stroma cells or in the ovarian surface epithelium. However, αvβ6 integrin, E-cadherin, EpCAM, EMA, Her/2neu and FR-α were always present at the plasma membrane of epithelial cells surrounding columnar and cuboidal inclusion cysts. On the breast tumor tissues E-cadherin showed the highest number of tumors in which the suitability thresholds were reached: 19, 17 and 5 out of 24 tumors, respectively.

CONCLUSION: Anti-E-cadherin seems a suitable target to detect breast cancer cells in ovaries prior to autotransplantation of ovarian tissue using a NIRF-conjugated antibody and an appropriate imaging system. Further investigations are needed to evaluate the feasibility of NIRF imaging in the field of fertility preservation.

Supported by: This research was Supported by a grant from DSW Health Insurance.

P-54 Tuesday, October 21, 2014

EXPERIENCE WITH OOCYTE THAW (OT) CYCLES INVOLVING METAPHASE I (MI) OOCYTES IN A HIGH VOLUME OOCYTE CRYOPRESERVATION PRACTICE. J. D. Kofinas, D. H. McCulloh, A. Berkeley, J. A. Grifo, N. Noyes, F. Licciardi. NYU Fertility Center, New York, NY.

OBJECTIVE: To evaluate the utility and efficiency of freezing M1 oocytes as measured by post-thaw fertilization, blastulation and implantation rates. Outcomes are compared to metaphase 2 (M2) oocytes.

DESIGN: Retrospective Cohort.

MATERIALS AND METHODS: Autologous oocyte thaw cycles at NYU medical center between 2008 and 2013 were reviewed. 99 patients thawed a total of 144 (MI) and 1008 (M2). Survival, fertilization, cleavage stage and blastocyst formation rate and live birth rates were analyzed and compared to M2 outcomes. Subgroup analysis was completed comparing patients under age 37 to age 37 and older. T-tests of means, were employed.

RESULTS: M1 oocytes display low survival, fertilization, cleavage and blastocyst formation rates (Table 1). 19/144 M1 oocytes developed into cleavage stage embryos and two developed to blastocysts. Three M1 derived embryos were transferred, 2 in SET cycles resulting in negative pregnancy tests (5 cells, day 3 transfer and stage 4BB blast, day 5). One M1 oocyte was transferred combined with two M2 oocytes on day 5, resulting in a single pregnancy. It is not clear, however, if the M2 or M1 oocyte was responsible for the pregnancy. The M1 was blast stage 1, the M2 oocytes were blastocyst stages 1 and 4Bc. Overall, 1% of M1 oocytes developed into blastocysts. Subgroup analysis revealed no difference in MI or M2 outcomes in respect to age.

Autologous oocyte thaw outcomes 2008-2013

<table>
<thead>
<tr>
<th>All Patients:</th>
<th>Frozen</th>
<th>Thawed</th>
<th>Survived</th>
<th>Fertilization</th>
<th>Cleavage</th>
<th>Blastocysts</th>
<th>Transferred</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>199</td>
<td>144</td>
<td>50(35%)</td>
<td>25(17%)</td>
<td>19(13%)</td>
<td>2(1%)</td>
<td>3(2%)</td>
</tr>
<tr>
<td>M2</td>
<td>1020</td>
<td>1008</td>
<td>805(80%)</td>
<td>643(64%)</td>
<td>557(55%)</td>
<td>197(20%)</td>
<td>111(11%)</td>
</tr>
<tr>
<td>Patients by Age*</td>
<td>Survived</td>
<td>2PN</td>
<td>Cleavage</td>
<td>Blastocyst</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age less than 37 M1</td>
<td>20%</td>
<td>5.5%</td>
<td>5.5%</td>
<td>0%</td>
<td>2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age 37 and older M1</td>
<td>24%</td>
<td>13%</td>
<td>11%</td>
<td>0.38</td>
<td>22%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-value**</td>
<td>0.62</td>
<td>0.19</td>
<td>0.29</td>
<td>0.55</td>
<td>0.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age less than 37 M2</td>
<td>78%</td>
<td>64%</td>
<td>53%</td>
<td>19%</td>
<td>19%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age 37 and older M2</td>
<td>83%</td>
<td>69%</td>
<td>60%</td>
<td>19%</td>
<td>19%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-value**</td>
<td>0.28</td>
<td>0.23</td>
<td>0.18</td>
<td>0.55</td>
<td>0.55</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*results in percentages **T-test of means.

CONCLUSION: Cryopreservation of M1 oocytes has yet to show benefit (no confirmed clinical pregnancy). In our center approx. 23,000 oocytes have been frozen, 14.3% of which are M1. M1 oocytes are therefore responsible for a significant proportion of the work and materials associated with egg freeze/thaw laboratory services, including added storage burden. In addition, patients with stored M1s become hopeful that those eggs will contribute to their success. M2 oocytes, however, continue to show excellent survival, blastocyst formation and clinical pregnancy rates. As more clinical data materializes, the utility of preserving M1 oocytes should be regularly assessed.

P-55 Tuesday, October 21, 2014


OBJECTIVE: Limited data exists on physician training in fertility preservation (FP). The oncology literature suggests variable compliance with current ASCO and ASRM guidelines that recommend discussing FP with patients and referring to FP providers when indicated. Among the key factors for non-compliance is a reported lack of education in FP methods. There are no studies specifically examining FP training for physicians expected to execute FP treatment. Our study objective was to evaluate the FP training experience among current REI fellows.

DESIGN: Cross-sectional mixed methods study.

MATERIALS AND METHODS: The study included a 38-item online survey with an optional interview component. Coordinators at each training program were contacted and asked to distribute the survey to their fellows via email. The survey link was emailed twice between 4/2014-5/2014. Data were collected and analyzed using Research Electronic Data Capture (REDCap) software (version 5.7.1) hosted at the University of Iowa.

RESULTS: 146 REI fellows are currently enrolled in 42 accredited fellowship programs. 9 fellows were excluded due to program restrictions or personal involvement with the study (n=1). At the time of abstract submission, 43 responses had been received (response rate 31.4%). Responses were evenly distributed across year of training (1st year 32.6%, 2nd year 32.6%, 3rd year 34.8%). 48% of respondents rated their FP training experience as 'excellent,' with the remaining 40% rated as 'good' and 12% as 'fair.' Although 95% of fellows felt that discussing FP options was 'very important,' only 42% felt their current level of knowledge was adequate to counsel their patients ‘all of the time.’ A majority of fellows felt they needed more education in FP (68%), and 98% were interested in a standardized curriculum. When asked if FP training was felt to be necessary in fellowship, 98% felt it was necessary and should be required.

CONCLUSION: There is significant variability in the perceived quality of FP training among REI fellows nationwide. Our study demonstrates the desire for further education in FP, with a majority of respondents feeling that it should be a required and standardized part of fellowship training.

Supported by: NIH 5K12HD00127-15.
IVM OOCYTE CRYOPRESERVATION AFTER EX-VIVO OOCYTE RETRIEVAL IN GYNECOLOGIC CANCER PATIENTS UNDERGOING RADICAL SURGERY. C. W. Park, a S. H. Lee, a J. Y. Kim, b M. K. Koong, c K. M. Yang, c I. H. Lee, b K. T. Lim, b K. H. Lee, b T. J. Kim. aDivision of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Cheil General Hospital and Women's Healthcare Center, Kwandong University College of Medicine, Seoul, Korea; bDivision of Gynecology Oncology, Department of Obstetrics and Gynecology, Cheil General Hospital and Women's Healthcare Center, Kwandong University College of Medicine, Seoul, Korea; cLaboratory of Reproductive Biology and Infertility, Cheil General Hospital and Women's Healthcare Center, Kwandong University College of Medicine, Seoul, Korea.

OBJECTIVE: In case of advanced gynecologic malignancy, radical surgery is necessary and reproductive organs such as uterus and ovaries could not be spared. Here we report novel approach for fertility preservation (FP) by means of ex-vo extravagant retrieval of immature oocytes from macroscopically normal ovarian tissue harvested during radical surgery in endometrial or ovarian cancer patients with advanced stage.

DESIGN: Case reports.

MATERIALS AND METHODS: Six advanced gynecology cancer patients referred for FP who were planned to undergo radical surgery the next day: endometrial (n=2), ovarian (n=3) and double primary endometrial and ovarian cancer (n=1). Ex-vivo retrieval of immature oocytes from the macroscopically normal ovarian tissue, followed by oocyte freezing after in vitro maturation (IVM) or embryo freezing with intracytoplasmic sperm injection (ICSI).

RESULTS: Oocyte retrieval was failed in one ovarian cancer patient No.5 who had decreased ovarian reserve with AMH < 0.1 ng/ml. A total of 53 oocytes were retrieved in five patients with a mean 10.6 oocytes. After IVM a total of 36 mature oocytes were obtained, which showed 67.9% maturation rate.

CONCLUSION: Immature oocytes can be successfully retrieved ex-vivo from the macroscopically normal ovarian tissue harvested during radical surgery. Oocyte or embryo freezing after IVM provides a possible strategy for FP in patients with advanced stage endometrial or ovarian cancer without the risk of cancer cells spillage and time delay.

---

P-57 Tuesday, October 21, 2014

THE NATIONAL PHYSICIANS COOPERATIVE (NPC) TISSUE STUDY: OVARIAN TISSUE FROM PEDIATRIC PATIENTS REMOVED FOR CRYOPRESERVATION CONTAINS FOLLICLES REGARDLESS OF TREATMENT HISTORY. M. E. Pavone, a F. Duncan, a C. Gracia, a J. Ginsberg, d S. Badawy, b B. Lockart, b Y. Gosiengfiao, a K. Smith, a T. K. Woodruff, a Obstetrics and Gynecology, Northwestern University, Chicago, IL; bHematology, Oncology & Stem Cell Transplant, Ann & Robert H. Lurie Children’s Hospital of Chicago, Chicago, IL; cObstetrics and Gynecology, University of Pennsylvania, Philadelphia, PA; dPediatric Oncology, Children’s Hospital of Philadelphia, Philadelphia, PA.

OBJECTIVE: To examine ovarian tissue of pediatric patients undergoing ovarian tissue cryopreservation (OTC) for fertility preservation for the presence of follicles.

DESIGN: A histological analysis was done from 24 pediatric patients undergoing OTC for fertility preservation as part of the oncofertility National Physicians Cooperative (NPC) protocol.

V: virgin, S: single, M: married

---

P-58 Tuesday, October 21, 2014

OOCYTE CRYOPRESERVATION: SLOW-COOLING VERSUS VITRIFICATION USING CLOSED SYSTEMS IN A NON-DONOR COHORT. J. K. Jain, a D. R. Albini, a J. Chen, a J. J. Stachecchi, b "Santa Monica Fertility, Santa Monica, CA; "Innovative Cryo Enterprises, Linden, NJ.

OBJECTIVE: An investigational clinical protocol was created to assess various methods of oocyte cryopreservation (OC) and to establish clinic-specific data as recommended by ASRM. Slow-cooling (SC) and vitrification (VT) were performed using closed storage devices to avoid theoretical viral contamination. Clinical pregnancy and live birth rates were the major outcomes.

DESIGN: Prospective clinical trial.

MATERIALS AND METHODS: Women ≤38 years of age with tubal factor as the sole cause of infertility had mature (MII) oocytes obtained from a single ovarian stimulation cycle and cryopreserved using 1) sodium-reduced medium (Sage CSC, Cooper Surgical) with propanediol and sucrose in 0.25cc straws, or vitrified using 2) ICE Oocyte Vitrification media (Innovative Cryo Enterprises LLC) a DMSO-free medium containing ethylene glycol in 0.25cc straws or flexipette tips sealed inside 0.5cc straws (micro-secure) or 3) EG/DMSO media (Vit Kit, Irvine Scientific). Standard protocols used were per manufacturer recommendations. Approximately 1-4 months later, oocytes were warmed, fertilized, and transferred.

RESULTS: 47 women with a mean age of 32 years (range 26-37) underwent 59 thaw cycles consisting of 605 oocytes (mean 10/cycle). Overall survival and fertilization was 71.2% and 80.2% respectively. Cleavage rate was 83.3%. All but one of the 57 embryo transfers were performed on Day 3 with a mean of 3.4±1.0 embryos. The exception was a single D5 blastocyst transfer. Overall implantation rate of 18.4%, clinical pregnancy rate of 43.8% and live birth rate of 36.8% was observed per embryo transfer. The...
overall miscarriage rate was 16%. Of the 27 live born infants, there were 4 twin and 1 triplet pregnancy. There was no difference between VIT storage device or media therefore the VIT data was combined (Table 1).

CONCLUSION: Overall live birth rates were similar between SC and VIT despite a trend toward higher implantation and pregnancy rates with VIT. Live birth rates shown here are comparable to our 2012 rate of 50%, for frozen non-donor embryos. The experience gained by systematic testing of various OC regimes, using only closed storage devices, has allowed us to become proficient in and successful counseling women for OC.

P-59 Tuesday, October 21, 2014

OBJECTIVE: To evaluate if gonadotropin releasing hormone agonist (GnRH) treatment during chemotherapy prevents ovarian failure and maintains women’s fertility.

DESIGN: Meta-analysis conducted according to recommendations of the Meta-analysis of Observational Studies in Epidemiology group. MEDLINE (Jan 1979 - Dec 2013) English language abstracts were searched for the keywords: GnRH agonist, fertility, ovarian failure or preservation, amenorrhea, chemotherapy.

MATERIALS AND METHODS: Twenty-seven clinical trials and case series were selected based on abstract content; 19 studies containing controls were included. Numbers varied by prospective and retrospective study.

RESULTS: Blumenfeld et al. performed 4/19 (21%) prospective, but non-randomized studies including a follow-up study with retrospective historical controls. The 11 remaining prospective studies were randomized. The patient populations of the studies varied in age, type of malignancy, and duration/type of chemotherapy received. Additionally, the types/routes of administered GnRH varied, as did the outcome measures. In studies with better-defined outcomes, the measures included examination of ovarian reserve, return of spontaneous menstruation/ovulation, hormonal changes during/after treatment, evaluation of cyclic ovarian function versus development of premature ovarian failure (POF), and pregnancy rates. Ovarian function/reserve was evaluated differently among studies using patient reports, resumption of menses, or laboratory analyses of pituitary gonadotropins and estradiol, and in some cases inhibin B or anti-Muellerian hormone; antral follicle count by ovarian sonography was rarely used. With all 19 studies included in the meta-analysis, resumption of menses favored co-administration of a GnRH during chemotherapy (OR 1.83; 1.08-3.10). Excluding studies by Blumenfeld et al. resulted in a reduced benefit; when all studies by Blumenfeld et al. were excluded from the meta-analysis, no benefit was seen with GnRH co-administration (OR 1.13, 0.78-1.64). Although some data sets were limited, examining all studies no differences (OR, 95% CI) were seen in amenorrhea (OR 0.46; 0.06-3.82), pregnancy rates (OR 1.05; 0.61-1.81), laboratory measures of ovarian function (OR 0.77; 0.45-1.33), restoration of ovarian function (OR 1.48; 0.37-5.96), maintenance of menses (OR 0.37; 0.078-1.98) and diagnosis of POF (OR 0.37; 0.09-1.02).

CONCLUSION: The benefit of administering GnRH with chemotherapy to preserve ovarian function in reproductive-age women is at best limited to maintaining menses, with a marginally positive result driven by studies from one group.

Supported by: NCI Hematology/Oncology Fellowship Program.

P-60 Tuesday, October 21, 2014
ADOLESCENT SPERM BANKING IN NORTH AMERICA: RESULTS FROM THE SBANK10 STUDY. J. L. Klosky, a K. M. Russell, a H. Zhang, a Q. Huang, a W. H. Kutteh, b P. Brezina, a J. L. Simmons, a L. R. Schover, a W. H. Salem, c J. L. Klosky, a H. Zhang, a Q. Huang, a W. H. Kutteh, b P. Brezina, a J. L. Simmons, a L. R. Schover, a W. H. Kutteh, b a National Cancer Institute, Bethesda, MD; b Fertility Associates of Memphis, Memphis, TN; c The Univ. of Texas MD Anderson Cancer Center, Houston, TX.

OBJECTIVE: To describe sperm banking practices among at-risk adolescents newly diagnosed with cancer in the US and Canada, and to identify factors predictive of banking outcome.

DESIGN: A prospective single group quasi-experimental study design was utilized to test the contributions of psychological, demographic, developmental, parent, provider, and medical factors on sperm banking outcome.

MATERIALS AND METHODS: At-risk adolescent males from 8 leading pediatric oncology centers (13-21 years of age, N = 146), their parents, and medical providers completed self-report questionnaires within one week of adolescent treatment initiation. A review of medical records was also conducted. Logistic regression with a single covariate was utilized to test each factor as a potential correlate of binary sperm banking outcome (bank attempt/no bank attempt), while multi-covariate logistic regression was utilized to model the overall contribution of these factors on sperm banking outcome.

RESULTS: Among adolescents (Mean age = 16.49 years, SD = 2.02), 43.8% banked sperm, 2.1% had azoospermic samples, and 7.5% attempted to provide a sample but were unsuccessful. When comparing these “attempters” (79/146) to “non-attempters” (67/146), uni-covariate tests revealed that higher Tanner stage, adolescent perceptions of fertility risk, banking self-efficacy, and benefits of banking, along with history of masturbation, communication of fertility risk, and recommendations to bank by providers and parents, associated with a banking attempt (ps range from .04 - <.001). Uni-covariate findings for maternal, paternal, and provider factors will be presented, along with an overall multi-covariate model for sperm banking outcome.

CONCLUSION: This is the first large nationally representative study to describe sperm banking practices among at-risk adolescents newly diagnosed with cancer. Although findings suggest that banking is underutilized in this group, modifiable factors associated with banking were identified. Implications for interventions designed to improve banking rates will be discussed.

Supported by: National Institute of Child Health and Human Development (NICHD), R21 HD061296 (Klosky, PI).

P-61 Tuesday, October 21, 2014
PHYSICIAN TRUCATION OF FERTILITY PRESERVATION COUNSELING LEADS TO DECREASED OPPORTUNITY FOR CANCER PATIENTS. W. H. Salem, a J. M. Letourneau, b J. L. Chan, c S.-W. Chan, a M. Cedars, a M. P. Rosen, a "Dept. of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, San Francisco, CA; b Dept. of Obstetrics and Gynecology, University of North Carolina at Chapel Hill, Chapel Hill, NC; c Dept. of Obstetrics and Gynecology, University of Pennsylvania, Philadelphia, PA.

OBJECTIVE: Women with gynecologic cancers (GYN) are counseled about Fertility Preservation (FP) at a significantly lower rate than women with non-GYN cancers. We sought to investigate the underlying themes for patient-perceived communication barriers with regards to FP among women with GYN cancers.

DESIGN: Cross sectional survey.

MATERIALS AND METHODS: A total of 2537 women with a GYN cancer diagnosis between 1993-2007, aged 18-40 at time of diagnosis were contacted from the California Cancer Registry. Information about demographics, cancer type, fertility desires and details regarding fertility counseling were collected. Framework and content analysis was conducted to categorize patient perception of physician-patient interactions involving FP counseling.
based on the free text response to, “What did your doctor tell you about how cancer treatment could affect your ability to have children?” Patients were included in more than one category if distinct communication barriers were expressed.

RESULTS: Among 2537 women contacted 1892 responses were received with 1686 reporting a treatment which may impact fertility. Among women with GYN cancers 51% received counseling regarding FP. This represents a lower rate of counseling than with women non-GYN cancers (OR 0.5-95% CI 0.4-0.6). 324 participants provided qualitative answers to the free text response (14% excluded). While 11% had a positive experience, 75% identified communication barriers and poor FP counseling. The reasons underlying poor counseling were due to 1) Physician truncation of information (i.e. “You will have a hysterectomy so you can no longer have children”) (59%), 2) Negative physician attitude (5%), 3) Incorrect knowledge (8%), 4) Nondisclosure/omission (29%), 5) Patient intimidated to present topic (6%) and 6) Perceived urgency to start treatment (7%).

CONCLUSION: These data suggest poor communication surrounding fertility preservation for women diagnosed with GYN cancers. Most notably, women felt that physician truncation was the largest communication barrier. Education surrounding FP counseling for these women could address the disparity to enable cancer survivors with positive counseling experiences and an opportunity to build a future family.

Supported by: NIH/NCCR/OD UCSF-CTSI TL1 RR024129.

P-62 Tuesday, October 21, 2014
THE EFFECT OF AMH SCREENING ON CHILDBEARING AND FERTILITY PRESERVATION ATTITUDES. A. E. Batcheller, S. Young, K. B. DiPaola, J. M. Sroga, M. A. Thomas. UC Center for Reproductive Health, University of Cincinnati, West Chester, OH.

OBJECTIVE: To determine awareness of ovarian reserve testing and fertility preservation between obstetrics and gynecology (OBG) and other surgical residents (OSR), and to determine the impact of ovarian reserve testing on childbearing and fertility preservation plans.

DESIGN: Descriptive study.

MATERIALS AND METHODS: 23 female OBG and 19 OSR residents completed a survey assessing childbearing plans, knowledge of reproductive decline with age, ovarian reserve testing, and fertility preservation. Subjects were offered anti-mullerian hormone (AMH) assessment of their ovarian reserve. Those who choose testing completed an additional follow-up survey. Chi Square, Students t-test, and logistic regression were performed using SPSS v 21. Significance was defined as p<0.05.

RESULTS: OBG were more aware of ovarian reserve testing and fertility preservation than OSR (91.3% vs 15.8%, p<0.01). There was no difference in age between the groups (OBG 28.9 yrs±2.1; OSR 29.3 yrs±2.5). Fewer OBG described their relationship status as single (21.7%) compared to OSR (68.4%, p<0.01). Fewer OBG (47.8%) than OSR (73.7%) stated their careers delayed their childbearing plans. OBG planned to initiate childbearing earlier (26-34yrs) than OSR (35-39yrs, p<0.03). More participants were interested in exploring fertility preservation (OBG 43.5%, OSR 47.4%) than altering their childbearing plans (OBG 34.8%, OSR 33.3%). Although 43.5% of OBG and 68.4% of OSR reported an interest in ovarian reserve testing, 69.6% of OBG and 47.4% of OSR actually completed testing. There was no difference in AMH levels for OBG (4.1ng/mL±3.4) and OSR (2.9ng/mL±3.6). In the follow-up survey, 12.5% of OBG and 11.1% of OSR stated that their AMH levels (knowing or not) affected their anticipated age of childbearing, while 18.8% of OBG and 33.3% of OSR felt their reserve testing would cause them to seek fertility preservation strategies.

CONCLUSION: Most women surveyed had an accurate knowledge of age related fertility decline, but OBG were more aware of ovarian reserve testing and fertility preservation options. Many participants choose to delay their careers for their personal career choices. This would consider fertility preservation rather than changing childbearing plans. Fertility preservation education should be offered to women in professional fields early in their training.

P-63 Tuesday, October 21, 2014
ABSTRACT WITHDRAWN

P-64 Tuesday, October 21, 2014
LIVE BIRTH WITH INTRAUTERINE INSEMINATION (IUI) AFTER ORTHOTOPIC TRANSPLANTATION OF CRYOPRESERVED OVARIAN TISSUE IN A YOUNG PATIENT PREVIOUSLY TREATED WITH CHEMOTHERAPY FOR ASKIN'S DISEASE. F. Lorenzo, M. Villamayor, P. J. Buzzi, G. Marconi, M. Tiberon, E. T. Young. Instituto de Ginecologia y Fertilidad(IFER), Buenos Aires, Bs.As., Argentina.

OBJECTIVE: To report the first case of live birth in South America after cryopreserved ovarian tissue retransplantation in a cancer survivor patient.

DESIGN: Case report.

MATERIALS AND METHODS: In 2005 a 28 year old patient was seen in consultation for fertility preservation before undergoing aggressive chemotherapy for a peripheral primitive neuroectodermal tumor (Askin tumor). Several strips of ovarian cortical tissue were obtained by laparoscopic approach and preserved by slow freezing technique. The patient underwent surgical excision of four left thoracic ribs, and chemotherapy (cyclophosphamide, vincristine, endoxan, actinomycin) completing 14 cycles, according to protocol EURO EWING99. Clinical and biochemical ovarian failure was established.

RESULTS: In 2009 orthotopic transplantation of frozen-thawed ovarian tissue was carried out by laparoscopy. Twelve tissue strips 1.5-2 mm thick were placed in the medullary portion of the right ovary, 2 tissue strips were left in culture and 2 tissue strips were sent to pathology for histology review. A biopsy procedure of the ovary confirmed absence of ovarian follicles. Six months after transplantation the patient resumed regular menses. As she requested fertility treatment four IVF cycles of were carried out. One mature oocyte was obtained at each retrieval, resulting in the transfer of one embryo each time with no clinical pregnancies. In April 2012 symptoms of ovarian failure began and in September 2012 amenorrhea was established. In February 2013 another laparoscopy was performed with the replacement of ovarian tissue, this time in the left ovary, following the same technique and strategy. In May 2013 spontaneous menses began. During ovulation monitoring an 18 mm follicle and an 8mm endometrium prompted an intrauterine insemination which resulted in pregnancy. In January 2014, and after an uneventful pregnancy, a healthy baby boy was born and delivered by cesarean section at 37 weeks of gestation, weighing 2,900 g.

CONCLUSION: This case highlights the importance of suggesting ovarian tissue cryopreservation to all young female patients who require potentially sterilizing treatment for cancer. Ovarian cortex cryopreservation can be performed at short notice for those patients who require immediate treatment and may allow resumption of normal cycles, natural conception and assisted reproductive techniques. This is the first report of the birth of a child following retransplantation of cryopreserved ovarian tissue and intrauterine insemination in South America.

P-65 Tuesday, October 21, 2014

OBJECTIVE: Lesbian, gay, bisexual, transgender (LGBT) rights in Sweden have been regarded as some of the most progressive in Europe. In 2013, a previously valid requirement of sterilization for legalisation of gender change was ruled unconstitutional in court. Hence, transsexuals may now be included in clinical programs of fertility preservation (FP) before sex-reassignment surgery. Our aim is to report a pilot experience with counseling and performance of FP in transsexual men, as standards of care for FP in this clinical setting are lacking.

DESIGN: Prospective study at a University Hospital Reproductive Medicine center.

MATERIALS AND METHODS: During 2013, nine transsexual men were referred for FP. Clinical characteristics, FP procedures and their outcomes are described. Participants’ psychological perceptions during the process were assessed. The study was approved by the human ethics committee in Stockholm.

RESULTS: Seven men received reproductive counseling (mean age 27.4 ± 6.0 yrs, range 19-35; mean time after diagnosis 4.0 ± 2.8 yrs, range 1-7). Two of the youngest patients were referred immediately after diagnosis and had not yet initiated testosterone treatment. Three of the treated men were on daily transdermal testosterone and two on intra-muscular injections every 2-3 months. Two men had previously undergone surgery of reproduc-
THE COMBINED PROCEDURE CAN BE USED AT ANY PHASE OF MENSTRUAL CYCLE. IN BREAST CANCER PATIENTS WHOSE OVARIAN TISSUE WAS CRYOPRESERVED FOR ONCOTHERAPY, THE COMBINED PROCEDURE CAN BE USED AT ANY PHASE OF MENSTRUAL CYCLE. IN BREAST CANCER PATIENTS WHOSE OVARIAN TISSUE WAS CRYOPRESERVED FOR ONCOTHERAPY, THE COMBINED PROCEDURE CAN BE USED AT ANY PHASE OF MENSTRUAL CYCLE.

OBJECTIVE: The goal of this study is to determine the factors that affect oocyte extraction efficiency when using the “combined procedure”, whereby oocyte retrieval follows cryopreservation of ovarian tissue.

DESIGN: Data were obtained retrospectively from the clinical records of 27 young breast cancer patients referred for fertility preservation.

MATERIALS AND METHODS: In the current study, all patients gave written, informed consent in keeping with the Declaration of Helsinki, and the study was approved by the institutional review board of St. Marianna University. Drawing from clinical records, data were collected on the following epidemiological parameters for breast cancer patients: the patient’s age, body mass index, marital status, phase of menstruation (follicular or luteal) at the time of operation, levels of anti-Mullerian hormone (AMH), phenotype of breast cancer (either Luminal A with B) versus non-Luminal A with B). Baseline ovarian reserve measurements, including use of ART (OR 15.1, 95% CI 2.1-107.5) and previous pregnancy (53.3%, n = 8). After adjustment, the only factor associated with disease regression was increased education level (OR 8.6, 95% CI 1.16-64.9). Age, race, BMI and parity were not statistically associated with odds of regression. Factors associated with increased odds of pregnancy included use of ART (OR 1.5, 95% CI 2.1-107.5) and previous pregnancy (OR = 1.58, 95% CI 1.2-190.4).

CONCLUSION: While fertility-sparing treatment for CAH and EC is a reasonable and safe alternative to hysterectomy in young women desiring pregnancy, clinicians should be aware that this patient population is at risk for uterine synechiae. To optimize pregnancy, prompt initiation of ART should be considered soon after disease regression.

OBJECTIVE: To evaluate treatment efficacy and fertility outcomes in women with complex atypical hyperplasia (CAH) or stage 1 grade 1 endometrial cancer (ECC) who are treated with oral progestins or other hormonal therapy rather than hysterectomy for fertility preservation.

DESIGN: Retrospective analysis.

MATERIALS AND METHODS: We performed an analysis of women, 18-42 years old, seen in consultation at an academic fertility center for assisted reproductive treatment (ART) from 2002 to 2014. Multivariate logistic regression models were constructed to identify factors associated with cancer regression as well as pregnancy; odds ratios (OR) with 95% confidence intervals (CI) were calculated.

RESULTS: 39 patients, ages 26-42, were treated with a mean age of 34.6 and mean body mass index (BMI) of 34.9. 76.9% of patients (n = 30) had EC and 23.1% had CAH (n = 9). Progestin treatment included mestrostrol acetate (73.6%, n = 28), levonorgestrel intrauterine device (7.8%, n = 3) or other progestin therapies (18.3%, n = 7). Pathologic regression was achieved in 78.9% (n = 30) with a mean time to regression of 6.8 months. Uterine synechiae were diagnosed by hysteroscopy in 17.9% of patients (n = 7). Pregnancy was achieved in 39.4% (n = 15) with live birth in 21.0% (n = 8). Among the pregnancies, 46.7% conceived with assisted reproductive technologies (ART), while the remainder were spontaneous pregnancies (53.3%, n = 8). After adjustment, the only factor associated with disease regression was increased education level (OR 8.6, 95% CI 1.16-64.9). Age, race, BMI and parity were not statistically associated with odds of regression. Factors associated with increased odds of pregnancy included use of ART (OR 1.5, 95% CI 2.1-107.5) and previous pregnancy (OR = 1.58, 95% CI 1.2-190.4).

CONCLUSION: While fertility-sparing treatment for CAH and EC is a reasonable and safe alternative to hysterectomy in young women desiring pregnancy, clinicians should be aware that this patient population is at risk for uterine synechiae. To optimize pregnancy, prompt initiation of ART should be considered soon after disease regression.

OBJECTIVE: Chemotherapeutic agents have a known gonadotoxic effect. However it can be difficult to predict the exact impact these drugs may have on response to ovarian stimulation. Few studies have evaluated the success of assisted reproductive treatment (ART) in patients who have been exposed to chemotherapy.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: 78 chemotherapy-naive and 31 post-chemotherapy patients who underwent ART between 1/2005 and 4/2014 were identified. Demographics, baseline ovarian reserve measurements, IVF stimulation parameters and embryo quality data were obtained from medical records. Demographic characteristics were summarized and compared using X², ranksum, and logistic regression modeling to identify risk factors for cancellation and low oocyte yield, adjusting for confounders.

RESULTS: Compared to chemo-naive patients, post-chemo patients were younger at consult (27 vs 32, p<.001) and at diagnosis (22 vs 31, p<.001). AFC was lower in post-chemo patients (8 vs 16, p=.0004) but there was no difference in baseline FSH levels (6.2 vs 5.1, p=.3). Breast cancer was the most common cancer in pre-chemo patients (56%), while hematologic cancers were most common in post-chemo patients (68%, p<.001). Those who were post-chemo were more likely to be cancelled during stimulation (29 vs 2%, p<.001). Among those that went to retrieval, there were no differences in total number of oocytes retrieved (10 vs 11, p=.6) or mature oocytes retrieved (8 vs 8, p=.8), despite higher starting (464 vs 313, p<.001) and total gonadotropin doses (4350 vs 3439, p=.03) in post-chemo patients. Comparing post-chemo patients who were cancelled with those who were not, median alkalylator score was higher in patients who were cancelled (4.7 vs 1.3, p=.047). AFC (2 vs 10, p=.003) and peak estradiol (149 vs 1946, p<.001) were significantly lower in the post-chemo group. In both pre- and post-chemo patients, all patients with AFC <3.

FERTILITY & STERILITY® e161
were cancelled. In multivariate models, AFC ≤6 was consistently associated with cycle cancellation and low oocyte yield (<6) across all models.

CONCLUSION: Patients exposed to chemotherapy are more likely to be cancelled during stimulation as compared with those that are chemotherapy-naive. Among patients who underwent retrieval, oocyte yield was similar in pre- and post-chemotherapy patients. AFC was the factor most strongly associated with cycle cancellation and oocyte yield. As expected, post-chemotherapy patients had higher rates of cycle cancellation but did equally well as chemotherapy-naive patients if they reached retrieval.

Supported by: NIH T32 HD007440 (LJ).

P-69 Tuesday, October 21, 2014
EXPERTING THE TIME FROM DECISION TO EGG RETRIEVAL IN THE ONCOFERTILITY PATIENT: USE OF LUTEAL PHASE STIMULATION. E. S. Constance, M. C. Goering, S. A. Krieg, Obstetrics and Gynecology, University of Kansas Medical Center, Kansas City, KS.

OBJECTIVE: The aim of this study is to describe the successful use of luteal stimulation to expedite ovaarian stimulation in the setting of urgent in vitro fertilization (IVF) for fertility preservation.

DESIGN: Retrospective case series.

MATERIALS AND METHODS: A retrospective case series of oncofertility patients treated at the University of Kansas Medical Center who presented during the postovulatory period. Under an IRB approved protocol, the records of all women presenting for ovarian stimulation and egg retrieval for the purpose of freezing oocytes or fertilized embryos prior to undergoing cancer treatment from 1/1/13 to 2/14/14 were reviewed. Patient demographics and clinical information regarding oocyte stimulation protocols and results were recorded. All patients who began stimulation during the postovulatory period were included in the study. Patients began stimulation with gonadotropin releasing hormone (GnRH) antagonist and recombinant follicle stimulating hormone on the day of presentation. Patient demographics were monitored by serial estradiol levels and ultrasound until they had at least two follicles measuring greater than 17mm, at which time they were triggered with 10,000 IU of human chorionic gonadotropin or 4 mg of GnRH agonist. Oocyte retrieval was performed 36 hours after trigger and either oocytes or blastocytes were frozen per patient preference.

RESULTS: During the study period, 5 oncology patients underwent IVF with ovarian stimulation post-ovulation. The mean age of patients in the study was 29.2 years and all patients were nulliparous. All 5 patients underwent oocyte retrieval with a median of 18 oocytes retrieved per patient (range 5-28) and a median of 9 MII oocytes per patient (range 2-24). All 5 women had either oocytes or fertilized embryos of adequate quality for cryopreservation. One woman underwent freezing of 8 unfertilized oocytes and 4 women underwent freezing of blastocysts with a median of 7 blastocysts per patient (range 1-12).

CONCLUSION: Luteal phase stimulation provides a safe and effective option for oocyte and/or embryo cryopreservation for patients requiring gonadotoxic medications. As the number of young cancer survivors increases, so does the need for efficient and effective methods of fertility preservation prior to the initiation of treatments that can cause infertility. By eliminating the need to wait until cycle day one to initiate ovarian stimulation, this protocol avoids potentially detrimental delays in cancer treatment while allowing young cancer survivors the opportunity to retain future fertility.

P-70 Tuesday, October 21, 2014
CHOLESTEROL AND DESMOSTEROL IN TWO SPERM POPULATIONS SEPARATED ON SIL-SELECT GRADIENT. A. H. Hassan, A. A. Kato, A. Christophe, E. Comhaire, T. Mostafa. Dermatology and Andrology, Mansoura Faculty of Medicine, Mansoura, Egypt. Medical Biochemistry, Mansoura Faculty of Medicine, Mansoura, Egypt. Endocrinology, Gent Faculty of Medicine, Gent, Belgium.

OBJECTIVE: To identify cholesterol and desmosterol composition of human spermatozoa of two sperm populations separated on Sil-Select gradient.

DESIGN: A prospective study.

MATERIALS AND METHODS: Forty-eight males were divided into four groups namely healthy men (n = 13), asthenozoospermia (n = 11), asthenoteratozoospermia (n = 10) and oligoasthenoteratozoospermia (n = 14). Sperm cholesterol and desmosterol were estimated in two human sperm population separated by centrifugation in a discontinuous Sirl-Select gradient.

RESULTS: cholesterol and desmosterol were the major steroids in human spermatozoa. Spermatozoa recovered from upper/lower layer interface (fraction I) had low fertilization potential, while those from the base (fraction II) had high fertilization potential. Median values of cholesterol and desmosterol in fraction I were 2.55 micromol and 0.77 micromol/10(9) spermatozoa and in fraction II were 1.16 micromol and 0.27 micromol/10(9) spermatozoa. Cholesterol/desmosterol ratio was significantly higher in fraction II than I (4.8 vs. 3.2, p < 0.01). Cholesterol, desmosterol, total phospholipids and sterols/phospholipids were negatively correlated with sperm concentrations, sperm motility, linear velocity, normal sperm morphology and acrosome reaction percentage whereas cholesterol/desmosterol ratio was positively correlated with these parameters.

CONCLUSION: The difference in sterol composition of sperm subpopulations separated on Sil-Select gradient suggests that composition of sterols is related to sperm functions.

Supported by: Mansoura Faculty of Medicine.

P-71 Tuesday, October 21, 2014

OBJECTIVE: Previous work has shown that among women diagnosed with cancer who seek pre-treatment FPC, those who pursue fertility preservation (FP) have decreased decisional regret. We sought to determine whether patient satisfaction was more closely related to decisional regret or undergoing FP itself.

DESIGN: Prospective survey.

MATERIALS AND METHODS: From January 2011 to February 2014, reproductive aged women with a new diagnosis of cancer who presented for FPC were consented. Women completed surveys at 4 timepoints (T: 1) before and 2) after a consultation, 3) after making a decision about FP, before initiating cancer treatment and 4) 6-8 months later. At T3 & T4, participants were administered a 5-item decisional regret scale and a decisional regret score (DRS) was obtained. Participants were also asked to rate their satisfaction related to decision-making and treatment outcomes at T3 & T4, respectively. Univariate analyses with Analysis of Variance (ANOVA) and linear regression were first used to determine if undergoing FP and/or decisional regret were associated with satisfaction scores. Next, Analysis of Covariance (ANCOVA) tests were used to determine the relationship between undergoing FP and DRS with satisfaction scores.

RESULTS: 189 women were recruited with a 94% accrual rate. The mean age was 33.6 yrs. 51.4% underwent FP. At T3, high participant satisfaction was no longer associated with satisfaction after controlling for decisional regret (P = 0.727). However, decisional regret remained a significant predictor of satisfaction regardless of whether or not a woman underwent FP (P < 0.0001). At T4, patient satisfaction was still predicted by DRS (r = 0.076, P = <0.0001) and undergoing FP (P < 0.0001), and again low decisional regret was strongly associated with high satisfaction even after controlling for whether or not a woman underwent FP (P < 0.0001).

CONCLUSION: These data demonstrate that low decisional regret is the best predictor of patient satisfaction, and that adequate counseling aimed at minimizing future regret can result in maximal satisfaction even in women who forgo FP. In order to maximize long-term satisfaction, FPC should focus on enabling patients to make an active decision about FP.

Supported by: NIH/NCCR/OD UCSF-CTSI Grant Number TL RR024129.
EFFICACY OF SERUM FOLLICLE-STIMULATING HORMONE LEVEL MONITORING DURING LETROZOLE-GONADOTROPIN OVARIAN STIMULATION CYCLES. J. Kim, a,b V. Turan, c G. Bedoschi, a,b K. Oktay. a,b New York Medical College, Valhalla, NY; a,b Innovation Institute for Fertility Preservation and IVF, New York, NY.

OBJECTIVE: To investigate the feasibility and efficacy of monitoring serum follicle–stimulating hormone (FSH) levels during letrozole-FSH ovarian stimulation cycles for fertility preservation (FP).

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: One-hundred and thirty eight women diagnosed with stage ≤3 breast cancer, who underwent ovarian stimulation with a letrozole-FSH protocol for FP were included. Serum FSH levels were measured throughout the cycle monitoring. Stimulation outcomes such as number of total and mature oocytes retrieved were recorded.

RESULTS: The multivariate analysis revealed that serum FSH levels both on 5th day of stimulation (FSH-5) and trigger day (FSH-t) had significant inverse relationship with number of total oocytes (p = 0.008 and p < 0.0001) and mature oocytes (p = 0.04 and p < 0.0001) retrieved after adjusting age, body weight and FSH dose administered. The risk of OHSS (>15 total oocytes) was significantly higher when FSH-5 ≤ 22 mIU/mL (OR:1.4, 95% CI: 1.2 - 1.6) and FSH-t ≤ 23 mIU/mL (OR:5.3, 95% CI: 1.8 - 16.0). When FSH-t was greater than 26 mIU/mL, the risk of poor response (<5 oocytes or <4 mature oocytes) was significantly higher (OR:3.7 (95% CI: 1.4, 10.1).

<table>
<thead>
<tr>
<th>TABLE 1. OR for poor response and high risk of OHSS at calculated cut-off values from ROC analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor response</td>
</tr>
<tr>
<td>Day 5 FSH &gt; 32 mIU/ml - OR (95% CI): 5.2 (0.8 - 34.1)</td>
</tr>
<tr>
<td>Trigger Day FSH &gt; 26 mIU/ml - OR (95% CI): 3.7 (1.4 - 10.1)</td>
</tr>
</tbody>
</table>

*Adjusted for age at stimulation, weight and starting FSH dose. a,b Adjusted for age at stimulation, weight and total FSH dose.

CONCLUSION: Serum FSH level monitoring may improve safety margin of ovarian stimulation. Serum FSH on the 5th and trigger day of stimulation may be predictive of oocyte retrieval outcomes. The additional information gained from serum FSH monitoring may enhance outcomes in FP cycles using letrozole and FSH.

Supported by: NIH R01 HD053112 and R21 HD061259.

P-74 Tuesday, October 21, 2014


OBJECTIVE: Graft ischemia due to suboptimal revascularization is a major hurdle towards implementation of efficient ovarian cryopreservation in clinical practice. Endothelial cells (ECs) exhibit unique paracrine signals that foster tissue regeneration and revascularization; co-engrafting ovarian-specific ECs with ovarian tissue in heterotopic locations may greatly foster viability, maximizing outcomes.

MATERIALS AND METHODS: Ovaries from RFP (red fluorescent protein) mice were engrafted into age-matched, oophorectomized VEGFR2-GFP (Vascular endothelial growth factor receptor 2) recipients in which endothelial cells fluoresce green; ovaries from VEGFR2-GFP mice were engrafted into oophorectomized RFP mice to assess the origin of new vessels (host versus graft). Tissue-specific ECs were isolated from adult mice, expanded in vitro, and labeled with a third fluorescent protein (Crimson) using lentiviral transduction. Wild-type B6 mouse ovaries (10-12 wk old) were transplanted into flanks of oophorectomized WT recipient B6 mice with co-transplantation of GFP-labeled ECs. Each mouse served as its own control, with a 10 mm flap. Endothelial cell grafts exhibited robust engraftment. Exogenous GFP-labeled ECs were isolated from ovarian tissue by histologic sections and analyzed using confocal microscopy.

RESULTS: Graft revascularization occurred in both directions, with new vessels arising in the graft originating from the host, and new vessels in the host originating from the graft. Ovarian transplants with co-transplantation of ECs exhibited robust engraftment. Exogenous GFP-labeled ECs were present throughout the area of transplantation, within ovarian stroma, and, notably, in theca and granulosa cell layers of the follicles themselves.

CONCLUSION: Exogenous ECs contribute to neovascularization and reperfusion of transplanted ovary. Further study is warranted to determine whether organ-specific subtypes of ECs preferentially benefit engrafted tissue. Moreover, genetically modified ECs engineered to secrete specific paracrine factors will establish a novel platform to test factors that foster or hinder optimal engraftment and, more broadly, to interrogate molecular influences that affect follicle viability, recruitment, and maturation.

Supported by: NIH R01 HD053112; R21 HD061259.

THE USE OF A “DOUBLE TRIGGER” FOR FERTILITY PRESERVATION IN POOR RESPONDERS. F. S. Pacheco, a,b A. Small, c C. Acosta, a K. Acosta, a S. Dunn, a K. Oktay, a,b Innovation Fertility Preservation & IVF, New York, NY; b New York Medical College, Valhalla, NY.

OBJECTIVE: In the practice of fertility preservation, poor responder patients present a special challenge, as often there is no time for repeated cycles. It is therefore crucial to investigate methods to improve mature oocyte yield from every follicle retrieved. Our hypothesis was to test a “double-trigger” approach to determine if oocyte maturity and D-3 embryo development can be improved in this population of patients.

DESIGN: Retrospective.

MATERIALS AND METHODS: Seventeen cycles from 6 women undergoing fertility preservation for various indications were deemed poor responders based on the Bologna criteria. All were stimulated with FSH (300-450 IU) with or without letrozole 5 mg added throughout. A GnRH antagonist was given when the lead follicle diameter reached ≥13 mm, and when the same reached ≥17 mm, hCG (1000 IU - 2500 IU) and leuprolide acetate (40-80 IU) were given 30-34 hours before retrieval. Trigger day was influenced by the follicle cohort, length of stimulation and in one case, cancer treatment start date. LH surge was confirmed next morning. During retrieval, each follicle was aspirated separately to identify the association of size with oocyte maturity. Those that were M-II were either vitrified (2 patients, 2 cycles) or subjected to ICSI (4 patients, 14 cycles) and cryopreserved on D-3 stage.

RESULTS: From the 17 cycles, 40/74 follicles were ≤13 mm on the trigger day. These yielded 47 oocytes of which 38 were mature (72%). Fourteen follicles originated from follicles ≤13 mm , resulting in 62% (13/21) maturity rate. This was compared to the maturity rate of 96% (25/26) from follicles ≥13-mm (p = 0.003). Strikingly, 8 MII oocytes were retrieved from follicles ≤10 mm (62% MII rate), showing a similar maturity rate to those 10-13 mm follicles. Of those MII, 7 were cryopreserved and remaining were ICSIed resulting in the cryopreservation of 27 D3-embryos (D3 embryo rate=87%). Of the 9 immature oocytes, 8 came from follicles ≤13-mm, 2 matured in vitro, further increasing the total mature oocyte yield to 71.4% for follicles ≤13 mm.

CONCLUSION: We presented novel evidence that mature oocytes can be obtained from very small follicles. The double trigger approach described here maybe helpful in improving mature oocyte yield from small follicles in poor responders and possibly when there is insufficient time to perform a full-length ovarian stimulation.

Supported by: NIH R01 HD053112; R21 HD061259.
WHOLE OVARY VITRIFICATION WITHOUT DMSO: A VIALBLE OPTION FOR IN VITRO MATURATION OF FOLLICLES?

OBJECTIVE: Random start controlled ovarian stimulation (COS), where a patient can be stimulated upon presentation regardless of the menstrual cycle phase, is an emerging method for oocyte/embryo cryopreservation in cancer patients.

RESULTS: Viable follicles were isolated from ovaries vitrified by both protocols. In Group A, 87% of oocytes in follicles (n=15) underwent GBV, resulting in a M1 or M2 oocyte (40% and 47%, respectively). In Group B, the rate of GV breakdown was 82%, with 27% of oocytes developing to M1 and 55% to M2 (n=11). Mean follicular diameter at start of culture was the same in Group A and B, 132 ± 24 µm and 119 ± 26 µm, respectively. Starting oocyte diameter was 61-69 µm in both groups. By the end of culture, Group A follicles measured 261 ± 125 µm as compared to 319 ± 110 µm in Group B. Overall growth of the follicle in culture was similar. Oocyte diameters were 69.4 ± 2.6 µm and 70.1 ± 5.6 µm in groups A & B, respectively. Histological assessment of representative tissue sections from both protocols failed to reveal any gross differences. In Group A, of the 152 follicles counted 43% were primordial, 28% primary and 29% were secondary as compared to 48%, 23% and 29%, respectively in Group B (n=156).

CONCLUSION: Vitrification methods without DMSO may be a viable alternative for vitrification of ovarian tissue in this animal model. However, more data is needed to determine if DMSO-free cryoprotectant solutions offer any clear advantage.

CRYPRESERVATION

THE EVOLUTION OF OCOCYTE CRYOPRESERVATION (OC): LONGITUDINAL TRENDS AT A SINGLE CENTER.

OBJECTIVE: To characterize changing trends in elective (E) and medical (M) OC at our institution.

RESULTS: 1,439 E-OC and 186 M-OC cycles (including 14 combined embryo/OC cycles) were performed from 2005-present. For E-OC, the steepest rise in cycle volume occurred from 2005-2006 (7:2005 vs. 381:2013). From 2009-2013, the no. of E-OC cycles/y increased ~36% while the number of M-OC cycles remained constant. Significantly, the no. of oocytes retrieved/cycle/y increased steadily in E-OC (2005:12 up to 2013:16) but was stable in M-OC, but not in M-OC. Although NS, the no. of oocytes retrieved/cycle/y increased steadily in E-OC (2005:12 up to 2013:16) but was stable in M-OC. The no. of MII oocytes were retrieved and cryopreserved in M-OC cycles compared to E-OC. 83 E-OC (excluding PGS after IVF) and 6 M-OC thaw cycles have resulted in live birth rates of 37% (E-OC, avg age 37.3y) and 50% (M-OC, avg age 35y).
CONCLUSION: The age of presentation for E-OC has decreased while demand steadily rises, likely reflective of improved technology in the context of heightened public awareness surrounding age-related infertility. Notably, outcomes for women undergoing E-OC and M-OC are comparable and have resulted in live birth rates commensurate with IVF national averages, confirming that both E-OC and M-OC represent viable and successful alternatives for postponing parenthood, regardless of indication.

P-78 Tuesday, October 21, 2014

OUTCOMES FROM FRESH IVF CYCLES ARE COMPARABLE TO OOCYTE WARMING CYCLES IN PATIENTS WHO CHOOSE TO ELECTIVELY CRYOPRESERVE THEIR OOCYTES. J. Linn, C.-C. Chang, S. Chhatriwala, T. Elliott, D. Shapiro, Z. P. Nagy. Reproductive Biology Associates, Atlanta, GA.

OBJECTIVE: Oocyte cryopreservation by vitrification has been proven to be an efficient approach; however there is limited data on cycle outcomes when performed in infertile patients who chose this approach to avoid embryo cryopreservation. Therefore, we collected and analyzed data from patients that chose to cryopreserve a portion of their oocytes for ethical / moral reasons and cryopreservation. Therefore, we collected and analyzed data from patients that chose to cryopreserve a portion of their oocytes for ethical / moral reasons and found no significant differences when compared with the fresh IVF cycle when performed in infertile patients who chose this approach to avoid embryo cryopreservation.

DESIGN: Retrospective data analysis.

MATERIALS AND METHODS: From October 2007 to October 2013, 114 patients chose to cryopreserve a portion of their oocytes for ethical/moral reasons and found no significant differences when compared with the fresh IVF cycle when performed in infertile patients who chose this approach to avoid embryo cryopreservation.

CONCLUSION: Our findings surprisingly indicate that GV vitrified oocytes recovered from unstimulated or after COH in cancer patients display similar SR as metaphase 2 oocytes of healthy women. Furthermore, in women suffering from cancer, the meiotic competence of vitrified GV oocytes is dramatically reduced when oocytes are recovered during an unstimulated cycle. Further investigation is needed to distinguish possible direct deleterious effects of vitrification procedures or an overall decreased intrinsic meiotic competence of oocytes retrieved from small antral follicles.

Supported by: University Hospital Jean Verdier, Paris 13.

P-80 Tuesday, October 21, 2014


OBJECTIVE: Sperm cryopreservation is an essential tool in preservation of fertility in oncology and vasectomy patients, and when male partners are unavailable during assisted reproductive technologies (ART). Test vials (TVs) are used to determine cryosurvival (CS) and are important for cryopreservation decision making and ART counseling; yet no agreement exist on optimal TV protocols. This study investigated efficacy of pre-freeze semen parameters and TVs in predicting CS of future ART procedure vials (ARTVs) and the necessity of obtaining a TV for every ejaculate a patient supplies.

DESIGN: Prospective study.

MATERIALS AND METHODS: Patients having semen cryopreservation between 2004-11 had one TV and ARTVs frozen per ejaculate. Test vials were thawed within 24h of freezing to calculate CS (post-thaw motility/ pre-freeze motility). The relative predictive importance (RPI) of pre-freezing parameters to CS of TVs was calculated using forward stepwise linear regression (ad.2). Mann-Whitney U test (MWU) and Spearman’s correlation coefficient (ρ) were calculated to determine the efficacy of TVs in predicting CS of ARTVs within the same ejaculate and CS of TVs and ARTVs in different ejaculates within the same patient.

Mean±SD. α=α<.0001; β=NS.

CONCLUSION: This study demonstrates that elective oocyte cryopreservation for ethical reasons provides comparable results to fresh cycle outcomes and may be used as an alternative to embryo cryopreservation.

P-79 Tuesday, October 21, 2014

PROSPECTIVE STUDY COMPARING SURVIVAL AND MEIOTIC RESUMPTION RATES OF IMMATURE OOCYTES FROM IN VITRO MATURATION AND STIMULATED CYCLES IN CANCER PATIENTS. C. Sifer, O. Sellam, N. Sermondade, C. Sonigo, J.-N. Hugues, A. Benoit, C. Poncelet, M. Grynenberg. IIVF Unit, Universitary Hospital Jean Verdier, Bondy, France.

OBJECTIVE: The present investigation aimed at assessing: (i) whether vitrification of immature oocytes retrieved from unstimulated cycles or after controlled ovarian hyperstimulation (COH) performed in cancer patients seeking fertility preservation have comparable survival (SR) and meiotic resumption rates (MRR) after in vitro maturation (IVM) (ii) the SR of germinal vesicle stage (GV) oocytes in cancer patients in comparison to those observed with metaphase 2 oocytes vitrified in healthy controls.

DESIGN: From November 2013 to January 2014, twenty-two patients suffering from cancer, candidates to oocyte vitrification in unstimulated (IVM group, n=10) or after COH (COH group, n=12) were prospectively studied.

RESULTS: SR of vitrified oocytes after warming were of 88.0, 86.3 and 88.1% in the IVM, COH and control groups, respectively. Although SR were similar in all groups (overall p value: p=0.88), the MRR of GV oocytes in IVM group was significantly lower when compared with COH groups (16.5 vs. 56.8%; p=0.018).

CONCLUSION: E-OC: age 37.6±4.5 - S.D. (n) 12) were prospectively

Table.

<table>
<thead>
<tr>
<th>Year</th>
<th>E-OC: age (no. of cycles)</th>
<th>M-OC: age (no. of cycles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>40±2 (7)</td>
<td>36.2±1 (3)</td>
</tr>
<tr>
<td>2006</td>
<td>39.7±3 (40)</td>
<td>29.9±8 (6)</td>
</tr>
<tr>
<td>2007</td>
<td>39.4±3 (35)</td>
<td>36.3±4 (5)</td>
</tr>
<tr>
<td>2008</td>
<td>39.1±3 (84)</td>
<td>31±7 (15)</td>
</tr>
<tr>
<td>2009</td>
<td>38.9±3 (125)</td>
<td>29±7 (21)</td>
</tr>
<tr>
<td>2010</td>
<td>38.5±3 (198)</td>
<td>32.3±6 (40)</td>
</tr>
<tr>
<td>2011</td>
<td>38.3±3 (262)</td>
<td>30.8±8 (30)</td>
</tr>
<tr>
<td>2012</td>
<td>38.1±3 (307)</td>
<td>28.6±7 (34)</td>
</tr>
<tr>
<td>2013</td>
<td>37.9±3 (381)</td>
<td>30.7±8 (32)</td>
</tr>
</tbody>
</table>

Mean±SD. α=α<.0001; β=NS.

<table>
<thead>
<tr>
<th>Year</th>
<th>E-OC: age (no. of cycles)</th>
<th>M-OC: age (no. of cycles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>40±2 (7)</td>
<td>36.2±1 (3)</td>
</tr>
<tr>
<td>2006</td>
<td>39.7±3 (40)</td>
<td>29.9±8 (6)</td>
</tr>
<tr>
<td>2007</td>
<td>39.4±3 (35)</td>
<td>36.3±4 (5)</td>
</tr>
<tr>
<td>2008</td>
<td>39.1±3 (84)</td>
<td>31±7 (15)</td>
</tr>
<tr>
<td>2009</td>
<td>38.9±3 (125)</td>
<td>29±7 (21)</td>
</tr>
<tr>
<td>2010</td>
<td>38.5±3 (198)</td>
<td>32.3±6 (40)</td>
</tr>
<tr>
<td>2011</td>
<td>38.3±3 (262)</td>
<td>30.8±8 (30)</td>
</tr>
<tr>
<td>2012</td>
<td>38.1±3 (307)</td>
<td>28.6±7 (34)</td>
</tr>
<tr>
<td>2013</td>
<td>37.9±3 (381)</td>
<td>30.7±8 (32)</td>
</tr>
</tbody>
</table>
RESULTS: Progressive motility was the best indicator of TV CS amongst initial semen parameters, yet was of modest RPI and correlation (68% of the effect; r2 = 0.3, p < 0.05; n = 703) and was not an indicator of ARTV CS (r2 = 0.1, p > 0.05; n = 30). Cryosurvival of TVs (81% ± 3%; mean ± SE) and ARTVs (83% ± 3%) from the same ejaculate were similar and correlated (p > 0.5, MWU; r2 = 0.8, p < 0.01). Cryosurvival of the first TV (73% ± 14%, n = 166) obtained in a series of cryopreserved ejaculates was comparable and correlated to subsequent TVs (72% ± 12%, n = 253) from different ejaculates within the same patient (p > 0.7, MWU; r2 > 0.6, p < 0.01). Similarly CS of the first TV (81% ± 5%, n = 9) to subsequent ARTVs (88% ± 3%, n = 22) was comparable and correlated (p > 0.1, MWU; r2 = 0.6, p < 0.01).

CONCLUSION: Cryosurvival calculation is essential for efficient and individualized fertility preservation and ART treatments. Test vial CS is an excellent predictor of ARTV CS and no pre-freeze semen parameters are better. Freezing a TV for each ejaculate a patient cryopreserves, in a short period of time, is unlikely not needed; as the first TV shows no significant difference in CS compared to subsequent TVs and ARTVs. Removing the need for multiple TVs per patient will make use of semen samples in ART and fertility preservation more efficient.

P-81 Tuesday, October 21, 2014

WOMEN PURSUING OOCYTE CRYOPRESERVATION FOR NON MEDICAL PURPOSES ARE MORE LIKELY TO ACHIEVE ≥10 OOCYTE IF THEIR FSH IS <11, REGARDLESS OF AGE. L. Schuman, a K. Bergin, b G. Wiltkin, ab A. B. Copperman, a,b bReproductive Medicine Associates of New York, New York, NY; aDepartment of OB/GYN and Reproductive Science, Mount Sinai School of Medicine, New York, NY.

OBJECTIVE: Women pursuing elective oocyte cryopreservation (EOC) hope to achieve an immediate favorable outcome to foster future goals of family building. A retrieval of ≥10 oocytes at vaginal oocyte retrieval (VOR) has been associated with ultimately achieving fertilization, implantation, and positive pregnancy. In order to prospectively identify realistic expectations, our study sought to evaluate baseline FSH level as a marker for achieving ≥10 oocytes at VOR.

DESIGN: Retrospective Analysis.

MATERIALS AND METHODS: EOC patients from 5/7/2005-3/26/2014 were included. Patient age and FSH were segregated and matched to oocyte counts at VOR. Mean oocyte counts were correlated with FSH levels to evaluate the number of cycles needed to achieve ≥10 oocyte outcome. Statistical analysis was conducted by ANOVA with significance set at p < 0.05.

RESULTS: Increasing baseline FSH levels correlated with decreasing oocyte counts among all age groups, and stabilizing at the lowest counts at FSH level <13. Patients ≥38 oocyte counts were significantly less when compared to <35 and 35-37 subsets (p < 0.05). Patients, regardless of age, required 1 cycle to attain ≥10 oocytes or if they reached an FSH level of <11.

CONCLUSION: EOC can be a physically, emotionally, and financially exhausting process, and patients should prospectively be provided with prognostic information. We demonstrated that patients who presented with a baseline FSH value of <11 mIU/mL and were <38 years of age at the time of retrieval had 77.8% chance of having ≥10 oocytes retrieved at VOR. Our data is reliable in offering realistic expectations to EOC patients; future research of these patients’ thaw-survival, fertilization rates and pregnancy outcomes will allow us to further formulate EOC cycle prognosticators.

P-82 Tuesday, October 21, 2014

MORPHOMETRIC QUANTIFICATION OF MICROFLUIDIC-REDUCED OSMOTIC STRESS IN OOCYTE AND ZYGOTE VITRIFICATION. G. D. Smith, a D. Lai, b J. Ding, c G. W. Smith, c S. Takayama. aObstetrics & Gynecology, University of Michigan, Ann Arbor, MI; bBiomedical Engineering, University of Michigan, Ann Arbor, MI; cAnimal Science, Michigan State University, East Lansing, MI.

OBJECTIVE: Cryosurvival for vitrification and warming is high, yet sub-lethal cellular damage can impede developmental competence. Automated gradual microfluidic cryoprotectant agent (CPA) exposure of murine zygotes promoted significantly higher quality blastocysts compared to manual stepwise CPA exposure. Studies investigated mechanisms and cellular characteristics that relate to differences in CPA exposure and developmental competence.

DESIGN: Controls were non-treated bovine oocytes or murine zygotes. Experimental groups were: i) pipetting of cells from base media directly to vitrification solution (VS; Direct), known to be detrimental to cryosurvival; ii) contemporary manual stepwise pipetting of cells from base media to equilibration solution (ES) and subsequently into VS (Stepwise); and iii) automated microfluidic gradual transition of cells from base media through ES to VS (Gradual). Vitrification, warming, and CPA removal were identical between groups.

MATERIALS AND METHODS: Zygote shrinkage rates were calculated with Kedem–Katchalsky equations. Zygote morphometric analysis was performed in VS using phase and confocal microscopy to quantify cell surface roughness index (RI) and sphericity. Oocyte cytoplasmic lipid content was quantified with Nile Red staining and confocal microscopy following CPA removal after treatment, vitrification, and warming. Statistical analyses were done using Student’s t-test.

RESULTS: Gradual zygotes shrank 14 times and 23 times slower than Stepwise and Direct, respectively; consequently shrinkage rate was inversely related to embryonic developmental competence. Zygotes had smoother RI (32.3 ± 5.2; n = 27; p < 0.001) cell surfaces in Gradual compared to Stepwise (RI = 33.3 ± 5.3; n = 26). Gradual zygotes also had higher sphericity (0.90 ± 0.1; n = 27; p < 0.001) than Stepwise (0.79 ± 0.1; n = 24), which had membrane buckling similar to Direct with the lowest sphericity (0.59 ± 0.1; n = 18). Oocyte cytoplasmic lipid content, known to be positively correlated to embryonic developmental competence, was higher in the Gradual (77.0 ± 1.0% of controls; n = 17; p = 0.01) compared to Stepwise (52.0 ± 1.0% of controls; n = 18) exposure.

CONCLUSION: Compared to contemporary Stepwise CPA exposure, Gradual CPA exposure results in reduced osmotic stress, lower cell shrinkage rate, improved morphometrics and cytoplasmic lipid retention, reduced sub-lethal damage, and enhanced embryonic developmental competence.

Supported by: NIH - GM096640, CA072005, and CA136829.

P-83 Tuesday, October 21, 2014

ULTRASTRUCTURAL AND MOLECULAR CHANGES AFTER OOCYTE VITRIFICATION PROTOCOL. V. Pineda, H. Uriondo, M. C. Franco, C. Alvarez Sedo. CEGYR - Genetics and Reproductive Medicine, Capital Federal, Buenos Aires, Argentina.

OBJECTIVE: Kuwayama (2007) describe the vitrification method for oocytes and embryo cryopreservation. The aim of the present study was to compare ultrastructural and molecular changes that are present in oocytes from egg donors after vitrification in compare with fresh oocytes.

DESIGN: Prospective and comparative cohort study.

MATERIALS AND METHODS: One hundred remanent oocytes from egg donation cycles were included in this study (2012-2013). Oocytes were selected from donors that had at least one previous donation cycle. Half of the oocytes were vitrified (V) by Cryotop method and the other half was evaluated in fresh (F). All oocytes were vitrified/thawed by the same operator. Ten oocytes of each group (V and F) were randomly selected for transmission electron microscopy in order to assess mitochondrial ultrastructural analysis.

<table>
<thead>
<tr>
<th>Fresh oocytes</th>
<th>Vitr/Thawed oocytes</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA fragmentation</td>
<td>8.6%</td>
<td>15.2%</td>
</tr>
<tr>
<td>Mitochondrial alterations</td>
<td>13.3%</td>
<td>20%</td>
</tr>
<tr>
<td>Metaphase plate disarrangements</td>
<td>5.7%</td>
<td>12.1%</td>
</tr>
<tr>
<td>Parthenogenetic activation</td>
<td>0%</td>
<td>3%</td>
</tr>
</tbody>
</table>

Fresh vs. Vitrified/Thawed oocytes

Vol. 102, No. 3, Supplement, September 2014
The rest of the oocytes were evaluated by immunocytochemistry for: DNA fragmentation (TUNEL), microtubules and metaphase plate organization (Tubulin antibodies and Hoechst).

**RESULTS:** The survival rate after vitrification was 96% (CI95% 86.3-99.5). The comparison of the assessed variables are shown in table 1. The percentage of the quantified variables seems to be higher in the vitrified oocytes group, however, the differences were not statistically significant. Mitochondrial alterations, evidenced by the inner plasma membrane dilation and electron-dense appearance, were associated with the activation of apoptotic mechanisms. In both groups, most of the oocytes that showed chromosomal alignment anomalies had DNA damage at the same time.

**CONCLUSION:** Regarding the ultrastructural and molecular changes assessed in this study, no significant differences were detected between fresh or vitrified/thawed oocytes. Thus, in our experience, the vitrification technique appears to be safe, confirming the previous reports by other authors.

*Supported by: CEGYR Foundation.*

**P-84** Tuesday, October 21, 2014


**OBJECTIVE:** Higher pregnancy rates can be achieved by frozen-thawed embryo transfer (FTET) compared to fresh embryo transfer for blastocysts. This results from the synchronization of embryo transfer with the time of endometrial implantation. FTET is particularly effective for growth-retarded embryos that develop into blastocysts on day 6. The size of the blastocyst at freezing and the timing of freezing differ slightly among institutions. At our clinic, Gardner’s classification is used for blastocyst assessment as follows: BL-3, diameter <160 μm; and BL-4, ≥160 μm. The size of the blastocyst at the time of freezing is ≥BL-3 and in order to minimize the stress from culture in vitro, we aim to freeze the blastocysts on day 5. To clarify the optimal time for blastocyst freezing, this study examined the influences of blastocyst size at freezing (BL-3 or BL-4) and the timing of blastocyst freezing (Day 5 or Day 6) on pregnancy rates.

**DESIGN:** This was a retrospective study of pregnancy rates.

**MATERIALS AND METHODS:** This study reviewed 883 cycles of FTET of good-quality embryos rated as at least BL-3BB based on Gardner’s classification. Pregnancy rates with FTET were compared for BL-3 frozen on Day 5 (D5-BL3), BL-4 frozen on Day 5 (D5-BL4), BL-3 frozen on Day 6 (D6-BL3), and BL-4 frozen on Day 6 (D6-BL4) blastocysts. Statistical analysis was performed using the chi-square test. Values of p<0.05 were considered statistically significant.

**RESULTS:** Pregnancy rates were 25.3% (59/233) for D5-BL3, 20.0% (5/25) D6-BL3, 40.0% (209/522) for D5-BL4, and 41.7% (43/103) for D6-BL4. Pregnancy rates did not differ significantly between D5-BL3 and D6-BL3, or between D5-BL4 and D6-BL4. Differences in the timing of freezing exerted no influence on pregnancy rates for blastocysts of the same size at freezing. Moreover, irrespective of the timing of freezing, pregnancy rates with FTET were significantly higher for embryos frozen at BL-4 than for those frozen at BL-3. These findings indicate that irrespectively of the day of freezing, blastocysts that have reached BL-4 should be frozen for use in FTET.

**CONCLUSION:** D6-BL4 showed higher pregnancy rates than D5-BL3. Even for embryos developed to blastocysts by day 5, our findings suggest that higher pregnancy rates can be achieved with FTET, depending on growth status, by continuing in vitro culture to day 6 and freezing more fully developed blastocysts. In other words, rather than freezing blastocysts at BL-3, blastocysts should be developed to BL-4 before freezing.

**P-85** Tuesday, October 21, 2014

**SUCCESSFUL VITRIFICATION OF MOUSE AND HUMAN EGGS USING LOWER PERCENTAGES OF CRYOPROTECTANTS BY MAINTAINING WEIGHT PERCENT OF THE VITRIFICATION SOLUTION.** T. Schlenker, R. L. Krisher, W. B. Schoolcraft.

**OBJECTIVE:** Cryoprotectants prevent ice crystal formation during vitrification, but can have toxic side effects. Our objective was to compare the efficacy of three vitrification solutions containing varying percentages of cryoprotectants on survival and subsequent development of vitrified mouse and human eggs.

**DESIGN:** Research study.

**MATERIALS AND METHODS:** Mature mouse (CF1) eggs were vitrified using: 1) A standard egg vitrification solution of TCM199 with 15% each DMSO and EG (15% total cryoprotectant) and 29% serum (M199); 2) an In House vitrification media with 12.5% each DMSO and EG (25% total cryoprotectants), 0.15% hydroxypropyl cellulose (HPC) with no serum (IH-MOD); or 3) an In House vitrification media with 10% each DMSO and EG (20% total cryoprotectants), 8% Ficoll, and 0.15% HPC with no serum (IH-LO). The weight % of IH-MOD was 49.1 and of IH-LO was 50.8. After warming, eggs were activated and cultured to assess development. GLM ANOVA with Tukey-Kramer test was used to analyze results (p<0.05, significant). To test these solutions for use in humans, 12 MII eggs collected from a single donor were split between M199 and IH-LO, and were vitrified and warmed a total of 4 times.

**RESULTS:** Eggs vitrified in IH-MOD and IH-LO survived better than those in M199 (95.7 ± 1.7%, 93.5 ± 2.1%, 75.7 ± 5.0%, respectively). Eggs vitrified in IH-MOD and IH-LO also cleaved at a higher frequency than those in M199 per total eggs vitrified (64.3 ± 4.1%, 62.3 ± 4.1%, 41.9 ± 5.8%, respectively), although as a percentage of surviving eggs there was no difference (67.2 ± 4.1%, 66.7 ± 4.2%, 55.4 ± 6.7%, respectively). Of total eggs vitrified, IH-MOD produced more blastocysts on D4 (51.4 ± 3.9%) than did M199 (49.4 ± 4.2%). There were no differences in D4 blastocyst development between treatments when calculated per surviving egg or per cleaved embryo (IH-MOD, 48.9 ± 5.3% IH-LO, 47.7 ± 5.4%; M199, 35.5 ± 8.7%). Human egg survival post vitrification-warming was similar between treatments (M199, 66% eggs, 100%; IH-LO, 56% eggs, 83%).

**CONCLUSION:** Cryoprotectant concentrations can be lowered in egg vitrification solutions while maintaining survival and viability by using additional compounds to maintain weight percent. Lower cryoprotectant concentration increased survival and blastocyst development. Solutions with lower cryoprotectant concentrations appear promising for application to human egg vitrification.

**P-86** Tuesday, October 21, 2014

**LIVE BIRTHS FOLLOWING OOCYTE VITRIFICATION USING DMOS-FREE CRYOPROTECTANTS.** C.-C. Chang, T. A. Elliott, J. R. Herrick, R. L. Krisher.

**OBJECTIVE:** Dimethyl sulfoxide (DMSO) has been widely used as a cryoprotectant (CPA) for oocyte cryopreservation. However, it has been indicated that DMSO could cause depolymerization of oocyte tubulin and malfunction of the meiotic spindle and trigger intracellular calcium increase, which may potentially cause oocyte activation and degeneration. Thus, our objective was to compare oocyte vitrification outcomes with vitrification solution containing DMSO vs. DMSO-free CPA.

**DESIGN:** Prospective study.

**MATERIALS AND METHODS:** Seventy-two recipients with an average age of 41.2±4.9 y were matched to use donor eggs from the cryo-egg bank (from 33 donors with an average age of 27.0±2.9 y). Cryopreservation on donor sibling oocytes was performed by two methods: CPAs containing 15% ethylene glycol, 15% DMSO and 0.5 M sucrose (group A containing DMSO) and CPAs containing 30% ethylene glycol and 0.5 M sucrose (group B-DMSO free). Sibling oocytes from both groups were warmed for each recipient. Oocytes were fertilized by ICSI 2-3 hours after warming. On day 5, blastocyst formation was assessed and embryo transfer was performed. Results were analyzed by the Chi-square (p<0.05) statistical test.

A total of 467 MII oocytes were warmed for 72 recipients. Two hundred eighty-five oocytes were warmed from group A (3.95±1.44), and

<table>
<thead>
<tr>
<th># of recipient</th>
<th>ET embryos only from A (EG+DMSO)</th>
<th>ET embryos only from B (EG)</th>
<th>ET embryos from A+B</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td># of ET</td>
<td>13 (6.5±0.48a)</td>
<td>17 (7.0±0.48a)</td>
<td>36 (21.1±0.31a)</td>
<td></td>
</tr>
<tr>
<td>(means±SD)</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Clinical</td>
<td>11/26 (42.3)</td>
<td>610 (60.0)</td>
<td>21/36 (58.3)</td>
<td>NS</td>
</tr>
<tr>
<td>pregnancy rate (%)</td>
<td>8/143 (54.8)</td>
<td>817 (47.0)</td>
<td>33/63 (43.4)</td>
<td></td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>9/26 (34.6)</td>
<td>4/10 (40.0)</td>
<td>18/56 (50.0)</td>
<td></td>
</tr>
<tr>
<td>Take home baby</td>
<td>15 (6)</td>
<td>26</td>
<td></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td># of ET</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a,b Significantly different (P<0.05)
185 oocytes were warmed from group B (2.52 ± 1.03). Survival rates were 90.1% (257/285) and 94.5% (172/182; NS) in groups A and B, respectively. Fertilization rates were 79.8% (229/285) in group A, and 81.3% (148/182; NS) in group B. Blastocyst formation rates were 57.9% (165/285) and 53.8% (98/182; NS) in groups A and B, respectively.

CONCLUSION: The data presented here suggest a CPA solution without DMSO can provide equivalent efficiency of oocyte vitrification to a common protocol that contains DMSO. Live births were also achieved using the cryoprotectant solution without DMSO.

P-87 Tuesday, October 21, 2014

TO FREEZE OR NOT TO FREEZE: THE COST-EFFECTIVENESS OF A FREEZE-ALL POLICY WHEN PROGESTERONE ON THE DAY OF HCG TRIGGER IS ELEVATED. G. Patounakis,<sup>a</sup> L. W. Sundheimer,<sup>b</sup> A. H. DeCherney,<sup>a</sup> M. J. Hill.<sup>a</sup> NIH/NICHD, Bethesda, MD; <sup>b</sup>UCLA, Los Angeles, CA.

OBJECTIVE: Estimate what progesterone level on the day of HCG trigger a freeze-all policy is cost-effective per live birth.

DESIGN: Cost analysis.

MATERIALS AND METHODS: Fresh and frozen IVF cycle costs were based on published US averages. The cost of a fresh IVF cycle was estimated at $10,500. The cost of a freeze-all cycle was estimated at $14,250 and included the costs of the initial fresh cycle without embryo transfer, freezing of embryos, and subsequent FET. Fresh IVF live birth rate with elevated progesterone was expressed as a fraction (alpha) of the FET rate. This modeling allowed for variations in progesterone assay and progesterone effects on live birth between practices. FET live birth rates were assumed to be 42% based on SART data. The primary analysis was based on a single IVF cycle with elevated progesterone. A second analysis modeled the cost with the availability of supernumerary embryos to allow for a 2nd frozen cycle if the initial fresh or freeze-all cycle failed. Given the wide range in reported costs and success rates in US IVF centers, sensitivity analyses were performed to adjust for cost and success rates of fresh IVF and FET. A final equation modeled costs and success rates to allow for variations between IVF programs.

RESULTS: For the single cycle cost analysis, alpha was 74% (about 0.74), indicating it was more cost effective to freeze-all once fresh transfer rates were less than 0.74 of the FET rates. Assuming average FET live birth rates of 42%, it was cost effective to freeze-all when elevated progesterone levels decreased fresh live birth rates below 31%. This corresponded to progesterone levels > 2 ng/mL in published data. In the case where supernumerary embryos were available, alpha decreased to 61%, indicating it was more cost effective to freeze-all once fresh transfer rates were less than 61% of the frozen rates. This corresponded to progesterone levels > 2.3 ng/mL in published data.

CONCLUSION: A freeze-all policy is more cost effective when elevated progesterone reduced the probability of live birth to less than 0.74 (74%) of an FET. If there were supernumerary embryos to freeze, the analysis shifted in favor of fresh transfer until the probability of live birth was reduced to 61% of an FET. These models provide general expressions that can be customized to any clinic even if costs, FET rates, and fresh rates with elevated progesterone do not match published data.

Supported by: This research was supported, in part, by Intramural research program of the Program in Reproductive and Adult Endocrinology, NICHD, NIH.

P-88 Tuesday, October 21, 2014

TIMING OF OOCYTE CRYOPRESERVATION FOR ELECTIVE INDICATIONS: A COST-EFFECTIVENESS ANALYSIS. T. B. Mesen, J. E. Mersereau, J. B. Kane, A. Z. Steiner. UNC Chapel Hill, Chapel Hill, NC.

OBJECTIVE: To determine the cost-effectiveness (CE) of oocyte cryopreservation (OC) for single women desiring fertility preservation for elective indications.

DESIGN: Decision-tree analysis.

MATERIALS AND METHODS: Using a Markov model, the CE of OC for elective indications between the ages of 25 and 40 was compared to the conventional strategy of expectant management followed by IVF if necessary. A 7-year horizon from OC decision to an attempt at pregnancy was assumed. Included in the decision tree were the probabilities of: natural conception (Time to Conceive, a prospective time-to-pregnancy study), miscarriage, live-birth (LB) with cryopreserved oocytes, and LB following IVF (CDC database). Costs were obtained from actual charge estimates at a private fertility center and are congruent with published data.<sup>1,4</sup> The overall probability of clinical pregnancy per LB at a given age by decision to cryopreserve oocytes was calculated. To account for differences in social practices two models were constructed: Model A: Women attempted pregnancy after 7 years regardless of marital/partner status, thus were willing to use donor sperm. Model B: Women only attempted pregnancy if married within 7 years (probability of marriage - National Survey of Family Growth 2006-10).

RESULTS: Compared to expectant management, OC at age 38 was most CE for women accepting of donor sperm ($13,804 per additional LB). However, at this age, only 44% of women who pursued OC had a LB, a 29% increase in LB compared to expectant management. The marginal effectiveness was first greater than 0.1 at age 31 (incremental cost-effectiveness ratio (ICER): $57,815) and was maximal at age 37 (0.30). At all ages, the ICER was significantly higher and the LB rate significantly lower in Model B (4.4 years regardless of marriage included) compared to Model A (donor sperm allowed). In Model B, when compared to expectant management, OC was most cost effective (ICER:$148,552) and had the highest marginal effectiveness (0.05) at age 35.

Supported by: Institutional support only.

P-89 Tuesday, October 21, 2014

ROLE OF CRYOSTRESS ON COMPETENCE OF TESTICULAR SPERMATOZOA IN NON-OBSTRUCTIVE AZOOSPERMIC MEN. Q. V. Neri,<sup>a</sup> D. Ryan,<sup>a</sup> S. Cheung,<sup>a</sup> T. Cozzubbo,<sup>a</sup> P. N. Schlegel,<sup>a</sup> Z. Rosenwaks,<sup>a</sup> G. D. Palermo,<sup>b</sup> Reproductive Medicine, Weill Cornell Medical College, New York, NY; <sup>b</sup>Urology, Weill Cornell Medical College, New York, NY.

OBJECTIVE: To determine the performance in terms of fertilization and clinical pregnancy rates of testicular specimens from NOA men before and after cryopreservation.

DESIGN: We categorized ICSI cycles of NOA men whose testicular spermatozoa were used fresh or following cryopreservation. To control for repeat-cycle bias, only first attempts for fresh and frozen biopsies were used.

MATERIALS AND METHODS: A total of 1329 NOA-TESE cycles were identified and included in the study. ICSI cycles were categorized according to whether fresh or cryopreserved testicular spermatozoa were used for injection. Sperm characteristics together with embryological and obstetrical outcomes were assessed and compared. Cryopreserved cycles performed following a failed cycle were omitted.

RESULTS: In an all-inclusive analysis (n=1329), a total of 1001 fresh TESE were compared to 329 frozen cycles, where the concentration (0.4±3 vs 0.2±4 million/mL) and motility (2.6±9 vs 1.2±3%) were comparable. Fertilization (57.4 vs 48.0%) and clinical pregnancy (42.0 vs 35.7%) rates appear higher in the fresh cycles in comparison to its cryopreserved counterpart (P=0.0001). When an exclusive analysis of first attempts only was carried out, 834 cycles included 700 with fresh and 134 with frozen spermatozoa (maternal age 32.8±6 vs 35.7±5yrs). The fresh versus frozen concentration (0.58±2 vs 0.7±4 million/mL) and motility (3.4±1 vs 2.9±14%), respectively, remained similar. Interestingly, the fertilization rose from 54.2% in the fresh versus 63.2% in the frozen group (P=0.0001). In the fresh cohort, the average transfer of 2.5 embryos per patient that yielded a clinical pregnancy rate of 40.0% with an implantation of 25.3% (395/1565) and delivery/ongoing of 37.0%. The cryopreserved group had an average embryo replacement of 2.7 that resulted in a clinical pregnancy of 39.6%, an implantation of 23.0% (79/343), and delivery/ongoing of 35.8%.

CONCLUSION: While the effect of cryostress is known to impair motility characteristics of spermatozoa, particularly those of severely oligozoospermic men, this remains particularly concerning when freezing testicular spermatozoa of men with compromised spermatogenesis where kinetic characteristics are used to gauge viability. While our initial analysis seems to endorse fresh testicular specimens, a controlled analysis on a first attempt basis normalized performance voiding the concern of cryostress on testicular spermatozoa of NOA men.

Supported by: Reproductive Medicine, Weill Cornell Medical College.
INTRAUTERINE HYPERGLYCEMIA ALTERED IMPRINTED GENES EXPRESSION IN GERM CELLS OF MALE OFFSPRING. J. Ren, a G.-L. Ding, b,c H.-F. Huang, b,c J. Sheng, a Department of Pathophysiology, School of Medicine, Zhejiang University, Hangzhou, Zhejiang, China; b,c The International Peace Maternity And Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China; a Key Laboratory of Reproductive Genetics, Ministry of Education, Hangzhou, Zhejiang, China.

OBJECTIVE: It has been reported that the expression of some imprinted genes were significantly altered in gestational diabetes mellitus (GDM) offspring. Gene imprinting erasure occurred during E11.5-13.5, while imprint reestablishment is completed at E18.5 in male mice fetus. The function of methyltransferases 1(DNMT1) is maintaining methylation, and DNMT3a/3b are responsible for de novo methylation. This study was designed to investigate which period of imprinting reprogramming process was changed during early fetal development in male germ cells of GDM offspring and what role DNMTs played in it.

DESIGN: Examine maintenance and de novo methylation process of GDM fetus mice male germ cells, by detecting imprinted genes and DNMTs expression.

MATERIALS AND METHODS: A GDM mouse model of intrauterine hyperglycemia was established. Primordial germ cells (PGCs) of E13.5 male fetus mice were collected to determine imprinting erasure process, while testes of E18.5 fetus mice were collected to examine imprinting reestablishment process by detecting the expression level of Igf2/H19 imprinted genes(real-time PCR). The DNMT levels were also tested in E13.5 and E18.5 male germ cells (real-time PCR) at the same time.

RESULTS: In E13.5 PGCs, Igf2 and H19 expression of GDM group were significantly decreased compared with control group (p<0.05, p<0.05). There was no significant difference in DNMT1 expression between two groups, DNMT3a and DNMT3b expression was higher in GDM group than control, respectively (p<0.05, p<0.01). In E18.5 PGCs, Igf2 and H19 expression of GDM group were significantly decreased than control (p<0.05, p<0.05). DNMT1 and DNMT3a expression were lower in GDM group (p<0.05, p<0.01), while DNMT3b expression was higher in GDM group than control (p<0.01).

CONCLUSION: Intrauterine hyperglycemia altered Igf2/H19 expression in germ cells of male offspring, associating with altered maintenance and de novo methylation.

Supported by: The research of the authors is Supported by the National Natural Science Foundation of China (31171444 and 81200485) and the Research Fund for the Doctoral Program of Higher Education (20120101120051).

P-93 Tuesday, October 21, 2014

WHICH IS BETTER FOR ROBERTSONIAN TRANSLOCATION'S PGD CYCLES,BLASTOMERE FISH OR TROPHECTODERM ARRAY? J. Huang, Y. Lian, N. Zhao, P. Liu, J. Qiao. Peking University Third Hospital, Beijing, China.

OBJECTIVE: To explore a better protocol of the PGD cycles for Robertsonian translocations.

DESIGN: Retrospective study.

MATERIALS AND METHODS: From January 2012 to June 2013, a total of 89 PGD cycles for Robertsonian translocations were done in our reproductive center, including 59 blastomere FISH cycles (defined as Group A) and 30 trophectoderm array cycles (defined as Group B). In group A, one blastomere was biopsied at D3, then FISHed by two chromosomal probes. After diagnosed and blastocyst culture, one or two blastocytes were transferred in each fresh cycle or one or two surplus blastocysts were transferred in several thawing cycles. In group B, several trophectoderm cells were biopsied at D5 or D6, then diagnosed by CGH or SNP arrays with 24 chromosomes. All the transfer cycles in group B were in thawing cycles with single blastocyst transferred. Compared the oocytes number, 2PN, D3 embryos, genetic balance embryos, good blastocysts, available embryos and ongoing pregnancy rates between the two groups.

Supported by: 1K12HD063086-01 (ARC).

P-90 Tuesday, October 21, 2014

FERTILITY & STERILITY®

TABLE 1. compared the results between the two groups

<table>
<thead>
<tr>
<th>Group A</th>
<th>Group B</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>cycles</td>
<td>59</td>
<td>30</td>
</tr>
<tr>
<td>Age of female</td>
<td>30.73±4.16</td>
<td>29.60±3.36</td>
</tr>
<tr>
<td>Cycles of no embryo</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>to transfer</td>
<td>D0 oocytes</td>
<td>17.44±8.03</td>
</tr>
<tr>
<td>2PN/D9 oocytes</td>
<td>57.05% (587/1029)</td>
<td>57.19% (318/556)</td>
</tr>
<tr>
<td>D3 embryos</td>
<td>491</td>
<td>249</td>
</tr>
<tr>
<td>D3 embryos/D0 oocytes</td>
<td>47.72% (491/1029)</td>
<td>47.78% (249/556)</td>
</tr>
<tr>
<td>good blastocysts</td>
<td>147</td>
<td>135</td>
</tr>
<tr>
<td>good blastocysts/D3 embryos</td>
<td>29.94% (147/491)</td>
<td>54.22% (135/249)</td>
</tr>
<tr>
<td>genetic balance embryos</td>
<td>142</td>
<td>82</td>
</tr>
<tr>
<td>genetic balance embryos/D3 embryos</td>
<td>88</td>
<td>82</td>
</tr>
<tr>
<td>available embryos</td>
<td>17.92% (88/491)</td>
<td>32.93% (82/249)</td>
</tr>
<tr>
<td>available embryos/D3 embryos</td>
<td>38.46% (20/52)</td>
<td>69.23% (18/26)</td>
</tr>
</tbody>
</table>

MATERIALS AND METHODS: Patient #1: 37yo G1, singleton pregnancy conceived after IVF utilizing endometrial cell coculture, derived from previously frozen maternal endometrial biopsy cells; cffDNA testing at 12.5 weeks. Patient #2: 36yo G4P0, heterotopic pregnancy conceived after the transfer of 3 embryos during a fresh IVF cycle. Underwent laparoscopic right salpingectomy at 7.6 weeks; cffDNA testing at 12.3 weeks. Patient #3: 33yo G1, spontaneously conceived dichorionic, triamniotic triplet pregnancy with demise of 2 of the fetuses diagnosed on ultrasound at 8.3 weeks. cffDNA testing at 20.3 weeks. Patient #4: 35yo G1P1, spontaneously conceived singleton pregnancy; cffDNA testing at 10.2 weeks.


CONCLUSION: ART pregnancies represent 1-2% of live births in the US. Yet, ART and multiple pregnancies are overrepresented in this case series. We postulate that this discordance may be a result of multiple implantation sites and continued slowing trophoblast despite removal or demise, under-lying mosaicism not clinically detected, or alterations in implantation from laboratory techniques and/or gamete/embryo micromanipulation. We believe this case series should heighten awareness, caution and further research in this unique patient population with regard to gender discordance in cffDNA testing.

Supported by: 1K12HD063086-01 (ARC).
RESULTS: There was no difference with oocytes number, 2PN, D3 embryos and genetic balance embryos between two groups. But, good blastocysts rate and ongoing pregnancy rates in group B were significantly higher than those in group A.

CONCLUSION: Although blastomere FISH protocol is very easy and it can be transferred in fresh cycles, trophoderm array protocol is much safer to embryos and it can be provided more genetic information for embryos. As a result, trophoderm array protocol is recommended for Robertsonian translocations’ PGD cycles.

Supported by: This project was Supported by Peking-Tsinghua Center for Life Sciences, the Beijing Municipal Science and Technology Commission (Z13110005213006) and the National Natural Science of China (31220047).

P-94 Tuesday, October 21, 2014

THE IMPACT OF FMR1 CARRIER TESTING IN REPRODUCTIVE DECISION-MAKING. V. L. Baker, A R. Walker, M. L. Clark, S. L. Young, L. M. Pastore, Stanford Univ, Stanford, CA; Univ of VA, Charlottesville, VA; Univ of NC, Chapel Hill, NC.

OBJECTIVE: The ACMG now recommends testing for premutations of the FMR1 gene (FXPM) in women with premature ovarian failure, elevated FSH for their age (which encompasses a dx of diminished ovarian reserve [DOR]), and/or a family hx of fragile X syndrome (FXS). This test may hold both welcome news (genetic explanation for DOR may provide comfort and closure) and unwelcome news (FXPM result has serious reproductive implications) for women with DOR. This study examined how women dx’d with DOR approach FXPM testing in their reproductive decision-making.

DESIGN: Mixed methods analysis

MATERIALS AND METHODS: 120 women clinically dx’d with DOR, with regular menses and w/o family hx of FXS, provided blood for FMR1 testing and completed pretest questionnaires (Q’s). Study-specific pretest genetic counseling reviewed FMR1 genetics and health implications. Prior to learning their test results, qualitative interviews were conducted with 7 of these women. Results from Q’s and interviews were analyzed separately

RESULTS: Participants were primarily white (73.3%) or Asian (18.3%), with a median age of 36 years. This investigation found that genetic carrier testing: (A) informs women’s view of an infertility diagnosis. Most (71.4%) projected that if they carried the FXPM, the knowledge that their DOR had a medical explanation would make them feel better about their dx. This sentiment was confirmed in the interviews, and was stronger among nulliparous women (p=0.02). (B) indicated that, if found to carry the FXPM, women were more concerned about the health consequences for future biological children than for themselves. Most (61.5%) reported upset at the possibility of affecting others due to the FXPM and were more likely to feel this if they perceived the FXPM to be a serious medical condition (p=0.003). (C) impacts women’s future reproductive decisions. 40% reported that a FXPM result would make them less likely to have biological children. 5 of the interviewees were waiting on the FMR1 results before moving forward.

CONCLUSION: FMR1 testing does inform a woman’s reproductive decision-making. Further research is needed to determine if the likelihood of being identified as a carrier is worth the distress of testing.

Supported by: Eunice K. Shriver National Center for Child Health and Human Development at the National Institutes of Health (grants HD52768, HD057485 and HD068440).

P-95 Tuesday, October 21, 2014

EMBRYO SELECTION VERSUS NATURAL SELECTION: HOW DO COMPREHENSIVE CHROMOSOME SCREENING (CCS) OUTCOMES COMPARE TO ANALYSIS OF PRODUCTS OF CONCEPTION (POC) FROM EARLY PREGNANCY LOSS (D&C). J. Rodríguez-Purata, a J. A. Lee, a M. C. Whitehouse, a T. Mukherjee, a, b L. Grunfeld, a, b S. Sandler, a, b A. B. Copperman, a, b Reproductive Medicine Associates of New York, New York, NY; Obstetrics, Gynecology and Reproductive Science, Icahn School of Medicine at Mount Sinai, New York, NY.

OBJECTIVE: Since the advent of preimplantation genetic screening (PGS), clinicians have questioned its use, as most numeric chromosome abnormalities (NCA) are considered to be lethal and without reproductive potential. The objective of this study was to assess the predictive value of all NCA reported after PGS compared to those reported after cytogenetic analysis from D&C.

DESIGN: Retrospective analysis.

MATeRIALS AND METHODS: Cytogenetic reports of patients who underwent an IVF cycle with CCS of ≥1 biopsied embryo were compared to cytogenetic analysis from patients who had a D&C after ART. Only conclusive results were included in the study. Frequencies for every numerical abnormality were compared.

RESULTS: A total of 1046 NCA were reported after CCS (monosomy, 47.1%; trisomy, 52.9%) and 444 after D&C (monosomy, 6.3%; trisomy, 82.6%; polyplody 11.0%). The top five most frequently affected chromosomes were 15, 16, 18, 21 and 22 in both PGS and D&C. Interestingly, after D&C, monosomies were rarely observed. All abnormalities per chromosome are reported below.

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Monosomy (%)</th>
<th>Trisomy (%)</th>
<th>Monosomy (%)</th>
<th>Trisomy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.29</td>
<td>1.2</td>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
<td>2</td>
<td>1.3</td>
<td>2.2</td>
<td>0</td>
<td>1.8</td>
</tr>
<tr>
<td>3</td>
<td>0.1</td>
<td>1.2</td>
<td>0</td>
<td>1.3</td>
</tr>
<tr>
<td>4</td>
<td>1.3</td>
<td>0.7</td>
<td>0</td>
<td>3.5</td>
</tr>
<tr>
<td>5</td>
<td>1.1</td>
<td>1.3</td>
<td>0</td>
<td>1.8</td>
</tr>
<tr>
<td>6</td>
<td>1.1</td>
<td>0.9</td>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>7</td>
<td>1.3</td>
<td>1.3</td>
<td>0.3</td>
<td>3.3</td>
</tr>
<tr>
<td>8</td>
<td>1.1</td>
<td>2.3</td>
<td>0</td>
<td>2.0</td>
</tr>
<tr>
<td>9</td>
<td>0.6</td>
<td>2.8</td>
<td>0</td>
<td>3.8</td>
</tr>
<tr>
<td>10</td>
<td>1.2</td>
<td>1.3</td>
<td>0.3</td>
<td>3.3</td>
</tr>
<tr>
<td>11</td>
<td>0.8</td>
<td>1.1</td>
<td>0</td>
<td>0.8</td>
</tr>
<tr>
<td>12</td>
<td>0.4</td>
<td>1.1</td>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>13</td>
<td>2.1</td>
<td>1.2</td>
<td>0</td>
<td>5.1</td>
</tr>
<tr>
<td>14</td>
<td>1.7</td>
<td>1.6</td>
<td>0</td>
<td>2.5</td>
</tr>
<tr>
<td>15</td>
<td>3.0</td>
<td>5.6</td>
<td>0</td>
<td>10.9</td>
</tr>
<tr>
<td>16</td>
<td>5.5</td>
<td>5.9</td>
<td>0</td>
<td>15.9</td>
</tr>
<tr>
<td>17</td>
<td>1.2</td>
<td>1.6</td>
<td>0</td>
<td>1.8</td>
</tr>
<tr>
<td>18</td>
<td>3.3</td>
<td>2.9</td>
<td>0</td>
<td>3.8</td>
</tr>
<tr>
<td>19</td>
<td>2.7</td>
<td>2.9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>1.1</td>
<td>2.2</td>
<td>0</td>
<td>4.6</td>
</tr>
<tr>
<td>21</td>
<td>5.4</td>
<td>4.1</td>
<td>2.0</td>
<td>8.1</td>
</tr>
<tr>
<td>22</td>
<td>9.3</td>
<td>6.7</td>
<td>0</td>
<td>15.4</td>
</tr>
<tr>
<td>X</td>
<td>1.1</td>
<td>0.7</td>
<td>4.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Y</td>
<td>0</td>
<td>0.1</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Triploid</td>
<td>0</td>
<td>26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetraploid</td>
<td>0</td>
<td>23</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CONCLUSION: Our analysis presents descriptive data of the most common abnormalities seen after PGS and D&C. PGS helps patients by eliminating most of the NCA and avoiding early pregnancy loss. Overall, the same common numerical alterations were observed. Although our data suggested that monosomies do not implant, they account for almost 50% of all abnormalities. As the use of IVF has evolved, so too has genomic assessment. Our study demonstrated the comparability in chromosomal abnormalities of naturally conceived fetuses; and highlights embryo selection of karyotypic embryos offer the possibility to patients to avoid invasive and potentially harmful procedures. As we move forward with CCS technology, patients will be courteous to its’ capabilities. In respecting our current technologies’ evolution, miscarriages due to chromosomal abnormalities could be minimized.

P-96 Tuesday, October 21, 2014

TO TEST OR NOT TO TEST? VARIABLE SPECTRUM MUTATIONS (VSMs) IN HIGH IMPACT DISEASES IDENTIFIED IN 3,208 CLINICAL SAMPLES SCREENED VIA AN EXPANDED CARRIER SCREENING PLATFORM. S. Rodriguez, a, b N. Kumar, a A. Bisignano, a, b S. Munne, a, b S. Chen, a J. Grifo, c M. Sury, c Recombine, New York, NY; Reprogenetics, Livingston, NJ; St. Barnabas Institute for Reproductive Medicine and Science, Livingston, NJ; NYU Langone Fertility Center, New York, NY; Southern California Reproductive Center, Beverly Hills, CA.

OBJECTIVE: Expanded carrier screening is now routinely performed at fertility centers. While such panels focus on identifying genetic diseases...
with high impact on quality of life or life expectancy, some vary in severity. Further, different mutations in the same gene can lead to variable expression of disease. The goal of this study was to explore VSMs and assess their impact on our positive screen rate.

DESIGN: Retrospective.

MATERIALS AND METHODS: The Illumina Infinium HD Custom Genotyping platform identified 1679 mutations associated with 213 recessive diseases. The analysis includes data from 3208 clinical referrals from reproductive endocrinologists, obstetricians, and genetic counselors. Documented informed consent to utilize data in a de-identified manner was obtained. VSMs were identified for several high impact recessive diseases and allele frequencies for each mutation were calculated. We measured the positive screen rate within our patient population for all mutations, high impact disease mutations and high impact disease mutations excluding the select VSMs.

RESULTS: We identified common variable spectrum mutations for high impact diseases, including p.D1270N in Cystic Fibrosis, p.A285X in β Thalassemia, the 4.2kb deletion in α Thalassemia, p.V37I and p.M347T in GJB2-Related Nonsyndromic Hearing Loss & Deafness, and p.D444H in Biotinidase Deficiency. The positive screen rate for all panel mutations was 59.4%. When looking only at high impact disease mutations, the positive screen rate was 42%. After excluding VSMs our positive screen rate reduced to 29.5%.

CONCLUSION: Our results indicate that VSMs within high impact diseases account for a significant portion of carriers on our panel (12.5%). We continue to report these mutations, as they can be clinically significant when in cis with other mutations or when in trans with a severe mutation. Therefore, genetic counseling for carrier screening results is critical. We could additionally consider removing VSMs, flagging these mutations on clinical reports or reporting these mutations only when a couple’s reproductive risk is high. Advances in genomic technology will lead to continued identification of VSMs; therefore, a consensus should be reached on how to manage these mutations in a clinically responsible way.

PREIMPLANTATION GENETIC DIAGNOSIS

P-97 Tuesday, October 21, 2014

TWICE THAWED EUPLOID (TTE) FROZEN EMBRYO TRANSFER (FET) REDUCES SPONTANEOUS ABORTION WITH SIMILAR PREGNANCY AND ON-GOING / DELIVERY RATES WITH FEWER EMBRYOS TRANSFERRED. A. Adler, H.-L. Lee, D. H. McCulloh, B. Hodes-Wertz, J. Grifo. Ob/Gyn, New York University Langone Medical Center, New York City, NY.

OBJECTIVE: To compare clinical pregnancy outcomes of standard FET cycles with TTE-FET for trophoderm transfer with biopsy from the fresh in vitro fertilization and embryo transfer (IVF-ET) for which these embryos originated. DESIGN: A retrospective cohort study.

MATERIALS AND METHODS: 39 patients who initially attempted IVF and embryo transfer without Preimplantation Genetic Screening (PGS) returned for their frozen embryos. Some of these patients did standard FET without PGS, all 39 then desired PGS due to an adverse outcome; either they did not get pregnant, or did, but resulted in miscarriage or termination, or they did conceive and delivered a healthy baby and desired a known euploid embryo for a subsequent attempt. In this study, embryos were thawed, biopsied and retrieved and then thawed and transferred for TTE-FET in a programmed cycle. The outcomes of cycles were then compared, fresh IVF, FET (no PGS) and TTE-FET.

<table>
<thead>
<tr>
<th>Clinical Pregnancy/sAB/ongoing and delivery rates IVF/FET/TTE-FET</th>
<th>Fresh IVF-ET</th>
<th>FET (no PGS)</th>
<th>TTE-FET</th>
</tr>
</thead>
<tbody>
<tr>
<td># patient (cycle)</td>
<td>39</td>
<td>7</td>
<td>26</td>
</tr>
<tr>
<td>Clinical Pregnancy (sAC)</td>
<td>26 (67%)</td>
<td>4 (57%)</td>
<td>12 (46%)</td>
</tr>
<tr>
<td>Spontaneous abortion</td>
<td>15 (57%)*</td>
<td>2 (50%)</td>
<td>1 (8%)* p&lt;0.01</td>
</tr>
<tr>
<td>Live birth/ongoing pregnancy</td>
<td>11 (28%)</td>
<td>2 (29%)</td>
<td>11 (42%)</td>
</tr>
</tbody>
</table>

RESULTS: 99/194 (51%) of the blastocysts thawed and biopsied were found to be euploid. Though no significant differences were seen among the three groups for pregnancy or ongoing/live birth rates, the Spontaneous abortion (sAB) rate was significantly lower when euploid blastocysts were transferred in TTE-FET compared to the fresh ET with no PGS. CONCLUSION: TTE-FET avoids transfer and implantation of aneuploid embryos (49% of the blastocysts available for FET with out PGS). TTE-FET avoids the pain and suffering associated with an abnormal conceptus, the cost of thawing and transferring abnormal embryos and the time lost with a non-viable pregnancy. Despite the invasive steps of additional thawing, vitrification and TE biopsy, the resulting euploid embryos establish pregnancies comparably with fresh IVF and FET (no PGS) and embryos retain the property of decreased pregnancy loss associated with once-thawed euploid embryos. Though this cohort is small, it suggests that TTE can still assure that patients will not receive an aneuploid embryo, and will have lower sAB, even when biopsy was not done initially in their fresh IVF cycle, TTE is an option that achieves viable pregnancy at least as well as IVF-ET and FET (no PGS).

P-98 Tuesday, October 21, 2014

PROPORTION OF ANEUPLOIDY DOES NOT IMPACT LIVE BIRTH RATE OR PREGNANCY LOSS RATE IN PATIENTS WITH RECURRENT PREGNANCY LOSS (RPL) UNDERGOING COMPREHENSIVE CHROMOSOME SCREENING. J. M. Franasiak, a, b R. Barnett, K. H. Hong, a, b M. D. Werner, a, b R. T. Scott, Jr. a, b RWJ Medical School, Rutgers University, New Brunswick, NJ; a, b RMA of New Jersey, Basking Ridge, NJ.

OBJECTIVE: There is limited evidence that aneuploidy disproportionately effects outcomes in patients with RPL, as compared to the general infertility population. In an effort to investigate the potential that patients with RPL without balanced translocations have a biologic mechanism related to aneuploidy behind their poor pregnancy outcomes, we sought to evaluate if the proportion of aneuploidy within a cohort of embryos impacts pregnancy outcomes. If an intrinsic biologic deficit exists, we suspect that patients with high aneuploidy rates in the RPL population would be disproportionately helped by CCS.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: Patients with RPL, defined as ≥ 2 failed pregnancies documented by ultrasound or histopathology, who underwent CCS from 2008-2014 were included. The percent aneuploid in the cohort was tabulated and patients were divided into quartiles of percent aneuploidy. Utilizing a chi-square test, successful outcomes (live birth) were compared to unsuccessful outcomes (negative pregnancy test, chemical pregnancy, failed clinical pregnancy). Subsequently, the difference between each pregnancy outcome was assessed between the aneuploidy quartiles. Finally, the overall loss rate (live birth/positive pregnancy tests) amongst the quartiles was assessed.

RESULTS: A total of 264 patients met the criteria for RPL and underwent CCS; 4 had 100% aneuploidy leaving 260 for analysis. The mean number of embryos evaluated was 5.8 (1-24). The aneuploidy rate for all patients was 26% (0-87.5%). The mean number of embryos transferred was 1.5 (1-3). The live birth rate was 58.5%. When analyzing aneuploidy by quartile, there was no difference in live birth rate between groups (Table 1) (p=0.18). There was no difference between quartiles when live birth, pregnancy loss, and negative pregnancy test were compared (p=0.10). Finally, loss rate was compared between quartiles in patients with a positive pregnancy test (n=211) and did not differ (p=0.65).

Differences in the proportion of aneuploid embryos does not affect live birth rate

<table>
<thead>
<tr>
<th>Cohort Aneuploid Quartile</th>
<th>No live birth, n (%)</th>
<th>Live birth, n (%)</th>
<th>Total, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>29 (27.9)</td>
<td>37 (38.1)</td>
<td>49</td>
</tr>
<tr>
<td>2nd</td>
<td>25 (27.9)</td>
<td>41 (38.1)</td>
<td>66</td>
</tr>
<tr>
<td>3rd</td>
<td>29 (33.4)</td>
<td>50 (45.6)</td>
<td>79</td>
</tr>
<tr>
<td>4th</td>
<td>27 (20.7)</td>
<td>22 (28.3)</td>
<td>49</td>
</tr>
<tr>
<td>Total</td>
<td>110</td>
<td>150</td>
<td>260</td>
</tr>
</tbody>
</table>

CONCLUSION: The proportion of aneuploid embryos patients with RPL is not associated with pregnancy outcome. Provided a euploid embryo exists,
the percent aneuploid rate of the cohort does not affect live birth rate or pregnancy loss rate.

P-99 Tuesday, October 21, 2014


OBJECTIVE: This study aimed to evaluate the feasibility and benefits of PGD via direct detection of fragile X syndrome (FraX) repeat expansion sizes in embryos at the blastocyst stage.

DESIGN: PGD for FraX was carried out on blastocysts from female pre-embryonic carriers. All TE samples were assessed using 3 different methods: direct detection of the FraX CGG repeat size, Karyomapping (Knapp) and STR analysis.

MATERIALS AND METHODS: 30 biopsied blastocysts derived from 9 couples were amplified using WGA. Aliquots of each product were used to perform the different 3 tests: PCR based direct mutation detection, STR-PCR and Knapp analysis using an Illumina beadarray.

RESULTS: Diagnostic results were obtained for 29/30 (96.7%) samples. Regarding mutation detection, 27/30 (90%) embryos showed amplification. Results obtained were 100% concordance with the 2 linkage analysis carried out in parallel. All 3 tests showed that 16 samples inherited the normal FMR1 allele from the female carrier and 11 embryos inherited a premutation or full expansion allele. Unlike the other 2 methods, direct mutation was able to distinguish which embryos inherited a premutation allele versus those that inherited full expansion.

CONCLUSION: Direct analysis of the FraX expansion mutation is rarely used in PGD due to the difficulties of amplifying large trinucleotide repeat expansion mutations in single cells. In this study, we present an efficient and robust method for detection of the FraX repeat size using a PCR approach applied on blastocyst embryos. As analysis via STR or Knapp does not test the mutation directly, embryos that inherit the allele with the expansion are not transferred even though it is not known if a premutation or full mutation is present. Direct analysis of FraX expansion provides a more comprehensive diagnosis and is expected to increase the number of embryos available for transfer after PGD. This method can be useful in couples with de novo mutations/no available family members for phase determination. It is important that before the methodology described above is applied to a clinical case, the couple is counseled appropriately and the expected PGD outcomes explained.

P-100 Tuesday, October 21, 2014


OBJECTIVE: To assess if the number of blastomeres on day 3 is associated with live birth following euploid blastocyst SET.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: All patients who underwent blastocyst culture, trophectoderm biopsy, array comparative genomic hybridization, and euploid single embryo transfer (SET) between 10/2010-11/2013 were identified. For patients with multiple cycles, only the first retrieval and ET were included. Embryos were grouped by number (no.) of cells on day 3: ≤ 5.6, or ≥ 8. Cycle parameters abstracted included age, day 2 estradiol (E2) and FSH, no. metaphase (MI) oocytes, no. 2PN embryos, no. embryos biopsied, no. euploid embryos, and other covariates. The primary outcome, live birth and ongoing pregnancy (LBR), was collected, as were secondary outcomes of biochemical pregnancy (BR), implantation rate (IR), and spontaneous abortion rate (SABR). Data were analyzed using chi-squared and ANOVA where appropriate, p < 0.05.

RESULTS: 251 patients (age range 34-37y) met inclusion criteria and embryos were analyzed by no. of cells on day 3. There were no differences in cycle parameters between patients who had euploid embryos with ≤ 5, 6, 7, or ≥ 8 cells on day 3. A significant difference in day 3 embryo grade was noted (embryos with ≤ 5cells: grade 2 ± 0.7; ≥ 8 cells: grade 1.5 ± 0.3, p < 0.001). No differences were noted in the no. of embryos biopsied (range 5.1 to 7, p = 0.05), no. of euploid embryos (range 2 to 3.4, p = 0.05), or in BR, IR, or SABR. Notably, euploid embryos with ≤ 5 cells on day 3 were less likely to result in live birth or ongoing pregnancy (p < 0.05).

Clinical outcomes following euploid blastocyst SET

<table>
<thead>
<tr>
<th>No. euploid biopsied</th>
<th>≤ 5 cells</th>
<th>6 cells</th>
<th>7 cells</th>
<th>≥ 8 cells</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>37.4 ± 4</td>
<td>36.6 ± 5</td>
<td>36.6 ± 4</td>
<td>36.5 ± 5</td>
<td>.96</td>
</tr>
<tr>
<td>No. embryos</td>
<td>5.7 ± 4</td>
<td>5.1 ± 3</td>
<td>6.1 ± 4</td>
<td>7 ± 5</td>
<td>.12</td>
</tr>
<tr>
<td>No. euploid embryos</td>
<td>2.8 ± 3</td>
<td>2 ± 1</td>
<td>3.2 ± 3</td>
<td>3.4 ± 3</td>
<td>.11</td>
</tr>
<tr>
<td>BR (%)</td>
<td>18.8</td>
<td>11.5</td>
<td>11.4</td>
<td>9.4</td>
<td>.51</td>
</tr>
<tr>
<td>IR (%)</td>
<td>47</td>
<td>73</td>
<td>63.6</td>
<td>70.5</td>
<td>.06</td>
</tr>
<tr>
<td>SABR (%)</td>
<td>20</td>
<td>10.5</td>
<td>14.3</td>
<td>10.5</td>
<td>0.7</td>
</tr>
<tr>
<td>LBR (%)</td>
<td>37.5</td>
<td>65.4</td>
<td>54.6</td>
<td>63.1</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Data presented in mean ± SD or %.

CONCLUSION: Few criteria exist to select the optimal euploid embryo for ET. Our data suggest that euploid embryos with ≤ 5 cells on day 3 may have decreased potential to produce live birth, and embryos with a greater no. of blastomeres on day 3 may be more likely to result in live birth. In patients with multiple euploid embryos available for ET, the number of cells on day 3 may be considered as a criterion for embryo selection.

P-101 Tuesday, October 21, 2014


OBJECTIVE: To examine morphokinetic data from euploid and aneuploid embryos to discern whether the data will satisfy assumptions required for use in discriminating euploid from aneuploid embryos.

DESIGN: Retrospective Analysis of Morphokinetic Data and PGS Data. MATERIALS AND METHODS: Morphokinetic parameters for 103 embryos from 20 patients were adjusted by subtraction of the time of syngamy and euploid embryos to discern whether the data will satisfy assumptions required for use in discriminating euploid from aneuploid embryos.

RESULTS: For 16MPs were examined using two-way ANOVA. The variability among patients (patient-to-patient variability) was significantly larger than the within group variability for most MPs. In contrast, the variability between ploidy groups (euploid versus aneuploid) was not significant for any MPs. Using one-way ANOVAs, no significant difference was found between euploid and aneuploid embryos within patients for most MPs, considering different differences in both means and variances. Exceptions were the time for completion of compaction and the duration of compaction. However, there was no significance between ploidy groups for these two MPs when considering their variability among patients.

CONCLUSION: The variability of MPs was greater from patient to patient than can be expected for variations in MPs with ploidy. The variability of morphokinetic parameters among patients was too large to permit discrimination of ploidy through the use of fixed thresholds. Therefore, we conclude that the morphokinetic parameters that we have examined will not be able to distinguish euploid from aneuploid embryos. Any significant associations seen with ploidy are likely to result from spurious ploidies in association with patient variability rather than due to ploidy itself. aCGH remains the most accurate method of distinguishing euploid from aneuploid embryos. Our observations do not discount the possibility that MPs may be useful in distinguishing the implantation potential of euploid embryos. Therefore, it may be useful to use morphokinetics in conjunction with aCGH to select the best euploid embryo for transfer.

OBJECTIVE: To examine a morphokinetic approach to predicting ploidy in blastocysts by examining each of the individual morphokinetic parameters (MPs) and its utility in distinguishing euploid from aneuploid blastocysts.

DESIGN: Retrospective Analysis of Morphokinetic and PGS Data. MATERIALS AND METHODS: Embryos cultured in the Embryoscope were retrospectively analyzed for MPs relative to the time of syngamy. Trophoeoderm biopsy was performed and blastocysts were vitrified. Trophoeoderm biopsies were analyzed using 24 chromosome array comparative genomic hybridization. MPs for each embryo were analyzed with reference to ploidy. The area under the curve (AUC) for the receiver operator characteristic (ROC) curve was used to determine whether MPs provided the ability to identify the embryo’s ploidy. We focused on the two MPs (time to blastocoe formation, and time to full blastocyst) used by Campbell et al. (2013) to distinguish euploid from aneuploid embryos.

RESULTS: 103 embryos from 20 patients were examined. All of the 16 MPs determined in our embryos were examined using ROC curves. Most parameters, including those identified (Campbell et al., 2013) had ROCs with AUCs less than 0.6. One of the parameters that provided poor discrimination of euploid from aneuploid embryos. Only one MP, the duration of compaction (DOC), had an AUC for its ROC curve that exceeded 0.6. Using a threshold value of 12.3 hours, we split the embryos into two groups, one with an incidence of euploidy of 55% (28/51) and the other with an incidence of euploidy of 25% (13/52). Use of Campbell’s criteria provided no significant enrichment observed in either grouped in unscreened embryos. CONCLUSION: The failure of Campbell’s MPs to have significant ROCs suggest that they are not universally applicable for segregating euploid from aneuploid embryos. The observation that DOC provided a significant enrichment for euploid embryos in our facility, but did not provide any enrichment in the Campbell lab (despite being considered) provides evidence that this MP is not universal. We conclude that there is no universal MP is present, to distinguish the ploidy of embryos. It remains unclear why MPs can yield significant, yet small, enrichment of euploidy in some facilities but are incapable of providing enrichment in other facilities.

P-103 Tuesday, October 21, 2014
SIMPLIFIED STATIC EMBRYO SCORE SYSTEM FOR THE PREDICTION OF BLASTOCYST FORMATION AND EUPLOIDY. L. Kaupert, a D. A. N. F. Januário, b C. E. Czeresnia, b M. G. Nisenbaum, b M. Maluf, a P. M. Perin, a aDivision of Reproductive Medicine, CEERH – Specialized Center for Human Reproduction, Sao Paulo, SP, Brazil; bDivision of Reproductive Medicine, Celula Mater, Sao Paulo, SP, Brazil.

OBJECTIVE: To determine parameters in early embryo development for the prediction of blastocyst formation and ploidy pattern evaluated through array comparative genomic hybridization (aCGH) in patients undergoing IVF/ICSI treatment.

DESIGN: One hundred and three patients (142 cycles) undergoing IVF/ICSI and aCGH preimplantation genetic screening were prospectively randomized into two groups. Only one MP, the duration of compaction (DOC), had an AUC for its ROC curve that exceeded 0.6. Using a threshold value of 12.3 hours, we split the embryos into two groups, one with an incidence of euploidy of 55% (28/51) and the other with an incidence of euploidy of 25% (13/52). Use of Campbell’s criteria provided no significant enrichment observed in either grouped in unscreened embryos. CONCLUSION: The failure of Campbell’s MPs to have significant ROCs suggest that they are not universally applicable for segregating euploid from aneuploid embryos. The observation that DOC provided a significant enrichment for euploid embryos in our facility, but did not provide any enrichment in the Campbell lab (despite being considered) provides evidence that this MP is not universal. We conclude that there is no universal MP is present, to distinguish the ploidy of embryos. It remains unclear why MPs can yield significant, yet small, enrichment of euploidy in some facilities but are incapable of providing enrichment in other facilities.

P-104 Tuesday, October 21, 2014
EARLY EMBRYO DEVELOPMENT AND PLOIDY IN WOMEN TREATED WITH CORIFOLLITROPIN ALFA FOR IVF/ICSI AND PREIMPLANTATION GENETIC SCREENING CYCLES. L. Kaupert, a P. D. S. Aranha, b C. E. Czeresnia, b M. G. Nisenbaum, b M. Maluf, a P. M. Perin, a CEERH – Specialized Center for Human Reproduction, Sao Paulo, SP, Brazil; bCelula Mater, Sao Paulo, SP, Brazil.

OBJECTIVE: To compare oocyte/embryo quality and ploidy in blastocysts from women undergoing IVF/ICSI using recombinant follicle-stimulating hormone (rFSH) or corifollitropin alfa (cFSH).

DESIGN: Forty-two patients (52 cycles) undergoing IVF/ICSI and array comparative genomic hybridization (aCGH) preimplantation genetic screening were prospectively randomized into two groups.

RESULTS: Patient demographics (age, body mass index, duration of stimulation and E2 and P levels on the day of oocyte retrieval) were similar in both groups. The mean number and MII oocytes at retrieval were 10.5±6.3 / 8.5±4.9 and 10.0±7.2 / 8.6±5.4 for groups A and B respectively, showing no difference between groups. The incidence of abnormal cytoplasmic organization and negative meiotic spindle visualization were similar in both groups. Polar body (p<0.001) and zona pellucida (p=0.012) abnormalities were significantly higher in group A when compared to group B. Normal fertilization rate was 72% in group A and 66% in group B, revealing no difference between groups. However, early cleavage rate was significantly lower (p=0.003) in group A (28.8%) than in group B (44.2%). No significant difference between groups was found for the cleavage and fragmentation rates on days two and three. However, the incidence of embryos with at least one multinucleated blastomere on day two was higher (p=0.005) in group B (36.7%) when compared to group A (22.5%). Time interval to reach the blastocyst stage, blastocyst rate and quality on days five and six were similar in both groups. Moreover, time interval to trophoderm biopsy showed no difference between groups. Blastocyst aneuploidy rate was 58.5% and 54.7% in groups A and B, respectively, showing that embryo ploidy was not influenced by the ovarian stimulation protocol (p=0.97).

CONCLUSION: The present study suggests that corifollitropin can be safely used for ovarian stimulation in women undergoing IVF/ICSI and aCGH preimplantation genetic screening.

P-105 Tuesday, October 21, 2014

OBJECTIVE: Goals of IVF include achieving a viable pregnancy and live birth of a healthy baby as quickly and efficiently as possible. This study analyzes cumulative live-birth outcomes with and without PGS and array comparative hybridization (aCGH) in patients where blastocysts were frozen and then thawed in subsequent frozen embryo transfers (FET) cycles.

DESIGN: Retrospective cohort study from 2011 through 2013.

MATERIALS AND METHODS: Patients underwent trophoderm biopsy (TE) biopsy on days 5 and/or 6 followed by blastocyst vitrification and aCGH. 273 patients underwent transfer of one or more euploid blastocysts. 45 patients subsequently frozen embryo transfer (FET-PGS) cycles. In the control group, 248 patients with supernumerary unscreened embryos cryopreserved on day 5 and/or 6 and underwent FET in cycles (FET-No PGS). Outcomes measured on
RESULTS: The outcomes were significantly better in the FET-PGS group than in the FET-No PGS (See Table). The number of FETs required to achieve the same OP-LB was 1.32 X longer for FET-No PGS than for FET-PGS.

<table>
<thead>
<tr>
<th></th>
<th>FET-PGS</th>
<th>FET (No-PGS)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Maternal age</td>
<td>37.0±4.51</td>
<td>34.6±4.84</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td># of Embryo transferred</td>
<td>1.13±0.36</td>
<td>1.72±0.69</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IR</td>
<td>60%</td>
<td>44%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CPR</td>
<td>64%</td>
<td>59%</td>
<td>0.16</td>
</tr>
<tr>
<td>SAB</td>
<td>12%</td>
<td>25%</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>mean OP-LBR</td>
<td>57%</td>
<td>43%</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>% Twin OP-LBR</td>
<td>1%</td>
<td>17%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>OP-LBR 1st FET attempt</td>
<td>57%</td>
<td>43%</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>OP-LBR 2nd FET attempt</td>
<td>87%</td>
<td>67%</td>
<td>NS</td>
</tr>
<tr>
<td>OP-LBR 3rd FET attempt</td>
<td>91%</td>
<td>85%</td>
<td>NS</td>
</tr>
</tbody>
</table>

CONCLUSION: Cumulative OP-LBRs indicate that OP-LB is achieved 1.32 X more rapidly when PGS was used to select euploid embryos for FET. Furthermore, the IR and mean OP-LB were significantly better when doing FET-PGS compared to FET. The goal of IVF to produce a healthy live birth can be better attained with fewer transfers (more efficiently) by selecting only euploid blastocysts for transfer. Our data suggests PGS testing of supernumerary embryos prior to cryopreservation of only reproductively competent embryos will assure better outcomes.

P-106 Tuesday, October 21, 2014


OBJECTIVE: To evaluate the correlation between blastocyst grade, time of blastocyst formation, maternal age and chromosomal aneuploidy.

DESIGN: Retrospective analysis.

MATERIALS AND METHODS: 377 patients who underwent IVF at our center from Jan 2013 to Dec 2013 were included. A total of 1403 blastocysts underwent trophectoderm biopsy and comparative genomic hybridization (CGH). The association of morphologic score, time of blastocyst formation, maternal age and the rate of aneuploidy were analyzed.

THE ASSOCIATION BETWEEN TIME OF BLASTOCYST FORMATION, EMBRYO GRADE, MATERNAL AGE AND RATE OF ANEUPLOIDY

Table 1 (A) Association between time of blastocyst formation and aneuploidy

<table>
<thead>
<tr>
<th>Age Groups</th>
<th>D5 Blastocyst</th>
<th>D6 Blastocyst</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>donor</td>
<td>29 (n=217)</td>
<td>38 (n=88)</td>
<td>0.17</td>
</tr>
<tr>
<td>&lt;35</td>
<td>24 (n=229)</td>
<td>46 (n=128)</td>
<td>0.00*</td>
</tr>
<tr>
<td>35-37</td>
<td>37 (n=139)</td>
<td>53 (n=100)</td>
<td>0.02*</td>
</tr>
<tr>
<td>38-40</td>
<td>55 (n=160)</td>
<td>68 (n=124)</td>
<td>0.04*</td>
</tr>
<tr>
<td>41-42</td>
<td>76 (n=80)</td>
<td>84 (n=45)</td>
<td>0.36</td>
</tr>
<tr>
<td>&gt;42</td>
<td>76 (n=42)</td>
<td>93 (n=51)</td>
<td>0.04*</td>
</tr>
</tbody>
</table>

Table 1 (B) Association between embryo grade and aneuploidy

<table>
<thead>
<tr>
<th>Aneuploidy Rate (%)</th>
<th>AA vs AB/BA</th>
<th>AA vs BB</th>
<th>AB/BA vs BB</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>D5 Blastocyst</td>
<td>0.00*</td>
<td>0.00*</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>D6 Blastocyst</td>
<td>0.12</td>
<td>0.00*</td>
<td>0.04*</td>
<td></td>
</tr>
</tbody>
</table>

Table 1 (C) Association between maternal age and aneuploidy

<table>
<thead>
<tr>
<th>Aneuploidy Rate (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;35</td>
<td>0.00*</td>
</tr>
<tr>
<td>35-37</td>
<td>0.00*</td>
</tr>
<tr>
<td>38-40</td>
<td>0.00*</td>
</tr>
<tr>
<td>41-42</td>
<td>0.00*</td>
</tr>
</tbody>
</table>

RESULTS: Embryos that progressed to blastocysts on day5 were less likely to be aneuploid than day6 blastocysts (Table 1A). A surprisingly high percentage of grade AA blastocysts were aneuploid. More than half of the grade AB and BB blastocysts were aneuploid (Table 1B). The aneuploidy rate increased significantly with maternal age (Table 1C).

CONCLUSION: Our data suggest a correlation between aneuploidy and the time of blastocyst formation, blastocyst quality and maternal age. A significant proportion of grade AA blastocysts were aneuploid suggesting morphologic analysis alone is insufficient to ensure transfer of normal embryos. Younger age groups had a high percentage of aneuploidy suggesting that PGS testing may be considered even in younger patients. Very few D6 blastocysts from patients >40 years were euploid. PGS-testing should be strongly considered in D6 embryos in this age group.

P-107 Tuesday, October 21, 2014

ALL 23 CHROMOSOME PAIRS DEMONSTRATE A RISK OF ANEUPLOIDY WHEN PERFORMING PREIMPLANTATION GENETIC SCREENING (PGS) ON DIFFERENTIATED BLASTOCYSTS. F. S. Chuong, K. J. Tobler, P. R. Brezina, A. T. Brenner, L. Du, X. Xu, B. Boyd, W. G. Kearns, Department of Gynecology and Obstetrics, Johns Hopkins Medical Institutions, Baltimore, MD. Center for Preimplantation Genetics, LabCorp, Rockville, MD. Vanderbilt University School of Medicine, Fertility Associates of Memphis, Memphis, TN.

OBJECTIVE: To determine the individual prevalence of aneuploidy in all 23 chromosomes in patients undergoing in vitro fertilization (IVF) and PGS.

DESIGN: Retrospective analysis.

MATERIALS AND METHODS: A retrospective review was conducted of blastocyst stage embryos that underwent comprehensive chromosome screening using single nucleotide polymorphism (SNP) microarrays from November 2009 to August 2011 performed in a single clinical laboratory. All evaluated embryos underwent a trophectoderm (TE) biopsy from differentiated blastocysts. TE cells underwent a whole genome amplification protocol followed by hybridization to HumanCytoSNP-12 DNA beadchips and analyzed by Illumina Genome Studio software. Differences in calculated proportions were evaluated using chi-square test. A p-value of <0.05 was considered statistically significant.

RESULTS: 1374 differentiated blastocysts resulting from 277 IVF/PGS resulted in a successful molecular karyotype. The overall aneuploidy prevalence was 35.7% (491/1374). Of the 491 aneuploid embryos, 1160 aneuploid chromosomes were identified including 300 (26%) monosomies to 860 (74%) trisomies. The prevalence of aneuploid chromosomes ranged from 2.6% in chromosomes 3 and 10 to 8.8% in chromosome 21 (p < 0.001) with a mean (standard deviation) value of 4.2% (+/- 0.02) for all chromosomes. See Table for the distribution of aneuploidies between all 23 chromosomes.
**DISTRIBUTION OF ANEUPLOIDIES**

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Total Monosity</th>
<th>Total Trisomy</th>
<th>Total Aneuploidy</th>
<th>Percent of 1160 Aneuploidy Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>31</td>
<td>36</td>
<td>3.1%</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>33</td>
<td>38</td>
<td>3.3%</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>27</td>
<td>30</td>
<td>2.6%</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>24</td>
<td>34</td>
<td>2.9%</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>33</td>
<td>39</td>
<td>3.4%</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>28</td>
<td>33</td>
<td>2.8%</td>
</tr>
<tr>
<td>7</td>
<td>13</td>
<td>30</td>
<td>43</td>
<td>3.7%</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>38</td>
<td>48</td>
<td>4.1%</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>46</td>
<td>47</td>
<td>4.1%</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>24</td>
<td>30</td>
<td>2.6%</td>
</tr>
<tr>
<td>11</td>
<td>14</td>
<td>29</td>
<td>43</td>
<td>3.7%</td>
</tr>
<tr>
<td>12</td>
<td>10</td>
<td>29</td>
<td>39</td>
<td>3.4%</td>
</tr>
<tr>
<td>13</td>
<td>12</td>
<td>67</td>
<td>79</td>
<td>6.8%</td>
</tr>
<tr>
<td>14</td>
<td>6</td>
<td>30</td>
<td>36</td>
<td>3.1%</td>
</tr>
<tr>
<td>15</td>
<td>21</td>
<td>39</td>
<td>60</td>
<td>5.2%</td>
</tr>
<tr>
<td>16</td>
<td>45</td>
<td>45</td>
<td>90</td>
<td>7.8%</td>
</tr>
<tr>
<td>17</td>
<td>8</td>
<td>49</td>
<td>57</td>
<td>4.9%</td>
</tr>
<tr>
<td>18</td>
<td>8</td>
<td>42</td>
<td>50</td>
<td>4.3%</td>
</tr>
<tr>
<td>19</td>
<td>14</td>
<td>18</td>
<td>32</td>
<td>2.8%</td>
</tr>
<tr>
<td>20</td>
<td>13</td>
<td>38</td>
<td>51</td>
<td>4.4%</td>
</tr>
<tr>
<td>21</td>
<td>27</td>
<td>75</td>
<td>102</td>
<td>8.8%</td>
</tr>
<tr>
<td>22</td>
<td>31</td>
<td>62</td>
<td>93</td>
<td>8.0%</td>
</tr>
<tr>
<td>X</td>
<td>27</td>
<td>23</td>
<td>50</td>
<td>4.3%</td>
</tr>
<tr>
<td>Y</td>
<td>n/a</td>
<td>0</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>Totals</td>
<td>300</td>
<td>860</td>
<td>1160</td>
<td>100%</td>
</tr>
</tbody>
</table>

CONCLUSION: Aneuploidy was observed in all chromosomes at the blastocyst stage. The broad distribution of aneuploidies illustrates the importance that a comprehensive chromosomal evaluation be used for PGS rather than only screening for the chromosomes that are most frequently identified at the time of miscarriage or obstetrical aneuploidy screening.

**P-109 Tuesday, October 21, 2014**

**CRYOPRESERVED OOCYTES UNDERGOING THAW, BLASTOCYST CULTURE (BC), AND TROPHECTODERM BIOPSY (TEB) DEMONSTRATE EUPLOIDY RATES EQUAL TO AGE-MATCHED FRESH OOCYTES.**


OBJECTIVE: To determine if a difference in euploidy exists between blastocysts derived from cryopreserved versus fresh oocytes.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: Oocyte thaw (OOT) cycles in which embryos underwent BC, TEB, and array comparative genomic hybridization (aCGH) for preimplantation genetic screening (PGS) were compared to in vitro fertilization (IVF) cycles in which TEB and aCGH were performed. IVF controls were age-matched 2:1 to match the mean 35.4y in the OOT group. Only each patient’s (pt) 1st cycle of OOT or IVF and 1st embryo transfer (ET) were included. The primary outcome was percent (％) euploidy. Additional parameters analyzed were day 2 estradiol (E2) and FSH, gonadotropin dosage, peak E2, no. of oocytes retrieved, metaphase-II (MII) oocytes, 2PN embryos, embryos biopsied, no. euploid embryos, implantation rate (IR), and live birth-ongoing pregnancy rate (LBR). Data were analyzed using Student’s t-test and Fisher’s exact test (p<0.05).

RESULTS: 25 OOT/PGS cycles were compared to 50 age-matched IVF/PGS controls. OOT/TEB and IVF/TEB cycles occurred between 4-2011 and 2-2014. Oocyte cryopreservation (OC) occurred between 2006 and 2014 using vitrification (75％) and slow freezing (25％) as was routine. There were no differences in cycle parameters between groups. 14 oocytes (avg) were thawed per pt in the OOT group. OOT/PGS and IVF/PGS groups were similar when comparing no. 2PN embryos, no. embryos biopsied, and no. euploid embryos, but the blastocyst formation rate (BFR) was lower in the OOT group (p<0.05). There was no difference in no. of euploid embryos or % euploidy between groups. 15 OOT pts and 26 IVF pts pursued ET, with no difference in IR or LBR.

CONCLUSION: Concerns have been posed regarding the risk of meiotic spindle disruption and aneuploidy in cryopreserved oocytes; however, our data suggest that cryopreserved oocytes, when thawed and biopsied for PGS, have equivalent euploidy rates compared to fresh oocytes. Our findings support the clinical application of PGS for cryopreserved oocytes when indicated and provide further evidence of oocyte competency following OC.

**Cycle parameters and outcomes**

<table>
<thead>
<tr>
<th></th>
<th>OOT/PGS (n=25)</th>
<th>IVF/PGS (n=50)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 2 FSH</td>
<td>5.8 ± 3</td>
<td>7.2 ± 2</td>
<td>0.15</td>
</tr>
<tr>
<td># Oocytes retrieved</td>
<td>17.2 ± 9</td>
<td>15.3 ± 10</td>
<td>0.44</td>
</tr>
<tr>
<td># MII</td>
<td>12.5 ± 8</td>
<td>12.9 ± 9</td>
<td>0.87</td>
</tr>
<tr>
<td># 2PN</td>
<td>8.6 ± 6</td>
<td>9.3 ± 7</td>
<td>0.69</td>
</tr>
<tr>
<td># embryos biopsied</td>
<td>4.5 ± 4</td>
<td>6.2 ± 5</td>
<td>0.12</td>
</tr>
<tr>
<td>BFR (%)</td>
<td>52.6</td>
<td>66.9</td>
<td>0.0005</td>
</tr>
<tr>
<td># Euploid embryos</td>
<td>2.2 ± 2</td>
<td>3.1 ± 3</td>
<td>0.2</td>
</tr>
<tr>
<td>Euploidy (%)</td>
<td>46 ± 25</td>
<td>45.6 ± 31</td>
<td>0.96</td>
</tr>
<tr>
<td>IR (%)</td>
<td>50</td>
<td>62.9</td>
<td>0.55</td>
</tr>
<tr>
<td>LBR (%)</td>
<td>53</td>
<td>65.4</td>
<td>0.52</td>
</tr>
</tbody>
</table>

Data presented in mean ± SD.
THAWED EUPLOID EMBRYO TRANSFER LEADS TO BETTER PREGNANCY OUTCOMES, IMPROVED MISCARRIAGE RATES AND NO ELECTIVELY TERMINATED ABNORMAL PREGNANCIES COMPARED TO FRESH IVF OR FROZEN EMBRYO TRANSFER. B. Hodes-Wertz, M. Smith, H.-L. Lee, A. Adler, G. A. Jamie. Ob-Gyn, NYU Langone Medical Center, New York, NY.

OBJECTIVE: To determine if trophoderm biopsy, array comparative genomic hybridization with frozen euploid embryo transfer (EuFET) impacts pregnancy outcomes and gestational age (GA) and/or birth weight (BW) center compared to routine fresh embryo fertilization (IVF) and unbiopsied frozen embryo transfer (UFET) at a large university-based fertility center.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: The implantation rate (IR), clinical pregnancy rate (CPR), spontaneous abortion per CP (SABR), ongoing-live birth rate (OP-LBR), and twin rate from EuFETs from 2011-2013 were compared to fresh embryo transfers (IVF) and unbiopsied frozen embryo transfers (UFET) during that time period. Any cycles that involved Day 3 embryo biopsy, donor oocyte, oocyte thaw or fresh EuFET transfers were excluded. In addition, all singleton livebirths (LB) of patients that underwent EuFET with available delivery data (BW and GA) were compared to all singleton livebirths from routine IVF and UFET. Independent t-test and chi-square were used for analysis.

RESULTS: In 2011-2013 EuFET, there were 339 transfers (n) with an average age (yrs) of 36.7±4.3. There was no difference in IR (59% vs 58%), CPR (64% vs 69%), or OP-LBR (31% vs 34%) compared to UFET. Singleton LBs (102 vs 361) were comparable with EuFET and UFET. There were no triplet pregnancies in EuFET but 6 in the IVF and 4 in UFET. Independent t-test and chi-square were used for analysis.

CONCLUSION: Overall, EuFET was associated with a higher IR, CPR and OP-LBR compared to UFET as well as higher BW at birth with EuFET compared to UFET.

P-112 Tuesday, October 21, 2014

HIGH CELLULARITY OF TROPHODERM BIOSPY ADVERSELY AFFECTS PREGNANCY OUTCOMES. S. A. Neal, J. M. Franasiak, E. J. Forman, M. D. Werner, D. Taylor, N. R. Treff, R. T. Scott, Jr., N. R., Medical University of South Carolina, Charleston, SC; RWJ SOM, Rutgers University, New Brunswick, NJ; RMA of New Jersey, Basking Ridge, NJ.

OBJECTIVE: Comprehensive chromosome screening (CCS) is becoming more frequently utilized to aid in embryo selection and optimize live birth rates. Level I data supports the safety and accuracy of trophoderm (TE) biopsy, but not all TE biopsies are equal. Whether the size of the biopsy impacts reproductive potential is unknown. Here, the association between TE biopsy cellularity and pregnancy outcomes is assessed.

DESIGN: Retrospective cohort of patients undergoing single embryo transfer (SET) after TE biopsy and CCS.

MATERIALS AND METHODS: 1,147 embryos undergoing TE biopsy on day 5 or 6 for CCS were included. A SET was performed on day 6 of the fresh cycle or in a subsequent frozen cycle. A standardized curve based upon quantitative real-time PCR amplification of single cell data was used to estimate the cellularity of the TE biopsies, which was then analyzed in quartiles from least (1) to most (4) cellular. Human chorionic gonadotropin (hCG) levels were measured at 4 weeks gestation and repeated 48 hours later. An ongoing pregnancy was defined as fetal cardiac activity at 9 weeks gestation.

RESULTS: The initial hCG and relative rise in hCG were similar for all quartiles. The quartile of highest cellularity had a significantly lower ongoing pregnancy rate when compared to the other three quartiles (Table). There was no difference between the quartiles regarding age, body mass index, ovarian response, or endometrial thickness.

CONCLUSION: TE biopsies with the highest cellularity are associated with lower ongoing pregnancy rates after euploid SET. Possible explanations

<table>
<thead>
<tr>
<th>Quartile</th>
<th>Ongoing Pregnancy Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>63.6% (a)</td>
</tr>
<tr>
<td>2</td>
<td>61.7% (a)</td>
</tr>
<tr>
<td>3</td>
<td>62.1% (a)</td>
</tr>
<tr>
<td>4</td>
<td>53.1% (b)</td>
</tr>
</tbody>
</table>

a vs. b, p < 0.05

P-113 Tuesday, October 21, 2014

IN VITRO FERTILIZATION (IVF) WITH 23-CHROMOSOME PAIR PREIMPLANTATION GENETIC SCREENING (PGS) FROM TROPHODERM BIOSPY IS MORE COST EFFECTIVE TO ACHIEVE A LIVE BIRTH COMPARED TO IVF ALONE. N. Resekova, K. J. Tobler, E. F. Werner, W. G. Kearns. Johns Hopkins Medical Institutions, Baltimore, MD; Warren Alpert Medical School of Brown University, Providence, RI; Center for Preimplantation Genetics, LabCorp, Rockville, MD.

OBJECTIVE: To determine if performing IVF/PGS using trophoderm biopsy from a differentiated blastocyst is cost effective in achieving a live birth as compared to IVF alone.

DESIGN: Retrospective.

MATERIALS AND METHODS: A decision model was created using TreeAge Pro 2013 (TreeAge Software, Williamstown, MA) to compare the cost of IVF/PGS performed by comparative genomic hybridization microarray versus IVF alone to achieve a live birth. The model was built from the patient perspective. A three pregnancy attempt maximum was used. Probabilities were derived from both published and internal data, including pregnancy rates from IVF with PGS involving 560 cycles. Clinical data for all maternal ages from the Society for Assisted Reproductive Technology (SART 2012) was used to calculate national IVF success rates. Cost data was derived from published literature, and converted to 2013 US dollars. The main outcome was the cost to achieve a live birth using IVF with microarray PGS from trophoderm stage biopsy compared to IVF alone. Other outcomes included the probability of success with IVF and PGS compared to IVF alone and the frequency of miscarriages using both methods. One-way, two-way, and Monte Carlo sensitivity analyses were performed to assess model robustness.

RESULTS: The model demonstrates that IVF with microarray PGS is cost effective for couples in their attempt to achieve a healthy baby versus IVF alone. The average cost of undergoing IVF with PGS is $25,242 with a 95% chance of achieving a live birth over three pregnancy attempts, compared to an average cost of $22,776 and a success rate of 64% for IVF alone. Evaluating embryos with biopsy at trophoderm stage has resulted in increasing pregnancy rates and reduced miscarriage rates, improving the effectiveness of PGS with IVF over previously presented data.

CONCLUSION: This model suggests that the addition of 23 chromosome microarray PGS to IVF is cost effective for couples across all maternal age groups compared to IVF alone over a wide range of probabilities and costs.
for this phenomenon include diminished accuracy of the euploid diagnosis (increased cellularity may cause assay saturation or dampen the detection of mosaicism) versus a mechanical impact of the biopsy. Further research is needed to determine whether CCS is less accurate for larger biopsies or if the biopsy itself impairs reproductive potential. Given the established role of TE biopsy and CCS in improving pregnancy outcomes, programs should implement quality control measures to monitor pregnancy outcomes after TE biopsy and consider limiting the size of TE biopsies.

P-113 Tuesday, October 21, 2014

SELECTING COMPETENT BLASTOCYTS FOR TRANSFER BY USING TIME-LAPSE MONITORING AND ARRAY CGH TESTING FOR PATIENTS UNDERGOING PREIMPLANTATION GENETIC SCREENING (PGS): A PILOT STUDY WITH SIBLING OCYCTES. Z. Yang, a,b,c J. Zhang, a,b X. Liu, c R. Salem, a, b J. Liu, a c *ART, Pacific Reproductive Center, Torrance, CA; b REI, New Hope Fertility Center, New York, NY; c IVF, Jia En de Yun Hospital, Beijing, China.

OBJECTIVE: Recent advances in time-lapse culture and monitoring have provided new morphokinetic markers for embryonic competence (1-3). However, there is still limited information about the relationship between morphokinetic parameters, chromosomal composition and implantation potential. Accordingly, this study aimed at investigating the effects of selecting competent blastocysts for transfer by using time-lapse monitoring and array CGH testing on pregnancy and implantation outcomes for PGS patients.

DESIGN: 1163 MII oocytes were retrieved from 138 PGS patients at a mean age of 36.6 ± 2.4 years. These sibling oocytes were randomized into two groups after ICSI: 1) Group A, oocytes (n = 582) were cultured in the time-lapse system and 2) Group B, oocytes (n = 581) were cultured in the conventional incubator.

MATERIALS AND METHODS: For both groups, fertilization was assessed at 18 hours post ICSI and embryos were cultured to blastocyst stage in a continuous culture medium. Following trophoderm biopsy on day 5, whole genomic amplification and aCGH testing were performed. One to two euploid blastocysts within the most predictive morphokinetic parameters (Group A) or with the best morphological grade available (Group B) were selected for transfer on day 6. Clinical pregnancy, ongoing pregnancy and implantation rates were compared between the two groups.

RESULTS: There were no significant differences in blastocyst formation rates between the time-lapse system (Group A) and the conventional incubator (Group B) (48.9% vs. 47.8%, respectively, p > 0.05). However, there were significant differences in clinical pregnancy rates between Group A and Group B (71.1% vs. 45.9%, respectively, p = 0.037). The observed implantation rate per embryo transfer significantly increased in Group A compared to Group B (66.2% vs. 42.4%, respectively, p = 0.011). Moreover, a significant increase in ongoing pregnancy rates was also observed in Group A compared to Group B (68.9% vs. 40.5%, respectively, p = 0.019). There was an insignificant trend in which miscarriage rates decreased in Group A compared to Group B (3.1% vs. 11.8%, respectively, p > 0.05).

CONCLUSION: Our data demonstrate that selecting competent blastocysts for transfer by using these two advanced technologies results in improved implantation and ongoing pregnancy rates for PGS patients. Further randomized clinical trials with a larger sample size are planned to verify these initial findings.

Supported by: This study is Supported by internal funds.

P-114 Tuesday, October 21, 2014


OBJECTIVE: Selecting embryos with the highest implantation potential is the most important challenges in the field of Assisted Reproduction. Previously, morphologic criteria were the most commonly used standards for embryo selection. The introduction of preimplantation genetic screen (PGS-24) provides a novel method to determine embryo competence. Our goal was to evaluate the clinical outcomes in patients with and without PGS-24 testing.

DESIGN: Retrospective study.

MATERIALS AND METHODS: 532 patients who underwent FET using vitrified-thawed blastocysts at our fertility center from Jan 2013 to Dec 2013 were included. 253 received PGS-24 testing and 279 patient did not. Patients were divided into 5 age groups (<35, 35-37, 38-40, >40 and donor). Clinical pregnancy rate and implantation rate were compared.

RESULTS: Clinical pregnancy rate and implantation rate for each age group were generally higher in the PGS compared to the non-PGS group (Table 1). The mean number of embryos transferred was significantly lower in PGS group vs non-PGS.

CONCLUSION: These data demonstrate significant improvements in clinical outcomes in patients with PGS-24 testing, suggesting that PGS-24 is an effective method to determine blastocyst competence. Furthermore, PGS-24 screening facilitates elective single embryo transfer while reducing the probability of ovarian hyperstimulation syndrome and avoiding multiple gestation.

P-115 Tuesday, October 21, 2014

OBJECTIVE: To re-investigate the morphokinetic characteristics of aneuploidy in embryos from randomly selected IVF-PGS (preimplantation genetic screening) patients whose ages are normally distributed.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: Data used were from our routine IVF-PGS patients (n=18) whose ages are normally distributed [25.8-43.6]. Autologous fresh oocytes (n=185) from these patients were incubated in Time-Lapse microscope (EmbryoScope; Unisense Fertiltech, Denmark) following insemination. After Day 3 biopsy (n=152), ploidy was analyzed using array-comparative genome hybridization (aCGH). According to PGS results, these embryos classified into two groups, euploid (n=46) and aneuploid (n=106). The morphokinetic parameters tested in this study, including three new parameters* (oocyte diameter, distance of pronuclei (PNs) and ratio of PN diameters) are listed in Table 1. Data were analyzed using one way ANOVA and χ²-test, and Pearson correlation test.

The Mean Differences of Morphokinetic Parameters within Groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Euploidy</th>
<th>Aneuploidy</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>34.1 ±4.2</td>
<td>37.5 ±4.6</td>
<td>0.000**</td>
</tr>
<tr>
<td>Oocyte diameter*</td>
<td>159.3 ±11.0</td>
<td>159.6 ±7.4</td>
<td>0.835</td>
</tr>
<tr>
<td>Distance of PNs*</td>
<td>44.2 ±5.0</td>
<td>49.7 ±49.5</td>
<td>0.462</td>
</tr>
<tr>
<td>Ratio of PN diameters*</td>
<td>1.1 ±0.2</td>
<td>1.0 ±0.2</td>
<td>0.386</td>
</tr>
<tr>
<td>Granular cytoplasm</td>
<td>10.9%</td>
<td>11.3%</td>
<td>0.935</td>
</tr>
<tr>
<td>tPB2 (time of polar body extrusion)</td>
<td>4.7 ±3.3</td>
<td>4.8 ±3.4</td>
<td>0.810</td>
</tr>
<tr>
<td>tPNa (time of PN appearance)</td>
<td>10.5 ±3.5</td>
<td>10.8 ±3.9</td>
<td>0.627</td>
</tr>
<tr>
<td>tPN (time of PN faded)</td>
<td>24.2 ±4.6</td>
<td>24.6 ±6.5</td>
<td>0.686</td>
</tr>
<tr>
<td>cc2 (time of 2nd cell cycle; t2-t3)</td>
<td>7.5 ±5.8</td>
<td>7.8 ±6.5</td>
<td>0.673</td>
</tr>
<tr>
<td>s2 (time of synchrony of 2nd cell cycle; t4+t3)</td>
<td>4.5 ±5.2</td>
<td>5.4 ±5.5</td>
<td>0.308</td>
</tr>
<tr>
<td>cc3 (time of 3rd cell cycle; t5+t3)</td>
<td>15.0 ±7.1</td>
<td>14.9 ±6.7</td>
<td>0.954</td>
</tr>
<tr>
<td>tM (time from insemination to formation of a morula)</td>
<td>89.6 ±12.3</td>
<td>89.6 ±12.4</td>
<td>0.984</td>
</tr>
<tr>
<td>tSB (time from insemination to start of blastulation)</td>
<td>100.6 ±11.6</td>
<td>99.9 ±8.9</td>
<td>0.750</td>
</tr>
<tr>
<td>tB (time from insemination to formation of a full blastocyst)</td>
<td>106.7 ±8.9</td>
<td>106.9 ±8.0</td>
<td>0.925</td>
</tr>
<tr>
<td>PN score grade I+II</td>
<td>80%</td>
<td>88.6%</td>
<td>0.332</td>
</tr>
<tr>
<td>Irregular Division</td>
<td>10.9%</td>
<td>21.7%</td>
<td>0.114</td>
</tr>
<tr>
<td>Recleavage</td>
<td>8.7%</td>
<td>6.6%</td>
<td>0.647</td>
</tr>
<tr>
<td>Multinucleation at 2 cell stage</td>
<td>32.6%</td>
<td>36.8%</td>
<td>0.621</td>
</tr>
<tr>
<td>Blastomere size (even)</td>
<td>37.0%</td>
<td>33.0%</td>
<td>0.268</td>
</tr>
</tbody>
</table>

determined definitions. The proportion of ODP and SDP embryos was determined for each ploidy group. Statistical comparisons were performed by means of a two-sided chi-squared test by an independent statistician.

MATERIALS AND METHODS: Seventy-eight cycles of PGS/PGD by blastocyst biopsy and aCGH, attempted between January 2012 and April 2014 at one clinic were included; 410 blastocysts were diagnosed. Translocation cases were excluded. ODP was defined as optimal morphology on all assessment days, including 2-5 cells on day 2; 6-12 cells on day 3; <10% fragmentation (days 2 and 3); symmetric division or stage appropriate unevenness (days 2 and 3); fully formed blastocyst with cohesive TE and distinct ICM on day 5 (and 6).

RESULTS: ODP varied significantly among the different ploidy categories, ranging from 11.5% to 50% (p<0.0001). Results are collapsed into two groups and summarized in Table 1. CONCLUSION: The relationship between aneuploidy and embryo morphology is tenuous, but the relationship between development profile and aneuploidy type has not been explored. Our data suggest that euploid embryos or those affected by trisomies that are compatible with life show optimal development significantly more frequently than sub-optimal development. By contrast, embryos carrying monosomies, complex and single aneuploidies incompatible with life show sub-optimal development during culture; this is consistent with the observation that the latter are rarely found in spontaneous abortions. The relatively high frequency of ODP among aneuploid embryos suggests that the only effective means by which such embryos may be excluded from transfer is genetic screening.

Supported by: Institutional.

P-116 Tuesday, October 21, 2014

DEVELOPMENTAL PROFILES OF EMBRYOS WITH ANEUPLIDIES COMPATIBLE AND INCOMPATIBLE WITH LIFE ARE DIFFERENT DURING 6 DAYS IN CULTURE. M. Alikani, Y. Pan, M. Bolkas, H. Yang, T. Singer, A. Herslag. Center for Human Reproduction, North Shore University Hospital, Manhasset, NY.

OBJECTIVE: To compare developmental profiles (DP) of embryos affected by aneuploidies compatible or incompatible with life.

DESIGN: Blastocysts with ploidy diagnosis were categorized in 5 groups based on compatibility of the ploidy type with life (potential for term development, early or late loss). The DP of the biopsied embryos was assessed as Optimal (ODP) or Sub-optimal (SDP) according to pre-
SERUM ANTI-MULLERIAN (AMH) LEVELS DO NOT PREDICT EMBRYO PLOIDY AFTER COMPREHENSIVE CHROMOSOMAL SCREENING (CCS) OF TROPHOBластODERM CELLS DURING PRE-IMPLANTATION GENETIC SCREENING (PGS).

MATERIALS AND METHODS: Patients who underwent an IVF cycle with PGS for aneuploidy were included in the study. In cases where ≥2 AMH measurements were recorded, the lowest level was selected. Serum AMH was measured either by Beckman-Coulter™ and/or Diagnostic Systems Laboratories’ assay, and an AMH level of 1 ng/mL was considered as normal. Embryo’s trophodermbs were biopsied on Day 5/6 and 23 chromosome PGS analysis was performed by quantitative PCR. Only embryos with an initial normal/abnormal result were included. We utilized generalized estimating equations (GEE) to understand the impact of AMH levels (predictor variables) in the model. Blood samples from the participants were collected before the initiation of the PGS procedure. The study was approved by the institutional review board (IRB). The lab protocol for the collection and storage of the samples was followed. To determine the maximum sample capacity allowed per run for accurate reporting, emulsion PCR was performed on the pooled amplified barcoded libraries (six runs). Sequencing was conducted on Ion Personal Genome Machine with 316 or 318 chips. Primary data alignment was performed using Torrent Suite Software v4.0.2. Post-alignment analysis was performed on Ion Reporter 4.0.

RESULTS: The average total of bases and reads outputted per run from the 316 chips were ~520M and ~2.4M, respectively, and ~730M and ~4.6M, respectively, from the 318 chip. Accurate copy number calls for sub-chromosomal deletion of 21.3Mb, del(4).arr p16.3p15.2, were made on all six specimens with a range of 37M bases to 205M bases and 225K reads to 955K reads, respectively, for the 316 chip, and a range of 57M bases to 80M bases and 347K reads to 450K reads, respectively, for the 318 chip. This method reported 100% accuracy for segmental deletions (6/6) as well as for whole chromosome aneuploidy detection (18/18).

CONCLUSION: This study further validated NGS-based methodology’s efficacy in detecting segmental gains/loss in addition to various whole chromosome aneuploidies with accurate diagnosis. With the potential advantages of reduced cost and enhanced precision, NGS-based comprehensive aneuploidy screening represents an improvement on current array-based PGS. Further assessment is required to test the relationship between the smaller lengths of segmental gain/loss and its sensitivity for detection, as well as to determine the maximum sample capacity allowed per run for accurate CNV detection.

Supported by: Institutional.

FERTILITY & STERILITY®
ANEUPLOIDY RATES IN DAY 5/6 EMBRYO SAMPLES TESTED WITH 24-CHROMOSOME SINGLE NUCLEOTIDE POLYMORPHISM (SNP) MICROARRAYS AND A BIOINFORMATICS TECHNIQUE. K. Merriion, M. Kiehl, B. Pettersen. Natera, Inc., San Carlos, CA.

OBJECTIVE: To report aneuploidy rates in Day 5/6 embryo samples, categorized by maternal age.

MATERIALS AND METHODS: Review of 24-chromosome preimplantation genetic screening (PGS) results on 6803 day 5/6 trophectoderm biopsies from 1242 cases. Patients were referred for PGS for reasons including: prior aneuploid pregnancy, prior failed in vitro fertilization (IVF) cycle, recurrent pregnancy loss, or advanced maternal age. Following in vitro fertilization, embryos underwent trophectoderm biopsy on day 5 or 6, according to each clinic’s standard procedures. Biopsy samples, along with parental blood or buccal samples, were shipped from IVF clinics to a single reference laboratory for analysis. Genotyping was performed using Illumina Cytos12 SNP microarray and an informatics technique which uses parental genetic data to improve the resolution and accuracy of the embryo sample chromosome measurements. Embryo samples that returned results were classified as ‘euploid’ if no chromosome abnormalities were detected, or ‘aneuploid’ if trisomy, monosomy, haploidy, triploidy, large deletions/duplications, and/or uniparental disomy was detected.

RESULTS: A total of 1223 patients agreed to undergo PGS at their IVF cycle start. Of those 259 (21%) produced 0 or 1 blastocyst, and 964 (79%) produced 2 or more (average 6.2 blastocysts). Of the first group 47 with one blastocyst decided to go on with the biopsy and 99 with two or more blastocysts did not proceed with biopsy.

Below shows the chance, by maternal age, to have 0, 1 or more than 2 blastocysts:

<table>
<thead>
<tr>
<th>Maternal Age</th>
<th># of cases</th>
<th># of embryos</th>
<th>Euploid Rate</th>
<th>Aneuploid Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;30 years</td>
<td>185</td>
<td>1221</td>
<td>69.1% +/- 1.32%</td>
<td>30.9% +/- 1.32%</td>
</tr>
<tr>
<td>30-34 years</td>
<td>259</td>
<td>1486</td>
<td>64.9% +/- 1.24%</td>
<td>35.1% +/- 1.24%</td>
</tr>
<tr>
<td>35-39 years</td>
<td>461</td>
<td>2285</td>
<td>50.5% +/- 1.05%</td>
<td>49.5% +/- 1.05%</td>
</tr>
<tr>
<td>≥40 years</td>
<td>337</td>
<td>1430</td>
<td>25.5% +/- 1.15%</td>
<td>74.5% +/- 1.15%</td>
</tr>
<tr>
<td>OVERALL</td>
<td>1242</td>
<td>6422</td>
<td>51.8% +/- 0.62%</td>
<td>48.2% +/- 0.62%</td>
</tr>
</tbody>
</table>

Day 5/6 embryo sample aneuploidy rates.

CONCLUSION: Aneuploidy rates in day 5/6 trophectoderm samples increased significantly with maternal age. Aneuploidy rates in cycles with egg donors <30 years were 29.5%, supporting that PGS should still be considered for IVF patients using egg donors to increase the chances of transferring an embryo with normal chromosome make-up. These maternal age-stratified aneuploidy risk figures can be used to counsel patients on the likelihood of having an embryo with a euploid result.

P-122 Tuesday, October 21, 2014

FROM CARRIER SCREENING TO SINGLE GENE PGD: AN ANALYSIS OF 762 COUPLES SCREENED VIA AN EXPANDED CARRIER SCREENING PLATFORM. N. Kumar, a A. Bissignano, a S. Asgari, b D. Hoffman, a M. Barrionuevo, e R. Prates, b bReprogenetics, Livingston, NJ; aNYU Langone Fertility Center, New York, NY; eSt. Barnabas Institute for Reproductive Medicine and Science, Livingston, NJ; aIVF Florida, Margate, FL.

OBJECTIVE: Carrier screening for recessive genetic diseases is offered to patients at fertility centers. Couples carrying the same genetic disease have a 25% chance of having an affected child. Such couples have several reproductive options available, including PGD. Our goal was to determine how many such couples pursue PGD.

DESIGN: Retrospective.

MATERIALS AND METHODS: The Illumina Infinium HD Custom Genotyping platform was used to identify 1578 mutations associated with 190 high impact recessive genetic diseases. This analysis includes data obtained from 762 couples referred by fertility centers. Informed consent to utilize data in a de-identified manner was obtained. Carrier couples were identified.

TABLE 1.

<table>
<thead>
<tr>
<th>Disease</th>
<th># Carrier Samples</th>
<th># PGD Samples</th>
<th>PGD Status/Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sickle Cell Disease</td>
<td>3</td>
<td>0</td>
<td>Preparation Complete</td>
</tr>
<tr>
<td>Cystic Fibrosis</td>
<td>4</td>
<td>2</td>
<td>Preparation Complete</td>
</tr>
<tr>
<td>Nonsyndromic Hearing Loss &amp; Deafness: DFNB1 Related</td>
<td>3</td>
<td>1</td>
<td>Initiated</td>
</tr>
<tr>
<td>Carnitine</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Palmitoyltransferase II Deficiency</td>
<td>10</td>
<td>1</td>
<td>Unaffected Embryos Transferred</td>
</tr>
<tr>
<td>Familial Dysautonomia</td>
<td>1</td>
<td>1</td>
<td>Unaffected/Non-Carrier Polar Bodies Selected</td>
</tr>
<tr>
<td>Niemann-Pick Disease Type A</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Hurler Syndrome</td>
<td>1</td>
<td>1</td>
<td>Unaffected/Non-Carrier Polar Bodies Selected</td>
</tr>
<tr>
<td>Spinal Muscular Atrophy: SMN1 Linked</td>
<td>2</td>
<td>1</td>
<td>Initiated</td>
</tr>
<tr>
<td>Familial Mediterranean Fever</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Tay-Sachs Disease</td>
<td>1</td>
<td>1</td>
<td>No Unaffected/ Chromosomally Normal Embryos Available</td>
</tr>
</tbody>
</table>

Total: 18 7
Couples that then pursued PGD through our partner laboratory were identified, and their PGD testing outcomes were reviewed.

RESULTS: Of the 762 couples, 18 (2.4%) were identified as carriers of the same genetic disease as shown. Of these, 7 couples pursued single gene PGD through our partner laboratory.

CONCLUSION: Results show that 11/18 carrier couples referred by fertility centers chose not to pursue PGD. There are several explanations for this. PGD may not be consistent with a couple’s personal beliefs or may not have the financial resources to pursue PGD. Additionally, it is possible that some couples pursued PGD through another laboratory, became pregnant without fertility treatment, or chose not to pursue fertility treatment in general. In this study, at least one affected embryo was detected for each couple that pursued and completed PGD testing, demonstrating the importance of performing PGD testing in these cases.

P-123 Tuesday, October 21, 2014

ARE ANEUPLOIDY RATES HIGHER IN EMBRYOS BIOPSIED ON DAY 6 COMPARED TO DAY 5 OF DEVELOPMENT? M. Alikani, E. Schenkman, Y. Pan, M. A. Cohen, A. Hershlag. Center for Human Reproduction, North Shore University Hospital, Manhasset, NY.

OBJECTIVE: To determine if aneuploidy rates are different in embryos biopsied on day 5 or 6 of development.

DESIGN: Retrospective data analysis. Aneuploidy rates were tabulated for blastocysts biopsied on day 5 and day 6 of development. The rates were compared for the total population of embryos, then stratified for two maternal age groups of ≤37 and >37 years. All statistical comparisons were performed by means of a two-sided chi-squared test.

MATERIALS AND METHODS: Seventy-eight cycles of PGS/PGD by blastocyst biopsy and aCGH, attempted between January 2012-April 2014 at one clinic were included; 414 blastocysts were biopsied. Indicators for PGS included AMA, RPL, RIF and family balancing. All embryos were subjected to zona opening on day 3 and cultured to day 5 or 6 for TE biopsy. A strict policy was followed with respect to the timing and selection of embryos for biopsy; only two embryologists made the final decision. Biopsy was delayed to day 6 for early day 5 blastocysts with limited number of TE cells. Blastocysts with poor quality ICM and TE were excluded from biopsy on both days. Biopsied cells were sent to one reference laboratory. Aneuploidy rates were stratified by day of biopsy and maternal age.

RESULTS: Overall and age-stratified aneuploidy rates on day 5 and 6 are summarized in Table 1. Equal proportions of blastocysts were biopsied on day 5 (213 or 51.4%) and day 6 (201 or 48.6%)(p=0.0401) and nearly 60% of the patients had a biopsy performed on both day 5 and day 6. The same proportion of females occurred among day 5 and 6 embryos.

Aneuploidy Rate in Day 5 v Day 6 Blastocysts

<table>
<thead>
<tr>
<th>Day of TE Biopsy</th>
<th>No. Euploid (%)</th>
<th>No. Aneuploid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Population</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 5 (N=213)</td>
<td>108 (50.7%)</td>
<td>105 (49.3%)</td>
</tr>
<tr>
<td>Day 6 (N=201)</td>
<td>91 (45.3%)</td>
<td>110 (54.7%)</td>
</tr>
<tr>
<td>p Value</td>
<td>0.269</td>
<td></td>
</tr>
<tr>
<td>≤ 37 Years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 5 (N=90)</td>
<td>52 (57.8%)</td>
<td>38 (42.2%)</td>
</tr>
<tr>
<td>Day 6 (N=93)</td>
<td>60 (64.5%)</td>
<td>33 (35.5%)</td>
</tr>
<tr>
<td>p Value</td>
<td>0.1092</td>
<td></td>
</tr>
<tr>
<td>&gt; 37 Years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 5 (N=123)</td>
<td>56 (45.5%)</td>
<td>67 (54.5%)</td>
</tr>
<tr>
<td>Day 6 (N=108)</td>
<td>31 (28.7%)</td>
<td>77 (71.3%)</td>
</tr>
<tr>
<td>p Value</td>
<td>0.0085</td>
<td></td>
</tr>
</tbody>
</table>

CONCLUSION: It has been suggested that the rate of aneuploidy is higher in day 6 blastocysts. Our data suggest that the correlation may be more nuanced than believed previously. Overall, aneuploidy rates were the same for day 5 and day 6 embryos. However, in patients older than 37 years, aneuploidy rate in day 6 embryos was indeed significantly higher than day 5 embryos, suggesting a relationship between delayed development and aneuploidy in older patients but not in younger patients. When counseling patients on the prognostic value of the day of TE biopsy, patient age should be taken into consideration.

Supported by: Institutional.

P-124 Tuesday, October 21, 2014

ROUTINELY DISTINGUISHING NORMAL AND BALANCED TRANSLLOCATION CARRIER EMBRYOS USING SNP ARRAYS. N. R. Treff, a,b E. J. Forman, a,b O. Bendarsky, a S. A. Neal, c R. T. Scott, Jr., a,b *RMA of New Jersey, Basking Ridge, NJ; bRWJ Medical School-Rutgers University, New Brunswick, NJ; cMedical University of South Carolina, Charleston, SC.

OBJECTIVE: Many translocation carrier couples convey a strong interest in preventing transmission to their offspring, sparing them the reproductive challenges they faced. However, contemporary methods of comprehensive chromosome screening (CCS) cannot reliably distinguish between normal embryos and those that carry a balanced translocation. This study was conducted to develop a method that can concurrently identify unbalanced and aneuploid embryos, and, for the first time, distinguish translocation carrier and truly normal embryos all from the same embryonic trophectoderm biopsy SNP array data.

DESIGN: Blinded evaluation of balanced/normal embryos from a translocation carrier patient.

MATERIALS AND METHODS: 262K SNP arrays were used on a family trio (parents and child) to phase the parental reciprocal translocation carrier. Informative SNPs 5 megabases (Mb) on each side of the chromosome breakpoints (heterozygous in the carrier and homozygous in the normal parent) were used to predict whether otherwise euploid embryos inherited the translocated chromosome and thus would be obligate balanced translocation carriers. Outcomes were evaluated against known genetic status from separate PCR analysis of a specific microdeletion (known to be associated with the translocation) in order to confirm results.

RESULTS: A total of 67 embryos were evaluated from a couple carrying 46,XX,t(2;20)(q21;p12.2); 12 were euploid and balanced or normal for the translocation based on conventional copy number analysis, 128 (chr2) and 191 (chr20) informative SNPs were identified within 5 Mb of the breakpoints. A child born without the translocation was used to phase the maternal chromosomes for the informative SNPs. When these SNPs were evaluated in blinded embryos, 2 were identified as carriers, and 10 as normal, which was 100% consistent with the known status of each embryo.

CONCLUSION: We have developed a proof of principle that phased informative SNPs can be evaluated to routinely distinguish balanced carrier and normal embryos in parallel with conventional CCS using SNP array technology. An ongoing clinical trial will continue to accumulate evidence of the predictive value for clinical outcomes and general applicability in additional cases. This may provide couples with a balanced translocation an additional selection criterion when considering which embryo to transfer.

P-125 Tuesday, October 21, 2014

ADAPTING NEXT-GENERATION DNA SEQUENCING TO DETECT ANEUPLOIDY. G. J. Porreca, J. Gole, A. Gore, M. A. Umbarger. Good Start Genetics, Inc., Cambridge, MA.

OBJECTIVE: We sought to evaluate the ability of a new next-generation DNA sequencing (NGS) based pre-implantation genetic screening (PGS) approach to determine chromosome copy number from limiting amounts of DNA.

DESIGN: PGS, or comprehensive chromosome screening (CCS), is used to assess the chromosome copy number of embryos. Although increasing evidence indicates that euploid embryo transfer increases implantation rates and reduces miscarriage rates, PGS adoption has been limited in part because traditional technologies make the procedure generally unaffordable. However, increased use of trophectoderm biopsy followed by vitrification and subsequent frozen embryo transfer, coupled with streamlined workflows employing NGS, are poised to enable broader PGS use. Here we evaluate the performance of a new NGS-based approach to determine chromosome copy number. We measure the performance of this method on both purified genomic DNA and isolated cells.

MATERIALS AND METHODS: We developed and implemented an automated PCR-based method that amplifies regions from each chromosome coupled with streamlined workflows employing NGS-based approach to determine chromosome copy number. We measured the performance of this method on both purified genomic DNA and isolated cells.

ADAPTING NEXT-GENERATION DNA SEQUENCING TO DETECT ANEUPLOIDY. G. J. Porreca, J. Gole, A. Gore, M. A. Umbarger. Good Start Genetics, Inc., Cambridge, MA.
purified from cell lines (~2 diploid cells) or lysate derived from 2-cell isolated cultured lymphocytes served as template for the PCR reactions. The PCR products were sequenced to generate count data for each chromosome, and this data was subsequently used to infer chromosome copy number.

RESULTS: A total of 37 true positive aneuploid chromosome calls were made across the DNA from 21 aneuploid cell lines. The method generated 789 correct diploid chromosome calls, 2 incorrect aneuploid (false positive) chromosome calls, and zero incorrect diploid (false negative) chromosome calls. Both incorrect aneuploid calls were in samples containing other aneuploid chromosomes, thus yielding perfect sample-level specificity and perfect chromosome-level sensitivity. Aneuploidies detected included: trisomy 2, 8, 9, 13, 18, 20, 21, 16+21, 2+21, monosomy X, tetrasomy X, XXY, and disomy Y. The technique also detected trisomy 21 and XXY when lysed lymphoblast cells were utilized as template.

CONCLUSION: Our automated, NGS-based method identified a variety of aneuploidies in DNA with high sensitivity and specificity, and detected common aneuploidies in isolated cells. The sample preparation process is designed to be compatible with either Illumina or Life Technologies NGS systems.

P-126 Tuesday, October 21, 2014


OBJECTIVE: Analyze cycle outcomes on a cohort of patients who pursued single gene preimplantation genetic diagnosis (PGD) for myotonic dystrophy type 1 (DM1) with concurrent 24-chromosome aneuploidy screening. DM1, an autosomal dominant disorder with a prevalence of 1 in 8000, is caused by an expansion of a CTG trinucleotide repeat in the DMPK gene on chromosome 19. This multisystemic disorder results in muscle wasting and weakness, with a spectrum of severity and age of onset depending on the length of the CTG repeat expansion.

DESIGN: Retrospective analysis.

MATERIALS AND METHODS: Chart review of all DM1 cases referred by in vitro fertilization (IVF) clinics to a reference lab for PGD was performed. For all cases, one member of the couple carried a confirmed DMPK repeat expansion. Due to the unreliable nature of direct detection of trinucleotide repeat size on embryo biopsy samples, test development was performed using DNA samples from both partners in addition to samples from the affected partner’s parent(s). Parent and relative single nucleotide polymorphism (SNP) microarray data was used to identify the chromosome 19 homolog containing the DMPK expansion. Each couple pursued an IVF cycle and embryo biopsies were performed on either day 3 or day 5/6 and shipped to the reference lab for PGD. Testing was performed using Illumina CytoSNP-12b microarrays and an informatics technique. All embryo samples were compared to parent samples across multiple SNP loci to determine chromosome 19 homolog phase, assess for crossovers, rule out DNA contamination, and establish chromosome copy number. Mutation and chromosome results were reported back to the IVF centers that performed the embryo transfers, and reported cycle outcomes to the reference lab.

RESULTS: Between 2011 and 2014, eight couples - five maternal carriers and three paternal carriers of a CTG repeat expansion - underwent PGD for DM1 with 24-chromosome aneuploidy screening. One couple cycled twice, giving a total of 9 cycles and 64 embryos tested. Eight of the nine cycles had at least one embryo that screened unaffected and euploid. Seven couples pursued a single or multiple embryo transfer. Chemical pregnancy rate was 86% (6/7), clinical pregnancy rate was 71% (5/7), and implantation rate was 73% (4/7).

CONCLUSION: Reliable single gene PGD for DM1 using homolog phasing with concurrent 24-chromosome aneuploidy screening showed excellent clinical outcomes, and can be considered a reproductive treatment option for couple’s trying to prevent passing on this condition to their offspring.

Supported by: NIH#R44HD054958.

P-127 Tuesday, October 21, 2014

THE PROBABILITY OF FINDING AT LEAST ONE EUPOID EM- BRYO BASED ON RETRIEVED OOCYTES IN PGD CASES FOR ANEUPLOIDY TESTING. A. P. Cil, S. Kahraman. ART and Reproductive Genetics Unit, Istanbul Memorial Hospital, Istanbul, Turkey.

OBJECTIVE: Clinical success of Preimplantation Genetic Diagnosis (PGD) depends on many factors such as age and ovarian reserve of the patient. Although age has been extensively studied, probability of finding at least one euploid embryo (POFEE) based on retrieved oocytes has not been evaluated extensively. For the individualization of counselling for PGD it is crucial to know the availability of transfer and inform the patient accordingly. In this study we aim to determine the optimal number of oocytes to find at least one transferrable euploid embryo in PGD cases for aneuploidy testing, in addition to determine POFEE based on retrieved oocytes.

DESIGN: Retrospective data analysis.

MATERIALS AND METHODS: ICSI cycles for PGD performed at Istanbul Memorial Hospital between years 2003 and 2013 were reviewed and 1588 PGD cycles resulting in 10035 biopsied embryos for aneuploidy were analyzed. Multivariate logistic regression analysis was done to determine predictors of success in finding euploid embryos. Receiver operating curve (ROC) analysis is done in order to find the optimal number of oocytes for finding at least one euploid embryo.

RESULTS: Age and number of retrieved oocytes are the two significant predictors of success in finding at least one euploid embryo for transfer. The probability of transfer increases steadily with the higher number of available oocytes and younger age. The plot graph showing POFEE (to be shown in the presentation) based on oocytes retrieved reaches a plateau after 10 oocytes. After this point we can say in confidence that over 90% chance of finding normal embryo is possible which is the best cut-off point according to ROC analysis with a sensitivity and specificity of 70% and 71%, respectively (AUC: 0.757 [0.722-0.791], p<0.001).

CONCLUSION: Every patient may benefit from aneuploidy testing. However with this study we showed that the probability of finding at least one euploid embryo increases with the higher number of available oocytes until 11 which is the best cut-off point for reaching over 90% chance of transfer. This data is important and may be helpful in individual counselling of PGD cases for aneuploidy testing. All patients should have appropriate counselling about the availability of transfer based on their age and oocyte number which will decrease patient anxiety.

P-128 Tuesday, October 21, 2014


OBJECTIVE: Recent publications have demonstrated significant improvement in IVF treatment outcomes by implementing aCGH or SNP-based preimplantation genetic screening (PGS) (Harper et. al., 2010). The objective of our study was to identify the relationship between blastocysts morphology and euploidy rates analyzed by SNP and aCGH-based PGS.

DESIGN: A retrospective study of PGS outcome data from blastocysts biopsied on day 5 or day 6 was conducted to identify differences in euploidy rates in embryos analyzed by SNP or aCGH-based PGS.

MATERIALS AND METHODS: 173 cycles of IVF treatment with PGS between January 2013 and March 2014 were included in the study: in 98 cases blastocysts were analyzed by SNP, and in 75 cases blastocysts were analyzed by aCGH PGS. 1003 embryos were analyzed, 5.8±3.3 per case, for euploidy rates and blastocyst morphology. Morphology of blastocysts was evaluated by Gardner classification (Gardner et al., 1985). Embryos were divided into two groups based on blastocyst expansion (expanded and not expanded) and into three groups based on blastocyst quality: good (AA/AB/BA), fair (BB), and poor (C or C-). Euploidy rates were assessed in each study group by SNP and aCGH PGS.
RESULTS: We observed a strong correlation between euploidy rates and embryo quality: good quality blastocysts had a much higher rate of euploidy than poor quality blastocysts (64.56±8.2 percent and 25.88±5.1 percent, respectively, p<0.05). Our data demonstrated reliable differences in the number of euploid blastocysts on day 5 and day 6 among non-expanded blastocysts (57.72±7.1 percent and 35.17±4.9 percent, respectively, p<0.05). There was no statistically significant difference in euploidy rates in expanded blastocysts on Day 5 and Day 6 (61.9±8.3 percent and 58.67±6.9 percent, respectively, p=0.4591). Overall, euploidy rates defined by SNP were very similar to aCGH (52.37±8.1 percent and 55.14±6.1 percent, respectively, p=0.19507) and were not statistically different in all study groups.

CONCLUSION: Comprehensive chromosome screening (CCS) provides us an opportunity to re-evaluate correlation between embryo euploidy and embryo morphology in order to create the best treatment strategy for the infertile couple undergoing IVF treatment. There is a significant difference in euploidy rates depending on quality of the blastocysts and time they become available for biopsy; however, our data demonstrated no difference between the use of SNP and aCGH-based PGS to determine euploidy rates in human blastocysts.

P-129 Tuesday, October 21, 2014
RAPID AND CONCURRENT COMPREHENSIVE CHROMOSOME SCREENING (CCS) AND SINGLE GENE DISORDER (SGD) PGS. X. Tao,a C. Jalas,a A. Fedick,a C. Bohrer,a H. Garney,a S. Kloskowski,b D. Gabriele,b B. Levy,b R. T. Scott, Jr.a,b N. R. Treff.a,b aRMA of New Jersey, Basking Ridge, NJ; bRWJ Medical School-Rutgers University, New Brunswick, NJ.

OBJECTIVE: Combining accurate CCS with SGD diagnosis from the same biopsy has presented many challenges. Current methods can either fail to detect all origins of aneuploidy, require excessive workup times, involve long PGD turn-around times, or are expensive to perform. The present study develops a method which overcomes some of these limitations using quantitative real-time (q)PCR.

DESIGN: Comparison of diagnostic performance.

MATERIALS AND METHODS: Workups involved identifying informative SNPs in the parents using SNP arrays and phasing the markers using qPCR on family members. Blastocyst biopsy samples underwent targeted multiplex amplification of several informative SNP-, mutation-, genetic identity-, and copy number-loci following by TaqMan-based qPCR for SGD, contamination, CCS analyses. SGD results were compared for consistency and reliability to conventional STR and sequencing based diagnoses which were obtained from a second biopsy. Additional linked informative markers were evaluated to resolve discordant cases.

RESULTS: 17 cases, including autosomal and X-linked, recessive and dominant, and compound heterozygosity disorders, provided 150 embryos for analysis. The typical workup involved approximately 4 weeks to complete, 150 (100%) gave a 4-hour diagnosis by SNP qPCR, while 139 (92.7%) gave a result by conventional STR analysis. Concordance in diagnosis was 97%, with 4 discrepancies. Subsequent analysis of additional markers confirmed the SNP qPCR diagnosis in all cases. The use of SNPs rather than STRs resulted in generally having markers nearer the mutation, enhancing the ability to avoid recombination-based misinterpretation or a failure to make a diagnosis in many of these cases.

CONCLUSION: This new approach to combined CCS and SGD PGD involves a short and inexpensive workup and the ability to reliably and rapidly produce accurate SGD PGS results in parallel with CCS from the same biopsy.

P-130 Tuesday, October 21, 2014

OBJECTIVE: Array CGH (aCGH) was long considered a standard test for detecting copy number abnormalities. However, previous reports aCGH have shown false positive rates as high as 2%. Here, we provide the first proof of concept comparing the ability of next generation sequencing and aCGH technologies to detect sex chromosome abnormalities, such as Turner Syndrome (XO), Triple X (XXX), Klinefelter (XXY), and XYY syndrome.

DESIGN: 10 well characterized clinical DNA samples representing four classes of sex chromosome abnormalities (Turner Syndrome, Triple X, Klinefelter, and XYY syndromes), and five control DNA samples representing normal male and female euploids were analyzed. This project was designed to test the ability of aCGH and NGS to discriminate between normal and abnormal XY chromosomes.

MATERIALS AND METHODS: Microarray was performed as follows: 300 ng of DNA sample were subject to Cy3/Cy5 labeling, labeled samples were hybridized to an array of BAC clones using BlueGnome microarray slides. Data analysis and reporting were performed using BlueFuse. For NGS analysis, 300 ng of each DNA sample were used for library preparation, followed by several steps of purification, enrichment and downstream sequencing steps (NGS) using an Ion PGM sequencer. Data analysis was performed using Ion Reporter software.

RESULTS: Of 15 samples, only 11 were identified with the correct XY copy number by microarray. The remaining four were identified as normal (false negatives), of which two Klinefelter and one XYY samples were missing an Y chromosome, while one Triple X was missing an X chromosome.

With NGS all 15 samples were identified correctly. Furthermore, an extra partial deletion Xq11.4q21.1 was detected in one XYY sample.

CONCLUSION: Our preliminary data suggest a high error rate associated with abnormal copy of XY chromosomes when aCGH was applied. In contrast, results generated using NGS were error-free. Additional studies employing larger sample sizes are required to confirm the accuracy of NGS for discriminating between normal and altered XY chromosome copy.

P-131 Tuesday, October 21, 2014
THE POTENTIAL USE OF BLASTOCOEOL FLUID (BF) FROM EXPANDED BLASTOCYSTS AS A LESS INVASIVE FORM OF EMBRYO BIOPSIES FOR PREIMPLANTATION GENETIC TESTING. K. J. Tohler,a Y. Zhao,a R. Ross,a A. T. Benner,a X. Xu,a L. Du,a K. Broman,a K. Thrill,a P. R. Brezina,b W. G. Kearns,ac,d Johns Hopkins Medical Institutions, Baltimore, MD; bFort Worth Fertility, Fort Worth, TX; cCenter for Preimplantation Genetics, LabCorp, Rockville, MD; dVanderbilt University School of Medicine, Fertility Associates of Memphis, Memphis, TN.

OBJECTIVE: To obtain embryonic molecular karyotypes from nuclear DNA obtained from the BF of expanded blastocysts and compare the karyotype with the remaining inner cell mass and trophectoderm (ICM/TE) cells of a differentiated blastocyst.

DESIGN: Prospective cohort study.

MATERIALS AND METHODS: Embryos were donated to research following IRB approval primarily due to their unsuitability for clinical use. All donated embryos were de-identified and patient demographic information was not available. 96 expanded blastocysts underwent aspiration of the BF using an intracytoplasmic sperm injection needle. The DNA was amplified and whole genomic karyotypes of the BF DNA and DNA from the ICM/TE were analyzed separately using comparative genomic hybridization (aCGH) microarrays.

RESULTS: A successful aCGH was completed on 63% (60/96) of BF samples analyzed. All ICM/TE samples successfully amplified and molecular karyotypes performed. The BF DNA karyotype was discordant from the ICM/TE karyotypes in 52% (31/60) of embryos analyzed. Only 2 embryos had a euploid karyotype obtained from the BF sample but an aneuploid ICM/TE. Diagnostic accuracy was quantified using the ploidy status (euploid or aneuploid) of the ICM/TE as the standard to determine true positive, true negative, false positive and false negative. We considered a “positive” test to be euploid and a “negative” test to be euploid. Probabilities for diagnostic accuracy were calculated and demonstrated the following: sensitivity: 0.88 (95% CI: 0.62-0.98), specificity: 0.55 (95% CI: 0.39-0.70), positive predictive value: 0.41 (95% CI: 0.25-0.60), negative predictive value: 0.92 (95% CI: 0.75-0.99).
CONCLUSION: Embryonic DNA within the BF can be isolated and analyzed. However, in this cohort of embryos, the high proportion of observed discordance between the BF DNA and the ICM/TE cells renders its use unacceptable at the present time. This may be due to the quality of the embryos donated for research. Further studies are required.

P-132 Tuesday, October 21, 2014
COPY NUMBER VARIATION SEQUENCING FOR DIAGNOSIS OF CHROMOSOME ABNORMALITIES IN PRE-IMPLANTATION EMBRYOS. Y. Yao, L. Wang, D. S. Cram, Obstetrics and Gynecology, Chinese PLA General Hospital, Beijing, China; Chinese PLA General Hospital, Beijing, China; Berry Genomics, C., Limited, Beijing, China.

OBJECTIVE: Our hypothesis is that a massively parallel shotgun sequencing based method could provide higher resolution chromosome copy number variation (CNV) testing at a cost more acceptable for preimplantation genetic diagnosis.

DESIGN: Whole Genome Amplification (WGA) products from 9 known and 14 blinded samples previously diagnosed with different chromosome abnormalities by array comparative genomic hybridization (aCGH) were subjected to CNV-Sequencing (CNV-Seq) analysis. The validated CNV-Seq method was applied in three clinical PGD cases.

MATERIALS AND METHODS: A low coverage CNV-sequencing method was developed to produce 2.7-3.0 million uniquely mapped chromosome reads from a whole genome amplification reaction performed on a single blastomere biopsy at day 3 of embryo development. Mean sequencing reads per sequential 20K chromosome bins in batched test samples were internally compared using bioinformatics algorithms to derive detailed CNV maps of all 24 chromosomes.

RESULTS: CNV-Seq diagnoses for chromosomal aneuploidies and imbalances caused by translocations were highly concordant with array CGH diagnoses. Terminal chromosome imbalances in embryos resulting from abnormal segregation of translocation chromosomes could be clearly detected by CNV-Seq, down to 1 Mb in size. The validated CNV-Seq method was successfully applied in three clinical PGD cases enabling the identification of balanced euploid embryos for transfer and resulting in clinical pregnancies.

CONCLUSION: CNV-Seq is highly sensitive and specific for detecting clinically significant CNVs in pre-implantation embryos. This method is promising for clinical translation to improve the efficiency and accuracy of PGD.

Supported by: This study was supported by the key research programs of the “Twelfth five-year Plan” of to Y.Q. Yao (BWS11J058).

P-133 Tuesday, October 21, 2014
ABSTRACT WITHDRAWN

P-134 Tuesday, October 21, 2014

OBJECTIVE: To determine the differences in blastocyst aneuploidy rates between three different comprehensive chromosome screening (CCS) platforms.

DESIGN: Retrospective, single center.

MATERIALS AND METHODS: All patients undergoing IVF with CCS at the blastocyst stage were included in this study. Assisted hatching was performed on day 3 for all cases. Embryos were cultured to the blastocyst stage and biopsied on either day 5 or day 6. Blastocysts were only biopsied when the trophectoderm was protruding from the zona pellucida. Only good quality blastocysts were biopsied and a piece of trophectoderm underwent array comparative genomic hybridization (aCGH), quantitative polymerase chain reaction (qPCR), or single nucleotide polymorphism array (SNP). Aneuploidy rates between the three techniques were compared utilizing non-parametric tests.

RESULTS: A total of 246 patients underwent IVF and CCS: 195 patients for aCGH, 24 patients for qPCR, and 27 patients with SNP. The average age was significantly different between aCGH, qPCR, and SNP, 35.8±4.2 years, 35.4±7.0 years, and 33.0±3.9 years, respectively (P=0.0030). The average number of blastocyst biopsied was not significantly different between aCGH, qPCR, and SNP, 4.3±2.7, 3.1±2.4, 4.5±3.3 blastocysts, respectively (P=0.0563). There was no difference in aneuploidy rates between aCGH (427/845, 50.5%), qPCR (42 of 74, 56.8%), or SNP (71 of 122, 58.2%) (P=0.1980).

CONCLUSION: Although small, our data suggests that there may differences between platforms. This difference could indicate that a platform overcalls aneuploidy or under calls euploidy. Ideally, a direct comparison between the same samples would yield a more definitive answer, however this is not possible. Larger studies are needed to confirm or refute our findings.

Supported by: Reproductive Endocrinology Associates of Charlotte.

P-135 Tuesday, October 21, 2014

OBJECTIVE: Next generation sequencing (NGS) is the latest technology available for DNA testing. It is highly accurate, fully scalable, and easy to automate. Here we evaluate the potential for the application of NGS in pre-implantation genetic screening, and compare its performance to array CGH.

DESIGN: 52 blinded samples were obtained using whole genome amplification from blastomere and trophectoderm samples. A total of 2024 chromosomes were analyzed by array CGH and NGS. Accuracy, amplification failure rate, and incidences of false positive/false negative outcomes were calculated prior to evaluating concordance between the two approaches.

MATERIALS AND METHODS: Whole genome amplifications (WGA) were obtained using PicoPlex™ (Rubicon Genomics). WGA products were quantified using a NanoDrop spectrophotometer prior to array CGH and sequencing. For array CGH, 100 ng of DNA sample with Cy3/Cy5 label were hybridized to an array of BAC clones using BlueGnome microarray slides. Data analysis and reporting were performed using BlueFuse. For NGS analysis, 100 ng of DNA sample were used for library preparation. NGS libraries were subject to several steps of purification, enrichment, qPCR quantification, equalization, and downstream sequencing steps using an Ion PGM sequencer. Data analysis was performed using Ion Reporter™ software.

RESULTS: Our preliminary data suggest a sensitivity of 100% for NGS compared to 95% for array CGH, and a specificity of 99.9% for NGS compared to 98% for array CGH. Lower specificity of array CGH was primarily attributed to additional gains and losses of chromosomes that were detected but not scored by array CGH.

CONCLUSION: In conclusion, NGS validation exhibited a high concordance with microarray data, with the further advantage of superior sensitivity and specificity obtained using the NGS method. This outcome suggests that NGS presents an attractive platform for preimplantation genetic screening and diagnosis.

P-136 Tuesday, October 21, 2014
THE USE OF NEXT-GENERATION SEQUENCING (NGS) FOR PRE-IMPLANTATION GENETIC SCREENING (PGS) AND DIAGNOSIS (PDD). K. J. Tobler, R. Ross, A. T. Benner, L. Du, P. R. Brezina, W. G. Kearns. Johns Hopkins Medical Institutions, Baltimore, MD; Fort Worth Fertility, Fort Worth, TX; Center for Preimplantation Genetics, LabCorp, Rockville, MD; Vanderbilt University School of Medicine, Fertility Associates of Memphis, Memphis, TN.
OBJECTIVE: Development and validation of NGS to perform PGS and FGD using trophectoderm (TE) cells previously diagnosed by comparative genomic hybridization microarrays (aCGH).

DESIGN: Retrospective blinded study.

MATERIALS AND METHODS: TE cells from 20 embryos underwent cell lysis and DNA amplification using a Klenow fragment and a modified random priming whole genome amplification protocol followed by aCGH microarray. The aCGH karyotype results were then blinded and NGS was performed on the same amplified DNA samples. For NGS, DNA libraries were prepared and DNA barcoding was performed which allowed simultaneous analysis of DNA from the 20 different samples. Sequencing was performed using paired-end dual index 2 x 36 base pairs reads and Burrows-Wheeler Alignment within the MiSeq Reporter software. Bioinformatic analysis was performed using alpha BlueFuse Multi software. Codons were broken and the NGS and aCGH results were compared for copy number concordance and structural chromosome imbalances.

RESULTS: All 20 TE samples resulted in a successful molecular karyotype by aCGH arrays and NGS. 100% concordance was observed between all aCGH arrays and NGS molecular karyotypes. Three of the samples were confirmed euploid and 11 of the karyotypes contained a single aneuploid chromosome (7 trisomy and 4 monosomy), 5 samples contained at least 2 chromosome aneuploidies. One sample successfully identified a deletion on chromosome 3 (46 XX del(3) q26.32), as the result of a 3:1 alternate segregation imbalance due to a parental translocation carrier.

CONCLUSION: Next generation sequencing demonstrated 100% concordance with aCGH arrays in diagnosing aneuploidy and structural chromosome imbalances. This study demonstrates the potential use of NGS as a platform to diagnose aneuploidy and structural chromosome imbalances as part of preimplantation genetic testing.

P-137 Tuesday, October 21, 2014
DIRECT DETECTION OF FMR1 CGG REPEATS CAUSATIVE FOR FRAGILE X SYNDROME COUPLED WITH 24-CHROMOSOME ANEUPLOIDY SCREENING IN SINGLE CELLS. K. Handschuh, C. Zhang, X. Qin, Z. Rosenwaks, K. Xu, Reproductive Medicine, New York Presbyterian Hospital-Weill Medical College of Cornell University, New York, NY.

OBJECTIVE: Fragile X syndrome (FXS) is caused by the expansion of CGG sequences in the 5' untranslated region of the Fragile X Mental Retardation 1 (FMR1) gene. Most current pre-implantation genetic diagnosis (PGD) protocols are based on indirect analysis of linkage markers because amplification of trinucleotide repeats is difficult. The aim of our study is to develop a dual test that can achieve direct diagnosis of FXS and screen for 24-chromosome aneuploidy from the same sample while being sensitive at the single cell level and applicable to PGD for IVF.

DESIGN: Experimental study.

MATERIALS AND METHODS: Four single-cell and five-cell samples of (1) a lymphocyte cell line carrying an intermediate mutation for FXS, (2) a fibroblast cell line carrying a full mutation for the FXS, (3) a trisomic cell line for chromosome 13, (4) a cell line monosomic for chromosome 21 and (5 and 6) two normal cell lines (46 XX and 46 XY) were subjected to whole genome amplification (WGA). All cell lines were established and commercially available. After WGA, a first aliquot was processed through the AmpliSeq™ protocol (Sasuragen) for CGG repeats assessment of the FMR1 gene. A second aliquot was used for 24-chromosome aneuploidy screening on array-CGH from BlueGene (Illumina).

RESULTS: Normal alleles (27 and 30), intermediate mutation (52) and full mutation of FMR1 (>200) were respectively detected in control cells (8/8), lymphocyte (8/8) and fibroblast (8/8) cell lines affected with FXS, in a very sensitive and reproducible manner. Concurrent aneuploidy screening was effectively achieved from the same WGA sample, as shown by the confirmation diagnosis of known trisomic 13, monosomic 21 or normal cell lines.

CONCLUSION: This is the first report of direct diagnosis for FXS coupled with 24-chromosome aneuploidy screening. The advantages of this new test are (1) it provides accurate sizing of FMR1 alleles up to 200 CGG repeats, allowing to define a pre-, intermediate or full Fragile X mutation, (2) it can be achieved from a unique biopsy specimen, offering the possibility to diagnose for multiple conditions on limited starting material, (3) it is reliable on a single cell, therefore applicable to pre-implantation genetic diagnosis on D3 blastomere as well as on D5/6 trophectoderm biopsy, (4) it can be completed within 24 to 48 hours, hence allowing for Day3 biopsy with Day5 fresh transfer.

Supported by: Institutional.

P-138 Tuesday, October 21, 2014

OBJECTIVE: To compare outcomes of IVF cycles with and without pre-implantation genetic screening (PGS) using array comparative hybridization (aCGH) in patients aged 40 through 43.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: From 2010-2013, 127 IVF cycles (maternal age range 40-43 years) with fresh embryo transfer (IVF-ET) on day 5 were compared to 49 cycles where patients elected trophectoderm (TE) biopsy on day 5 and or day 6 blastocysts with vitrification and aCGH. Only normal euploid blastocysts were transferred in a subsequent frozen embryo transfer (PGS-FET). In order to better compare these 2 groups of patients, we restricted our non-PGS group to patients who had fresh transfers of embryos that would have been biopsied (Graded 2BC or 2CB or better). Also compared were outcomes in these two groups with a third group of patients in an attempt to discern whether the frozen embryo transfer (FET) paradigm or the transfer of only euploid embryos was the basis for the difference between the PGS and non-PGS group. Outcomes measured included implantation rate (IR), live birth-ongoing pregnancy rate (LB-OPR) per transferred embryo, and Sacs loss rate (SLR). SLR was defined as total number of sacs lost per total number of embryos implanted as documented by a sac on ultrasound.

<table>
<thead>
<tr>
<th># Patients</th>
<th>Embryos Transferred</th>
<th>IR</th>
<th>LB-Op per transferred Embryo</th>
<th>Sac Loss Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVF-ET</td>
<td>127</td>
<td>2.38 (303/127)</td>
<td>23.8% (72/303)</td>
<td>15.8 (48/303)</td>
</tr>
<tr>
<td>FET</td>
<td>28</td>
<td>2.25 (63/28)</td>
<td>25.4% (16/63)</td>
<td>19% (12/63)</td>
</tr>
<tr>
<td>PGS-FET</td>
<td>49</td>
<td>1.15 (55/49)</td>
<td>21% (12/55)</td>
<td>10.7% (5/55)</td>
</tr>
</tbody>
</table>

P-value a: NS  
P-value b: <0.0001

RESULTS: There were no significant differences in outcomes between Non-PGS group (IVF-ET vs. FET). However, outcomes were significantly different when comparing the PGS-FET with FET groups. (See Table)

CONCLUSION: The present data provides conclusive evidence of the benefit of PGS with regard to implantation rate and miscarriage rate. Another advantage of PGS has been reduction in multiple gestation. Despite the high rate of aneuploidy after age 40, FET after PGS (predominantly single embryo transfer) result in significantly improved implantation and take home rates in similar groups of women aged 40-43.

P-139 Tuesday, October 21, 2014
CLINICAL STUDY OF MASSIVELY PARALLEL SEQUENCING ON CHROMOSOMAL ABNORMALITY DETECTION OF HUMAN CLEAVAGE-STAGE EMBRYOS. Z. Zhou,† L.-J. Li,† Y. Zhang,† J. Li,† W. Xu,† Y.-H. Huang,‡ K.-W. Cho,‡ W.-Y. Lu,‡ Y.-L. Ma.† "Hainan Provincial Key Laboratory for Human Reproductive Medicine and Genetic Research, Affiliated Hospital of Hainan Medical University, Haikou, Hainan, China; †BGI-Shenzhen, Shenzhen, Guangdong, China; ‡Department of Obstetrics and Gynecology, The Chinese University of Hong Kong, Shatin, NT, Hong Kong SAR, China.

OBJECTIVE: The aim of this study was to use massively parallel sequencing (MPS) for identification of aneuploidy and unbalanced chromosomal rearrangements after blastomere biopsy in preimplantation human cleavage-stage embryos.
Table 1. Clinical results from 11 MPS based PGD cycles.

<table>
<thead>
<tr>
<th>Clinical data</th>
<th>Couples with balanced translocations</th>
<th>Couples with numerical chromosomal abnormalities</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of couples treated</td>
<td>8</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>Maternal age (average, years)</td>
<td>29.4±4.0</td>
<td>31.7±1.8</td>
<td>30.0±3.7</td>
</tr>
<tr>
<td>No. of embryos diagnosed</td>
<td>76</td>
<td>22</td>
<td>98</td>
</tr>
<tr>
<td>Balanced (%)</td>
<td>9(11.9)</td>
<td>5(22.7)</td>
<td>14(14.3)</td>
</tr>
<tr>
<td>Unbalanced (%)</td>
<td>21(27.6)</td>
<td>0(0.0)</td>
<td>21(21.4)</td>
</tr>
<tr>
<td>Balanced + aneuploid (%)</td>
<td>20(26.3)</td>
<td>13(59.1)</td>
<td>33(33.7)</td>
</tr>
<tr>
<td>Unbalanced + aneuploid (%)</td>
<td>26(34.2)</td>
<td>4(18.2)</td>
<td>30(30.6)</td>
</tr>
<tr>
<td>No. of embryos transferable</td>
<td>9</td>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td>No. of embryos transferred</td>
<td>6</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>No. of pregnancies still ongoing</td>
<td>3</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>No. of babies born</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

DESIGN: Cell biopsies were performed on 6- to 8- cell cleavage-stage (day 3) embryos. Single blastomeres were lysed, processed by whole genome amplification (WGA) and then subjected to both MPS and array-CGH for double confirmation of the results. Euploid embryos identified as “balanced or normal” by both methods were selected for frozen thawed embryo transfer cycles.

MATERIALS AND METHODS: WGA was conducted using SurePlex DNA Amplification System (BlueGene, Cambridge, UK). High-throughput sequencing was performed using an Illumina HiSeq2000 platform (Illumina, CA, USA) with an average of 0.073 depth and 5.5% coverage of the human genome [1]. Array-CGH was performed according to the Agilent oligonucleotide array-based CGH for single cell analysis protocol (Agilent technologies, CA, USA).

RESULTS: Clinical results were shown in Table 1. The majority of embryos diagnosed by MPS were confirmed by array-CGH, with the exception of 4 samples, where different sizes of unbalanced rearrangements were detected, possibly due to chromosomal GC bias in array analysis.

CONCLUSION: Our study reported the first clinical study of applying MPS on chromosomal abnormality identification in preimplantation human cleavage-stage embryos, as well as the first clinical study of single copy based MPS for PGD of chromosomal abnormality, which got satisfactory pregnancy rate. This study indicated that MPS could be applied to accurately detect embryonic chromosomal abnormality in PGD.

Supported by: This work was supported by grants from NNSFC (No. 81060016, 31140021, 81260032), Hainan Provincial Department of Science and Technology (No. YJJC20120007, 2012-GH009, 8122003), Hainan Provincial Department of Health (No. 2011-38), Hainan Provincial Department of Education (No. HKJ2012-26) and Haikou Municipal Bureau of Science, Technology and Industrial Information (No. 2012-065, 2013-48, 2013-49).

P-140 Tuesday, October 21, 2014

DOES THE PROTOCOL FOR EMBRYO BIOSPY FOR ARRAY COMPARATIVE GENOMIC HYBRIDIZATION (aCGH) ASSOCIATED TO EMBRYO VITRIFICATION AFFECTS EMBRYO QUALITY? A PILOT STUDY. C. Madaschi, a P. Q. D’Elia, a P. Guilhaume, a G. Fassolas, a C. R. Izzo, a IVF and Andrology Laboratory, Origine - Centro de Reprodução Humana, São Paulo, SP, Brazil. bClinical and Laboratory Director, Origine - Centro de Reprodução Humana, São Paulo, SP, Brazil.

OBJECTIVE: The aim of this study was to evaluate the association of the protocol for embryo biopsy and trophoectoderm cells and embryos qualities on transfer day in aCGH cycles.

DESIGN: Pilot study. We retrospectively analyzed 19 embryo vitrification-thaw cycles with aCGH realized between 2012-2013, with two biopsy protocols: 7 cycles with embryos cultured and biopsied on blastocyst stage and then submitted to vitrification; in a subsequent endometrial preparation embryos were warmed and transferred (Group-CGH+FET); and 12 cycles with embryos cultured and vitrified on D3; in subsequent cycle embryos were warmed and culture until blastocyst stage, biopsied on blastocyst stage (day 5) and transferred in the following day (day 6) (Group-FET+CGH).

MATERIALS AND METHODS: Embryos were vitrified-warmed according to Irvine Scientific® vitrification kit. Variables studied included normal fertilization, embryo grade on day 3, and blastocyst expansion on day 5. The top-quality embryos rate was calculated by number of top-quality embryos on transfer day per number of euploid embryos biopsied. p ≤0.05 was considered statistically significant.

RESULTS: Groups are similar on age (CGH+FET:36.0±3.6; FET+CGH: 36.3±3.0; p=0.653), number of MII in stimulated cycle (CGH+FET:10.7±4.4; FET+CGH: 13.7±6.3; p=0.249), number of embryos biopsied (CGH+FET:4.9±4.3; FET+CGH: 3.7±1.7; p=0.565) and number of embryos transferred (CGH+FET:1.0±0.8; FET+CGH: 1.1±1.1; p=0.857). The top-quality euploid embryos rate were also similar (CGH+FET: 16%; FET+CGH: 17.9%; p=0.819) between groups. One clinical pregnancy occurred in each group.

CONCLUSION: This pilot study suggested there is no influence of biopsy time (previous or after embryo vitrification) on embryos quality at day transfer. However, we there is a important technical advantages on carrying out aCGH in fresh embryos at blastocyst stage, followed by vitrification, as it allow the maintenance of only normal embryos and better management on warmed embryos transfer cycles. Embryos were pre-selected for biopsy/vitrification on CGH- FE group, while all viable embryos were vitrified on day-3 on FET-CGHgroup. Hence, the biopsy previously to vitrification can prompt a economical advantage as well. Clinical comparisons weren’t possible due to small samples number. Further randomized studies are necessary confirm those findings regard embryo/biopsy/vitrification protocols in aCGH cycles.

P-141 Tuesday, October 21, 2014


OBJECTIVE: To address the oncofertility and preimplantation genetic diagnosis (PGD) information gap in the BRCA1/2 mutation positive population by studying reproductive endocrinologists’ (REI) attitudes and practices regarding BRCA1/2 positive oncofertility patients, including those who have been diagnosed with cancer and those at elevated risk of developing cancer. Specific objectives of this study include assessment of: family history elicitation and intervention timing; experience with genetic counselors and genetic testing; assessment and use of single gene PGD for BRCA1/2 mutations in oncofertility intervention timing; and recommendations regarding embryo selection based on gene mutation status.

DESIGN: Exploratory pilot study. Members of the ASRM affiliate Society for Reproductive Endocrinology & Infertility (SREI) whom are practicing REIs were invited to take a survey. The survey was mailed to 1000 REIs and responses were accepted for eight weeks in the fall of 2013/winter of 2014. A response rate of 14.5% was achieved.

MATERIALS AND METHODS: Data was analyzed using SPSS software and statistical significance was determined using chi squared statistic and Fisher’s exact test.

RESULTS: Participating REIs reported providing fertility preservation and/or PGD to BRCA1/2 positive patients both after a cancer diagnosis and in anticipation of a future cancer diagnosis. However, there was no age group in which 100% of participants stated they were willing to provide fertility preservation. The majority of participating REIs reported utilizing genetic counselors for PGD patient counseling, genetic testing, and general genetics questions. No consensus among participants was found regarding: willingness to offer PGD to BRCA1/2 mutation carriers (indeed 9%, either would not or did not respond), to implant a BRCA1/2 mutation positive embryo (46% either would not or did not respond), and to provide an embryo selection recommendation to the patient when all the embryos are BRCA1/2 mutation positive (49% selected other and 7% did not respond).

CONCLUSION: Our findings suggest the need for a decision aid to assist REIs and patients with embryo selection for BRCA1/2 mutations based on the lack of consensus and/or lack of ability to answer questions in the survey. Decision trees have been shown to lead to more informed clinical judgment of patients regarding medical decisions. Our findings can also be added to the existing literature base and used to create patient and provider educational materials regarding oncofertility and PGD.

Supported by: This study was completed in fulfillment of the Master of Science degree from Northwestern University Graduate Program in Genetic Counseling and received a $1000 budget from the program. Additional support from the SCCPIR NICHD U54 HD076188.
MALE REPRODUCTIVE ENDOCRINOLOGY

P-142 Tuesday, October 21, 2014

VARIABLE SERUM TESTOSTERONE LEVELS SUGGEST DIFFERENT TREATMENT_THRESHOLDS FOR HYPOGONADAL SYMPTOMATOLOGY. N. Wilken, R. Ramasamy, J. Scovell, J. R. Kovac, L. I. Lipshtultz, Urology, Baylor College of Medicine, Houston, TX.

OBJECTIVE: Hypogonadism is diagnosed by a combination of symptomatology and low serum testosterone. The current study assessed hypogonadal symptoms in men ≥40 years of age using the Androgen deficiency in Aging Male (ADAM) questionnaire.

DESIGN: Retrospective study.

MATERIALS AND METHODS: We surveyed a sample of 360 men (160 hypogonadal patients with total T ≤300ng/dL and 200 controls with total T=300-499ng/dL) not on testosterone therapy between the ages of 40 and 90 years old. Using the Androgen Deficiency in the Aging Male (ADAM) questionnaire, we collected data with regard to sexual, psychological, and physical health. T levels were measured in morning blood samples by radioimmunnoassay. Men who were currently on testosterone supplementation therapy (TST) or had received TST in the one month previous to reporting the survey were excluded from the analysis.

RESULTS: Of the 10 hypogonadal symptoms that are part of the ADAM questionnaire, the prevalence of 5 symptoms were significantly different (p<0.05) between men with serum testosterone levels of ≤ 300ng/dL and men with T >300ng/dL. These included one sexual symptom (decreased libido), one psychological symptom (lack of energy), and three physical symptoms (decreased strength and endurance, decreased ability to play sports, and falling asleep after dinner).

CONCLUSION: Among men >40 years of age presenting to a man’s health clinic, symptoms of decreased libido, decreased energy, decreased strength and endurance, decreased ability to play sports, and falling asleep after dinner were most associated with low total serum testosterone. The threshold serum testosterone for these hypogonadal symptoms ranged from 320 – 357ng/dL, rather than a solitary testosterone level, was found to differentiate between the five, most significantly different symptoms. Specifically, serum testosterone thresholds of 375ng/dL were identified for decreased libido, 350ng/dL for lack of energy, 320ng/dL for decreased ability to play sports, 340ng/dL for decreased strength and endurance, and 360ng/dL for increasingly falling asleep after dinner.

FERTILITY & STERILITY®

P-144 Tuesday, October 21, 2014

FUNCTIONAL PROTEOMIC ANALYSIS OF SPERM PROTEINS IN INFERTILE MEN WITH UNILATERAL VARICOCELE. A. Agarwal,1 R. Sharma,2 D. Durairajanaayagam,3 Z. Cui,4 A. Ayaz,5 S. Gupta,6 B. Willard,7 B. Gopalan,7 E. Sabanegh4 Center for Reproductive Medicine, Urology Department, Cleveland Clinic, Cleveland, OH;4Lerner Research Institute, Cleveland Clinic, Cleveland, OH.

OBJECTIVE: Varicocele is the most common correctable cause of male factor infertility is observed in 35% to 50% of adult men with primary infertility. Oxidative stress plays an important role in the pathogenesis of sperm DNA damage in patients with varicocele. The objective was to examine if proteomic analysis and bioinformatic tools can help in identifying proteins of interest in infertile men with unilateral varicocele.

DESIGN: This prospective study analyzed spermatozoa proteins from infertile men with unilateral varicocele (n=5) and healthy fertile men (n=5) who recently established a pregnancy to identify the proteins of interest involved in the pathophysiology of varicocele and male infertility.

MATERIALS AND METHODS: Spermatozoa were obtained from infertile men with unilateral varicocele and healthy men of proven fertility. Proteins were extracted and separated by 1-D gel. Bands were digested and on a LTQ-Orbitrap Elite hybrid mass spectrometer system. Functional annotations of proteins were obtained using bioinformatics tools and pathway database.

RESULTS: A total of 1035 proteins were identified in the spermatozoa of fertile group and 757 in the unilateral varicocele group. 369 proteins were differentially expressed. Of these, 120 were unique to the fertile group and 38 to the unilateral varicocele group. 114 were overexpressed and 97 were underexpressed in the unilateral varicocele group. Apoptosis, post-translational modification, protein ubiquitination, mitochondrion dysfunction, oxidative stress response were some of the major functional categories observed for the differentially expressed proteins. Additionally, we have identified about 30 proteins of interest that play a role in sperm motility, capacitation, acrosome reaction and fertilization and may be compromised or altered in infertile men with unilateral varicocele.

CONCLUSION: We identified significant differences in the distribution of differentially expressed proteins (DEP) in fertile men and infertile men with unilateral varicocele. Distinct proteins are involved in various biological processes, molecular function and cellular location.

MALE REPRODUCTIVE UROLOGY

P-143 Tuesday, October 21, 2014

SURVIVAL OF MALE GERM CELLS FROM APOPTOSIS IN HYPERPROLACTINEMIC RATS IS THROUGH MODIFICATION OF TNF RECEPTOR EXPRESSION AND NF-κB SIGNALING PATHWAY. W. J. Huang a,b,c d H.-H. Chen, b Z.-L. Wang, b H.-H. Lin, b Y.-C. Chen, a L.-W. Chen a Department of Urology, National Yang-Ming University, Taipei, Taiwan; bDepartment of Physiology, National Yang-Ming University, Taipei, Taiwan; cShu-Tien Urology Research Institute, National Yang-Ming University, Taipei, Taiwan; dDepartment of Urology, Taipei Veteran General Hospital, Taipei, Taiwan.

OBJECTIVE: Hyperprolactinemia (hyperPRL) induced hypogonadism is known caused by tumor necrosis factor (TNF) -α-related inhibition of Leydig cell. Furthermore, TNF-α also induces germ cell apoptosis via TNF receptors to activate the caspase pathway. Administering anti-TNFα antibody (Ab) in vitro can reverse hyperPRL-related germ cell apoptosis. This study was aimed to investigate whether NF-κB is activated to rescue the germ cells from apoptosis through treatment with TNF-α inhibitors, including anti-TNF-α antibody and antagonist etanercept in hyperPRL.

DESIGN: Animal study using Sprague-Dawley rats (8-12 weeks old), and hyperPRL was induced by anterior pituitary (AP) glands graft into the renal subcapsular space.

MATERIALS AND METHODS: HyperPRL rats were grafted with 2 AP, and the control group was grafted with similar amount of cerebral cortex (CX). Six weeks later, rats received intratesticular injection with TNF inhibitors, including anti-TNF-α Ab (12.5 μg/testis/kg) or etanercept (0.4 mg/testis/kg). One week after injection, testicular tissue was examined by TUNEL assay, weten blot and immunohistochemical (IHC) staining. IHC studies included the use of Ab against p65, TNF-α receptor type 1 (TNFR-1) and type 2 (TNFR-2). The scoring was done by two independent observers who were blind to group assignment.

RESULTS: The AP-grafted group presented more apoptotic germ cells, lower expression of NF-κB signals and greater expression of TNFR-1 in germ cells than the CX-grafted control group (all P<0.05). The TNFR-1 was especially significantly expressed in spermagonia of AP-grafted group. Injection of anti-TNF-α Ab or etanercept both significantly decrease the number of apoptotic germ cells. Furthermore, after TNF inhibitors treatment, TNFR-1 expression was decreased and TNFR-2 expression increased in AP-grafted group, and NF-κB signals were similar between groups.

CONCLUSION: The survival signals, NF-κB, were shown decreased in AP-grafted group. TNF inhibitors might contribute to improve the survival of germ cells in hyperPRL rats. The mechanism was suggested to involve the dynamics of TNFR-1, 2 and NF-κB signaling pathways. It implies that TNF inhibitors show a therapeutic potential in treating hyperPRL-induced male subfertility or infertility.

Supported by: National Science Council, Taiwan; Taipei Veterans General Hospital.
These proteins may serve as potential biomarkers that are involved in the pathophysiology of varicocele and male infertility. 

Supported by: Cleveland Clinic.

P-145 Tuesday, October 21, 2014


OBJECTIVE: Fertilization and pregnancies can be obtained with spermatozoa recovered not only from the ejaculate, but seminiferous tubules (testicular sperm extraction, TESE). Microdissection (Micro-TESE), in combination with intracytoplasmic sperm injection (ICSI), is presently used to treat infertility in cases of NOA including Klinefelter syndrome (KS). KS is the most common sex-chromosome disorder among infertile males, with a prevalence of 1 in 660 men and is a frequent cause of hypogonadism and infertility. The aim of this study is to assess the prevalence and the significance including sperm retrieval rate (SRR) by micro-TESE in non-obstructive azoospermic patients including KS.

DESIGN: Retrospective clinical analysis.

MATERIALS AND METHODS: The records were retrospectively evaluated for 1028 NOA patients (including 123 non-mosaic KS patients) who were underwent micro-TESE. Chromosomal analysis was performed on all patients on cultured lymphocytes from peripheral blood. Micro-TESE was used in which seminiferous tubules are directly examined throughout the testicle using an operating microscope and selectively biopsied for all of the NOA patients in modified Schlegel’s method. We did not undergo preoperative hormonal therapy for KS patients. In our study, KS patients did not have microdeletion of Y chromosomes.

RESULTS: Testicular sperm were successfully retrieved by micro-TESE in 69 of 123 (56.1%) non-mosaic KS and 380 of 905 (42.0%) not KS NOA patients. Of these, 48 (69.6%) KS and 344 (90.5%) not KS NOA patients had sperm identified through the initial wide incision alone. For patients with KS, the chance of sperm retrieval on the contralateral side after a negative unilateral microdissection was 28.0% (21/75) and significant higher than not KS NOA patients (36/561–6.4%) (p < 0.001). In almost all patients in whom micro-TESE was successful we could identify focal spermatogenenesis in dilated and opaque seminiferous tubules surrounded by shrunken tubules or fibrous tissue. No correlation was found between serum FSH, LH, and T level with the DFI relationship was found between serum FSH, LH, and T level with the DFI. Micro-TESE was successful for cases of KS is significantly younger (33.5 ± 4.2 years) than that in failed cases (37.4 ± 3.9 years) (p < 0.05).

CONCLUSION: Micro-TESE is particularly helpful for successful sperm retrieval in KS cases. In NOA, the absence of uniformity in testicular tissue is a critical key to succeed and rationale in micro-TESE.

P-146 Tuesday, October 21, 2014


OBJECTIVE: To assess the DFI of spermatozoa retrieved in different areas of the male genital tract. To measure embryo developmental performance of surgically retrieved spermatozoa with lower DFI.

DESIGN: Men with extremely high DFI in their ejaculates (n = 24) were counseled to undergo surgical sampling. DFI analysis was carried out on the spermatozoa from the ejaculate, vasoal fluid, epididymis, and testis. To determine whether a testicular biopsy would yield spermatozoa with healthier chromatin and superior embryo developmental competence, men underwent ICSI with these specimens.

MATERIALS AND METHODS: We identified infertile men (n = 24) with high DNA fragmentation in their ejaculates that agreed to undergo surgical sampling for this indication. Chromatin fragmentation index was assessed by SCSA and/or TUNEL on specimens isolated from all sites. ICSI outcomes with ejaculated and testicular spermatozoa were analyzed and compared.

RESULTS: In ejaculated spermatozoa the average DFI was 38.9 ± 21% (range 26-96) assessed in 31 occasions. In some of these men, aspiration of the vas deferens (n = 2) yielded a DFI of 16.5 ± 1% (range 15.7-17.3) while spermatozoa from the epididymis (n = 12) has a DFI of 15.1 ± 5.2% (range 8.4-25.9) and testicular spermatozoa (n = 24) was 11.3 ± 7.3% (range 2-26.2). This topographic representation of the DFI found the DNA integrity of testicular spermatozoa and encouraged us to utilize these gametes for ICSI. In a paired analysis of 8 couples where testicular spermatozoa were used, a fertilization of 50% (26/52) and a cleavage rate of 100% (26/26) resulted in a clinical pregnancy of 25.0% (2/8). This finding appears superior to their respective ICSI cycles carried out with ejaculated spermatozoa that resulted in fertilization of 89.0% (33/37), embryo cleavage of 63.6% (21/33), and clinical pregnancy rate of 12.5% (1/8).

CONCLUSION: The topographic assessment of sperm chromatin integrity throughout the male genital tract suggested that there is a disruption in DNA packaging during spermiogenesis that does not allow sperm chromatin to withstand oxidative stressors, possibly compounded by a compromised total antioxidant capacity in the seminal fluid. The utilization of testicular spermatozoa may represent a viable option for men with high sperm chromatin fragmentation in their ejaculates.

Supported by: Reproductive Medicine, Weill Cornell Medical College

P-147 Tuesday, October 21, 2014

PREOPERATIVE ULTRASOUND VARICOCELE VEIN DIAMETER UNDERESTIMATES INTRAOPERATIVE ASSESSMENT. M. S. Wosnitzer, A. Babaja, M. Goldstein. Center for Male Reproductive Medicine and Microsurgery, Department of Urology and Institute for Reproductive Medicine, Weill Cornell Medical College of Cornell University, New York, NY.

OBJECTIVE: Varicocele assessment may include preoperative ultrasound with varicoceole cutoff accepted as ≥ 2.3 mm vein diameter (1). Because ultrasound measures blood-vein interface (inner vein diameter) which may not reflect the thickened varicose vein wall outer diameter, we sought to compare accuracy of preoperative ultrasound vein diameter to intraoperative vein measurement.

DESIGN: Prospective cohort study.

MATERIALS AND METHODS: 57 consecutive men underwent subinguinal microsurgical varicocelectomy with intraoperative largest vein (outer diameter) measurement following preoperative ultrasound (supine, standing +/- Valsalva, reversal of flow, ROF) measuring blood-vein interface (inner diameter).

RESULTS: Of 57 men (mean age 36 years +/-8.3), left varicocele had clinical grade III: 48%, II-III: 5%, II: 28%; I: 7% and right: III: 7%, II-III: 2%, II: 19% and I: 32%. 12% of men had left and 17% had right varicocele identified only by ultrasound. 75% had bilateral and 21% had left varicocele. Mean ultrasound left vein diameter was 3.63 (+/-1.35) mm (standing + Valsalva), 3.35 (+/-1.28) (standing - Valsalva), 3.14 (+/- 0.82) (supine + Valsalva), and 2.88 (+/-0.82) (supine - Valsalva). Mean ultrasound right vein right values were 2.95 (+/-1.00), 2.67 (+/-1.03), 2.56 (+/-0.89), and 2.30 (+/-0.83) respectivly. Intraoperative mean largest vein was 4.65 (+/-1.44) mm (left) and 3.98 (+/-1.18) (right).

Mean varicocele ultrasound values (supine, standing, +/- Valsalva) were significantly lower than mean intraoperative largest vein diameter (p ≤ .001) with mean discrepancy 1.40 mm (left) and 1.39 mm (right). In 9 men, a single measurement (left standing + Valsalva) overestimated actual vein diameter. Clinical varicocele grade correlated with left ultrasound supine +/- Valsalva measure (p=0.002, p=.004). Higher right varicocele clinical grade (grade II-III vs. I), but not left, correlated with larger intraoperative vein diameter (p=.04). Ultrasound ROF correlated with higher right varicocele clinical grade (II-III vs. I), larger left vein diameter on ultrasound (standing +/- Valsalva, p≤.001, p=.003), but not intraoperative vein diameter. Intraoperative vein number was not associated with vein diameter.

CONCLUSION: Preoperative ultrasound significantly underestimates actual vein diameter, possibly due to ultrasound measurement of inner vein luminal diameter rather than thickened outer varicose vein wall. Additional parameters should be considered in clinical decision-making.

Supported by: Center for Male Reproductive Medicine and Microsurgery, Department of Urology
IS CLOMIPHENE CITRATE A SAFE DRUG TO USE IN PATIENTS WITH INFERTILITY AND HYPOGONADISM? S. Luján, C. Vendryes, C. Niederberger. Urology, University of Illinois. College of Medicine, Chicago, IL.

OBJECTIVE: To assess the safety of clomiphene citrate (CC) treatment in patients with infertility and hypogonadism (HG).

DESIGN: Retrospective review.

MATERIALS AND METHODS: We reviewed HG male patients referred for infertility (Testosterone, T, <300 ng/dL and Bioavailable Testosterone, BT, <154 ng/dL) between January 2009 to March 2014. Patients were treated with CC 50 mg every other day. To monitor patients’ response and potential side effects, we scheduled clinical follow up two weeks after treatment, then every four months for the first year, and then annually. Laboratory evaluation included hormone endocrine profiles, prostate specific antigen (PSA), and hemoglobin (Hg) levels. Clinical and biochemical adverse events (AE) were noted, and treatments given for AEs were reviewed.

RESULTS: One hundred and forty patients with a mean age of 36.3 (±5.96) years were analyzed. The median follow up was 143.5 (range=23-1942) days. The most common cause of infertility was non obstructive azoospermia (43.6%) followed by oligospermia (17.9%). AEs are presented in table 1. A total of 40 (28.5%) patients had AEs. Thirty (21.4%) AEs were classified as biochemical and 10 (7.1%) as clinical.

<table>
<thead>
<tr>
<th>AE Classification and number</th>
<th>Clinical components</th>
<th>Biochemical components</th>
</tr>
</thead>
<tbody>
<tr>
<td>AE BIOCHEMICAL</td>
<td>n (%)</td>
<td>20 (66.6%)</td>
</tr>
<tr>
<td>Paradoxical decrease testosterone</td>
<td>7 (23.3%)</td>
<td></td>
</tr>
<tr>
<td>Excessive increase T/BT</td>
<td>3 (10%)</td>
<td></td>
</tr>
<tr>
<td>Excessive Estradiol increase</td>
<td>20 (66.6%)</td>
<td></td>
</tr>
<tr>
<td>AE CLINICAL</td>
<td>n (%)</td>
<td>7 (23.3%)</td>
</tr>
<tr>
<td>Areola fullness</td>
<td>2 (1.4%)</td>
<td></td>
</tr>
<tr>
<td>Emotional changes</td>
<td>2 (1.4%)</td>
<td></td>
</tr>
<tr>
<td>Muscle ache</td>
<td>1 (0.7%)</td>
<td></td>
</tr>
<tr>
<td>Sweat</td>
<td>1 (0.7%)</td>
<td></td>
</tr>
<tr>
<td>Rash</td>
<td>1 (0.7%)</td>
<td></td>
</tr>
<tr>
<td>Unfocused vision</td>
<td>1 (0.7%)</td>
<td></td>
</tr>
<tr>
<td>Hot flash</td>
<td>2 (1.4%)</td>
<td></td>
</tr>
</tbody>
</table>

AE treatments included using an alternative drug (29, 20.7%), discontinuing CC (4, 2.9%), titrating CC (6, 4.3%), or no change in medication (11, 7.9%). The alternative drug prescribed was anastrozole (18, 62%) or hCG (11, 39.2%). All patients recovered from their AE.

CONCLUSION: CC is a safe drug in the treatment of infertile, hypogonadal men; however, a periodic follow up with hormone profile evaluation is mandatory. The most common AE in patients prescribed CC was an increase of estradiol in the serum. Patients who have an AE following CC may recover without sequelae.

P-148 Tuesday, October 21, 2014

COMPARISON OF PATIENTS SEEKING MALE INFERTILITY EVALUATION AND UNDERGOING VASECTOMY: DATA FROM THE NATIONAL SURVEY OF FAMILY GROWTH. J. M. Hotaling; W. O. Brant, J. B. Myers, M. R. Cullen, M. L. Eisenberg; Surgery(Urology), University of Utah, Salt Lake City, UT; Urology, Stanford, Palo Alto, CA; Internal Medicine, Stanford, Stanford, CA.

OBJECTIVE: An estimated 7 million American couples per year seek infertility care in the United States whereas 3.7 million men ages 18-45 have undergone a vasectomy. We sought to evaluate the differences in these two populations of men.

DESIGN: We analyzed data from cycles 5 to 7 of the National Survey of Family Growth performed by the Centers for Disease Control to determine the associated reproductive and demographic factors of men seeking a male infertility evaluation versus those undergoing a vasectomy.

MATERIALS AND METHODS: A total of 25,846 women and 11,067 men were surveyed. In comparison to men seeking infertility care, men undergoing vasectomy were significantly older, more likely to be married, Caucasian, have multiple children and less likely to have a college degree. No differences in BMI, health status, insurance, income or religion were identified between the two groups.

RESULTS: A total of 25,846 women and 11,067 men were surveyed. In comparison to men seeking infertility care, men undergoing vasectomy were significantly older, more likely to be married, Caucasian, have multiple children and less likely to have a college degree. No differences in BMI, health status, insurance, income or religion were identified between the two groups.

CONCLUSION: Education but not income or insurance status appears to be a significant factor associated with infertility care seeking in comparison to men undergoing a vasectomy. Otherwise, these two groups are very similar. These findings warrant further investigation.

P-150 Tuesday, October 21, 2014

ABSTRACT WITHDRAWN

P-151 Tuesday, October 21, 2014

MODIFICATION AND OUTCOME IN SURGICAL RECONSTRUCTION OF ACQUIRED SEMIAL DUCT OBSTRUCTION. H. Jiang, Q. Yuan, K. Xiao, J. Yang. Department of Urology, Shenzhen People’s Hospital, Shenzhen, Guangdong, China.

OBJECTIVE: To evaluate the overall outcome of surgical treatment for acquired obstructive azoospermia by using or combining modified microsurgery, laparoscopic surgery and endoscopic surgery.

DESIGN: We analyzed the outcome of surgical treatment of 51 cases of suspected acquired obstructive azoospermia in our single center between January 2009 and May 2013.

MATERIALS AND METHODS: Modified microscopic vasoepididymostomy(VE), laparoscopically assisted microscopic vasovasostomy(VV) and transurethral incision of the ejaculatory duct (TUIED) with holmium laser were performed depending on the different obstruction sites. Semen analyses were initiated at 4 weeks postoperatively, followed by trinomially(month 3, 6, 9, 12) semen analyses until no sperm was found at 12 months or pregnancy was achieved. Obstruction sites, postoperative patency and natural pregnancy rate were recorded.

<table>
<thead>
<tr>
<th>TABLE 1. Obstruction sites and Postoperative outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obstruction sites</td>
</tr>
<tr>
<td>--------------------</td>
</tr>
<tr>
<td>Bilateral Ejaculatory Duct Obstruction</td>
</tr>
<tr>
<td>Unilateral Ejaculatory Duct Obstruction and contralateral CAVD</td>
</tr>
<tr>
<td>Unilateral pelvic vas &amp; Bilateral inguinal Vas (iatrogenic injury)</td>
</tr>
<tr>
<td>Unilateral epididymis</td>
</tr>
<tr>
<td>Unilateral intratesticular tubule with contralateral cryptorchidism</td>
</tr>
<tr>
<td>Unilateral epididymis and contralateral pelvic vas</td>
</tr>
<tr>
<td>Unilateral epididymis and contralateral scrotal vas</td>
</tr>
<tr>
<td>Unilateral epididymis and contralateral CASV**</td>
</tr>
</tbody>
</table>

*CAVD: congenital absence of vas deferens **CASV: congenital agenesis of seminal vesicle
RESULTS: Of 51 consecutive patients, 47 underwent bilateral or unilateral surgical reconstruction, the other four were unable to be treated with surgical reconstruction due to pelvic varus and intratesticular obstruction. Reconstruction rate was 92.2% (47/51), patency rate and natural pregnancy rate were 89.4% (42/47) and 38.1% (16/42) respectively. The incidence of epididymal obstruction and ejaculatory duct obstruction (EDO) were 55.6% and 21.2% respectively.

CONCLUSION: By using multiple advanced surgical techniques, most of acquired obstructive azoospermia could be repaired, a favorable patency and pregnancy rate can be achieved for properly selected patients.

P-152 Tuesday, October 21, 2014

SALVAGE HORMONAL THERAPY AFTER FAILED MICRODISSECTION TESTICULAR SPERM EXTRACTION: A MULTI-INSTITUTIONAL PROSPECTIVE STUDY. K. Shiraiishi, T. Ishikawa, N. Watanabe, T. Iwamoto, H. Matsuyama. Department of Urology, Yamaguchi University, Ube, Yamaguchi, Japan; Reproduction Clinic Osaka, Osaka, Japan; Reproduction Center, Akasaka Sanno Hospital, Tokyo, Japan; Center for Infertility and IVF, International University of Health and Welfare Hospital, Nasushiobara, Tochigi, Japan.

OBJECTIVE: We have reported an hCG-based salvage hormonal therapy for men with nonobstructive azoospermia (NOA) who failed to retrieve sperms by microdissection testicular sperm extraction (micro-TESE) and 20% of men could retrieve sperms at the 2nd micro-TESE (Hum Reprod 2012). To validate the efficacy of the salvage hormonal therapy, a multi-institutional prospective study has been done by recruiting NOA men who could not retrieve sperms with various histologies because our prior study included a number of patients with late maturation arrest and hypospermatogenesis.

DESIGN: A prospective study at reproduction centers.

MATERIALS AND METHODS: NOA men who could not retrieve sperms by micro-TESE and required further treatment, excluding, chromosomal abnormalities, AZFa or b deletions, extremely small testes (less than 2 ml), patients coming from North American institutions, NOA with specific karyotype abnormalities, severe idiopathic azoospermia, or patients coming from North American institutions.

RESULTS: Twenty-one men were eligible to our inclusion criteria and all the participants completed the hormonal treatment without any severe adverse effects. At the first micro-TESE, 13 and 5 patients showed Sertoli cell only or early maturation arrest, respectively. Serum testosterone significantly increased and endogenous gonadotropin secretions significantly decreased after one month of treatment. With the second micro-TESE, sperms were successfully obtained from two patients (9%). Patern age, testicular volume, and hormone profiles did not associated with the results of the 2nd micro-TESE, however, the testicular histologies of the two patients were late maturation arrest and hypospermatogenesis.

CONCLUSION: The effectiveness of hCG-based salvage hormonal therapy preceding a second micro-TESE has been validated for limited cases. No one of the patients was eligible to the second micro-TESE.

P-153 Tuesday, October 21, 2014

VALIDATION OF NOMOGRAMS PREDICTING SEMEN PARAMETERS FOLLOWING VARICOCELE REPAIR. M. K. Sampalis, C. Yu, M. Kattan, H. Yan, K. C. Lo, E. D. Grober, A. Babajia, M. Goldstein, K. Alrabeeah, A. Zini, K. A. Jarvi. Mount Sinai Hospital, University of Toronto, Toronto, ON, Canada; Cleveland Clinic Foundation, Cleveland, OH; Weil Cornell Medical College, New York, NY; McGill University, Montreal, QC, Canada.

OBJECTIVE: We recently developed nomograms using clinical features (age, laterality and grade of varicocele) and pre-varicocele repair semen parameters, to predict semen parameters following varicocele repair. The aim of this study was to externally validate these nomograms using data from patients coming from North American institutions.

DESIGN: Retrospective review of prospectively collected data.

MATERIALS AND METHODS: Complete data from 375 patients undergoing varicocele repairs from 1 American and 2 Canadian centers were available for analysis as a validation cohort. The estimated post-repair semen parameters were generated using the previously developed nomograms and compared with the actual values in the validation cohort. The predictive accuracy was quantified by concordance correlation coefficient and Pearson’s correlation coefficients, and calibration was evaluated.

RESULTS: The semen parameters after varicocele repair were overall similar between the nomogram development and validation arms, with p values for concentration (p=0.153) being not significantly different, and total motile sperm count (TMC) being marginally different, p=0.028. Nomograms (for sperm concentration, motility, morphology and TMC) demonstrated good model predictive performance on the validation data with respect to concordance correlation coefficient and Pearson’s correlation coefficient (Table 1), indicating good reproducibility and generalizability of the nomograms with respect to their predictive value.

Model predictive performances of the validation data.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Concordance correlation coefficient</th>
<th>Pearson correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>0.709</td>
<td>0.729</td>
</tr>
<tr>
<td>Motility</td>
<td>0.549</td>
<td>0.690</td>
</tr>
<tr>
<td>Morphology</td>
<td>0.668</td>
<td>0.704</td>
</tr>
<tr>
<td>TMC</td>
<td>0.490</td>
<td>0.586</td>
</tr>
</tbody>
</table>

CONCLUSION: Our previously published nomograms demonstrate good model predictive performance when validated on a group of patients from different centers. Clinical factors and pre-varicocele repair semen parameters provide substantial ability to predict post-varicocele repair semen parameters. These nomograms may be used by clinicians to predict post-varicocele repair semen parameters. An online predictive tool has been developed for clinicians to better counsel their patients, and is available at: http://www.r-calc.com/calculator.aspx?calculator_id=BIASWNPH.

P-154 Tuesday, October 21, 2014


OBJECTIVE: To examine the rate of inflammatory bowel disease (IBD) in the male fertile patients in Japan and to investigate whether mesalazine known as 5-aminosalicylates has any effects on fertility.

DESIGN: Retrospective analysis.

MATERIALS AND METHODS: After obtaining approval from the institutional review board, we analyzed the records of 1225 male infertile patients who had visited the male infertility clinic at Dokkyo Medical University Koshigaya Hospital between January 2010 and December 2012. We reviewed the cases of IBD in the medical records and evaluated the prevalence of the disease. Specifically, we examined IBD patients who ceased mesalazine during male infertility treatment, and compared the seminogram of these patients before and after discontinuation of mesalazine. We also analyzed pregnancy outcome after discontinuation.

RESULTS: Of 1225 male infertile patients, 2 had Crohn’s disease and 6 had ulcerative colitis at the first visit to our clinic. Therefore, the prevalence rate of Crohn’s disease and ulcerative colitis in our male infertile patients was 163 per 100,000 men and 490 per 100,000 men, respectively. Seven patients had taken mesalazine and 6 of them subsequently stopped the medication. The mean values of sperm concentration, sperm motility, percentage of normal formed sperm, semen volume, and total motile sperm count before discontinuation were all increased after discontinuation of mesalazine. Among these parameters, the sperm motility and total motile sperm count were significantly improved (P < 0.05) after discontinuation.
Comparison of semen parameters before and after discontinuation of mesalazine.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before</th>
<th>After</th>
<th>Change</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration (million/ml)</td>
<td>19.3 ± 15.5</td>
<td>43.3 ± 55.9</td>
<td>24.0 ± 61.3</td>
<td>NS</td>
</tr>
<tr>
<td>Motility (%)</td>
<td>31.2 ± 10.1</td>
<td>48.3 ± 15.7*</td>
<td>17.2 ± 11.9</td>
<td>0.03</td>
</tr>
<tr>
<td>Normal form (%)</td>
<td>52.8 ± 15.1</td>
<td>63.3 ± 10.3</td>
<td>10.5 ± 19.0</td>
<td>NS</td>
</tr>
<tr>
<td>Volume (mL)</td>
<td>2.25 ± 0.42</td>
<td>2.40 ± 0.52</td>
<td>0.15 ± 0.43</td>
<td>NS</td>
</tr>
<tr>
<td>TMC (million)</td>
<td>14.2 ± 13.2</td>
<td>64.0 ± 88.4*</td>
<td>49.7 ± 91.3</td>
<td>0.03</td>
</tr>
</tbody>
</table>

NS, not significant; TMC, total motile sperm count. Changes are expressed as values after discontinuation minus values before discontinuation of mesalazine. *Statistically significant

Of the 6 patients who stopped the drug, 4 achieved pregnancy with their partners, with 2 of the 4 men showing significant improvement in not only the percentage of normal shaped sperm but also sperm motility and total motile sperm count.

CONCLUSION: In Japan, the prevalence of IBD is higher in male infertility patients than in the general population. Mesalazine may have gonadotoxic effects on fertility in men with IBD.

P-155 Tuesday, October 21, 2014

ABSTRACT WITHDRAWN

P-156 Tuesday, October 21, 2014

BENEFICIAL EFFECT OF MICROSURGICAL VARICOCELECTOMY ON SEMEN PARAMETERS AND CLINICAL OUTCOME IN SEVERE MALE FACTOR INFERTILITY. T. Takeuchi, K. Honda, Y. Mori, N. Aono, Y. Nakaio,a K. Kyono,a,b

OBJECTIVE: Microsurgical varicocelectomy is considered as an effective treatment option for infertile men with clinical varicoceles. The objective of this study was to assess the effect of surgical repair of varicoceles on spermatogenesis and clinical outcome of infertile couples, with a particular focus on severe male factor infertility.

DESIGN: Comparison of semen parameters before and after varicocelectomy and assessment of clinical outcome.

MATERIALS AND METHODS: A total of 37 male infertile patients with either grade II (48.6%) or III (51.4%) clinical varicoceles underwent microsurgical varicocelectomy. Their pre-operative semen analyses revealed impaired spermatogenesis with at least one semen parameter compromised. Ejaculate samples were obtained and semen analysis was performed before and 3 months following the surgical repair. In some couples, clinical outcome was assessed as long as follow-up data was available.

RESULTS: The average paternal age was 35.6 ± 6 years. Prior to the surgery, average total motile sperm count was 14.4 ± 19 million, while 3 months after it was remarkably improved to 35.2 ± 50 million (p < 0.01). Total of 15 patients with less than 5 million motile sperm were categorized as severe male factor infertility. Their average total motile sperm count was significantly improved from 1.3 ± 1 million to 18.8 ± 20 million (p < 0.01), with 7 (46.7%) being over 15.6 million (WHO normal limit). Among these patients, two were non-obstructive azoospermic (NOA), and 4 were cryptozoospermic men and considered as most severe cases. After varicocelectomy, a few spermatozoa was found in ejaculates in one NOA patient, while in 4 cryptozoospermic cases, 3 (75%) had more than 2.9 million motile sperm in their ejaculates, 1 became normal normozoospermic and 1 with 9.8 million motile sperm underwent conventional IVF and his wife successfully conceived. Overall, in 19 couples with follow-up data available, 8 (42.1%) became pregnant in a 6 months period after the surgery.

CONCLUSION: In severe forms of male infertility, simply classified as ICSI indication, surgical repair of varicoceles appears to improve semen parameters often reaching to even normal limits. Particularly in severely compromised spermatogenesis with extreme low sperm count, such as azoospermia or cryptozoospermia, by varicocelectomy some patients can avoid unnecessary testicular sperm retrieval or even ICSI.

Baseline vs. 5 months Post-CC Therapy

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>5 months</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH (mIU/mL)</td>
<td>6.3 ± 1.2</td>
<td>9.6 ± 2.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LH (mIU/mL)</td>
<td>5.0 ± 0.5</td>
<td>7.1 ± 0.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TT (ng/dL)</td>
<td>284.0 ± 14.0</td>
<td>501.3 ± 27.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>BT (ng/dL)</td>
<td>167.8 ± 8.6</td>
<td>300.6 ± 25.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>E (pg/mL)</td>
<td>24.0 ± 1.3</td>
<td>39.0 ± 3.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>PSA (ng/mL)</td>
<td>0.60 ± 0.04</td>
<td>0.77 ± 0.12</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Sperm Concentration (M/mL)</td>
<td>21.3 ± 3.4</td>
<td>23.3 ± 5.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TMC (M)</td>
<td>31.4 ± 5.6</td>
<td>47.6 ± 17.3</td>
<td>0.05</td>
</tr>
</tbody>
</table>

A positive trend was seen in total motile count (TMC), from 31.4 Million to 47.6 Million (p=0.05). Four of 59 men (6.8%) reported minor headaches during the 1st month of CC therapy, which subsequently resolved. Twelve of 38 subjects (31.6%) achieved pregnancy, including 5/8 (62.5%) conceiving through in vitro fertilization, 5/11 (45.5%) through intrauterine insemination and 2/19 (10.5%) through natural intercourse.

CONCLUSION: Significant improvement in testosterone levels and sperm concentration is seen with the use of clomiphene citrate in the treatment of the obese, subfertile male. Clomiphene citrate is well tolerated with minimal side effects in obese male subjects. Concomitant clomiphene citrate therapy in conjunction with assisted reproductive techniques appears to be associated with successful conception.

P-157 Tuesday, October 21, 2014

EFFICACY AND TOLERABILITY OF CLOMIPHENE CITRATE IN THE TREATMENT OF OBSESE, SUBFERTILE MALES. D. Shin,a P. Khandge,a,b Urology, Hackensack University Medical Center, Hackensack, NJ; aUrology, Rutgers New Jersey Medical School, Newark, NJ.

OBJECTIVE: Elevated body mass index has been shown to be negatively correlated with total testosterone (TT) and bioavailable testosterone (BT) levels and semen parameters in infertile males. Clomiphene citrate (CC), a selective estrogen receptor modulator, is used in the empirical treatment of subfertile males to increase endogenous testosterone levels and improve semen concentration. We sought to study the efficacy and tolerability of CC for treatment of subfertile males who are obese (BMI>30 kg/m²).

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: This is a retrospective cohort study of 59 obese, subfertile men who were treated with CC between 2009 and 2014. FSH (follicle stimulating hormone), LH (luteinizing hormone), TT, BT, E (estradiol), and PSA (prostate specific antigen) levels were recorded at baseline and measured at 1, 3 and 5 months during therapy. Follow-up semen analysis or pregnancy status was recorded when available. Paired t-test analysis was used to compare pre- and post-treatment biochemical and semen parameters.

RESULTS: The study included 59 men, mean age 35.8±0.8 years (SEM). After 5 months of CC therapy, FSH, LH, TT, BT, E, PSA and sperm concentration increased significantly (p<0.05).

Baseline vs. 5 months Post-CC Therapy

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>5 months</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH (mIU/mL)</td>
<td>6.3 ± 1.2</td>
<td>9.6 ± 2.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LH (mIU/mL)</td>
<td>5.0 ± 0.5</td>
<td>7.1 ± 0.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TT (ng/dL)</td>
<td>284.0 ± 14.0</td>
<td>501.3 ± 27.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>BT (ng/dL)</td>
<td>167.8 ± 8.6</td>
<td>300.6 ± 25.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>E (pg/mL)</td>
<td>24.0 ± 1.3</td>
<td>39.0 ± 3.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>PSA (ng/mL)</td>
<td>0.60 ± 0.04</td>
<td>0.77 ± 0.12</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Sperm Concentration (M/mL)</td>
<td>21.3 ± 3.4</td>
<td>23.3 ± 5.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TMC (M)</td>
<td>31.4 ± 5.6</td>
<td>47.6 ± 17.3</td>
<td>0.05</td>
</tr>
</tbody>
</table>

A positive trend was seen in total motile count (TMC), from 31.4 Million to 47.6 Million (p=0.05). Four of 59 men (6.8%) reported minor headaches during the 1st month of CC therapy, which subsequently resolved. Twelve of 38 subjects (31.6%) achieved pregnancy, including 5/8 (62.5%) conceiving through in vitro fertilization, 5/11 (45.5%) through intrauterine insemination and 2/19 (10.5%) through natural intercourse.

CONCLUSION: Significant improvement in testosterone levels and sperm concentration is seen with the use of clomiphene citrate in the treatment of the obese, subfertile male. Clomiphene citrate is well tolerated with minimal side effects in obese male subjects. Concomitant clomiphene citrate therapy in conjunction with assisted reproductive techniques appears to be associated with successful conception.

P-158 Tuesday, October 21, 2014

GENE EXPRESSION PATTERNS IN NON-OBSTUCTIVE AZOOSPERMIC MEN WITH VARICOCELES REVEAL UNIQUE PATIENT-SPECIFIC CHARACTERISTICS. J. R. Kovač,a,b C. Cengiz,a J. Addai,a L. I. Lipshultz,a D. J. Lamb,a Urology, Baylor College of Medicine, Houston, TX; aUrology of Indiana, Carmel, IN.

OBJECTIVE: Varicoceal repair in men with NOA can result in improved spermato genesis. The genetic difference between NOA men with and without varicoceles has never been reported. Results may yield important information about the nature of the testicular changes seen in

FERTILITY & STERILITY® e191
these two populations of NOA men and what men may benefit from surgical correction.

DESIGN: Tissues and blood were obtained from men with NOA (n=16) and subdivided into those with varicoceles (n=9) and those without (n=7).

MATERIALS AND METHODS: Gene expression microarray (Agilent SurePrint G3 8x60K) screened for gene expression with candidate genes identified using Ingenuity Pathway Analysis (IPA) software. Results were validated using qPCR.

RESULTS: NOA men with, and without varicoceles had similar ages (34±0.4 vs. 32±2 years) and testicular volumes (Left, 15±2 vs. 13±1 mL; Right, 14±2 vs. 13±1 mL). Serum levels for FSH (20±7 vs. 22±4 mIU/L), LH (7±1 vs. 8±1 mIU/L) and testosterone (313±43 vs. 296±30 ng/dL) were not different. IPA revealed 39 genes preferentially expressed in men with varicoceles (IPA threshold=12 fold) while network plotting identified ‘Cellular’ and ‘Reproductive System Development’ as the most perturbed bio-function in NOA men with varicoceles. Expression of top candidates was validated using qPCR with fold change (FC; relative to control) for genes involved in apoptosis (PLAT, FC=2.1±1.1; CAV1, FC=1.5±0.2; VASN, FC=0.3±0.7), hypoxic angiogenesis (ANGPTL4, FC=-2.4±0.7; UXT, FC=-0.6±0.5) and spermatogenesis (MEA1; FC=0.2±0.5). When sub-categorized by histological subtype (i.e. hypoxic spermatogenesis, maturation arrest, Sertoli Cell Only), distinct patient-specific expression patterns were observed suggesting unique gene expression contributes to varicocele pathogenesis. IPA data-filteration revealed the serum biomarkers CAV1 and PLAT could be important in differentiating these patient populations.

CONCLUSION: The current study has identified numerous genes associated with the presence of varicoceles in men with NOA. Unique patient-specific expression patterns suggest varicocele pathogenesis is typically multifactorial.

Supported by: Supported by a Male Reproductive Health Research Career (MRHR) Development Physician Scientist Award (K12) (HD073917-01) from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) Program and by NIH grants P01HD036289 from the Eunice Kennedy Shriver NICHD and 1ROI1DK078121 from the National Institute of Kidney and Digestive Diseases.

P-159 Tuesday, October 21, 2014

THE POTENTIAL APPLICATION OF URINE DERIVED STEM CELLS IN MALE INFERTILITY: G. Liu,¹ T. Li,² J. Zhang,³ X. Yang,³ X. Liang,³ Y. Zhang.¹ ¹Center for Reproductive Medicine, Sixth Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China; ²Wake Forest Institute for Regenerative Medicine, Winston Salem, NC.

OBJECTIVE: Urine derived cells can be obtained non-invasively and may represent a potentially significant source of autologous cells for tissue engineering. One goal of this study was to test the hypothesis that the cell population obtained from urine contains cells that meet the defining criteria of stem cells (self-renewal and multipotency). In particular, the ability of these cells to give rise to induced pluripotent stem cells (iPS) for the potential sperm progenitor cell generation was tested.

DESIGN: Human urine derived cells were characterized and induced into iPS.

MATERIALS AND METHODS: Human urine derived cells from 9 individual donors (ages 5 to 40 years) were plated on multi-well plates. Single cell growth was monitored using time-lapse microcinematography. The cells were analyzed for expression of canonical reprogramming factors and pericytes/mesenchymal stem cell (MSC) markers. Expression of telomerase in the isolated cells was assessed by ELISA. Next, urine derived cells were cultured in various induction media for 21 days and assessed for evidence of differentiation into various cell types, including adipocytes, osteocytes, chondrocytes, SMC, and UC. USC's were induced into iPS by polyacrylamide lentiviral vector encoding human Oct-3/4, Sox2, Klf4 and c-Myc (OSKM).

RESULTS: Some urine derived cells grew rapidly from a single cell clone for over 25 population doublings. Six of seven independent clones of urine derived cells expressed detectable levels of telomerase. USC's express the canonical reprogramming factors c-myc and klf4, and positive for pericytes/ MSC markers such as CD146, NG2 and PDGF-receptor beta. When placed in appropriate induction media, these cells differentiated towards adipogenic, osteogenic and chondrogenic lineages. Pluripotency of urine-derived iPS clones was confirmed by immunocytochemistry, RT-PCR and teratoma formation.

CONCLUSION: Urine-derived cells expressed the phenotypic features of pericytes/MSC, including self-renewal and multipotency. One urine-derived cell clone can differentiate to multiple cell lineages. These results demonstrate the feasibility of generating iPS from a urine sample and that urine-derived iPS might be further exploited for potential sperm progenitor cells generation study.

P-160 Tuesday, October 21, 2014

ASSOCIATION OF SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) IN METHYLENETETRAHYDROFOLATE REDUCTASE (MTHFR) AND MALE INFERTILITY. R. Ng, K. Louie, K. Poon, V. Chow, S. Ma. Department of Obstetrics and Gynaecology, University of British Columbia, Vancouver, BC, Canada.

OBJECTIVE: To determine whether C677T SNPs in the MTHFR gene are associated with an increased risk of infertility in a Canadian population.

DESIGN: We compared the frequencies of the SNPs obtained from genotyping the MTHFR gene in men with oligospermia, azoospermia, and fertile men. We also investigated the possible risk of infertility in different ethnic populations.

MATERIALS AND METHODS: Peripheral blood samples were obtained from oligospermic (N=17) and azoospermic (N=22) patients. Blood samples for controls (N=19) were from men who had fathered a child within the previous year and from men with proven fertility undergoing vasectomy reversals. DNA was extracted from blood using the Gentra Puregene Blood Kit (Qiagen) according to their protocol. Polymerase chain reaction (PCR) was used to amplify the region of interest at MTHFR. Restriction fragment length polymorphism (RFLP) with HinfI enzyme (New England Biolabs) was used on the amplified DNA to digest the DNA according to its genotype. Following RFLP, the samples were electrophoresed on a 3% gel stained with SYBR safe (Invitrogen) to separate the fragments and the bands were visualized using a UV illuminator. Fisher’s exact test was used to determine significance between groups.

RESULTS: Frequencies of the wild type 677CC genotype and the 677CT/677TT genotypes associated with infertility in fertile controls were 42.1%, 26.3%, and 31.6%; 70.6%, 23.5%, and 5.9% in oligospermic men; and 45.5%, 45.5%, and 9.1% in azoospermic men, respectively. Comparing 677CT polymorphism, controls vs. azoospermia (P=0.330) and controls vs. oligospermia (P=1.000) were not significant. Comparing 677TT polymorphism, controls vs. azoospermia (P=0.115) and controls vs. oligospermia (P<0.092) were not significant. Ethnicity data were available for 15 oligospermic (11 Asian, 4 Caucasian) and 12 azoospermic men (4 Asian, 8 Caucasian). Comparing Asian vs. Caucasian populations, the 677CT polymorphism (P=1.000) and the 677TT polymorphism (P=1.000) were not significant.

CONCLUSION: There does not seem to be a correlation between 677CT and 677TT polymorphisms and an increased risk of oligospermia or azoospermia. Furthermore, these polymorphisms are not significantly different in infertile Asian vs. infertile Caucasian populations, contrary to current literature. More cases in all the groups are necessary to further the investigation.

Supported by: This study was Supported by the Canadian Institutes of Health Research (CIHR, grant to Sai Ma).

P-161 Tuesday, October 21, 2014

ASSOCIATION OF RS3129878 AND RS498422 IN THE HLA REGION WITH NON-OBSTRUCTIVE AZOOSPERMIA IN THE HAN CHINESE POPULATION. S. Zou,¹ P. Song,¹ T. Chen,² J. Chen,² X. He,² P. Xu,² M. Liang,² K. Luo,² X. Zhu,² E. Tian,² Q. Du,² Z. Wen,² Z. Li,³ M. Wang,³ Y. Sha,³ Y. Cao,³ Y. Shi,³ Z. Li,³ H. Hu.¹ ¹Department of Urology, Shanghai, China; ²BIO-X Center, Shanghai, China; ³Reproduction Center, Hefei, Anhui, China; ⁴Reproduction Center, Shenyang, Liaoning, China; ⁵Second Affiliated Hospital of Shandong, Jinan, Shandong, China; ⁶Reproduction Center, Nanning, Guangxi, China; ⁷Reproduction Center, Nanchang, Jiangxi, China; ⁸Sheng Jing Hospital of China Medical University, Shenyang, Liaoning, China; ⁹Xiamen Women and Children Health Care Hospital, Xiamen, Fujian, China.

ASSOCIATION OF RS3129878 AND RS498422 IN THE HLA REGION WITH NON-OBSTRUCTIVE AZOOSPERMIA IN THE HAN CHINESE POPULATION. S. Zou,¹ P. Song,¹ T. Chen,² J. Chen,² X. He,² P. Xu,² M. Liang,² K. Luo,² X. Zhu,² E. Tian,² Q. Du,² Z. Wen,² Z. Li,³ M. Wang,³ Y. Sha,³ Y. Cao,³ Y. Shi,³ Z. Li,³ H. Hu.¹ ¹Department of Urology, Shanghai, China; ²BIO-X Center, Shanghai, China; ³Reproduction Center, Hefei, Anhui, China; ⁴Reproduction Center, Shenyang, Liaoning, China; ⁵Second Affiliated Hospital of Shandong, Jinan, Shandong, China; ⁶Reproduction Center, Nanning, Guangxi, China; ⁷Reproduction Center, Nanchang, Jiangxi, China; ⁸Sheng Jing Hospital of China Medical University, Shenyang, Liaoning, China; ⁹Xiamen Women and Children Health Care Hospital, Xiamen, Fujian, China.
OBJECTIVE: The previous genome-wide association study (GWAS) of non-obstructive azoospermia (NOA) in the Han Chinese populations identified two NOA-risk loci (rs498422 and rs3129878) within the HLA Region, and provided strong evidence for the genetic influence of male infertility. A further case-control study found that only rs3129878 remained to be significantly associated with NOA in the Japanese population. Therefore, we conducted the association study to further validate whether the risk of these two SNPs still existed in an independent Han Chinese male population, consisting of 550 NOA cases and 555 normal controls.

DESIGN: A case-control study.

MATERIALS AND METHODS: These two SNPs were analyzed in 550 NOA patients and 555 controls of Chinese origin using direct sequencing. Then, the genotype and allele distributions of them were further analyzed using the online software SHEsis (http://analysis.bio-x.cn).

RESULTS: The association studies strongly supported the significant association of rs498422 and rs3129878 with NOA for both genotype and allele distributions (p=0.047 and p=1.87 x 10-10, respectively).

CONCLUSION: In our replication study of Chinese samples, we provided genetic evidence for these two NOA-risk SNPs within the HLA genes region, contributing to predicting males at high risk of NOA in Han Chinese population. Considering genetic differences among populations, future validating studies in independent samples are suggested.

Supported by: The work was Supported by the National Natural Science Foundations of China [grant number 30973069, 30672146] and the Foundation of Pujiang [grant number 08PJ14100].

P-162 Tuesday, October 21, 2014

UP REGULATION OF MIR-630 BY HEAT SHOCK INHIBITS SERTOLI CELL PROLIFERATION, MIGRATION AND IMMATURE SERTOLI CELL MARKER EXPRESSION. Y.-M. Lin, C.-W. Lu, H.-Y. Ma. Urology, National Cheng Kung University College of Medicine and Hospital, Tainan, Taiwan.

OBJECTIVE: Our previous study has demonstrated that up-regulation of miR-630 by heat shock leads to decreased SOX30 expression in spermatogenesis. Since miR-630 and SOX30 have been shown to be expressed in the Sertoli cells and heat shock affects the morphology and function of Sertoli cells, this study was conducted to explore the impacts of miR-630 on Sertoli cell function.

DESIGN: The effects of miR-630 on Sertoli cell proliferation, migration and the expression of immature and mature Sertoli cell markers were investigated in vitro.

MATERIALS AND METHODS: Human Sertoli cells and HeLa cells were treated with different doses and combinations of miR-630 mimic and miR630 inhibitor, and then the cells were subjected to cell proliferation assay by using a CellTiter 96 AQueous One Solution Cell Proliferation Assay kit. Human Sertoli cells were treated with different doses of miR630 mimic or SOX30, and then the cells were subjected to cell migration assay by using Transwell migration assay. The effects of heat shock and miR-630 on the expressions of immature (cytokeratin 18 and M2A) or mature (SGP2, Laminin and p27Kip1) markers of Sertoli cells were investigated by qRT-PCR.

RESULTS: miR-630 mimic treatment at both 20 nM and 40 nM significantly decreased human Sertoli cell and HeLa cell proliferations, and this effect could be partially recovered by the addition of miR-630 inhibitor. Similarly, miR-630 mimic treatment at both 20 nM and 40 nM significantly decreased human Sertoli cell migration, and this effect could be partially recovered by the addition of miR-630 inhibitor or SOX30. In addition, SOX30 could significantly induce human Sertoli cell migration. After heat shock or miR-630 mimic treatment, the cytokeratin 18 was significantly decreased by heat shock at 24 hours; however, no significant change was found in the expressions of M2A, SGP2, Laminin and p27Kip1.

CONCLUSION: Upregulation of miR-630 by heat shock significantly inhibit Sertoli cell proliferation, migration and immature Sertoli cell marker expression, which may contribute to one of the causes of spermatogenic failure.

Supported by: This work was Supported by the National Science Council of Taiwan (NSC 99-2628-B-006-019-MY3).

P-163 Tuesday, October 21, 2014

SEMN-DERIVED AMYLID FIBRILS AFFECT SPERMATOZOA MOTILITY AND FERTILIZATION. N. L. Sandi-Monroy, S. M. Usmani, N. N. Roan, A. Gawanbacht, O. Sakk, T. Wirth, F. Gagstein, F. Kirchhoff, J. Munch. Institute of Molecular Virology, Ulm University Medical Center, Ulm, BW, Germany; Kinderwunsch-Zentrum Ulm, Ulm, BW, Germany; Department of Urology, University of California at San Francisco, San Francisco, CA; J. David Gladstone Institutes, San Francisco, CA; Institute of Physiological Chemistry, Ulm University, Ulm, BW, Germany.

OBJECTIVE: Semen contains amyloid fibrils derived from fragments of prostatic acid phosphatase (PAP) and semenogelin (SEMs) that potentially increase human immunodeficiency virus infection1,2. The purpose of this study was to evaluate the effect of seminal amyloid on spermatozoa function.

DESIGN: The effect of monomeric and fibrillar forms of PAP248-286 and SEM peptides entrapped in vitro fertilization (IVF). Human semen samples were obtained from patients undergoing infertility treatment after informed consent. The project registration number was O.185.

MATERIALS AND METHODS: Isolated human and mouse spermatozoa were incubated with monomeric peptides or the respective fibrils and analyzed by confocal imaging or video microscopy over time. For IVF, mouse spermatozoa were incubated with increasing fibril concentrations, and oocytes isolated from superovulated mice were added. Fertilization rates were determined by counting the number of 2-cell embryos. One way analysis of variance was used to compare groups.

RESULTS: Amyloid fibrils formed by PAP248-286 and SEM peptides entrap spermatozoa in a dose and time-dependent manner without exerting cytotoxic effects. This entrapment resulted in a loss of progressive motility that may have implications in fertility. Spermatozoa immobilization was dependent on the cationic properties of the fibrils, since treatment with heparin, or use of non-cationic amyloid-beta fibrils, did not immobilize the sperm. IVF studies with spermatozoa treated with increasing concentrations of fibrils revealed that concentrations of fibrils above 50 mg/ml resulted in significantly fewer 2-cell embryos 24 hours after IVF (p<0.05). At 250 mg/ml, fertilization was completely inhibited.

CONCLUSION: Our findings suggest that mature amyloid fibrils in semen may have patho-physiological relevance for infertility in men when present at high concentrations in the ejaculate.

Supported by: This work was Supported by the DFG to J.M. (Ma 3115/2-1) and S.M.U. (US116/1-1).

P-164 Tuesday, October 21, 2014

THE SEMINAL PLASMA PROTEOME REFLECTS ALTERATION IN SPERMATOGENESIS. P. Intasqui, M. Camargo, M. Antoniassi, K. H. M. Cardozo, V. M. Carvalho, D. S. Zylbersztajn, R. P. Bertolla. Department of Urology, University of California, San Francisco, CA; Human Reproduction Section, Division of Urology, Sao Paulo Federal University, Sao Paulo, Brazil; Fleury Group, Sao Paulo, Brazil.

OBJECTIVE: To analyze seminal plasma protein profile associated with sperm functional aspects.

DESIGN: Cross sectional study including 156 normozoospermic patients.

MATERIALS AND METHODS: Sperm functional tests were performed: mitochondrial activity, acrosome integrity, and DNA fragmentation, by a Comet assay. Groups were: (i) Normal (bottom 15%, control group, n=26) x high (top 15%, altered group, n=23) mitochondrial alteration; (ii) High (control group, n=23) x Low (altered group, n=22) acrosome integrity; and (iii) Low (control group, n=22) x High (altered group, n=22) DNA fragmentation. The remaining semen was centrifuged and the seminal plasma was utilized for proteomics analysis using label-free quantification (LC-MS/MS). For identification of potential biomarkers, a multivariate statistical analysis was performed.

RESULTS: Mitochondrial activity (%) altered was 5.9 ± 1.42 in controls and 22.4 ± 4.12 in altered group. Acrosome integrity (%) normal
was 86.2 ±4.01 in controls, and 63.2 ±3.54 in altered group. Sperm DNA fragmentation was 26.2 ±2.72 in controls and 63.9 ±4.99 in the altered group. In total, 571 proteins were quantified. Four proteins were absent, 36 were downregulated, 3 were exclusive, and 61 were overexpressed in the altered mitochondrial activity group, with the following enriched functions: endopeptidases inhibition, glycosaminoglycans catabolism, and immune response. Five proteins were absent, 22 were downregulated, 2 were exclusive, and 47 were overexpressed in the altered acrosome integrity group. Enriched functions were lysosomal transport, exocytosis, arachidonic acid metabolism, response to hydrogen peroxide, and phospholipase inhibition. Four proteins were absent, 104 were down-regulated, 3 were exclusive, and 23 were overexpressed in increased DNA fragmentation. The enriched functions were prostaglandin synthesis and fatty acid binding, and alterations in carbohydrate metabolism, lipoprotein regulation, and apoptosis. The suggested biomarkers are: mitochondrial activity: ACBP, ANX7, BPIFB1, CST2, ERP44, GSTM3, and HEXA; acrosome integrity: COL12A1, LCN15, PLTP, S100A10, SHISA5, and ZNF236; and DNA fragmentation: CRIPSLD1, CRIPSLD2, APOLD1, RARRES1, SPAG11B, SERPINF2, EDIL3, and PSMA5.

CONCLUSION: Seminal plasma protein profile and post-genomic pathways are associated to sperm functional quality, with the detection of several proteins related to sperm damage and apoptosis.

Supported by: FAPESP (process 2011/14631-7) and CNPq (472941/2012-7).

A COMPARISON OF REPORTED MALE REPRODUCTIVE HORMONE REFERENCE RANGES IN THE UNITED STATES. M. Le, D. Flores, E. Gourley, D. May, A. K. Nangia. Dept of Urology, University of Kansas Medical Center, Kansas City, KS.

OBJECTIVE: Reference values for male reproductive hormones are poorly defined as they relate to male reproductive dysfunction. The objective was to compare reported reference ranges and testing methods of follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), prolactin (PR), free and total testosterone (FT & TT) by clinical laboratories in the US.

DESIGN: Cross-sectional study.

MATERIALS AND METHODS: Upper and lower reference values, assay methodology and source of published reference ranges were obtained from 2-3 laboratories from each state across the USA. A standardized questionnaire was reviewed with laboratory technicians and supervisors by direct phone contact. Descriptive statistics were used.

RESULTS: 122 independent & hospital based laboratories from 45 states were surveyed: 14 labs sent all hormone assays to larger reference labs, such as ARUP, Quest Diagnostics, & LabCorp. Of those remaining, 103 had complete data on FSH, LH, E2 on E2, 97 on PR, 115 on FT, and 114 on TT. Reference values are tabulated. The majority of labs measured their hormones in-house on high throughput analyzers. All but one lab determined their hormone reference values for FSH & LH. Reported reference ranges for each hormone often came from the instrument's reference package insert values. These ranges were often validated using a local but small test group with limited known demographic information. A greater percent of labs sent out FT to large reference labs for measurement, 94/115 (82%), compared to the other hormones. 70% of labs used immunoassay technique to measure TT & only 23% age stratified their TT reference ranges.

CONCLUSION: There is a wide difference in reference ranges among commonly evaluated hormones in the evaluation of male reproductive dysfunction as it relates to known literature on normal hormonal/testicular function. Our study demonstrates that reported reference values are largely based on small population studies of men with undefined sexual or reproductive function. To create a reference standard, an appropriate comparison group with defined normal sexual and reproductive function must be developed.

P-166 Tuesday, October 21, 2014

HARMFUL EFFECTS OF SMOKING TO MALE FERTILITY. M. Antoniassi, P. Intasqui, M. Camargo, D. S. Zyibersztejn, A. P. Cedenho, R. F. Bertolla. Human Reproduction Section, Division of Urology, Sao Paulo Federal University, Sao Paulo, Brazil.

OBJECTIVE: To verify the effects of smoking on sperm functional aspects and seminal plasma lipid peroxidation levels in patients with and without varicocele.

DESIGN: Cross-sectional study.

MATERIALS AND METHODS: Semen was analyzed according to the WHO 2010. The study included 17 controls (non-smokers without varicocele), 17 smokers without varicocele (S group), 24 non-smokers with varicocele (V group), and 22 smokers with varicocele (SV group). Mitochondrial activity was analyzed by a colorimetric stain (DAB I – all mitochondria active to DAB IV – all inactive); acrosome integrity was evaluated by a fluorescent stain (PNA-FITC) and lipid peroxidation was assessed (TBARS). Groups were compared using one-way ANOVA, followed by a Tukey’s HSD test.

RESULTS: In the V group, 22 presented bilateral grade II varicocele and 2 presented left grade II and right grade I varicocele, and in the SV group, 18 presented bilateral grade II varicocele and 4 presented left grade II and right grade I varicocele. Semen and sperm functional analysis results are presented in table 1.

Semen and sperm functional analysis and lipid peroxidation levels. Data are mean ±standard deviation.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>S group</th>
<th>V group</th>
<th>SV group</th>
<th>P (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Count</td>
<td>354.5 ±307.0</td>
<td>163.8 ±116.1</td>
<td>283.5 ±154.4</td>
<td>199.1 ±198.2</td>
<td>0.026</td>
</tr>
<tr>
<td>Proactive Motility (%)</td>
<td>54.2 ±9.7 a, b</td>
<td>46.6 ±14.6 a</td>
<td>53.6 ±8.9 a</td>
<td>41.2 ±15.4 b</td>
<td>0.003</td>
</tr>
<tr>
<td>Morphology (% normal)</td>
<td>6.9 ±2.6 a, b</td>
<td>5.6 ±3.5 a, b</td>
<td>7.9 ±2.8 b</td>
<td>4.9 ±3.6 a</td>
<td>0.009</td>
</tr>
<tr>
<td>Neutrophils (x106/ml)</td>
<td>0.1 ±0.1 a, b</td>
<td>0.2 ±0.5 a, b</td>
<td>0.1 ±0.1 b</td>
<td>0.7 ±1.4 a</td>
<td>0.023</td>
</tr>
<tr>
<td>PNA (%)</td>
<td>77.1 ±7.1 a</td>
<td>67.9 ±8.8 a</td>
<td>72.8 ±10.7 a</td>
<td>64.8 ±12.2 b</td>
<td>0.002</td>
</tr>
<tr>
<td>DAB I (%)</td>
<td>16.5 ±13.7 a</td>
<td>8.7 ±14.4 b</td>
<td>16.5 ±14.9 a</td>
<td>4.2 ±5.5 b</td>
<td>0.004</td>
</tr>
<tr>
<td>DAB II (%)</td>
<td>57.7 ±10.8 a</td>
<td>44.6 ±17.7 b</td>
<td>58.2 ±12.6 a</td>
<td>50.1 ±15.4 a</td>
<td>0.008</td>
</tr>
<tr>
<td>DAB III (%)</td>
<td>20.9 ±11.0 a</td>
<td>35.5 ±14.7 b</td>
<td>20.6 ±6.2 a</td>
<td>34.6 ±13.9 b</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>DAB IV (%)</td>
<td>4.8 ±10.0 a</td>
<td>11.0 ±7.0 b</td>
<td>4.7 ±2.7 a</td>
<td>11.1 ±6.3 b</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TBARS (ng/ml)</td>
<td>0.4 ±0.3 a</td>
<td>1.4 ±1.6a, b</td>
<td>0.5 ±0.3a, b</td>
<td>2.9 ±4.5 b</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Different letters in a same line indicate significant difference in a post-hoc test.

CONCLUSION: In men with varicocele, smoking is associated to important alterations in semen quality (motility, morphology, and neutrophil count), sperm function (mitochondrial activity and acrosome integrity), and semen oxidative stress. However, it is noteworthy that men without...
varicoceles are at a greater risk for sperm alterations than men with varicoceles who do not smoke, indicating an important environmental component to male infertility.

Supported by: CNPq (160857/2012-0) (Brazil).

P-167 Tuesday, October 21, 2014

METHYLATION ANALYSIS OF TESTIS AND BRAIN SPECIFIC GENES ON THE X CHROMOSOME IN KLINEFELTER SYNDROME (KS) PATIENTS PROVIDES INSIGHT INTO PARTIAL ANDROGEN RESISTANCE AND BEHAVIORAL ISSUES IN MEN WITH KS. A. Melnik,1 A. Mehta,1 L. J. Dow,2 M. Funaro,3 P. N. Schlegel,4 D. A. Paduch,5 “Well Cornell Medical College, New York, NY; 6Emory University School of Medicine, Atlanta, GA.

OBJECTIVE: This study examined the inactivation status of genes spanning the X chromosome in Klinefelter Syndrome (KS) patients. We hypothesized that gene inactivation further from the X chromosome inactivation center will be less stringently controlled, inducing the wide phenotypic spectrum seen in KS. Our analysis focused on the inactivation status of 2 genes important to Androgen Receptor (AR) signaling, AR and Filamin A (FLNA), to assess the reasons for partial androgen resistance and underdetermination in KS.

DESIGN: We executed a cohort analysis of gene inactivation from methylated on the X chromosome, comparing KS to non-KS patients.

MATERIALS AND METHODS: We chose 8 genes spanning the entire X chromosome for methylation analysis, selecting genes expressed in the testis and brain: VCX, FAM9A, DDX5, AR, XIST, ESX1, GLUD2, FLNA. Methylated and unmethylated primer pairs for each gene were designed using Methyl Primer Express and MethPrimer. Methylation specific PCR was performed on bisulfite treated DNA extracted from five 47, XXY males, five Methyl Primer Express and MethPrimer. Methylation specific PCR was performed on bisulfite treated DNA extracted from five 47, XXY males.

RESULTS: XIST followed a similar inactivation pattern in KS patients and females, with one copy of XIST methylated (inactive), the other unmethylated (active). The ratio of methylated to unmethylated was measured using qRT-PCR and Western Blot, respectively. The ratio of methylated to unmethylated products was measured.

RESULTS: XIST followed a similar inactivation pattern in KS patients and females, with one copy of XIST methylated (inactive), the other unmethylated (active). The ratio of methylated to unmethylated was measured using qRT-PCR and Western Blot, respectively. The ratio of methylated to unmethylated products was measured.

CONCLUSION: Our analysis of the methylation status of 8 genes on the X chromosome found clear differences in the inactivation patterns in KS patients. Hypermethylation of FLNA may explain the partial androgen resistance in KS, providing possible new treatment avenues for KS. Activation of DDX5 in KS suggests an underlying cause of developmental issues in KS, but this hypothesis requires further study.

Supported by: Robert S Dow Foundation. Lindsay Dow was Supported by Eric M. Smith scholarship in Andrology and Business.

P-168 Tuesday, October 21, 2014

MICROFLUIDIC ISOLATION OF SPERM FOR MICRO-TESTICULAR SPERM EXTRACTION (MTESE). K. Murphy,1 J. Son,1 J. Hotaling,2 B. Gale,3 D. Carrell,4 “Andrology, University of Utah, Salt Lake City, UT; 2Mechanical Engineering, University of Utah, Salt Lake City, UT.

OBJECTIVE: The purpose of this study is to develop a system for separating limited numbers of non-motile human sperm from somatic testicular cells.

DESIGN: A Sperm separation device was designed based on concepts of inertial microfluidic forces in order to separate cell types by size and shape. The separation efficiency of the design was evaluated by comparing the contents of the device outputs to the device inputs.

MATERIALS AND METHODS: Spiral channel prototypes were fabricated using plastic laminates and laser cutting to contain four outlet channels corresponding to different flow lanes within the spiral. Device characterization was performed by flowing solutions of fluorescent microsphere particles and quantifying particle separation into specific outlet channels. Device prototypes were further tested for cell separation efficiency by flowing immobilized mouse sperm, mouse red blood cells, and mixtures of sperm and red blood cells (RBCs). The sperm enrichment in output channels compared to input was calculated, and statistical analyses were performed using student t-tests.

RESULTS: We found that microsphere particles with a diameter of nine micrometers could be selectively focused towards the innermost outlet channel of the spiral device at a flow rate of 0.5 ml/minute. Similarly, when mouse RBCs were flowed through the device, they were more concentrated within the innermost outlet channel. Immobilized mouse sperm cells, however, were selectively concentrated within the outermost outlet channel, likely the result of their unique morphology. In experiments where sperm cells were mixed with RBCs, the sperm:RBC ratio was enriched by more than three fold in the outermost outlet channel, suggesting that our spiral microfluidic design is capable of separating sperm cells from non-sperm cells.

CONCLUSION: Our results show that inertial microfluidic forces can be applied to viable cells, and used to enrich sperm cells from a mixed sperm-somatic cell population. This proof-of-principle study will serve as the groundwork for further microfluidic device optimization in order to achieve isolation of sperm cells from testicular somatic cell types. Because our design aims to isolate sperm cells without the use of cell lysis or enzymes, it will be amenable to use in a clinical in vitro fertilization (IVF) setting following micro-testicular sperm extraction (mTESE) surgery.

P-169 Tuesday, October 21, 2014

VAS DEFERENS ANASTOMOSIS AND EPIDIDIMOVARCOSTOMY. MALE FERTILITY SURGICAL TREATMENT SIMULATION MODEL USING HUMAN AND BOVINE PLACENTA. A. B. Reis, M. M. R. Oliveira, Surgery, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.

OBJECTIVE: Different types of surgical simulations are described, and the most used in urology are cadavers, animals (pigs), and synthetic models. Each one has its advantages and disadvantages. Description of vas deferens and epididymo vasostomy microsurgery training model that is ex vivo, biological, cheap, and has high fidelity is the aim of this study.

DESIGN: Urology microsurgery procedures are mostly done for male fertility treatment. Vas deferens anastomosis and epididymo-varicocelectomy are high technically demanding procedures that require continuing surgical practice to maintain good quality work. A description of an ex vivo, cheap, high fidelity model using human and bovine placenta was done.

MATERIALS AND METHODS: The research project was approved by the ethical committee of the Federal University of Minas Gerais, Belo Horizonte, Brazil. Twelve placentas, six human and six bovine were collected from the pathology department of the University hospital, and from Arro farm, respectively. They were kept at the microsurgical laboratory of that university where the work was developed. All placentas were cleaned in normal salting solution and had their main vessels canulated with urinary catheters, and the intravascular blood clots removed.

RESULTS: All human and bovine placenta has had biological properties that allowed the simulation of the proposed surgical exercises. The vas deferens has the exact diameter, vessel wall size, and lumen of medium size bifurcation of the bovine placenta artery. The human placenta vessels have a plethora of different caliber arteries and veins that resembles the vessels needed to be anastomosed to the epididymus in real surgery.

CONCLUSION: Urology microsurgery simulation has been used with proved benefits. A description of an ex vivo, high fidelity, low cost, easily available model for microsurgery training in male infertility is done. Further studies with validation methods will be needed to prove its efficacy.

Sperm Biology

P-170 Tuesday, October 21, 2014


OBJECTIVE: The purpose of this study is to examine the two treatments of in-vitro conception, viz. conventional IVF and ICSI, and assess the hypothesis that ICSI should be performed in cases with increased sperm DNA damage.
RESULTS: FR was comparable between Group A vs. Group B (77.7 ± 2.7 vs. 83.5 ± 3.0, p = 0.21, NS). PR was similar between Group A and B (43.4% vs. 42.5%, p = 1, NS). Differences between both groups. Regarding good-quality embryos (grade I and II, Lucinda Veeck criteria) there was a significant difference between Group A and Group B (1.98 ± 0.1 vs. 1.5 ± 0.1, p = 0.005). Pregnancies from Group A resulted in 22 healthy babies and in Group B 10 healthy babies.

CONCLUSION: Paternal miRNA analysis suggest that sperm DNA damage is an important factor to consider when using ICSI. Further work is needed to determine the effect of sperm DNA damage on embryonic development.

**ABSTRACT WITHDRAWN**

**P-173 Tuesday, October 21, 2014**

SPERMATOZOA MIR-34C LEVELS ARE RELATED WITH INTRACYTOPLASMIC SPERM INJECTION OUTCOMES. Y. Ye, L. Cui. Women’s Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang, China.

OBJECTIVE: Paternal miRNAs, one of the important epigenetic factors, can be delivered to the oocyte during fertilization. It has been reported that sperm-borne miR-34c is important for the first cleavage division of mouse embryo. The objective of our study os to investigate whether the expression of miR-34c in human sperm is related with intracytoplasmic sperm injection (ICSI) outcomes, such as fertilization rate, early cleavage rate, good quality embryo rate, pregnancy rate and implantation rate.

RESULTS:FR was comparable between Group A vs. Group B (77.7 ± 2.7 vs. 83.5 ± 3.0, p = 0.21, NS). PR was similar between Group A and B (43.4% vs. 42.5%, p = 1, NS). Differences between both groups. Regarding good-quality embryos (grade I and II, Lucinda Veeck criteria) there was a significant difference between Group A and Group B (1.98 ± 0.1 vs. 1.5 ± 0.1, p = 0.005). Pregnancies from Group A resulted in 22 healthy babies and in Group B 10 healthy babies.

CONCLUSION: Paternal miRNAs, one of the important epigenetic factors, can be delivered to the oocyte during fertilization.

**TABLE 1. Differences (%) and P values comparing embryo quality between ICSI and IVF methods**

<table>
<thead>
<tr>
<th>Embryo quality</th>
<th>Low</th>
<th>Intermediate</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Two Good</td>
<td>1.5</td>
<td>0.786</td>
<td>17.8</td>
</tr>
<tr>
<td>Fair</td>
<td>12.5</td>
<td>0.793</td>
<td>2.03</td>
</tr>
<tr>
<td>Poor</td>
<td>-14.0</td>
<td>0.002</td>
<td>-19.8</td>
</tr>
<tr>
<td>Three Good</td>
<td>9.5</td>
<td>0.085</td>
<td>20.5</td>
</tr>
<tr>
<td>Fair</td>
<td>1.2</td>
<td>0.785</td>
<td>2.8</td>
</tr>
<tr>
<td>Poor</td>
<td>-10.7</td>
<td>0.041</td>
<td>-23.3</td>
</tr>
<tr>
<td>Five Good</td>
<td>1.6</td>
<td>0.677</td>
<td>4.5</td>
</tr>
<tr>
<td>Fair</td>
<td>21.4</td>
<td>&lt;0.001</td>
<td>12.9</td>
</tr>
<tr>
<td>Poor</td>
<td>-23.0</td>
<td>&lt;0.001</td>
<td>-17.4</td>
</tr>
</tbody>
</table>

**Difference = % ICSI embryos - % IVF embryos**

**CONCLUSION: ICSI improves prognosis for patients with increased sperm DNA damage.**

Supported by: This study was Supported by University of Utah internal funds.
A BIOASSAY TO MEASURE FERTILIZATION COMPETENCE OF HUMAN SPERMATOCOA. T. Paniza, V. Q. Neri, Z. Rosenwaks, G. D. Palermo. Reproductive Medicine, Weill Cornell Medical College, New York, NY.

OBJECTIVE: To test a biomarker-based assay to diagnose sperm function which specifically predicts the ability to capacitate and fertilize.

DESIGN: We assessed ganglioside GM1 localization in sperm as a biomarker to quantify the sub-population that could respond to capacitating stimuli and become fertilization competent. Specific patterns of sperm GM1 localization were quantified in basal and capacitating conditions on semen samples of consenting men with the aim of predicting fertilizing ability.

MATERIALS AND METHODS: Semen parameters were evaluated according to WHO 2010 criteria. Samples were scored via fluorescence microscopy for GM1 localization patterns reflecting capacitation status in at least 200 sperm incubated under both standard and capacitating media. Based on preliminary data and normal timing of human sperm capacitation within 4hrs, we made observations at 1, 2, and 3hrs. Men were categorized as having normal or abnormal capacitation based on pattern frequencies compared to our reference ranges, and then clinical outcomes followed to assess predictive ability.

RESULTS: In all tested men (n = 63), average semen parameters were 58.1 ± 20 x10^6/ml, motility of 47.8 ± 8%, and normal morphology of 2.7 ± 1%. We identified 31 men with scores matching the normal reference group, with baseline GM1 patterns of 17%-22%-28% in standard and 26%-31%-38% in capacitating media, respectively. We identified 32 men with below reference values of 15%-20%-24% in standard and 20%-25%-29% in capacitating media. Semen parameters were comparable between the two groups. The population with normal range GM1 patterns had an IUI pregnancy rate of 45.2% (14/31; P = 0.03). In this cohort, 13 underwent ICSI and 6 became pregnant (46.2%).

CONCLUSION: The GM1 assay reflected sperm fertilizing ability and could identify men prone to IUI failure irrespective of semen parameters. Assay results may guide selection of optimal ART treatment.

Supported by: BioAccelerate NYC Prize through the Partnership Fund of New York City (A.J. Travis, Cornell). Dr. Travis taught methods to the Palermo lab, who independently recruited patients, performed assays and analyzed results.

OVEREXPRESSION OF SOX9 IN ABSENCE OF SRY SUPPORTS SPERMATOGENESIS PROGRESSION AND MALE FERTILITY IN THE MOUSE. E. A. Ortega, V. A. Ruthig, Y. Yamauchi, M. A. Ward. Institute for Biogenesis Research, Department of Anatomy, Biochemistry, and Physiology, University of Hawaii John A. Burns School of Medicine, Honolulu, HI.

OBJECTIVE: The Y chromosome gene Sry is responsible for sex determination in mammals. It acts briefly during fetal development and induces the development of testes rather than ovaries by turning on the expression of its autosomal downstream target Sox9. The activation of Sox9 in the absence of Sry is sufficient for the initiation of male specific sex determination but the effects of such replacement on adult male fertility remain unclear. Here we tested for Sry-to-Sox9 replacement effects on spermatogenesis and fertility by examining transgenic mice with deleted endogenous Sry and testis determination driven by either Sry or Sox9 transgene, and comparing them to males with an intact Y chromosome.

DESIGN: Research study.

MATERIALS AND METHODS: Males with Y chromosome carrying a deletion removing the endogenous Sry (Y\textsuperscript{tdym1}) and testis determination driven either by the Sox9 (XY\textsuperscript{tdym1Sox9}) or the Sry (XY\textsuperscript{tdym1Sry}) transgenes, as well as wild-type males (XY) were examined. Fertility was assessed by mating 4-6 males of each genotype with wild-type females for 10 wks, and assessing the numbers of litters and offspring produced. In vitro fertilization (IVF) experiments were also performed to sperm ability to fertilize oocytes and embryo development to the blastocyst stage. The normalcy of testis formation and spermatogenesis progression were evaluated by sperm count, motility, and morphology analyses and examination of testicular vasculature and testicular sections.

RESULTS: All tested males were fertile. XY\textsuperscript{tdym1Sry} and XY\textsuperscript{tdym1Sox9} mice and XY males yielded similar average number of litters and pups per male. In vitro, sperm from XY\textsuperscript{tdym1Sry} and XY\textsuperscript{tdym1Sox9} males fertilized oocytes with a similar efficiency but were less effective than sperm from XY males. For all tested genotypes, the majority of 2-cell embryos (>70%) developed to normal, healthy blastocysts. Sperm analyses revealed no differences between XY\textsuperscript{tdym1Sry} and XY\textsuperscript{tdym1Sox9} males in sperm number, motility and morphology. XY\textsuperscript{tdym1Sox9} males had altered testis vasculature pattern and increased incidence of defects in seminiferous epithelium adjacent to coelomic blood vessel. These abnormalities may be due to altered Sox9 expression, which in adult XY\textsuperscript{tdym1Sox9} gonad was ~2-fold higher than in XY.

CONCLUSION: Male lacking the testis determinant Sry can be fertile supporting that Sry does not play a role in a mature gonad. Sry-independent upregulation of Sox9 is sufficient to yield a fertile male.

Supported by: HCF13ADVC-60314 & NIH HD072380 grants to MAW.

P-176 Tuesday, October 21, 2014

PROGRESS IN DEFINING WHETHER LACK OF THE Y CHROMOSOME LONG ARM (NPYq) ENCODED GENE SLY IS THE SOLE OR THE CONTRIBUTING CAUSE OF SPERMIOGENIC DEFECTS IN MICE WITH SEVERE NPYq DELETIONS. J. Riel, V. Ruthig, Y. Yamauchi, M. Ward. Institute for Biogenesis Research, Department of Anatomy, Biochemistry and Physiology, University of Hawaii, John A. Burns School of Medicine, Honolulu, HI.

OBJECTIVE: The Y-specific, non-pairing region of the mouse Y chromosome long arm (NPYq) encompasses 99% of the Y-specific DNA content and encodes multiple copies of a gene expressed in spermatids: Sry, Sly, Asty, Orly. Mice with NPYq deletions have sperm defects and are sub- or infertile, with the severity of the phenotype increasing proportionally to the deletion size. Mice with transgenically (RNAi) silenced Sly have a similar phenotype but less severe. If sperm abnormalities in NPYq deficient mice are a consequence of Sly deficiency, then there should be a correlation between the extent of Sly reduction and the severity of sperm defects. We have shown earlier that Sly transcript levels correlated well with the phenotype. However, the analysis of Sly protein expression was hampered by lack of a suitable antibody. Here we aimed to develop a new anti-Sly antibody and clarify whether milder phenotype of shSly mice is due to insufficient Sly knockdown, involvement of another NPYq gene, or both.

DESIGN: Research study.

MATERIALS AND METHODS: SLY-specific peptide present in both SLY isoforms, SLY1 and SLY2, was used to immunize mice. The serum was tested in ELISA, dot-blot, and western blot with protein lysates from HEK293 cells transfected with SLY1 and SLY2 ORFs fused to FLAG tags. The serum was SLY specific, recognized both SLY1 and SLY2, and was transformed into a monoclonal anti-SLY antibody. The antibody was used in western blots with testes from mice with NPYq deficient and shSly mice.

RESULTS: Mice with a partial NPYq deletion and a slight ‘sperm phenotype’ had most of SLY1 (98%) and SLY2 (70%) retained. Mice with extensive NPYq deletions and severe phenotype had no identifiable SLY1/2. Two shSly lines were examined. Line sh344, with no phenotype, retained 5% of SLY1 but almost entire SLY2 (96%). Line sh367, with a moderate phenotype, retained 4% of SLY1 and 6% of SLY2.

CONCLUSION: The milder phenotype of sh367 is due, at least partially, to retention of residual SLY1/2. It remains possible that lack of another NPYq-encoded gene contributes to the severe phenotype of mice with extensive NPYq deletions.

Supported by: Supported by NIH RR024206 HD072380 to MAW.
P-177 Tuesday, October 21, 2014

TRANSGENERATIONAL EFFECTS OF DNA METHYLATION INHIBITOR TREATMENT TO MALE MICE. K. Murphy, a T. Jenkins, a C. Pfueger, a K. I. Aston, a B. Cairns, a D. Carrell. aAndrology, University of Utah, Salt Lake City, UT; aOncological Sciences, University of Utah, Salt Lake City, UT.

OBJECTIVE: The purpose of this study is to determine the effects of mouse sperm exposure to 5-Azacytidine on offspring and subsequent generations in vivo.

DESIGN: Male mice were treated with 5-Azacytidine, a methyltransferase inhibitor commonly used in chemotherapy. The effects on DNA methylation, fertility, and sperm count were assessed in the drug-treated mice as well as the F1 and F2 generations produced from the exposed animals.

MATERIALS AND METHODS: Male C57BL/6 mice were treated for seven weeks with either saline or 5-Azacytidine, and then mated with a highly polymorphic mouse strain in order to generate F1 and F2 offspring. Fertility, sperm count, and sperm DNA methylation alterations on a genome-wide level were assessed through bisulfite conversion followed by sequencing in the treated lineage compared to the control group. Changes in fertility factors were statistically evaluated using the student t-test, and genome methylation alterations were subjected to either fisher exact or chi-square tests.

RESULTS: We found that DNA methylation levels were globally reduced in the sperm of 5-Azacytidine treated animals, but to a lesser extent than previously reported by alternative genome-wide methylation quantification techniques. Furthermore, 5-Azacytidine treated males were strikingly less fertile than control males. Fewer drug-treated animals sired F1 offspring (23% compared to 100% for controls), F1 litter sizes were significantly smaller for drug-treated animals (an average of 2.3 pups/litter compared to 4.3 pups/litter for controls), and sperm counts in the drug-treated males were significantly reduced. This fertility effect, however, did not persist into the F2 generation. The offspring of 5-Azacytidine treated animals, both males and females, were able to produce similar numbers of pups to offspring of saline-treated males.

CONCLUSION: The chemotherapeutic agent 5-Azacytidine has detrimental effects on male fertility following a drug regimen that exceeds one spermatogenic cycle. However, offspring of drug-treated males have normal fertility. Future work will determine whether the methylation alterations observed in the sperm of the F0 generation are inherited transgenerationally.

P-178 Tuesday, October 21, 2014

AN INTEGRATED PROTEO-BIOINFORMATICS APPROACH TO GAIN INSIGHTS IN THE PATHOPHYSIOLOGY OF VARICOCELE ASSOCIATED MALE INFERTILITY. A. Agarwal, a R. Sharma, a D. Durairajanayagam, a Z. Cui, a A. Ayaz, a S. Gupta, a B. Willard, a B. Gopalan, a E. Sabanegh, a Center for Reproductive Medicine, Urology Department, Cleveland Clinic, Cleveland, OH; aLerner Research Institute, Cleveland Clinic, Cleveland, OH.

OBJECTIVE: Varicoceles appear to affect later stages of spermatogenesis and cause scrotal hyperthermia, hypoxia, hormonal imbalances, and re-flow of metabolites from renal and/or adrenal glands leading to oxidative stress. The objective was to study the major differences in the distribution of spermatozoa proteins in infertile men with varicocele compared to fertile men.

DESIGN: his prospective proteomic study analyzed proteins in spermatozoa from infertile men with unilateral (n = 5) or bilateral (n = 3) varicocele and men of proven fertility (n = 5) to study the proteins involved in the pathophysiology of varicocele-associated male infertility.

MATERIALS AND METHODS: Spermatozoa proteins were extracted from infertile men with unilateral and bilateral varicoceles and men with proven fertility. Proteins were separated by 1-D gel electrophoresis and bands were digested and identified on a LTQ-Orbitrap Elite hybrid mass spectrometer system. Bioinformatics tools were used to identify pathways and functions of proteins of interest.

RESULTS: Of the 99 proteins that were differentially expressed in the varicocele group, 9 proteins were uniquely expressed in the fertile group compared to 2 proteins that were unique to the varicocele group. 12 proteins were overexpressed and 76 were underexpressed in the varicocele group. In the varicocele group, the top networks were energy production, lipid metabolism, post-translational modification and protein folding. While proteins such as outer dense fiber protein 2 isoform 3 and tektnin-3, that are known to play a role in sperm motility were overexpressed in the varicocele group, a majority of proteins, such as, acrosin binding protein precursor, calmodin precursor that are involved in spermatic differentiation, spermatid development, spermatogenesis, reproductive cellular processes, and fertility were observed to be underexpressed.

CONCLUSION: Proteomics and bioinformatic analysis are powerful tools in understanding the pathology of varicocele associated male infertility. We have identified key proteins that are altered or modified in the presence of varicocele and may result in male infertility. Infertile men with unilateral or bilateral varicocele have a large number of spermatozoal proteins that are differentially expressed compared to those of fertile men. These protein alterations may be a contributing factor in male infertility.

Supported by: Cleveland Clinic.

P-179 Tuesday, October 21, 2014

IMPACT OF SPERM DNA DAMAGE ON IMPLANTATION FOLLOWING ART. L. Simon, a K. I. Aston, a J. A. Dorais, a J. M. Hotaling, a M. Link, a E. B. Johnston, a A. K. Moore, a O. Hammoud, a C. M. Peterson, a D. T. Carrell. Andrology and IVF, University of Utah School of Medicine, Salt Lake City, UT.

OBJECTIVE: The purpose of this study is to examine the effect of sperm DNA damage and female age on blastocyst implantation rate following assisted reproductive treatment (ART).

DESIGN: Cross-sectional study of 215 couples undergoing ART.

MATERIALS AND METHODS: Sperm from men undergoing ART were analyzed for DNA damage using the alkaline Comet assay and classified into three groups 'Low' (0 to 30%), 'Intermediate' (31 to 70%) and 'High' (71 to 100%). Couples' infertility was categorized into male, female or unexplained. To identify blastocysts that successfully implanted following transfers, the following inclusion criteria were followed: a) If a single blastocyst was transferred, b) If more than one blastocyst was transferred and all the transferred blastocysts are of the same stage and grade, c) If multiple blastocysts types are transferred, then either all the blastocysts implanted or none of the blastocysts implanted. Chi-square and logistic regression were used to test for differences in implantation rates between age groups, couple's infertility classification and level of sperm DNA damage.

RESULTS: The implantation rate was lower in the high DNA damage group compared with intermediate and low DNA damage groups (P < 0.001; Table 1). Implantation was higher when the female partner age was < 35 years when compared with > 35 age group (52.8% vs. 35.4%; P = 0.008). The high DNA damage group had a lower implantation rate when the female partner age was > 35 years when compared with ≤ 35 age group (5.6% vs. 44.8%; P < 0.001). Couples with unexplained infertility had a lower implantation rate (32.7%) when compared with male (60.0%, P < 0.001) and female (54.0%, P = 0.001). The implantation rate for each embryo type decreased with increased sperm DNA damage.

CONCLUSION: Implantation rate following ART was influenced by sperm DNA damage, couples infertility type and female partner’s age.

Supported by: This study was supported by University of Utah internal funds.

| TABLE 1. Comparison between level of sperm DNA damage and implantation rate |
|-------------------------------------|--------|--------|--------|
| All embryos transferred (%)         | 65.0   | 55.3   | 33.3   |
| Blastocyst (%)                      | 74.1   | 58.8   | 48.9   |
| Early blastocyst (%)                | 66.7   | 57.1   | 41.7   |
| Cavitating morulae (%)              | 57.9   | 41.1   | 23.5   |

CONCLUSION: Implantation rate following ART was influenced by sperm DNA damage, couples infertility type and female partner’s age.
OOCYTE MATURATION

P-180 Tuesday, October 21, 2014


OBJECTIVE: To describe an intrinsic defect of oocyte maturation and determine its prevalence among women undergoing assisted reproductive technologies.

DESIGN: Retrospective cohort.

MATERIALS AND METHODS: Data were collected by review of electronic medical records and an IVF database of all women enrolled in the ART program at Walter Reed Army Medical Center from 1999 to 2009. Women underwent conventional ovarian stimulation with either the standard long luteal-phase GnRH agonist protocol or the mini dose flare (MDF) protocol. Oocytes were counted and the morphology assessed with maturation grading assigned as GV, MI, or MII. Fertilization was defined as the number of oocytes with 2 pronuclei and the fertilization rate was determined by dividing the number of fertilized oocytes by the number of mature oocytes retrieved. Morphologic assessment of embryo quality was performed utilizing a modified Veeck grading scale and the number of embryos transferred was determined based upon the ASRM guidelines. Main outcome measures were fertilization and live birth rates. Live birth and fertilization rates were compared across categories of oocyte maturity rates, and odds ratios for live birth were estimated adjusting for age.

RESULTS: Of 2511 women undergoing their first cycle of IVF, 25 women produced fewer than 25% mature oocytes, suggesting a prevalence of 1% for impaired oocyte maturation. Two women had no mature oocytes. Women with low maturity (<25% maturity) had significantly lower fertilization rates (52% vs. 70%) and live birth rates (17% vs. 40%, adjusted odds ratio 0.29, 95% confidence interval 0.10, 0.87) compared to women with higher oocyte maturity (76-100% maturity).

<table>
<thead>
<tr>
<th>Oocyte Maturity</th>
<th>Fertilization Rate %</th>
<th>Clinical Pregnancy %</th>
<th>Live Birth %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate %</td>
<td>n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-25</td>
<td>26</td>
<td>52.1 (SD 4.2)</td>
<td>6/25 (24)</td>
</tr>
<tr>
<td>26-50</td>
<td>185</td>
<td>64.4 (SD 1.6)</td>
<td>72/179 (40)</td>
</tr>
<tr>
<td>51-75</td>
<td>859</td>
<td>66.8 (SD 0.7)</td>
<td>320/585 (54)</td>
</tr>
<tr>
<td>76-100</td>
<td>1445</td>
<td>70.1 (SD 0.6)</td>
<td>761/1425 (53)</td>
</tr>
</tbody>
</table>

CONCLUSION: Both NE and DA are playing a role in oocyte maturation. In the study higher DA and NE levels in PCOS group supports that oocyte maturation is not diminished through catecholamine pathway in PCOS patients.

P-182 Tuesday, October 21, 2014

ASSESSMENT BY TIME-LAPSE OF THE DEVELOPMENT POTENTIAL OF MI OOCYTES MATURED IN VITRO AFTER CUMULUS CELL REMOVAL. H. Kitasaka, N. Fukunaga, T. Yoshimura, F. Tamura, M. Katou, K. Nakayama, H. Oono, S. Misaki; Y. Hashiba, Y. Asada, TVF Laboratory, Asada Ladies Nagoya Clinic, Nagoya, Aichi, Japan; TVF Laboratory, Asada Ladies Kachiwaga Clinic, Nagoya, Aichi, Japan; Asada Institute for Reproductive Medicine, Asada Ladies Clinic, Nagoya, Aichi, Japan.

OBJECTIVE: Previously we have routinely cultured for a few hours metaphase I (MI) oocytes following cumulus cell removal. In cases where the first polar body (PB) was released, we have performed intra cytoplasmic sperm injection (ICSI), and have cases of successful deliveries. Therefore, we considered the development potential of oocytes which matured to MI just after cumulus cell removal.

DESIGN: Prospective cohort study.

MATERIALS AND METHODS: 103 patients having 103 cycles (n=172) were included from July to December 2012 in which MI oocytes were observed immediately after cumulus cell removal. MI oocytes were observed over a three hour period using the EmbryoScope time-lapse system. For oocytes where release of the first PB was confirmed, ICSI was performed one hour after PB release. Over the same time period, in the control group (n=3720), metaphaseII(MII) oocytes were observed immediately after cumulus cell removal, and ICSI was performed.

RESULTS: The overall rate of maturation from MI to MII was 32.0%. The PB was released within less than 1 hour from cumulus cell removal in 18.2% of cases, 25.6% from 1 to 2 hours, 38.2% for 2 to 3 hours and 18.2% for 3 to
P.183 Tuesday, October 21, 2014

PREDICTION OF EMBRYONIC DEVELOPMENT AND CLINICAL OUTCOME FOLLOWING ICSI ACCORDING TO THE STAGES OF NUCLEAR MATURATION DIVISION. A. Tanaka, M. Nagayoshi, I. Tanaka, Y. Takemoto. Saint Mother Hospital, Kitakyushu, Fukuoka, Japan.

OBJECTIVE: Pre-ovulatory oocytes collected after controlled ovarian stimulation are considered mature with the extrusion of the first polar body at ICSI. However embryonic development varies following ICSI for morphologically mature oocytes. We conducted this study to investigate whether it is possible to predict embryonic development and clinical outcome according to the stages of nuclear maturation division.

DESIGN: Retrospective study to investigate the relationship between the clinical outcome and various nuclear stages of M-II oocytes.

MATERIALS AND METHODS: After obtaining their informed consent a total of 53 patients (53 cycles) oocytes were retrieved. The 237 freshly ovulated M-II oocytes were examined. The proportion of grouped oocytes according to the stages of M-II chromosomes were 69.6% (165/237) in group A, 6.3% (15/237) in group B, 13.1% (31/237) in group C and 11.0% (26/237) in group D. The fertilization, cleavage, blastocyst stage rates and pregnancy, miscarriage and birth rates in four groups are listed in table.

CONCLUSION: Identification of chromosomal stages has a high potential to predict the embryonic development and clinical outcome. Longer pre-incubation (5–6 hours) might be beneficial for prometaphase II oocytes to advance to Metaphase II oocytes. Irregularly arranged chromosomes might be a sign of nuclear degradation.

Clinical outcome following ICSI according to the stages of nuclear maturation division

<table>
<thead>
<tr>
<th>Group</th>
<th>Fertilization rates (%)</th>
<th>Cleavage rates (%)</th>
<th>Blastocyst rates (%)</th>
<th>Pregnancy rates (%)</th>
<th>Miscarriage rates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>78.4 (131/167)</td>
<td>80.2/105 (131)</td>
<td>58.9/76 (131)</td>
<td>44.7/34 (76)</td>
<td>11.8/4 (34)</td>
</tr>
<tr>
<td>B</td>
<td>73.8 (11/15)</td>
<td>63.6/7 (11)</td>
<td>36.3/4 (11)</td>
<td>25.8/1 (4)</td>
<td>100/1 (1)</td>
</tr>
<tr>
<td>C</td>
<td>54.8 (7/13)</td>
<td>70.3/12 (17)</td>
<td>17.6/17 (17)</td>
<td>0/0 (0)</td>
<td>0/0 (0)</td>
</tr>
<tr>
<td>D</td>
<td>(0/026)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

P.184 Tuesday, October 21, 2014

THE STEROID INHIBITOR TRILOSTANE DECREASES GONADOTROPIN-, BUT NOT PROGESTIN E2-, STIMULATED HUMAN EMBRYONIC DEVELOPMENT. A. Y. Ting, J. D. Hennebold, R. L. Stouffer. Division of Reproductive & Developmental Sciences, Oregon National Primate Research Center, Oregon Health & Science University, Beaverton, OR.

OBJECTIVE: To determine whether the inhibition of steroid synthesis with trilostane (TR) affects synthesis of the cumulus expansion-associated glycosaminoglycan, hyaluronan (HA), in macaque cumulus-oocyte-complexes (COCs) in the absence or presence of gonadotropins or progesterone E2 (PGE2).

DESIGN: Nonhuman primate model, 3x2 factorial design, randomized, controlled study.

MATERIALS AND METHODS: Healthy macaque (n=5) COCs (n=3-6/group/animal) were used. Twenty-five small antral follicles were recruited and stimulated to reach the metaphase II oocyte stage with either independent of steroid hormones or downstream of the steroid hormone signaling pathway during PGE2 induction of cumulus expansion. This study supports an important role of steroid hormones in the intrafollicular environment for cumulus expansion during oocyte maturation in primates, and has implications for improving in vitro fertilization techniques and fertility preservation in humans. Ongoing studies include hormone replacement to determine which steroid hormone is required for HA synthesis.

Supported by: NIH U54HD071836, R01HD020869.

P.185 Tuesday, October 21, 2014

AGING IS ASSOCIATED WITH DIFFERENTIAL GENE EXPRESSION IN HUMAN GRANULOSA AND CUMULUS CELLS LEADING TO RAPID PROGRESSION OF MEIOTIC AND OOCYTE MATURATION IN HUMAN GRANULOSA AND CUMULUS CELLS LEADING TO RAPID PROGRESSION OF MEIOTIC AND OOCYTE MATURATION COMPETENCE. H.-J. Lee, Y.-G. Wu, E. Lazzaroni-Tealdi, D. H. Barad, V. A. Kushnir, N. Gleicher.

OBJECTIVE: Age related decline of ovarian function results in diminished oocyte number and quality. To elucidate the effect of maternal aging on interaction between oocyte and follicle cells, we examined gene expression in granulosa cells (GCs) and cumulus cells (CCs) in relation to meiotic and oocyte maturation competence in women at different age.

DESIGN: Prospective laboratory study of two patient cohorts at different ages.

MATERIALS AND METHODS: GCs from human follicular fluid and CCs from human oocyte complex (COC) were from 8 younger (<38; mean 30.8 ± 4.3 years) and 10 older (>38, mean 42.6 ± 2.1 years) patients were investigated. Among those, 23 GV-stage oocytes were retrieved in both groups. We performed real-time reverse-transcription polymerase chain reaction (RT-qPCR) to analyze mRNA, and evaluated gene expression of follicular stimulating hormone receptor (FSHR), luteinizing hormone receptor (LHR), progesterone receptor (PR), prolactin receptor (PRLR), natriuretic peptide receptor 2 (NPR2), connexin 37, 43 (Cox37, Cox 43), normalized to ß-actin mRNA levels. GV-stage oocytes were in vitro matured (IVM), evaluating
meiotic maturation (indicated by germinal vesicle break down, GVBD, within 24 hours) and oocyte maturation competence (by maturation to MII stages within 48 hours).

RESULTS: In older women FSHR expression was significantly lower (P<0.01), while LHRR, PR, PRLR expression were significantly lower (all P<0.05) in GCs. No difference in expression of any of these receptors was found in CCs based on female age. Older patients also demonstrated significantly reduced expression of NFR2 and Cox 43 in GCs and CCs (P<0.05), but no differences was found in Cox 37. Time to reach GVBD and MII was 11.5 ± 0.9 hours and 9.3 ± 0.5 hours (P<0.05), and 28.5 ± 1.0 and 23.8 ± 1.2 (P<0.01) in younger and older patients, respectively.

CONCLUSION: Up-regulation of LHRR, PR RLR in GCs in older women appears to induce premature luteinization and reduction of NFR2 and Cox43 expression in GCs and CCs, likely resulting in more rapid meiotic as well as oocyte maturation. With advancing female age, follicle and oocyte maturation, therefore, appear regulated by increasingly differential and impaired gene expressions in GCs as well as CCs, an observation which may be utilized to modify clinical management.

Supported by: Foundation for Reproductive Medicine, Center for Human Reproduction.

P-186 Tuesday, October 21, 2014

IN VITRO MATURATION (IVM) WITH A FREEZE-ALL EMBRYO PROTOCOL MAY BENEFIT PATIENTS WITH POLYCYSTIC OVARIAN SYNDROME (PCOS). M. Walls, T. Hunter, J. P. Bryan, J. Keelan, E. Nathan, R. Hart. Fertility Specialists of WA, Claremont, WA, Australia; School of Women’s and Infant’s Health, University of WA, Subiaco, WA, Australia; Biostatistics and Research Design Unit, Women and Infants Research Foundation, Subiaco, WA, Australia.

OBJECTIVE: To determine the success rates of fresh vs frozen single blastocyst transfers for IVM and to assess if a freeze-all embryo protocol might achieve success rates similar to those for in vitro fertilisation (IVF).

DESIGN: Retrospective case-control.

MATERIALS AND METHODS: Patients diagnosed with PCOS who underwent IVM or IVF from 2007-2012 were included in the study. IVM patients received 3-5 days of 100-150U follicle stimulating hormone (FSH) priming, no human chorionic gonadotrophin (hCG) trigger and a 24 hour maturation period in G2 plus culture media (Vitrolife) supplemented with 10% maternal serum, 0.1 IU/ml FSH and 0.5 IU/ml hCG. Endometrial preparation was achieved using hormone replacement therapy (HRT). IVM patients received either a gonadotrophin releasing hormone (GnRH) antagonist protocol or a long GnRH agonist protocol. For both groups the protocol for a frozen embryo transfer (FET) cycle was either low dose FSH stimulation or HRT. All fresh cycles involved day 5-6 blastocyst culture and patients with failed absorption/response were excluded. All dual trigger trigger patients received estrogen supplementation beginning on cycle day 15. Patients were stratified into SART age categories for analysis. Outcomes measured included total and mature oocyte yield, fertilization rate, implantation rate, and clinical pregnancy/live birth rates. Statistical analysis included χ2 and t tests. P<0.05 was deemed statistically significant.

RESULTS: We analyzed 1223 IVF cycles from January 2007 to October 2013. 883 patients received low-dose hCG and 340 received dual trigger. Peak E2 levels were significantly higher in the low-dose hCG group in patients younger than 35 years old. There was no difference in peak E2 levels between groups in all other age categories. The number of mature oocytes retrieved was significantly higher in the dual trigger groups in all patients 42 and younger. In patients older than 42, there was no difference in oocyte yield between trigger type. There were 3 cases of OHSS requiring hospitalization, all in the low-dose hCG group. Across all age groups, there were no significant differences in fertilization, implantation, clinical pregnancy, or live birth rates between trigger types.

CONCLUSION: By reducing the incidence of miscarriage, a freeze-all strategy may improve the efficiency of IVM while lowering the risks and cost associated with IVF treatment.

Supported by: Women’s and Infant’s Research Foundation.

P-187 Tuesday, October 21, 2014


OBJECTIVE: To compare the effect of triggering ovulation with low dose hCG versus a combination of low dose hCG and GnRH analog (GnRH-a) on IVF cycle and pregnancy outcomes.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: Patients undergoing IVF at our center with elevated E2 who received either low dose hCG (3300iu) according to our hCG sliding scale or dual trigger with GnRH-a and low dose hCG (2 mg leuprolide acetate + 1500iu hCG) were included. Absorption of serum hCG and response to lupon were assessed on the morning following injection and patients with failed absorption/response were excluded. All dual trigger patients received estrogen supplementation beginning on cycle day 15. Patients were stratified into SART age categories for analysis. Outcomes measured included total and mature oocyte yield, fertilization rate, implantation rate, and clinical pregnancy/live birth rates. Statistical analysis included χ2 and t tests. P<0.05 was deemed statistically significant.

RESULTS: We analyzed 1223 IVF cycles from January 2007 to October 2013. 883 patients received low-dose hCG and 340 received dual trigger. Peak E2 levels were significantly higher in the low-dose hCG group in patients younger than 35 years old. There was no difference in peak E2 levels between groups in all other age categories. The number of mature oocytes retrieved was significantly higher in the dual trigger groups in all patients 42 and younger. In patients older than 42, there was no difference in oocyte yield between trigger type. There were 3 cases of OHSS requiring hospitalization, all in the low-dose hCG group. Across all age groups, there were no significant differences in fertilization, implantation, clinical pregnancy, or live birth rates between trigger types.

CONCLUSION: The use of a combination GnRH-a and low dose hCG trigger in patients with high E2 levels yields results comparable to low-dose hCG and may result in higher oocyte yield in some age groups.

Outcomes of Fresh and Frozen Embryo Transfer Cycles.

<table>
<thead>
<tr>
<th>IVM</th>
<th>IVF</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>N = 64</td>
<td>N = 58</td>
</tr>
<tr>
<td>Biochemical Pregnancy</td>
<td>28/64</td>
<td>43.8%</td>
</tr>
<tr>
<td>Clinical Pregnancy</td>
<td>19/64</td>
<td>29.7%</td>
</tr>
<tr>
<td>Live Birth</td>
<td>12/64</td>
<td>18.8%</td>
</tr>
<tr>
<td>Miscarriage</td>
<td>7/19</td>
<td>36.8%</td>
</tr>
</tbody>
</table>

Frozen | N = 62 | N = 117 | 0.488 |
| Biochemical Pregnancy | 26/62 | 41.9% | 59/117 | 50.4% |
| Clinical Pregnancy | 22/62 | 35.5% | 43/117 | 36.8% |
| Live Birth | 21/62 | 33.9% | 35/117 | 29.9% |
| Miscarriage | 1/22 | 4.5% | 8/43 | 18.6% |

*includes 2 twin live births

Mature oocytes retrieved, Clinical pregnancy per retrieval/ Clinical pregnancy per transfer (%)

<table>
<thead>
<tr>
<th>Age</th>
<th>3300iu</th>
<th>Dual</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;35</td>
<td>(N=355)</td>
<td>*13.5±5.9, 54.5/59.9 (N=131)</td>
</tr>
<tr>
<td>35-37</td>
<td>(N=197)</td>
<td>*12.7±5.4, 47.5/52 (N=76)</td>
</tr>
<tr>
<td>38-40</td>
<td>(N=182)</td>
<td>*12±5.3, 39.5/43.4 (N=72)</td>
</tr>
<tr>
<td>41-42</td>
<td>(N=88)</td>
<td>*12±4.8, 38.6/40.5 (N=35)</td>
</tr>
<tr>
<td>&gt;42</td>
<td>(N=61)</td>
<td>11.0±5.0, 24.6/26.8 (N=26)</td>
</tr>
</tbody>
</table>

>0.05

CONCLUSION: The use of a combination GnRH-a and low dose hCG trigger in patients with high E2 levels yields results comparable to low-dose hCG and may result in higher oocyte yield in some age groups.

FERTILIZATION

P-188 Tuesday, October 21, 2014


OBJECTIVE: Oligospermia is a leading cause of infertility. While severe oligospermia requires ICSI, it is not clear whether mild oligospermia needs ICSI or conventional IVF suffices. The purpose is to evaluate whether there are differences in fertilization rate and pregnancy rate when subnormal sperm are treated with conventional IVF without ICSI.

CONCLUSION: The use of a combination GnRH-a and low dose hCG trigger in patients with high E2 levels yields results comparable to low-dose hCG and may result in higher oocyte yield in some age groups.
MATERIALS AND METHODS: Patients were< 41 years old or using donor oocytes, had > 8 oocytes retrieved, <2 previous cycles, and used fresh ejaculated sperm. Oocytes were divided equally into four different concentrations of motile sperm: (A) 0.15x10^6/mL, (B) 0.10x10^6/mL, (C) 0.075x10^6/mL, and (D) 0.05x10^6/mL. Odd numbers of oocytes went to the greatest sperm concentration first. Sperm was added 6 hours post retrieval and fertilization assessed 18 h later. Fertilization took place in 200ul drops of P-1 +5mg/mL HSA (Irvine) and embryos were cultured in G1/G2 (Vitrolife) + 10% SSS (Irvine) all under oil in humidified 6%CO2 and 5%O2. Normal and abnormal fertilization, day 5 blastocyst formation per 2PN, number of freeze quality blastocysts per 2PN, and number transferred per 2PN were analyzed by ANOVA. Differences between treatments were assessed using Fisher’s LSD. Odds of obtaining low fertilization, defined as <50% 2PN, were calculated using logistic regression.

RESULTS: A total of 533 oocytes were retrieved from 25 patients. Overall normal and abnormal fertilization rates were 73.7 and 14.3%; respectively. Overall, implantation and clinical pregnancy rates were 57.5% and 62.5%; respectively, with an average number of embryos transferred of 1.7. The odds of inscription resulting in low fertilization were not increased by decreasing sperm concentration. Treatment group means ± SE are shown in the table below. No significant differences between treatment groups were observed for any variable.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Oocytes</th>
<th>2PN</th>
<th>Abnormal Blastocysts</th>
<th>ET</th>
<th>Cryo</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>144</td>
<td>74±8.5±6</td>
<td>48.5±6.4</td>
<td>18.4±4.9</td>
<td>13.4±4.4</td>
</tr>
<tr>
<td>B</td>
<td>137</td>
<td>79±5.0±5</td>
<td>10.5±3.5</td>
<td>52.2±6.5</td>
<td>11.4±3.2</td>
</tr>
<tr>
<td>C</td>
<td>130</td>
<td>68±5.1±1</td>
<td>16±3.9</td>
<td>41.7±3.9</td>
<td>11.5±4.7</td>
</tr>
<tr>
<td>D</td>
<td>122</td>
<td>72±5.3±7</td>
<td>14±2.5</td>
<td>43.4±5.7</td>
<td>11.9±4.8</td>
</tr>
</tbody>
</table>

CONCLUSION: Decreasing sperm concentration to only 50,000 motile sperm/mL for conventional insemination does not significantly affect normal or abnormal fertilization. However, decreased sperm concentration did not result in any improvement in embryo development either.

P-190 Tuesday, October 21, 2014

THE EFFECTS OF SPERM CONCENTRATION ON IN VITRO EMBRYO DEVELOPMENT

DESIGN: Prospective sibling oocyte study.

MATERIALS AND METHODS: Patients were< 41 years old or using donor oocytes, had > 8 oocytes retrieved, <2 previous cycles, and used fresh ejaculated sperm. Oocytes were divided equally into four different concentrations of motile sperm: (A) 0.15x10^6/mL, (B) 0.10x10^6/mL, (C) 0.075x10^6/mL, and (D) 0.05x10^6/mL. Odd numbers of oocytes went to the greatest sperm concentration first. Sperm was added 6 hours post retrieval and fertilization assessed 18 h later. Fertilization took place in 200ul drops of P-1 +5mg/mL HSA (Irvine) and embryos were cultured in G1/G2 (Vitrolife) + 10% SSS (Irvine) all under oil in humidified 6%CO2 and 5%O2. Normal and abnormal fertilization, day 5 blastocyst formation per 2PN, number of freeze quality blastocysts per 2PN, and number transferred per 2PN were analyzed by ANOVA. Differences between treatments were assessed using Fisher’s LSD. Odds of obtaining low fertilization, defined as <50% 2PN, were calculated using logistic regression.

RESULTS: A total of 533 oocytes were retrieved from 25 patients. Overall normal and abnormal fertilization rates were 73.7 and 14.3%; respectively. Overall, implantation and clinical pregnancy rates were 57.5% and 62.5%; respectively, with an average number of embryos transferred of 1.7. The odds of inscription resulting in low fertilization were not increased by decreasing sperm concentration. Treatment group means ± SE are shown in the table below. No significant differences between treatment groups were observed for any variable.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Oocytes</th>
<th>2PN</th>
<th>Abnormal Blastocysts</th>
<th>ET</th>
<th>Cryo</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>144</td>
<td>74±8.5±6</td>
<td>48.5±6.4</td>
<td>18.4±4.9</td>
<td>13.4±4.4</td>
</tr>
<tr>
<td>B</td>
<td>137</td>
<td>79±5.0±5</td>
<td>10.5±3.5</td>
<td>52.2±6.5</td>
<td>11.4±3.2</td>
</tr>
<tr>
<td>C</td>
<td>130</td>
<td>68±5.1±1</td>
<td>16±3.9</td>
<td>41.7±3.9</td>
<td>11.5±4.7</td>
</tr>
<tr>
<td>D</td>
<td>122</td>
<td>72±5.3±7</td>
<td>14±2.5</td>
<td>43.4±5.7</td>
<td>11.9±4.8</td>
</tr>
</tbody>
</table>

CONCLUSION: Decreasing sperm concentration to only 50,000 motile sperm/mL for conventional insemination does not significantly affect normal or abnormal fertilization. However, decreased sperm concentration did not result in any improvement in embryo development either.

P-191 Tuesday, October 21, 2014

SPERM DEATH AFTER FERTILIZATION ON CONVENTIONAL IVF CAN BE A GOOD PREDICTION OF IVF OUTCOMES

OBJECTIVE: Live and motile sperm is usually observed after fertilization in the culture dish, but interestingly dead and immotile sperm was sometimes observed. However, the association of sperm death after fertilization in the culture dish and IVF outcomes have not yet reported. In this study, we investigated relationship association of IVF outcomes and sperm death in the culture dish after fertilization on conventional IVF.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: We analyzed 472 cycles from patients aged under 40 years undergoing conventional IVF from January 2012 to December 2013. After fertilization, the patients were classified depending on sperm condition in the culture dish into two groups : group A (n=435): high percentage of the living sperm with hyperactivated motility, group B
EMBRYO BIOLOGY

P-193 Tuesday, October 21, 2014

ACCURATE QUANTIFICATION OF SPECIFIC PROTEINS OF INTEREST IN SINGLE HUMAN BLASTOCYSTS USING TARGETED MASS SPECTROMETRY. M. Poli, M. Beck, M. P. Zappacosta, A. Valcarcel, F. Lorenzo, IFER Instituto de Ginecologia y Fertilidad, Buenos Aires, Argentina.

OBJECTIVE: In this study we investigate the feasibility of detecting and quantifying specific protein targets secreted within single human blastocysts using Targeted Mass Spectrometry (MS).

DESIGN: Prospective Cohort Study.

MATERIALS AND METHODS: Blastocyst fluid was removed from 80 patients during day 5 of the embryo transfer cycle. Blastocoel fluid was digested, spiked with synthetic peptides and analyzed by targeted tandem mass spectrometry.

RESULTS: Analysis of combined tandem MS runs identified 287 proteins that exist within the human blastocyst (false discovery rate of <1%). Comparison of transcriptomic and proteomic data suggests that >80% of proteins found in blastocyst fluid are derived from genes activated through development. The data presented is likely to be of relevance to the phenotype of the embryo and may therefore have clinical value. The use of protein concentration ratios as embryo viability/implantation markers is currently under investigation. Supported by: Institutional funding.

P-194 Tuesday, October 21, 2014

ORC4 PLAYS A ROLE IN POLAR BODY EXTRUSION IN THE MOUSE OOCYTE AND ZYGOTE. H. Nguyen, M. Ko, M. A. Ortega, J. Marh, W. S. Ward. Institute for Biogenesis Research - John A Burns School of Medicine, Honolulu, HI.

OBJECTIVE: Six proteins, ORC1-6, make up the origin recognition complex (ORC) that prepares DNA replication origins for licensing. In somatic cells, the ORC1-6 proteins bind to an unlicensed origin to form a complex, and allow licensing to occur by loading Mcm2-7. We tested if ORC1-6 proteins in the mouse oocyte and zygote behave the similarity to the licensing pathways in somatic cells.

DESIGN: At different stages of meiosis and the first cell division cycle, embryos were stained for one of five ORC proteins, and MCM7 which associate to the chromosomes at metaphase, but appear on both sets of separating chromosomes at telophase. At this point, the ORC4 in the polar body also migrated into the nuclei.

Supported by: Institutional funding.
modified during the first embryonic cell cycle from a protein that is primarily localized to cytoplasmic, perinuclear structure to one that binds to DNA.

OBJECTIVE: The Barker hypothesis holds that alterations to homeostasis during critical periods of development predispose individuals to adult-onset chronic diseases. Previously, we found that mice conceived by IVF display altered growth and altered glucose homeostasis. Further, it appears that epigenetic alterations might be the molecular mechanism responsible for the observed phenotypic alterations. To explore this possibility, we have assessed epigenetic alterations at selected loci in embryos and adult tissue of IVF and naturally conceived offspring.

DESIGN: Experimental study in inbred (C57Bl/6J) and outbred (CF1xB6D2F1) mice.

MATERIALS AND METHODS: In vitro-fertilized embryos were cultured in KSOM with amino acids (5% O2; 5% CO2) until the blastocyst stage. In vivo-conceived blastocysts flushed out of the uterus were used as control. Some of the blastocysts were used for molecular analysis while others were transferred into pseudopregnant recipient dams; the resulting IVF (n=26) and control offspring (n=19) were assessed for metabolic alteration and their tissues were collected for molecular and epigenetic analysis. RT PCR, Western blot, DNA promoter methylation using bisulfite sequencing and Chromatin immunoprecipitation of selected histone marks (H4ac; H3K20me3) were performed at selected loci in blastocysts and adult tissues.

RESULTS: We identify thio-redoxin-interacting protein (TXNIP)—a key molecule involved in integrating cellular nutritional and oxidative states with metabolic response—as a marker for preimplantation stress. Analysis by qPCR confirmed that IVF induces a nearly four-fold increase in Tnpi transcription with a comparable increase in TXNIP protein relative to in vivo-generated blastocysts. Examination of the chromatin architecture at the Tnpi promoter in IVF blastocysts revealed an enrichment for the active modification acetylated H4 (H4ac). Bisulfite sequencing of the Tnpi promoter, detected no changes in CpG methylation between conception conditions. The adipose tissue of IVF mice replicated the molecular and epigenetic findings in the blastocysts, while beta cells, adipose tissue and muscle did not.

CONCLUSION: We have identified Tnpi as a molecular marker of preimplantation stress in both inbred and outbred mice. Importantly histone modifications as opposed to DNA methylation promoter changes were observed in IVF blastocysts and maintained in adult adipose tissue of inbred mice. This indicates that an epigenetic memory following preimplantation disturbance is maintained in selected non-imprinted loci.

Supported by: R01 HD 062803 - 01A1 to PFR.

P-195 Tuesday, October 21, 2014

OBJECTIVE: The Barker hypothesis holds that alterations to homeostasis during critical periods of development predispose individuals to adult-onset chronic diseases. Previously, we found that mice conceived by IVF display altered growth and altered glucose homeostasis. Further, it appears that epigenetic alterations might be the molecular mechanism responsible for the observed phenotypic alterations. To explore this possibility, we have assessed epigenetic alterations at selected loci in embryos and adult tissue of IVF and naturally conceived offspring.

DESIGN: Experimental study in inbred (C57Bl/6J) and outbred (CF1xB6D2F1) mice.

MATERIALS AND METHODS: In vitro-fertilized embryos were cultured in KSOM with amino acids (5% O2; 5% CO2) until the blastocyst stage. In vivo-conceived blastocysts flushed out of the uterus were used as control. Some of the blastocysts were used for molecular analysis while others were transferred into pseudopregnant recipient dams; the resulting IVF (n=26) and control offspring (n=19) were assessed for metabolic alteration and their tissues were collected for molecular and epigenetic analysis. RT PCR, Western blot, DNA promoter methylation using bisulfite sequencing and Chromatin immunoprecipitation of selected histone marks (H4ac; H3K20me3) were performed at selected loci in blastocysts and adult tissues.

RESULTS: We identify thio-redoxin-interacting protein (TXNIP)—a key molecule involved in integrating cellular nutritional and oxidative states with metabolic response—as a marker for preimplantation stress. Analysis by qPCR confirmed that IVF induces a nearly four-fold increase in Tnpi transcription with a comparable increase in TXNIP protein relative to in vivo-generated blastocysts. Examination of the chromatin architecture at the Tnpi promoter in IVF blastocysts revealed an enrichment for the active modification acetylated H4 (H4ac). Bisulfite sequencing of the Tnpi promoter, detected no changes in CpG methylation between conception conditions. The adipose tissue of IVF mice replicated the molecular and epigenetic findings in the blastocysts, while beta cells, adipose tissue and muscle did not.

CONCLUSION: We have identified Tnpi as a molecular marker of preimplantation stress in both inbred and outbred mice. Importantly histone modifications as opposed to DNA methylation promoter changes were observed in IVF blastocysts and maintained in adult adipose tissue of inbred mice. This indicates that an epigenetic memory following preimplantation disturbance is maintained in selected non-imprinted loci.

Supported by: NIH grant HD060722.

P-197 Tuesday, October 21, 2014

OBJECTIVE: To investigate the relationship between cleavage stage mouse embryo kinetics and subsequent blastocyst metabolism and viability.

MATERIALS AND METHODS: In vitro fertilized mouse zygotes were observed using a time-lapse imaging system and zygotes were identified as ‘fast’ or ‘slow’. Blastocysts were analyzed for carbohydrate and amino acid metabolism followed by assessment of quality and viability.

RESULTS: Embryos observed with a faster time of first cleavage (first quartile, designated ‘fast’) were found on average to be 2.7h ahead of the slower embryos (fourth quartile, designated ‘slow’), 11.5±0.1h vs. 14.2±0.1h, p<0.0001). Subsequently on day 5, blastocysts developed from ‘fast’ embryos had more cells in the ICM (17.4±2.1 vs. 13.7±4.2, p<0.001), a higher glucose consumption (21.2±1.2 pmol/h vs. 13.6±1.0 pmol/h, p<0.0001) and a lower glycolytic rate (54.5±3.1% vs. 67.3±5.1%, p<0.05) compared to ‘slow’ embryos. Further LC-MS analysis revealed that ‘fast’ blastocysts consumed more aspartate than ‘slow’ blastocysts (2.2±0.1 pmol/embryo/h vs. 1.8±0.1 pmol/embryo/h, p<0.05). Blastocyst outgrowth area was significantly higher in ‘fast’ embryos compared to ‘slow’ embryos (491370.8±28975m vs. 393385.5±289728 pixels, p<0.05). There was no significant difference in implantation rate; however ‘fast’ embryos showed higher fetal development per implantation as ‘slow’ embryos (69.6% vs. 40.4%, p<0.01).

CONCLUSION: These findings suggest that kinetically different embryos develop into blastocysts with differences in metabolic profiles and potential viability. Ongoing work is now using these viability markers in combination to improve embryo selection efficiency and further improve pregnancy rates.

Supported by: NIH Grant HD060722.
IMPACT OF ENDOPLASMIC RETICULUM STRESS ON THE POST-OVULATORY AGING OF THE MOUSE OOCYTE. H. Igarashi, T. Takahashi, M. Amita, A. Hasegawa. Obstetrics and Gynecology, Yamagata University Faculty of Medicine, Yamagata, Japan.

OBJECTIVE: The endoplasmic reticulum (ER) stress is activated in response to an accumulation of unfolded protein in the lumen of the ER. ER stress is critical for cell survival, but chronic or excess ER stress can lead to apoptosis in various cell types. ER stress is known to cause various aging related diseases such as diabetes and neurodegenerative diseases. In this study, the participation of the ER stress in the postovulatory aging of oocyte was examined.

DESIGN: Animal model study.

MATERIALS AND METHODS: In this study, mouse oocytes released from the oviduct at 12.5 hour and 18.5 hour post-hCG were designated as “fresh” and “aged” oocytes, respectively. Experiments were done as follows: (1) Analysis of expression of BiP (chaperone induced at ER stress) level by western blotting with embryo development in the fresh oocytes. (2) Comparison of expression of BiP at protein level in the fresh and the aged oocytes. (3) Analysis of the effects by ER stress inducer, thapsigargin (Tg: calcium pump inhibitor of ER) on BiP expression and on fertilization rate and embryo development after in vitro fertilization. Animals were treated in accordance with the NIH Guide for the Care and Use of Laboratory Animals, as approved by the Animal Care and Use Committee of Yamagata University.

RESULTS: (1) Expression of BiP was the highest in the MI oocytes, and decreased with embryo development after in vitro fertilization. (2) Expression of BiP in the aged oocytes was increased compared with that in the fresh oocytes. (3) Tg treated oocytes showed increase in expression of BiP, low fertilization rate and deteriorated embryo development. Furthermore, Tg treatment in the fresh oocytes deteriorated embryo quality.

CONCLUSION: Expression of BiP in mouse oocyte/embryo decreased with the embryo development. The result that excessive expression of BiP in the aged oocyte was observed in association with the poor embryo development suggested that aged oocytes were exposed to excess ER stress. Because excess ER stress induced by Tg led to low fertilization rate and poor embryo development, these data suggested that ER stress might participate in postovulatory aging process of the mouse oocyte.

Supported by: This study was Supported by JSPS KAKEN research grants 20591905 to H.I.

P-199 Tuesday, October 21, 2014

BLASTOCOEFL FLUID (BF) HARBORS EMBRYONIC DNA THAT MAY RESULT FROM THE MARGINALIZATION OF ANEUPLOID CELLS DURING EMBRYOGENESIS. K. J. Tobler, Y. Zhao, R. Ross, A. T. Benner, X. Xu, D. Du, K. Bromann, K. Thrift, P. R. Brezina, W. G. Kearns, Johns Hopkins Medical Institutions, Baltimore, MD; Fort Worth Fertility, Fort Worth, TX; Center for Preimplantation Genetics, LabCorp, Rockville, MD; Vanderbilt University School of Medicine, Fertility Associates of Memphis, Memphis, TN.

OBJECTIVE: To determine if aneuploic cleavage stage embryos can marginalize aneuploid cells into the BF.

DESIGN: Prospective cohort study.

MATERIALS AND METHODS: 38 cleavage stage embryos had a single blastomere removed and were then cultured to the expanded blastocyst stage. BF was removed from the blastocyst cavity using an intracytoplasmic sperm injection needle and stained using 4',6-diamidino-2-phenylindole to confirm blastomere removal. BF was removed from the blastocoel cavity using an intracytoplasmic sperm injection needle to confirm blastomere removal. BF was analyzed. Of the 20 aneusomic cleavage stage embryos, 70% (14/20) developed into a euploid blastocyst (ICM/TE). Of these 14 normalized blastocysts, 86% (12/14) had euploid DNA within the BF (Table 1). All 10 euploid cleavage stage embryos remained euploid during differentiation to blastocysts. Three of these nine euploid blastocysts harbored aneuploid DNA within the BF.

Resolution of aneusomy during differentiation from an aneuploid cleavage stage embryo to a euploid blastocyst with euploid DNA within the blastocoeal fluid

CONCLUSION: Aneuomics cleavage stage embryos can differentiate into euploid blastocysts using a mechanism that involves marginalizing aneuploid cells or nuclei into the blastocoeal cavity.

P-200 Tuesday, October 21, 2014

A GENOME-WIDE STUDY OF RECOMBINATION EVENTS IN HUMAN PREIMPLANTATION EMBRYOS. M. Konstantinidis, N.-N. Goodall, W. Caswell, G. Rosen, G. Celia, J. Meriano, E. Yebosh, E. Mills, Reprogenetics, Livingston, NJ; Fertility Centers of New England, Reading, MA; Reproductive Partners Medical Group, Redondo Beach, CA; Dominion Fertility, Atlanta, VA; LifeQuest Centre for Reproductive Medicine, Toronto, ON, Canada.

OBJECTIVE: To carry out an in depth investigation of recombination events in human preimplantation embryos.

DESIGN: Single nucleotide polymorphism (SNP) arrays were used to genotype 73 blastocyst embryos. The number of recombination events was determined for each chromosome.

MATERIALS AND METHODS: Each embryo included in this study was biopsied once and cells obtained were processed using the Infinium Karyomap-12 BeadChip. Wilcoxon Signed-Rank Test (SPSS v19.0.0, USA) was used for statistical evaluation.

RESULTS: In total, 2,238 chromosomes and 3,582 recombination events were investigated. Regarding autosomes, the recombination rate was determined to be 40.5±1.1 (standard error of the mean) for female meiosis and 24.0±0.7 for male meiosis. Number of cross-over events was calculated for each autosome inherited from father and mother. Cross-over events for autosomes inherited from the father ranged from 0.5 to 1.8 per chromosome, while maternal meiosis events ranged from 0.7 to 3.3. Recombination rate at pseudoautosomal PAR1 region for paternally inherited X and Y chromosomes was calculated to be 0.3. Recombination rate for maternally inherited X chromosome was 1.8. Importantly, mean recombination frequencies for chromosomes 21 (0.6) and 22 (0.7) were found to be the lowest amongst autosomes; the difference in rate from all the other autosomes being statistically significant (P<0.001). No correlation of maternal or paternal age with recombination rate could be calculated.

CONCLUSION: The technology used in this study offers the potential of performing genome-wide investigation of recombination events in human preimplantation embryos and is expected to assist research carried out on this important subject. Studies carried out in previous years had indicated that altered meiotic recombination is associated with genesis of aneuploidy. This study provides further evidence towards this indication and suggests that the high aneuploidy rates seen in chromosomes 21 and 22 in embryos might be linked to reduced recombination rates observed for these chromosomes.

P-201 Tuesday, October 21, 2014

THE IMPACT OF FOOD INTAKE AND SOCIAL HABITS ON EMBRYO QUALITY AND THE LIKELIHOOD OF BLASTOCYST FORMATION. G. Halpern, D. P. A. F. Braga, A. S. Setti, R. C. S. Figueira, A. Iaconelli, Jr., E. Borges, Jr., Fertility - Centro de Fertilização Assistida, São Paulo, SP, Brazil; Instituto Sapientiae, São Paulo, SP, Brazil.

OBJECTIVE: To evaluate whether patients’ lifestyle factors and eating habits can influence embryo quality, the likelihood of blastocyst formation and intracytoplasmic sperm injection (ICSI) outcomes.
MATERIALS AND METHODS: The study included 269 embryos recovered from 269 cytoplasts undergoing ICSI cycles between January 2012 and July 2013. All patients completed a questionnaire with multiple-choice questions prior to the beginning of the treatment. The women were asked about the frequency of their consumption of many food items and about their social habits. The effects of dietary and social habits on embryo quality on day three and the likelihood of blastocyst formation were evaluated. Moreover, the influence of dietary and social habits on pregnancy and miscarriage rates was also investigated.

RESULTS: The consumption of cereals (OR: 1.34, CI: 1.09-1.59), vegetables (OR: 1.25, CI: 1.06-1.38) and fruits (OR: 1.38, CI: 1.07-1.71) positively influenced the embryo quality at the cleavage stage. The quality of the embryo at the cleavage stage was negatively correlated with the consumption of alcoholic drinks (OR: 0.75, CI: 0.62-0.95) and smoking habits (OR: 0.95, CI: 0.82-0.99). The consumption of fruits influenced the likelihood of blastocyst formation (OR: 1.32, CI: 1.08-1.63), which was also positively affected by the consumption of fish (OR: 1.31, CI: 1.05-1.66). Being on a weight-loss diet (OR: 0.78, CI: 0.62-0.98), the consumption of red meat (OR: 0.81, CI: 0.65-0.99) and alcoholic drinks (OR: 0.75, CI: 0.62-0.95), as well as smoking habits (OR: 0.76, CI: 0.56-0.94) had negative influences on the likelihood of blastocyst formation. The consumption of red meat and the body mass index had a negative impact on the likelihood of pregnancy (OR: 0.68, CI: 0.48-0.89 and OR: 0.43, CI: 0.25-0.93, respectively). The occurrence of miscarriage was not influenced by any food consumption or social habit.

CONCLUSION: Our evidence suggests a possible relationship between environmental factors and ovary biology. Therefore, couples seeking assisted reproductive technology must be advised about the adverse effects of female lifestyles on treatment success.

P-203 Tuesday, October 21, 2014

MORPHOKINETICS OF CLEAVAGE STAGE PREDICTS DEVELOPMENT TO THE BLASTOCYST STAGE. H. J. Kim, J. M. Jang, W. D. Lee, S. H. Yoon, J. H. Lim. Maria Fertility Hospital, Seoul, Republic of Korea; Fertility Research Center, Maria Medical Foundation, Seoul, Republic of Korea.

OBJECTIVE: The aim of our study was to compare the early embryo morphokinetics parameters of the embryos that developed into blastocyst or arrested, in order to find out possible differences in between two groups.

MATERIALS AND METHODS: This study was conducted between April 2013 and March 2014 in a analysis of fertilized embryos (n=724, 185 patients) were cultured in time-lapse incubator (EmbryoScope, Unisense Fertilitech, Denmark) until day 6 and annotated for pattern time of cleavage. Student’s t-test was used for statistical analysis. Average hours±SD from ICSI insemination are reportage for all stages.

RESULTS: The mean timings of the pPB2, pPNF, t2, t3 and t4 for embryos that developed into blastocysts were faster than the arrested embryos. Moreover, the length of the second cell cycle (cc2=t2-t3) was significantly longer in the embryos that developed into blastocysts compared with arrested embryos.

<table>
<thead>
<tr>
<th>TABLE 1.</th>
<th>Blastocyst (n=298)</th>
<th>Arrested embryo (n=426)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>CI 95%</td>
</tr>
<tr>
<td>pPB2</td>
<td>3.0 ± 1.1</td>
<td>(2.9-3.2)</td>
</tr>
<tr>
<td>pPNF</td>
<td>24.0 ± 3.0</td>
<td>(23.7-24.3)</td>
</tr>
<tr>
<td>t2</td>
<td>26.6 ± 3.2</td>
<td>(26.2-26.9)</td>
</tr>
<tr>
<td>t3</td>
<td>36.4 ± 4.8</td>
<td>(35.8-36.9)</td>
</tr>
<tr>
<td>t4</td>
<td>38.5 ± 5.2</td>
<td>(37.9-39.1)</td>
</tr>
<tr>
<td>t5</td>
<td>49.0 ± 7.5</td>
<td>(48.1-49.8)</td>
</tr>
<tr>
<td>cc2</td>
<td>9.8 ± 3.9</td>
<td>(9.3-10.2)</td>
</tr>
<tr>
<td>s2</td>
<td>2.2 ± 3.9</td>
<td>(1.7-2.6)</td>
</tr>
</tbody>
</table>

CONCLUSION: Significant differences were shown in embryo morphokinetics between two groups. The various time-lapse parameters in our analysis were identified as predictive for development to blastocysts.

P-204 Tuesday, October 21, 2014


OBJECTIVE: Recently, some study showed to limited implantation potential of direct cleavage (DC) embryos. Extremely short cell cycles could be due to that incomplete DNA replication may be associated with unequal distribution of DNA to blastomeres. The aim of this study was to investigate that fertilization methods and patients age were affected DC rate.

MATERIALS AND METHODS: Retrospective analysis of fertilized embryos (n=1757, 306 patients) were cultured in time-lapse incubator (EmbryoScope, Unisense Fertilitech, Denmark) at 37 °C, 5% CO2 and 5% O2 between March 2013 and March 2014. In order to investigate ovarian factor infertility, the evaluation of DC formation according to fertilization method was conducted by age (younger versus older patients). DC from two to three cells was defined as the second cell cycle being shorter than 5 hours (cc2=t3-t2 ≤ 5 hours). All embryos were cultured and assessed a time-lapse incubator (EmbryoScope, Unisense Fertilitech, Denmark) and annotated for pattern time of cleavage.

RESULTS: Among the 1757 fertilized embryos, 398 (22.7%) were DC embryos. DC rate of ICSI fertilized embryos (23.3%, 283 of 1217) was slightly higher than IVF fertilized embryos (21.3%, 115 of 540), although there was no significant difference. As a result of comparing DC rate between fertilization by ICSI and IVF, there was no difference in younger (≤ 35 years) patients group (20.9% and 20.6%). In older (≥ 36 years) patients
group, DC rate of ICSI (26.3%, 140 of 532) is statistically significantly higher than IVF (21.8%, 69 of 317) (P < 0.05).

**CONCLUSION:** In this study, DC formation did not affected by fertilization method, but it of older patients was higher in ICSI group. It means that ovarian factor could have detrimental effect on embryo development.

**P-205 Tuesday, October 21, 2014**

THE EVALUATION OF HUMAN EMBRYO DEVELOPMENT IN IVF/ICSI FERTILIZED OOCYTES: A TIME-LAPSE STUDY. H. J. Kim, a H. J. Yoon, a J. M. Jang, a W. D. Lee, a S. H. Yoon, b J. H. Lim. a Maria Fertility Hospital, Seoul, Republic of Korea; bFertility Research Center, Maria Medical Foundation, Seoul, Republic of Korea.

**OBJECTIVE:** To compare the dynamics of early development between embryos cultured in IVF and ICSI fertilized oocytes.

**DESIGN:** Retrospective analysis.

**MATERIALS AND METHODS:** A total of 1714 normal fertilized embryos obtained from 267 IVF/ICSI patients who underwent ovum retrieval culture using a time-lapse incubator (EmbryoScope, Unisense Fertilitet, Denmark) at 37°, 6% CO2 and 5% O2 between April 2013 and March 2014. All embryos were investigated by detailed time-lapse analysis that measured the developmental events in the hours after IVF/ICSI insemination.

**RESULTS:** Embryos cultured in ICSI fertilized oocytes were advanced from the first mitosis cycle and reached 2 to 5-cell stages earlier. However, there was not any difference between the length of the second cell cycle (cc2 = t3 – t2) and the synchrony in the division from two to four cells (s2 = t4 – t3).

**CONCLUSION:** Morphokinetics of embryo development vary between IVF and ICSI fertilized oocytes at least until the 5-cell stage. However, the overall embryological parameters remain similar regardless of the fertilization method.

**P-206 Tuesday, October 21, 2014**

SYNCHRONICITY OF CLEA VAGE CYCLES PREDICTS BLASTOCYST FORMATION AND QUALITY. C. Pirkevi, M. Cetinkaya, Y. Kuntepe Colakoglu, H. K. Yelke, S. Kahraman. ART and Reproductive Genetics Center, Istanbul Memorial Hospital, Istanbul, Turkey.

**OBJECTIVE:** The aim of the study is to predict high quality blastocyst formation potential from day 3 embryos using morphokinetics.

**DESIGN:** This retrospective cohort study was conducted between August 2011 and March 2013 in a private ART Center and approved by the institutional review board. The study included initially 1340 embryos having a (t8) and belonging to patients transferred on day 5. The cohort studied involved only infertile patients (female, male or combined) and is in this respect a heterogeneous population.

**MATERIALS AND METHODS:** Blastocysts were scored before transfer according to Gardner’s classification (115-120h post ICSI). The cleavage synchronicity from 2 to 8 cells was expressed by the following equation: 

\[
CS2-8 = \frac{((t3-t2) + (t5-t4)) / (t8-t2))}{(t8-t2)}
\]

that was subsequently applied to score embryos and to predict high quality blastocyst formation. The algorithm built was then verified in two other independent sets of day 5 embryos (n=688; n=787, respectively).

**RESULTS:** Embryos should mainly stay in even cell stages, which should be balanced when compared to each other, ideally reflecting cleavage synchronicity. The formula was applied and embryos were classified in 10% groups and good/ top quality blastocyst rate (BR) was computed for the 10 intervals. Each class had a mean BR, which was subtracted from the initial BR, giving a positive or negative score. Obtained scores were used for the final prediction of the blastocyst formation potential. The logistic regression model built gave an AUC=0.799 (95% CI: 0.776 to 0.820; Sensitivity: 84.0; Specificity: 63.6). In the second dataset of 688 day 5 embryos, the formula gave an AUC=0.802 (95% CI: 0.770 to 0.831; Sensitivity: 87.9; Specificity: 61.7). Finally, in the third dataset of 787 day 5 embryos the equation yields an AUC=0.879 (95% CI: 0.759 to 0.817; Sensitivity: 76.9; Specificity: 71.7).

**CONCLUSION:** Time points defining precise embryo cleavage events may not be generalized to infertile patients with different etiologies. However, using ratios based on cleavage cycles defining synchronicity of embryos, allows in this retrospective cohort study an individualized analysis giving high predictivity of blastocyst formation and quality in three independent sets of data. The proposed model has to be further tested in a randomized prospective trial to evaluate its efficacy, and may in the future improve embryo selection in IVF treatments.

**P-207 Tuesday, October 21, 2014**

POOR OVARIAN RESERVE, IN ACCORDANCE WITH THE BOLOGNA CRITERIA, AND MULTINUCLEATED BLASTOMERS – A PROSPECTIVE RANDOMIZED STUDY. O. Radin, a Y. Yuchi Lahav, a I. Izhaki, b S. Bar-Ami, a M. Ben-Ami. a J. S. Younis. b Reproductive Medicine, Department of Obstetrics and Gynecology, Faculty of Medicine in Galilee, Bar Ilan University, Poria Medical Center, Tiberias, Lower Galilee, Israel; aDepartment of Evolutionary and Environmental Biology, University of Haifa, Haifa, Israel.

**OBJECTIVE:** Aim: To gain insight into the pathophysiology of ovarian aging, as defined by the Bologna criteria for poor ovarian response, by evaluating its association to MNB.

**DESIGN:** Introduction: In recent years multinucleated blastomeres (MNB) has been suggested as a morphological scoring parameter. MNB has been shown to be a predictor of low embryo implantation, poor pregnancy rate and aneuploidy. Since high rate of aneuploidy is anticipated in low ovarian reserve patients, we expect to find a positive correlation between low ovarian reserve women undergoing ART and high rate of MNB in their embryos.

**MATERIALS AND METHODS:** Infertile women eligible for IVF/ICSI treatment were prospectively investigated throughout their IVF cycles. Basal ovarian reserve studies including antral follicle count (AFC) and basal hormone profile were performed in all women three months before starting the treatment. At the end of treatment, 51 women with poor ovarian response in accordance with the Bologna criteria were chosen as the study group. The control group included 51 women with normal ovarian response that were treated at the same time period. All embryos were graded before transfer in accordance with the routine embryo scoring systems and for the presence or absence of MNB.

**RESULTS:** The number of women which had at least one embryo with MNB was significantly higher in the study group compared to the control group; 49% vs. 31%, respectively. In addition, the average ratio of MNB embryos to total number of embryos was significantly higher in the poor ovarian response group compared to the normal ovarian response group, 0.20 ± 0.27 vs. 0.10 ± 0.23, respectively. Moreover logistic regression analysis has found that the rate of MNB was significantly and inversely dependent on the number of total embryos achieved following treatment i.e. ovarian reserve.

**CONCLUSION:** Our study reveals for the first time that the rate of MNB is higher in infertile low ovarian reserve women. This finding supports the notion that multinucleation is a phenomenon associated with chromosomal aneuploidy and that MNB can be employed as a simple morphological measure for grading embryos’ quality in IVF laboratories. **Key words:** multinucleation, poor ovarian response, low ovarian reserve, ovarian aging, IVF.

**P-208 Tuesday, October 21, 2014**


**OBJECTIVE:** To determine the relevance of cell count and capacity to progress to blastocyst as predictors of ploidy in tripronucleated embryos derived from ICSI (TPN).
P-210 Tuesday, October 21, 2014

INFLUENCE OF THE MATERNAL AGE ON THE EMBRYO DEVELOPMENT AND EUPLOIDY IN EMBRYOS ANALYZED BY FISH OR ACGH. P. Villanueva, a S. Sepulveda, a L. Noriega, b R. Lopez, c J. Portella, c L. Guzman. a Embryology Laboratory, Grupo PRANOR, Lima, Peru, b Reprogenetics Latinoamerica, Lima, Peru.

OBJECTIVE: To determine the influence of the maternal age on embryo development by using oocyte donor IVF cycles undergoing PGS by FISH on day 3 or aCGH on day 5.

DESIGN: Retrospective cohort analysis.

MATERIALS AND METHODS: In total, 613 oocyte donor cycles were included. The study groups were classified according to the maternal age: group 1, <40 years (n = 231); group 2, from 40 to 49 years (n = 292) and group 3 males ≥ 50 years (n = 90). Data was collected from the computerized database of the IVF Centers.

RESULTS: Fertilization rate was lower in group 2 compared to group 1 and 3 (p<0.006). There were no differences on embryo quality on day 3 among the groups. Blastocyst rate was 45.8% (852/1861) in group 1 and it was significantly higher to group 2 (42% (955/2276)) and group 3 (41.5% (299/721)) (p<0.025). In group 1, top quality blastocyst rate was 49.6% (325/655) and was significantly higher to group 2 and 3 (36.8% (264/717) and 40.2% (104/259) respectively) (p<0.001) when embryos were analysed by FISH. Although the same tendency was observed on embryos analysed by aCGH no significant differences were found. No differences were found on the euploid rate either by FISH (group 1 = 50.2%, group 2= 53.8% and group 3= 51.9%; p=0.68) or aCGH (group 1= 64.8%, group 2= 71.2% and group 3= 63.2%; p=0.48).

CONCLUSION: The increased paternal age significantly decreased the blastocyst rate and the morphology of the blastocyst in oocyte donor cycles. However, the euploid rate was similar among the groups.

P-209 Tuesday, October 21, 2014

PREDICTION BLASTOCYST FORMATION BY OXYGEN CONSUMPTION IN HUMAN D3 THAWED EMBRYOS SUPERNUMERARY AFTER FROZEN EMBRYO TRANSFER. S. B. Han, N. H. Chung, Maternity and Children Health Care Hospital, Chongqing, China.

OBJECTIVE: Compare oxygen consumption of supernumerary thawed human D3 TPN embryos between which development to blastocyst and not by scanning electrochemical microscopy (SECM).

DESIGN: Cohort study.

Human supernumerary thawed D3 FET embryos were obtained from clinical FET cycles in Chongqing Reproduction and Genetics Institute. D3 FET embryos cultured 24 hr after thawed; after culture the embryos do not meet the standard of transplantation were performed by oxygen consumption analysis. After oxygen consumption analysis, embryos were cultured untilled at D6 and analysis the rate of blastocyst formation.

MATERIALS AND METHODS: Human supernumerary thawed D3 FET embryos were obtained from clinical FET cycles in Chongqing Reproduction and Genetics Institute. After cultured 24 hr thawed, the embryos who do not meet the standard of transplantation were performed by oxygen consumption analysis. For the measurement of oxygen consumption, medium was transferred into a plate with cone-shaped micro-wells. A micro-disk electrode scanned in the Z-direction from the bottom of a micro-well. The motor driven XYZ stage was located on the microscope stage for electrode tip scanning. The XYZ stage and potentiostat were controlled by the computer. The oxygen consumption rate of the embryos was calculated by software, using an algorithm based on spherical diffusion theory. The measurements of oxygen consumption of each embryo took approximately 40 sec. It took less than 3 min to perform three measurements, which were used to calculate the average respiratory activity of each embryo. After oxygen consumption analysis, embryos were cultured untilled at D6 to Statistical blastocyst formation rate.

RESULTS: A total of 108 D3 embryos were measured by SECM, after culture 24 hr, 56 embryos have development into blastocyst (blastocyst formation group), and 52 were not (blastocyst formation fault group). The respiration rates (F =10-14 mol O2 mmol -1/hr) of blastocyst group formation and blastocyst formation fault group were 0.38±0.15 vs 0.23±0.13 respectively (p=0.0308).

CONCLUSION: Our data demonstrate that oxygen consumption could as a predictor of blastocyst formation for thawed supernumerary FET embryos.
A statistically significant difference in blastocyst and hatching rates was noted, with p-values <0.05 at a 95% confidence interval. No significant difference was noted at the cleavage stage for these incubators.

CONCLUSION: Late embryo growth and development is greatly improved in the non-humidified conventional box incubator when compared to a non-humidified top-load bench incubator. This significant disparity could be caused by the difference in mechanisms of maintaining environmental control of these incubators, however further study into the temperature and environment control of these incubators is needed.

P-212 Tuesday, October 21, 2014

METABOLIC PROFILING OF EMBRYO CULTURE MEDIUM ON DAY 3 IS ASSOCIATED WITH SUBSEQUENT BLASTOCYST DEVELOPMENT. R. L. Krisher, a A. F. Greene, b J. Stevens, a A. Heuberger, c W. B. Schoolcraft, d National Foundation for Fertility Research, Lone Tree, CO; e Fertility Laboratories of Colorado, Lone Tree, CO; f, Colorado State University, Ft. Collins, CO; g Colorado Center for Reproductive Medicine, Lone Tree, CO.

OBJECTIVE: Determine metabolic biomarkers of blastocyst development and quality based on changes in media metabolites following culture of single embryos to D3.

DESIGN: Retrospective analysis of media samples from individually cultured D3 embryos based upon outcome of embryo development on D5.

MATERIALS AND METHODS: Media (Quinn’s Advantage®; Cooper Surgical) samples were collected from wells of an EmbryoSlide™ following culture of individual embryos to D3. Samples from wells without an embryo were collected as controls (n=7). Development of each embryo was recorded on D5/6. D3 samples were grouped based upon subsequent embryo development: no blastocyst (NoBl; n=8), poor quality blastocyst (PoorBl; n=4) and good quality blastocyst (GQBl; n=13). Samples were analyzed for 27 selected metabolites using GC-MS. Metabolites were quantified using the mean peak area of selected ions (n=2 injection replicates) against a 5-point standard curve. Values for each metabolite were analyzed using ANOVA with Fisher’s LSD test (p<0.01 considered significant).

RESULTS: Several amino acids significantly altered in the culture media by D3 embryos were associated with subsequent developmental success. Interestingly, embryos that did not make a blastocyst were found to have an intermediate profile to embryos that made a blastocyst of good or poor quality for all of these amino acids. There was not a typical amino acid metabolic profile of high quality D3 embryos. Some amino acids were taken up in greater, or lesser extent by good quality compared to poor quality embryos.

P-213 Tuesday, October 21, 2014

PROLONGED TIME TO FIRST CYTOKINESIS AND THE INTERVAL BETWEEN THE FIVE CELL STAGE AND EARLY CAVITATION ARE ASSOCIATED WITH EMBRYONIC MOSAICISM. K. H. Hong, a M. D. Werner, a J. M. Fraunsiak, a E. J. Forman, a,b K. Upham, b N. R. Treff, a,b R. T. Scott, Jr., a,b REI, RMA NJ, Basking Ridge, NJ; 3RMA NJ, Basking Ridge, NJ.

OBJECTIVE: The use of time lapse imaging through day 3 of development provides insight into the probability a cleavage stage embryo will blastulate. This raises the question of whether these parameters are correlated with other biologic phenomena occurring during the same time interval. One such parameter is mosaicism. Mosaicism is a particularly problematic abnormality as it may result in misdiagnoses from comprehensive chromosome screening (CCS) and is a potential reason why some embryos designated as euploid fail to deliver. Parameters identifying embryos at increased or decreased risk for mosaicism would, therefore, be useful during embryo selection.

DESIGN: Prospective observational study.

MATERIALS AND METHODS: Embryos that tested aneuploid and had undergone time-lapse imaging from the 2PN to the blastocyst stage were studied. The embryos were biopsied 4 times, placed into individual PCR tubes, blinded as to their origin, and submitted for NextGen based CCS. If any of the biopsies yielded different CCS results, the embryo was deemed mosaic. If all biopsies agreed it was deemed non-mosaic. Traditional time lapse parameters shown to predict blastulation and an additional parameter, time from 5-cell stage to the first sign of cavitation, were compared between mosaic and non-mosaic embryos.

RESULTS: 12 of the 53 (23%) embryos were mosaic. Mosaic embryos took longer to undergo first cytokinesis (P0) (p<0.02). Other time-lapse parameters evaluated through the cleavage stage and development were equivalent. Interestingly, the time to early cavitation from the 5-cell stage was longer in mosas (P<0.03).

CONCLUSION: These data demonstrate that two time-lapse parameters related to first cytokinesis and time to first cavitation are associated with embryonic mosaicism. The former may reflect abnormalities in formation of the first mitotic spindle. The latter may indicate that cellular processes leading to mosaicism also result in delayed rates of development after genomic activation. With further investigation, such parameters may help avoid the transfer of mosaic embryos.

Supported by: Auxogyn, equipment.

Time-lapse parameters in mosaic and non-mosaic embryos

<table>
<thead>
<tr>
<th>Time to Caviation</th>
<th>P0</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mosaic</td>
<td>21.6</td>
<td>0.42</td>
<td>11.54</td>
<td>0.79</td>
<td>13.77</td>
</tr>
<tr>
<td>Non-Mosaic</td>
<td>22.22</td>
<td>0.37</td>
<td>11.16</td>
<td>1.30</td>
<td>12.25</td>
</tr>
<tr>
<td>P Values</td>
<td>0.02</td>
<td>0.81</td>
<td>0.70</td>
<td>0.50</td>
<td>0.15</td>
</tr>
</tbody>
</table>

P-214 Tuesday, October 21, 2014


OBJECTIVE: This study explores the relationship between abnormal cleavage such as direct cleavage from 1-3 cells and 2-5 cells, of developing embryos and its correlation to blastocyst formation.

DESIGN: A clinical retrospective study.

MATERIALS AND METHODS: Embryo development was recorded and analyzed using Embryoscope, Unisense Fertilitech, Denmark. All morphokinetic observations were made with special attention to the time at which the pronuclei disappears, first and second cleavage times. 14 couples (maternal age ≤38) underwent ICSI (n=263), of which 45 cycles led to an embryo transfer between December 2012 and September 2013. All transfers were frozen cycles and embryos were transferred at either cleavage or blastocyst stages.

RESULTS: Conducted t-tests illustrate the significant difference between embryos that developed into blastocysts and those that did not (TABLE 1). Of 263 cases, 194 embryos were kept in continuous culture till Day 5. Thirteen of these embryos had an abnormal division from 1-3 cells and another 11 embryos divided directly from 2-5 cells. Whereas embryos dividing from 1-3 cells directly could not form any blastocysts, three embryos with abnormal cell division from 2-5 cells successfully developed into blastocysts. One of three however, led to a successful fetal heart beat.
**TABLE 1.**

<table>
<thead>
<tr>
<th>Blastocysts</th>
<th>Blastocysts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not Blastocyst</td>
<td>Quality</td>
</tr>
<tr>
<td>Blastocysts (MEAN±SD)</td>
<td>Blastocysts Quality (MEAN±SD)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>The number of Embryos</th>
<th>90/149</th>
<th>104/149</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of the pronuclei disappearance</td>
<td>24.5±2.8</td>
<td>28.0±4.8</td>
</tr>
<tr>
<td>Time of the first cleavage</td>
<td>27.3±2.9</td>
<td>30.5±5.0</td>
</tr>
<tr>
<td>Time of the second cleavage</td>
<td>38.9±4.3</td>
<td>42.2±8.4</td>
</tr>
<tr>
<td>The Zcl at the first cleavage</td>
<td>96.7±%</td>
<td>59.6±%</td>
</tr>
</tbody>
</table>

| p-value | 28/90 | 62/90 |

CONCLUSION: These results indicated that good quality blastocysts developed slightly faster than others; thereby supporting the fact that the divisions must take place during a narrow window to maintain the viability of the embryo. Focusing on the first cleavage times, our study concludes that embryos divided from one to two cells had approximately 35% increased chance of successfully growing into a blastocyst than those that cleaved from one to three cells, therefore recognizing it as an imperative marker. Lastly, our results show that abnormally dividing embryos can potentially result in a positive pregnancy suggesting that if the embryo does develop into blastocyst after the 2-5 cells, there may be a chance to applying transfer.

**EMBRYO CULTURE**

**P-215** Tuesday, October 21, 2014

**NOVEL AUTOMATED DEVICE WITH THE CHIP ELECTRODE FOR MONITORING RESPIRATORY ACTIVITY OF EMBRYOS, H. Kurosawa, a H. Utsunomiya, a N. Shiga, a A. Takahashi, a M. Ishibashi, a Z. Watanabe, a H. Abe, a Y. Terada, a T. Takahashi, a A. Fukui, a R. Suganuma, b N. Yaegashi, a Tohoku University Graduate School of Medicine, Sendai, Japan; a Yamagata University, Yonezawa, Japan; a Akita University Graduate School of Medicine, Akita, Japan; a Yamagata University Faculty of Medicine, Yamagata, Japan; b Hiroaki University School of Medicine, Hiroaki, Japan; a Fukushima Medical University, Fukushima, Japan.

OBJECTIVE: Morphological evaluation has been generally used to evaluate embryo quality. However, it is subjective and frequently different between investigators. We previously reported that the oxygen consumption rate of embryos measured by the scanning electrochemical microscopy was a useful parameter for evaluating embryo quality1). Although this method is an epoch for high sensitivity and non-invasiveness, popularization of the device is stagnant because it takes a lot of time to handle the device. We have developed a new device that measures the oxygen consumption rate automatically. In this study, we investigated the measurement of oxygen consumption using the new device.

DESIGN: Experimental work.

MATERIALS AND METHODS: The oxygen consumption rates of splashed medium of MCF-7 breast carcinoma cells and bovine embryos were measured by the new device. There were 4 embryo cavities in its electrode, and oxygen reduction current was measured with electrodes. A sample was transferred into the cavity filled with embryo respiratory assay medium, and then measured in incubator. The oxygen consumption rate was calculated from the difference of the oxygen reduction current with custom software based on spherical diffusion theory. It took within 5 minutes to measure the amount of oxygen consumption.

RESULTS: The oxygen consumption rates of spheroids (n=22) were 5.25-16.8±1014mol/sec and correlate with the radius of the spheroids. This result accorded with previous report2), indicating that measurement of the oxygen consumption rates was correctly performed by the chip electrode. The oxygen consumption rates of bovine embryo (n=4) were 1.30-2.89±1014mol/sec, these values were similar levels of that of human embryos previously reported3).

CONCLUSION: We could measure the oxygen consumption rate simply in a short time using new device including the chip electrode. These results indicated that we could measure the range of the oxygen consumption rates of human embryos using the chip electrode. In addition, measurement of embryonic respiration together with morphological evaluation could improve the accuracy of predicting pregnancy rate.

**Supported by:** A Grant-in-Aid from the Ministry of Health, Labor and Welfare, Japan.

**P-216** Tuesday, October 21, 2014

**IMPACT OF EMBRYO GENDER ON MORPHOKINETIC BEHAVIOUR. F. Bronet,a M. Mogales,a E. Martiienza, a M. Ariza,a A. Liñan,a C. Rubio,b J. A. Garcia-Velasco,a M. Meseguer. a IVF-PGD, b IVI Madrid, Madrid, Spain; b IVIOMICS, Valencia, Comunidad Valenciana, Spain; c IVI, Valencia, Comunidad Valenciana, Spain.

OBJECTIVE: To determine if it is possible to estimate embryo gender according to the embryo cleavage timings.

DESIGN: A retrospective and observational study including 369 embryos from our Comprehensive Chromosome Screening program conducted from January 2013 to December 2013. Embryos were grouped according to their sex: male (176 embryos) or female (161 embryos). Additionally another group was included with 32 embryos with Turner Syndrome (TS).

MATERIALS AND METHODS: All embryos were cultured in an incubator with time lapse technology, Embryoscope. Cleavage timing from insemination to day 3 was studied and all kinetic parameters that have been described in previous studies by our group have been taken into account and compared among the three groups.

RESULTS: Chromosomal abnormal rate was similar in both groups (male: 62.5%; female: 58.4%; p=0.4418). When morphokinetic parameters were separated in different quartiles and grouped we found statistical differences between male or female embryos. By logistic regression analysis we found that two specific kinetic variables were relevant; second synchrony (s2-t3) (>2 hours) and time for morula formation (Tm) (60.8±90.9 hours). By using these parameters we can propose an algorithm with different categories from A to D according to the likelihood to be a female embryo ranging from 71% to 42%. Additionally, we found statistical differences in cleavage time from one cell to two cells (t2) in TS embryos compared with male or female embryos (TS: 27.6±3.7; male: 26.3±3.0; female: 26.3±2.8; p=0.032).

CONCLUSION: Embryo development is affected by embryo gender and sex ratio could be affected by the embryo selection method for transfer based on kinetic parameters.

**P-217** Tuesday, October 21, 2014

**EMBRYOS DEVELOPING INDIVIDUALLY IN THE EMBRYOSCOPE SYSTEM ARE OF COMPARABLE QUALITY TO EMBRYOS GROWN IN GROUP CULTURE IN LARGE CELL CULTURE INCUBATORS. T. Elliott, J. Linn, S. Chhatiriwala, M. Best, P. Brahima, Z. P. Nagy. Reproductive Biology Associates, Atlanta, GA.

OBJECTIVE: The EmbryoScope has shown great promise as a tool for recording the development of human embryos and then allowing the retrospective examination of markers of embryo quality, ultimately enabling the embryologist to make a fully informed decision about which embryos to transfer and freeze. However, there is a school of thought that embryos grown individually, as in the EmbryoScope slide cells, may not benefit from paracrine factors available from group culture. Therefore, we collected and analyzed developmental data from patients whose sibling embryos were cultured in either traditional incubators in group culture or embryos in the EmbryoScope in single culture.

DESIGN: Retrospective data analysis.

MATERIALS AND METHODS: From April 2010 to February 2014, 18 patients had 221 sibling embryos split between traditional incubators in group culture and the EmbryoScope in single culture. Embryos from each group were assessed on day 5 and cell count and grade was noted. The grade was then given a numerical value from 4 to 1 (4=top quality “A” grade embryo;1=single cell on day 3 after 2PN seen on day 1). The embryos were assessed again on day 5 and our regular grading system was converted to a numerical scale where a top quality embryo was awarded 4 points, through to an arrested or degenerated embryo which would score 0 points. Finally an outcome score was given as 1 for transferred or day 5 frozen embryos.
or 0 for all outcomes. The cell numbers, grade conversions and outcomes were tallied and divided by the number of embryos in each group. Unpaired t-test was used to evaluate statistical significance.

RESULTS: See table.

<table>
<thead>
<tr>
<th></th>
<th>Group Culture EmbryoScope</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of embryos</td>
<td>109</td>
<td>112</td>
</tr>
<tr>
<td>Average number of cells on day 3</td>
<td>6.70 ± 2.019</td>
<td>7.09 ± 1.802</td>
</tr>
<tr>
<td>Day 3 grade conversion</td>
<td>3.08 ± 1.201</td>
<td>3.32 ± 0.945</td>
</tr>
<tr>
<td>Day 5 grade conversion</td>
<td>1.52 ± 1.737</td>
<td>1.58 ± 1.755</td>
</tr>
<tr>
<td>Outcome Score</td>
<td>0.43 ± 0.497</td>
<td>0.47 ± 0.501</td>
</tr>
</tbody>
</table>

CONCLUSION: This study demonstrates that embryos that develop in the EmbryoScope are not disadvantaged by the lack of group culture in terms of cell count on day 3, embryo quality grade on day 3, blastocyst grade quality on day 5 or outcome.

P-218 Tuesday, October 21, 2014


OBJECTIVE: This study was to evaluate the effect of autologous cumulus cell mass on embryo coculture in patients with a history of previous IVF failure.

DESIGN: Prospective cohort study between April 2013 and March 2014.

MATERIALS AND METHODS: A total of two hundred and forty-four patients who underwent GnRH long or antagonist protocol with fresh embryo transfer were analyzed. The cycles with severe male factor and single embryo transfer were excluded. Patients were divided into two groups. Patients who had embryos cultured in 20 ul of defined medium with autologous cumulus cell mass (ACC) (n = 101) and without autologous cumulus cell mass (No ACC) (n = 143). Well dispersed autologous cumulus cell mass directly put into the culture medium without hyaluronidase treatment. We compared the rates of good-quality embryos, implantation, multiple pregnancy, clinical pregnancy, and ongoing pregnancy between ACC and No ACC.

RESULTS: There were no differences between ACC and No ACC regarding mean female age (35.3 ± 3.3 vs. 34.5 ± 3.3, p = 0.134), mean number of retrieved oocytes (10.1 ± 6.1 vs. 9.8 ± 6.0, p = 0.736), maturation rate (87.8% vs. 92.4%, p = 0.798), fertilization rate (78.5% vs. 77.3%, p = 0.941), and mean number of transferred embryos (2.5 ± 0.6 vs. 2.4 ± 0.6, p = 0.130). We also observed similar rates of good-quality embryos (85.5% vs. 10.1%, p = 0.323), multiple pregnancy (23.4% vs. 30.4%, p = 0.445), and implantation (23.0% vs. 17.9%, p = 0.125) in ACC and No ACC, respectively. Although an increased mean previous IVF failure in ACC (2.4 ± 1.4 vs. 1.7 ± 1.1, p = 0.000), ACC achieved significantly higher rates of clinical pregnancy (46.5% vs. 32.2%, p = 0.023) and ongoing pregnancy (38.6% vs. 25.9%, p = 0.034) than those of No ACC.

CONCLUSION: These results suggested that coculture with autologous cumulus cell mass could improve the rates of clinical pregnancy and ongoing pregnancy in patients with a history of previous IVF failure.

P-219 Tuesday, October 21, 2014

FERTILIZATION AND EMBRYO DEVELOPMENT AT SPINDLE LOCATED OOCYTE FROM IMMATURE OOCYTE IN VITRO MATURATION CYCLES. Y. Nagase, M. Ikegami, Y. Yamamoto, T. Matsuura. ACT Tower Clinic, Hamamatsu, Shizuoka, Japan; Center for Reproductive & Developmental Medicine, Hamamatsu, Shizuoka, Japan.

OBJECTIVE: We occasionally obtain germinal vesicle (GV) oocytes by IVF cycles with mild ovarian stimulation. Immature oocytes were cultured in vitro maturation (IVM), and matured oocytes used for IVF-ET. We checked spindle before performed ICSI. It was aimed to evaluate the normal fertilization rate, to reach blastocyst rate and good blastocyst cryopreservation rate in vitro matured oocytes with or without spindle.

DESIGN: A reproductive analysis of patients undergoing IVM cycles.

MATERIALS AND METHODS: We evaluated 572 IVF cycles from January 2010 to February 2014 at ACT tower clinic, Shizuoka, Japan. We obtained 670 GV oocytes by needle aspiration with mild ovarian stimulation. We collected GV oocytes were assessed under the microscope with high magnification(×>200) using the spreading method. GV oocytes were cultured by IVM medium contain hyaluronan supplemented with 75μIU/ml recombinant FSH and HCG for 20~24 hours. The oocytes were demided of cumulus cells with hyaluronidase and pipetting after cultured. Mature oocytes were inseminated by Spindle Located ICSI (SL-ICSI).

RESULTS: We obtained 387 matured oocytes (maturatin rate is 57.8%). We performed SL-ICSI for 219 matured oocytes. In 178 oocytes, it was located spindle (81.3%). In normal fertilization rate, there were significantly higher achieved after ICSI at spindle located oocytes (SLs) than at non spindle located oocytes (nSLs) (70.8% vs.48.8%, P<0.05). SLs developed blastocyst rate and good blastocyst cryopreservation rate were 24.4% and 16.5%. In nSLs group was 5.6%, 5.6%.

CONCLUSION: Our results suggest that in vitro matured oocytes with spindle are good quality embryos. We will suspect development embryo to reach blastocyst by spindle located ICSI with in vitro matured oocytes. However, there are lower good quality blastocyst rate in vitro maturation cycles yet. We will study more good condition in vitro maturation with Spindle Located ICSI.

P-220 Tuesday, October 21, 2014

ADDITIVE EFFECT OF DIBUTYRYL cAMP TO IN-VITRO MATURATION RADIUM MEDIUM ON DEVELOPMENTAL COMPETENCE OF BOVINE AND HUMAN OOCYTES AFTER ICSI. C. Kani, M. Sakurai, N. Yamanaka, H. Yonaha, T. Matsuura. Yume Clinic Nagoya, Nagoya, Aichi, Japan; Prefectural University of Hiroshima, Shoubara, Hiroshima, Japan.

OBJECTIVE: Developmental competence of in-vitro-matured human oocytes has been still lower than that of in vivo-matured oocytes. It is well known that cyclic AMP (cAMP) is one of key signals during in vitro maturation (IVM) of oocytes. The aim of this study was to evaluate the effect of dibutylryl cAMP (dbcAMP), a transmembrane-type reagent during IVM on developmental competence of bovine, and human GV-oocytes with cumulus cells.

MATERIALS AND METHODS: As a basic medium, TC199 supplemented with 1 mg/ml albumin, 1 lU/ml FSH and 50 ng/ml EGF of recombinant human proteins was used. Cumulus-oocyte complexes (COCs) were cultured in the basic medium supplemented with 0, 25, 50 and 100 μM dbcAMP for 21 h (bovine) and 27 h (human). Human GV-oocytes with cumulus cells collected from clomiphene stimulation cycles were used for IVM, followed by Piezo-ICSI using fluorinert and culture with EmbryoScope for blastocyst development. Then, we examined the effect of dbcAMP during IVM on nuclear maturation and the blastocyst development after ICSI.

RESULTS: Treating bovine COCs with 0 to 100 μM dbcAMP during IVM did not affect percentage of MI oocytes (73.8 to 81.7%). Blastocyst rate in the group of 50 μM dbcAMP (48.3%) was significantly higher than that in the control group (0 μM: 25.9%), but not different between the other groups (25 μM: 28.9%, 100 μM: 40.6%). On the other hand, treating human COCs with 50 and 100 μM dbcAMP during IVM significantly reduced the percentage of MI-oocytes compared to 0 μM (81.3% vs. 57.7% and 52.8%). Blastocyst rate in each group of 25 and 50 μM dbcAMP was significantly higher than that in the group of 0 μM dbcAMP (0% vs. 15.4% and 24.4%).

CONCLUSION: Addition of dbcAMP to IVM medium increases the developmental competence of bovine immature oocytes without loss of nuclear maturation. On the other hand, treating of human immature oocytes with dbcAMP improves blastocyst rate, but the percentage of MI-oocyte decreases. Therefore, in human, shorter treating of dbcAMP during IVM may be useful for the improvement of developmental competence without loss of nuclear maturation.
P-221 Tuesday, October 21, 2014

INTERACTION OF AIR QUALITY AND CULTURE ENVIRONMENT: ROLE OF PROTEIN CONCENTRATION AND OIL QUALITY ON EFFECTS OF VOLATILE ORGANIC COMPOUNDS (VOCs) ON EMBRYO DEVELOPMENT. G. Karougou,a J. R. Fredrickson, b D. E. Morbeck, a,b 1,2 Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology and Infertility, Mayo Clinic, Rochester, MN; 2Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN.

OBJECTIVE: High quality indoor air is an important component of a successful IVF program, yet evidence on the impact of VOCs is anecdotal and therefore industry standards for filtration are lacking. Since VOC exposures are variable and effects of short exposures are unknown, we developed a mouse embryo model to study short-term VOC exposure. Our objective was to test the hypothesis that effects of an airborne VOC on mouse embryo development are exacerbated by toxic oil and mitigated by protein.

DESIGN: Animal study.

MATERIALS AND METHODS: Fresh mouse zygotes were collected from superovulated FVB mice and were allocated to four treatment groups: A) HTF-PVA (polyvinyl alcohol) + 5 mg/ml Human Serum Albumin (HSA), B) HTF-PVA, C) HTF-PVA + 1 mg/ml HSA, and D) HTF-PVA + 1 mg/ml HSA cultured with oil that contained peroxides at a level that passed the standard mouse embryo assay. The embryos were cultured individually for 96h in an Embryoscope and exposed to acrolein (ACR) at 500 ppb for the first 24h of culture. ACR is an airborne VOC with widespread environmental prevalence produced during lipid peroxidation and by burning of tobacco or liquid fuels. Endpoints included blastocyst stage and cell cycle timinng and number of cells. Results: ACR had an effect on blastocyst development that was dependent on protein concentration and oil quality. Embryos in media with 5 mg/ml HSA developed to blastocysts at a normal rate (80%) whereas most embryos in low protein (group C) arrested at the 4-cell stage (63.1%) with the remainder arresting at the morula stage. In contrast, embryos in groups B (protein-free) and D (low protein + peroxide oil) arrested and lysed at the 1-cell stage within 24h of culture. Timings of cell cycles for embryos in groups A and C were not affected by protein concentration.

CONCLUSION: Protein protects embryos from VOCs in a concentration dependent manner within the range used by clinical IVF labs. Toxicity from oil peroxides and VOCs were additive, illustrating how air quality may alter lab-to-lab observations when a sub-optimal batch of oil or protein is used for clinical IVF. Further studies are ongoing to determine latent effects of VOCs on implantation and miscarriage rates.

P-222 Tuesday, October 21, 2014

DOES CULTURE IN A TIME-LAPSE INCUBATOR IMPROVE BLASTOCYST OUTCOMES? P. L. Wale,a,b D. H. Edgar,a,b,c Melbourne IVF, East Melbourne, Australia; cUniversity of Melbourne, Parkville, Victoria, Australia; bReproductive Services, Royal Women's Hospital, Parkville, Victoria, Australia.

OBJECTIVE: Time-lapse monitoring allows flexible embryo evaluation and potentially provides new dynamic markers of embryo viability, but in the basic sense also allows ultra-minimal disturbance culture. This study compares outcomes from embryos cultured to the blastocyst stage in a mini-incubator (MINC) to embryos cultured in a time-lapse incubator (EmbryoScope).

DESIGN: Retrospective analysis of cycles from women under 36 years of age between September 2012 and December 2013 which featured 135 cycles cultured in MINCs and 36 cycles cultured in an EmbryoScope.

MATERIALS AND METHODS: IVF or ICSI embryos were cultured individually in either NUNC plates/MINC incubators or EmbryoSlides/EmbryoScope. Both culture incubators had a gas phase of 89%N2/6%CO2/5%O2. For embryos cultured in the EmbryoScope, images were recorded every 7 minutes by time-lapse microscopy. For both systems, embryo development was assessed and selection performed using standard protocols.

CONCLUSION: Protein protects embryos from VOCs in a concentration dependent manner within the range used by clinical IVF labs. Toxicity from oil peroxides and VOCs were additive, illustrating how air quality may alter lab-to-lab observations when a sub-optimal batch of oil or protein is used for clinical IVF. Further studies are ongoing to determine latent effects of VOCs on implantation and miscarriage rates.

P-223 Tuesday, October 21, 2014

DIRECT INJECTION MASS SPECTROMETRY REVEALS UNIQUE METABOLITE PROFILES FROM SPENT HUMAN EMBRYO CULTURE MEDIA DUE TO ALBUMIN SOURCE AND PREGNANCY OUTCOME. J. R. Sheedy,a A. Yoshida,a,b D. K. Gardner,c 1Department of Zoology, The University of Melbourne, Parkville, Victoria, Australia; bKiba Park Clinic, Kiba, Koto-ku, Japan.

OBJECTIVE: Identification of biomarkers associated with embryo viability will facilitate the move to single embryo transfer. Here we employed a rapid, high-throughput direct injection-mass spectrometry (DI-MS) method to analyze spent human embryo culture media samples to resolve a unique metabolic signature related to pregnancy outcome, and media supplemented with either human serum albumin (HSA), or a high/low dose of recombinant human serum albumin (rHSA).

DESIGN: Spent media samples from embryos cultured in 5 mg/ml HSA (n=68 treated; n=30 control), 2.5 mg/ml rHSA (n=88 treated; n=37 control) were measured in duplicate by DI-MS in positive and negative ion modes. As a pilot study, the DI-MS profiles were also matched to patients’ pregnancy outcomes (n=17 no pregnancy; n=5 pregnancy).

MATERIALS AND METHODS: Aliquots (1 µl) of spent media samples (10 µl) were diluted 1:10 with 95% formic acid (0.1% v/v) and 5% acetonitrile. Spent media samples were analyzed by electrospray ionization time-of-flight mass spectrometry, over a mass range of 50 – 450 m/z. Metabolite peak areas were quantified in each sample in negative ion mode (n=88) and positive ion mode (n=146). Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA) identified distinct metabolite profiles between sample groups. Analyses were validated using duplicate samples, Kruskal-Wallis one-way ANOVA with Dunn’s post test identified biomolecular differences between groups, where P < 0.05 was significant.

RESULTS: DI-MS profiles exhibited differences in media composition due to supplementation with HSA or rHSA. Embryos exposed to each of these media types were metabolically distinct from their controls and albumin supplementation groups (neg m/z = 245.1035, 303.0531, 310.9707, 328.9268, 350.8983; and pos m/z = 81.5218, 247.1116, 269.0940, 291.0832; P < 0.05 to P < 0.001). Detection of novel biomarkers in spent culture media additional to the media composition, related to albumin source and pregnancy outcome. Embryos that resulted in pregnancy versus no
EMBRYO KINETICS IN BRIGHT FIELD VS DARK FIELD TIME-LAPSE IN EMBRYO SELECTION FOR TRANSFER.  A. Azzarello, T. Hoest, A. L. Mikkelsen. Holbaek Fertility Clinic, Holbaek, Region Sjaeland, Denmark.

OBJECTIVE: Dark field time-lapse (DF TL) is used to assess embryo kinetics for selection for transfer, observing solely the cytokinesis. Bright field time-lapse (BF TL) shows both the cytokinesis and the presence of the nuclei, which distinguish large fragments and blastomeres. We evaluated the potential of nucleus observation vs solely cytokinesis to predict live birth potential.

DESIGN: Prospective study.

MATERIALS AND METHODS: Embryos were obtained by ICSI. By a single observation at 45h post-fertilization, 1 or 2 embryos with ≥2 blastomeres, and ≤25% fragmentation were transferred. Live birth of healthy babies was reported. Double transfers resulting in a single live birth were excluded. Interval between 1st and 2nd mitoses and between 2nd and 3rd mitoses were observed in bright field time-lapse records of all embryos according to the following DF TL and BF TL models. In DF TL, we reported the embryo kinetic as the timing of cytokinesis resulting in cyttoplasmic structures larger than 45μm. In BF TL, embryo kinetics was the timing of cytokinesis resulting in cytoplasmatic structures presenting a clear nucleus; consequently, large anucleated structures (ie large fragments) were included in the total fragmentation and highly fragmented embryos (>25% fragmentation) were defined as poor quality embryos (PQ), and cytokineses resulting in 3 nucleated blastomeres were considered abnormal (ABN). In the BF TL model, PQ and ABN embryos were excluded. In each model, timing of embryos resulting in a live birth (LB) were compared to embryos resulting in non-live birth (NLB).

RESULTS: In BF TL model, kinetics of LB embryos did not differ from regular NLB (p=NS), and PQ+ABN resulted in no LB. In DF TL model, kinetics of LB embryos was significantly different from NLB (p<0.01). However, in BF TL we could recognized and excluded 68 PQ+ABN. In DF TL, 60.3% of PQ+ABN embryos showed similar kinetics of LB embryos.

CONCLUSION: In BF TL, embryo kinetics is not relevant in predicting LB, since the observation of the nuclei can distinguish and exclude PQ+ABN embryos. DF TL, observing solely the cytokinesis, can distinguish only part of the PQ+ABN embryos recognized in BF TL. Therefore, BF TL is more sensitive in recognizing embryos with no LB potential.

P-225 Tuesday, October 21, 2014


OBJECTIVE: Compare embryo development and clinical outcome for poor prognosis patients in two culture systems: autoologous endometrial culture (CC) and time lapse culture system (EmbryoScope®).

DESIGN: A controlled study on sibling oocytes in IVF patients with poor prognosis and repeated implantation failure comparing embryo development and clinical outcome.

MATERIALS AND METHODS: Sibling zygotes (n=693) from 67 cycles were randomized to CC culture system (standard incubator, 20% O2) and time-lapse system (EmbryoScope®, Unisense Fertitech, E. 5% O2). ET was performed on Day 3 (D3) and remaining embryos were cultured to day 5/6 for potential freezing. Embryo developmental parameters and clinical outcome were analyzed by Chi square and ANOVA tests (P<0.05).

RESULTS: Embryo development and morphology were enhanced in embryos cultured in our time-lapse system. Average cell numbers on day 2 and 3 were significantly lower in CC than E (3.4 vs. 3.7; 5.7 vs. 6.5, respectively, both P<0.001). Proportionally, significantly more 4-cell embryos on D2 were found in E than CC (70.2% vs. 56.1%, P<0.001). Similarly, the proportion of 8-cell embryos on D3 were significantly higher in E vs. CC (48.1% vs. 24.2%, P<0.001). The non-cleavage rate was 4.1% in CC and 1.8% in E (P=0.085). Day 3 fragmentation was slightly higher in the CC group as well (9.6% vs 5.3%). Moreover, more good quality embryos (≥6cells, <20% fragments) were observed in E (57.4%) vs. CC (38.1%) (P<0.001). Marginally more blastocysts were frozen from E system compared to CC (10% vs. 7.1%). Implantation rate was 4.8% in CC, 7.7% in E (P=0.49), and 16.8% in mixed embryo transfers.

CONCLUSION: Culture in our time-lapse system (5% O2) improved embryo development and clinical outcome compared to our endometrial coculture system in standard incubator. The use of time-lapse culture system with reduced oxygen represents a good alternative to the labor-intensive CC system.

Supported by: Institutional.

P-226 Tuesday, October 21, 2014

DOES ADVANCING MATERNAL AGE AFFECT MORPHOKINETIC PARAMETERS DURING EMBRYO DEVELOPMENT? N. Watcharaneranee, S. D. Ploskonka, J. Goldberg, T. Falcone, N. Desai. OB-GYN/Women’s Health Institute, Cleveland Clinic, Beachwood, OH.

OBJECTIVE: Age related decline in fertility is associated with an increase in oocyte aneuploidy and may affect embryo growth patterns and kinetics (1-3). Time lapse imaging offers an opportunity to compare kinetic parameters in embryos from women with advancing maternal age to their younger counterparts.

DESIGN: Retrospective analysis.

MATERIALS AND METHODS: Time lapse imaging data was collected from 221 patients having either a Day 3 (n=111) or Day 5 (n=110) transfer. Morphokinetic data were analyzed from zygotes (n=1654) cultured in the Embryoscope until transfer. Kinetic markers assessed were time to syngamy (tPNf), 2 cell (t2), 3 cell (t3),4 cell (t4),5 cell (t5) and 8 cell (t8). For embryos being cultured to blastocyst we also monitored time to morula (tM ), start of cell cycles (t2c, t3c) and time to complete synchronous divisions (s2,s3) were calculated. Data was stratified in to three groups by patient age: (A) <35, (B) 35-37 (C) ≥38. Blastocyst formation, clinical pregnancy (CPR) and implantation (IR) rates were calculated. Mean timings of cell division and cell cycle intervals were compared between transfers where all embryos implanted (KID+) and those in which all embryos failed to implant (KID-) using the ANOVA analysis. P-values of <0.05 were considered statistically significant.

RESULTS: Clinical pregnancy and implantation rates in the three groups were: A- CPR 60%, IR, 48%; B- CPR 56%, IR 37% and C- CPR 48% and IR
The proportions of embryos dividing on day 1 were similar between all conditions (p=0.33). There was a trend in decreased embryo degeneration in media supplemented with 1% HSA (p=0.052). Pair-wise analysis revealed a lower rate of embryo degeneration in the 1% HSA group compared to the control (Odds Ratio (OR) 0.27, 95% confidence interval (CI) 0.13-0.55; p=0.001). The 1% group had significantly greater blastulation compared to the control (OR 2.38, 95% CI 1.38-4.15; p<0.001) and the 2.5% group (OR 2.75, 95% CI 1.56-4.76; p<0.001). Rates of embryo hatching were also different between the 4 groups (p<0.001). The 1% group demonstrated the highest hatching rate compared to the control (OR 1.89, 95% CI 1.38-2.62; p=0.001). There was a trend in decreased embryo degeneration (OR 2.75, 95% CI 1.56-4.76; p<0.001), and the 2.5% group (OR 3.30, 95% CI 1.75-6.59; p<0.001).

CONCLUSION: While higher HSA concentrations in human embryo culture have been well established, there is a paucity of information on optimal HSA concentrations in mouse embryo culture. Our findings support the concept of lowering the HSA concentration in mouse embryo culture medium. Medium supplemented with 1% HSA appears to improve outcomes for blast formation and hatching while decreasing mouse embryo degeneration.

P-229 Tuesday, October 21, 2014

OPTIMIZING EMBRYO VITRIFICATION - DETERMINING THE IMPACT OF SUCROSE IN EQUILIBRATION SOLUTION. B. Patel, S. Thakore, M. Perri, L. Lam, J. Liu, J. Goldfarb, A. Ahmady, Obstetrics and Gynecology, Case Western Reserve University, Cleveland, OH.

OBJECTIVE: To assess the effects of sucrose in equilibration solution (ES) on mouse embryo survival and development.

MATERIALS AND METHODS: Two-cell mouse embryos were cultured to the 6-8-cell stage. In a two-step vitrification process, using Sage vitrification kits (Cooper Surgical, CT), embryos were exposed to 50µL of MOPS-buffered ES consisting of either 0.25M (mol/L) sucrose, 0.5M sucrose, or no sucrose (control) for 6 minutes (min). In step 2, all embryos underwent serial exposure to 2 x 50 µL drops of vitrification media (VS) containing 0.6M sucrose. Embryos were subsequently loaded into Cryotip, a closed vitrification system (Irvine Scientific, CA). The maximal duration of step 2 was 110 seconds. Thawing consisted of exposure to media with 1M sucrose.
for 1 min, 0.5M sucrose for 4 min, and MOPS-buffered media for 6 min. Sur-
ival, blastulation, and hatching rates were monitored daily. Chi-square test was 
used for statistical analyses, with post-hoc Tukey testing for multiple compara-
isons.

RESULTS: A total 302 embryos were vitrified and recovered. Results are 
summarized below.

Effects of Sucrose in ES on Mouse Embryo Development

<table>
<thead>
<tr>
<th>Control (n%)</th>
<th>0.25M (n%)</th>
<th>0.5M (n%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival</td>
<td>94 (93%)</td>
<td>99 (99%)</td>
<td>98 (97%)</td>
</tr>
<tr>
<td>Blast stage</td>
<td>57 (56%)</td>
<td>69 (69%)</td>
<td>60 (59%)</td>
</tr>
<tr>
<td>Hatch stage</td>
<td>37 (36%)</td>
<td>46 (46%)</td>
<td>45 (45%)</td>
</tr>
</tbody>
</table>

*0.25M vs. control = 0.037

Post-thaw survival rates were similar among all three groups. However, 
pair-wise analysis revealed a significant increase in survival between the 
0.25M group vs. control (p = 0.037). There was also a trend towards increased 
blast formation in the 0.25 M group compared to control (p = 0.06). While 
there were no differences in hatching rates between all three groups, there 
was a trend towards increased hatching in the 0.25M group compared to 
control (p = 0.09). No differences in survival, blastulation, and hatching rates 
were noted between the 0.25M and 0.5M groups. All three outcomes were 
also similar between the 0.5M and control groups.

CONCLUSION: The addition of sucrose, a non-permeating osmolyte, to 
ES may enhance intracellular dehydration at an earlier stage. This may pro-
 mote a higher efficiency of dehydration during rapid exposure to VS within 
the requirements of high osmolality permeating cryoprotectants. Addition of 0.25M sucrose to ES resulted in higher embryo survival and a 
trend towards increased blastulation. Further study is warranted to deter-
mine optimal concentrations of disaccharides in ES to optimize vitrifica-
tion.

P-230 Tuesday, October 21, 2014
NON-INVASIVE TEST FOR EMBRYO COMPETENT SELECTION BY 
QUANTIFICATION OF CELL-FREE NUCLEIC ACIDS IN EM-
BRYO CULTURE MICRO DROP.

S. Assou, a A. Gala, b T. Al-Edani, a A. Ferrières, a A. R. Thierry, b S. Hamamah a Université Montpellier 1, 
Inserm U1040, IRMB, Montpellier, Hérault, France. b INSERM U986, Montpelli-
er, Hérault, France. c ART-PGD Department, Université Montpellier 1, 
Inserm U1040, IRMB, Montpellier, Hérault, France.

OBJECTIVE: In order to increase the success rates of IVF cycles, improved methods for embryo selection to produce a baby are required. 
We determined the cell-free nucleic acids (cfNA) levels in human embryo spent culture media and we evaluated their possible use as biomarkers for embryo competent selection.

DESIGN: Human fertilized oocytes were individually cultured from 
zygote to blastocyst stage. A total of 60 spent culture media were collected 
on day 3 (6-8 cells) and day 5/6 blastocyst stage.

MATERIALS AND METHODS: MicroRNAs (miRNAs) such as MIR-21 and Let-7b were extracted from drops with the QIAamp kit and quantified by RT-qPCR using TaqMan technology. Cell-free DNA (cfDNA) was quantified using Bio-Rad Supermix SYBR Green. Statistical analyses defined relationship between nucleic acid content and embryo outcome.

RESULTS: We demonstrate that the embryo culture medium samples, during in vitro early embryo development, contained embryonic cfDNAs and miRNAs. The concentration values of cfDNA are lower in the culture medium in which emerge top quality embryo compared to no top (p = 0.05). In the embryos that reached good blastocyst quality and leading to preg-
nancy, the variation in the cfDNA concentration between day 3 and day 5/6 is significantly decreased significantly and drastically of 88% (22.16 ng/ml and 2.75 ng/ml at day 3 and day 5/6 respectively). This variation is very low between day 3 and day 5/6 in the no good blastocyst quality (6.46 ng/ml and 3.78 ng/ml at day 3 and day 5/6 respectively, 41% decrease). Relate to the expression of the miRNAs which identified in spent media and was correlated with embryo outcome, MIR-21 and Let-7b were more highly concentrated in both day-3 and day-5 media samples when compared with day-0 samples (cycle threshold = 33 and 34 versus 39.5, respectively).

CONCLUSION: Under in vitro IVF/ICSI conditions, changes in the nu-
cleic acids levels in the embryo culture medium on day 3 and day 5/6 predict 
the embryo quality and may be used as a new potential biomarker for select-
ing top quality embryos. Our data strongly open the possibility to develop a 
new quick and low-cost test for the selection of the embryos viable with the 
highest implantation potential.

Supported by: Ferring and Geneverrier companies.

P-231 Tuesday, October 21, 2014
DO EMBRYO DESCRIPTORS ON CULTURE DAYS 2 AND 3 AID IN 
SELECTION OF BLASTOCYSTS WITH IMPLANTATION 
POTENTIAL? D. H. McCalli0h, K. Goldman, B. Hodes-Wertz, 
C. McCaffrey, J. A. Grifo. NYU Fertility Center, NYU Langone Medical 
Center, New York, NY.

OBJECTIVE: To determine if embryo descriptors on days 2 and 3 provide 
selective value over and above the quality descriptors on day 5.

DESIGN: Retrospective Analysis of Embryo Scores and Implantation.

MATERIALS AND METHODS: Data from our electronic medical record system were accumulated for patients who became pregnant between 1/1/ 
2003 and 12/31/2012 but for whom all or only some of the transferred em-
byos implanted. This method was used previously to assess embryo charac-
teristics associated with implantability, greatly reducing the impact of uterine factors on outcome. Embryos were selected for transfer by their characteris-
tics (Stage, ICM and TE scores) on Day 5. Multiple logistic regression was 
used to determine if the number of cells, the fragmentation, or the embryo 
grade on days 2 and/or 3 provided any further predictability of implantation. 
Significant parameters were chosen using the Akaike Information Criterion 
(AIC) and the ability to predict outcome was assessed using Receiver Oper-
ator Characteristic (ROC) curves, and in particular the Area Under the ROC 
Curve.

RESULTS: 3971 embryos transferred to 2111 patients who experienced a 
clinical pregnancy were analyzed. ROC curves were constructed using the 
best fit MLR equations using Day 5 Blastocyst Scores alone, Day 5 Blas-
tocyst scores plus day 3, plus day 2 and plus days 2 and 3 descriptors. The 
area under the ROC curve assessing the ability of Blastocyst scores on day 5 
to predict implantation was 0.57. The AUC was not improved by adding day 
2, day 3 or both day 2’s and day 3’s descriptors to the MLR best fit equation.

CONCLUSION: We conclude that consideration of embryo descriptors 
on days 2 and 3 for embryos without preimplantation genetic screening, 
provides no additional selective value over the blastocyst descriptors on day 
5 alone. Therefore, neither the number of cells on day 2 or 3, the frag-
mentation nor the grades provide additional information about the embryos 
implantation potential that is not already present in the description of the 
blastocyst on day 5. Since we only considered blastocysts on day 5, we 
cannot determine if day 2 and day 3 descriptors will predict which embryos 
will become blastocysts. Our results indicate to us that descriptions of blas-
tocysts on day 5 provide discrimination of morphological features associ-
ated with implantability that are superior to any features that we observed 
on days 2 and 3.

P-232 Tuesday, October 21, 2014
DO MICROMANIPULATION TECHNIQUES AND CRYOPRESER-
VATION ALTER PRE-IMPLANTATION DEVELOPMENT 
AND GENE EXPRESSION IN CULTURED EMBRYOS? M. Paczkowski, 
M. Reyes, D. Jones, T. J. Kuehl. Baylor Scott & White Healthcare, Temple, 
TX.

OBJECTIVE: To examine the effects of micromanipulation and cryopres-
ervation of mouse blastocysts cultured in vitro using morphologic and gene 
expression endpoints.

DESIGN: Research study.

MATERIALS AND METHODS: Two-cell mouse embryos were cultured 
for 42 hrs until the blastocyst stage and divided into 3 treatment groups: 
control, laser assisted hatching (LAH), and biopsy. A subset of the LAH (LAH-cryo) and biopsied (biopsy-cryo) embryos was cryopreserved via slow 
cooling and subsequently thawed for analysis. After manipulation, or 
thaw, embryos were cultured for an additional 24, 28 and 40 hrs and blasto-
cyst development was recorded. Fully hatched blastocysts were collected at
28 hrs for quantitative PCR and transcript abundance of 18s rRNA, Ptpa, Oct4, Plac8, Cox2, and Glut1 was determined. Data were analyzed using ANOVA with Duncan’s post-hoc test and significance was defined as a P-value less than 0.05.

RESULTS: Development to fully hatched blastocyst stage was significantly increased in biopsied embryos compared to LAH embryos, which was significantly increased compared to the unmanipulated controls at 24, 28 and 40 hrs post manipulation. While the percentage of fully hatched blastocysts in the biopsied and biopsy-cryo groups seemed to lag in development compared to their fresh counterparts, by 40 hrs there was no significant difference. Transcript abundance for 18s rRNA, Ptpa, and Oct4 were significantly higher in biopsied embryos compared to all other treatment groups. Glut1 was significantly higher in the biopsied groups compared to all other treatments except for the biopsy-cryo, which was not significantly different. Plac8 was not significantly different in any of the treatment groups.

CONCLUSION: The short-term and long-term effects of embryo manipulation are still unknown. This study suggests that survivability of biopsied embryos may be improved compared to unmanipulated embryos, contrary to the belief that ‘the least amount of manipulation is best.’ Biopsied embryos, regardless of whether the embryos were cryopreserved post-manipulation, had better developmental capabilities compared to control and LAH embryos. The increase in expression of essential developmental genes further suggests that biopsied embryos are able to compensate for the insult that occurred during manipulation, and may actually have advanced faster.

Supported by: Baylor Scott & White Healthcare.

---

P-234 Tuesday, October 21, 2014

THE USE OF MORPHOKINETICS AS A PREDICTOR OF EMBRYO IMPLANTATION. H. L. Wu, W. Han, H. Ye, N. G. Huang. Chongqing Maternity and Children Health Care Hospital, Chongqigin, China.

OBJECTIVE: The prospective single arm study was to identify the morphokinetic parameters specific to embryos that were capable of implanting.

DESIGN: Diagnostic study.

MATERIALS AND METHODS: fifty-three- patients ( age ≤ 35 years; Body Mass Index: 18-25, 5-15 oocytes; pure tubal factor infertility) were included in the study. All patients underwent first IVF treatment. The Primo Vision Time-Lapse Imaging System was used in this study. Embryos were scored and selected by traditional morphology assessment. Transfers were performed on day3. The clinical pregnancy confirmed by the presence of gestational sacs with fetal heart beat by transvaginal ultrasound examination in week 7. only cycles with evidence of 100 % implantation or 100 % implantation failure were included. Fifty implanted embryo from twenty-five patients and fifty-eight no-implanted embryo from twenty-eight patients were analyzed. P < 0.05 were regarded as significant difference.

RESULTS: There were no significant difference of patients characteristics. In the implanted group, the t2, t4, t6 and t8 were significant shorter than non-implanted group. No significant difference were observed in the other dynamic parameters.

---

P-233 Tuesday, October 21, 2014

ESTABLISHING A CONTINUOUS BLASTOCYST CULTURE SYSTEM WITHOUT DIRECT OBSERVATION AND EXCHANGE OF CULTURE MEDIUM BY EMPLOYING A TIME-LAPSE INCUBATION SYSTEM. N. Fukunaga, a,b, c H. Kitasaka, a,b T. Yoshimura, a,b N. Hasegawa, a,b Y. Asada, a,b c IVF Laboratory, Asada Ladies Nagoya Clinic, Nagoya, Aichi, Japan; 1IVF Laboratory, Asada Ladies Kochiwa Clinic, Nagoya, Aichi, Japan; 2Asada Institute for Reproductive Medicine, Asada Ladies Clinic, Nagoya, Aichi, Japan.

OBJECTIVE: Human embryo culture is still far from being perfected. Blastocyst culture conventionally involves daily direct observation and 2-3 exchanges of culture medium. It is important that the culture environment maintains low oxygen concentration for embryo culture. This study was designed to establish a continuous blastocyst culture system using a Time-Lapse Incubator system. It will reduce the need for direct observation and the exchange of culture medium and result in an improvement of embryo development by Time-Lapse system. Furthermore, we examined the performance of different culture medium for continuous blastocyst culture.

DESIGN: prospective cohort study.

MATERIALS AND METHODS: A total of 53 pronuclear embryos from 4 patients who consented to post-thaw culture were studied. Embryos were cultured without direct observation or exchange of culture medium using the Time-Lapse system(EmbryoScope:FertiliTech:Denmark). We compared Continuous Single Culture Medium(CSC: Irvine Scientific:USA) and One Step Medium (OS:NAKA IVF Medium:Japan) which is the suitable medium for continuous blastocyst culture. Images were recorded automatically every 10 min. Culture time was set at 120 h. Time-Lapse analyses were made Day3 and Day5, at the same time point as our standard observation.

RESULTS: The rate of good embryo formation at Day3 was 38.5% (10/26) in CSC group and 63.0% (17/27) in OS group and was significantly higher in OS group vs. CSC group (P < 0.05). The rate of blastocyst formation at Day5 was 50.0% (13/26) in CSC group and 55.6% (15/27) in OS group. The rate of blastocyst formation in the LAH-cryo and biopsy-cryo groups seemed to lag in development compared to their fresh counterparts, by 40 hrs there was no significant difference. Transcript abundance for 18s rRNA, Ptpa, and Oct4 were significantly higher in biopsied embryos compared to all other treatment groups. Glut1 was significantly higher in the biopsied groups compared to all other treatments except for the biopsy-cryo, which was not significantly different. Plac8 was not significantly different in any of the treatment groups.

CONCLUSION: The preliminary study result indicated that the shorter cytokinesis duration in implanted embryos than non-implanted. Significant difference exit in the time of t2, t4, t6 and t8. We need further study to confirm and clarify the results.

---

P-235 Tuesday, October 21, 2014

VALIDATING IMPROVED BLASTOCYST QUALITY AND DEVELOPMENTAL ATTRIBUTES IN SINGLE CULTURE MEDIUM COMPARE TO SEQUENTIAL CULTURE MEDIA USING A MURINE MODEL. J. M. Hennings,a R. L. Zimmer,a b J. W. Davis,b P. Sutovsky,b K. L. Sharpe-Timms,b Obsterics, Gynecology and Women's Health, The University of Missouri School of Medicine, Columbia, MO; Department of Health Management and Informatics, The University of Missouri Biostatistics, Columbia, MO; Animal Science, The University of Missouri College of Agriculture, Food and Natural Resources, Columbia, MO.

OBJECTIVE: Determine effects of single versus sequential culture media on murine embryo quality and developmental attributes, which are not accessible in human embryos.

DESIGN: Murine embryos cultured in commercially available single and sequential media were used to determine initial preimplantation embryo quality and development attributes.
MATERIALS AND METHODS: Murine zygotes (n=40) were cultured in commercially available single or sequential media for 5 days. Images (200x) were acquired every 5 minutes using inverted phase contrast microscopes with differential interference contrast on developmental day 3 (d3) to facilitate quantification of the number of embryos developing to the 8-cell stage or greater in the two media. On d5, images were made and embryos fixed. Comparing and contrasting embryo images from the two types of media facilitated identification of an inner cell mass (ICM), nuclear quality assessment, and quantification of the total number of cells per embryo, ICM and trophoblast. The least square means (LSM) of total nuclei number, ICM nuclei number, and presumptive TE nuclei number between the blastocysts were analyzed using a general linear mixed model. An approximate t-test was used to compare differences in nuclei number (SAS 9.22 PROC MIXED, SAS Institute Inc, Cary, NC). Logistic regression analysis (SAS PROC LOGISTIC) was used to compare the total embryo numbers per stage per day on d3 and d5. Hatching/hatched to unhatched blastocysts, TE: ICM and abnormal nuclei: normal nuclei were compared through this same analytical approach. A P < 0.05 was considered significant. Deubiquitinating enzymes localizing in the ICM served as a point of reference for morphometric analysis.

RESULTS: Equivalent numbers of embryos reached the 8C stage on d3 in both single and sequential embryo culture media. More d5 embryos reached the blastocyst stage, had greater numbers of normal nuclei, hatched and had significantly more trophoblast cells, but not ICM cells, when cultured in the single media compared to the sequential media.

CONCLUSION: Single medium yields higher quality blastocyst embryos on day 5 in sequential media. This supports the idea that single stage medium is more beneficial in human assisted reproduction procedures when compared to sequential media.

P-236 Tuesday, October 21, 2014

FIRST MORPHOKINETIC ANALYSIS OF BLASTOCYST EXPANSION IN HUMAN EMBRYOS OF KNOWN POSITIVE IMPLANTATION USING AN EMBRYOSCOPE. T. T. F. Huang. Pacific IVF Institute, Honolulu, HI.

OBJECTIVE: Using an Embryoscope, the objective was to morphokinetically describe features of the expanding human blastocyst cavity in embryos yielding sustained ongoing pregnancies.

DESIGN: Retrospective descriptive study in a private practice setting.

MATERIALS AND METHODS: Data were obtained from 27 sequential egg donation cycles using blastocysts selected for transfer using an Embryoscope. Day 5 embryos were ranked and selected for transfer by the greatest degree of blastocyst cavity expansion (BE) with otherwise normal cleavage, ICM and TE characteristics. BE was determined from sequential hourly 2D cross sectional area (CSA) measurements starting from blastocyst formation up until ET. Sustained pregnancy was defined as clinical detection of a beating heart.

RESULTS: Of 5 single blastocyst transfers (SBT), 4 (80%) resulted in a sustained pregnancy (IR=80%). Of 21 double blastocyst transfers (DBT), 21 (100%) resulted in a sustained pregnancy with an IR of 80.4%. (One patient had a triple BT but did not become pregnant). Of the DBT’s, 12 (57.1%) were twins (100% IR) and 9 (42.9%) were singleton (50% IR). The study confirmed a correlation between the time of the start of blastocyst cavitation (Tsb) and the time of blastocyst formation (Tb) for both the 100% (r2 = 0.830) and 50% (r2 = 0.893) IR groups. In the 100% IR group, the average hourly rate of BE increased over consecutive 3 hour intervals (557.4 u2 /hr; 916.2 u2/hr; 1276.3 u2/hr), then tapered to 870.4 u2/hr during the fourth interval (hours 10-12). This was similar in the 50% IR group. Two morphokinetically distinguishable events characterized BE. The first was a novel pulsatile, oscillatory pattern of accelerations and decelerations with a periodicity of 2-3 hours resulting in continuous BE. The second was an occasional, acute contraction of the cavity, ranging from 5-50% in CSA, often occurring several times in blastocysts monitored for up to 22 hours. Remarkably, contractions recovered completely within 1-3 hours. Interestingly, the rates of expansion varied to such given CSA milestones at 1.5, and 10 hours from Tb did not correlate with the initial time of blastocyst formation in the cohort of 100% IR blastocysts.

CONCLUSION: This reports the first quantitative description of BE in human embryos yielding sustained pregnancies. Expansion typically accelerates in an oscillatory manner, with occasional but reversible collapses in the cavity. Results supports the hypothesis that time-lapse technology identifies novel biological information useful in embryo selection.

P-237 Tuesday, October 21, 2014


OBJECTIVE: Embryos in vivo are not present in a static condition, since in the fallopian tube they are exposed to continuous movement, compression caused by the cilia and peristaltic movements, and shear stress from tubal fluid flow. In this study, we compared the outcome of human oocytes and embryos cultured in a micro-vibration culture system for In Vitro Fertilization (IVF) patients in comparison to the traditional static culture environment.

DESIGN: Retrospective analysis.

MATERIALS AND METHODS: Oocytes and embryos from 1943 patients enrolled in IVF treatment from January 2010 to February 2013 were cultured in a traditional static culture, while 497 patients’ embryos from March 2013 to March 2014 were cultured in a micro-vibration culture. In the micro-vibration group, culture dishes were placed on the top of a platform producing a three-dimensional vibration of 56 Hz for 5 seconds every 60 minutes. Patients in both groups were compared according to their age (< 30; 30-35; 36-38 and 39 years old), the outcomes of micro-vibration culturing were compared for fertilization, blastulation, pregnancy and implantation rates. Variables were analyzed by chi-square test and Fisher’s exact test.

RESULTS: Pregnancy rates significantly increased in the micro-vibration culture in younger patient groups compared to the static culture groups (< 30: 59.4% vs. 38.4%, P=0.0006; 30-35: 50% vs. 36.3%, P<0.0005; 36-38: 42.7% vs. 31.2%, P=0.03) as did the implantation rate (<30: 54.2% vs. 25.5%, P<0.0001; 30-35: 48.1% vs. 30.1%, P<0.0001; 36-38: 40.3% vs. 26.5%, P<0.0001). The number of transferred embryos and day of transfer did not differ between these groups. For patients >39-years-old, micro-vibration culture tended to increase both pregnancy and implantation rates over the static culture but did not reach statistical significance (20.6% vs. 17.2%, P=0.41 and 21.1% vs. 15.3%, P=0.08, respectively). Micro-vibration culturing produced no effect on the fertilization rate. In addition, the overall blastulation rate was significantly higher in the micro-vibration culture compared to the static culture (40.5% vs. 31.2%, P<0.0001).

CONCLUSION: These results demonstrate clearly that the three-dimensional micro-vibration culture of oocytes and embryos significantly increases pregnancy and implantation rates. Mechanical vibration of the embryo may mimic the embryo’s in vivo environment and may cause movement of media around the embryo aiding in refreshing the media surrounding the embryos and diffusion of waste material.

P-238 Tuesday, October 21, 2014

UNDISTURBED EMBRYO CULTURE IN A TIME-LAPSE MONITORING SYSTEM IMPROVE EMBRYO QUALITY: A PROSPECTIVE COHORT STUDY. W. Han, H. L. Wu, H. Ye, N. G. Huang. Chongqing Maternity and Children Health Care Hosp, Chongqing, China.

OBJECTIVE: To quantify the benefit of culturing embryos in a time-lapse monitoring system.

DESIGN: Prospective cohort study.

MATERIALS AND METHODS: One hundred and sixty patients (age ≤ 35 yrs; BMI: 18-25; 5-15 oocytes; pure tubal factor infertility) were included in this prospective cohort study, and were randomly assigned to the control group (80 patients) or the time-lapse(TL) group (80patients) from April 22 to December 25, 2013. Fertilized oocytes in the TL group were cultured in microwell culture dishes and monitored on compact digital inverted microscopes (Primo Vision, Vitrolife). Images were captured every 5 minutes .In the control group, fertilized oocytes were cultured in conventional dishes (one embryo in each microdroplet). In both groups, embryo were selected for transfer on day 3, by traditional morphology assessment. The clinical pregnancy confirmed by the presence of...
P-239 Tuesday, October 21, 2014

LOW OXYGEN TENSION INCREASE MITOCHONDRIAL MEMBRANE POTENTIAL AND IMPACTABILITY BY ENHANCED EXPRESSION OF ANTIOXIDANT GENES IN MOUSE BLASTOCYST CULTURED IN VITRO.  Y.-L. Ma, a J.-W. Wang, b C.-R. Tseng, a Center for Reproductive Medicine & Sciences-Taipei Medical University, Taipei, Taiwan; aDepartment of Obstetrics & Gynecology Taipei Medical University Hospital, Taipei, Taiwan.

OBJECTIVE: In human IVF, many studies have shown that embryos cultured in lower oxygen tension (5% O2) have higher pregnancy rate, when compared with normoxic condition (20% O2). However the beneficial effect of low oxygen tension in embryogenesis remains unclear. This study aimed to investigate the expression of oxygen and antioxidant genes in mouse embryo cultured under hypoxic and normoxic conditions.

DESIGN: A control study of animal experiment in a university hospital.

MATERIALS AND METHODS: The 2-cell ICR mouse embryos were cultured in hypoxic atmosphere (5% O2) and normoxic condition (20% O2). The 5% O2 group was cultured in low oxygen condition for 72 hours. The expression of oxygen-related genes (HIF-1α, HIF-2α) and antioxidant genes (MnSOD and LIFR) were analyzed for 7 growth factors (EGF, FGF-1, FGF-2, VEGF-A, VEGF-C, FGF-2, FGF-2) using Real-time PCR. Protein levels were validated by Immunofluorescence analysis. The apoptosis and mitochondrial membrane potential (mtpM) were assessed by TUNEL and JC-1 stain, respectively. Student's t test or one-way ANOVA test was used to evaluate statistical significance.

RESULTS: The blastocyst formation and hatching rate (Mean ± SE) increased significantly in 5% O2 group when compared to 20% O2 group (90.5±3.3% vs. 77.8±2.4% and 82.9±6.9% vs. 70.7±2.3%, respectively. P<0.05). The transcription levels of MnSOD and PRDX5 were also significantly increased seven to eight fold in 3% O2 group, compared to 20% O2 group (P<0.05). Immunofluorescence staining showed the intensity of MnSOD and LIFR was higher in 3% O2 than 20% O2 group, respectively. Protein levels of HIF-2α was detected higher in the nucleus of 3% O2 group. Although HIF-1α and HIF-2α mRNA level was similar in two groups, apoptotic index was significantly higher in 20% O2 group, compared with 3% O2 group (P<0.05). The 3% O2 blastocyst also showed a significantly higher mtpM compared with 20% O2 group.

CONCLUSION: This study has proposed that lower O2 tension may improve embryo development and viability by increased antioxidant enzymes, it provides more conducive environment by up-regulation of LIFR to increase implantation ability and by increased mtpM to stimulate mitochondrial activity against apoptosis during implantation period. All these effects are initiated and regulated by HIF-2α, which acts as key mediator in hypoxic environment.

Supported by: NCNS-2314-B-038003-MY3.
OVARian Stimulation

P-241 Tuesday, October 21, 2014

FOLLICLE NUMBER AND SIZE AS PREDICTORS OF PREGNANCY OUTCOME IN UNEXPLAINED INFERTILITY COUPLES TREATED WITH CLOMPHENE CITRATE AND INTRAUTERINE INSÉMINATION. G. M. Barché,* R. D. Robinson,* H. Caddell,* T. Falcone,* R. R. Schenken.* *Reproductive Endocrinology and Infertility, University of Texas Health Science Center, San Antonio, TX; *Reproductive Endocrinology and Infertility, Cleveland Clinic Foundation, Cleveland, OH.

OBJECTIVE: Ovulation induction with intrauterine insemination (IUI) increases pregnancy rates in couples with unexplained infertility. However, the optimal number or size of follicles required to achieve pregnancies and live births is unknown. Our objective was to evaluate whether the number and/or size of mature follicles in patients with unexplained infertility treated with Clomphene Citrate (CC) and intrauterine insemination are predictors of pregnancy and live birth rates.

DESIGN: Retrospective cohort study from two academic institutions.

MATERIALS AND METHODS: 414 treatment cycles of couples from the infertility clinics at UT Medicine San Antonio, Texas and the Cleveland Clinic, Cleveland, Ohio were included. All couples were diagnosed with unexplained infertility and were treated with CC and IUI between 2005-2012. Primary outcomes were pregnancy rate and live birth rate and analyzed by the number and size of mature follicles. The secondary outcome analyzed was the multiple pregnancy rate. Statistical analysis using Chi Square, T tests and logistic regression was performed as appropriate. P < 0.05 was considered statistically significant.

RESULTS: Pregnancy rates significantly increased in women with two dominant follicles (defined as >16 mm). Similarly, there was a higher live birth rate in patients found to have two or three mature follicles in comparison to a single follicle, P<0.05. In addition, pregnancy rates significantly increased when the size of the mature follicle was ≥ 18-20 mm (OR = 1.92, 95% CI [1.35, 2.72]; P = 0.0003) and > 20 mm (OR 1.56, 95% CI [1.12, 2.18]; P = 0.0084). The probability of a live birth also increased when the size of the mature follicle was ≥ 18-20 mm (OR = 1.90, 95% CI [1.28, 2.81]; P = 0.0014) and >20 mm (OR 1.86, 95% CI [1.28, 2.72]; P = 0.0012.) A higher rate of multiple gestations was found with three or more mature follicles but this was not statistically significant.

CONCLUSION: Higher pregnancy and live birth rates are achieved in patients with unexplained infertility treated with CC and IUI when patients have at least two mature follicles with size ≥18 mm. A prospective randomized trial is needed to confirm our findings.

P-242 Tuesday, October 21, 2014

INTRAUTERINE INSÉMINATION (IUI) IN PATIENTS WITH SINGLE PATENT TUBE. A RETROSPECTIVE REVIEW TO ANALYZE CANCELLATION RATE, PREGNANCY RATE AND TO IMPROVE INTRAUTERINE INSÉMINATION TREATMENT OUTCOME IN UNEXPLAINED INFERTILITY COUPLES. A. Chakraborty,* L. Helingaard,† J.-C. Arce.† Global Clinical R&D (Biopharmaceuticals), Ferring Pharmaceuticals, Copenhagen, Denmark; †Global Clinical R&D (Reproductive Health), Ferring Pharmaceuticals, Copenhagen, Denmark.

OBJECTIVE: To explore the impact of female body weight on serum FSH concentration and ovarian response parameters following controlled ovarian stimulation with HP-hMG in IVF/ICSI patients.

DESIGN: Retrospective analysis of 737 IVF/ICSI patients undergoing controlled ovarian stimulation with HP-hMG in two large randomized, assessor-blind, controlled, multicenter trials conducted with the long GnRH agonist protocol (MERIT trial)* and the GnRH antagonist protocol (MEGASET trial).†

MATERIALS AND METHODS: Patients were stimulated with a fixed starting dose of HP-hMG (MENOPUR, Ferring Pharmaceuticals) of 225 IU/day in the long agonist protocol and 150 IU/day in the antagonist protocol for the first five days and adjusted individually thereafter. In both trials, rHCG (250 µg) was given when ≥3 follicles were ≥17 mm. ANCOVA models adjusted for AMH at start of stimulation were used to assess the influence of body weight on serum FSH and ovarian response parameters (estradiol and number of oocytes retrieved). Serum AMH, FSH and estradiol were analyzed by a central laboratory. Body weight at start of stimulation was 62.7±8.5 kg and 60.6±6.8 kg, respectively, in the long agonist and antagonist trials.

RESULTS: Serum FSH concentrations at stimulation day 6 (i.e., around the time of reaching steady state) and at the end of stimulation were in both trials significantly (p<0.001) inversely related with the patient’s body weight, meaning that serum FSH concentrations decreased by increasing body weight. Body weight also had a significant inverse relation with serum estradiol at the end of stimulation (p<0.01) and with the number of oocytes retrieved (p<0.05) in both trials. One oocyte less was retrieved for every 12 kg increase in body weight when using 150 IU/day as starting dose in the antagonist protocol or 225 IU/day as starting dose in the long agonist protocol.

CONCLUSION: Body weight has a significant impact on serum FSH concentrations and consequently ovarian response in IVF/ICSI patients undergoing controlled ovarian stimulation with HP-hMG. Therefore, further considerations should be given to the patient’s body weight when developing individualized gonadotropin starting regimens.

Supported by: Ferring Pharmaceuticals.

P-244 Tuesday, October 21, 2014


OBJECTIVE: To assess if clinical pregnancy rates are affected by a decrease in FSH dose on day 5 of stimulation in women that undergo IVF/ICSI in an antagonist protocol when compared to dose reduction on day 6 or further.
CONCLUSION: The number of TF on the day of hCG represents the best predictor of severe OHSS in IVF cycles before triggering final oocyte maturation. The presence of more than 15 TF is associated with a dramatic increase in the risk for severe OHSS and in such cases the use of specific countermeasures against OHSS should be strongly considered.

P-246 Tuesday, October 21, 2014

OPTIMAL USAGE OF DUAL TRIGGER TO PREVENT OHSS IN A LONG-PROTOCOL IVF CYCLE. Y. H. Jung, Y. Y. Kim, M. H. Kim, Y. J. Yoo, J. D. Jo. Infertility Clinic, ElleMedi OB/GYN, Chang-Won, GyeongNam, Republic of Korea.

OBJECTIVE: When a patient undergoing an long protocol IVF cycle has high risk of severe OHSS, rescuing the cycle by withdrawing the agonist, replacing (adding) it with an antagonist and triggering with a GnRH agonist has been considered. However, we underwent lower the number of oocytes retrieval in GnRH agonist trigger group. Therefore we aimed to compare outcomes between trigger with GnRH agonist and dual trigger (GnRH agonist and low dose hCG). In addition, optimal bCG dosage is investigated in dual trigger with combination of gonadotropin-releasing hormone agonist and human chorionic gonadotropin.

DESIGN: A prospective clinical study.

MATERIALS AND METHODS: We evaluated total 26 high responder patients with a long protocol during 2009~2013. We monitored by measuring the FSH dose and the duration of stimulation as significant predictors of severe OHSS. ROC analyses indicated that the TF had the best capacity to discriminate between cycles with or without severe OHSS.

Cycles in which more that 15 TF were observed on the day of hCG had markedly increased risk of being complicated with severe OHSS (OR: 37.4, 95% CI: 11.2-124.2).

P-247 Tuesday, October 21, 2014

DUAL TRIGGER WITH GNRH AGONIST (GNusra) AND VARYING DOSES OF hCG INCREASES THE BLASTULATION RATE AMONGST HIGH RESPONDERS. M. D. Werner, a E. J. Forman, a,b K. H. Hong, a J. M. Fransasiak, a S. A. Neal, b R. T. Scott, Jr., a,b "REI, RWJ, Rutgers, Basking Ridge, NJ; "RMA NJ, Basking Ridge, NJ.

OBJECTIVE: GnRHa trigger reduces ovarian hyperstimulation syndrome (OHSS) risk while maintaining acceptable clinical outcomes. The impor-

<table>
<thead>
<tr>
<th>Marker</th>
<th>Logistic regression OR (95% CI)</th>
<th>ROC analysis AUC (95% CI)</th>
<th>Optimal cut-off (Sens/Spec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicles ≥ 10 mm</td>
<td>1.18 (1.15-1.20)</td>
<td>0.898 (0.892-0.903)</td>
<td>&gt;15 (88.8/92.4)</td>
</tr>
<tr>
<td>E2 on the day of hCG (1000 pg/mL)</td>
<td>2.3 (1.69-3.14)</td>
<td>0.776 (0.769-0.783)</td>
<td>≥ 2.18 (88.9/92.0)</td>
</tr>
<tr>
<td>Duration of stimulation (days)</td>
<td>0.91 (0.83-0.99)</td>
<td>0.576 (0.567-0.584)</td>
<td>≤ 10 (66.7/54.1)</td>
</tr>
<tr>
<td>Total dose of gonadotrophins (1000 IU)</td>
<td>0.18 (0.09-0.36)</td>
<td>0.813 (0.806-0.820)</td>
<td>≤ 1.65 (81.5/72.7)</td>
</tr>
</tbody>
</table>
tance of sufficient LH stimulation is evidenced by improved oocyte yield and outcomes when adding hCG. However, there are little data addressing the issue as to whether the addition of an agonist induced FSH surge increases yield, or whether the presence of the endogenous surge allows a reduction in hCG without diminishing outcomes. This study evaluates the outcomes in GnRH-a trigger cycles with varying dose of hCG augmentation, comparing them to cycles triggered with hCG alone to determine if a dual trigger benefits high responders.

DESIGN: Matched case-control.

MATERIALS AND METHODS: All first GnRH antagonist cycles from 2012-2014 in which a GnRH-a trigger was used were analyzed. These patients received varying doses of hCG (0, 1500, 2500, 5000, and 10000 IU) at the time of the 1st GnRH-a injection. The following criteria were used to select controls: 1) full dose hCG; 2) retrieval < 6 months of study case; 3) age within 1 year; and 4) follicles > 14 mm at time of trigger (=3 follicles). Usable blastocysts are those embryos deemed suitable for transfer or vitrification. The usable blastocyst rate is the number of usable blastocysts divided by the number of follicles >14 mm at time of trigger. Paired t-tests were performed for all comparisons.

RESULTS: 365 GnRH-a cases and 365 matched controls were identified. Dual trigger with GnRH-a and hCG resulted in more usable blastocysts and a higher conversion rate from mature follicle to usable blastocysts in the groups receiving 1,500, 2,500, and 5000, of hCG.

Comparison in usable blast rate between hCG alone vs. Lupon & varying doses of hCG

<table>
<thead>
<tr>
<th>Dose of hCG added to GnRH-a trigger (IU)</th>
<th>Usable Blasts Group (N; % of mature follicles)</th>
<th>Usable Blasts Group (N; % of mature follicles)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>16 7.4, 40%</td>
<td>17 7.4, 40%</td>
<td>0.9</td>
</tr>
<tr>
<td>1500</td>
<td>233 5.6, 38%</td>
<td>233 5.6, 38%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2500</td>
<td>33 5.2, 37%</td>
<td>33 5.2, 37%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>5000</td>
<td>43 4.9, 36%</td>
<td>43 4.9, 36%</td>
<td>0.05</td>
</tr>
<tr>
<td>10000</td>
<td>40 4.0, 36%</td>
<td>40 4.0, 36%</td>
<td>0.3</td>
</tr>
</tbody>
</table>

CONCLUSION: The addition of GnRH-a to hCG when triggering high responders increases usable blastocyst yield. A randomized dose ranging study evaluating both GnRH-a use and optimal hCG dose is needed to verify these results and confirm that these blastocysts maintain equivalent implantation rates when transferred.

P-249 Tuesday, October 21, 2014


OBJECTIVE: There is a lack of consensus regarding the ideal management of patients with poor response to ovarian stimulation. Letrozole (Lz) increases endogenous gonadotropin (GND) release, without competing with endometrial estradiol (E2) receptors. We seek to elucidate whether adding Lz in the early follicular phase of a GnRH antagonist (GA) stimulation cycle improves IVF outcome in poor responders.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: We included 90 poor responders, comparing baseline characteristics and IVF outcomes from prior GA cycle vs. subsequent GA cycle with added follicular phase Lz (LzGA). Student’s t-test and chi-square analysis were used with significance at p<0.05.

RESULTS: An interval of 0.36±0.5 years passed between GA and subsequent LzGA cycles. Patient age, BMI and basal antral follicle count were similar among both cycles. LzGA had higher Day 3 (D3) FSH (9.1±0.5 vs. 7.6±4.5, p<0.05) and lower peak E2 levels (515.7±377.7 vs. 917.9±639, p<0.05). The proportion of follicles reaching >14mm (53.6% vs. 56.3%) and number of mature oocytes retrieved (232 vs. 216) were comparable between LzGA and GA, respectively. LzGA had a lower fertilization rate (15.2% vs. 24.1%, p<0.05). There was a non-significant trend towards higher implantation rate (25.5% vs. 19.6%) and ongoing pregnancy rate (35.7% vs. 28.1%) in LzGA. LzGA had thicker endometrium at time of embryo transfer (9.8±2.4mm vs. 8.9±2.1mm, p<0.05) and yielded greater high quality D3 (132 vs. 69) and Day 5 embryos (D5) (32 vs. 24). LzGA received a mean Lz dose of 22.95±7.2mg and required less total amounts of FSH (9237.1±3869 vs. 11714±3755, p<0.05) and HMG (5451.2±3382.1 vs. 6528.7±4232, p<0.05). LzGA and Lz were cancelled vs. 45.6% of GA. Of cancelled GA (n=41), 56.1% yielded 115 mature oocytes with LzGA stimulation, of which 46.1% fertilized. 21 D3 and 11 D5 embryos were transferred, with 18.8% implantation rate and 15.6% ongoing pregnancy rate.

CONCLUSION: LzGA reduced GND requirement and demonstrated a trend towards improved implantation and ongoing pregnancy rates. 56% of the poorest responders (with <4 mature follicles in previously cancelled GA cycles) had oocytes retrieved with LzGA, resulting in 18.8% implantation. While Lz did not improve overall oocyte quality, it may improve oocyte quantity in poorly responding patients without adversely affecting the endometrium.
transdermal testosterone for the last 4 weeks and late luteal phase start GH supplementation before the commencement of COH in DOR patients who had previously cancelled/failed IVF/ICSI cycles.

DESIGN: Retrospective analysis.

MATERIALS AND METHODS: We compared 44 cycles in 33 females that had resulted in cancellation or pregnancy failure with 37 cycles where a novel treatment protocol was applied which we called ISIK protocol (IP), consisting of 12 weeks of DHEA 75 mg/d in combination with 25 mg transdermal testosterone gel daily for 4 weeks and late luteal start 3IU GH administration before the start of COH. We also define a control which was totally 51 patients that had 102 conventional IVF/ICSI cycles.

RESULTS: The duration of COH cycles, number of follicles >14 mm, number of oocytes, number of metaphase 2 oocytes and fertilization rate were significantly higher in the IP. The clinical pregnancy rate per embryo transfer of the IP was 38.2% (13/34). The cancellation rate of cycles decreased significantly from 54.5 % (24/44) to 8.1 % (3/37) with the IP, while the ongoing pregnancy rate was 35.3% (12/34 embryo transfer). However, CPR and OPR in control group were significantly lower than IP (22%, 10% respectively).

TABLE 1. Basal characteristics of study groups

<table>
<thead>
<tr>
<th>Protocol Control</th>
<th>Conventional</th>
<th>Isik Protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (n=126)</td>
<td>Group (n=44)</td>
<td>Group (n=37)</td>
</tr>
<tr>
<td>Age (mean±SD)</td>
<td>33.94±3.38</td>
<td>33.34±4.29</td>
</tr>
<tr>
<td>FSH (mean±SD)</td>
<td>14.49±1.48</td>
<td>11.7±1.29</td>
</tr>
<tr>
<td>AMH (mean±SD)</td>
<td>0.26±0.15</td>
<td>0.17±0.09</td>
</tr>
<tr>
<td>Duration of infertility (mean±SD)</td>
<td>5.67±4.33</td>
<td>4.5±2.91</td>
</tr>
<tr>
<td>E2 level on the day of MCO (mean±SD)</td>
<td>598.15±683.09</td>
<td>491.68±684.34</td>
</tr>
<tr>
<td>P4 level on the day of MCO (mean±SD)</td>
<td>0.84±1.55</td>
<td>0.77±1.14</td>
</tr>
<tr>
<td>Endometrium thickness (mean±SD)</td>
<td>9.4±1.86</td>
<td>9.43±2.02</td>
</tr>
<tr>
<td>Duration of COH (mean±SD)</td>
<td>8.96±2.21</td>
<td>8.0±3.23</td>
</tr>
</tbody>
</table>

CONCLUSION: Our study has shown that even the poorest responders could achieve clinical pregnancy during ovarian folliculogenesis with a combination of transdermal testosterone, DHEA and GH.

P-251 Tuesday, October 21, 2014

TEN YEARS EXPERIENCE WITH POOR RESPONDENT PATIENTS FULFILLING BOLOGNA CRITERIA. B. Ozmen,1 G. E. Pabuccu,2 O. Kan,3 M. Sonmezer,3 C. S. Atabekoglu,4 R. Pabuccu.1 Obh-Gyn, Ankara University, Ankara, Turkey; 2Obh-Gyn, Uluk University, Ankara, Turkey.

OBJECTIVE: To compare the cycle outcomes of different regimens in poor responders undergoing IVF/ICSI.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: Computerized data concerning COH and embryological outcomes of 534 patients who underwent COH for IVF/ICSI at Ankara University School of Medicine and at Centrum Clinic IVF Center between 2004 and 2013 were retrospectively analyzed. All patients had at least two of the defined features of bologna criteria. Patients were allocated to group 1 (Microdose flare-up, MF), group 2 (luteal estrogen protocol, LE), group 3 (Aromatase Inhibitor, AIs) and group 4 (GnRH antagonist, Gant). A sample size of 75 cases (totally 300 cases) per group is essential detect a difference in retrieved oocyte means (power of 81% and a P value set at 0.05) using a one-way ANOVA study and F-Test.

RESULTS: All data was demonstrated in Table 1. The pregnancy rates and COS outcomes were all found as lower as half in all groups when female partner age > 40 years-old.

Cycle and Stimulation Outcomes among Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Conventional Protocol Control (n=110)</th>
<th>Conventional Isik Protocol (n=75)</th>
<th>Group 3: AIs (n=165)</th>
<th>Group 4: Gant (n=184)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>36.1±5.8</td>
<td>36.6±6.2</td>
<td>36.5±5.7</td>
<td>36.5±5.9</td>
</tr>
<tr>
<td>Body mass index</td>
<td>24.6±2.6</td>
<td>24.1±1.8</td>
<td>25±2.4</td>
<td>24±2.2</td>
</tr>
<tr>
<td>FSH (IU/mL)</td>
<td>10.4±4.4</td>
<td>11.2±3.1</td>
<td>10.3±3.3</td>
<td>10.8±3.2</td>
</tr>
<tr>
<td>AMH (ng/mL)</td>
<td>1.3±0.7</td>
<td>1.2-1.6</td>
<td>1.1±0.1</td>
<td>1.3±0.8</td>
</tr>
<tr>
<td>Gonadotropins used (IU)</td>
<td>396.5±1628</td>
<td>3612.±1155</td>
<td>3532.±1107</td>
<td>3531.±1077</td>
</tr>
<tr>
<td>Peak E2 (pg/mL)</td>
<td>1564±703</td>
<td>1936±701</td>
<td>839±534</td>
<td>1785±901</td>
</tr>
<tr>
<td>Cycle cancellation</td>
<td>25.4%</td>
<td>18.6%</td>
<td>20.6%</td>
<td>25%</td>
</tr>
</tbody>
</table>

(a) Statistical importance found between group 3 vs others (b) In cases where AMH was studied and taken as a criteria (42.8%, 229/534)

CONCLUSION: Albeit innovations, still clinical and ongoing PRs were disappointing in poor responders fulfilling Bologna criteria.

P-252 Tuesday, October 21, 2014

IS THERE ANY CLINICAL UTILITY TO MEASURING THE ESTRADIOL FLARE DURING MICRODOSE (MCD) FLARE CYCLES? T. G. Nazem, J. D. Kofinas, D. McCulloh, J. A. Grifo, A. Berkeley. Obstetrics and Gynecology, NYU School of Medicine, New York, NY.

OBJECTIVE: To determine if measuring the estrogen flare effect after two days of MCD Lupron in fresh IVF cycles is predictive of cycle outcome.

DESIGN: Retrospective study in an academic institution.

MATERIALS AND METHODS: A retrospective analysis of fresh MCD flare cycles (n=326) performed during the years 2006 through 2013 was undertaken. Subgroup analyses were performed on a cohort of patients who did not receive oral contraceptives (OCPs) prior to initiation of the cycle (n=89) versus those who did receive OCPs (n=237). MCD was usually initiated day 1 of the patient’s menstrual cycle with baseline estradiol levels obtained. Estradiol levels were then repeated 48 hours later before the initiation of gonadotropins. The percent change in estradiol between these two time points was calculated. Main outcome measures included live birth, number of mature oocytes, number of grade 2 or better blasts and cycle cancellation. Receiver operator characteristic (ROC) curves were used to determine the predictive value of each measure.

RESULTS: Of the 326 patients, 72(22%) were cancelled and did not undergo retrieval. Subgroup analyses were performed on a cohort of patients who did not receive oral contraceptives (OCPs) prior to initiation of the cycle (n=89) versus those who did receive OCPs (n=237). MCD was usually initiated day 1 of the patient’s menstrual cycle with baseline estradiol levels obtained. Estradiol levels were then repeated 48 hours later before the initiation of gonadotropins. The percent change in estradiol between these two time points was calculated. Main outcome measures included live birth, number of mature oocytes, number of grade 2 or better blasts and cycle cancellation. Receiver operator characteristic (ROC) curves were used to determine the predictive value of each measure.

(e222) ASRM Abstracts
>6, 2 or blastocysts grade 2 or better were 0.75, 0.59, 0.59, 0.54 respectively. Patients with OCP priming showed AUCs for cycle cancellation, live birth, number of mature eggs > 6 and (2 or more) blastocysts grade 2 or better of 0.68, 0.59, 0.52, and 0.55 respectively.

CONCLUSION: Measuring the estradiol flare during MCD cycles can help to predict cancellation but is not predictive of live births, number of mature eggs > 6 and (2 or more) blastocysts grade 2 or better. Either the test can be abandoned in MCD cycles, or caution must be exercised when interpreting the test for cycle cancellation as the AUC values indicate poor performance of this measure.

P-253 Tuesday, October 21, 2014


OBJECTIVE: In previous retrospective studies (Patrizio 2009, Stoop 2012) overall oocyte utilization following conventional ovarian stimulation was found to be constantly low (<5%) and declining even further (<1%) in >37 years old women. It was suggested that mild ovarian stimulation could yield eggs of better quality (Ikeg. 2005). However to date there are no large-scale analyses available from well-controlled trials on the reproductive potential of MII oocytes obtained via mild IVF in comparison to conventional stimulation.

DESIGN: Data from an RCT involving 564 patients randomized to mild (n=285) (minimal ovarian stimulation with clomiphene, bclastocyst culture, delayed vitrified single embryo transfers) or conventional IVF treatment (n=279) (long GnRH agonist protocol, blastocyst culture, fresh and vitrified transfers of two embryos) in a private infertility center between 2009 and 2013 was analyzed.

MATERIALS AND METHODS: The reproductive potential of MII oocytes (cumulative ongoing pregnancy rate per mature MII oocyte) was calculated separately for the mild versus conventional IVF group and also according to three ovarian response categories (minimal: 1-2, mild: 3-6, normal to high response: 7-38 mature MII eggs).

RESULTS: In the mild IVF treatment arm a total 1024 MII eggs (mean 3.7±2.8, range: 0-18) originated resulting in 144 (51%) ongoing pregnancies whereas in the conventional IVF arm 2527 MII eggs (mean 10±6.7, range: 0-38) were retrieved resulting in 185 (66%) ongoing pregnancies. Whereas in the mild IVF arm the majority of eggs originated from cycles with minimal (14%) or mild (54%) ovarian response in the conventional arm most oocytes (88%) originated from normal to high ovarian response cycles. Overall MII oocyte utilization rate was twice as high in the mild IVF group compared with conventional treatment (14% versus 7%, OR: 2.07 95%CI: 1.64-2.61). Egg efficiency was inversely related to ovarian response categories (22, 15, 10% with mild IVF and 36, 13, 6% with conventional treatments).

CONCLUSION: In <38 year old patients overall MII oocyte utilization rate was twice as high following mild IVF suggesting that oocytes might be biologically more effective compared to eggs obtained via the standard long agonist protocol. Egg efficiency was also inversely related to ovarian response categories both with mild and conventional IVF treatment. Further studies involving embryo aneuploidy screening are needed to determine underlying causes to this phenomenon.

P-254 Tuesday, October 21, 2014

SELECTION OF AN OPTIMAL CONTROLLED OVARIAN HYPERSTIMULATION METHOD IN RELATION TO THE NUMBER OF ANTRAL FOLLICLES IN PATIENTS LESS THAN 40 YEARS OLD. A. Tanaka, M. Nagayoshi, I. Tanaka. Saint Mother Hospital, Kitakyushu, Fukuoka, Japan.

OBJECTIVE: It is widely known that one of the most important factors for the success of assisted reproductive technology treatments is the quality of the collected oocytes. It is therefore crucial to choose the right method for controlled ovarian stimulation (COH). We performed this study in order to choose the optimal COH method based on the number of antral follicles from January 2011 to December 2012.

DESIGN: Controlled prospective study to choose the optimal controlled ovarian hyperstimulation method according to the number of antral follicles.

MATERIALS AND METHODS: A total of 1652 patients (2256 cycles) under 40 years of age randomly received one of three kinds of COH: GnRH-antagonist + HMG (a), GnRH-agonist + FSH (HMG), short protocol (b), long protocol (c) after counting the numbers of small antral follicles on day 3 and randomly choosing fresh or thawed embryo transfer. The total number of antral follicles were classified into 3 types, few (<5) (I), intermediate (5-14) (II) and many (>15) (III). Embryos were cultured for 3-5 days, and single embryo was transferred. Luteal function was supported by the progesterone suppository 60mg/day + oestradiol valerate 2mg.

RESULTS: Pregnancy and miscarriage rates according to the number of antral follicles ([<5]-I, (5-14)-II, (>15)-III] among the GnRH-antagonist + HMG (a), GnRH-agonist + HMG (FSH)-Short (b) GnRH-agonist + HMG (FSH)-Long (c) protocols were summarized in table.

<table>
<thead>
<tr>
<th>Protocols (a)</th>
<th>The number of antral follicles I</th>
<th>The number of antral follicles II</th>
<th>The number of antral follicles III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy rates</td>
<td>56.3%(36/64)</td>
<td>39.0%(128/328)</td>
<td>34.6%(36/104)</td>
</tr>
<tr>
<td>Miscarriage rates</td>
<td>44.5%(16/36)</td>
<td>18.8%(24/128)</td>
<td>33.3%(12/36)</td>
</tr>
<tr>
<td>Protocols (b)</td>
<td>Pregnancy rates</td>
<td>26.9%(28/84)</td>
<td>31.0%(228/736)</td>
</tr>
<tr>
<td>Miscarriage rates</td>
<td>28.6%(8/28)</td>
<td>14.0%(32/228)</td>
<td>25.0%(8/32)</td>
</tr>
<tr>
<td>Protocols (c)</td>
<td>Pregnancy rates</td>
<td>0%(0/4)</td>
<td>28.6%(16/56)</td>
</tr>
<tr>
<td>Miscarriage rates</td>
<td>0%(0/0)</td>
<td>50.0%(8/16)</td>
<td>10.0%(4/40)</td>
</tr>
</tbody>
</table>

The number of antral follicles are [(<5)-I, (5-14)-II, (>15)-III] and the protocols are [GnRH-antagonist + HMG (a), GnRH-agonist + HMG (FSH)-Short (b), GnRH-agonist + HMG (FSH)-Long (c)].

CONCLUSION: (1) In the group with a small number of antral follicles (<5), GnRH-antagonist + HMG produced the highest pregnancy rates among the three methods of COH. (2) In the intermediate group (5-14), GnRH-antagonist + HMG or GnRH-agonist + HMG (FSH)-Short were preferable. (3) In the group with the large number of antral follicles (>15), GnRH-agonist + HMG (FSH)-Long produced the highest pregnancy rates.

P-255 Tuesday, October 21, 2014

DOES ANTI-MULLERIAN HORMONE (AMH) LEVEL PREDICT PREGNANCY OUTCOME IN PATIENTS WITH UNEXPLAINED INFERTILITY UNDERGOING CLOMPHENE CITRATE/INTRAUTERINE INSEMINATION (CC/IUI)? V. Libby, C. Mullin, A. Ulrich, M. Lesser, A. Hershlag. 4Center for Human Reproduction, North Shore Long Island Jewish Health Systems, Manhasset, NY; 4Biostatistics Unit, The Feinstein Institute for Medical Research, Manhasset, NY.

OBJECTIVE: AMH has become an important predictor of ovarian reserve. Low AMH levels forecast reduced response to gonadotropins in IVF cycles as well as inferior clinical pregnancy rates (CPR). This study was conducted in order to determine whether AMH has the same predictive value in patients with unexplained infertility undergoing CC/IUI.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: Of 502 IUI cycles between 2012-2013, there were 122 IUI cycles in 48 couples with unexplained infertility which were included in our study. Patients were categorized into groups based on AMH levels. The number of cycle days was defined as the number of days from the first day of menses till the day of IUI. A mature follicle was defined as any follicle >17mm. Patients underwent IUI on two sequential days following either a LH surge or hCG trigger when the lead follicle was >20 mm. CPR was defined as a positive fetal heart rate detected by ultrasound. CPR was adjusted for couples who underwent multiple IUI cycles. Depending on the outcome variable, the normal, Poisson, or logit link functions were used.
RESULTS: CPR per cycle ranged between 15 and 20%. CPR was not affected by AMH level. CPR remained high despite AMH level. Moreover, AMH was not predictive of follicular response.

Ovarian Response and Pregnancy Outcomes in Couples with Unexplained Infertility Undergoing CC/IUI

<table>
<thead>
<tr>
<th>AMH Level (ng/mL)</th>
<th>0-0.9</th>
<th>1-2.9</th>
<th>3-4.9</th>
<th>≥5</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td># patients/ cycles</td>
<td>7/20</td>
<td>27 / 65</td>
<td>10 / 20</td>
<td>4 / 21</td>
<td></td>
</tr>
<tr>
<td>Mean Age (yrs)</td>
<td>35.1 ± 1.6</td>
<td>34.6 ± 0.8</td>
<td>31.7 ± 1.3</td>
<td>34.0 ± 2.1</td>
<td>0.26</td>
</tr>
<tr>
<td>Mean CC Dose</td>
<td>103.7 ± 11.9</td>
<td>86.0 ± 5.8</td>
<td>84.1 ± 9.7</td>
<td>106.6 ± 13.7</td>
<td>0.32</td>
</tr>
<tr>
<td>Mean # of Cycle Days</td>
<td>15.0 ± 0.7</td>
<td>14.7 ± 0.3</td>
<td>15.0 ± 0.6</td>
<td>16.9 ± 0.8</td>
<td>0.09</td>
</tr>
<tr>
<td># Mature Follicles</td>
<td>1.6 ± 0.2</td>
<td>1.5 ± 0.1</td>
<td>1.5 ± 0.2</td>
<td>1.2 ± 0.3</td>
<td>0.83</td>
</tr>
<tr>
<td>CPR (%)</td>
<td>17.3</td>
<td>17.7</td>
<td>15.4</td>
<td>19.5</td>
<td>0.99</td>
</tr>
</tbody>
</table>

CONCLUSION: AMH levels do not predict pregnancy outcome in couples with unexplained infertility undergoing CC/IUI cycles, nor are they predictive of follicular response. Therefore, patients with unexplained infertility and AMH levels <1 should still be treated with CC/IUI prior to IVF.

P-256 Tuesday, October 21, 2014

EFFECTS OF GNRH AGONIST OR GNRH ANTAGONIST ON EXPRESSION OF GDF9 AND BMP15 IN CONTROLLED HYPER-STIMULATED OVARY IN MICE. J.-Y. Zhang, X.-Y. Guo, Reproductive Medicine Center, Guangzhou, Guangzhou Province, China; 

OBJECTIVE: To compare the gonadotropin releasing hormone antagonist protocol to agonist long protocol during COS cycles with human-derived gonadotropins on IVF outcomes.

DESIGN: Forty-one women ages 21-40 with anti-Mullerian hormone ≥ 1 ng/ml were randomized between the treatments. Continuous variables were expressed as mean ± standard deviation. Student's t-tests and Fisher's exact tests were used, statistical significance was set at p<0.05, and data were analyzed with SAS/STAT 12.1 software.

MATERIALS AND METHODS: All study patients underwent a cycle with oral contraception pills (OCP) before starting the COS cycle for IVF. All patients received a fixed protocol of human-derived gonadotropins at a 3:1 ratio (225/75 IU) for the first 4 days of stimulation followed by a flexible protocol, which was adjusted by the physician to optimize response. Twenty patients received the agonist (Luprolide Acetate), starting on day 18 of the OCP cycle, and 21 patients received the antagonist starting when follicle size reached 12 mm during the COS cycle. Human chorionic gonadotropin (hCG) was given when at least three follicles reached 17 mm to induce oocyte maturation 36 hours prior to oocyte retrieval.

RESULTS: The mean estradiol (E2) concentration ([207.6 ± 791.2 pg/ml]) on the day of hCG was lower (p=0.003) in the antagonist group (n=21) compared to the E2 (3045.4 ± 1219.5 pg/ml) in the agonist group (n=20). The luteinizing hormone (LH) concentration (0.41 ± 0.27 IU/ml) on the day of hCG was less (p<0.001) in the antagonist group compared to the LH (1.66 ± 0.92 IU/ml) in the agonist group. There was no difference in age, duration of stimulation, number of oocytes retrieved, number of mature oocytes, fertilization rate, implantation rate, and pregnancy rate.

Ovarian Response and Pregnancy Outcomes in Couples with Unexplained Infertility Undergoing CC/IUI

<table>
<thead>
<tr>
<th>AMH Level (ng/mL)</th>
<th>0-0.9</th>
<th>1-2.9</th>
<th>3-4.9</th>
<th>≥5</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td># patients/ cycles</td>
<td>7/20</td>
<td>27 / 65</td>
<td>10 / 20</td>
<td>4 / 21</td>
<td></td>
</tr>
<tr>
<td>Mean Age (yrs)</td>
<td>35.1 ± 1.6</td>
<td>34.6 ± 0.8</td>
<td>31.7 ± 1.3</td>
<td>34.0 ± 2.1</td>
<td>0.26</td>
</tr>
<tr>
<td>Mean CC Dose</td>
<td>103.7 ± 11.9</td>
<td>86.0 ± 5.8</td>
<td>84.1 ± 9.7</td>
<td>106.6 ± 13.7</td>
<td>0.32</td>
</tr>
<tr>
<td>Mean # of Cycle Days</td>
<td>15.0 ± 0.7</td>
<td>14.7 ± 0.3</td>
<td>15.0 ± 0.6</td>
<td>16.9 ± 0.8</td>
<td>0.09</td>
</tr>
<tr>
<td># Mature Follicles</td>
<td>1.6 ± 0.2</td>
<td>1.5 ± 0.1</td>
<td>1.5 ± 0.2</td>
<td>1.2 ± 0.3</td>
<td>0.83</td>
</tr>
<tr>
<td>CPR (%)</td>
<td>17.3</td>
<td>17.7</td>
<td>15.4</td>
<td>19.5</td>
<td>0.99</td>
</tr>
</tbody>
</table>

CONCLUSION: The GnRH antagonist protocol resulted in lower E2 and LH concentrations on the day of hCG. However, IVF outcomes were not affected. Larger clinical trials are necessary to study the clinical significance of these findings.

Supported by: Ferring Pharmaceuticals Inc.

P-258 Tuesday, October 21, 2014

IS THERE AN ASSOCIATION BETWEEN GONADOTROPIN DOSING AND ANEUPLOIDY RATES? J. D. Kort,a J. Zolton,b B. Behr,b R. B. Lathi, B. L. Baker,a Obstetrics and Gynecology, Stanford University Hospital, Stanford, CA; aObstetrics and Gynecology, Summa Akron City Hospital, Akron, OH.

OBJECTIVE: While high doses of gonadotropins are associated with increases in oocyte yield, excessive dosing may be detrimental to oocytes. This study sought to assess whether there is an association between gonadotropin doses and aneuploidy rates by using the ovarian sensitivity index (total gonadotropin dose/ # oocytes retrieved) as a measure of gonadotropin dosing...
that controls for ovarian response as poor responders may have higher baseline aneuploidy rates.

**DESIGN:** We conducted a retrospective analysis of all in vitro fertilization (IVF) cycles utilizing pre-implantation generic screening (PGS).

**MATERIALS AND METHODS:** All IVF-PGS cycles with 24 chromosome analyses between 2012 and 2013 at a single academic IVF clinic were reviewed. Charts were reviewed for maternal age at oocyte retrieval, oocyte yield, PGS results and total gonadotropin doses. Ovarian sensitivity indices were calculated, and chi-squared analyses were used to compare euploid rates of patients with a lower ovarian sensitivity index to patients with a higher ovarian sensitivity index after stratifying for patient age. The implantation rates of patients who underwent a fresh embryo transfer were compared.

**RESULTS:** After stratifying patients by age, there was a trend that patients with an ovarian sensitivity index ≤ 300 IU/oocyte retrieved had a lower aneuploidy rate than those with an ovarian sensitivity index > 300 IU/oocyte retrieved (Table I). This trend reached statistical significance among patients ages 35 to 40. Among both age groups, those patients with an ovarian sensitivity index ≤ 300 IU/oocyte retrieved were significantly more likely to have at least one euploid embryo available for transfer. There was no difference between the implantation rate of fresh euploid embryo transfers after stratifying by patient age and ovarian sensitivity index.

**CONCLUSION:** When stratified by age, patients requiring higher gonadotropin doses to yield a particular number of oocytes may have higher aneuploidy rates than patients yielding a similar number of oocytes after exposure to a lower dose of gonadotropins. Prospective studies are needed to assess the incremental yield of euploid embryos resulting from very high dose gonadotropin regimens in IVF cycles.

**TABLE I.**

<table>
<thead>
<tr>
<th>Age</th>
<th>Ovarian Sensitivity Index</th>
<th>Aneuploidy Rate</th>
<th>≥ 1 euploid embryo for transfer p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>35 to 40yo</td>
<td>≤ 300 IU/egg retrieved</td>
<td>65%</td>
<td>89%</td>
</tr>
<tr>
<td>&gt; 300 IU/egg retrieved</td>
<td>&gt; 40yo</td>
<td>75%</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

**P-259 Tuesday, October 21, 2014**

**ADMINISTRATION OF THE FIRST 2 DOSES OF GONADOTROPIN AT TWICE THE DOSE DURING CONTROLLED OVARIAN HYPERSTIMULATION DECREASES TOTAL GONADOTROPIN ADMINISTRATION DURING IN VITRO FERTILIZATION (IVF) CYCLE.** T. Charkviani, a L. Chkonia, a N. Karashvili, a N. Katchalikidze, a T. Zhorzholadze, a D. McCulloh, b Georgia American Center for Reproductive Medicine RepronAT, Tbilisi, Georgia; aNYU Fertility Center, NYU Langone Medical Center, NY, NY.

**OBJECTIVE:** Blood levels of Gonadotropins(Gnd) change slowly in response to repeated administration of the same daily dose(SDD). Typical controlled ovarian hyperstimulation(COH) involves administration of the SDD of Gnd throughout the cycle with dose adjustments after 3-4 days and every 1-3 days.

**DESIGN:** We tried a different approach, administering higher doses for the first 2 days in order to raise Gnd levels to a stable state(SS) more rapidly and compared it with the typical approach to determine if IVF outcomes improve.

**MATERIALS AND METHODS:** Ovarian response and IVF outcomes were compared between 2 administration methods:Group1 – with administration of Gnd at twice the standard dose for 2 days at one facility; Group2 – historical data from another facility in which administration of Gnd began with repeated SDD. We examined 3 age groups(30-34, 35-40, 41-42 years). Dose adjustments were permitted following the 3rd day of administration. Outcome parameters included blood levels of FSH(FSHb), total gonadotropin administered(U), days of stimulation(days), numbers of follicles larger than 16 mm(F16), oocytes retrieved(eggs), number of eggs fertilized(Fert) as well as clinical pregnancy rate(CPR), implantation rate(IR) and live birth rates(LBR).

**RESULTS:** Results compare Group1 relative to Group2. SS levels of FSHb were attained earlier in Group1. Significantly fewer days (~1) were required in Group1 and resulted in ≥2F16 at trigger. Significantly less IU(600-1000 IU) were administered in Group1 in association with indistinguishable maximum levels of FSHb in 2 younger groups of Group1, but with significantly lower FSHb in the oldest group. Fewer Eggs and Fert in Group1 were associated with indistinguishable totals of embryos transferred and/or cryopreserved. Despite the transfer of significantly fewer embryos(0.3, 0.35, 1.26) in ascending age groups of Group1 CPR and IR were not significantly different except in the older group(with lower FSHb) in which these were lower than for Group2.

**CONCLUSION:** Augmented administration of Gnd for the first 2 days of COH was associated with similar outcomes(number of transferable/cryopreparable embryos,CPR,IR,LBR) while achieving SS levels of Gnd sooner, utilizing less Gnd with fewer days. The administration of higher doses of Gnd on days 1 and 2 surprisingly was associated with a decreased requirement for Gnd. Briefier stimulation and decreased IU requirement improved the tolerability and affordability of IVF treatment.

**P-260 Tuesday, October 21, 2014**

**THE IMPACT OF CONTROLLED OVARIAN STIMULATION PROTOCOL ON EMBRYO TRANSFER RATE IN PGD CYCLES FOR ANEUPLOIDY TESTING.** A. P. C1, N. Dokuzeul, A. Karahasanoglu, S. Kahraman. ART and Reproductive Genetics Unit, Istanbul Memorial Hospital, Istanbul, Turkey.

**OBJECTIVE:** To determine whether there is a difference between long agonist and short antagonist protocols in reaching embryo transfer in PGD cycles for aneuploidy testing.

**DESIGN:** Retrospective data analysis.

**MATERIALS AND METHODS:** Overall 1529 PGD cycles performed in Istanbul Memorial Hospital between years 2003 and 2013 were analyzed. The data was subdivided into Long agonist and short antagonist protocols for comparison. Our primary endpoint was embryo transfer rate (ETR). Besides ETR, other parameters such as number of retrieved, mature, fertilized oocytes, biopsied embryos, total gonadotrophins used and euploidy rate were compared. Student’s t-test, chi-square and UNIANOVA were used for comparison of the data where appropriate.

**RESULTS:** Of 1529 cycles for aneuploidy testing, in 48 and 42% of the cycles antigenist and agonist suppression were used, respectively. The mean age and BMI of the patients using antagonist protocol were significantly higher than the patients undergoing long agonist protocol. The number of retrieved, mature and fertilized oocytes were significantly higher in long agonist group despite the use of similar amount of gonadotrophins. As this group was younger euploidy rate was higher as expected. Accordingly biopsied and diagnosed embryos were also higher in long agonist group. In order to exclude the effect of age and BMI on the outcome parameters, we compared the two groups after adjusting for age and BMI, and the results were still in favor of Long agonist protocol. Higher number of available oocytes for biopsy and higher euploidy rate in this group resulted in significantly higher transfer rate (88 vs 95%) in long agonist group. However live birth rate per cycle and per transfer were similar in both groups.

**TABLE I.**

<table>
<thead>
<tr>
<th>Age</th>
<th>Ovarian Sensitivity Index</th>
<th>Aneuploidy Rate</th>
<th>≥ 1 euploid embryo for transfer p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>35 to 40yo</td>
<td>≤ 300 IU/egg retrieved</td>
<td>65%</td>
<td>89%</td>
</tr>
<tr>
<td>&gt; 300 IU/egg retrieved</td>
<td>&gt; 40yo</td>
<td>75%</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

**P-260 Tuesday, October 21, 2014**

**THE IMPACT OF CONTROLLED OVARIAN STIMULATION PROTOCOL ON EMBRYO TRANSFER RATE IN PGD CYCLES FOR ANEUPLOIDY TESTING.** A. P. C1, N. Dokuzeul, A. Karahasanoglu, S. Kahraman. ART and Reproductive Genetics Unit, Istanbul Memorial Hospital, Istanbul, Turkey.

**OBJECTIVE:** To determine whether there is a difference between long agonist and short antagonist protocols in reaching embryo transfer in PGD cycles for aneuploidy testing.

**DESIGN:** Retrospective data analysis.

**MATERIALS AND METHODS:** Overall 1529 PGD cycles performed in Istanbul Memorial Hospital between years 2003 and 2013 were analyzed. The data was subdivided into Long agonist and short antagonist protocols for comparison. Our primary endpoint was embryo transfer rate (ETR). Besides ETR, other parameters such as number of retrieved, mature, fertilized oocytes, biopsied embryos, total gonadotrophins used and euploidy rate were compared. Student’s t-test, chi-square and UNIANOVA were used for comparison of the data where appropriate.

**RESULTS:** Of 1529 cycles for aneuploidy testing, in 48 and 42% of the cycles antigenist and agonist suppression were used, respectively. The mean age and BMI of the patients using antagonist protocol were significantly higher than the patients undergoing long agonist protocol. The number of retrieved, mature and fertilized oocytes were significantly higher in long agonist group despite the use of similar amount of gonadotrophins. As this group was younger euploidy rate was higher as expected. Accordingly biopsied and diagnosed embryos were also higher in long agonist group. In order to exclude the effect of age and BMI on the outcome parameters, we compared the two groups after adjusting for age and BMI, and the results were still in favor of Long agonist protocol. Higher number of available oocytes for biopsy and higher euploidy rate in this group resulted in significantly higher transfer rate (88 vs 95%) in long agonist group. However live birth rate per cycle and per transfer were similar in both groups.

**TABLE I. Comparison of the protocols**

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Long agonist</th>
<th>Short antagonist</th>
<th>p value*</th>
<th>p value after adjustments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycles, %</td>
<td>42</td>
<td>48</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>33.8±5.3</td>
<td>35.9±5.8</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>25.1±4.4</td>
<td>25.9±4.8</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>Total gonadotropin dose</td>
<td>2715±1102</td>
<td>2795±1279</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Retrieved oocytes</td>
<td>16.5±7.1</td>
<td>12.8±7.2</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Mature oocyte</td>
<td>12.7±5.8</td>
<td>9.9±5.6</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Fertilized oocyte</td>
<td>9.8±4.7</td>
<td>8.1±4.5</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Euploidy rate, %</td>
<td>46.3±</td>
<td>35±25.9</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Biopsied embryos</td>
<td>7.1±3.8</td>
<td>6.6±3.8</td>
<td>0.013</td>
<td></td>
</tr>
<tr>
<td>Diagnosed embryos</td>
<td>6.2±3.4</td>
<td>5.9±3.4</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Embryo transfer rate, %</td>
<td>95.5</td>
<td>87.7</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

* Student’s t-test, aUNIANOVA
CONCLUSION: In PGD cycles for aneuploidy testing Long agonist protocol is beneficial compared to Short antagonist protocol in retrieving more oocytes which results in higher euploidy and transfer rate.

P-261 Tuesday, October 21, 2014


OBJECTIVE: LET use for OI and COH has widely increased in the past few years. Several studies have compared its' effectiveness in terms of pregnancy outcomes to the more formerly used CC. The objective of this study was to compare the multiple pregnancy rates between LET and CC protocol.

DESIGN: Retrospective cohort analysis.

MATERIALS AND METHODS: All patients <40 years with normal ovarian reserve (normal FSH, normal AMH, normal AFC) and normal semen parameters undergoing a LET or CC cycle from January 2010 - November 2013 were analyzed. LET or CC was administered on days 3-7 with US monitoring initiated on cycle day 12 and/or until a follicle >20mm was observed. Intratubal insemination or sexual intercourse was recommended 24 to 36 hours after hCG trigger. The number of sacs was evaluated at first ultrasound (US) 14 days later. Categorical variables were assessed by Fisher's exact test for small frequencies with significance at a p-value of <0.05. RESULTS: A total of 7,064 cycles were included in the study. No significant differences were observed in age, basal FSH or clinical PR in LET (n=1829) compared to CC cycles (n=5235). Notwithstanding, the multiple pregnancy rate was significantly increased in the CC cohort (9.4%), nearly doubled that to LET cycles (4.8%).

<table>
<thead>
<tr>
<th>LET</th>
<th>CC</th>
<th>Stat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrial thickness</td>
<td>7.6 (±2.4)</td>
<td>8 (±2.4)</td>
</tr>
<tr>
<td>Fols &gt;14mm</td>
<td>2.09 (±1.2)</td>
<td>2.39 (±1)</td>
</tr>
<tr>
<td>Preg rate (%)</td>
<td>13.5% (247/1829)</td>
<td>12.8% (674/5235)</td>
</tr>
<tr>
<td>Twins (n)</td>
<td>12</td>
<td>58</td>
</tr>
<tr>
<td>Triples (n)</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Multiple PR (%)</td>
<td>4.8%</td>
<td>9.4%</td>
</tr>
</tbody>
</table>

CONCLUSION: Our pooled data demonstrated a lower multiple pregnancy rate in LET cycles (4.8%) versus CC cycles (9.4%) in spite of similar pregnancy rates between both agents. Notably, 6 triplets were observed in CC cycles and none were observed after LET use. Although pregnancy rates are similar, our analysis suggests a clear difference in multiple pregnancy rates between these two agents. Prospective studies are needed to confirm our findings.

P-262 Tuesday, October 21, 2014

WHAT IS THE SAFE AND EFFECTIVE FIRST LINE THERAPY FOR WOMEN WITH UNEXPLAINED INFERTILITY? A. Fukui, A. Funamizu, R. Fukuhara, T. Sakamoto, S. Fujii, H. Mizumura. Obstetrics and Gynecology, Hirotsuki University Graduate School of Medicine, Hirotsuki, Aomori, Japan; Sakamoto Tomomi Clinic, Hirotsuki, Aomori, Japan; “ef.clinik, Aomori, Japan.

OBJECTIVE: To compare the patients' responses to clomiphene citrate (CC), 37.5IU and 50IU recombinant human FSH (rhFSH) protocols for the treatment of unexplained infertility.

DESIGN: A prospective, unblinded, randomized controlled multi-center study.

MATERIALS AND METHODS: Unexplained infertile women aged <40 years with normal menstrual period, basal FSH and LH < 10mIU/mL, BMI of 18-28kg/m2 and gave consent to this study were included. Infertile patients with polycystic ovary syndrome were excluded. Patients were randomized to non-stimulation (control), CC (CC 30mg/day for 5 days from Day 3), rhFSH (rhFSH 37.5IU/day for 5 days) or rhFSH (rhFSH 50IU/day from Day 5). The changes of serum FSH and E2 levels (primary endpoint), the number of follicles and pregnancy rate (PR) in each group were assessed. Statistical differences among the groups were analyzed by one-way ANOVA. The difference of PR of each group was analyzed by χ² test. Differences were considered significant for probability <0.05.

RESULTS: Patients (n=277) cycles were randomized to Control (n=17: 59 cycles), CC (n=22: 100 cycles), rhFSH (37.5) (n=24: 79 cycles) or rhFSH (50) (n=22: 79 cycles). There were no significant differences with baseline characteristics (age, serum LH, FSH, PRL, E2 and T levels) in each group. No incidence of ovarian hyperstimulation syndrome and multiple pregnancies were reported. The serum FSH level in rhFSH (50) group was increased at the time of hCG injection (inj.) compared with control and rhFSH (37.5) groups (p<0.05). The CC group showed the highest E2 level at the time of hCG inj. (CC: 712±360pg/ml, rhFSH (50): 572±294, rhFSH (37.5): 371±169, control: 353±183, p<0.001). The number of follicles and E2 level at the time of hCG inj. were not significant differently among each group (CC: 1.4±0.7, rhFSH (50): 1.5±0.8, rhFSH (37.5): 1.1±0.3, and control: 1.1±0.3, p<0.001). PR was higher in rhFSH (50) group (7.6%: 6/79) compared with CC (3%: 3/100), rhFSH (37.5) (5.1%: 4/79) and control (3.4%: 2/59) groups.

CONCLUSION: This study showed that the patients treated with 37.5IU or 50IU rhFSH exhibited acceptable changes of serum hormone levels. The number of follicles and E2 level at the time of hCG inj. were not high in rhFSH groups, suggesting this may be the safer treatment for unexplained infertility. Moreover, PR in rhFSH groups, especially rhFSH (50) group was acceptable.

EMBRYO TRANSFER

P-263 Tuesday, October 21, 2014

ELECTIVE CRYOPRESERVATION OF ALL EMBRYOS AND SUBSEQUENT CRYOTHAW EMBRYO TRANSFER RESULTS IN BETTER OUTCOMES THAN FRESH EMBRYO TRANSFER IN SHARED EGG DONOR PROGRAM. A. S. Cambiaghi, B. F. Leao, A. V. Alvarez, P. B. Martins, P. F. Nascimento. Reproductive Medicine, IPGO, São Paulo, SP, Brazil.

OBJECTIVE: To compare if cryopreservation of all embryos and subsequent cryothaw embryo transfer (ET) results in better outcomes compared with fresh ET in shared egg donor program and recipients.

DESIGN: Open randomized controlled trial.

MATERIALS AND METHODS: From November 2012 to March 2013, 100 women from shared egg donor program that were submitted to oocyte retrieval were selected. The inclusion criteria: age between 21 and 34 years-old, number of retrieved oocytes between 10 and 20, normal karyotype, no signs of endometriosis, no severe male factor and normal ovarian reserve. Each women shared half of their oocytes with a recipient. In the day of oocyte retrieval, the women were allocated in two groups. In group one, all the embryos were cryopreserved in day 5 and 2 blastocysts transferred in a subsequent cycle. In group two, 2 blastocysts were transferred in the same cycle. The exclusion criterion was less than two blastocysts five days after oocyte retrieval. Recipients that received fresh embryos from both groups were included as a third group. All recipients transferred 2 blastocysts in day 5. The outcomes were clinical pregnancy rate, implantation rate, miscarriage rate and multiple pregnancy rate. The comparisons among the three groups were performed two by two using chi-square test.

RESULTS: Egg donors with fresh ET presented significantly lower clinical pregnancy rate (50%, n=47) than egg donors with frozen ET (72%, n=50) or recipients (70.1%, n=9; p=0.026 and 0.049, respectively). The implantation rate were also significantly lower in this group (28%) than frozen ET group (42%; p=0.042) and lower but not significantly than recipients group (39%; p=0.064). There was no differences comparing these outcomes between donors that cryopreserved embryos and recipients. No differences in miscarriage or multiple pregnancy rates was demonstrated among the groups. Three donors with fresh embryo transfer presented ovarian hyperstimulation syndrome.

CONCLUSION: In shared egg donors, cryopreservation of all embryos with cryothaw transfer in subsequent cycle improves clinical pregnancy.

ASRM Abstracts Vol. 102, No. 3, Supplement, September 2014
and implantation rate comparing to donors with fresh embryo transfer. In shared egg program, the policy of frozen all embryos seems to be a good choice.

P-264 Tuesday, October 21, 2014


OBJECTIVE: The objective of this study is to examine the clinical significance of blastocyst grade at post-thawing.

DESIGN: Retrospective case-control study.

MATERIALS AND METHODS: In this study, we collected data from the patients who underwent single frozen-thawed blastocyst transfer in hormone replacement cycle at our clinic. We included a total of 2425 cycles from January 2011 to December 2012. We evaluated blastocyst grade before vitrification and after thawing according to Gardner’s classification and examined clinical pregnancy rate (CPR) and delivery rate (DR).

RESULTS: The results are summarized in Table 1.

<table>
<thead>
<tr>
<th>TABLE 1.</th>
<th>3-hr post thaw blastocyst grade</th>
<th>2 h</th>
<th>166(72.5)</th>
<th>37.2</th>
<th>30</th>
<th>18.1</th>
<th>18.1</th>
<th>10.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade1 n=229</td>
<td>1</td>
<td>63(27.5)</td>
<td>37.4</td>
<td>18</td>
<td>28.6</td>
<td>12</td>
<td>19.0</td>
<td></td>
</tr>
<tr>
<td>Grade2 n=576</td>
<td>&gt;1</td>
<td>200(30.3)</td>
<td>37.0</td>
<td>40</td>
<td>20.0</td>
<td>23</td>
<td>11.5</td>
<td></td>
</tr>
<tr>
<td>Grade3 n=760</td>
<td>2</td>
<td>264(45.8)</td>
<td>36.6</td>
<td>69</td>
<td>26.1</td>
<td>45</td>
<td>17.0</td>
<td></td>
</tr>
<tr>
<td>Grade4 n=860</td>
<td>3</td>
<td>112(19.5)</td>
<td>37.1</td>
<td>53</td>
<td>47.3</td>
<td>40</td>
<td>35.7</td>
<td></td>
</tr>
<tr>
<td>Grade5 n=940</td>
<td>&gt;3</td>
<td>339(44.6)</td>
<td>36.5</td>
<td>118</td>
<td>34.8</td>
<td>29</td>
<td>23.3</td>
<td></td>
</tr>
<tr>
<td>No. of blastocysts(% patient(y)</td>
<td>3</td>
<td>240(31.6)</td>
<td>36.3</td>
<td>108</td>
<td>45.0</td>
<td>75</td>
<td>31.3</td>
<td></td>
</tr>
<tr>
<td>No. of clinical pregnancies(transfer rate(%</td>
<td>&gt;3</td>
<td>181(23.8)</td>
<td>35.9</td>
<td>107</td>
<td>59.1</td>
<td>86</td>
<td>47.5</td>
<td></td>
</tr>
<tr>
<td>Grade6 n=1040</td>
<td>&lt;4</td>
<td>406(46.7)</td>
<td>35.3</td>
<td>179</td>
<td>44.1</td>
<td>143</td>
<td>35.2</td>
<td></td>
</tr>
<tr>
<td>Grade7 n=1200</td>
<td>4</td>
<td>264(30.9)</td>
<td>35.5</td>
<td>163</td>
<td>61.3</td>
<td>129</td>
<td>48.5</td>
<td></td>
</tr>
<tr>
<td>Grade8 n=1300</td>
<td>&gt;4</td>
<td>200(18.9)</td>
<td>34.9</td>
<td>107</td>
<td>56.9</td>
<td>90</td>
<td>47.9</td>
<td></td>
</tr>
</tbody>
</table>

As a total, 38.6% of the blastocysts revealed equivalent grade both at pre-freezing and post-thawing, 39.0% of the blastocysts decreased their grade and 22.4% of blastocysts increased their quality at post-thawing observation. In the group of blastocysts those increased their morphological grade after thawing, CPR and DR tended to be high when it compares to the group of blastocysts those decreased their grade after thawing. This tendency was common irrespective of the grade at pre-freezing.

CONCLUSION: The degree of post-thaw recovery of blastocyst could be one of the predictive factors for CPR and DR in frozen-thawed blastocyst transfer. Viable blastocysts may recover their quality during freeze-thaw process.

P-265 Tuesday, October 21, 2014


OBJECTIVE: To investigate compared with 2 h and overnight (16 h) the association of culture instruction time for embryo transfer after frozen-thawed with day3 and 5 embryos.

DESIGN: Retrospective study on embryo from IVF/ICSI cycles.

MATERIALS AND METHODS: A total of 164 infertility patients were included all the patients who received frozen-thawed single blastocyst transfer (n=374) at our center between Dec 2010 and May 2013. The morphological evaluation of blastocysts was performed according to the Gardner and Schoolcraft grading system and included three different parameters: expansion and hatching (EH) stage, ICM grade and TE grade. For blastocysts with grade of 3BB or better were frozen. The mean age of patients was not significantly different between the groups (ICM grade A group vs. ICM grade B group, 31.9 vs. 32.3 years and TE grade A group vs. TE grade B group, 31.6 vs. 32.4 years). AR and LBR were compared between the groups using chi square Test.

RESULTS: The AR of frozen-thawed SBT cycles was 23.4% (26/111) vs. 24.0% (18/75) for ICM grade A vs. B groups (P=0.927) and 21.7% (20/92) vs. 25.5% (24/94) for TE grade A vs. B groups (P=0.542), neither of which did not differ significantly. The corresponding LBR of frozen-thawed SBT cycles was 47.0% (85/181) vs. 29.5% (57/193) for ICM grade A vs. B groups (P<0.001) and 51.4% (72/140) vs. 29.9% (72/234) for TE grade A vs. B groups (P<0.001), both of which were statistically significant.

CONCLUSION: Our study demonstrated that higher live birth rate was obtained after transfer with AA grade blastocyst compared with BB grade evaluations, and the numbers of gestational sacs with cardiac activity were determined 4-5 weeks after embryos transfer.

RESULTS: Listed below.

<table>
<thead>
<tr>
<th>TABLE 1.</th>
<th>Day 3</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultured time</td>
<td>2 h</td>
<td>O/N</td>
</tr>
<tr>
<td>Average age</td>
<td>34.3</td>
<td>33</td>
</tr>
<tr>
<td>of patients(y)</td>
<td>&lt;35 patients</td>
<td>28</td>
</tr>
<tr>
<td>&gt;35 patients</td>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td>Factor</td>
<td>Female</td>
<td>7</td>
</tr>
<tr>
<td>Male</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>Combined</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Unknown</td>
<td>19</td>
<td>17</td>
</tr>
<tr>
<td>No. of embryos</td>
<td>114</td>
<td>90</td>
</tr>
<tr>
<td>served and transferred</td>
<td>40</td>
<td>56</td>
</tr>
<tr>
<td>Mean blastocysts transferred</td>
<td>2.85 ± 0.60</td>
<td>2.72 ± 0.45</td>
</tr>
<tr>
<td>Clinical pregnancy / transfer cycles (%)</td>
<td>22.5</td>
<td>46.4</td>
</tr>
<tr>
<td>Implantation Rates / embryo transfer (%)</td>
<td>14.9</td>
<td>45.5</td>
</tr>
<tr>
<td>Multiple (n)</td>
<td>2</td>
<td>7</td>
</tr>
</tbody>
</table>

O/N is overnight as 16h culture. * Values are significant at P < 0.05.

CONCLUSION: Cryopreservation embryo transfer has greatly increased the chance of cumulative pregnancy as a useful tool in assisted reproductive technology. This study has the effect of extended culture on frozen-thawed embryo transfer. The findings suggest that 16 h culture as an overnight is not harmful to clinical outcome in this study.

P-266 Tuesday, October 21, 2014

BLASTOCYST MORPHOLOGY GRADE DOES NOT INFLUENCE THE ABORTION RATE OF FROZEN-THAWED SINGLE BLASTOCYST TRANSFER. L. Jia, C.-M. Yue, C. Fang. Sixth Affiliated Hospital of Sun Yan-Sen University, Guangzhou, Guangdong, China.

OBJECTIVE: To estimate the effect of inner cell mass (ICM) morphology grade and trophoectoderm (TE) morphology grade on abortion and live birth in frozen-thawed single blastocyst transfers (SBT).

DESIGN: Retrospective study. Outcome was evaluated by abortion rate (AR), defined as the number of abortion per clinical pregnancy and live birth rate(LBR), defined as the live birth per embryo transfer(ET).

MATERIALS AND METHODS: This study included all the patients who received frozen-thawed single blastocyst transfer (n=374) at our center between Dec 2010 and May 2013. The morphological evaluation of blastocyst was performed according to Gardner’s and Schoolcraft grading system and included three different parameters: expansion and hatching (EH) stage, ICM grade and TE grade. For blastocysts with grade of 3BB or better were frozen. The mean age of patients was not significantly different between the groups (ICM grade A group vs. ICM grade B group, 31.9 vs. 32.3 years and TE grade A group vs. TE grade B group, 31.6 vs. 32.4 years). AR and LBR were compared between the groups using chi square Test.

RESULTS: The AR of frozen-thawed SBT cycles was 23.4% (26/111) vs. 24.0% (18/75) for ICM grade A vs. B groups (P=0.927) and 21.7% (20/92) vs. 25.5% (24/94) for TE grade A vs. B groups (P=0.542), neither of which did not differ significantly. The corresponding LBR of frozen-thawed SBT cycles was 47.0% (85/181) vs. 29.5% (57/193) for ICM grade A vs. B groups (P<0.001) and 51.4% (72/140) vs. 29.9% (72/234) for TE grade A vs. B groups (P<0.001), both of which were statistically significant.

CONCLUSION: Our study demonstrated that higher live birth rate was obtained after transfer with AA grade blastocyst compared with BB grade.
blastocyst. However, the abortion rate did not differ with different morphology grade, indicating the morphology evaluation of the blastocyst would only predict the implantation potential of the blastocyst but not the ongoing pregnancy.

P-267 Tuesday, October 21, 2014

CLINICAL OUTCOMES OF DAY 7 VITRIFIED-THA WED BLASTOCYST TRANSFER IN PATIENTS WITH SLOW DEVELOPMENT OF EMBRYOS. W. Chai, Q. Chen, Z. Yan, S. Xue, Q. Lyu, Y. Kuang. Department of Assisted Reproduction, Shanghai, China.

OBJECTIVE: We analyzed the pregnancy outcomes of transfer of human day 7 blastocyst vitrified-thawed in an attempt to confirm the value of such transfer.

DESIGN: This article reports the pregnancy outcome in 94 cycles of 154 vitrified day 7 blastocysts for ART between January 2007 and May 2012 in department of Assisted Reproduction, the Ninth People’s Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China.

MATERIALS AND METHODS: Subjects: The pregnancy outcomes in 94 cycles of 154 vitrified day 7 blastocysts for ART were analyzed retrospectively between January 2007 and May 2012.

Blastocyst grading system: The morphological evaluation of blastocysts was performed according to the Gardner grading system (Gardner and Schoolkraft, 1999) and included three different parameters: EH stage, ICM grade and TE grade.

Blastocyst vitrification: Vitrification of blastocysts was performed according to the Cryotop method described by Kuwayama et al. Warming of blastocyst: The blastocyst to be thawed was quickly moved to the thawing liquid 1 for 1 min. The blastocyst was then gently moved to the thawing liquid 2 for 3 min, then moved to the thawing liquid 3 and 4 for 6 min. The embryo was moved to equilibrated culture drops for continuous washing for 5-10 drops and cultured continuously for 2-3 h for transfer.

Blastocyst survival: The thawed blastocysts were cultured for 1-3 h to observe expansion of the cavity. Full or partial expansion of the blastocystic cavity was considered surviving and used for transfer.

RESULTS: A total of 153 blastocysts survived with thawing with a survival rate of 99.4%. Transfer of the 151 blastocysts resulted in clinical pregnancy in 25 patients, with a clinical pregnancy rate of 26.6% and an implantation rate of 17.9%. Of them, eight patients delivered healthy babies successfully.

CONCLUSION: Transfer of vitrified day 7 blastocysts is especially valuable for patients who failed to get ideal embryos after repeated IVF cycles of day 3, day 5 and days 3 or those whose embryos have a slow developmental potential. Transfer of day 7 vitrified-thawed blastocyst has a clinically important potential.

Supported by: The study was Supported by National Natural Science Foundation of China (No. 81270749 and No. 31101070).

P-269 Tuesday, October 21, 2014

DOES EXTENDED CULTURE REDUCE EMBRYO EFFICIENCY? A COMPARISON OF OUTCOMES FOR WOMEN WITH 6 ZYGOTES UNDERGOING DAY 3 AND DAY 5 EMBRYO TRANSFER. R. Mejia, E. H. Duran, A. Sparks, B. Van Voorhis. Obstetrics and Gynecology, University of Iowa, Iowa City, IA.

OBJECTIVE: To assess clinical outcomes and embryo efficiency between day 3 and day 5 embryo transfer (ET) in women with 6 zygotes to culture.

DESIGN: Retrospective cohort analysis.

MATERIALS AND METHODS: We analyzed all women 36 years or younger who had 6 zygotes following IVF at our program between 2003 and 2013. Per policy, in women with 6 embryos to culture, fresh ETs were performed on culture day 3 before 2009 (day 3 ET group) and on culture day 5 after 2009 (day 5 ET group). In both groups, non-transferred embryos were cultured to day 5 or 6 and cryopreserved if they reached the blastocyst stage. Main outcome measures were fresh and cumulative live birth rate, and embryo efficiency. Cumulative live birth rate was censored for delivery and calculated on a per patient basis. Embryo efficiency was calculated based on proportion of live born babies during both fresh and frozen cycles per total number of embryos. Number of embryos transferred, implantation rate and cumulative twin delivery rate were also analyzed. Chi-square, Fisher’s exact and Mann-Whitney U tests were used for statistical analysis.

RESULTS: A total of 185 patients were included, 95 in day 3 ET group and 90 in day 5 ET group. From this total, 23 women have remaining cryopreserved embryos. 3 women in day 3 group with 5 embryos cryopreserved and 20 women in day 5 group with 40 embryos cryopreserved. Results are summarized in the table.

TABLE ID.

Clinical pregnancy outcomes of vitrified blastocysts

<table>
<thead>
<tr>
<th>Number of HCG-positive pregnancies</th>
<th>34 (36.2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(% per transferred cycle)</td>
<td></td>
</tr>
<tr>
<td>Number of clinical pregnancies</td>
<td>25 (26.6)</td>
</tr>
<tr>
<td>(% per transferred cycles)</td>
<td></td>
</tr>
<tr>
<td>Number of implanted embryos</td>
<td>27 (17.9)</td>
</tr>
<tr>
<td>(% per transferred blastocyst)</td>
<td></td>
</tr>
<tr>
<td>Number of miscarriages</td>
<td>3 (12.0)</td>
</tr>
<tr>
<td>(% per clinical pregnancy)</td>
<td></td>
</tr>
<tr>
<td>Number of ectopic pregnancies</td>
<td>0</td>
</tr>
<tr>
<td>(% per clinical pregnancy)</td>
<td></td>
</tr>
<tr>
<td>Number of ongoing pregnancies</td>
<td>14 (56)</td>
</tr>
<tr>
<td>(% per clinical pregnancy)</td>
<td></td>
</tr>
</tbody>
</table>

CONCLUSION: Transfer of vitrified day 7 blastocysts is especially valuable for patients who failed to get ideal embryos after repeated IVF cycles of day 3, day 5 and days 3 or those whose embryos have a slow developmental potential. Transfer of day 7 vitrified-thawed blastocyst has a clinically important potential.

P-268 Tuesday, October 21, 2014


OBJECTIVE: To investigate the efficacy of the transfer of blastocysts cultured from vitrified-thawed cleavage stage embryos in patients with low quality frozen embryo compared with vitrified-thawed cleavage stage embryo transfer.

DESIGN: Retrospective compared analysis.

MATERIALS AND METHODS: This study included 204 vitrified-thawed embryo transfer cycles (age, < 43) with poor quality cleavage stage frozen embryo between February 2012 and January 2014. Patients with transfer of thawed embryo were divided into two groups; the transfer of vitrified thawed cleavage stage embryo on day 3 (CS, n = 168) and the transfer of blastocysts derived from vitrified-thawed cleavage stage embryos on day 5, (B-CS, n = 36). Main clinical outcome, survival rate, clinical and ongoing pregnancy rate and implantation rate were compared.

RESULTS: Between CS and B-CS, patient age (35.5 ± 3.5 yrs vs. 34.4 ± 3.1yrs), number of thawed cleavage stage embryos (4.8 ± 1.2 vs. 5.0 ± 1.0) and survival rate (89.0%, 720/809 vs. 89.0%, 162/182) showed no statistical differences. Clinical pregnancy (CS, 63/168 (37.5%) vs B-CS, 20/36, 55.6%, p < 0.05) and implantation rate per transfer (CS, 77/501 (15.4%) vs B-CS, 26/70 (37.1%), p < 0.00001) were significantly higher in transfer of blastocysts developed from vitrified-thawed cleavage stage. Higher ongoing pregnancy (CS, 34/168 vs. 16/36, P = 0.0136), Higher implantation rate per number of thawed embryo was presented in B-CS (9.5%, 77/809 vs. 14.3%, 26/182; P = 0.0569).

CONCLUSION: This study showed that the clinical pregnancy and implantation rate were significantly increased in the transfer of blastocysts, cultured and developed from vitrified-thawed cleavage stage embryos in patients with poor quality frozen cleavage stage embryos.
Numbers represent mean ± standard deviation or percentage. Clinical pregnancy is defined by ultrasonographic demonstration of fetal cardiac activity. Implantation rate is defined as total gestational sacs divided by total embryos transferred in each category.

CONCLUSION: These data suggest that extended embryo culture to day 5 does not adversely affect embryo efficiency in a select group of women with only 6 zygotes and does not pose a detrimental effect on cumulative live birth rate. Additionally, a significant decrease in cumulative twin delivery rate is achieved by this strategy if single embryo transfer is used frequently for day 5 ETs. This may translate to a safer IVF practice strategy in prevention of multiple pregnancies, including twins.

**P-270 Tuesday, October 21, 2014**

**CLINICAL AND ONGOING PREGNANCY RATES ARE IMPROVED BY THE ADDITION OF LETROZOLE IN THE HORMONE REPLACEMENT CYCLES UNDERGOING THE FROZEN-THAWED SINGLE BLASTOCYST TRANSFER.** K. Matsuura, 2,3 M. Ikegami, 2 Y. Nagase, 2 T. Wada, 2 T. Matsuura, 3,4 ACT Tower Clinic, Hamamatsu, Shizuoka, Japan; 3The Center for Reproductive and Development, ACT Tower Clinic, Hamamatsu, Shizuoka, Japan; 4OneART Laboratories Japan, Hamamatsu, Shizuoka, Japan.

OBJECTIVE: The objective of this study was to examine the effect of letrozole on clinical and ongoing pregnancy rates in the hormone replacement cycles undergoing the frozen-thawed single blastocyst transfer.

DESIGN: A prospective randomized controlled study in a private ART center.

MATERIALS AND METHODS: 102 infertile women under 42 years of age were treated with hormone replacement cycle undergoing the frozen-thawed single blastocyst transfer. Group A (n=55) : The estradiol supplementation was initiated on day 2 from the menstruation cycle, thereafter the dydrogesterone was added. The frozen-thawed single blastocyst was transferred to uterine cavity on day 20. Group B (n=47) : Taking the estradiol initiated with letrozole (2.5mg), 3 consecutive days together. Both groups followed the same protocol. The hormone levels serum were measured by the enzyme immunoassay. Blastocysts were cryopreserved using a vitrification methods. All cases were transferred single blastocyst. In pregnant patient, estradiol and progesterone was continued until placental steroidogenesis was done. Statistical analysis was performed using the Chi square test and Student’s T test. A P-value <0.05 was considered statistically significant.

RESULTS: There were no significant difference between the two groups in the age (Group A 35.2 ± 4.1 vs Group B 35.0 ± 3.8). Group A was 55 cases, and Group B was 47 cases that the blastocyst was transferred. The clinical pregnancy rate was significantly higher in the Group B with Group A (52.0% vs 27.2%, P<0.05). The ongoing pregnancy rate was also significantly higher in the Group B with Group A (38.5% vs 20.0%, P<0.05) . All clinical and ongoing pregnancies were single.

CONCLUSION: Our findings suggest that the letrozole improved the clinical and ongoing pregnancy rates in the hormone replacement cycles undergoing the frozen-thawed single blastocyst transfer. Additional studies are needed to evaluate the role of letrozole in endometrial receptivity and subsequent pregnancy.

**P-271 Tuesday, October 21, 2014**

**DECIDING BETWEEN SINGLE AND DOUBLE BLASTOCYST TRANSFER IN THE GOOD-PREDISSION PATIENT: A MODEL OF MATERNAL PREFERENCES TOWARD FETAL REDUCTION AND ITS EFFECT ON PREGNANCY AND NEONATAL OUTCOMES.** R. A. Pilliod, 2,3 D. J. Kaser, 2 A. B. Caughey, 2 Obstetrics & Gynecology, Brigham & Women’s Hospital, Boston, MA; 3Reproductive Endocrinology & Infertility, Brigham & Women’s Hospital, Boston, MA; 4Obstetrics & Gynecology, Oregon Health & Science University, Portland, OR.

OBJECTIVE: To compare maternal preferences and neonatal outcomes for single vs. double embryo transfer (SET vs. DET) with or without fetal reduction in a hypothetical cohort of women.

DESIGN: Decision analytic model.

MATERIALS AND METHODS: Published data were used to create a decision analytic model comparing pregnancy and neonatal outcomes after one cycle of fresh SET vs. DET among women who were willing to consider fetal reduction of a twin gestation and those who were not. In our model we excluded higher-order multiple pregnancies. We assumed a population of healthy women ≤37 years of age with >1 good quality blastocyst available for day 5 transfer. Our primary outcome was maternal quality-adjusted life years (QALYs) per transfer, and our secondary outcomes were preivable loss, preterm or term delivery, all live births, moderate and severe disabilities and neonatal mortality. Sensitivity analyses were performed for maternal preferences regarding these outcomes.

RESULTS: DET with reduction was the dominant strategy to optimize maternal QALYs (Table 1). Robust sensitivity analysis demonstrated that our model was exquisitely sensitive to clinical pregnancy rate: with a rate of at least 65.4% (a 2.4% increase from the baseline rate), SET becomes favorable in terms of optimizing maternal QALYs. Rates of neonatal morbidity and disability are highest among women undergoing DET without reduction; surprisingly, overall QALYs were still higher in this group relative to SET due to total live birth rate. Overall neonatal outcomes were optimized with SET which was not driven by a woman’s willingness to reduce.

**TABLE 1. Maternal & Neonatal Outcomes by Transfer Strategy & Willingness to Reduce**

<table>
<thead>
<tr>
<th>Strategy</th>
<th>QALYs Birth/100 Transfers</th>
<th>Preterm Birth/100 Transfers</th>
</tr>
</thead>
<tbody>
<tr>
<td>SET reduce</td>
<td>24.97</td>
<td>49</td>
</tr>
<tr>
<td>DET no reduce</td>
<td>24.97</td>
<td>49</td>
</tr>
<tr>
<td>DET reduce</td>
<td>25.02</td>
<td>51</td>
</tr>
<tr>
<td>DET no reduce</td>
<td>24.99</td>
<td>56</td>
</tr>
</tbody>
</table>

CONCLUSION: Maternal QALYs are optimized with a strategy of DET with reduction, followed by DET without reduction, despite excess neonatal morbidity and mortality compared to SET. Ultimately, the goal should be improving SET clinical pregnancy rates such that maternal QALYs and neonatal outcomes are both optimized. Until that time this model may be useful in counseling patients on the day of transfer.

**P-272 Tuesday, October 21, 2014**


OBJECTIVE: Despite recommendations to consider single embryo transfer to reduce the multiple pregnancy rate, single blastocyst transfer is used in less than 10% of IVF cycles in the United States. Following universal funding for assisted reproductive treatment (ART) in Quebec, Canada in 2010, in our center, blastocyst culture and single embryo transfer has been performed for cycles where there is more than one good quality embryos on day 3 after egg retrieval and have done more than 50% of IVF cycles. The purpose of our study was to evaluate the clinical outcomes of single blastocyst transfer depending on age of patients.

DESIGN: Retrospective analysis.

MATERIALS AND METHODS: All fresh blastocyst stage embryo transfers (ET) from August 2010- December 2013 among women up to 43 years
old were analyzed. Pregnancy outcomes in 2,440 single-blastocyst transfers were analyzed without any omissions or exclusions depending on cycles with/without freezable embryos (SBT/eSBT) and women’s age. Clinical pregnancy and implantation rates were compared. Chi Square test or Fisher Exact Test was used for statistical analysis of categorical variables. P < 0.05 was considered statistically significant.

RESULTS: Compared to SBT, elective SBT (eSBT) was associated with higher rates of implantation and pregnancy (P=0.0001). There were similar clinical outcomes between women < 35 and 35-37 years old in SBT and eSBT. In both SBT and eSBT, groups with women under 38 years old had higher rates of clinical pregnancy and implantation compared to the women who were 38-43 years old.

<table>
<thead>
<tr>
<th></th>
<th>SBT</th>
<th>eSBT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of patient</td>
<td>&lt; 38</td>
<td>&lt; 38</td>
</tr>
<tr>
<td># ET</td>
<td>566</td>
<td>183</td>
</tr>
<tr>
<td>β-hCG (+)</td>
<td>46.5%</td>
<td>34.4%</td>
</tr>
<tr>
<td>Clinical PR</td>
<td>39.8%</td>
<td>24.6%</td>
</tr>
<tr>
<td>Implantation</td>
<td>40.1%</td>
<td>24.6%</td>
</tr>
</tbody>
</table>

\( a vs. b: P<0.01, c vs. d: P<0.01 \)

CONCLUSION: Cycles with supernumerary vitrified blastocysts (eSBT) correlated positively with the clinical outcomes in single-blastocyst transfers. Women age under 38 years old who have supernumerary blastocysts are predictive of successful clinical outcomes for single-blastocyst transfer.

P-273 Tuesday, October 21, 2014

TRAINING OF REPRODUCTIVE ENDOCRINOLOGY AND INFERTILITY FELLOWS FOR EMBRYO TRANSFER DID NOT RESULT IN A DECREMENT IN PREGNANCY. S. M. Zarek, a P. R. Brezina, a S. L. Mumford, a T. C. Plowden, a C. C. Coddington, a J. H. Segars. a NIH, Bethesda, MD; *Fertility Associates of Memphis, Memphis, TN; #Mayo Clinic, Rochester, MN.

OBJECTIVE: Although the majority of fellows report that adequate training in embryo transfer technique is an important component of fellowship, the question of whether skill acquisition can be attained without a reduction in pregnancy outcome remains unclear. The objective was to assess if embryo transfers performed by fellows demonstrated a pregnancy rate comparable to staff physicians.

DESIGN: Retrospective analysis.

MATERIALS AND METHODS: The study group was defined as all fresh and cryopreserved/thawed embryo transfers from an academic assisted reproductive technology center from 2012-2013 by fellows and staff. Fellows underwent a training program of thirty mock embryo transfers under staff supervision for instruction in the procedure of embryo transfer. All transfers were performed with ultrasound guidance with a soft transfer catheter (Cook) using the mock “afterload method” as described (Neithardt et al. 2005). Fellows initially performed transfer of cleavage stage, or poor grade embryos to confirm the absence of blood and only after proficiency was extension of blastocyst culture does not appear to increase the risk of monozygotic twinning. The deliberate extension of blastocyst culture does not appear to increase the risk of monozygotic twinning.

CONCLUSION: Fellow performed embryo transfers demonstrated similar pregnancy outcomes to staff performed transfers after controlling for potential confounders. Using this method of instruction, similar pregnancy rates were observed between trainees with fewer than, or greater than, 25 transfers.

Supported by: Intramural Research, PRAE, NICHD, NIH.

P-274 Tuesday, October 21, 2014

PREDETERMINED DAY 6 EMBRYO TRANSFER DOES NOT DECREASE CLINICAL PREGNANCY RATES. J. D. McCarthy, K. Pomeroy, M. Bustillo, I. Collazo, J. Ricard, M. Johnson, J. Eisermann. South Florida Institute for Reproductive Medicine, Miami, FL.

OBJECTIVE: To compare clinical pregnancy rates when embryo transfer is determined by embryo development versus a predetermined day 6 transfer.

DESIGN: Retrospective clinical study.

MATERIALS AND METHODS: Fresh blastocyst transfers performed at a private ART center between January 2009 and December 2013 were categorized as follows:

- Day 5 (D5): women were transferred 5 days after oocyte retrieval if they had expanded blastocysts on that day.
- Day 6 (D6): women were transferred 6 days after oocyte retrieval because they did not have expanded blastocysts on D5.
- Predetermined Day 6 (pD6): women were transferred 6 days after oocyte retrieval regardless of embryo development on D5. The predetermination was made at the start of ovarian stimulation and based on physician and/or patient availability.

Women undergoing PGD/PGS were excluded from the analysis. Patient characteristics were compared using ANOVA. Clinical pregnancy, implantation and monzygotic twinning rates were compared with chi-square analysis.

RESULTS: A total of 1717 blastocyst transfers were included in the analysis. There were 1268 transfers in the D5 group, 388 transfers in the D6 group and 61 transfers in the pD6 group. Mean age, number of oocytes retrieved, and number of blastocysts transferred did not statistically differ between the groups. Women whose embryo transfer date was arbitrarily determined at stimulation start (pD6) had similar clinical pregnancy rates to patients transferred on D5 (60.7% vs 53.9%, NS). Both D5 and pD6 patients had clinical pregnancy rates that were significantly higher than D6 patients (43.6%, p<0.001 and p<0.01, respectively). Implantation rates were also similar with pD6 patients having an implantation rate of 46.1%, compared with 40.6% in D5 patients (NS). Both D5 and pD6 patients had higher implantation rates than D6 patients (31.1%, p<0.0001 and p<0.01 respectively). Rates of monzygotic twinning was similar between groups.

CONCLUSION: Previous studies have suggested that when embryo transfer date is determined by embryo development, women receiving D6 transfers have lower pregnancy rates than those receiving D5 transfers. Our data confirms this observation, however, women undergoing predetermined D6 embryo transfer have similar clinical pregnancy rates compared with those having a transfer as soon as expanded blastocysts are available, allowing for more predictable scheduling of embryo transfers. The deliberate extension of blastocyst culture does not appear to increase the risk of monzygotic twinning.
OBJECTIVE: To determine if a prior Cesarean delivery (CD) makes embryo transfer more difficult and impacts pregnancy rate.

DESIGN: Prospective cohort study.

MATERIALS AND METHODS: Patients with a history of at least one delivery after 20 weeks gestation were eligible for the study. The patients were grouped by exposure to CD. They were approached for consent while waiting for their transvaginal oocyte retrieval (TVOR). Patients who consented to the study had the timing of their embryo transfer recorded. The total time of the embryo transfer included the following: mock transfer, embryo transfer, evaluating the transfer catheter for retained embryos, and any time required for re-transfer of retained embryos. The presence of blood included both microscopic and gross blood. Pregnancy was defined as having a positive serum hCG 14 days after TVOR. Frequency data was analyzed with Fisher’s Exact test. Data that did not satisfy the Shapiro test for normality was analyzed with the Mann-Whitney U test.

RESULTS: 195 patients met the inclusion criteria and had properly recorded embryo transfer times. The results are summarized below:

<table>
<thead>
<tr>
<th>Vaginal Deliveries Only</th>
<th>History of CD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>109</td>
<td>86</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>35.3±4.2</td>
<td>35.7±4.3</td>
</tr>
<tr>
<td>Endometrial thickness</td>
<td>11.2±2.4</td>
<td>11.4±2.4</td>
</tr>
<tr>
<td>at hCG trigger (mm)</td>
<td>1.95±0.74</td>
<td>1.95±0.77</td>
</tr>
<tr>
<td>Embryos transferred</td>
<td>9/109 (8%)</td>
<td>18/85 (21%)</td>
</tr>
<tr>
<td>Mucous on catheter</td>
<td>29/109 (27%)</td>
<td>39/85 (46%)</td>
</tr>
<tr>
<td>Blood on catheter</td>
<td>9/109 (8%)</td>
<td>18/85 (21%)</td>
</tr>
<tr>
<td>Transfer time (sec)</td>
<td>157 [74-750]</td>
<td>187 [76-945]</td>
</tr>
<tr>
<td>Pregnant</td>
<td>59/107 (55%)</td>
<td>45/84 (54%)</td>
</tr>
</tbody>
</table>

Note: Values are listed as mean±standard deviation and median [range]

CONCLUSION: Embryo transfers performed on patients who have a history of CD take 30 seconds longer. They are also more likely to have blood or mucous on the catheter. However, pregnancy rates were not different between the two groups despite the more difficult transfers in the CD group.

Supported by: This work was supported, in part, by the Program in Reproductive and Adult Endocrinology, NICHD, NIH, Bethesda, MD.

P-277 Tuesday, October 21, 2014

PREIMPLANTATION GENETIC TESTING WITH ARRAY CGH AND THE TRANSFER OF EUPLOID EMBRYOS DOES NOT DECREASE THE RATE OF BIOCHEMICAL PREGNANCY AFTER IVF. S. Ghazal, a W. G. Kears, a,b K. J. Tobler, b M. R. Maduro, a P. Patrizio,a,b Obstetrics, Gynecology, & Reproductive Sciences, Yale School of Medicine, New Haven, CT; bGynecology and Obstetrics, The Johns Hopkins Medical Institutions, Baltimore, MD; a Center for Preimplantation Genetics, Laboratory Corporation of America, Rockville, MD.

OBJECTIVE: Biochemical pregnancies are still a mystery and are often attributed to chromosomal abnormalities even though there are no objective data to support this contention. The aim of this study was to determine whether the transfer of euploid embryos affects the rate of biochemical pregnancies compared to unselected patients undergoing in vitro fertilization (IVF).

DESIGN: Retrospective chart review.

MATERIALS AND METHODS: We identified 65 patients from a single academic IVF center between December 1, 2012 and December 1, 2013 who underwent 69 IVF cycles with fresh transfer of euploid embryos following comparative genomic hybridization (aCGH) microarray preimplantation genetic screening (PGS) on trophectoderm cells. A biochemical pregnancy was defined as a serum positive h-hCG that never reaches a level that allows visualization of a gestational sac by ultrasound. As controls, we reviewed 322 unselected fresh IVF cycles during the same time period.

RESULTS: Of the 69 IVF cycles with the transfer of euploid embryos after PGS with aCGH, 24 (34.8%) had a negative pregnancy test, 7 (10.2%) experienced biochemical pregnancies, 5 (7.2%) had missed abortions, and 33 (47.8%) had delivered or had ongoing pregnancies. In the control group of 322 unselected IVF cycles during the same time period, 29 (9.0%) resulted in a biochemical pregnancy.

CONCLUSION: Despite the transfer of aCGH-screened euploid blastocysts, the rate of biochemical pregnancy is not improved compared to the background rate of biochemical pregnancy for patients undergoing IVF. This suggests that biochemical pregnancies may represent some genetic defect in the embryo leading to early developmental arrest or may be due to factors interfering with the early implantation. Biochemical
pregnancies after transfer of euploid embryos by aCGH should prompt clinicians to investigate possible uterine factors that may impact implantation, embryo transfer techniques, and further research on genetic (non-chromosomal) abnormalities responsible for early embryo development.

**Supported by:** Departmental

---

**P-278 Tuesday, October 21, 2014**

**THE USEFULNESS OF ULTRASOUND IN PREDICTING PREGNANCY RATE IN AN OOCYTE DONATION PROGRAM.** M. G. Yuri, A. Valcarcel, E. T. Young, P. J. Buzzi, L. V. Sicaro, A. E. Kenny. Sonography, Biology, Gynecology and Fertility Department, IFER, CABA, Buenos Aires, Argentina.

**OBJECTIVE:** One of the limiting steps in ART success, is the relatively low embryo implantation rate. Among important factors in the implantation process, embryo quality and endometrial adequacy are key factors. Many ART programs have correlated endometrial morphology and width, and the uterine perfusion as measured by transvaginal ultrasound and color Doppler (TV-US) with pregnancy. In previous studies differences ultrasound parameters of endometrial receptivity in patients with ovarian stimulation were evaluated with good correlation between them. Which ones are the better predictors of optimal endometrial receptivity in a oocyte donor cycle? The aim of this study was to evaluate four good sonographic parameters as a useful predictor of endometrial receptivity in terms of pregnancy rate in an oocyte donation program.

**DESIGN:** Prospective, controlled, study.

**MATERIALS AND METHODS:** We evaluated sonographic parameters of uterine receptivity, measured on the day when Progesterone supplementation should begin. 78 consecutive patients whose stimulation endometrial cycle resulted in embryo transfer from fresh oocyte donor cycle, were included in this study. Four ultrasonographic parameters of good prognosis were considered as follow:

- Endometrial thickness ≥ 7mm, trilaminar endometrial pattern, uterine artery Pulsatility index (PI) ≤ 3 and presence of subendometrial vessels ≥ 4

Two groups were formed accordingly to the number of parameters founded (PF) Group I: 62 patients with ≥ 3 PF Group II: 16 patients with <3 PF Epidemiological data, age, number and quality of embryo transfer, Pregnancy rate and clinical pregnancy rate were evaluated between groups. Student and Fisher Test were used.

**RESULTS:** Epidemiological data between groups were comparable. Patients age was in Gr I 42.6±5.4, in Gr II 43.7±6.8 (pNS). The number of embryo transferred was 3.2 ± 0.7 in Group I and 3.1 ± 0.9 in Group II (pNS). Pregnancy rate 71% (44 / 62) in Gr I and 12.5% (2/16) in Gr II (p 0.0001). Clinic pregnancy rate 61.3% (38 / 62) in GrI and 12.5 % (2/16) in Gr II (p<0.0001) This data shows sensitivity 75.8%, specificity 43.75%, positive predictive value 70.9% and negative predictive value 87.5%.

**CONCLUSION:** When three or more parameters of good prognosis were present, a higher pregnancy rate was obtained. The sum of good prognostic sonographic criteria offers a useful tool for predicting success in oocyte donation cycles.

---

**P-279 Tuesday, October 21, 2014**


**OBJECTIVE:** Mechanical endometrial injury is proposed to induce and modulate expression of genes for factors required for improving implantation. Hysteroscopy also provides reassurance and opportunity for treatment of uterine pathology. However, with a lack of large sized RCTs, recommendation is based on meta-analysis, using non randomized or quasi random-ized studies, which have used non-matched controls. We sought to identify whether a normal hysteroscopy in the cycle preceding transfer (fresh/frozen) would have a beneficial impact on the implantation and clinical pregnancy rate after matching for variables known to affect pregnancy rates.

**DESIGN:** Case -controlled study.

**MATERIALS AND METHODS:** Case controlled study of women under-going hysteroscopy, during IVF treatment in the last 4 years in one clinician’s practice. All patients with no abnormality detected at hysteroscopy followed by transfer in the next cycle (n= 87) were matched with controls in a 3:1 ratio(no=263). Matching included age, cycle and treatment number, stimulation protocol, IVF/ICSI and Fresh/Frozen embrios transferred, and day of transfer. Statistical analysis was performed with Fischer’s exact test.

**RESULTS:** Mean age in the hysteroscopy and control group was comparable: 36.2 y, and 36.3 years respectively. The only biochemical positive pregnancy rate per transfer in the intervention group was 5.9, in controls 7.1 (p<NS), pregnancy rate per transfer 28.7% in the intervention group versus 29.0%p< NS, stratification of fresh versus frozen was non-signifi-

---

**P-280 Tuesday, October 21, 2014**


**OBJECTIVE:** To investigate the relationship between the pregnancy rates and the medications used during and after an IVF cycle among patients with recurrent implantation failure (RIF).

**DESIGN:** A retrospective cohort study of 854 IVF cycles in 183 patients was conducted. It has included patients of less than 35 years old with at least three failed fresh IVF cycles.

**MATERIALS AND METHODS:** We have analyzed the relation between the probability of pregnancy and the following criteria: IVF protocol, types of gonadotropins, type of luteal support and IVF outcome.

**RESULTS:** Pregnancy rates were similar regardless of the protocol employed. Nevertheless, while comparing a small sub-group of 8 patients who conceived using follitropin beta (Puregon) for ovarian stimulation with 25 patients treated with follitropin alpha (Gonal F), a significantly increased pregnancy rate was noted in the follitropin beta group (22.2% vs. 9.8%, p=0.03). There was no difference in pregnancy rates between the groups of patients stimulated by recombinant or urinary gonadotropins, or combination of these medications. Combining estrogen and progesterone in the luteal support significantly increased pregnancy rates compared to progesterone only (16.1% vs. 10.4%, p=0.04). No differences were observed between the patients receiving vaginal versus intramuscular luteal support and there was no added value for the use of hCG for luteal support.

**CONCLUSION:** Combining estrogen and progesterone in the luteal support regime in cases of RIF, increases pregnancy rate. Moreover, when using recombinant agents for ovarian stimulation, follitropin beta resulted in better pregnancy rates as compared to tofollitropin alpha.
THE TP53 CODON 72 POLYMORPHISM (RS1042522) IN WOMEN IS NOT ASSOCIATED WITH PREGNANCY OUTCOMES AFTER ART. R. L. R. Barrafi,a,b C. G. Petersen,a,b L. D. Vagnini,a,b A. Renzi,a,b G. R. Oliveira-Pelegrin,a,b A. L. Mauri,a,b F. C. Massaro,a,b M. Cavagna,a,b J. B. A. Oliveira,a,b J. G. Franco, Jr.,a,b Center for Human Reproduction Prof. Franco Jr, Ribeirao Preto, Sao Paulo, Brazil; 4Paulista Center for Diagnosis Research and Training, Ribeirao Preto, Sao Paulo, Brazil; 5Women’s Health Reference Centre, Perola Byington Hospital, Sao Paulo, Brazil.

OBJECTIVE: The literature provides evidence that the genotype for the TP53 codon 72 polymorphism (encoding Arginine [Arg] or Proline [Pro]) in women is associated with repeated implantation failures after IVF/ICSI or recurrent miscarriages (the Arg allele is correlated with the best fertility prognosis). However, the data are not definitive, and the relationship between the TP53 polymorphism and clinical outcomes after IVF/ICSI requires additional evaluation. This study analyzed whether the TP53 codon 72 polymorphism in women can predict pregnancy outcomes in ART.

DESIGN: Prospective cohort.

MATERIALS AND METHODS: A total of 390 couples subjected to IVF/ICSI protocols were recruited. The women were genotyped for the TP53 polymorphism Arg/Arg (n=192), Arg/Pro (n=170) and Pro/Pro (n=28). DNA was extracted from peripheral blood samples taken from each participant, and the TP53 codon 72 single nucleotide polymorphism (SNP / rs1042522) (Arg/Pro) was genotyped using real-time PCR. Cumulative results, including fresh and frozen cycles, were analyzed.

RESULTS: Hardy-Weinberg genotype distributions of the entire sample indicated concordance between the observed and expected frequencies. No correlation was observed between the genotype for the TP53 codon 72 SNP Arg/Pro polymorphism in women and clinical outcomes after IVF/ICSI. Table 1 summarizes the results.

**Table 1. Results**

<table>
<thead>
<tr>
<th>WOMEN’S GENOTYPES</th>
<th>ARG/ARG</th>
<th>ARG/PRO</th>
<th>PRO/PRO</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women age(years)</td>
<td>35.1 ±4.2</td>
<td>35.8±4.4</td>
<td>36.3±4.2</td>
<td>0.18</td>
</tr>
<tr>
<td>Men age(years)</td>
<td>37.7±6.6</td>
<td>38.3±6.7</td>
<td>39.2±7.6</td>
<td>0.10</td>
</tr>
<tr>
<td>Transfer(n)</td>
<td>1.5±0.9</td>
<td>1.4±0.8</td>
<td>1.4±0.6</td>
<td>0.86</td>
</tr>
<tr>
<td>Total embryos transferred (fresh+frozen)(n)</td>
<td>3.0±2.1</td>
<td>3.1±1.9</td>
<td>2.6±1.6</td>
<td>0.15</td>
</tr>
<tr>
<td>Implantation rate</td>
<td>19.6%</td>
<td>18.6%</td>
<td>13.9%</td>
<td>0.49</td>
</tr>
<tr>
<td>Clinical pregnancy rate/patient</td>
<td>48.4%</td>
<td>45.3%</td>
<td>28.6%</td>
<td>0.14</td>
</tr>
<tr>
<td>Miscarriage rate</td>
<td>30.1%</td>
<td>24.7%</td>
<td>37.5%</td>
<td>0.29</td>
</tr>
<tr>
<td>Ongoing pregnancy rate/patient</td>
<td>33.8%</td>
<td>34.1%</td>
<td>17.9%</td>
<td>0.21</td>
</tr>
</tbody>
</table>

CONCLUSION: There appears to be no relationship between this TP53 codon 72 Arg/Pro (rs1042522) polymorphism and implantation or pregnancy rates after IVF/ICSI. The genotype for the TP53 codon 72 polymorphism in women may not be a useful susceptibility factor for the prediction of the chances of achieving pregnancy. However, increasing the number of analyzed cases will be important to provide more information about the potential use of this polymorphism.

MATERIALS AND METHODS: After established in-vitro model of embryo implantation by co-cultivating human blastocysts with human endometrial decidualization cell monolayers, β-catenin was detected by immunofluorescence analysis on endometrial cells at pre-, peri- and post-implantation stages. β-catenin,Wnt4,Fzr26 were detected by real time fluorescent quantitative PCR analysis on endometrial cells at pre-/peri- and post-implantation stages.

RESULTS: The blastocysts adhered to the decidualization cell layer within 5-10h, attached and invasion into the decidualization cell layer after 48h of coculture. The expression of β-catenin protein was observed on endometrial cells and mainly located at the cellular membrane at pre-implantation stage; more β-catenin protein was observed at the membrane and cytoplasm on decidualization cells at peri-implantation stage; the expression of β-catenin was detected in cellular nucleus after 48 h of implantation. The expression of β-catenin,Wnt4,Fzr26 mRNA was significantly increased on decidualization cells at peri-implantation stage compared with pre-implantation stage (P<0.05), and significantly increased at post-implantation stages compared with peri-implantation stage (P<0.05).

CONCLUSION: Human blastocysts co-cultured with human endometrial decidualization cell monolayers could be used to study the mechanism of embryo implantation. Wnt/β-catenin pathway was activated and played an important role in the procedure of embryo implantation.

Supported by: This study was Supported by grants from Beijing New Star Technology Project (H200281200190), Beijing Natural Science Foundation (5122015), and Beijing Obstetrics and Gynecology Hospital Foundation (201114).

P-282 Tuesday, October 21, 2014

THE EXPRESSION OF WNT/β-CATENIN PATHWAY FACTORS ON ENDOMETRIUM CELLS OF IN-VITRO MODEL OF EMBRYO IMPLANTATION. Y. Liu H. Chao. Department of Reproductive Medicine, Beijing Obstetrics and Gynecology Hospital, Capital Medical University, Beijing, China.

OBJECTIVE: To explore the role of Wnt/β-catenin pathway in the procedure of embryo implantation.

DESIGN: Prospective experimental study.

P-283 Tuesday, October 21, 2014


OBJECTIVE: To investigate whether low-molecular-weighted-heparin (LMWH) is effective in improve assisted reproductive technique (ART) outcomes in RIF patients with decreased uterine blood flow.

DESIGN: The prospective study population included 41 RIF patients with decreased uterine blood flow and control were 48 RIF patients with normal uterine blood flow.

MATERIALS AND METHODS: Uterine color-pulsed transvaginal Doppler ultrasound was performed to evaluate uterine radial artery resistance index (RI) in RIF patients on late follicular phase and hCG administration day. In RIF patients with decreased uterine radial artery blood flow (RI > 0.6), LMWH was daily injected subcutaneously (range 40-80mg/ day) from embryo transfer day to the day of checking the level of serum b-hCG. We compared the pregnancy rate and clinical pregnancy rate between RIF patients with LMWH treatment and RIF patients without treatment.

RESULTS: The mean age of LMWH treatment group and without treatment group was 35.8 ± 3.8 and 36.9 ± 3.9 (yrs), respectively. Pregnancy rate was significantly higher in RIF patients with LMWH treatment than without treatment group (58.5% vs. 31.3%, p =0.009). Also clinical pregnancy rate was significantly higher in RIF group with LMWH treatment (53.7% vs. 27.1%, p =0.009).

CONCLUSION: LMWH seemed to be effective treatment to improve pregnancy rate and clinical pregnancy rate in RIF patients with decreased uterine blood flow.

P-284 Tuesday, October 21, 2014

A COMPREHENSIVE ANALYSIS OF THE PHARMACODYNAMIC EFFECTS OF NT100, A NOVEL G-CSF. L. Kozhaya,a D. Carter,a J. Tong,b D. Unutmaz. NYU School of Medicine, New York, NY; 2Nora Therapeutics, Inc., Palo Alto, CA.

OBJECTIVE: NT100 is a novel rhG-CSF being developed specifically for use in the indications of repeated implantation failure and recurrent
m miscarriage. The hypothesized mechanism of action in these indications is promotion of maternal-fetal tolerance. Because maternal-fetal tolerance en-
compasses a series of immunologic changes affecting many cell types, analyses of
individual proteins or cell types may not provide a complete picture. We there-
fore undertook a comprehensive flow cytometric analysis of more
individual proteins or cell types may not provide a complete picture. We
therefore undertook a comprehensive flow cytometric analysis of more

**RESULTS:** NT100 induced broad and complex immunomodulatory
changes within each compartment consistent with a net toleragenic profile. The
effects were apparent after multiple-dose administration of NT100, and
were not observed after a single dose. These effects were persistent dur-
ing the period of drug administration and rapidly reversed on cessation of
drug treatment.

**CONCLUSION:** NT100 induces a complex and temporary set of
changes consistent with a state of enhanced maternal-fetal tolerance. Two
and a half sets of double-blind, placebo-controlled trials of NT100
are ongoing in unexplained recurrent pregnancy loss (RESPONSE trial)
and in repeated implantation failure patients undergoing IVF (Thrive-
IVF trial). These will provide the opportunity to correlate the observed
pharmacodynamics effects of NT100 treatment with changes in preg-
nancy outcomes.

Supported by: This study was Supported by Nora Therapeutics, Inc.
endometrial biopsy timing affects implantation rates and pregnancy outcomes in patients undergoing IVF with AECC.

RESULTS: All patients underwent luteal phase endometrial biopsy in preparation for IVF. Biopsy samples were either utilized for IVF in a consecutive menstrual cycle or were frozen for use in a future cycle. Embryos were cultured in AECC media and transferred on day 3. Outcomes included embryo grade, implantation rate, clinical pregnancy rate, and live birth rate. Statistical analysis included Mann Whitney U and χ² tests. P<0.05 was deemed statistically significant.

RESULTS: 2568 cycles of 1764 patients who underwent an AECC/IVF cycle between May 2004 and November 2013 were analyzed. 1453 biopsies were performed in the cycle prior to IVF and 1133 were performed more than one cycle prior to IVF. The two groups were similar in age, BMI, number of mature oocytes retrieved, number of embryos transferred, and best embryo grade. There were significant differences in implantation, clinical pregnancy, or live birth rates with adjusted relative risks of 1.0 (95%CI 0.90-1.11), 1.00 (95%CI 0.89-1.11), and 0.98 (95%CI 0.85-1.14) respectively.

CONCLUSION: Coculture biopsy in the cycle preceding IVF does not increase implantation, clinical pregnancy, or live birth rates when compared to biopsies performed more than one cycle prior to IVF. Previously demonstrated improvements in embryo quality and pregnancy outcomes in patients undergoing IVF with AECC are not attributable to biopsy-induced endometrial injury.

P-289 Tuesday, October 21, 2014

IMPLOMATION FAILURE OF TRANSLOCATED EMBRYOS CAN BE EXPLAINED BY IMPAIRED TROPHOBLASTIC DIFFERENTIATION. Y. Kalma, a, b A. Shipz, a, b T. Frumkin, a M. Telias, a, b T. Shwartz, a T. Cohen, a A. Amit, b D. Ben-Yosef, a, b Wolfe PGD-Embryonic Stem Cell Laboratory, Racine IVF Unit, Lis Maternity Hospital, Tel-Aviv Sourasky Medical Center, Tel Aviv, Israel; bDepartment of Cell and Developmental Biology, Sackler Medical School, Tel-Aviv University, Israel, Tel Aviv, Israel.

OBJECTIVE: Carriers of translocation (11;22), the most common recurrent reciprocal translocation in humans, have high risk of repeated miscarriages. Current research models for studying the biological basis for implantation failure in translocated embryos are limited. The aim of this work is to study the reason for repeated miscarriages of unbalanced translocated embryos using a human embryonic stem cell line (hESC) carrying the unbalanced translocation t(11;22).

DESIGN: The novel hESC line we have recently derived from an embryo with unbalanced t(11;22) following PGD, was induced to differentiate into trophoblast, and the results were compared to those obtained from control hESCs with normal karyotype. Gene expression of trophoblastic markers and hCG secretion were analyzed and compared in the translocated and the control hESCs at different time points of in vitro differentiation into trophoblasts.

MATERIALS AND METHODS: Fluorescent in-situ hybridization and karyotype analysis were performed to analyze t(11;22). The hESC line was characterized by RT-PCR and FACS analysis for pluripotent markers. Differentiation potential was assessed by spontaneous differentiation into teratomas, as well as by in-vitro differentiation into trophoblasts. Trophoblast development was assessed by measuring hCG secretion, hCG immunostaining and by gene expression of trophoblastic markers.

RESULTS: We derived the first hESC line carrying an unbalanced t(11;22) which we labeled Lis05_t(11;22). This hESC line showed the typical morphological and molecular characteristics of a hESC line. It was functionally pluripotent, giving rise to derivatives of all three germ layers. Interestingly, Lis05_t(11;22) hESCs failed to differentiate into trophoblasts as evidenced by their failure to secrete hCG. Gene expression analysis demonstrated reduced and delayed expression of the trophoblastic genes (CDX2, KRT7, GCM1, PPARG, KLF4, TP63 and CGA), concomitant with their failure to secrete hCG.

CONCLUSION: Our findings provide evidence that the failure in trophoblastic differentiation in t(11;22), correlating with implantation failure in unbalanced translocated embryos.

Supported by: This study was supported, in part, by the intramural research program of the Program in Reproductive and Adult Endocrinology, NICHD and NHLBI, NIH.
THE OPTIMAL ENDOMETRIAL PREPARATION FOR FROZEN-THAWED SINGLE EMBRYO TRANSFER IN A NATURAL CYCLE: A RETROSPECTIVE STUDY


OBJECTIVE: Single embryo transfer (SET) is the best method for preventing multiple births. The purpose of this study is to evaluate pregnancy outcomes with or without luteal phase support (LPS) of frozen-thawed single blastocyst transfer (SBT) treatment performed in natural cycles.

DESIGN: The study retrospectively analyzed 96 frozen-thawed SBT in natural cycles with or without LPS performed between the periods January 2010 to April 2013.

RESULTS: There were no significant differences in the patients' demographic data (age, basal FSH and embryo quality), clinical pregnancy rate (42.5% vs. 38.0%, p=0.150) and miscarriage rate (24.7% vs. 20.3%, p=0.296), between the groups. On the other comparison (383 cycles), a strong tendency of increased clinical pregnancy rates were observed in the high P4 levels group compared to the low P4 levels group (51.1% (H-) vs. 31.9% (L-), p=0.059) and (42.1% (H+) vs. 28.0% (L+), p=0.131), but no difference in the miscarriage rates were noted (26.7% (H-) vs. 25.0% (L-), p=0.908) and (37.5% (H+) vs. 23.8% (L+), p=0.367).

CONCLUSION: The pregnancy outcomes of natural cycle frozen-thawed SBT were similar regardless of LPS. Our results suggest that LPS with oral chlomadinone acetate may not improve the clinical pregnancy nor decrease miscarriage rate. Patients with low levels of P4 might need additional interventions in order to achieve higher pregnancy rates. Our findings suggest that 2-day post ovulation levels ≥ 7.6 ng/mL of P4 provide an optimal endometrial environment. However, we need further studies to determine the optimal treatment for patients with low P4 levels to undergo frozen thawed SBT in a natural cycle.
CONCLUSION: There were no significant differences in IR, CPR, LBR or SABR regardless of the route of progesterone for luteal support in this cohort of DER who underwent fresh ET.

P-293 Tuesday, October 21, 2014

LUTEAL PHASE SUPPLEMENTATION WITH VAGINAL PROGESTERONE IN WOMEN WITH POLYCYSTIC OVARY SYNDROME AND OVULATORY DYSFUNCTION UNDERGOING OVULATION INDUCTION WITH LETROZOLE: A RANDOMIZED CONTROLLED TRIAL. T. Pakrashi, a H. Baydoun, b S. Bocca, c S. Okanlami,c *Stadlerbauer, a*Obstetrics and Gynecology, Jones Institute for Reproductive Medicine/Eastern Virginia Medical School, Norfolk, VA; bGraduate Program in Public Health, Eastern Virginia Medical School, Norfolk, VA.

OBJECTIVE: Polycystic Ovary Syndrome (PCOS) is a leading cause of anovulatory infertility in women. Aromatase inhibitors such as letrozole are routinely used in the treatment of anovulatory women to induce ovulation (OI). These patients are often supplemented with progesterone in the luteal phase. The aim of this study is to determine if there is benefit to the use of vaginal progesterone (8% Crinone gel) in women with PCOS and ovulatory dysfunction undergoing OI with letrozole.

DESIGN: Ongoing randomized controlled trial.

MATERIALS AND METHODS: PCOS patients with ovulatory dysfunction less than 40 years of age who met inclusion criteria and were being treated with letrozole (2.5-7.5 mg) were consented for this study between November 2012 and April 2014. They were randomized (1:1) to the treatment group (8% Crinone vaginal gel once a day) or no treatment upon achieving at least 1 dominant follicle ≥ 17mm to be started 3 days after hCG trigger. Subjects underwent intrauterine insemination or timed intercourse depending on their semen parameters. An interim analysis was carried out on demographic, cycle characteristics and pregnancy rates between those with and without treatment. Data were analyzed using Student’s t-test and chi-square as appropriate. For a medium size (25%) difference in pregnancy rates and a 16% pregnancy rate per cycle, 51 cycles in each group would achieve a significance level of α=0.05 and β=0.20.

RESULTS: A total of 62 completed cycles out of 102 targeted cycles were analyzed. The primary outcome measure was pregnancy rate per cycle (total 8 pregnancies). Patients in the treatment group were older than the no treatment group (31.4±4.3 years vs. 28.8±4 years, p<0.01). There were no significant differences noted in any other cycle and demographic characteristics between the two groups including BMI, day 3 hormones, number of dominant follicles, endometrial thickness and semen parameters. Although the pregnancy rate was higher in treatment group compared to the no treatment group (21.4% vs. 6.1%, p<0.07), this difference is not statistically significant at this time.

CONCLUSION: The interim analysis suggests that routine vaginal progesterone (8% Crinone gel) supplementation of women with PCOS and ovulatory dysfunction undergoing OI with letrozole in the luteal phase demonstrates a trend towards higher pregnancy rates.

Supported by: Actavis Inc.

P-294 Tuesday, October 21, 2014

STARTING TIME OF PROGESTERONE LUTEAL PHASE SUPPORT IN IVF: A SYSTEMATIC REVIEW AND META-ANALYSIS. M. T. Connell,a A. M. Propst,b S. Bocca,c S. Okanlami,c *Stadlerbauer, a*Obstetrics and Gynecology, Jones Institute for Reproductive Medicine/Eastern Virginia Medical School, Norfolk, VA; aGraduate Program in Public Health, Eastern Virginia Medical School, Norfolk, VA.

OBJECTIVE: To assess the starting time of progesterone luteal support in IVF and its effect on clinical pregnancy and live birth.

DESIGN: Systematic review and meta-analysis.

MATERIALS AND METHODS: A systematic literature search in Embase and MEDLINE was performed for published randomized controlled trials comparing different starting points of progesterone for luteal support in IVF. Random effects models were used for statistical synthesis due to clinical heterogeneity in the studies. Statistical heterogeneity was assessed with Q and I2 tests.

RESULTS: 714 abstracts and 14 full text papers were screened. Six randomized controlled trials were identified that met inclusion criteria with a total of 1154 patients. Progesterone luteal support start times ranged from 36 hours prior to oocyte retrieval to six days post retrieval. Five trials utilized vaginal progesterone and one trial intramuscular. Three studies compared progesterone started before oocyte retrieval versus the day of oocyte retrieval. Starting progesterone prior to oocyte retrieval resulted in a decreased likelihood of clinical pregnancy (OR 0.65, 95% CI 0.44-0.94, Q=0.75, I2=0%). One study compared starting progesterone on post retrieval day 3 versus day 6, with a trend towards a decreased likelihood of pregnancy in the day 6 group (OR 0.51, 95% CI 0.25-1.05). Four trials comparing progesterone start times on the day of oocyte retrieval versus one, two, or three days post retrieval showed no significant differences in pregnancy.

CONCLUSION: Starting progesterone before oocyte retrieval and after day 5 post retrieval results in lower pregnancy rates. There appears to be a window for progesterone start time between the night after oocyte retrieval and day 5. Retrospective studies have suggested a potential benefit in delaying vaginal progesterone start time to 1-2 days after oocyte retrieval, but this review could not find randomized controlled trials to adequately assess this. Further randomized clinical trials are needed to better define progesterone start time for luteal support, particularly for vaginal progesterone which may more rapidly advance the endometrium.

Supported by: This work was supported, in part, by PRAE, NICHD, NIH.

P-295 Tuesday, October 21, 2014


OBJECTIVE: To determine if routine estradiol and progesterone measurements on day of frozen euploid embryo transfer predict clinical outcome.

DESIGN: Retrospective Cohort.

MATERIALS AND METHODS: A retrospective analysis was performed of 213 patients undergoing single euploid embryo transfer during a programmed frozen embryo transfer cycle. Time frame of the study was between 2010 and 2013. Exclusion criteria included recipients of donor oocytes, patients with embryos that had not been screened for ploidy and patients whose progesterone dose was increased. The cycle protocol used in all patients was oral estrace (max dose of 6mg/day) and intramuscular progesterone (P4) (50-75mg/day). The main outcome measures were live birth + ongoing pregnancy (LBR/OPR) and clinical pregnancy (defined as positive fetal heart tones, CPR) as well as missed abortion and biochemical pregnancy rates. Receiver operator characteristic (ROC) curves and chi-squared tests were used for statistical analysis.

RESULTS: The abilities of P4, E2/P4 ratio on the day of embryo transfer and its effect on clinical pregnancy and live birth.

CONCLUSION: Starting progesterone before oocyte retrieval and after day 5 post retrieval results in lower pregnancy rates. There appears to be a window for progesterone start time between the night after oocyte retrieval and day 5. Retrospective studies have suggested a potential benefit in delaying vaginal progesterone start time to 1-2 days after oocyte retrieval, but this review could not find randomized controlled trials to adequately assess this. Further randomized clinical trials are needed to better define progesterone start time for luteal support, particularly for vaginal progesterone which may more rapidly advance the endometrium.

Supported by: This work was supported, in part, by PRAE, NICHD, NIH.

FERTILITY & STERILITY®

e237
TABLE 1. P4 ranges and percent outcomes

<table>
<thead>
<tr>
<th>P4</th>
<th>P4</th>
<th>P4</th>
<th>P4</th>
<th>P4</th>
</tr>
</thead>
<tbody>
<tr>
<td>(10-15)</td>
<td>(15-20)</td>
<td>(20-30)</td>
<td>(30-40)</td>
<td>(&gt;40)</td>
</tr>
<tr>
<td>LBR, OPR (%)</td>
<td>70</td>
<td>62</td>
<td>52</td>
<td>50</td>
</tr>
<tr>
<td>Missed Abortion, Biochemical pregnancies (%)</td>
<td>7</td>
<td>15</td>
<td>27</td>
<td>32</td>
</tr>
</tbody>
</table>

*P4 is in ng/ml

CONCLUSION: P4 levels greater than 20 ng/ml on day of transfer (during FET cycles) are associated with increased incidence of missed abortion and biochemical pregnancies and decreased incidence of live birth/ongoing pregnancy.

EARLY PREGNANCY

P-296 Tuesday, October 21, 2014

MICRON flirt THE EXPRESSION OF MATRIX METALLOPROTEINASE (MMP-2 AND MMP-9 IN HUMAN TROPHOBLAST CELL LINE JEG-3 VIA TGF-β/SMAD3 PATHWAY. Z. Huang, S. Li, Q. Ma, W. Fan, Y. Wang, Z. Xiao. Obstetrics and Gynecology, West China 2nd Hospital, Sichuan University, Chengdu, Sichuan, China.

OBJECTIVE: Our previous study showed that TGF-β1 treatment upregulated the expression of miR-224 and the invasive-associated genes MMP-2/9 in human trophoblast cell line JEG-3 cells. The objectives of this study is to investigate whether miR-224 is involved in TGF-β induced up-regulation of MMP-2/9 in JEG-3 cells and the possible mechanisms.

DESIGN: A controlled experiment.

MATERIALS AND METHODS: JEG-3 cells were transfected with miR-224 mimics, miR-224 mimics control, pretreated with SB431542 (a TGF-β/Smad3 inhibitor) for 30 minutes and then transfected with miR-224, or co-transfected with miR-224 mimics and siRNA targeting Smad3 using Lipofectamine following the manufacturer’s protocol. After transfection for 6h, the media were changed into medium with TGF-β and then harvested for quantitative real-time PCR and Western blot analysis.

RESULTS: Over-expression of miR-224 by transfection with miR-224 mimics led to the up-regulation of MMP-2/9 in JEG-3 cells.

CONCLUSION: The up-regulation of miR-224 could enhance TGF-β induced the up-regulation of MMP-2/9 in JEG-3 cells and the possible mechanisms.

P-297 Tuesday, October 21, 2014

THROMBIN ALTERS DECIDUALIZATION AND MATRIX HOMEOSTASIS IN HUMAN ENDOMETRIAL STROMAL CELLS. S. N. Babayev, O. Bukulmez, B. R. Carr, R. A. Word. OB/Gyn, UT Southwestern Medical Center, Dallas, TX.

OBJECTIVE: Vaginal bleeding and subchorionic hematoma are associated with increased risk of both early and late pregnancy loss. Previous work suggests that thrombin generation may play a pivotal role in development of these complications. Specifically, in human endometrial stromal cells (HESC), thrombin acts via protease-activated receptors (e.g. PAR-1) to induce matrix metalloproteinases (MMPs) and inflammatory factors. Our hypothesis is that thrombin activates MMPs and disrupts expression of genes involved in matrix homeostasis. Further, we sought to determine how thrombin alters the decidualization process.

DESIGN: HESCs (precursor of decidua) were used as an in vitro model system.

MATERIALS AND METHODS: HESC were isolated from proliferative phase endometrium of premenopausal women undergoing benign hysterectomy. Cells were treated with vehicle control or thrombin at baseline or during decidualization using cAMP and medroxyprogesterone acetate (MPA). Thereafter, mRNA levels were analyzed by qPCR.

RESULTS: As expected, two marker of decidualization (IGF-1 and prolactin (PRL)) were induced 15 and 9-fold 72 h after initiation of cAMP/MPA. Treatment with human plasma thrombin (6 U/ml) x 24 h decreased mRNA expression of IGF-1 and PRL at baseline (71-79%), but not when thrombin completely blocked expression of IGF-1 in decidualized cells and decreased PRL mRNA from 9.7±1.48 to 5.47±0.63 R.U./GAPDH (p=0.057). Thrombin induced MMP1 at baseline (7-fold, p<0.01) and during decidualization (11.4-fold, P<0.001). COX-2 gene expression was also induced 8-fold under baseline conditions and amplified in cells undergoing decidualization (from 4- to 7.7-fold). Thrombin also decreased expression of collagen Iα1 50-65%, but not fibronectin. Further, thrombin decreased mRNA of lysyl oxidase (LOX), the major collagen cross-linking enzyme. Decidualization increased LOX mRNA 6.8-fold which was inhibited significantly by thrombin. Recombinant thrombin mimicked the effects of purified plasma thrombin suggesting that these effects were not due to contamination of plasma thrombin. Like thrombin, PAR-1 agonist induced inhibited IGF-1, PRL, LOX, and Collα1.

CONCLUSION: Taken together, our data indicate that thrombin adversely affects decidualization in vitro primarily through PAR-1. Furthermore, thrombin not only activates genes involved in matrix degradation (MMPs) and inflammation (COX-2), but also suppresses genes encoding factors important for collagen formation (Collα1, LOX). The results suggest that intrauterine bleeding and genetic defects of thrombin impairs decidualization and endometrial support of early pregnancy.

Supported by: Study was Supported by Division of Reproductive Endocrinology and Infertility, UT Southwestern Medical Center.

P-298 Tuesday, October 21, 2014

“NEGATIVE” SERUM HCG CAN FAIL TO DIAGNOSE ECTOPIC AND ABNORMAL INTRAUTERINE PREGNANCY AT LEVELS BETWEEN 1.0-5.0MIU/ML. B.-S. L. Maslow, A. Bartolucci, C. M. Sueldo, L. Engmann, C. A. Benadiva, J. C. Nelsen. Center for Advanced Reproductive Services/University of Connecticut Health Center, Farmington, CT.

OBJECTIVE: To assess the negative predictive value (NPV) of a “negative” first post-embryo transfer (ET) quantitative serum hCG with respect to ectopic pregnancy (EP) or abnormal intrauterine pregnancy (IUP).

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: We collected all first post-ET “negative” serum hCG results processed at a large fertility center from 6/2009-2/2014 and recorded clinical outcomes. “Negative” serum hCG was defined as ≤5mIU/mL. All first post-ET serum hCG were measured 14 days after oocyte retrieval and processed at our in-house laboratory with Siemens Immulite 2000. Associations were analyzed using χ2 test or Fisher’s Exact test for categorical variables and student’s t-test for continuous variables.

RESULTS: 1495 first post-ET serum hCG <5mIU/mL were collected. 1,343 (90%) were ≤1.0mIU/mL and 152 results (10%) were 1.0-5.0mIU/mL. 5.3% (8/152) of those with hCG 1.0-5.0mIU/mL were subsequently diagnosed with a pregnancy. There were 4 EP, of which 3 required salpingectomy, and 4 spontaneous abortions, one who presented with hemorrhage and was diagnosed with a pregnancy. There were 4 EP, of which 3 required salpingectomy, and 4 spontaneous abortions, one who presented with hemorrhage and was diagnosed with a pregnancy. There were 4 EP, of which 3 required salpingectomy, and 4 spontaneous abortions, one who presented with hemorrhage and was diagnosed with a pregnancy. There were 4 EP, of which 3 required salpingectomy, and 4 spontaneous abortions, one who presented with hemorrhage and was diagnosed with a pregnancy.

CONCLUSION: Our study demonstrates that serum hCG levels ≥1.0mIU/mL are associated with increased risk of both early and late pregnancy loss. Serum hCG levels ≥1.0mIU/mL were collected. 1,343 (90%) were ≤1.0mIU/mL and 152 results (10%) were 1.0-5.0mIU/mL. 5.3% (8/152) of those with hCG 1.0-5.0mIU/mL were subsequently diagnosed with an abnormal pregnancy. This is especially true for women at high risk for abnormal pregnancy, such as those undergoing assisted reproductive technologies. We propose that a first post-ET serum hCG 1.0-5.0mIU/mL should be considered “indeterminate,” and recommend repeat testing 48 hours later. Serial serum hCG levels in those whose
second value is not <1.0mIU/mL may identify abnormal pregnancies with low hCG levels.

P-299 Tuesday, October 21, 2014


OBJECTIVE: To assess the cost-effectiveness of three initial approaches to pregnancy of unknown location (PUL): methotrexate, dilation and curetage (D&C) or observation.

DESIGN: Cost-effectiveness analysis using decision tree modeling.

MATERIALS AND METHODS: A decision tree was constructed using TreeAge Pro 2014 comparing methotrexate, D&C, and observation as initial treatment for PUL. A systematic search was performed in PubMed to define probabilities at chance nodes and direct costs (adjusted to 2014 US dollars using the medical care component of the U.S. Consumer Price Index), with a base case probability of ectopic of 35%. Total costs, quality-adjusted life weeks (QALWs) over a 6-week horizon, and incremental cost-effectiveness ratios (ICER) were calculated and one- and two-way sensitivity analyses performed.

RESULTS: Direct costs were significantly lower in the observation group ($1087.61) compared to the other two groups ($1175.41 for methotrexate and $2482.00 for D&C) and the observation group had the highest number of QALWs (5.96); observation therefore dominated the other two strategies in cost-effectiveness. Sensitivity analysis revealed that increasing the probability of ectopic by 25% or decreasing the probability of spontaneous resolution of ectopic pregnancy below 60% could change the preferred strategy to methotrexate. Observation remained the preferred strategy even when the probability of ectopic was varied up to 99%. Substituting the cost of manual vacuum aspiration for D&C reduced the overall cost of this strategy to $1455.80, but it was still dominated by observation on analysis.

CONCLUSION: Based on currently available literature, observation is the most cost-effective strategy for initial treatment of PUL and appears to result in the best patient outcome based on QALWs. As observation is not a commonly used strategy in the United States for PUL, further clinical study comparing the three approaches is warranted.

P-300 Tuesday, October 21, 2014


OBJECTIVE: Previous studies have demonstrated aberrant serum cytokine levels in abnormal pregnancies after IVF. We investigated a series of 12 serum biomarkers in IVF patients to determine their possible association with pregnancy outcome and to develop a panel that was predictive of an EP in the early luteal phase.

DESIGN: We evaluated a retrospective cohort (matched by age, BMI, IVF response and number of embryos replaced) of 278 pregnant women undergoing IVF, utilizing sera from days 24 (7 days after embryo transfer) and 28 (initial pregnancy test), IVF outcomes were 60 women (21.5%) with a biochemical pregnancy (BC), 55 (19.8%) with an ectopic pregnancy (EP), and 51 (18.3%) with a first trimester miscarriage (SAB) and 112 (40.3%) with a live birth (LB).

MATERIALS AND METHODS: Serum levels of 12 different compounds were determined by quantitative ELSAs by investigators blinded to pregnancy outcome. Data was analyzed by Fisher’s exact test for categorical variables and non-parametric tests for continuous variables.

RESULTS: On day 24, the following biomarkers independently predicted a subsequent ectopic pregnancy – interleukin (IL)-11, IL-13, IL-1b, insulin-like growth factor binding protein-2, brain derived neurotrophic factor (BDNF) and secretory leukocyte protease inhibitor. On day 28, the following biomarkers independently predicted the outcome of an ectopic pregnancy – IL-1b, IL-13, IL-11, human epididymal protein-4, BDNF and neurotrophic factor-4. The day 24 panel of 6 compounds was the most predictive of ultimate development of an ectopic pregnancy. Utilizing this panel, if at least 4 of the biomarkers were in the predictive quartile, the women were 4 times more likely to have an EP versus another type of pregnant outcome (BC, SAB or LB) (30.4% vs 7.2%, p < 0.01). When restricting the analysis to only pregnancies that progressed beyond 5 weeks, a highly significant percentage of pregnancies with at least 4 markers positive on day 24 ultimately had an EP as compared to women with three or fewer markers (65.2% vs 21.1%; p < 0.0001).

CONCLUSION: A panel of select biomarkers measured as early as 4 days before the initial pregnancy test in patients undergoing IVF is highly associated with prediction of a subsequent EP.

Supported by: Institutional.

P-301 Tuesday, October 21, 2014

IMMUNITY AGAINST MALE SPECIFIC HY-ANTIGENS IS PROGNOSTICALLY IMPORTANT IN SECONDARY RECURRENT MISCARRIAGE. O. B. Christiansen, A. M. Kolte, E. C. Larsen, H. S. Nielsena, *Fertility Clinic, Rigshospitalet, Copenhagen, Denmark; bDept. Obstetrics and Gynecology, Aalborg Hospital, Aalborg, Denmark.

OBJECTIVE: Immune reactions against male-specific minor histocompatibility (HY) antigens may be a causal factor in secondary recurrent miscarriage (SRM) defined as consecutive miscarriages after a birth. We wanted in a new cohort of SRM patients to confirm or reject previous findings that in those with a firstborn boy, HLA-class II alleles (DRB1*15 and DQB1*0501/0502 called risk HLA alleles) predisposing to anti-HY immunity have prognostically negative impact.

DESIGN: In SRM patients included in a randomized placebo-controlled trial (RCT)3 of intravenous immunoglobulin (IvIg) we assessed pregnancy outcome in the RCT and cumulatively afterwards according to the sex of the firstborn child and maternal carriage/non-carriage of risk HLA alleles.

MATERIALS AND METHODS: Eighty-two patients with unexplained SRM and a mean of 5.0 miscarriages were between 2008-13 allocated to repeated infusions of IvIg or placebo from 5th to 14th gestational week. The last patient has now given birth but the randomization code is still not broken. Patients who miscarried in the RCT were offered IvIg treatment in their next pregnancy/ies without knowledge of their allocation in the trial. Patients (N = 71) with one firstborn or two firstborns of the same sexes were HLA class II typed using peripheral blood DNA.

RESULTS: In the RCT, the overall live birth rate was 52.4%. Patients with a firstborn boy carrying a risk HLA allele (group I, N = 17) had a live birth rate of 29.4%, which was significantly (p < 0.05) lower than the live birth rates in patients with a firstborn boy without risk HLA: 66.7% (group II, N = 18) and patients with a firstborn girl: 52.8% (group III, N = 36). After up to three pregnancies with IvIg treatment offered to patients who miscarried in the RCT, 8 (47.1%) in group I compared with 16 (88.9%) in group II had given birth (p < 0.01). In group III there was no significant difference in the cumulative chance of live birth between patients carrying or not carrying risk HLA alleles (70.6% and 78.9%).

CONCLUSION: In a new independent cohort of patients with SRM treated with IvIg or placebo we confirmed previous findings that in those with a firstborn boy maternal carriage of HLA class II alleles predisposing to immunity against HY-antigens is associated with a significantly poorer prognosis in subsequent pregnancies compared with SRM patients without these features. Anti-HY immunity developed during a first ongoing pregnancy with a boy may harm subsequent conceptions resulting in repeated miscarriage.

Supported by: Supported by public funds and the Sven Andersen foundation.

P-302 Tuesday, October 21, 2014

HIGH SERUM ESTRADIOL LEVELS ON THE DAY OF HCG ADMINISTRATION DURING CONTROLLED OVARIAN HYPERSTIMULATION IS ASSOCIATED WITH THE INCREASED RISK OF ECTOPIC PREGNANCY IN A DOSE-RESPONSE MANNER. J. Wang, W. Luo, F. Diao, Y. Mao, J. Liu. The State Key Laboratory of Reproductive Medicine, Clinical Center of Reproductive Medicine, First Affiliated Hospital, Nanjing Medical University, Nanjing, Jiangsu, China.

OBJECTIVE: To investigate whether high serum estradiol levels on the day of HCG administration increase the risk of ectopic pregnancy (EP) after controlled ovarian hyperstimulation (COH).

DESIGN: A retrospective cohort study was conducted at Reproductive Medicine Center of Nanjing Medical University (China) from 01/2007-12/2011.
MATERIALS AND METHODS: We included 3303 women achieving clinical pregnancies in fresh embryo transfer cycles undergoing COH. The cut points of serum estradiol levels on hCG administration day were set between 3000 pg/mL (the 50th percentile) and 5000 pg/mL (the 90th percentile) with a step of 500 pg/mL. The primary outcome measure was the occurrence of EP. Chi-square test was used for univariate analysis. Multivariate logistic regressions were performed to estimate the independent association between each estradiol level and EP with adjustment of important confounders, including previous EP, infertility factors, number of embryos transferred, endometrial thickness, days of stimulation, gonadotropins dosage, ovarian stimulation protocol and progesterone level on hCG day.

RESULTS: EP rates between women with estradiol level above and below 3000 or 3500 pg/mL on hCG day were comparable [2.6% (44/1676) vs 2.4% (39/1627), P=0.675; 3.0% (39/1308) vs 2.2% (44/1995), P=0.163]. The EP rates in women with estradiol level above 4000, 4500 and 5000 pg/mL were significantly higher compared with that in women with estradiol level below the value respectively [3.5% (30/867) vs 2.2% (53/2436), P=0.038; 3.7% (24/656) vs 2.2% (59/2647), P=0.036; 4.4% (13/295) vs 2.3% (70/3008), P=0.029]. Multivariate logistic regression showed a similar result (summarized in table below). The risk effect exhibited statistical significance when the cut points were at 4000 pg/mL or above. The adjusted odds ratios for EP were gradually increased following the increase of serum estradiol levels from 3000 to 5000 pg/mL.

Adjusted odds ratio of EP risk influenced by different estradiol levels

<table>
<thead>
<tr>
<th>Estradiol levels on hCG day (pg/mL)</th>
<th>AOR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3000</td>
<td>1.15 (0.71-1.85)</td>
<td>0.570</td>
</tr>
<tr>
<td>3500</td>
<td>1.48 (0.92-2.39)</td>
<td>0.110</td>
</tr>
<tr>
<td>4000</td>
<td>1.82 (1.11-2.97)</td>
<td>0.018</td>
</tr>
<tr>
<td>4500</td>
<td>1.82 (1.08-3.05)</td>
<td>0.024</td>
</tr>
<tr>
<td>5000</td>
<td>1.97 (1.04-3.73)</td>
<td>0.038</td>
</tr>
</tbody>
</table>

CONCLUSION: High estradiol levels on hCG administration day during controlled ovarian hyperstimulation is associated with increased risk of EP in a dose-response manner. Women undergoing COH with estradiol levels above 4000 pg/mL need increased surveillance on EP. Controlling estradiol levels in COH treatment may be helpful in preventing EP.

Supported by: China 973 Program.

P-305 Tuesday, October 21, 2014

PRACTICE PATTERNS OF INFERTILITY EVALUATION AND REFERRAL AMONG GENERAL OBSTETRICIAN-GYNECOLOGISTS WITHIN A LARGE HEALTH SYSTEM: A PRELIMINARY REPORT. S. S. Rothenberg, a, b, N. Bhatte, b, S. R. Nayak, a, M. N. Menke. a

OBJECTIVE: To investigate which attributes of Reproductive Endocrinology and Infertility (REI) fellowship applicants are most valued by fellowship program directors during the match process.

DESIGN: Electronic survey.

MATERIALS AND METHODS: An electronic survey administered to REI fellowship directors to determine applicant characteristics most favored in the REI fellow selection process. Characteristics were ranked on a five-point Likert scale, with 1 being "most important (highly likely to affect match)" and 5 being "least important (least likely to affect match)." The main outcome measure was factors highly desired by REI fellowship directors.

RESULTS: The overall response rate was 61% (27/44). Objective factors ranked most important were clinical research experience (1.81 ± 0.40), followed by training at a competitive obstetrics and gynecology residency program (1.81 ± 0.56), and basic science research experience (2.04 ± 0.60). Personal interview (1.15 ± 0.40) and perceived ability to work well with others (1.27 ± 0.45) were subjective factors considered highly favorable by REI fellowship directors.

CONCLUSION: When selecting REI fellows for interviews, fellowship directors value candidates that have trained at a competitive obstetric and gynecology residency program and who have clinical or basic science research experience. When subsequently ranking fellowship applicants, however, the most important factors are those found in the interview process.

P-303 Tuesday, October 21, 2014


OBJECTIVE: Report on products of conception (POC) samples showing single chromosome uniparental disomy (UPD). UPD of select chromosomes has been attributed as a cause for miscarriage, but UPD can occur for any chromosome, largely due to trisomy rescue events. Unlike other methods, molecular chromosome analysis of POC samples using SNP microarray with bioinformatics allows for identification of UPD.

DESIGN: Retrospective analysis.

MATERIALS AND METHODS: Review of 11,318 consecutive fresh POC samples sent to a reference lab along with maternal blood samples. Genotyping was performed using Illumina CytosNP-12b microarray and bioinformatics.

RESULTS: Fetal results were obtained on 9632 (85%) of the POC samples. 18 cases (0.2%) of UPD without aneuploidy of other chromosomes were identified. The UPD was of maternal origin in 17 of 18 (94%) cases.

CONCLUSION: SNP microarray with bioinformatics is a unique method for POC analysis that allows for detection of single chromosome UPD, which may be the cause of miscarriage or a finding worth causal consideration. While the mechanism and underlying etiology cannot be confirmed through SNP array testing, UPD originating from a trisomy rescue event is likely. In this cohort of samples, 94% of cases had maternal UPD; a finding supported by the higher rate of nondisjunction in oogenesis. UPD originating from a trisomy rescue event can result in mosaicism of aneuploid chromosomes as reported here in two cases. The original aneuploidy may be the cause of the pregnancy loss, or UPD could be the sole cause by altering critical developmental pathways and/or placental function or by unmasking a lethal autosomal recessive mutation.

PRACTICE MANAGEMENT

P-304 Tuesday, October 21, 2014


OBJECTIVE: To investigate which attributes of Reproductive Endocrinology and Infertility (REI) fellowship applicants are most valued by fellowship program directors during the match process.

DESIGN: Electronic survey.

MATERIALS AND METHODS: An electronic survey administered to REI fellowship directors to determine applicant characteristics most favored in the REI fellow selection process. Characteristics were ranked on a five-point Likert scale, with 1 being "most important (highly likely to affect match)" and 5 being "least important (least likely to affect match)." The main outcome measure was factors highly desired by REI fellowship directors.

RESULTS: The overall response rate was 61% (27/44). Objective factors ranked most important were clinical research experience (1.81 ± 0.40), followed by training at a competitive obstetrics and gynecology residency program (1.81 ± 0.56), and basic science research experience (2.04 ± 0.60). Personal interview (1.15 ± 0.40) and perceived ability to work well with others (1.27 ± 0.45) were subjective factors considered highly favorable by REI fellowship directors.

CONCLUSION: When selecting REI fellows for interviews, fellowship directors value candidates that have trained at a competitive obstetrics and gynecology residency program and who have clinical or basic science research experience. When subsequently ranking fellowship applicants, however, the most important factors are those found in the interview process.
analysis in the initial evaluation of a 29 year old nulliparous patient (92%); however, 54% would not include an assessment of tubal patency (p<0.01). Age was not a factor in evaluation, but did prompt earlier referral. Evaluation of a 29 year old nulliparous patient included female hormonal assessment, evaluation of tubal patency and semen analysis in 46% of respondents compared to 58% in a 35 year old nulliparous female patient (p=0.6). However, physicians were more likely to refer a 38 year old patient with primary infertility to a fertility specialist compared to a 29 year old (OR 0.1, [95%CI: 0.03,0.38]).

CONCLUSION: Although most generalist obstetrician gynecologists report they evaluate patients for infertility in their general practice, our data suggest a need to focus continuing medical education efforts on early assessment and treatment amongst generalist obstetricians and gynecologists.

Supported by: K12 HD 063087.

P-306 Tuesday, October 21, 2014

EFFECTIVE ANALYSIS COMPARING A FREEZE-ALL PROTOCOL TO FRESH BLASTOCYST EMBRYO TRANSFER IN NORMAL RESPONDERS, S. M. Zarek, S. L. Mumford, J. H. Segars, A. Y. Armstrong, Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, MD; Division of Intramural Population Health Research, NICHD, Rockville, MD.

OBJECTIVE: Although recent studies demonstrate high pregnancy rates and improved perinatal outcomes in freeze-all cycles, the financial considerations of the increased cost associated with an initial freeze-all cycle may affect decision making. The aim of this study was to identify the most cost-effective procedure in patients undergoing fresh blastocyst transfer versus a “freeze-all” protocol.

DESIGN: Decision tree mathematical model with sensitivity analysis.

MATERIALS AND METHODS: PubMed was searched to determine pregnancy rates in randomized controlled trials of freeze all blastocyst transfer versus fresh blastocyst transfer in normal responders (8-15 follicles). Charges in United States dollars for an initial freeze-all cycle ($17,000), a pregnancy resulting in spontaneous abortion ($14,000), a pregnancy resulting in spontaneous abortion ($1,500) and a supernumerary frozen embryo transfer (FET) ($4,000) were used as a surrogate to direct societal costs, in 2013 dollars, and obtained from individual clinics as well as published United States averages. The cost of a freeze-all cycle included the cost of the initial stimulation cycle, embryo cryopreservation and thaw, and frozen embryo transfer. A decision tree comparing a freeze-all protocol versus fresh embryo transfer was created from the perspective of societal costs per ongoing pregnancy. Modeling was constructed to include an initial fresh or freeze-all blastocyst transfer, as well as second cost analysis of a subsequent frozen embryo transfer of available supernumerary embryos if the initial fresh or freeze-all transfer failed. Sensitivity analyses were performed over the range of costs and pregnancy rates.

RESULTS: A single freeze-all cycle was more cost effective compared to a single fresh cycle when the cost of a freeze-all cycle was less than $21,500. For a single cycle, if the ongoing pregnancy rate (OPR) of a fresh cycle was set at 51%, then a freeze-all approach was cost effective when the OPR for frozen cycles was 62% or greater. The freeze-all strategy was also more cost effective with the inclusion of a secondary supernumerary FET when the cost of the freeze-all cycle was less than $16,000.

CONCLUSION: In normal responder patients, the model suggested it was more cost effective from a societal perspective to proceed with a freeze-all approach rather than fresh blastocyst embryo transfer when the OPR was greater than 62% and the cost of the freeze-all cycles was less than $21,500.

Supported by: Intramural Research, PRAE.

P-307 Tuesday, October 21, 2014

MANAGEMENT OF NEW MEDICAL INFORMATION INVOLVING GAME DONORS. P. Callum, L. Isley, M. Gillespie. California Cryobank, Los Angeles, CA.

OBJECTIVE: To summarize the last 5 years of experience with management of new donor medical information and discuss recommendations for communication to recipients.

DESIGN: Records from April 2009 - March 2014 were reviewed to summarize the genetic factors that led to changes in the distribution of donor specimens after initial qualification. The time lapse from initial release of a donor’s specimens to the change in distribution was calculated for each donor.

RESULTS: Distribution of specimens from 147 donors was restricted due to genetic indications between April 2009 and March 2014. These restrictions arise due to new medical diagnoses in donors, their family members, and donor-conceived offspring, as well as changes to genetic testing practices and internal company standards (Table 1).

In most cases, specimens from the donors remain available for future use by clients who used vials from those donors previously. The client and her physician are required to provide written documentation that they were informed about the new information and that genetic counseling is recommended prior to use of those specimens.

CONCLUSION: New medical information may be obtained at any time after a donor has been qualified. Documentation of medical issues involving the donor and offspring is critical for an ongoing risk assessment for management of the health risks to the donor and offspring. There are no established industry protocols for communicating this information to recipients or adult offspring. We recommend that clients report their pregnancies and births to the gamete facility and update their contact information so the facility can attempt to contact them and share significant new information that may be important to their family’s health. In addition, gamete providers do not know which clients have vials or embryos stored at external facilities. It is recommended that clients or their physicians contact the gamete facility prior to use of stored specimens and embryos to check for new information.

P-308 Tuesday, October 21, 2014

RACIAL DIFFERENCES IN FERTILITY KNOWLEDGE AND AWARENESS AMONGST REPRODUCTIVE AGE WOMEN IN THE U.S. J. C. Yano, S. L. Sundsberg, L. Pal, OB/Gyn, Mount Sinai Beth Israel, New York, NY; OB/Gyn, Yale University School of Medicine, New Haven, CT.

OBJECTIVE: To determine if fertility knowledge and reproductive awareness amongst reproductive age women varies by race/ethnicity.

DESIGN: Cross-sectional survey of 1,000 U.S. women.

MATERIALS AND METHODS: Using quotas for ethnicity and geographic region, an online survey was conducted among reproductive age (18-40 years old) women in March 2013. To analyze racial discrepancies as a sub-analysis using Chi-square tests, specific questions were selected from an original study (1) reflecting 2 domains: 1) risk factor awareness for infertility; 2) factors that may delay pregnancy susceptibility.
RESULTS: Across ethnic groups, knowledge about smoking and advancing age were statistically significant. Overall, <16% correctly understood the fertility window with the majority reporting that it occurs after ovulation. Less than 40% of women know that egg production does not continue throughout reproductive years. Ethnic differences were noted in demographics. Asian women reported highest levels of: education (75%), full-time employment (54.6%), and income >50K/year (67.4%). However, their fertility knowledge was not greater than other ethnic groups.

Risk Factors and Pregnancy Susceptibilities - Percent Answered Correctly

<table>
<thead>
<tr>
<th>Question</th>
<th>White</th>
<th>Hispanic</th>
<th>Black</th>
<th>Asian</th>
<th>Other</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>College Degree or More</td>
<td>37.9%</td>
<td>38.5%</td>
<td>42.9%</td>
<td>37.5%</td>
<td>38.1%</td>
<td>0.0004</td>
</tr>
<tr>
<td>Employment Status*</td>
<td>36.3%</td>
<td>33.6%</td>
<td>37.2%</td>
<td>54.6%</td>
<td>27.3%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Full-time</td>
<td>46.5%</td>
<td>41.2%</td>
<td>32.5%</td>
<td>67.4%</td>
<td>38.9%</td>
<td>0.0045</td>
</tr>
<tr>
<td>Income*</td>
<td>73.5%</td>
<td>78.0%</td>
<td>70.5%</td>
<td>63.6%</td>
<td>72.2%</td>
<td>0.04</td>
</tr>
<tr>
<td>Smoking</td>
<td>60.0%</td>
<td>69.6%</td>
<td>80.8%</td>
<td>63.6%</td>
<td>68.0%</td>
<td>0.059</td>
</tr>
<tr>
<td>STDs</td>
<td>29.3%</td>
<td>28.0%</td>
<td>30.8%</td>
<td>45.5%</td>
<td>50.0%</td>
<td>0.05</td>
</tr>
<tr>
<td>Dysmenorrhea</td>
<td>75.0%</td>
<td>72.0%</td>
<td>75.6%</td>
<td>59.0%</td>
<td>0.045</td>
<td></td>
</tr>
<tr>
<td>Obesity</td>
<td>81.9%</td>
<td>82.4%</td>
<td>74.4%</td>
<td>79.6%</td>
<td>59.1%</td>
<td>0.047</td>
</tr>
<tr>
<td>Intercourse within 2 days after ovulation</td>
<td>81.9%</td>
<td>82.4%</td>
<td>74.4%</td>
<td>79.6%</td>
<td>0.047</td>
<td></td>
</tr>
<tr>
<td>A woman’s ovaries continue to create new eggs during the reproductive years</td>
<td>39.5%</td>
<td>27.2%</td>
<td>30.8%</td>
<td>36.4%</td>
<td>31.8%</td>
<td>0.068</td>
</tr>
</tbody>
</table>

n = 1,000; * = Only a portion of the data presented

CONCLUSION: Amongst reproductive age women in the U.S., carry the greatest subjective burden of fertility related concerns. Although reported stress related to getting pregnant was similar across ethnic groups, Asian women are hesitant to participate in fertility related discussions. The observed differences could reflect potential roles of cultural nuances in the perception of fertility. These findings underscore a need for inclusion of both mothers and daughters in discussions related to reproductive health and wellbeing, particularly in the Asian communities. Health care providers can help mitigate the disproportionate level of psychological stress around fertility concepts that this population apparently endure.

Supported by: Sampling and data collection conducted by Edelman-Berland and funded by Church & Dwight Inc. Yale received an unrestricted educational grant for collaboration on project. Data analysis and interpretation conducted independently by Yale.

P-310 Tuesday, October 21, 2014

IMPACT OF THE INTRODUCTION OF A CAP ON MEDICARE FUNDING ON THE UTILIZATION OF ART IN A LARGE AUSTRALIAN FERTILITY CENTRE. D. R. Listijono, X. Q. Wang, G. M. Chambers, M. G. Chapman. “School of Women and Children’s Health, University of New South Wales, Sydney, New South Wales, Australia; ’Perinatal and Reproductive Epidemiology Research Unit, University of New South Wales, Sydney, New South Wales, Australia; ’IVF Australia Southern Sydney, Sydney, New South Wales, Australia.

OBJECTIVE: To examine the policy impact of a decrease in the contribution of public reimbursement for ART treatments on the relative utilization of intratubal insemination (IUI) and in vitro fertilization and intracytoplasmic sperm injection (IVF/ICSI).

DESIGN: Policy impact analysis using time series analysis of all ART cycles (IUI and IVF/ICSI) undertaken at a large Australian fertility network (IVFAustralia) pre and post the introduction of the policy in 2010.

MATERIALS AND METHODS: Data from all fresh non-donor ART cycles between 2006 and 2013, retrieved from the IVFAustralia database, were analysed before (January 2006 – December 2009) and after (January 2010 – December 2013) the implementation of the Extended Medicare Safety Net Caps Policy which significantly reduced patient reimbursement for IUI cycles relative to IVF/ICSI cycles. Total cycle numbers as well as the relative usage of IUI and IVF/ICSI were assessed. IVF/ICSI cancellation rates were also compared. GraphPad InStat was used for statistical analysis; P<0.05 was considered significantly different.

RESULTS: Of the 18,818 ART cycles undertaken in 2006-2009, 4,893 were IUI cycles and 13,925 were IVF/ICSI cycles, compared to 19,688 ART cycles in 2010-2013; 3,198 IUI cycles and 16,490 IVF/ICSI cycles. There was a significant decrease in the proportion of IUI cycles (26% vs 16.2%, P<0.001) and an increase in the proportion of IVF/ICSI cycles (74% vs 83.8%, P<0.001) before and after the policy introduction. There was also a significant decrease in the IVF/ICSI cancellation rate after 10 (10.2% vs 7.0%, P<0.05). A notable short term impact of the funding policy change was a substantial reduction in all ART usage from 2009 to 2010 (total ART, 21.5% decline; IVF/ICSI, 15.1% decline) but most markedly a 43.1% decline in IUI usage in 2010.

CONCLUSION: This study suggests that a change in public funding impacts the treatment modality in ART, as evidenced by a relative decrease...
in IUI and increase in IVF/ICSI usage at a large Australian fertility clinic. Further econometric analysis of data from the Medicare Benefit Schedule (MBS) is required to determine the full impact of this policy change on a nationwide level.

P-311 Tuesday, October 21, 2014


OBJECTIVE: With advances in the digital age, an increasing number of infertile couples are turning to the Internet as their primary source of medical information. However, there is little data assessing the quality and content of this information on fertility websites. We sought to evaluate the information and options available on Society for Assisted Reproductive Technologies (SART) member websites.

DESIGN: Cross-sectional evaluation.

MATERIALS AND METHODS: Between 2/2014-4/4014, 396 SART member websites were evaluated by 2 independent researchers. Websites were surveyed for the following parameters: information on female and male factor infertility, success rates, egg cryopreservation, cost of treatment, surgical treatment, psychosocial resources and wellness information. \( \chi^2 \) tests were used to assess for differences between groups.

RESULTS: 98% (387/396) of clinics had a website, of which 80% were private and 20% were academic centers. 26% of practices performed \( \geq 500 \) cycles/year while 74% performed <500 cycles/year. 94% of websites provided general information on female factor infertility, while 88% offered material on male factors. 54% reported both cycle number and live birth rate, with large practices reporting more than smaller ones to report this data (76 vs 50%, \( p<0.001 \)). Large practices were more likely to offer egg cryopreservation than smaller ones (81 vs 65%, \( p=0.003 \)), but rates were similar between academic and private practices (72 vs 68%, \( p=0.5 \)). Academic and private practices were equally likely to report cost of treatment (32 vs 36%, \( p=0.5 \)) but private ones were more likely to offer shared risk financing (43 vs 28%, \( p=0.01 \)). 19% of practices promoted robotic surgery and 44% offered tubal reversals. Only 55% publicized an on-site mental health specialist and 45% advertised wellness programs. Academic practices were more likely to offer experimental procedures (44 vs 14%, \( p<0.0001 \)) and participation in research studies (37 vs 12%, \( p<0.0001 \)) as compared to their private counterparts.

CONCLUSION: Though the majority of SART clinic websites offer general infertility information, there is substantial variability in the quality and completeness of content presented. Although a high success rate is commonly cited as the top reason patients choose an infertility clinic, only 54% of websites had accurate success rate reporting. The majority of practices offer surgical treatment, including robotic surgery and tubal reversals. Although anxiety and depression levels are high amongst infertility patients, a limited number of practices offer on-site psychosocial resources.

P-312 Tuesday, October 21, 2014

CONTROVERSY OVER RIGHTS OF CHILDREN BORN FROM OOCYTE DONATIONS, IS FULL DISCLOSURE OF DONOR’S IDENTITY NECESSARY? A. Tanaka. Saint Mother Hospital, Kita-kyushu, Fukuoka, Japan.

OBJECTIVE: In 2003 the Japanese Ministry of Health, Labor and Welfare approved and set the guidelines for egg donation, they established the donor’s right to remain anonymous but also the child born from oocyte donation’s right to know his/her origin. These guidelines contradict each other. As of this date no donors without family or friendly ties have appeared. The main reason for this lack of donors seems to be the fear of the identity disclosure. In Japan, recipients must tell their offspring that the genetic mother is different from the biological mother at an early age. When children are fifteen years old, they can require full disclosure about the donor. That is, donor must be ready to disclose her identity. We report here the current status of oocyte donation in Japan.

DESIGN: A retrospective analysis of questionnaire and clinical outcome after oocyte donation at Saint Mother Hospital.

MATERIALS AND METHODS: We gave a questionnaire to 347 ART patients to inquire whether they would consent to give one or two oocytes for free to other woman waiting for an egg donation before their oocyte collection. There were two scenarios, one where they would remain anonymous and the other one where they would permit the disclosure of their identity. The results were that 51% (108/217) in the first group and 0% (0/347) in the second group respectively would agree to a donation. In 2004 the JISART (Japanese Institution for Standardizing Assisted Reproductive Technology) established an ethics committee and loosened the rules to allow the oocyte donation from sisters or friends. Guidelines require that recipients are females who have no chance to become pregnant without oocyte donation. Donors must be married, less than 40 years old and have more than one child.

EXCHANGE OF MONEY IS FORBIDDEN.

RESULTS: There were 29 cases oocyte donation offers from April 2007 to March 2014. 2 cases canceled the oocyte donation during counseling for fear of the detrimental effect on their children. 26 donors were interviewed and 2 sisters-in-law and 1 was a close friend. Pregnancy rates, miscarriage rates and birth rates were 29.4% (15/51), 6.7% (1/15) and 17.6% (9/51). 9 were normally delivered and 3 ongoing, including 2 cases of tubal pregnancies. So far, no major problems have been reported.

CONCLUSION: Oocyte donation under the current guidelines is very different because the right to anonymity and right to identity disclosure for the child are basically incompatible. Donor’s disclosure information should be limited to information that does not reveal their identity completely.

P-313 Tuesday, October 21, 2014

THE CORRELATION BETWEEN SERUM ESTRADIOL LEVELS AND TOTAL PROPOFOL DOSE USED FOR ULTRASOUND GUIDED FOLLICLE ASPIRATION: MOVING TOWARDS A “PROTOCOL BASED APPROACH.” L. Xiaojie, a S. Reddy, a, 1 Raman, b, S. Varadan, b, R. Odem, a, C. Cooper, a Obstetrics and Gynecology, Washington University, St. Louis, MO, aAnesthesiology, Washington University, St. Louis, MO.

OBJECTIVE: Transvaginal ultrasound guided follicle aspiration (USFA) is the most common outpatient surgical procedure in assisted reproduction & its use is increasing annually. The anesthetic goals are to minimize patient anxiety, pain, & movement while avoiding any deleterious effect on oocyte quality and the potential for pregnancy. Our center utilizes a propofol based protocol though dosing can vary greatly between patients. Our primary objective was to determine the relationship between serum levels of estradiol (E2), which can theoretically alter medication metabolism, & requirements for propofol (P).

DESIGN: IRB approved cohort study.

MATERIALS AND METHODS: Patients who underwent USFA in 2012 & were entered into the electronic anesthesia database at Washington University were included. Patient demographics were collected in addition to peak E2 levels 2-3 days prior to USFA (16% of patients have a blind trigger I day after peak E2 in our unit). Anesthesia data including total P dose, ASA score, procedure time, episodes of desaturations (SpO2 <90%), bradycardia (HR<60), mean arterial pressure (MAP)<65, use of sympathomimetics, total intravenous fluids (IVF), use of oral airway & need for intubation were obtained from the anesthesia electronic medical record. Multivariate correlation analyses were performed (SPSSv22).

RESULTS: 273 women (mean 34.4+4.4 years) were included. Indications for IVF included male (28%), tubal (21%), PCOS (20%), endometriosis (15%), diminished ovarian reserve (13%) & unexplained (18%). Peak E2 (2527+1220 ng/mL) & patient data including mean BMI (267+7), current smoking (5%), rFSH dose (2912+1080 IU), oocytes retrieved (14+6), surgical time (32+11 min), total P requirement (3294+149 mg), IVF (605+188 mL), oral airway requirement (17%), postoperative nausea (15%), & need for O2 increase(17%) was calculated. Controlling for age, BMI, day of peak E2 measurement, & gonadotropin dosage, E2 levels were highly correlated with P requirements (r=0.23, p=0.001). For each 1% increase in BMI 14 mg of additional P was needed, & for each 100 ng/mL increase in E2, an additional 2.3 mg of P was required.

CONCLUSION: Our data demonstrates a correlation between E2 levels & P dosing for otherwise healthy women undergoing ART oocyte retrieval procedures. This has led to a further prospective clinical study in our institution in an attempt to optimize dosing, minimize postoperative resuscitation, & improve patient safety & well-being.

Supported by: 1K12HD063086-01 (ARC).

P-314 Tuesday, October 21, 2014

UTERINE FIBRIN ASPIRATION IN IVF CYCLES: OUTCOME & TWO DIFFERENT APPROACHES. M. E. Eid, a, b Obstetrics/Gynecology & Infertility, Cairo University, School of Medicine, Cairo, Eastern, Egypt, bIVF Unit, Fakih IVF Fertility Center, Duida, Jumeirah 1, United Arab Emirates.

OBJECTIVE: Although fluid accumulation within the uterine cavity during ovarian stimulation in IVF cycles is not a common complication, it is
detrimental to embryo implantation. It is mostly associated with hydrosalpinges, polycystic ovarian disease and subclinical uterine infections. We aimed to examine the website content into Spanish. Methods for removing the fluid with an embryo transfer catheter, either at time of egg collection or before embryo transfer, on the success rate of IVF-ET.

DESIGN: Twenty-eight patients were recruited for this prospective controlled study. Patients with evident hydrosalpinges, endometriosis or cervical stenosis were excluded.

MATERIALS AND METHODS: Fluid aspiration was done using an embryo transfer catheter (laboject). Patients were divided into 2 groups; Group I (16 patients), where the aspiration was done immediately after egg collection plus supplementation with Doxycycline antibiotic till the day of embryo transfer (5 days), and Group II (12 patients), where the aspiration was done immediately before embryo transfer. The outcome of IVF-ET cycles, (pregnancy rate, implantation rate and ectopic pregnancy), was compared between the two groups.

RESULTS: Improved cycle outcome was observed in Group I where early fluid aspiration was done compared to Group II where late aspiration was done as seen in the pregnancy rate (50% vs 33%), implantation rate (22% vs 13%) and ectopic pregnancy (0% vs 6%) respectively.

CONCLUSION: The approach of early fluid aspiration was proved to be superior as it gives enough time for the endometrium to recover and the uterine cavity to be quiet. The antibiotic supplementation may have a positive effect against the subclinical endometritis or salpingitis. This novel protocol needs further studies to test its real effect on the IVF-ET outcome.

P-315 Tuesday, October 21, 2014

SE HABLA ESPANOL? HOW REPRODUCTIVE ENDOCRINOLOGY PRACTICES REACH OUT TO SPANISH SPEAKING PATIENTS IN THE UNITED STATES.

L. Londra,1 K. Tobler,2 K. Omurtoglu,3 M. Donohue3 GYN/OB, REI Division, Johns Hopkins School of Medicine, Baltimore, MD; 2OB/GYN, REI Division, Johns Hopkins School of Medicine, St. Louis, MO.

OBJECTIVE: To analyze the use of Spanish language translation on the websites of Reproductive Endocrinology and Infertility (REI) practices in the context of the projected growth of Hispanics as health consumers and evidence of underutilization of infertility services by minority populations.

DESIGN: Cross sectional survey of websites from REI practices and assessment of the relationship between Spanish-translated website content and REI practice characteristics and location.

MATERIALS AND METHODS: We reviewed the websites of all REI practices that report to the national database of in vitro fertilization (IVF) in the US. We used descriptive statistics and logistic regression analysis to report on the characteristics of the websites and the propensity to translate website content into Spanish according to characteristics of the practice and location. The method used for translation was classified in automatic and human translation, and accuracy of the content was evaluated in 30 randomly selected websites.

RESULTS: Of the 376 REI practice websites analyzed, 101 websites (27%) offered at least some information in Spanish. We identified 97 Spanish speaking practitioners at 71 REI Practices. A Spanish-translated website was significantly associated with using social media, having an out of town/international webpage and a Spanish speaking physician in the practice. Location in a state with mandated insurance for reproductive services and being a private or university-based practice was not associated with increased odds of translating the website. In practices located in the top 60 metropolitan areas by Hispanic population the odds of having a translated website were only by 4% compared to the rest of the US. In practices located in the top 60 metropolitan areas, the odds of having a translated website were only by 7% compared to the rest of the US.

CONCLUSION: An important minority population in the context of projected growth of Hispanics as health consumers and evidence of underutilization of infertility services. These results suggest that translation efforts are needed to ensure equal access to infertility care for Spanish speaking consumers.

P-316 Tuesday, October 21, 2014

NODAL REGULATES THE DIFFERENTIATION OF IPS CELLS TO MALE GERM CELLS VIA SMAD2/3, OCT4 AND FOXHI ACTIVATION.

S. Yang, R. Tian, J. Wang, Z. Zhu, P. Li, Z. Li, Shanghai Human Sperm Bank, Ren Ji Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China.

OBJECTIVE: The differentiation of male germ cells from IPS cells provides an ideal model for unveiling molecular mechanisms of spermatogenesis. Nodal could promote proliferation of mouse spermatogonial stem/progenitor cells via Smad2/3 and oct-4 activation. The objective of this study was to determine the role of nodal signaling in the differentiation of IPS cells to male germ cells.

DESIGN: Comparative and controlled study.

MATERIALS AND METHODS: In this study, embryoid body (EB) formation and the exposure of Nodal in new were applied to induce the male germ cells from mouse IPS in vitro. Germ cell-specific genes and proteins were assessed using real-time PCR, immunoblotting and flow cytometry. The moleculars of Nodal signaling pathway were detected by immunoblotting.

RESULTS: We found that Nodal and its receptors Alk4, Actr-IB except Alk7 were expressed in the mouse IPS cells, whereas both Nodal and its receptors were detected in the EBs. Nodal could promote the propagation of EBs and nodal RNAi disrupted the proliferation of IPS cells. The results of real-time PCR and western blots showed that Nodal could up-regulate the expression of germ-cell marker genes and proteins in IPS-derived EBs. Moreover, the level of Smad2/3 phosphorylation, Oct4 and Foxhi transcription, and cyclin D1 and E were increased with graded Nodal signaling.

CONCLUSION: Collectively, the above results suggest that Nodal promotes the generation of male germ cells from IPS cells via the activation of Smad2/3 and Oct4 and Foxhi transcription. This study offers novel insights into molecular mechanisms of male germ cell development.

Supported by: This work was Supported by National Science Foundation of China (31201109) and China National Key Project (2010CB945200).

P-317 Tuesday, October 21, 2014

CHROMOSOME MICRODUPPLICATION IN SOMATIC CELLS DECREASES THE GENETIC STABILITY OF HUMAN SOMATIC CELLS DURING REPROGRAMMING.

Y. Yu, L. Chang, H.-C. Zhao, R. Li, J. Qiao. Peking University Third Hospital, Beijing, China.

OBJECTIVE: Human pluripotent stem cells offer a limitless source of cells for regenerative medicine, however, derivation efficiency is limited and a large proportion of cells are arrested during reprogramming. We explored the microduplication in arrested and established reprogrammed cells, and investigated the correlation between derivation efficiency and somatic cells with pre-existed chromosome aberrant.

DESIGN: Three somatic cell lines were obtained and one of them showed microduplication in chromosome 1 during culture in vitro. These three somatic cell lines were acted as donor cells to be reprogrammed by nuclear transfer and pluripotent transcript factor transfection. The reprogramming efficiency was evaluated by cloned blastocyst formation and IPS cell establishment.

MATERIALS AND METHODS: Nuclear transfer technology was performed to produce cloned embryos, and IPS cell lines were obtained from the three same somatic cell lines. The arrested developed embryos, primary colonies were collected. SNP methods were used to analyze the karyotyping and real-time PCR method were used to analyze the apoptosis-related gene expression.

RESULTS: Our results showed that aneuploidy induced by nuclear transfer is a key factor in the development failure of human cloned blastocysts and resulted primary colonies, and expression patterns of apoptosis-related genes are dynamically altered. Overall, ~20%–53% arrested primary colonies in IPS cells displayed aneuploidy, and unprogrammed of p53 and Bax genes occurred in all arrested primary colonies. When somatic cells with pre-existing chromosome mutations were used as donor cells, no cloned blastocysts were obtained and additional chromosome mutations were detected in the resulting IPS cells, which was not observed in the two IPS cell lines reprogrammed from somatic cells with normal karyotype. In conclusion, aneuploidy induced by the reprogramming process restricts the derivation of pluripotent stem cells, and more importantly, pre-existing chromosome mutations enhance the risk of genome instability, which limits their clinical utility.

Supported by: This work was Supported in part by the Ministry of Science and Technology of China Grants (973 program: 2011CB944504 and...
DEFINING DIFFERENTIATED METHYLATION REGIONS ESPECIALLY FOR PLURIPOTENCY ACQUISITION AND MAINTENANCE IN HUMAN STEM CELL VIA HIGH PROBE DENSITY MICROARRAY. Y. Fan, W. He, X. Sun. The Third Affiliated Hospital of Guangzhou Medical University, Guangzhou, Guangdong, China.

OBJECTIVE: Epigenetic regulation is a critical event in the maintenance of human pluripotent stem cells. It had been shown that pluripotent stem cells, such as embryonic stem cells and induced pluripotent stem cells, appeared hyper-methylated status compared to differentiated cells. However, epigenetic mechanisms of “stemness maintaining” and “reprogramming” remain confused since the limitation of former detection platform.

MATERIALS AND METHODS: we determined the DNA methylation profiles of 12 human cell lines, including 2 ESC lines, 4 virally-delivered iPS lines, 2 episcopal-delivered iPS lines, and 2 parent cell lines that iPSs were derived from using Illumina’s Infinium HumanMethylation450.

RESULTS: The iPSCs exhibited a hypermethylated status similarly to ESCs but with distinct differences from the parent cells. Genes of common methylation pattern between iPSCs and ESCs were regarded as critical factors for stemness, whereas differences existing between iPSCs and ESCs implied that iPSCs partly retained the parental characteristics and gained de novo methylation aberrations during cell reprogramming. No significant variant was identified between virally- and episcopal-systems. Based on microarray platform of higher probe density and greater genome coverage, de novo differentiated methylation regions as the signature of particular stem cell lines were emerged in detail.

CONCLUSION: This study figure out DNA methylation profiles of human iPSCs generated in virally- and episcopal-system, the responding somatic cells as well as hESCs. Series of ss-DMRs and ES-iPS-DMRs were defined in a high resolution. Knowledge of epigenetic information could be used as a signature for “stemness” and “self-renewal” and provided potential of selecting optimum pluripotent stem cells for human regenerative medicine.

TROPHOBLAST CELLS GENERATED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS (iPSC) DERIVED FROM UMBILICAL CORD BY USING BMP4 CAN BE DISEASE MODELS FOR EARLY PLACENTAL DISORDERS. M. Amita, b T. Ezashi, a L. C. Schulz, a, b D. J. Schust, a R. M. Roberts, b Obstetrics, Gynecology, Yamanagata University Faculty of Medicine, Yamagata, Japan; bBond Life Sciences Center, University of Missouri, Columbia, MO; University of Missouri, Columbia, MO.

OBJECTIVE: Poor placentation in early pregnancy leads to placental pathogenesis, such as preeclampsia (PE). Understanding the pathogenesis of the disease has been hampered by inaccessible to early stage placental tissue where the initial causal changes take place. Recently we reported that human embryonic stem cells (hESC) treated with inhibitors of activin A (A83-01) and FGF2 (PD173074) signaling in presence of BMP4 (termed BMP4/BAP) differentiate unidirectionally into trophoblast (TB) cells, which progress to the EVT stage, where the initial causal changes take place. Recently we reported that both the V lines and P lines treated with BMP4/BAP expressed TB markers. FACs analysis revealed that KRT7 positive cells had emerged more slowly in V lines treated with BMP4 than in P lines by d 2, whereas no differences were observed in response to BAP, with almost all cells converting to KRT7+ by d 2. BAP also drove differentiation of both lines to TB without expression of mesoderm markers.

CONCLUSION: iPSC from umbilical cords of newborn babies treated with BAP, differentiate into TB cells and provide a potential model system to uncover the mechanisms of PE.

MAINTENANCE OF FUNCTIONAL EMBRYO BODY IN CRYOPRESERVED, MICROFLUIDIC CHIPS: A PLATFORM FOR PERSONALIZED MEDICINE. R. M. Anchan, a S. Guven, b J. Lindsey, a M. Nickerson, a,b S. Chinthala, a B. Gerami-Naini, c U. Demirci, d, e Center for infertility and Reproductive Surgery, Obstetrics, Gynecology and Reproductive Biology, Brigham and Women’s Hospital, Harvard Medical School, Boston, MA; aBio-Acoustic-MEMS in Medicine Laboratories, Division of Biomedical Engineering, Department of Medicine, Brigham and Women’s Hospital, Harvard Medical School, Boston, MA; dDepartment of Oral and Maxillofacial Pathology, Oral Medicine and Craniofacial Pain, School of Dental Medicine, Tufts University, Boston, MA; eHarvard-MIT Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, MA.

OBJECTIVE: Employ microfluidic cassettes as a novel platform for long-term culture and cryopreservation of functional, differentiated mouse embryoid bodies.

DESIGN: Basic Research Study.

MATERIALS AND METHODS: Embryoid bodies (EBs), grown in suspension from mouse embryonic stem cells (ESCs), were embedded in Matrigel-coated channels with a constant 1 µl/min flow of culture media for 21 days. EB viability, differentiation, and functionality were assessed as measures of the culture system’s efficacy. Viability was assessed with Live/Dead stains and BrdU proliferation assays. Differentiation was analyzed with immunocytochemistry (ICC) for markers of endoderm, ectoderm, and mesoderm, as well as ovarian tissue. Hormone synthesis served as an indicator of EB differentiation and functionality. Conditioned media collected over 24 hr interval period was assayed by ELISA for estradiol (E2), progesterone (P4), and testosterone (T) synthesis. We also slow-froze sealed microfluidic cassettes in isopropanol, thawed these, and measured viability and functionality of the EBs.

RESULTS: 1. EBs grown in microfluidic cassettes maintain long-term viability and proliferation after 21 days. 2. Differentiation of EBs in the microfluidic system was verified, as shown by ICC of cell markers from all three germ layers and expression of ovarian cell markers (inhibin, Cyp19a1, and AMHR). 3. Functional analyses show increasing synthesis of E2 (15 pg/ml on Day 1 to 31 pg/ml on Day 20). 4. Cryopreserved EB-laden microfluidic chips recovered upon thawing and continued hormone synthesis.

CONCLUSION: 1. Microfluidic culture of functional EBs is a promising system that can maintain EB viability, differentiation, and functionality, even after recovery from cryopreservation. 2. This system affords an opportunity to develop patient-specific cassettes of differentiated human ESCs that may be stored, used in drug testing, or harvested for hormones.

SUPPORT: NIH 1 R01 EB015776-01A1 (SG, RMA, UD), Michael Cassidy and Caroline Wang Stem Cell Research Fund (RMA).

ENVIRONMENTAL PHTHALATE EXPOSURE IS ASSOCIATED WITH LOW INTEREST IN SEXUAL ACTIVITY IN PREMENOPAUSAL WOMEN. E. S. Barrett, a L. E. Parlett, b S. H. Swan c Obstetrics and Gynecology, University of Rochester School of Medicine and Dentistry, Rochester, NY; cEpidemiology, Johns Hopkins University, Baltimore, MD; aPreventive Medicine, Icahn School of Medicine at Mount Sinai, New York, NY.

OBJECTIVE: Several studies have found that occupational exposure to high levels of endocrine disrupting chemicals can interfere with sexual
function in men. Here, our objective was to determine whether environmental exposure to phthalates, which interfere withtestosterone and estradiol activity, are associated with altered sexual function in women.

DESIGN: The Study for Future Families (SFF) measured urinary phthalate metabolite concentrations in pregnant women in four American cities. In addition, subjects completed questionnaires including items on sexual problems in the months prior to conception.

MATERIALS AND METHODS: We fit multivariable logistic regression models to examine two dimensions of sexual dysfunction (lack of interest in sexual activity and vaginal dryness) in relation to phthalate metabolite concentrations. Our primary models focused on three metabolites of diethylhexyl phthalate (DEHP), a phthalate ester with particularly potent endocrine-disrupting properties. Analyses were adjusted for covariates including age, parity, education, race, stress, and antidepressant use.

RESULTS: Of 360 subjects, 46 expressed lack of interest in sexual activity in the months prior to conception and 37 reported vaginal dryness. Women in the highest quartile of exposure to Mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP), a DEHP metabolite, had 2.58 (95% CI 1.33, 5.00) times the adjusted odds of reporting a lack of interest in sexual activity, and results were similar for mono-2-ethyl-5-oxohexyl phthalate (MEOHP; aOR: 2.56, 95% CI 1.32, 4.95), another DEHP metabolite. Similar, but non-significant, associations were seen for all other phthalate metabolites measured. Self-reported vaginal dryness was not associated with any phthalate metabolite concentration.

CONCLUSION: Our data suggest that environmental exposure to phthalates may be associated with reduced sexual interest in stored sperm. Better understanding how adult exposure to phthalates may affect reproductive health, including sexual function, is of utmost public health importance given that virtually all Americans show measurable levels of exposure.

Supported by: NIH K12 ES019852-01, ES01247, R21ES015509, R01ES09916.

P-322 Tuesday, October 21, 2014

A NEW PARADIGM FOR SEXUALLY TRANSMITTED INFECTION (STI) SCREENING IN THE HAMPTON ROADS COMMUNITY OF SOUTHEASTERN VIRGINIA.

T. D. Kimble,*,† Eastern Virginia Medical School, Norfolk, VA; *Planned Parenthood of Southeastern Virginia, Virginia Beach, VA.

OBJECTIVE: Southeastern Virginia has the highest rates of STIs in the State. With funding from the Elton John AIDS Foundation (EJAF), PPSEV screened 966 individuals in Hampton Roads. Objectives were to examine costs of the program compared to scheduling visits with providers; and compare time to notification of abnormal results.

DESIGN: Retrospective, Cohort Study.

MATERIALS AND METHODS: The study group was 966 patients seen in the EJAF program from January 2013 to December 2013. Screening was performed either by non-licensed staff at LGBT community events or by healthcare assistants in the office on a walk-in basis with no provider visit. Urine NAAT was used to screen for Gonorrhea/Chlamydia. Blood was collected for HIV and syphilis. Control group was 966 patients screened by traditional method of scheduling appointments with providers. We used the equal variance left tailed t-test to determine if the average of notification days for study group is shorter than controls. JMP® Pro 10.0.2.64-bit Edition (SAS Institute Inc.) was used.

RESULTS: EJAF program resulted in $10,731 savings. Study group were notified on an average of 4 days after testing and 5 days for controls. Both population means follow a normal distribution. The test of equal variance failed to reject the null hypothesis (Brown-Forsythe p-value = 0.4162 > 0.05). Equal variance t-test shows difference in time to notification between groups (t=43.92, df=84, p-value<0.05 95% CI [-12.0,30]). Estimate of this difference is 0.6. Study group did not experience a longer time to notification of abnormal results than control group.

CONCLUSION: There were decreased costs in the EJAF program having non-licensed staff do STI screening and counseling compared to the traditional method of an in-office appointment with a provider. The total savings were $24/participant. Participants in the EJAF program were notified of positive results 24 hours quicker. This could expedite treatment and decrease transmission.

Supported by: This program was generously funded by the Elton John AIDS Foundation.

P-323 Tuesday, October 21, 2014

CARDIOVASCULAR ANALYSIS IN PATIENTS WITH ERECTILE DYSFUNCTION (ED).

P. Khandge,*,† J. V. DiTrollo,*,† M. LaSalle,† Rutgers New Jersey Medical School, Newark, NJ; *St Barnabas Medical Center, Livingston, NJ.

OBJECTIVE: The Cardiovascular system, a new non-invasive test of peripheral vasculature function, allows physicians to attain an accurate representation of the peripheral vasculature and autonomic nervous system (ANS) in patients with ED.

DESIGN: Prospective trial of 40 patients presenting to a single urologist.

MATERIALS AND METHODS: Patients were evaluated with a 3 minute evaluation performed with the patient sitting with a pulse oximeter type device applied to the finger. Height, weight, blood pressure and abdominal circumference were measured. Information regarding age and comorbidities such as hypertension (HTN), diabetes mellitus, ED and smoking status was collected. The report gives the biological arterial age compared to the patient’s chronological age in the form of a “wave type”. Wave type scores range from 1-7 with 1 indicating arteries with minimal atherosclerosis and 7 indicating a high degree of atherosclerosis and decreased compliance. The analysis of ANS also gives scores indicating mental stress, physical stress and stress resistance (ability to cope with stressors). Scores from results were analyzed for potential association using the Student’s T-test.

RESULTS: Men with ED were found to have a higher wave type in comparison with men without ED (p < 0.01). Also, men with ED and co-morbidites such as HTN or diabetes had a higher wave type when compared to men without ED diagnosed with either HTN or diabetes (p < 0.01). The evaluation found that former smokers with ED had a higher wave type and therefore biologically older arteries than those men with ED who were non-smokers (p < 0.05). When comparing men with ED based on abdominal circumference that were diagnosed with either HTN or DM, there was a substantial decrease in their stress resistance (p < 0.01) with an abdominal circumference > 40 inches.

CONCLUSION: The ability to evaluate the vascular system quickly and non-invasively gives the urologist a more complete evaluation of the ED patient. Our results demonstrated the device’s ability to determine if patients with ED were affected by either the autonomic nervous system or the status of the vasculature. This device allows for an improved and appropriate treatment selection in a cost effective manner. Lifestyle modifications, if indicated by results, can be emphasized with initial treatment followed by efficient re-assessment of disease progression. This system deserves further evaluation.

MENTAL HEALTH

P-324 Tuesday, October 21, 2014

STRESS AND ANXIETY ON EMBRYO TRANSFER DAY DO NOT AFFECT IN VITRO FERTILIZATION (IVF) OUTCOME.

L. Leis,* C. E. Busso,* G. Zampieri,† J. B. Soares,‡ S. Glina,§ B. S. Oliveira,‖ R. Wonchockier,‖ C. Velloso,§ N. E. Busso,* Projeto Alfa- Assisted Reproduction, São Paulo, SP, Brazil; †Salomo Zoppo Diagnoses, São Paulo, SP, Brazil.

OBJECTIVE: To evaluate whether stress and/or anxiety, at the moment of embryo transfer, affect IVF outcome.

DESIGN: Prospective cohort study.

MATERIALS AND METHODS: Eighty five women undergoing IVF/ICSI (intracytoplasmic sperm injection) treatment, up to 35 years-old and with good prognosis for conception (between 5 and 20 oocytes recovered, sperm collected by masturbation and at least one good quality embryo for transfer) were selected.

As measuring instruments for stress we used Lipp Inventory of Stress and a salivary cortisol assay. To evaluate anxiety we used IDATE State Anxiety Inventory. Inventories were applied a few minutes before embryo transfer. Saliva samples for cortisol assay were collected right after embryo transfer.

Statistical analysis of salivary cortisol results was done using generalized linear model with distribution gamma and link identity. The analysis of anxiety and stress inventories were done using student t test for means of two populations with equal variance and chi-squared test, respectively.

RESULTS: Five patients (5.8%) presented with high anxiety levels. Sixteen patients (18.8%) presented stress symptoms and 15 patients (17.6%) had salivary cortisol levels above reference values. However, no statistical differences were found in salivary cortisol averages between patients
who did and did not get pregnant (P=0.846). No statistical differences were found between the state of anxiety and the occurrence of pregnancy (P=0.695), nor between the presence of stress and the occurrence of pregnancy (P=1.000).

CONCLUSION: Anxiety and stress, at the moment of embryo transfer, do not seem to interfere on pregnancy rates in IVF/ICSI cycles. These results could help patients to overcome the feeling of guilt related to stress and anxiety, very common in these treatments.

P-325 Tuesday, October 21, 2014
DOES FERTILITY KNOWLEDGE AFFECT LIFE PLANNING AND CHILDBEARING? E. Maeda, 1 F. Nakamura, 1 J. Boivin, 2 H. Sugimori, 1 H. Saito, 2 1Graduate School of Medicine, the University of Tokyo, Bunkyo, Tokyo, Japan; 2School of Psychology, Cardiff University, Cardiff, South Wales, United Kingdom; 3Graduate School of Sports and Health Sciences, Daito Bunka University, Higashimatsuyama, Saitama, Japan; 4National Center for Child Health and Development, Setagaya, Tokyo, Japan.

OBJECTIVE: To investigate whether fertility knowledge affects life planning and successful childbearing.

DESIGN: Cross-sectional study.

MATERIALS AND METHODS: Given the fertility decline in developed countries and the importance of fertility knowledge, we conducted an online survey in Japan (n=4,946). In Study 1, we analyzed the data from participants aged 18 and 24 years of age who had children or hoped to have children in the future (n=403) and compared participants’ scores on the Japanese version of the Cardiff Fertility Knowledge Scale (CFKS-3 J) depending on their consideration of the ideal age to start a family (considered vs. did not consider). In Study 2, we tested the relation between childbearing and past fertility knowledge in participants between 55 and 40 years of age (n=370).

RESULTS: In Study 1, women who had thought about their ideal age for starting families had significantly more fertility knowledge than women who did not consider their ideal childbearing age (47.2 vs. 39.0, P=0.005). In Study 2, the OR of past fertility knowledge to having children was 5.23 (p=0.016) in women. Study 3 showed that women who knew about age-related infertility during their 20s were less likely to give birth to their first child at age 35 or later (0% vs. 13.7%; p=0.007). Across studies, there was not a significant relation between fertility knowledge and life planning or childbearing in men.

CONCLUSION: Our findings show that fertility knowledge could help women to successfully have children, although causality was inconclusive due to the study design. These findings will provide policy-makers with important background to guide educational initiatives.

Supported by: This study was supported by National Center for Child Health and Development, Seiiku Medical Study Grant (24-6).

P-326 Tuesday, October 21, 2014
IMPROVEMENT IN HEALTH RELATED QUALITY OF LIFE (HRQOL) FOLLOWING WEIGHT LOSS AND/OR NORMALIZATION OF HYPERANDROGENISM WITH CONTINUOUS ORAL CONTRACEPTIVES (OC) IN WOMEN WITH PCOS. L. Milman, 1 A. Dokras, 2 K. C. Allison, 1 D. B. Sarwer, 2 C. Coutifaris, 2 P. M. Kris-Etherton, 2 A. R. Kuszelnick, 2 C. Sletter, 1 W. Dodson, 3 R. S. Legro, 2 Obstetrics/Gynecology, University of Pennsylvania, Philadelphia, PA; 3Obstetrics/Gynecology, Hershey Medical Center, Hershey, PA.

OBJECTIVE: To examine if HRQOL improves with clinically significant weight loss, normalization of hyperandrogenism with continuous oral contraceptives(OC) or both in women with PCOS.

DESIGN: Randomized, 2-site study of women (n=133) with PCOS(BMI 27-42 kg/m^2) who received 1 of 3 interventions for 16 weeks including Lifestyle (LS) consisting of caloric restriction with the use of meal replacements, use of a weight loss medication, and increased physical activity, OC, and OC+Lifestyle (COMB).

MATERIALS AND METHODS: Validated measures of HRQOL(SF-36) and disease specific quality of life(PCOSQ) were completed at baseline & after 16 weeks of treatment. Linear regression was used to assess differences in outcomes within and between the treatment arms.

RESULTS: The mean % weight loss was 6.2±3.9, 1.3±3 and 6.6±4.1 and % decline in testosterone (T) levels was 0.9±28.8, 51.3±27 and 52±29.8 in the LS, OC and COMB groups respectively. On the PCOSQ survey the baseline scores in the weight and infertility domains were the lowest in all 3 groups. After 16 weeks of treatment, there were significant improvements in 3 of 5 domains (weight, infertility, and menstrual symptoms) within all 3 groups(p<0.01), emotion and body hair domains improved in the OC and COMB groups(p≤0.01). Physical well-being scores improved in all 3 arms(p<0.05), general well-being scores improved in the LS and COMB groups(p<0.01) and emotional well-being scores improved only in the COMB group(p<0.03). The COMB group had more improvement in weight scores than the other 2 groups(p<0.02). The correlation between change in BMI and weight domains was significant in the LS and COMB group (p<0.05). There was no correlation between change in T and body hair scores. On the SF-36, women in all three groups reported significant improvements in general health(p<0.05); OC and LS groups reported improvements in vitality(≥0.05); and the OC group reported improvements in emotional role, bodily pain and overall mental health(≥0.05). There was no significant difference across the three groups.

CONCLUSION: Women with obesity and PCOS treated with an intensive weight loss intervention reported significant improvements in most domains of disease-specific QOL as well as general health related QOL. In addition, physical and some mental domains improved with the use of continuous OCs alone, a common therapy in PCOS.

Supported by: This project was supported by the Eunice Kennedy Shriver National Institutes of Child Health and Human Development, National Center for Research Resources, and the National Center for Advancing Translational Sciences at the National Institutes of Health, through Grants R01 HD056510, UL1 TR000127 and U54 HD29834 (UVA Core Ligand Assay Core of the Specialized Cooperative Centers Program in Reproduction). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

P-327 Tuesday, October 21, 2014

OBJECTIVE: To investigate the levels of psychological distress in Korean infertile women, and to investigate the correlation between the psychological distress and fertility quality of life (Ferti-Qol).

DESIGN: Prospective cohort study.

MATERIALS AND METHODS: One hundred eleven infertile women and 64 healthy women participated in this study. Psychological distress (emotional depression, anxiety, stress) of them was measured by DASS 42, and quality of life (mind-body, emotional, social, relational scores) of infertile women was evaluated by Ferti-Qol inventory. The participants voluntarily performed the on-line psychological tests prepared on the internet homepage of Mizmedi hospital.

RESULTS: The mean age of participants was 35.4±4.3 years and years of marriage was 5.2±4.3. Scores of emotional depression (13.8±8.5), anxiety (10.8±6.9) and stress (18.4±8.7) of infertile women were statistically significantly higher than the scores of fertile women (9.5±8.4), anxiety (6.8±6.8), and stress (12.6±7.7, P<0.001) of fertile women. Scores of mind-body (47.4) and relational (61.4) subscales in Korean infertile women were lower than those (54.8 and 68.7) in western infertile women. According to the increase of patient’s age, psychological distress was reduced but relational score (relative to husband) was increased. Significant negative correlation was found between DASS scores (depression, anxiety and stress) and Ferti-Qol subscales (mind-body, emotional, social).

CONCLUSION: This study found that the negative correlation between quality of life as measured by Ferti-Qol and psychological distress as measured by DASS 42. This is the first validation study of Korean Ferti-Qol. These data serve an valuable information about psychological distress for infertile women and suggest an importance of psychological counseling for infertile women.
DOES SELF-REPORTED, INFERTILITY-ASSOCIATED ANXIETY CORRELATE WITH PERCEIVED PAIN FOLLOWING OOCYTE RETRIEVAL DURING IN VITRO FERTILIZATION? S. Arian, a K. McKnight, a C. Sams, a L. McKenzie, a T. Hickman, a The University of Texas Health Sciences Center at Houston, Houston, TX; bHouston IVF, Houston, TX.

OBJECTIVE: To determine if women reporting increased baseline anxiety prior to infertility treatment reported more perceived pain following transvaginal oocyte retrieval for in vitro fertilization.

DESIGN: A prospective cohort design with 157 women undergoing IVF at our institution between November 2013 and March 2014.

MATERIALS AND METHODS: Women aged 21-45 years, who underwent IVF, were included. Age, parity, and duration of infertility were obtained from patients’ charts. Patients not reporting baseline anxiety scores were excluded. Patients self-reported baseline anxiety levels (0 - 10 scale) as it pertained to infertility at the initial consultation prior to treatment. All patients received conscious sedation and analgesia with intravenous midazolam, fentanyl, and propofol during oocyte retrieval. Following retrieval, patients rated their pain on a 0 to 10 visual analog scale at 0, 5, 10, 15, and 30 minutes post-procedure. Post-procedure pain scores in women with baseline anxiety scores ≥ 5 (high-anxiety group) were compared to those reporting scores < 5 (low-anxiety group) at each time point. Proportion of women requiring post-operative analgesics was also compared. A Mann Whitney U test was used to compare pain scores in each group for each time point. Chi square analysis was used to evaluate post-operative analgesic use between groups.

RESULTS: 157 women were included in the study, 112 with baseline anxiety scores ≥ 5 and 45 women with scores < 5. No significant differences were seen in reported pain scores between the high and low-anxiety groups at 0, 5, and 10 minutes following oocyte retrieval. Women in the high-anxiety group reported increased pain at 15 and 30 min following retrieval (P = 0.01) [Table 1]. 48.2% of high-anxiety women required pain medication after the procedure, compared with 28.9% in the low-anxiety group (P = 0.03).

CONCLUSION: Women with higher baseline anxiety surrounding infertility reported more perceived pain beginning 15 minutes after oocyte retrieval and were more likely to require analgesics post-procedure. These women may benefit from empiric oral or intravenous analgesic medication immediately after retrieval.

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Anxiety</td>
<td>0.85 ± 1.98</td>
<td>2 ± 2.28</td>
<td>2 ± 2.34</td>
<td>2 ± 2.14</td>
<td>2 ± 1.86</td>
</tr>
<tr>
<td>Low Anxiety</td>
<td>0.62 ± 1.39</td>
<td>1 ± 1.57</td>
<td>1.11 ± 1.64</td>
<td>1.31 ± 1.83</td>
<td>1.07 ± 1.62</td>
</tr>
<tr>
<td>P-value</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

NS= Nonsignificant

P-330 Tuesday, October 21, 2014

DOES THE HISTORY OF INDUCED ABORTION AFFECT THE OUTCOMES OF ASSISTED REPRODUCTION? E. V. Espirito Santo, a J. B. A. Oliveira, a C. G. Petersen, b,a A. L. Mauri, a F. C. Massaro, a M. Cavagna, a,b R. L. R. Baruff, b,c 1 G. J. Franco, Jr., a,b,c Center for Human Reproduction Prof. Franco Jr, Ribeirão Preto, Sao Paulo, Brazil; bPaulista Center for Diagnosis Research and Training, Ribeirão Preto, Sao Paulo, Brazil; cWomen’s Health Reference Centre, Perola Byington Hospital, Sao Paulo, Brazil.

OBJECTIVE: Particularly in countries where it is forbidden, it is not uncommon for some type of sequelae to appear after an abortion is induced, which leads to difficulties in getting pregnant. However, women often experience guilt after undergoing an induced abortion. This guilt may resurface, sometimes more strongly, if the woman presents fertility problems. In fact, many women attribute treatment failures to some type of "punishment". To try to quantify the effects of IVF/ICSI cycle in women with and without a history of induced abortion.

DESIGN: Matched control study.

MATERIALS AND METHODS: A total of 276 couples submitted to IVF/ICSI were divided into two groups matched by female age according to the women’s history:

- with at least one induced abortion (n = 138)
- without history of induced abortion (n = 138)

Patients with endometrial pathologies or hydrosalpinx were excluded. The procedures were performed under the same laboratory conditions for both groups. The cumulative results from fresh and frozen cycles were analyzed.

RESULTS: There was no significant difference in the number and quality of embryos transferred between the two groups. No differences in clinical outcomes after IVF/ICSI were observed between the groups with or without a history of induced abortion. Table 1 summarizes the results.

Table 1: Results

<table>
<thead>
<tr>
<th>Induced abortion</th>
<th>yes</th>
<th>no</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women age(years)</td>
<td>37.4±4.4</td>
<td>37.4±4.4</td>
<td>0.98</td>
</tr>
<tr>
<td>Men age(years)</td>
<td>41.0±7.1</td>
<td>41.0±7.4</td>
<td>0.95</td>
</tr>
<tr>
<td>Induced abortions (n)</td>
<td>1.2±0.4</td>
<td>1.2±0.4</td>
<td></td>
</tr>
<tr>
<td>Transfers(n)</td>
<td>2.2±1.6</td>
<td>2.0±1.2</td>
<td>0.32</td>
</tr>
<tr>
<td>Pregnancy rate/transfer</td>
<td>20.1%</td>
<td>18.0%</td>
<td>0.60</td>
</tr>
<tr>
<td>Pregnancy rate/patient</td>
<td>44.2%</td>
<td>35.5%</td>
<td>0.17</td>
</tr>
<tr>
<td>Miscarriage rate</td>
<td>22.9%</td>
<td>18.4%</td>
<td>0.72</td>
</tr>
<tr>
<td>Patient with at least on live birth</td>
<td>33.3%</td>
<td>33.3%</td>
<td>1.0</td>
</tr>
</tbody>
</table>
CONCLUSION: Induced abortion does not appear to be an important factor that influences the clinical outcomes of IVF. Psychological sequelae following induced abortion may be responsible for the exacerbated negative emotional feelings that patients experience with treatment failure.

MENOPAUSE

P-331 Wednesday, October 22, 2014


OBJECTIVE: Vasomotor symptoms are prevalent in surgically menopausal women. Other than a beneficial role for HRT, little is known about other factors influencing vasomotor symptoms in surgically menopausal women.

DESIGN: Cross-sectional analysis of 599 women with BRCA mutations after RRSO.

MATERIALS AND METHODS: BRCA carriers were recruited through national advocacy groups and the Cancer Risk Evaluation Program at Penn. Online questionnaires assessed demographic data, medical history, and depression/anxiety (Hospital Anxiety Depression Scale). A validated questionnaire (Greene Scale) was utilized to assess hot flashes and night sweats. Wilcoxon rank sum and X2 were utilized for univariate comparisons, and logistic regression models were constructed to evaluate associations between vasomotor symptoms and other variables (reported as adjusted odds ratios, AOR).

RESULTS: 67% and 61% of women reported hot flashes and night sweats, respectively. 22% reported current use of systemic HRT. Hot flashes and night sweats were less common in HRT users. Hot flashes were significantly more common with younger age, recent RRSO, obesity, depression, anxiety, antidepressant use, and selective estrogen receptor modulator (SERM) use. Night sweats were more common with recent RRSO, obesity, depression, anxiety, and SERM use. In multivariable models, HRT use was strongly protective against hot flashes, while younger age, obesity, anxiety, antidepressant use, and SERM use were associated with higher odds of hot flashes. Odds of night sweats were decreased with HRT use and increased with recent RRSO, obesity, and depression. The results were similar after restricting the analysis to women not using HRT.

<table>
<thead>
<tr>
<th>Hot flashes</th>
<th>AOR (95%CI)</th>
<th>p value</th>
<th>Night sweats</th>
<th>AOR (95%CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRT use</td>
<td>0.41 (0.26-0.63)</td>
<td>&lt;0.01</td>
<td>HRT use</td>
<td>0.47 (0.31-0.71)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Age&lt;50</td>
<td>1.72 (1.17-2.55)</td>
<td>&lt;0.01</td>
<td>RRSO within 2 yrs</td>
<td>1.44 (1.02-2.03)</td>
<td>0.04</td>
</tr>
<tr>
<td>Overweight</td>
<td>1.49 (0.97-2.30)</td>
<td>0.07</td>
<td>Overweight</td>
<td>1.12 (0.76-1.67)</td>
<td>0.57</td>
</tr>
<tr>
<td>Obese</td>
<td>1.70 (1.05-2.80)</td>
<td>0.04</td>
<td>Obese</td>
<td>1.83 (1.15-2.94)</td>
<td>0.01</td>
</tr>
<tr>
<td>Anxiety</td>
<td>1.97 (1.9-3.26)</td>
<td>&lt;0.01</td>
<td>Depression</td>
<td>1.70 (1.08-2.69)</td>
<td>0.02</td>
</tr>
<tr>
<td>Antidepressants</td>
<td>2.8 (1.41-5.37)</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SERM use</td>
<td>2.89 (1.48-5.64)</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CONCLUSION: HRT use remains the strongest predictor of vasomotor symptoms after surgical menopause though utilization is low in this population. Our findings highlight the importance of lifestyle modification, optimizing medical approaches, and addressing mental health concerns to improve quality of life.

Supported by: Basser Research Center for BRCA (SD); NIH T32 HD007440 (LJ); NIH SK12HD01265-14 (SS); Susan G. Komen SAC10003 (SD); NIEHS 5P30ES013508-07 (SB).

FERTILITY & STERILITY® e249

P-332 Wednesday, October 22, 2014

ASSOCIATION BETWEEN POLYMORPHISMS IN PERIOD (PER) GENE AND BONE RESPONSE TO HORMONE THERAPY IN POST-MENOPAUSAL KOREAN WOMEN. J. G. Kim, H. Kim, S.-Y. Ku, Y. M. Choi, H. J. Kim.

OBJECTIVE: To explore the association between polymorphisms in PER gene and bone response to hormone therapy (HT) in postmenopausal Korean women.

DESIGN: Prospective study.

MATERIALS AND METHODS: In tertiary university hospital, five hundred and nine postmenopausal women received sequential estrogen plus progestogen therapy. The PER1 c.2284C>G, c.2247T>C, PER2 c.3731G>A, PER3 c.2592G>A, c.3083T>C polymorphisms, and 54bp variable number of tandem repeat VNTR were analyzed. BMD at the lumbar spine and femoral neck before and after 1 year of HT and serum levels of osteoprotegerin (OPG), soluble receptor activator of the nuclear factor-sB ligand (sRANKL) and bone turnover markers were measured after 6 months of HT.

RESULTS: Among SNPs measured, the PER c.2884 C>G polymorphism and PER3 54bp VNTR were associated with annual percent changes in BMD of femoral neck after 1 year of HT (P<0.05). Changes in BMD at femoral neck in the non-CG genotype of the PER c.2884 C>G polymorphism and in the 4 repeat homozygote of PER 34bp VNTR were significantly lower than those in CC genotype and non-4 repeat homozygote respectively. When a non-respondor was defined as a woman who had lost more than 3% of BMD per those years after HT, the PER1 c.2884 C>G polymorphism only was associated with the risk of non-response of HT. The non-CG genotype of the PER c.2884 C>G polymorphism showed a 1.92-times higher risk of non-response at the lumbar spine and/ or femoral neck (P=0.11), as compared with the CC genotype. However, no significant changes in bone markers after 6months of HT were noted according to the PER1 c.2884 C>G polymorphism.

CONCLUSION: The PER c.2884 C>G polymorphism may be associated with risk of non-response to HT in postmenopausal Korean women.

Supported by: This research was Supported by Basic Science Research Program through the National Research Foundation of Korea(NRF) funded by the Ministry of Education, Science and Technology (2012000119).

P-333 Wednesday, October 22, 2014


OBJECTIVE: To estimate whether intima-media thickness (IMT) of carotid artery is associated with serum estradiol (E2) level in postmenopausal women.

DESIGN: A retrospective study.

MATERIALS AND METHODS: We performed a doppler ultrasonography of the carotid artery and measured intima-media thickness (IMT) in 124 postmenopausal women who did not take postmenopausal hormone therapy. Women were divided into two groups according to the IMT (< 1.0mm, n = 59 and ≥ 1.0 mm, n = 65). Serum estradiol (E2) level, lipid profile, bone mineral densities (BMD) of the lumbar vertebrae and femoral neck, current statin treatment status, and other cardiovascular risk factors were analyzed in these two groups.

RESULTS: Compared to women with higher IMT (≥ 1.0mm), women with lower IMT (< 1.0mm) had a significantly higher level of serum E2 (23.01 ± 7.08 vs. 8.47 ± 1.25 pg/ml, P = 0.04), lower tendency of BMI, mean age, time to since menopause (23.48 ± 0.33 vs. 24.14 ± 0.44 kg/cm2, 56.19 ± 0.76 vs. 58.00 ± 0.84 years, 6.59 ± 0.77 vs. 8.34 ± 0.88 years) and were significantly less likely to have dyslipidemia (15.3 % vs. 32.3 %, P = 0.03). Women with IMT < 1mm were also more likely to have a serum E2 level greater than 20 pg/ml (13.6 % vs. 4.6 %, P = 0.08). After adjusting risk factors by multiple logistic regression analysis, women with a higher serum E2 level were less likely to have a higher IMT. (OR 0.97, 95% confidence interval: 0.95 to 1.00, P = 0.08, borderline significant).

CONCLUSION: Postmenopausal women with a higher serum E2 level were less likely to have a higher IMT of carotid artery. This study suggests that a higher level of endogenous E2 might lower IMT of carotid artery in postmenopausal women.
FEMALE REPRODUCTIVE ENDOCRINOLOGY

P-334 Wednesday, October 22, 2014

CREAM INGESTION PROMOTES THE EXPRESSION OF TOLL-LIKE RECEPTOR-2 (TLR-2) AND MATRIX METALLOPROTEINASE-2 (MMP-2) IN POLYCYSTIC OVARY SYNDROME. F. González, R. V. Considine, S. L. Pardue, A. J. Acton. Obstetrics and Gynecology and Internal Medicine, Indiana University School of Medicine, Indianapolis, IN.

OBJECTIVE: Lipid-stimulated oxidative stress and circulating heat shock protein 70 (Hsp70) are increased in Polycystic Ovary Syndrome (PCOS). Hsp70 is induced by oxidative stress and can bind the TLR-2 receptor to stimulate proatherogenic inflammation. MMP-2 promotes atherosclerotic plaque rupture. We examined the effect of cream ingestion on serum Hsp70 and TLR-2 and MMP-2 protein content in women with PCOS compared with ovulatory controls; and its relation to the ovarian androgen response to HCG administration and abdominal adiposity (AA).

DESIGN: Cross sectional study

MATERIALS AND METHODS: We studied 16 women with PCOS (8 lean, 8 obese) between ages 18-40, diagnosed on the basis of oligomenorrhea and hyperandrogenemia, and 16 ovulatory controls (8 lean, 8 obese) of similar age. Subjects ingested 100 ml of dairy cream and received a 5000 IU IM HCG injection within 5-8 days of menses. Serum Hsp70 was measured by ELISA and TLR-2 and MMP-2 protein content were quantified by Western blotting in mononuclear cells (MNC) isolated from blood samples drawn while fasting and 2 hours after cream ingestion. Androgens were measured from blood samples drawn at 0, 24, 48 and 96 hours after HCG administration. AA was defined as the % ratio of truncal fat to total body fat measured by DEXA.

RESULTS: Compared with lean controls, obese controls and lean and obese women with PCOS exhibited a greater change from baseline in Hsp70 (-1.11.6±4.2 vs. 2.4±1.9, p<0.002) and MMP-2 (-1.4±3.3 vs. 11.2±2, 15±3, 19±4 %; p<0.0001). Compared with weight-matched controls, women with PCOS exhibited a greater area under the curve (AUC) following HCG administration for testosterone (T) (lean: 693±715 vs. 4010±340, p<0.03; obese: 7079±1236 vs. 3173±853, p<0.007), and androstenedione (A) (lean: 471±30 vs. 317±26, p<0.0001; obese: 511±14 vs. 294±19, p<0.0001). Androgen AUC was positively correlated with the change from baseline in Hsp70 (T: r=0.42, p<0.05, A: r=0.41 p<0.05), TLR-2 (T: r=0.42, p<0.04, A: r=0.47, p=0.02) and MMP-2 (T: r=0.40, p<0.04, A: r=0.58, p<0.02), AA was also positively correlated with the change from baseline in Hsp70 (r=0.37, p<0.05), TLR-2 (r=0.42, p<0.03) and MMP-2 (r=0.41, p<0.03).

CONCLUSION: In PCOS, cream ingestion increases Hsp70, TLR-2 and MMP-2 independent of obesity. Lipid-stimulated inflammation may promote atherogenesis in PCOS. This phenomenon may be perpetuated by hyperandrogenism and excess abdominal adiposity.

Supported by: NIH grant HD048535 to FG.

P-336 Wednesday, October 22, 2014

SYNCHRONIZATION OF WOMEN’S CYCLES: A BIG DATA AND CROWDSOURCING APPROACH TO MENSTRUAL CYCLE ANALYSIS. P. Chenette a, C. Martinez. b "Pacific Fertility Center, San Francisco, CA; aGlowing, San Francisco, CA."

OBJECTIVE: The ubiquity of mobile devices offers a novel paradigm for fertility study, enabling data collection from thousands of users to provide an unprecedented view of menstrual cycles. Glow, a women’s health app available on iOS and Android platforms, collects menstrual cycle parameters from users. Over time a very large collection of cycles emerges, with the challenge of sifting data to provide meaningful analysis. In this phase one study, we studied the synchronization of cycles across a large sampling of users, and its relationship to the phase of the moon.

DESIGN: The Glow app was developed to track menstrual cycles, and to provide insights into health for women (www.glowing.com). Glow records data related to fertility and offers algorithms to predict optimal fertile periods. Women’s statements of the date menses began were compared across the population of Glow users.

MATERIALS AND METHODS: Glow was offered for free download to iOS and Android users beginning in Aug 2013. A daily calendar records menstrual cycle start, ovulation symptoms, and BTB. Cycles included for analysis were from users who had an average cycle length greater than 28 days in length and less than 31 days in length. Menses start date was recorded relative to the date of the nearest full moon (FM) and the distribution of daily menses start relative to all menses calculated. Start dates were grouped into 5-day ranges.

RESULTS: 39,541 cycles were selected for analysis from 8,233 users. The mean cycle length was 29.41 days, + 0.659. Menses start date was related to the phase of the moon, with a distribution that plateaued at 3.5 to 4.0% on days FM-11 through FM+3 then declined over 4 days to a lower plateau of 2.2-2.8% of cycles. Peak menses start date was FM-9 and nadir FM-14. Peak incidence (4.0%) exceeded nadir (2.2%) by 81.8%.

Menses start distribution relative to Full Moon (FM)

<table>
<thead>
<tr>
<th>Days from FM</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>-2 to 2</td>
<td>18.6%</td>
</tr>
<tr>
<td>3 to 7</td>
<td>16.8%</td>
</tr>
<tr>
<td>8 to 12</td>
<td>13.9%</td>
</tr>
<tr>
<td>13 to -13</td>
<td>13.2%</td>
</tr>
<tr>
<td>-12 to -8</td>
<td>18.9%</td>
</tr>
<tr>
<td>-7 to -3</td>
<td>18.8%</td>
</tr>
</tbody>
</table>

CONCLUSION: Menstrual cycles showed evidence of global synchronization in this dataset. There was a correlation of menses start to the phase of the moon, with most starting in a range of 11 days before to 3 days after the full moon. The big Data approach to menstrual cycle analysis provides unique and powerful insights into population fertility, and potent opportunities for future study of menstrual cycle dynamics, and patient counseling on fertility, and fertility avoidance.

ASRM Abstracts Vol. 102, No. 3, Supplement, September 2014
P.337 Wednesday, October 22, 2014

PREDICTORS OF A SUBSEQUENT DETECTABLE ANTI-MÜLLERIAN HORMONE (AMH) LEVEL AFTER AN UNDETECTABLE AMH LEVEL IN POPULATION-BASED COHORT. M. S. M. Lanham, C. Kim, C. Karvonen-Gutierrez, S. Harlow, J. Randolph, D. McConnell. University of Michigan, Ann Arbor, MI.

OBJECTIVE: To determine the effects of age, smoking, and reproductive exogenous hormone use on the odds of whether a woman with an undetectable serum AMH level will have a detectable AMH level in the future.

DESIGN: Population-based case-control study of longitudinally collected AMH levels.

MATERIALS AND METHODS: The Michigan Bone Health and Metabolism Study, a population-based longitudinal study with annual data ranging from 1992 to 1995, 1998 to 2002, and 2005 to 2009, included physical measures, health and social questionnaires, and serum evaluations. AMH assays were performed on 1886 samples from 391 women (Beckman-Coulter Gen II) if an individual woman had two available frozen serum samples collected prior to age 43 years. Women were eligible for this secondary analysis if they had an undetectable AMH level (<0.16 ng/ml, the level of quantitation of the assay), a subsequent AMH level, and no history of hysterectomy, oophorectomy, fertility medication use, or prior chemotherapy. Multivariable logistic regression models were used to examine predictors of a subsequent detectable AMH level, including age dichotomized at various levels in order to optimize model fit.

RESULTS: Of the 66 women who had an AMH level below the limit of detection (<0.16 ng/ml), 39 had subsequent serum available for AMH analysis; 18 of these women had at least one subsequent detectable AMH level. The subjects had between 1 and 9 levels drawn after the initial undetectable level. The age of first undetectable level ranged from 28 to 42 years, and dichotomizing at age 39 years optimized model fit. Predictors of a repeat level that was detectable included age under 39 years at the time of the initial undetectable level (OR 4.61, 95%CI 1.36 – 15.6, P = 0.0154), and increasing age at the time of the subsequent serum measure (OR 0.69 for each increased year of age, 95%CI 0.53 – 0.91, P = 0.0095). Associations with smoking status and exogenous reproductive hormone use at the time of the initial undetectable level or the subsequent level were not significant.

CONCLUSION: Women under age 39 who have an undetectable AMH level are more likely to have a subsequent detectable AMH level than those who are at or older than age 39, but the odds of a subsequent detectable level falls as women continue to age.

Supported by: Unrestricted Grant from Beckman-Coulter; DP3 DK098129; University of Michigan Department of Obstetrics and Gynecology, NIAMS, AR051384 – Change in Bone, Arthritis, Function: Hormone & Obesity.

P.338 Wednesday, October 22, 2014

ULTRASTRUCTURE OF PLACENTA OF GRAVIDAS WITH GESTATIONAL DIABETES MELLITUS. Q. Meng,1,a L. Shao,1,a Y. Luo,1,a Y. Mu,1,a W. Xu,1,a C. Gao,1,a L. Gao,1,a J. Liu,1,a Y. Cui.1,a 1The State Key Laboratory of Reproductive Medicine, Center of Clinical Reproductive Medicine, First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, China; 2Department of Obstetrics, Lianyungang Maternity and Child Health Care Hospital, Lianyungang, Jiangsu, China.

OBJECTIVE: Gestational diabetes mellitus (GDM) leads to an abnormal placental environment which may cause some structural alterations of placenta, and affect placentation development and function. Here we conducted the study to determine whether the structural alterations exist in GDM placenta.

DESIGN: In this study, the ultrastructural appearance of term placentas from women with GDM and normal pregnancy were meticulously compared.

MATERIALS AND METHODS: The placenta tissues of term birth from 10 women with GDM and 10 women with normal pregnancy were applied with the signed informed consent. The morphology of fetomaternal interface of placenta was examined using light microscopy (LM) and transmission electron microscopy (TEM).

RESULTS: On LM, the following morphological changes in villous tissues were found in the GDM placentas when compared with the control placentas: edematous stroma, apparent increase in the number of syncytial knots and perivillous fibrin deposition. On TEM, the distinct ultrastructural alterations indicating the degeneration of terminal villi were found in the GDM placentas as follows: thickening of the basal membrane (BM) of vasculosyncytial membrane (VSM) and the VSM itself, significantly fewer or even absent syncytiotrophoblastic microvilli, swollen or completely destroyed mitochondria and endoplasmic reticulum, and syncytiotrophoblasts with multiple vacuoles.

CONCLUSION: Ultrastructural differences exist between GDM and control placentas. The differences of placenta ultrastructure are likely responsible for the impairment of placental barrier and function in GDM.

Supported by: This work was Supported China 973 Program (2012CB8244703, 2012CB8244902), a project from State Key Laboratory of Reproductive Medicine, Nanjing Medical University (SKLRM-KF-1110), and Jiangsu Clinical Center Program (BL2012009), China. We sincerely thank Liling Zhou and Qin Lu for their help to prepare TEM samples.

P.339 Wednesday, October 22, 2014

PREGNANCY PROGNOSIS IN WOMEN WITH LOW AND EXTREMELY LOW SERUM ANTI-MÜLLERIAN HORMONE LEVELS. M. Fujita, K. Takahashi. Takahashi Women’s Clinic, Chiba, Japan.

OBJECTIVE: To estimate and compare the clinical and ongoing pregnancy rates in women with low (0.1–1.0 ng/ml) and extremely low (<0.1 ng/ml) serum anti-Müllerian hormone (AMH) levels after the infertility treatment, such as natural conception, intrauterine insemination (IUI) and in vitro fertilization (IVF).

DESIGN: Retrospective study.

MATERIALS AND METHODS: Serum AMH and follicle-stimulating hormone (FSH) levels were measured at initial clinic visits and prior to all following infertility treatment cycles in 134 women (385 cycles) with AMH levels < 1.0 ng/ml. The main outcome measures were clinical and ongoing pregnancy rates classified into two groups: low AMH (0.1–1.0 ng/ml) and extremely low AMH (<0.1 ng/ml). Statistical analyses were performed using the analysis of variance and chi-square test.

RESULTS: In the low AMH group, 113 patients underwent 349 cycles, whereas in the extremely low AMH group, 21 patients underwent 36 cycles. The age distribution of both groups was similar (mean ± SD = 39±3.4 vs 40±4.3, P = 0.106). In the extremely low AMH group, more patients had increased FSH levels (>10 mIU/ml) than in the low AMH group. With regard to clinical pregnancy rate, it was much higher in the low AMH group than in the extremely low AMH group (41.7% vs 14.3%, P = 0.016). In the low AMH group, a negative linear correlation existed between gravidity and patients’ age (r = -0.28). Ongoing pregnancy rates were not significantly different between the groups (21.7% vs 9.5%, P = 0.197). With regard to cycles, there was no significant difference between clinical and ongoing pregnancy rates between the two groups (15.8% vs 8.3%, P = 0.1 and 8.6% vs 5.6%, P = 0.53, respectively). Almost half of pregnant patients miscarried in both groups. In particular, for IVF treatment, clinical pregnancy rates were 22.7% in the low AMH group and 9% in the extremely low AMH group.

CONCLUSION: For patients in their thirties, low AMH still led to a 40–50% pregnancy rate after the infertility treatments; however, pregnancy rate decreased with increasing age. An early decision on treatment is therefore important. On the other hand, the extremely low AMH group had significantly lower pregnancy rates. The sample size was small; therefore, further investigation is needed to confirm the findings.

P.340 Wednesday, October 22, 2014

GnRH ANALOGS INHIBIT THE REGULATORY EFFECTS OF 17β-ESTRADIOL TREATMENT ON EXTRACELLULAR MATRIX PRODUCTION IN LEIOMYOMA 2D AND 3D CULTURES. J. L. Britten,a M. Malik,a J. Cox,a A. Patel, a J. Deng,a W. H. Catheroına, b Obstetrics and Gynecology, Uniformed Services University of the Health Sciences, Bethesda, MD. aProgram in Reproductive and Adult Endocrinology, Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, MD.

OBJECTIVE: The GnRH analogs cetrorelix acetate and leuprolide acetate have clinical efficacy in decreasing fibroid size and symptoms, and directly regulate extracellular matrix (ECM) production in 2-dimensional (2D) and 3-dimensional (3D) leiomyoma cell cultures. However, it is unknown whether the regulatory effects of GnRH analogs occur in the presence of estradiol (E2). Our objective in this study was to assess E2-mediated ECM expression and the impact when E2 was used in combination with cetrorelix acetate or leuprolide acetate.

DESIGN: Laboratory study.

MATERIALS AND METHODS: Leiomyoma cells grown in 2D and 3D cultures were exposed to E2 at 10^{-8} M alone and in combination with FERTILITY & STERILITY® e251
cetrorelix acetate (10^{-8}M) and leuprolide acetate (10^{-7}M and 10^{-6}M) for 72 hours. Western blot analysis was used to evaluate ECM protein production of both fibronectin and collagen-1A (COL1A).

RESULTS: The production of fibronectin in 2D cultures was increased following treatment with E2 (1.60+/−0.52-fold), but fibronectin expression was unchanged from controls when treated with either cetrorelix (1.2+/−0.23-fold) or leuprolide (0.85+/−0.24-fold). The production of fibronectin when exposed to combined treatment of E2 and cetrorelix in 2D cultures (1.4+/−0.37-fold) was compared to the level of controls, but the combination of E2 and leuprolide showed a greater than 2-fold decrease in fibronectin production (0.39+/−0.12-fold) compared to leuprolide-treated leiomyoma cells. In 3D cultures, E2 increased fibronectin protein production (2.2+/−0.16-fold). Treatment with cetrorelix (1.3+/−0.25-fold) or combined with E2 showed no significant change compared to controls (1.45+/−0.37-fold). However, leiomyoma cells in 3D culture exposed to leuprolide demonstrated a decrease in fibronectin production (0.78+/−0.18-fold). When E2 and leuprolide were combined, fibronectin concentrations approached untreated expression levels (1.4+/−0.10-fold). COL1A was markedly decreased in 2D cultures with leuprolide plus E2 treatment (0.3+/−0.14-fold). In 3D cultures, COL1A production showed a decrease with cetrorelix (0.64+/−0.15-fold), and E2 plus cetrorelix (0.76+/−0.17-fold).

CONCLUSION: GnRHa inhibits the stimulatory effect of E2 on ECM production. Our observations suggest a feasible use for GnRHa analogs as a localized therapy, thereby avoiding systemic side effects.

Supported by: This research was supported by Intramural grant from Uniformed Services University of the Health Sciences, QPSG13F and NICHD, NIH R21, HD070152-01A. The research was also supported, in part, by the intramural research Program in Reproductive and Adult Endocrinology, NIH.

P-341 Wednesday, October 22, 2014


OBJECTIVE: We established an in vivo xenograft model of human uterine leiomyoma (UL) in non-obese diabetic severe combined immunodeficient (NOD/SCID) interleukin-1L-2R-γ-null mice. In this model, a xenograft was grown beneath the kidney capsule by co-administration of estrogen (E) and progesterone (P); however, the low fecundity and high cost of the host together with the difficulty of the grafting procedure in the subrenal space have prohibited the use of this model as a research tool. Here, we modified the xenograft model using a severely immunocompromised host that is more suitable for UL research.

DESIGN: Experimental study.

MATERIALS AND METHODS: All protocols including animal experiments were approved by the IRB at Chiba University, NOD/SCID, SCID, and BALB/c nude mice were used as alternative hosts, where the subcutaneous (SC) space was considered as an alternative grafting space for the xenograft. We grafted xenografts, comprising 5 × 10^6 primary cultured UL or myometrial cells, into the subrenal and SC spaces in ovariectomized mice followed by E plus P administration. After 4–8 weeks, we evaluated the viability, volume, histology, and sex steroid receptor expression of xenografts in response to E plus P administration. The viability and volume of the xenograft were compared with those of NOD/SCID IL-2-R-γ-null mice. We further measured mRNA expression levels of 12 genes representative of UL in the xenograft to characterize the model.

RESULTS: The rate of successful subrenal UL xenograft transplantation was highest in NOD/SCID (55.1%), followed by SCID (33.3%) and nude mice (0%). The rate of successful UL xenograft transplantation in NOD/SCID mice was comparable with that in NOD/SCID IL-2-R-γ-null mice (56.3%). Although the implantation rates of subrenal and SC UL xenografts were similar in NOD/SCID mice (54.2% vs. 50.0%, respectively), the volume of subrenal UL xenografts was significantly larger than that of SC UL xenografts (3.07 ± 1.61 mm vs. 1.10 ± 0.43 mm^3, respectively). The volume of the xenograft comprising myometrial cells never increased. Xenografts reproduced the histology and maintained the expression of both estrogen and progesterone receptors, and showed similar expression patterns of the representative genes to the original UL tissue.

CONCLUSION: The modified UL xenograft model in NOD/SCID mice reproduced most characteristics of the original UL tissue, including gross appearance, histology, sex steroid receptor expression, and representative gene expression. Our model provides a more convenient research tool to investigate UL pathogenesis.

Supported by: Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science.

P-342 Wednesday, October 22, 2014

REPRODUCTIVE FUNCTIONING AFTER SURGICAL AND NON-SURGICAL WEIGHT LOSS: STABLE OVARIAN RESERVE AND IMPROVED SEXUAL FUNCTIONING AT 12 MONTH FOLLOW UP. S. Butts, K. Allison, J. Spitzer, R. Moore, A. Dokras, D. Sarver.* Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; *Statistics, North Carolina State University, Raleigh, NC.

OBJECTIVE: Limited data exists regarding weight loss in extremely obese women and subsequent sexual functioning or ovarian reserve. We compared sexual functioning according to method: surgical (Roux-en-Y gastric bypass or adjustable gastric banding) or non-surgical and compared changes in Anti-Mullerian Hormone (AMH) over 12 months according to weight loss method.

DESIGN: Cohort Study.

MATERIALS AND METHODS: Outcome variables included AMH at baseline and 12 months and the Female Sexual Function Index (FSFI), an index of sexual functioning in six domains, at baseline and 12 months. A total score of 26.55 or lower indicates sexual dysfunction. Student’s t-tests were used to compare differences in means. Changes in sexual functioning and AMH were assessed with the use of repeated measures.

RESULTS: Mean age and body mass index (BMI) of the non-surgical weight loss group (n=48) were 32.3 ± 7.3 yrs, and 38.8 ± 7.3 kg/m2, respectively. Mean age and BMI of the surgical weight loss group (n=29) was 34.3 ± 5.1 years and 48.8 ± 9.3 kg/m2 respectively. Mean baseline total FSFI score for the cohort was 26.2 ± 7.8. While overall FSFI score did not significantly change with weight loss over time, improvements in desire were highly significant and improvements in arousal reached borderline significance.

Changes in sexual functioning were comparable in both groups. Weight loss was not associated with a statistically significant decline in AMH in either group.

Post Treatment Changes in Sexual Function and Ovarian Reserve

<table>
<thead>
<tr>
<th>Post Treatment Changes in Sexual Function and Ovarian Reserve</th>
<th>Non-Surgical Therapy, Mean ± SE</th>
<th>Surgical Therapy, Mean ± SE</th>
<th>Change Over Time</th>
<th>P-Value</th>
<th>Time*Group</th>
<th>P Value for Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desire</td>
<td>3.48 ± 0.18</td>
<td>3.73 ± 0.22</td>
<td>0.49 ± 0.28</td>
<td>0.014</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>Arousal</td>
<td>3.7 ± 0.29</td>
<td>4.48 ± 0.37</td>
<td>0.76 ± 0.49</td>
<td>0.047</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>Total Score</td>
<td>24.51 ± 1.27</td>
<td>28.34 ± 1.55</td>
<td>3.83 ± 1.52</td>
<td>0.001</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>AMH</td>
<td>3.95 ± 0.41</td>
<td>2.44 ± 0.54</td>
<td>-1.51 ± 0.65</td>
<td>0.003</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>(ng/ml)</td>
<td>—</td>
<td>—</td>
<td>-3.66 ± 2.1</td>
<td>0.003</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>% Weight Change</td>
<td>—</td>
<td>—</td>
<td>-30.36 ± 2.47</td>
<td>0.003</td>
<td>0.0001</td>
<td></td>
</tr>
</tbody>
</table>

* P = 0.025 for difference in baseline sexual functioning; Lubrication, orgasm, satisfaction, and pain domains showed no significant change over time.

CONCLUSION: Regardless of method, weight loss over 12 months in extremely obese women is associated with improvements in multiple domains of sexual functioning. Ovarian reserve did not decline significantly over time. These findings support the positive role of weight loss on reproductive outcomes in obese women.

Supported by: NIEHS 5P03ES013508-07 (SB), American Society for Metabolic and Bariatric Surgery Pilot Grant (DS).

P-343 Wednesday, October 22, 2014

IVF PREGNANCY RATES WITH PRECONCEPTION SUBCLINICAL HYPOTHYROIDISM. T. N. Crawford, N. L. Bossert, M. Danich, P. H. Leonard. Department of Reproductive Endocrinology and Infertility, Reproductive Medicine Center, University of Minnesota, Minneapolis, MN.

OBJECTIVE: Treatment of subclinical hypothyroidism in infertility patients is debated. Abnormal TSH cutoff values for subclinical hypothyroidism are unclear with some practitioners using 2.5 mIU/L and others using
FERTILITY & STERILITY®

P-345 Wednesday, October 22, 2014

BASAL PROGESTERONE LEVELS REPRESENT THE ONLY INDEPENDENT RISK FACTOR PRIOR TO INITIATION OF STIMULATION FOR ELEVATED PROGESTERONE ON THE DAY OF HCG. C. A. Venetis, a E. M. Kolibianakis, b G. T. Lainas, b I. A. Stountouris, a B. C. Tarlatzis, b T. G. Lainas. aWomen’s & Children’s Health, St. George Hospital, University of New South Wales, Kogarah, New South Wales, Australia; bUnit for Human Reproduction, 1st Dept. of OB/Gyn, Medical School, Aristotle University of Thessaloniki, N. Efkarpia, Thessaloniki, Greece; cEugonia Unit of Assisted Reproduction, Athens, Attica, Greece.

OBJECTIVE: The detrimental role of progesterone elevation (PE) on the day of human chorionic gonadotrophin (hCG) for pregnancy achievement after a fresh IVF cycle has been confirmed by many studies. The aim of this study is to evaluate whether there are early markers that could identify women at risk for PE prior to the completion of their stimulation.

DESIGN: A retrospective analysis of all fresh IVF cycles performed using gonadotrophins and GnRH analogues during the period 2001-2013 in a single IVF center (N=3,174).

MATERIALS AND METHODS: Stepwise logistic regression analyses were performed in order to identify potential early predictors (female age, body mass index, baseline progesterone, estradiol and progesterone) of PE (>15 ng/mL) on the day of hCG and to assess their predictive capacity independently of the intensity of ovarian stimulation, i.e. total dose of FSH and duration of stimulation, E2 on the day of hCG, the number of follicles ≥11 mm on the day of hCG (TF) and the number of embryos retrieved.

RESULTS: Two hundred thirteen cycles with PE (6.7%, 95% CI: 5.9-7.6) were identified. Mean female age (34.4 vs. 36.3 p<0.001), mean concentration of basal progesterone (0.70 vs. 0.59, p<0.005) and basal FSH (7.9 vs. 10; p<0.001) were significantly different between cycles with or without PE, respectively. Duration of ovarian stimulation (11.0 vs. 10.1 days; p<0.001), the total dose of FSH (3106 vs. 2786; p<0.001), E2 on the day of hCG (2645 vs. 1423; p<0.001), TF (20.7 vs. 13.0; p<0.001) and the number of oocytes retrieved (19.5 vs. 11.2; p<0.001) were significantly different between cycles with or without PE, respectively. A backward stepwise regression process including all the aforementioned potential predictors led to the construction of a model in which basal progesterone (OR: 1.22, 95% CI: 1.07-1.38), E2 on the day of hCG (OR: 1.9, 95% CI: 1.65-2.19; for every 1000 pg/mL increase) and the number of oocytes retrieved (OR: 1.05, 95% CI: 1.05-1.06) and the total dose of FSH (OR: 1.03, 95% CI: 1.02-1.04; for every additional ampoule of 75IUs used) were associated with the occurrence of PE.

CONCLUSION: Basal progesterone levels represent the only risk factor prior to initiation of stimulation for PE on the day of hCG independently of the intensity or the result of the ovarian stimulation.

P-346 Wednesday, October 22, 2014

VARIABLE GENE EXPRESSION IN TURNER SYNDROME PATIENTS WITH BICUSPID AORTIC VALVE. N. K. Banks, a P. S. Krusska, a C. Cheng, a A. Elkahalom, b C. A. Bony, b M. Muenke. aNHGRI, Bethesda, MD; bNICHHD, Bethesda, MD.

OBJECTIVE: Turner syndrome (TS) is caused by the partial or complete loss of the second sex chromosome in females and affects 1 in 2500 live female births. Thirty percent of women with non-mosaic classic TS (45,X) have bicuspid aortic valve (BAV), as compared to 1-2% of individuals in the general population. BAV and aortic coarctation have been associated with loss of the p arm of the X chromosome in TS. Importantly, the presence of cardiac anomalies in TS greatly increases the risk of cardiac related death.
in women with TS who pursue donor egg pregnancy. Gene expression profiling studies may identify specific genes on the X chromosome associated with BAV, or may highlight ongoing differences in gene transcription related to the presence of BAV.

DESIGN: Gene expression profiling analysis.

MATERIALS AND METHODS: Sixteen women with 45,X detected on peripheral karyotype in 50 cells underwent DNA expression array profiling of peripheral blood lymphocytes using Affymetrix GenChip Human Genome U133 Plus 2.0 arrays as part of the IRB approved NICHD protocol “Turner Syndrome: Genotype and Phenotype.” All women underwent cardiac MRI to accurately access aortic valve anatomy and all 16 had unambiguous cardiac MRI results. Eight women had BAV and eight women had tricuspid aortic valve (TAV). Analysis comparing TS with TAV and TS with BAV gene expression was performed with Partek Genomics Suite, using a 1% ANOVA test.

RESULTS: Comparing TS with BAV and TS with TAV, 2747 genes show a significant difference in expression of 1.2 fold or greater (p<0.05) and 155 genes were differentially expressed with p-value less than 0.001. Genes with greater than a 2 fold difference are enriched for immunoglobulin genes, with 11 immunoglobulin (Ig) genes showing greater than 2 fold up-regulation in BAV versus TAV (of 67 genes). CD38, a gene encoding for a cell surface marker, is up-regulated 1.9 fold in BAV versus TAV (p=0.0011).

CONCLUSION: There are differences in gene expression comparing TS with BAV versus TAV. It is unclear why expression of Ig genes would be up-regulated with BAV; however, this finding may have potential as a biomarker in TS for BAV through measurement of quantitative IgGs or Ig light chain levels. Additionally, flow cytometry may detect different levels of CD38 expression, another possible biomarker. These findings require confirmation using a different expression profiling modality. Further study is needed to explore whether these or possibly other biomarkers suggested by transcriptomic data, may be useful clinically in TS.

Supported by: NICHD, NICHD Intramural Research Programs.

P-347 Wednesday, October 22, 2014

FIRST REPORT OF A SUCCESSFUL SINGLETON LIVE BIRTH IN A FEMALE WITH 17-HYDROXYLASE DEFICIENCY THROUGH IVF FROZEN-THAWED EMBRYO TRANSFER AFTER ADEQUATE ENDOMETRIAL PREPARATION

P. H. M. Bianchi, a P. Serafini, b P. A. A. Monteleone, a S. Domenice, b E. E. M. Costa, b B. B. Mendonca, b E. Baracat, a P. R. da Silva, a

CONCLUSION: An increase in RMR was seen in young, healthy women 3 weeks after receiving 150 mg DMPA for contraception. The effect was augmented when the drug was administered during the luteal phase of the menstrual cycle, and was independent of changes in weight.

Supported by: Financial support for this project was provided by the Charles B. Hammond Research Fund, Department of Obstetrics and Gynecology, Duke University Medical Center.

P-349 Wednesday, October 22, 2014

SUCCESSFUL PREGNANCIES AND LIVE BIRTHS AFTER ADEQUATE HORMONE REPLACEMENT AND OVARIAN STIMULATION IN FOUR PATIENTS WITH CONGENITAL HYPOPITUITARISM

P. H. M. Bianchi, a F. A. Correa, b R. J. M. Rodrigues, a L. R. S. Carvalho, b E. Baracat, a P. Serafini, b

CONCLUSION: A live birth was achieved through IVF, cryopreservation of embryos and frozen thawed embryo transfer after the reduction of P synthesis in a patient with 17-hydroxylase deficiency.

P-348 Wednesday, October 22, 2014

THE EFFECT OF DEPOT-MEDROXYPROGESTERONE ACETATE ON RESTING METABOLIC RATE IN PREMENOPAUSAL WOMEN

R. G. Stewart, a P. Z. Stanczyk, b T. M. Price, c

OBJECTIVE: To examine the short-term effect of depot-medroxyprogesterone acetate (DMPA) on resting metabolic rate (RMR) in healthy, premenopausal women without weight change.

DESIGN: A prospective cohort study was performed at an academic medical center.

MATERIALS AND METHODS: Thirteen women, previously dispositioned to receive DMPA for contraception, completed 3 testing sessions. Inclusion criteria were age 18-35 and body mass index (BMI) 20-35. Recent pregnancy or use of hormonal contraceptives, hypertension, thyroid disease, impaired glucose tolerance, smoking, use of metabolism-altering drugs, and intending dietary change were reasons for exclusion. DMPA (150 mg intramuscularly) was given after assessment of baseline anthropometrics, RMR, body composition, and serum hormone status. Subjects were similarly tested 3 and 9 weeks after DMPA. The primary outcome was longitudinal change in RMR from baseline to week 3 to week 9. Secondary endpoints included changes in anthropometric values, body composition, and serum estradiol (E2), luteinizing hormone (LH), progesterone (P), and MPA.

RESULTS: The percent change in RMR from baseline to week 3 (9% ± 0.05) was greater than the percent change from baseline to week 9 (1.6% ± 0.06) (95% CI [0.002, 0.145]; p = 0.045). A trend toward significance remained after RMR adjustment for weight and BMI (both 9% ± 0.04 vs. 3.4% ± 0.05, respectively) (95% CI [-0.004, 0.115]; p = 0.064). A significant percent change from baseline to the 3 (12.6% ± 0.09) and 9 (6.4% ± 0.09) week time points was seen, after adjustment for weight, in the women who received DMPA in the luteal phase (95% CI [0.015, 0.108]; p = 0.021). An MPA effect was demonstrated as significant main effects for treatment were seen for E2 (p = 0.002), LH (p = 0.007), P (p = 0.036), and body temperature (p = 0.044).

CONCLUSION: An increase in RMR was seen in young, healthy women 3 weeks after receiving 150 mg DMPA for contraception. The effect was augmented when the drug was administered during the luteal phase of the menstrual cycle, and was independent of changes in weight.

Supported by: NICHD, NICHD Intramural Research Programs.

P. H. M. Bianchi, a F. A. Correa, b R. J. M. Rodrigues, a L. R. S. Carvalho, b E. Baracat, a P. Serafini, b

CONCLUSION: An increase in RMR was seen in young, healthy women 3 weeks after receiving 150 mg DMPA for contraception. The effect was augmented when the drug was administered during the luteal phase of the menstrual cycle, and was independent of changes in weight.

Supported by: NICHD, NICHD Intramural Research Programs.

P-347 Wednesday, October 22, 2014

FIRST REPORT OF A SUCCESSFUL SINGLETON LIVE BIRTH IN A FEMALE WITH 17-HYDROXYLASE DEFICIENCY THROUGH IVF FROZEN-THAWED EMBRYO TRANSFER AFTER ADEQUATE ENDOMETRIAL PREPARATION

P. H. M. Bianchi, a P. Serafini, b P. A. A. Monteleone, a S. Domenice, b E. E. M. Costa, b B. B. Mendonca, b E. Baracat, a

OBJECTIVE: To report a singleton live birth in a female patient with congenital adrenal hyperplasia (CAH) due to 17-hydroxylase deficiency. CAH due to 17-hydroxylase deficiency in 46,XX patients is characterized by low serum levels of estradiol (E2) and high levels of progesterone (P). Therefore it is associated with ovariary dysfunction, ovarian cysts and inadequate endometrial development for embryo implantation. No other infertility factors could be identified. IVF was recommended, considering the disorder of the complex of the disorder. Ovulation induction (OI) was achieved with a long GnRH agonist protocol and recombinant FSH (rFSH). All embryos were electrolyte cryopreserved (vitrification) after 5 days of development. Goserelin acetate was administered after menstruation to suppress ovarian synthesis of P. The patient also received 0.5 mg/day of dexamethasone acetate to reduce adrenal synthesis of P. Once P was < 1 ng/mL, endometrial preparation (EP) with estradiol valerate (6mg/day orally) was initiated.

RESULTS: OI was accomplished with 1237.5 IU of rFSH over 11 days. At the end of OI, P was 13 ng/mL and E2 < 13pg/mL. Four mature oocytes were retrieved and fertilized (ICSI) by a fresh semen sample. On the fifth day of embryo culture, two morulae were cryopreserved. Goserelin acetate (10mg) was administered subcutaneously one month after oocyte retrieval. Three months later, when P < 1 ng/mL, EP was started. When endometrial thickness reached 8 mm (18th day of EP), 200mg of micronized progesterone were administered vaginally t.i.d. Five days later, embryos were thawed and transferred into the uterus. β hCG tested positive 10 days later. A singleton pregnancy was confirmed with transvaginal ultrasound at the 6th week of gestational age. Hormonal support (estradiol + progesterone) was maintained until 8 weeks of GA. A live birth occurred at 30 weeks of GA due to fetal distress (true umbilical knot). The neonate was discharged in good conditions after 30 days of neonatal ICU.

CONCLUSION: A live birth was achieved through IVF, cryopreservation of embryos and frozen thawed embryo transfer after the reduction of P synthesis in a patient with 17-hydroxylase deficiency.
patients underwent the basic infertility work up. Prior to ovulation induction (OI), GH doses were titrated until serum IGf1 achieved normal values (median ± 1DP). OI was performed with Menotropins starting with 75 IU/day; increases in the daily doses were made according to follicular growth. When at least one follicle reached 18 mm, 250 mcg of recombinant hCG was used to trigger final follicular maturation. Since multiple pregnancies in women with CPHD are associated with a poor outcome, when OI resulted in monofollicular growth, patients were elected to timed intercourse (TI/fintruterine insemination (IUI)), and if resulting in multifollicular growth, IVF and elective single embryo transfer (eSET) at the cleavage stage were performed.

RESULTS: The first OI cycle in Patient 1 (25 yrs) (20 days of OI, 2025 IU) resulted in a singleton pregnancy and live birth after IUI. Patient 2 (27 yrs) had the first cycle of OI cancelled, but the second attempt (26 days of OI, 326.5 IU) resulted in a singleton pregnancy and live birth after TI. Patient 3 (26 yrs) underwent 3 attempts of OI and TI (15-28 days, 1550 ±610.83IU) that did not result in pregnancy. In the 4th OI attempt (20 days, 2175 IU), the cycle was converted to IVF due to multifollicular growth, but no pregnancy was achieved after 3 consecutive eSETs. The 5th attempt (IVF; 1200 IU; 9 days) resulted in 3 embryos; 1 was transferred fresh resulting in a singleton ongoing pregnancy. Patient 4 (35 yrs) had tubal abnormalities and IVF and eSET were promptly indicated. The OI (9 days, 1800 IU) resulted in 3 embryos. The first attempt of eSET did not result in pregnancy. The second attempt (frozen-thawed embryo) resulted in an ongoing pregnancy.

CONCLUSION: GH administration along with treatment of other hormonal deficiencies appears to play a role in OI in patients with CPHD. Longer OI periods were observed to achieve monofollicular growth.

P-350 Wednesday, October 22, 2014

OBJECTIVE: Suboptimal cellular conditions result in the accumulation of unfolded proteins in the Endoplasmic Reticulum (ER) and trigger ER stress. ER stress has been implicated as a determinant of oocyte and granulosa cell viability. We hypothesized that FSH may down-regulate ER stress to improve follicle viability, while ER stress may negatively affect FSH response. We investigated the effects of FSH on ER stress response in vivo and in vitro and determined how ER stress affects FSH response in granulosa cells in culture.

DESIGN: Experimental study.

MATERIALS AND METHODS: (1) To determine the effect of FSH on ER stress response in vivo, 3 week-old female C57BL6 mice were injected intraperitoneally with 5IU pregnant mare’s serum gonadotropin (PMSG). Ovaries and granulosa cells were collected 24 or 48 hours later, and the expression of ER stress associated genes (Atf4, Atf6, Chop, Caspase-12, Xbp1s) was determined by quantitative real-time RT-PCR (qRT-PCR). (2) To determine the effect of FSH on ER stress response in vitro, primary mouse granulosa cells in culture were treated with 3 different doses of ovine FSH (10 IU/ml, 30 mIU/ml, 100 mIU/ml) for 24 and 48 hours, and the expression of ER stress associated genes were determined by qRT-PCR. (3) To determine how ER stress affects FSH response, ER stress was induced in mouse granulosa cells in culture using 2 different models (Tunicamycin-induced and Thapsigargin-induced) and response to FSH was evaluated by detecting cellular catechol (Cyp19a1) expression by PCR, and measuring estradiol in the culture media by ELISA.

RESULTS: (1 and 2) FSH attenuated ER stress in mouse granulosa cells in vivo and in vitro. mRNA levels of Atf6, Chop, Caspase-12 and Xbp1s was decreased by 2- to 7-fold (P<0.05) upon exposure to FSH/PMSG. (3) The induction of ER stress via 2 different treatments inhibited FSH response of mouse granulosa cells. Estradiol production increased 3-fold in untreated granulosa cells after incubation with FSH for 60 hours (p<0.05), whereas ER stress induced cells did not show any change in estradiol levels. Moreover, ER stress induced cells failed to demonstrate aromatase expression upon exposure to FSH.

CONCLUSION: Our findings suggest that FSH decreases ER stress in granulosa cells under physiologic conditions, promoting cell survival and proliferation. However, under condition that cause a significant increase in ER stress, FSH response is attenuated. These findings may help better understand the mechanisms of FSH response variations under physiological conditions and during IVF.

P-352 Wednesday, October 22, 2014

OBJECTIVE: Suboptimal cellular conditions result in the accumulation of unfolded proteins in the Endoplasmic Reticulum (ER) and trigger ER stress. ER stress has been implicated as a determinant of oocyte and granulosa cell viability. We hypothesized that FSH may down-regulate ER stress to improve follicle viability, while ER stress may negatively affect FSH response. We investigated the effects of FSH on ER stress response in vivo and in vitro and determined how ER stress affects FSH response in granulosa cells in culture.

DESIGN: Experimental study.

MATERIALS AND METHODS: (1) To determine the effect of FSH on ER stress response in vivo, 3 week-old female C57BL6 mice were injected intraperitoneally with 5IU pregnant mare’s serum gonadotropin (PMSG). Ovaries and granulosa cells were collected 24 or 48 hours later, and the expression of ER stress associated genes (Atf4, Atf6, Chop, Caspase-12, Xbp1s) was determined by quantitative real-time RT-PCR (qRT-PCR). (2) To determine the effect of FSH on ER stress response in vitro, primary mouse granulosa cells in culture were treated with 3 different doses of ovine FSH (10 IU/ml, 30 mIU/ml, 100 mIU/ml) for 24 and 48 hours, and the expression of ER stress associated genes were determined by qRT-PCR. (3) To determine how ER stress affects FSH response, ER stress was induced in mouse granulosa cells in culture using 2 different models (Tunicamycin-induced and Thapsigargin-induced) and response to FSH was evaluated by detecting cellular catechol (Cyp19a1) expression by PCR, and measuring estradiol in the culture media by ELISA.

RESULTS: (1 and 2) FSH attenuated ER stress in mouse granulosa cells in vivo and in vitro. mRNA levels of Atf6, Chop, Caspase-12 and Xbp1s was decreased by 2- to 7-fold (P<0.05) upon exposure to FSH/PMSG. (3) The induction of ER stress via 2 different treatments inhibited FSH response of mouse granulosa cells. Estradiol production increased 3-fold in untreated granulosa cells after incubation with FSH for 60 hours (p<0.05), whereas ER stress induced cells did not show any change in estradiol levels. Moreover, ER stress induced cells failed to demonstrate aromatase expression upon exposure to FSH.

CONCLUSION: Our findings suggest that FSH decreases ER stress in granulosa cells under physiologic conditions, promoting cell survival and proliferation. However, under condition that cause a significant increase in ER stress, FSH response is attenuated. These findings may help better understand the mechanisms of FSH response variations under physiological conditions and during IVF.

P-351 Wednesday, October 22, 2014
ENDOPLASMIC RETICULUM (ER) STRESS INHIBITS FSH RESPONSE AND FSH MODULATES THE EXPRESSION OF ER STRESS ASSOCIATED GENES IN MOUSE GRANULOSA CELLS. E. Babayev, M. D. Lalioti, E. Seli. Obstetrics, Gynecology and Reproductive Sciences, Yale School of Medicine, New Haven, CT.

OBJECTIVE: Suboptimal cellular conditions result in the accumulation of unfolded proteins in the Endoplasmic Reticulum (ER) and trigger ER stress. ER stress has been implicated as a determinant of oocyte and granulosa cell viability. We hypothesized that FSH may down-regulate ER stress to improve follicle viability, while ER stress may negatively affect FSH response. We investigated the effects of FSH on ER stress response in vivo and in vitro and determined how ER stress affects FSH response in granulosa cells in culture.

DESIGN: Experimental study.

MATERIALS AND METHODS: (1) To determine the effect of FSH on ER stress response in vivo, 3 week-old female C57BL6 mice were injected intraperitoneally with 5IU pregnant mare’s serum gonadotropin (PMSG). Ovaries and granulosa cells were collected 24 or 48 hours later, and the expression of ER stress associated genes (Atf4, Atf6, Chop, Caspase-12, Xbp1s) was determined by quantitative real-time RT-PCR (qRT-PCR). (2) To determine the effect of FSH on ER stress response in vitro, primary mouse granulosa cells in culture were treated with 3 different doses of ovine FSH (10 IU/ml, 30 mIU/ml, 100 mIU/ml) for 24 and 48 hours, and the expression of ER stress associated genes were determined by qRT-PCR. (3) To determine how ER stress affects FSH response, ER stress was induced in mouse granulosa cells in culture using 2 different models (Tunicamycin-induced and Thapsigargin-induced) and response to FSH was evaluated by detecting cellular catechol (Cyp19a1) expression by PCR, and measuring estradiol in the culture media by ELISA.

RESULTS: (1 and 2) FSH attenuated ER stress in mouse granulosa cells in vivo and in vitro. mRNA levels of Atf6, Chop, Caspase-12 and Xbp1s was decreased by 2- to 7-fold (P<0.05) upon exposure to FSH/PMSG. (3) The induction of ER stress via 2 different treatments inhibited FSH response of mouse granulosa cells. Estradiol production increased 3-fold in untreated granulosa cells after incubation with FSH for 60 hours (p<0.05), whereas ER stress induced cells did not show any change in estradiol levels. Moreover, ER stress induced cells failed to demonstrate aromatase expression upon exposure to FSH.

CONCLUSION: Our findings suggest that FSH decreases ER stress in granulosa cells under physiologic conditions, promoting cell survival and proliferation. However, under condition that cause a significant increase in ER stress, FSH response is attenuated. These findings may help better understand the mechanisms of FSH response variations under physiological conditions and during IVF.
P-353 Wednesday, October 22, 2014

VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) AND PLACENTAL GROWTH FACTOR (PLGF) DIRECTLY CORRELATE WITH OVARIAN FOLLICLE SIZE IN WOMEN UNDERGOING IN VITRO FERTILIZATION (IVF). L. Hou, R. N. Taylor, Y. Shu, E. B. Johnston-MacAnanny, T. M. Yalcinkaya. Section of Reproductive Endocrinology-Infertility, Wake Forest School of Medicine, Winston-Salem, NC.

OBJECTIVE: To assess VEGF and PI GF concentrations in FF from women undergoing IVF, stratified according to follicle size, and to correlate these with VEGF and PI GF mRNA expression in corresponding luteinized granulosa cells (GC), as a follow-up of our previous demonstration that these genes are expressed in GC.

MATERIALS AND METHODS: 18 consenting subjects with normal ovarian function (IVF being undertaken due to male factor) were recruited prior to oocyte retrieval (mean age of the women = 31 years). Only blood-free FF obtained with the first puncture of each ovary was collected, without the use of flushing media to avoid dilution of the FF contents. All specimens were categorized and analyzed as FF derived from small follicles (>8 mm diameter) or large follicles (>14 mm diameter), VEGF and PI GF concentrations in the FF were measured using validated commercial ELISAs (Ray-Bio®). Levels of FF estradiol (E2) and progesterone (P4) were determined by RIA. VEGF and PI GF mRNA expression was evaluated by real time RT-PCR, using cultured GC derived from the small and large follicles as indicated above. We also evaluated the expression of other relevant ovarian genes.

RESULTS: FF VEGF concentrations from large follicles were markedly higher (6788 ± 2863 pg/ml) compared to those in small follicles (1942 ± 879 pg/ml, p = 0.0003). PI GF concentrations in FF from large follicles also was higher (2004 ± 816 pg/ml) than in small follicles (1144 ± 898 pg/ml, p = 0.002). By RT-PCR, the expression of VEGF mRNA was ∼2.5 fold greater in GC from large follicles than from small follicles. However, we observed no significant statistical differences in PI GF mRNA expression relative to follicle size. As expected, expression of mRNAs encoding steroi-dogenic acute regulatory protein (StAR), anti-mullerian hormone (AMH) and aromatase P450 (CYP19) were all increased in cells from large follicles as compared with those from small follicles.

CONCLUSION: VEGF and PI GF protein concentrations in FF are correlated with follicle size; this relationship also applied to E2 concentration and StAR, AMH and CYP19 expression. Moreover, VEGF and PI GF levels were significantly higher in FF than in matched serum. Similar trends were noted for VEGF mRNA, but not PI GF mRNA expression from cultured GC. We conclude that local VEGF and PI GF gene expression are likely to mediate ovarian follicle angiogenesis and may contribute to oocyte development, maturation and selection of dominant follicle.

Supported by: Ob/Gyn departmental research fund.

P-354 Wednesday, October 22, 2014

THE FUNCTION OF MITOCHONDRIA IN CUMULUS CELLS OF PCOS PATIENTS. H.-C. Zhao, Y. Yu, J. Qiao. Peking University Third Hospital, Beijing, China.

OBJECTIVE: To assess the the changes of mitochondrial function in cumulus cells of PCOS patients; to explore the potential impact of PCOS on follicular microenvironment; to improve the growth and development of PCOS patients' follicle and oocyte.

DESIGN: Cumulus cell was taken on oocyte retrieval day from 80 PCOS women and 91 controls underwent intracytoplasmic sperm injection (ICSI) treatment. The function of mitochondrial in cumulus cells was assessed.

MATERIALS AND METHODS: The ultrastructure of mitochondria was observed with transmission electron microscopy (TEM). The mitochondrial membrane potential was assessed using MitoPT-JC1 assay kit by flow cytometry. The localization of mitochondrial was assessed by immunofluorescence with Mito Tracker Green. mRNA content and expression of PI GF-1a were detected by real-time PCR. The staining of PGC-1a promoter was detected by methylation-specific real-time PCR. The levels of ATP were detected by chemiluminescence.

RESULTS: The mitochondria in cumulus from PCOS patients showed an increased frequency of fragmented mitochondria, a decreased transmembrane potential, an aggregated distribution of mitochondria and a decreased mtDNA content with a decrease PGC-1a mRNA expression and hypermethylation of PGC-1a promoter. However, the level of ATP in cumulus from PCOS patients showed no change.

CONCLUSION: The dysfunction of mitochondria in cumulus cells from PCOS patients may play a role in the pathogenesis of PCOS and may affect the follicle growth and oocyte development.

Supported by: This work was Supported in part by the Ministry of Science and Technology of China Grants (973 program; 2011CB944504) to J.Q., and by the National Natural Science Funds for Young Scholars (31000661) to Y.Y., and by the National Natural Science Foundation of China (General Program) (81070534 to P.L.; 31371521 to Y.Y.).

OBESITY AND METABOLISM

P-355 Wednesday, October 22, 2014

EVALUATION OF SONOGRAPHIC AND BIOCHEMICAL MARKERS OF CLOMIPHENE CITRATE RESISTANCE IN POLYCYSTIC OVARY SYNDROME. A. F. Amen, M. Salah, H. Abozeid, E. Badran." "OB/Gyn, Assiut University, Assiut, Egypt; "Radiodiagnostics, Assiut University, Assiut, Egypt; "Biochemistry, Assiut University, Assiut, Egypt.

OBJECTIVE: To investigate potential hormonal, metabolic and vascular markers implicated in resistance of polycystic ovary syndrome (PCOS) patients to clomiphene citrate.

DESIGN: Cross sectional observational study.

MATERIALS AND METHODS: 90 PCOS patients diagnosed by the Rotterdam criteria were recruited in a university affiliated fertility clinic. 49 patients were clomiphene citrate( CC) resistant ( failed to ovulate in response to CC 150 mg /day for 5 days for 3 successive cycles) and 41 patients were CC responders. History was taken and body mass index (BMI) was calculated. Hormonal and metabolic markers including serum luteinizing hormone (LH), follicle stimulating hormone (FSH), total testosterone, fasting insulin, fasting glucose and homeostatic model assessment-insulin resistance (HOMA-IR) and 25 hydroxy vitamin D were evaluated in both groups. Sonographic evaluation of ovarian and uterine blood flow and carotid intima-media thickness (CIMT) were compared between the groups.

RESULTS: The mean age was comparable while the BMI was significantly higher in the CC resistant group. Total testosterone, serum LH, fasting serum insulin, and HOMA-RI were significantly higher in the CC resistant group ( P values were 0.000),while serum 25 hydroxy vitamin D was significantly lower in CC resistant patients (16.25 ± 9.95 ng/ml) than in the CC responders(31.88 ± 7.24 ng/ml). Relevant sonoraphic vascular markers were examined. Ovarian artery RI (resistive index),and PI (pulsatility index) were significantly lower in CC resistant patients,RI (2.30 ± 0.48) compared to ( 2.00 ± 0.58 ), PI (0.45 ± 0.50) compared to (2.17 ± 0.61). Uterine artery PI in CC resistant patients were significantly higher(2.30 ± 0.48) compared to(2.00:±0.58) (P values were 0.0001 ). CIMT as a marker for early atherosclerosis, was significantly higher in the CC resistant group (P value 0.0001 ). Four patients of the CC resistant group had evidence of atheromatous plaques. There was no significant difference between both groups in the following (FSH, fasting glucose, uterine artery RI , and ovarian volume).

CONCLUSION: The resistance of PCOS patients to CC may be affected by multiple metabolic and vascular factors. Lower levels of vitamin D may influence the potency of CC in ovulation induction in PCOS patients. The
association between CC resistance and increased CIMT could be further evaluated as a prognostic indicator of the cardiovascular risk in women with PCOS.

P-356 Wednesday, October 22, 2014

BODY MASS INDEX AND BREAST CANCER: A NATIONWIDE POPULATION-BASED PROSPECTIVE COHORT STUDY ON 1,393,985 TAIWANESE WOMEN. M.-J. Chen, a Y.-Y. W. Wu,b,c M.-F. A. Yen,a C.-Y. J. Fann,a L.-S. S. Chen,d Y.-H. S. Chiu,e Y.-S. Yang,f H.-N. Ho,g H.-H. Chen,h S.-T. Chiu.i 1Department of Obstetrics and Gynecology, National Taiwan University Hospital, Taipei, Taiwan; 1Graduate Institute of Epidemiology and Preventive Medicine, Taipei, Taiwan; 1Institute of Public Health, Taipei, Taiwan; 1School of Oral Hygiene, College of Oral Medicine, Taipei, Taiwan; 1Department of Health Industry Management, School of Healthcare Management, Tao-Yuan, Taiwan; 1Department of Health Care Management, College of Management, Tao-Yuan, Taiwan; 1Health Promotion Administration, Taipei, Taiwan.

OBJECTIVE: Asian women have a younger age at onset of breast cancer and a lower body mass index (BMI) than Western women. The linkage between obesity and the risk of breast cancer in Asian women remains elusive. This study aimed to investigate the effect of BMI on the risk of incident breast cancer on Taiwanese women.

DESIGN: This prospective cohort study enrolled women from the different phases of nationwide Taiwanese breast cancer screening program between 1999 and 2009.

MATERIALS AND METHODS: A total of 1,393,985 women who were cancer-free before recruitment, public health nurses or health care providers conducted interviews at recruitment to collect data. The incidence of breast cancer during follow-up was assessed through the linkage of the entire cohort with the National Cancer Registry and the National Death Certification System.

RESULTS: Though morbid obesity (BMI exceeding 35 kg/m2 at baseline) appeared to be associated with a reduced risk of breast cancer in premenopausal women, especially among those diagnosed at younger ages, this association was not statistically significant. BMI was not significantly associated with the occurrence of incident breast cancer among women who were enrolled before menopause, but BMI was significantly associated with the risk of incident breast cancer among postmenopausal women after adjustment for extraneous risk factors.

CONCLUSION: Obesity acts mainly as an influential promoter of the development of late-onset breast cancer after menopause in Taiwanese women.

Supported by: This study is supported by the Health Promotion Administration, Ministry of Health and Welfare in Taiwan.

P-357 Wednesday, October 22, 2014


OBJECTIVE: Given the uncertainties regarding safety and efficacy of ART in the obese patient, the purpose of this study was to survey the IVF centers across the United States to determine what policies, if any, have been instituted in response to an increasing overweight and obese population.

DESIGN: Survey.

MATERIALS AND METHODS: An anonymous survey was sent to Medical Directors at 395 IVF centers in the SART database regarding recommendations, policies, and restrictions for overweight/obese patients who desire infertility treatment. 77 surveys were received.

RESULTS: Of the respondents, 58.4% perform 100-499 fresh ART cycles per year, and 19.7% practice in mandated states. 50 centers (64.9%) state they have a policy for obesity and offering ART, including in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI), intratuterine insemination (IUI), and donor egg recipient cycles. Of those with policies, 64.0% depend specifically on BMI; 30.0% include BMI combined with other criteria. Other criteria included weight, neck/abdomen/waist circumference, waist-to-hip ratio, fat%, inadequate trial transfer, anesthesia clearance, and medical morbidities such as hypertension, diabetes, and sleep apnea. 84% of programs with policies state they have a maximum BMI at which they will perform IVF, with 28.6% setting a limit at 40 and 9.5% setting a limit at 45. Similarly, 62.0% of programs reported a maximum BMI for donor egg recipients (mode BMI 40), but only 38% had a maximum BMI for IUI. When asked what criterion primarily dictates the exclusion of patients in ART, 62% reported anesthesia requirements, 14% selected safety during ongoing pregnancy and 14% selected IVF outcomes and success/miscarriage rates as their primary consideration. About half (50.6%) of centers have an affiliated outpatient anesthesiology department with a policy regarding BMI, often setting a limit at 40 (35.9%); many require anesthesia consultation from an anesthesiologist. Weight loss in and above ART, 7, 8, 9, 10 is a priority for patients to a nutritionist/dietician, and 67.5% refer to another specialist such as bariatric surgery, endocrine, or psychology. When asked the highest BMI the center collects treating, the maximum reported was 62, minimum 31, and mode 45.

CONCLUSION: While some centers do not have policies regarding obesity and access to ART, those that do consider BMI and other criteria related to efficacy, procedural safety, safety in pregnancy, and overall health status. Policies vary widely and the topic requires continued discussion.

P-358 Wednesday, October 22, 2014

ELEVATED MATERNAL BMI RESULTS IN SEVERELY DISRUPTED BlastOCYST METABOLISM. R. L. Krisher,a A. F. Greene,a J. Stevens,a A. Heuberger,b W. B. Schoolcraft,b 1National Foundation for Fertility Research, Lone Tree, CO; 1Fertility Laboratories of Colorado, Lone Tree, CO; 1Colorado State University, Ft. Collins, CO; 1Colorado Center for Reproductive Medicine, Lone Tree, CO.

OBJECTIVE: Compare the metabolism of blastocysts from patients with elevated BMI to those of women with normal BMI using metabolicomics technology.

DESIGN: Research study.

MATERIALS AND METHODS: Media (Quinn’s Advantage®; Cooper Surgical) were collected from wells of an EmbryoSlide™ following culture (25 μL) of individual embryos that developed into good quality blastocysts on D5. Media samples from wells that did not contain an embryo were collected as controls. Three samples from each of three patients (n=9 per treatment) were collected in both elevated (38.4, obese, OB) and normal (24.1, N) BMI groups. Patients were matched for age (OB and N, 35.7 yrs). Average blastocyst stage (OB and N, 3.6), ICM grade (A=1, B=2, OB 1.7, N 1.3) and TE grade (OB, 1.8; N, 1.9) were similar between treatments. Samples were analyzed using GC-MS. Metabolites were quantified using the mean peak area of selected ions against a 5-point standard curve. Values for each metabolite were analyzed using ANOVA with Tukey-Kramer multiple comparison test (p<0.05 considered significant).

RESULTS: Of the 27 metabolites analyzed, 12 were metabolized differently (p<0.05) between N and OB blastocysts and 2 others tended (p<0.09) to be different. A majority of these metabolites (arginine, citrate, cysteine, glucose, isoelucine, lysine, methionine, phenylalanine, threonine, tyrosine) displayed a similar pattern, in which N blastocysts produced a small amount of the metabolite (not different than control) while OB blastocysts used the metabolite compared to control medium. Three nonessential amino acids (glycine, proline, serine) displayed a different pattern in which OB blastocysts produced a small amount of the metabolite while N blastocysts did not alter the metabolite compared to control medium. Pyruvate was taken up from the medium (42 μM) by N embryos, but was not used by OB embryos.

CONCLUSION: Although embryos from normal and elevated BMI patients both developed into good quality blastocysts on D5, a morphological assessment usually associated with high quality human blastocysts, they differed significantly in both amino acid and carbohydrate metabolism. The perturbed metabolism observed in blastocysts from women with elevated BMI may lead to impaired implantation, fetal development, or offspring health.

P-359 Wednesday, October 22, 2014

IMPACT OF VARIOUS DOSES OF URINARY HCG ADMINISTERED TO TRIGGER FINAL OCYTE MATURATION IN CONTROLLED OVARIAN STIMULATION FOR IVF ON THE CLINICAL PREGNANCY AND LIFE BIRTH RATES IN OBESE WOMEN. L. R. Hoyos, S. Khan, J. Bolnick, M. Singh, E. Puscheck, A. A Wong. Obstetrics & Gynecology, Wayne State University, Detroit, MI.

OBJECTIVE: To determine the impact of various doses of urinary human Chorionic Gonadotropin (u-hCG) administered to trigger final oocyte
maturation in controlled ovarian stimulation for in vitro fertilization (IVF) on the clinical pregnancy and life birth rates in obese women.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: Four hundred women who underwent conventional in-vitro fertilization (IVF)/ICSI cycles from January 2003 to December 2010 were included in the study and divided into three groups based on Body Mass Index (kg/m²): < 25 (n=176), 25-30 (n=120) and > 30 (n=104). Following oral contraceptive pill, patients underwent stimulation protocols with gonadotropins and gonadotropin releasing hormone agonists or antagonists. Urinary hCG was administered when ≥ 2 follicles reached > 18 mm whereas varying doses of hCG namely 5,000 IU, 10,000 IU or 15,000 IU were administered intramuscularly and oocytes retrieved 36 h later. Demographic variables, serum hCG concentration 12-14 h after ovulation trigger, response to superovulation, fertilization rates, number of embryos transferred, clinical pregnancy rates (CPR), and live birth rates (LBR) were compared between the three groups. Chi Square test and Fisher’s exact, one-way analysis of variance and Kruskal-Wallis tests with Bonferroni and Dunn multiple comparisons respectively, were used as appropriate. Significance was defined as p < 0.05.

RESULTS: Administration of 10,000 and 15,000 IU u-hCG for ovulation trigger did not impact the CPR and LBR significantly irrespective of patients’ Body Mass Index (BMI). Significantly lower serum hCG levels were recorded 12-14 h after u-hCG in those who received 5,000 IU. Although demographic variables and response to superovulation were similar, patients with BMI > 30 compared with those with a BMI < 30 achieved the lowest CPR and LBR.

CONCLUSION: The use of doses of u-hCG < 5,000 may lower the clinical pregnancy and live birth rates in women with BMI > 30. Given similar results with 15,000 and 10,000 IU of u-hCG, there is little justification for the use of the 15,000 IU in Assisted Reproductive Technology.

P-360 Wednesday, October 22, 2014


OBJECTIVE: To assess the association between oocyte donor BMI and recipient pregnancy outcome.

DESIGN: Cohort study.

MATERIALS AND METHODS: Two hundred twenty consecutive fresh IVF donor oocyte cycles from 2004-2013 at the Massachusetts General Hospital Fertility Center were reviewed. Analyses included 189 oocyte donors (219 total cycles). Logistic regression models using generalized estimating equations (GEE) were fit to investigate the relationship between donor body mass index (BMI) and clinical pregnancy per oocyte retrieval while controlling for confounders and within-person correlations in cycle outcome. Multivariate results were adjusted for donor TSH, recipient TSH, donor prior cycles, donor age, recipient age, recipient BMI, donor AFC, and donor status (known vs. anonymous).

RESULTS: Mean oocyte donor age was 26.9 years. Mean donor BMI was 23.4 ± 2.3 kg/m² at time of first cycle, with a donor BMI range of 17.8 – 34.0 kg/m². Recipient mean age was 41.4 years, mean BMI was 24.2 ± 2.7 kg/m². There was no association between recipient BMI and pregnancy outcome. Crude clinical pregnancy rate per oocyte retrieval was 36/53 (68%), 40/54 (74%), 32/57 (56%), and 30/55 (55%) among cycles with donor BMI of 17.8-21.1 kg/m², 21.2-22.8 kg/m², 22.9-25.0 kg/m², and 25.1-34.0 kg/m², respectively. In multivariate regression, donor BMI was negatively associated with clinical pregnancy per oocyte retrieval (p-linear trend 0.04). Using a retrospective cohort approach, adjusted odds ratios of (95% CI) were 0.9 (0.4-2.3), 0.5 (0.2-1.1), and 0.5 (0.2-1.1) for BMI groups 17.8-21.1 kg/m², 22.9-25.0 kg/m², and 25.1-34.0 kg/m², respectively.

CONCLUSION: This analysis may suggest that peri-fertilization donor BMI range of 21.2-22.8 kg/m² as the reference category, adjusted odds ratios with clinical pregnancy per oocyte retrieval (p-linear trend 0.04). Using a retrospective cohort approach, adjusted odds ratios of (95% CI) were 0.9 (0.4-2.3), 0.5 (0.2-1.1), and 0.5 (0.2-1.1) for BMI groups 17.8-21.1 kg/m², 22.9-25.0 kg/m², and 25.1-34.0 kg/m², respectively. In multivariate regression, donor BMI was negatively associated with clinical pregnancy per oocyte retrieval (p-linear trend 0.04). Using a retrospective cohort approach, adjusted odds ratios of (95% CI) were 0.9 (0.4-2.3), 0.5 (0.2-1.1), and 0.5 (0.2-1.1) for BMI groups 17.8-21.1 kg/m², 22.9-25.0 kg/m², and 25.1-34.0 kg/m², respectively.

CONCLUSION: The use of doses of u-hCG < 5,000 IU of u-hCG, there is little justification for the use of the 15,000 IU in Assisted Reproductive Technology.

P-362 Wednesday, October 22, 2014

REDUCTION IN CIRCULATING TRIACYLGLYCEROLS AND FATTY ACIDS IN A DIET INDUCED MODEL OF OBESITY IMPROVES GLUCOSE TOLERANCE YET HAS NO EFFECT ON REPRODUCTIVE FUNCTION. M. N. Menke, a R. Willis, a G. Schoiswohl, b M. Basanti, b V. L. Reeves, a E. E. Kershaw. a Department of Obstetrics, Gynecology, and Reproductive Sciences, Magee-Womens Hospital, Pittsburgh, PA; b Department of Medicine, University of Pittsburgh, Pittsburgh, PA.

OBJECTIVE: Elevated circulating lipids and fatty acids have been implicated in both metabolic dysfunction and reduced fertility in diet-induced animal models of obesity. Our objective was to determine the contribution of circulating triacylglycerols (TAGs) and fatty acids to reproductive function in a diet-induced model of obesity.

DESIGN: In vivo mouse model.

MATERIALS AND METHODS: Through use of the Cre/LoxP conditional knockout system, we generated mice with an adipocyte-specific target gene mutation of adipose triglyceride lipase (ATGL), the rate-limiting enzyme mediating TAG hydrolysis. Adipocyte-specific ATGL knockout (AAKO) mice and littermate controls were given ad libitum access to high-fat diet (HFD, 45 kcal% fat). Metabolic and reproductive function was assessed longitudinally for 20 weeks. Parallel breeding studies were performed amongst AAKO and littermate controls for 20 weeks following weaning with males of proven fertility. Data is presented as means and standard error of the means.

RESULTS: Control and AAKO mice exhibited similar gains in gross weight and body fat; however, AAKO mice had significant reductions in serum TAGs (p<0.05) and NEFAs (p<0.05) as well as improved glucose tolerance (p<0.001). No differences in age at first estrus, age at vaginal opening and estrous cyclicity were noted amongst AAKO HFD mice. Continuous breeding studies demonstrated a non-significant reduction in cumulative number of deliveries in AAKO mice on HFD (3.43 ± 0.30 versus 2.57 ± 0.14, p=0.10). No differences in age at first delivery or average litter size were seen.

CONCLUSION: Obesity-associated elevations in serum lipids and NEFAs have been implicated in both metabolic and reproductive dysfunction. Glucose intolerance is also independently associated with infertility; however, despite improved glucose tolerance as well as decreased serum TAGs and NEFAs, mice with adipocyte-specific inhibition of TAG hydrolysis showed no increases in cumulative deliveries whilst on HFD. Rather, a non-significant reduction was noted.

Supported by: NIH K12 HD 063087, NIH R01 DK090166.

1.8, P

9.8 81.8

1.8, P

S. K. Jindal, 9.4 0.28 9.2 70.8 12.7 46.7

a

7.5 0.25 1.6 0.24 0.3 0.08

Y. Aydin, D. Burkankulu, 0.4 2.1

5.5 0.88

1.5, P

6.5 0.69

12) fed HF diet. Group 4: Agouti

2 vs 37.2

a

2.0 14.8 0.9 vs 6.7

Hassa, Y. Aydin, D. Burkankulu, D. Arslantas, D. Sayiner, N. Ozerdogan. Obstetrics and Gynecology Department, Eksiehir Osmangazi University-Medical Faculty, Eksiehir, Turkey; Department of Public Health, Eksiehir Osmangazi University-Medical Faculty, Eksiehir, Turkey; Eksiehir Osmangazi University-Nursing College, Eksiehir, Turkey.

OBJECTIVE: The purpose of this study was to evaluate the effects of polycystic ovary syndrome (PCOS) on the prevalence of metabolic syndrome (MBS) in adolescents with normal body mass index (BMI).

DESIGN: Cross-sectional study conducted in a university hospital adolescent clinic.

MATERIALS AND METHODS: We studied with 63 adolescent girls with PCOS and 159 matched controls. The diagnosis of PCOS was based on the recent ESHRE/ASRM proposal and required that all three of the Rotterdam criteria for diagnosing PCOS in adolescents be met (1). In all of the participants BMI was less than 25 kg/m2. Indices of insulin sensitivity, metabolic variables, circulating androgen levels, lipidemic markers were measured and blood pressures (BP) were assessed. To diagnose the cases with MBS, Cook modified criteria were used and patients who had at least 3 of the 5 criteria were diagnosed with MBS (2).

RESULTS: Adolescent girls with PCOS had higher blood pressure parameters (P<0.01), insulin (P=0.007), LDL (P=0.017), triglycerides (P=0.045) and total (P<0.001) and free testosterone (P=0.001) levels compared to the control group. More cases with at least one of Cook’s criteria were found among girls with PCOS compared to the control group (P=0.05). The prevalence of MBS was significantly higher in girls with PCOS compared to control group (P=0.02).

Prevalence of metabolic risk factors and metabolic syndrome in adolescents with and without PCOS

Table 1. Embryo morphokinetic parameters

<table>
<thead>
<tr>
<th>BMI 18-23</th>
<th>BMI &gt;23</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n=62 embryos)</td>
<td>(n=44 embryos)</td>
</tr>
<tr>
<td>p-value</td>
<td></td>
</tr>
<tr>
<td>t-2 furrow</td>
<td>2.3 ± 0.4</td>
</tr>
<tr>
<td>t-3</td>
<td>2.9 ± 1.3</td>
</tr>
<tr>
<td>t-4</td>
<td>13.3 ± 2.7</td>
</tr>
<tr>
<td>t-5</td>
<td>14.4 ± 2.0</td>
</tr>
<tr>
<td>t-8</td>
<td>26.8 ± 4.2</td>
</tr>
<tr>
<td>t-comp 1</td>
<td>32.8 ± 6.2</td>
</tr>
<tr>
<td>t-comp 2</td>
<td>48.5 ± 12.7</td>
</tr>
<tr>
<td>t-cav</td>
<td>60.1 ± 10.9</td>
</tr>
<tr>
<td>t-blast</td>
<td>71.5 ± 9.2</td>
</tr>
<tr>
<td>t-blast</td>
<td>83.9 ± 9.8</td>
</tr>
</tbody>
</table>

Data are presented in Mean ± S.D.

CONCLUSION: There was no relationship between BMI and embryo morphokinetic parameters in this otherwise homogeneous cohort of patients, suggesting that the negative impact of being overweight on IVF and reproductive outcomes may not be related to timing of early embryonic events.

POLYCYSTIC OVARY SYNDROME

P-365 Wednesday, October 22, 2014

WHAT IS THE RISK OF METABOLIC SYNDROME IN ADOLESCENTS WITH NORMAL BMI WHO HAVE POLYCYSTIC OVARY SYNDROME? H. Hassa, Y. Aydin, D. Burkankulu, D. Arslantas, D. Sayiner, N. Ozerdogan. Obstetrics and Gynecology Department, Eksiehir Osmangazi University-Medical Faculty, Eksiehir, Turkey; Department of Public Health, Eksiehir Osmangazi University-Medical Faculty, Eksiehir, Turkey; Eksiehir Osmangazi University-Nursing College, Eksiehir, Turkey.

OBJECTIVE: To determine if a relationship exists between BMI and timing of early embryonic events using Embryoscope time-lapse imaging.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: We studied with 63 adolescent girls with PCOS and 159 matched controls. The diagnosis of PCOS was based on the recent ESHRE/ASRM proposal and required that all three of the Rotterdam criteria for diagnosing PCOS in adolescents be met (1). In all of the participants BMI was less than 25 kg/m2. Indices of insulin sensitivity, metabolic variables, circulating androgen levels, lipidemic markers were measured and blood pressures (BP) were assessed. To diagnose the cases with MBS, Cook modified criteria were used and patients who had at least 3 of the 5 criteria were diagnosed with MBS (2).

RESULTS: Adolescent girls with PCOS had higher blood pressure parameters (P<0.01), insulin (P=0.007), LDL (P=0.017), triglycerides (P=0.045) and total (P<0.001) and free testosterone (P=0.001) levels compared to the control group. More cases with at least one of Cook’s criteria were found among girls with PCOS compared to the control group (P=0.05). The prevalence of MBS was significantly higher in girls with PCOS compared to control group (P=0.02).

Prevalence of metabolic risk factors and metabolic syndrome in adolescents with and without PCOS

<table>
<thead>
<tr>
<th>Cases with</th>
<th>Cases without</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCOS (n=63)</td>
<td>PCOS (n=159)</td>
</tr>
<tr>
<td>P value</td>
<td></td>
</tr>
<tr>
<td>Cases with ≥1 metabolic risk factor, n (%)</td>
<td>31 (50.1)</td>
</tr>
<tr>
<td>Cases with ≥2 metabolic risk factor, n (%)</td>
<td>7 (11.1)</td>
</tr>
<tr>
<td>Cases with ≥3 metabolic risk factor (metabolic syndrome), n (%)</td>
<td>5 (7.9)</td>
</tr>
</tbody>
</table>

NS: non-significant
CONCLUSION: The prevalence of MBS was higher in adolescent girls with normal BMI and PCOS, compared to an age- and BMI-matched control group. Thus, as clinicians, we must determine the criteria for MBS in girls with PCOS, even if they have a normal BMI. In addition to the most important complaints of adolescent girls with PCOS, such as hirsutism, acne and oligomenorrhea, we must consider the criteria for MBS, which can determine long-term quality of life.

Supported by: This study was funded by the Scientific Investigations Department of our University with the number of 201211008.

P-366 Wednesday, October 22, 2014

SUBJECTIVE PERCEPTION OF HAIR EXCESS RELIABLY PREDICTS OBJECTIVE HIRSUTISM IN WOMEN WITH PCOS. K. W. Keefe, a M. A. Khan, a S. Alaparth, a V. Snegovskikh, a J. Williams, a L. Pal. a Obstetrics, Gynecology & Reproductive Sciences, Yale University School of Medicine, New Haven, CT; bZiauddin Medical College, Karachi, Sindh, Pakistan; cLehigh University, Bethlehem, PA; dObstetrics and Gynecology, Brown University School of Medicine, Providence, RI.

OBJECTIVE: To ascertain how closely subjective perception of hirsutism correlates with objective assessment utilizing Ferriman Galway (FG) pictorial scoring system, and to determine if this relationship varies by race.

DESIGN: Cross sectional study of women who were evaluated at an academic reproductive endocrine practice by a single reproductive endocrinologist for complaints relating to polycystic ovary syndrome (PCOS).

MATERIALS AND METHODS: At initial evaluation, patients were asked to identify subjective perception of hirsutism severity utilizing a modified 11 site FG pictorial scoring system. An objective evaluation of the hair excess severity was then performed by a trained reproductive endocrinologist who was blinded to the subjective scoring at the time of physical examination. Degree of congruence between subjective and objective FG scores was assessed in the context of age and self identified race (White, African American, South Asian, Other).

RESULTS: Of the 101 women diagnosed with PCOS (Rotterdam criteria), subjective assessment of degree and distribution of hair excess highly correlated with objective evaluation by a trained reproductive endocrinologist (r=0.79, p<0.001). The magnitude of correlation between subjective and objective FG scores was highest for AA race (r=0.82, p<0.01), intermediate for Whites (r=0.78, p<0.01), and South Asians (r=0.76, p=0.01) and lowest for women of "other race" (r=0.62, p=0.03). Logistic regression analysis adjusting for patient age and race identified a 33% increased likelihood for hirsutism (FG score ≥8) for each unit increase in subjective FG score (OR 1.13, 95% CI 1.17-1.50).

CONCLUSION: In women with PCOS, patient self-scoring of hair excess intensity and distribution by utilizing FG pictorial chart allows reliable assessment of hirsutism severity.

P-367 Wednesday, October 22, 2014

WOMEN WITH MALE-ASSOCIATED HIP ABNORMALITIES (CAM FEMOROACETABULAR IMPINGEMENT) HAVE HIGHER ANTRAL FOLLICLE COUNTS. T. C. Flodwen, a A. Napoli, a A. H. DeCherney, a A. B. Wolff, b E. F. Wolff. a PRAE, NICHD, NIH, Bethesda, MD; bWashington Orthopaedics & Sports Medicine, Washington, DC.

OBJECTIVE: Hip pain in young people is often the result of Cam type femoroacetabular impingement (FAI), which is known to develop during puberty at the time of proximal femoral physeal closure. There is no way to prevent this abnormal hip development, and patients must undergo complex surgery to correct severe hip damage. Interestingly, Cam FAI is typically seen in men, as the objective of this study was to determine if androgenic hip morphology (defined as cam FAI) was associated with androgenic gynecologic features such as polycystic ovaries, menstrual irregularity and hyperandrogenism.

DESIGN: Prospective cohort of reproductive aged women with hip pain requiring arthroscopic hip surgery.

MATERIALS AND METHODS: Reproductive aged women with indication for arthroscopic hip surgery were assessed. Measurements of alpha angles on 45 degree Dunn lateral radiographs were done. Cam FAI was defined as an alpha angle of >55 degrees. MRI was used to assess antral follicles and the average number per ovary was recorded. In a subset of patients, menstrual irregularity and clinical hyperandrogenism were assessed by history and physical exam. Means were compared using students t test and correlations using Pearson’s. All continuous data was expressed as Mean±SD.

RESULTS: 15 women with cam FAI and 13 without were found to have median alpha angle of 62 and 46 respectively (P<0.0001). Average ages were similar between groups, but antral follicle counts per ovary were significantly higher in women with cam FAI than controls, respectively (13.7±5.3 vs 8.5±2.9, P=0.004). Univariate analysis revealed a statistically significant correlation between alpha angle measurements and antral follicle counts per ovary (R=0.30, P=0.03), indicating that Cam FAI appears to be more consistent with a continuum rather than cut-point with respect to antral follicle counts. Clinical symptoms of PCOS were assessed in a subset of women (n=10), but no association of Cam FAI was detected with a history of clinical hyperandrogenism or menstrual irregularity.

CONCLUSION: Androgenic hip morphology (cam FAI) was found to be strongly associated with antral follicle numbers, but not menstrual irregularity or clinical hyperandrogenism. Further study is needed to characterize biochemical evidence of hyperandrogenism related to this disorder as well as assess hormonal influence such as PCOS on hip development during puberty. Abnormal female hip development during puberty may be amenable to anti-androgenic treatments to prevent the development of Cam FAI.

Supported by: Program in Reproductive and Adult Endocrinology, NICHD, NIH.
CONCLUSION: Serum AMH concentration is significantly elevated in non-obese women compared to obese women with PCOS. Elevated serum AMH is associated with a decrease in number of mature follicles with oral OI therapy. Elevated serum AMH in non-obese women with PCOS may be predictive of decreased odds of ovulation with oral OI agents.

P-369 Wednesday, October 22, 2014

OBJECTIVE: Insulin resistance (IR) is central in the pathophysiology of PCOS. Fasting and 2-hour post-glucose challenge insulin levels are frequently assessed in women with PCOS, but their clinical significance is uncertain. We sought to characterize clinical and biochemical traits of PCOS patients in groups categorized by results of these tests.

DESIGN: Prospective cross-sectional.

MATERIALS AND METHODS: 113 women with PCOS-Rotterdam were systematically evaluated in a multidisciplinary clinic. IR was evaluated by fasting and 2-hour insulin values following 75g oral glucose tolerance test, with cutoffs of 12 and 50 mU/L, respectively. Patients were subdivided into four groups based on results of these tests: G1) both tests normal G2) 2-hour insulin elevated G3) fasting insulin elevated, and G4) both tests elevated. Clinical and metabolic features were compared across groups with the non-parametric test for trend or Fisher’s exact as indicated.

RESULTS: 41% of PCOS patients had normal insulin tests, while 22%, 6% and 31% had abnormal tests categorized into Groups 2, 3 and 4, respectively. Significant differences were noted between BMI waist circumference, fasting triglycerides and HDL. Acanthosis and MFG scores were highest in Groups 3 and 4.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI, kg/m²</td>
<td>25.5 (5.4)</td>
<td>28.2 (7.6)</td>
<td>33.6 (7.0)</td>
<td>35.0 (7.9)</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Age, yrs</td>
<td>28.5 (5.2)</td>
<td>26.9 (7.5)</td>
<td>27.0 (6.0)</td>
<td>28.8 (7.4)</td>
<td>0.931</td>
<td></td>
</tr>
<tr>
<td>Waist, cm</td>
<td>30.9 (4.8)</td>
<td>32.6 (7.1)</td>
<td>36.6 (8.2)</td>
<td>38.6 (10.2)</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>SHBG, nmol/L</td>
<td>57.2 (28.3)</td>
<td>62.0 (55.6)</td>
<td>55.2 (34.3)</td>
<td>51.0 (16.4)</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>177.5 (29.7)</td>
<td>185.4 (44.1)</td>
<td>180.5 (29.8)</td>
<td>179.3 (28.6)</td>
<td>0.765</td>
<td></td>
</tr>
<tr>
<td>Triglycerides</td>
<td>75.8 (37.3)</td>
<td>93.5 (60.1)</td>
<td>106.2 (64.6)</td>
<td>142.6 (91.7)</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>HDL</td>
<td>640.1 (14.9)</td>
<td>593.1 (18.1)</td>
<td>522.4 (14.4)</td>
<td>463.9 (35.0)</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Total testosterone, ng/dL</td>
<td>52.8 (20.6)</td>
<td>46.9 (13.9)</td>
<td>50.3 (28.5)</td>
<td>52.5 (34.5)</td>
<td>0.236</td>
<td></td>
</tr>
<tr>
<td>Androstenedione</td>
<td>1649 (94.7)</td>
<td>1515 (66.5)</td>
<td>1317 (51.2)</td>
<td>1850 (78.2)</td>
<td>0.359</td>
<td></td>
</tr>
<tr>
<td>Acanthosis (%)</td>
<td>26.7</td>
<td>32.0</td>
<td>83.3</td>
<td>77.1</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>MFG Score</td>
<td>6.1 (4.4)</td>
<td>8.0 (6.4)</td>
<td>11.1 (4.6)</td>
<td>10.1 (5.5)</td>
<td>0.000</td>
<td></td>
</tr>
</tbody>
</table>

G1: Both tests normal; G2: 2-hour insulin abnormal; G3: Fasting insulin abnormal; G4: Both tests abnormal; SHBG: sex hormone-binding globulin; MFG: Modified Ferriman Gallway

CONCLUSION: Elevated insulin levels on glucose challenge tests are associated with multiple adverse health indices in PCOS and indicate a more severe metabolic phenotype. Patients with isolated abnormal fasting insulin had slightly worse clinical and metabolic abnormalities than those with isolated 2-hour insulin elevations, while patients with both tests abnormal had the most severe derangements.

P-370 Wednesday, October 22, 2014
EMOTIONAL STRESS AND ASSOCIATED FACTORS IN WOMEN WITH POLYCYSTIC OVARY SYNDROME. J. C. Park, S. M. Kim, J. H. Rhee, J. I. Kim. Department of Obstetrics and Gynecology, School of Medicine, Keimyung University, Daegu, Korea.

OBJECTIVE: The aim of this preliminary study was to evaluate anxiety, depression and stress in women with polycystic ovary syndrome (PCOS) and to investigate the risk factors related to psychological difficulties.

DESIGN: prospective cross-sectional study.

MATERIALS AND METHODS: Sixty women with PCOS were evaluated for level of psychological stress using Beck depression inventory (BDI) and Depression anxiety stress scale (DASS) questionnaire. Serum anti-Mullerian hormone (AMH), total testosterone (T), luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E2), lipid profile and 75g OGTT were also measured. Thirty women seeking general health screening served as the control. Paired T test, Mann-Whitney test, Fisher’s exact test and Pearson’s correlation coefficients test were performed.

RESULTS: Fifty two women with PCOS and twenty nine healthy women completed a questionnaire. The mean age, parity, and status of marriage were comparable. Mean BDI score in women with PCOS was significantly higher (10.15±9.64) compared with control (3.62±2.57). Using DASS, depression (7.08±7.51), anxiety (5.69±5.99), and stress (10.46±7.48) were significantly higher in women with PCOS compared with control. Women with depression who scored more than 13 by BDI and more than 10 by DASS were 58.5%, women with anxiety who scored more than 8 by DASS were 23.1%, and women with stress who scored more than 15 by DASS were 39.5%, which were significantly higher than control. In PCOS women, age, parity and hirsutism (Ferriman-Gallwey score>8) were not correlated with BDI or DASS scale. Serum AMH was significantly correlated with depression (Pearson’s coefficient r=0.83, p<0.01) and anxiety (r=0.43, stress (r=0.53, p<0.01). LH was significantly correlated with depression (r=0.43, stress (r=0.50) and anxiety (r=0.43). Total testosterone was also significantly correlated with depression (r=0.42) and stress(r=0.39). FSH, E2, TSH, and prolactin had a significance with BDI or DASS scale. TG (r=-0.36), BMI (r=0.43) and waist-hip ratio (r=0.43) were also significantly correlated with depression. In women diagnosed as diabetes by 75g OGTT, depression and stress were significantly prevalent.

CONCLUSION: Women with PCOS seemed to be more vulnerable to emotional stress such as depression, anxiety and stress. AMH, LH, dyslipidemia, diabetes, BMI and waist-hip ratio were significantly correlated with emotional stress.

P-371 Wednesday, October 22, 2014

OBJECTIVE: To examine the effect of serum AMH on FR in women with PCOS. In some studies, the IVF outcome was better in groups with lower levels of AMH in patients with PCOS. We evaluated whether age-related decline in FR was still effective in women with PCOS undergone ART when serum AMH level was high.

DESIGN: Single-center retrospective study.

MATERIALS AND METHODS: This study included 26 fresh cycles of in-vitro fertilization (IVF)/ Intracytoplasmic sperm injection (ICSI) at the Seoul National University Hospital from January 2010 to December 2013 in women with PCOS. All patients were nulligravida. PCOS was diagnosed according to the Rotterdam criteria. The relationship between serum AMH level and age with the number of total oocytes, good quality embryo rate and FR was assessed.

RESULTS: The mean number of oocytes retrieved was higher in women with higher serum AMH level (≥10µg/ml) compared with women with lower serum AMH level (23.0 vs. 8.0, P=0.015). The FR was significantly higher in young (<35 years) women compared with old aged women (64.9% vs. 33.0%, P=0.029). However, this difference in FR following age was not significant in case the AMH level was above 10µg/ml. The FR was similar between young and old aged women when serum AMH level was above 10µg/ml (50.0% vs. 52.1%, P=0.099), whereas the FR was significantly higher in young women compared with old aged women (75.1% vs. 18.8%, P=0.025) when serum AMH level was below 10µg/ml.

CONCLUSION: When serum AMH level was high (≥10µg/ml), the age-related decline in FR after ART was insignificant in women with PCOS.
OBJECTIVE: Proteolytic processing of amyloid precursor protein (APP) by α- or β-secretase results in two soluble metabolites, sAPPα and sAPPβ, respectively. Previous studies have shown that sAPP shows similarities with growth factors and increases the in vitro proliferation of many types of cells. The aim of this study was to compare the levels of sAPP in follicular fluid (FF) between polycystic ovarian syndrome (PCOS) women and non-PCOS women.

DESIGN: Prospective comparative study.

MATERIALS AND METHODS: Twenty-five women with PCOS (study group) and thirty non-PCOS women (control group) undergoing IVF-ET at the Center for Assisted Reproduction of Nanfang hospital from January, 2013 to January, 2014, were included in this study. FF sample from a single dominant follicle form each patient was collected during oocyte retrieval. FF was centrifugated for 10min at 1000 rmp and the supernatant was stored at −20°C. Serum samples for the control and study groups were obtained from 45 non-PCOS and 41 PCOS women, respectively. The serum total testosterone, LH/FSH ratio, AMH and sAPP levels were determined by using the commercially available, sAPP enzyme immunoassay ELISA kit. Statistical analysis was made with Student’s t-test. The data are expressed as mean±SE. P<0.05 was considered statistically significant. The study was approved by the Ethics Committee and each woman filled a written informed consent before to participate to it.

RESULTS: There were no statistically significant differences in the mean age, BMI, serum basal FSH levels, fasting glucose between the groups. The women with PCOS had significantly higher antral follicle count (AFC) (30.4 ± 6.7 VS 14.0 ± 4.9, P=0.000), total testosterone (0.512 ± 0.208 VS 0.278 ± 0.128, P=0.000), serum basal LH levels (9.87 ± 4.93 VS 4.26 ± 1.35, P=0.000), LH/FSH ratio (1.73 ± 0.91 VS 0.63 ± 0.19, P=0.000), number of collected oocytes (150 ± 467 VS 2458 ± 772, P=0.000) and lower total gonadotrophin dosage used (1500 ± 467 VS 2458 ± 772, P=0.000) than controls. sAPPβ concentrations were significantly higher in the FF of PCOS patients compared with controls (143.96 ± 25.60 ng/mL VS 126.31 ± 33.37 ng/ml, P=0.035). PCOS women also had higher levels of sAPPα compared with the control group (179.11 ± 31.58 ng/mL VS 167.35 ± 36.87 ng/ml), but this difference did not achieve significance (P=0.215).

CONCLUSION: The present study demonstrates that sAPP levels in FF is increased in PCOS patients compared with non-PCOS women, may suggest that either sAPP play a contributin role in the pathogenesis of PCOS or they may be the by-products of the pathogenetic mechanisms leading to the condition.

Supported by: Science Foundation of Nanfang Hospital (G201206); National Natural Science Foundation of China (81170754, 31571517).

P-374 Wednesday, October 22, 2014


OBJECTIVE: Anti-mullerian hormone (AMH) is an established marker of ovarian reserve. AMH levels tend to be higher in patients with PCOS. AMH has been shown to have an inverse relationship with body mass index (BMI) in PCOS patients. The impact of AMH and BMI levels on pregnancy outcomes in CC/IUI is unknown and its understanding can promote evidence-based IUI recommendations.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: We reviewed 103 cycles in 45 women diagnosed with PCOS as defined by the Rotterdam criteria who underwent CC/IUI between 2012-2013. Patients were subdivided into groups based on AMH level and BMI category to determine the differences in clinical pregnancy rates (CPR). CPR was defined as a fetal heart rate detected by ultrasound. The generalized linear mixed model was used to determine clinical significance.

RESULTS:

Clinical Pregnancy Rates (n) According to BMI (kg/m²) and AMH (ng/mL)

<table>
<thead>
<tr>
<th>BMI &lt; 19</th>
<th>BMI 19-24</th>
<th>BMI 25-29</th>
<th>BMI &gt; 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMH &lt; 0.9</td>
<td>0(0)</td>
<td>20.0(5)</td>
<td>0(0)</td>
</tr>
<tr>
<td>AMH 0.9-1.9</td>
<td>50.0(2)</td>
<td>0(8)</td>
<td>0(3)</td>
</tr>
<tr>
<td>AMH 1.9-3.9</td>
<td>0(1)</td>
<td>33.3(9)</td>
<td>0(1)</td>
</tr>
<tr>
<td>AMH ≥ 4</td>
<td>0(0)</td>
<td>25.8(31)</td>
<td>33.3(3)</td>
</tr>
</tbody>
</table>

Most patients had a high AMH, consistent with previous reports that AMH in PCOS patients are two fold higher in PCOS than controls (1). Pregnancies were encountered at all BMI ranges and BMI values and did not decline with obesity. Previous studies have concluded that the AMH level in PCOS patients is a potential marker for recruited non-growing follicles rather than a marker for ovarian reserve (1).

CONCLUSION: In women diagnosed with PCOS undergoing CC/IUI, AMH levels are not predictive of CPR when adjusting for BMI. While commonly much higher in this patient population, no “infertile threshold” can be determined for either AMH or BMI for PCOS patients. These findings may indicate that the success of ovulation induction in PCOS does not correlate with the size of the cohort of non-growing follicles.

P-375 Wednesday, October 22, 2014

TRIGGERING OF FINAL OOCYTE MATURATION WITH GONADOTOXIN RELEASE IN HORMONE AGONIST IN PATIENTS WITH POLYCYSTIC OVARIAN SYNDROME UNDERGOING IN VITRO FERTILIZATION, T. B. Tarlatzi, E. M. Kolibianakis, J. K. Bosdou, C. A. Venetsis, A. Makedos, K. Chatzimeletiou, L. Zepiridis, G. Lainas, I. Sfoutouris, T. Lainas, B. C. Tarlatzi. "Unit for Human Reproduction, 1st Dept. of Obstetrics and Gynaecology, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece; "Eugonia Unit of Assisted Reproduction, Athens, Greece; "Fertility Clinic, Erasmus Hospital, Universite Libre de Bruxelles, Anderlecht, Brussels, Belgium.

OBJECTIVE: To assess the safety and efficacy of triggering final oocyte maturation with gonadotrophin releasing hormone (GnRH) agonist and performing embryo transfer in a frozen embryo transfer (FRET) cycle in 121
patients with polycystic ovaries (PCO), at high risk for ovarian hyperstimulation syndrome (OHSS) (≥ 14 follicles ≥ 11 mm).

DESIGN: Prospective, observational study (September 2011-January 2014).

MATERIALS AND METHODS: Ovarian stimulation was performed with 200 IU of recombinant follicle stimulating hormone (FSH) and GnRH antagonists. Triggering of final oocyte maturation was performed with 0.2 mg triptorelin, when at least three follicles of 17 mm were present at ultrasonography 2-3 hours before triggering. Oocytes were retrieved after human chorionic gonadotropin (10000 IU) injection and following fertilization, 966 2pn oocytes were retrieved (mean number 10.7±5.5). None of the patients experienced severe OHSS or was hospitalised. Furthermore, none of the patients reported nausea, abdominal pain, oliguria or feeling of unwellness. Until today, from the 121 patients triggered with GnRH agonist, 114 have performed at least one FRT cycle (max. number of FRT cycles=3). Overall 165 cycles have been performed. Ongoing pregnancy rate in the first FRT cycle (29/114 patients) was 25.4% [95% confidence interval (CI): 18.3-34.1%]. Cumulative ongoing pregnancy rate after the third FRT cycle was 62.7% [95% CI: 44.5-80.7%]. Currently, 633 embryos have been thawed, while 333 2pn oocytes are still frozen.

CONCLUSION: Triggering of final oocyte maturation with GnRH agonist in women who fail to develop a mature follicle predicts OHSS and results in a high cumulative probability of ongoing pregnancy after transfer of embryos in subsequent FRT cycles. This is a non-comparative study and thus conclusions regarding the relative efficacy and safety of agonist versus human chorionic gonadotropin triggering cannot be drawn. On the other hand, a comparative study addressing the above question might be ethically challenging.

P-376 Wednesday, October 22, 2014

ELEVATED SERUM ANTI-MULLERIAN HORMONE (AMH) LEVELS ARE ASSOCIATED WITH A DIMINISHED RESPONSE TO OVULATION INDUCTION WITH LETROZOLE (LZ) IN WOMEN WITH POLYCYSTIC OVARY SYNDROME (PCOS). D. Torres, a A. A. Delaney, a C. Salyer, a C. H. Ziegler, a S. T. Nakajima, a H. Bohler, a Obstetrics, Gynecology, and Women’s Health, University of Louisville, Louisville, KY; b School of Medicine, University of Louisville, Louisville, KY; c Obstetrics and Gynecology, Stanford School of Medicine, Palo Alto, CA.

OBJECTIVE: AMH is known to diminish response of follicles to follicle stimulating hormone (FSH)1. High levels of AMH, found in PCOS patients may confer a resistance to ovulation induction2. Our objective was to see whether serum AMH levels were increased in PCOS patients who failed to develop a mature follicle during ovulation induction with LZ.

DESIGN: Retrospective chart review.

MATERIALS AND METHODS: This is a retrospective chart review conducted at the University of Louisville Infertility clinic. It included 49 women with PCOS who received ovulation induction with LZ. Inclusion criteria included: age between 18 and 40 years, BMI ≤ 35, and diagnosis of PCOS based on the Rotterdam criteria. Variables analyzed included: age, body mass index (BMI), AMH, thyroid stimulating hormone, luteinizing hormone, FSH, free and total testosterone, and prolactin. Patients received LZ per standard protocol. Mean follicular size was assessed via transvaginal ultrasonography. A mature follicle was defined as a mid-cycle follicle 17mm or larger in greatest dimension. Non-responders to LZ were defined as patients who failed to develop a mature follicle after receiving LZ 5.0mg. Statistical analysis included chi-squared (x2) analysis, x2 tests for linear trend, independent sample t-tests, receiver operating characteristic curves (ROC), and logistic regression analysis. Statistical significance was set at p < 0.05.

RESULTS: Clinical characteristics between responders and non-responders were not significantly different, P > 0.05. Our ROC curve analysis showed the area-under-the-curve was 0.69 in predicting non-responders and showed the optimal cut point as AMH ≥ 4.53 ng/mL (sensitivity 0.67; specificity 0.71; positive predictive value 0.57; negative predictive value 0.79). A significant difference between those with AMH ≥ 4.53 ng/mL and <4.53 ng/mL was found in those who developed a mature follicle, [AMH ≥ 4.53 ng/mL, 43% (9/21)]; AMH < 4.53 ng/mL, 79% (22/28), P = 0.01). Logistic regression showed no interaction between BMI (≥ or < 30) and AMH cut point values, P = 0.905. A linear trend existed in development of a mature follicle with increasing age, P = 0.015.

CONCLUSION: PCOS women with AMH ≥ 4.53 ng/mL are less likely to develop a mature follicle after attempting ovulation induction with LZ.

P-377 Wednesday, October 22, 2014

DECREASED MTDNA COPY NUMBER AND GLUTATHIONE PEROXIDASE IN GRANULOSA CELLS OF POLYCYSTIC OVARY SYNDROME WOMEN. H.-H. Lin, a R.-H. Hsieh, a J.-W. Wang, b C.-H. Chen, a Y.-F. Chen, c R. T. Reeg, d Center for Reproductive Medicine and Sciences, Taipei Medical University Hospital, Taipei, Taiwan; e Department of Obstetrics and Gynecology, Taipei Medical University Hospital, Taipei, Taiwan; f School of Nutrition and Health Sciences, Taipei Medical University, Taipei, Taiwan.

OBJECTIVE: Granulosa cells (GCs) produce steroidal hormones and growth factors, and are endowed with antioxidant and detoxifying enzymes, which are essential for normal follicular maturation process. To analyze oxidative stress and mitochondrial function in GCs, and whether oxidative stress and mitochondrial function is associated with embryo quality in IVF patients.

DESIGN: To analysis mitochondria function and oxidative stress in GCs and to distinguish the relationship between mitochondria functions and oocyte quality in IVF program of a university hospital.

MATERIALS AND METHODS: GCs were obtained from follicular aspirates of 63 women undergoing in vitro fertilization. The levels of malondialdehyde (MDA), glutathione peroxidase (Gpx), mitochondrial oxygen consumption and mitochondrial DNA (mtDNA) copy number were analyzed. The number of oocytes, fertilization rate, embryo morphology and embryo quality were also assessed. Besides, we also analyzed the relationship between mtDNA copy number and two IVF protocols including gonadotropin releasing hormone antagonist and agonist, respectively.

RESULTS: The concentration of MDA in GCs of women with polycystic ovary syndrome (PCOS) (1.60 ±0.05 mg/g) was significantly higher (p<0.05) than that in control subjects (0.86 ±0.06 mg/g). The activity of Gpx was significantly lower in PCOS (1270.27 ±0.05 U/g) than that in control patients (1327.35 ±0.06 U/g) and other subfertile groups with female factors compared with that of the controls (2183.75 ±0.05 U/g). The maximal respiration and mtDNA copy number were lower in the PCOS group than in control group. Gpx in GCs had no correlation with IVF outcome. However, MDA was negatively correlated with the quality of embryo (R = 0.502, p = 0.017). Compared the difference between two drugs, mtDNA copy number in antagonist group was significantly higher than agonist group.

CONCLUSION: When compared PCOS group with control group, the oxidative stress was relatively higher whereas the level of antioxidant enzyme and mitochondrial function were relatively lower. MDA level was negatively correlated with the quality of embryo. Increased mtDNA copy number was correlated with higher quality of embryo. Therefore, mtDNA copy number in GCs might be an indicator of good quality of embryo.

P-378 Wednesday, October 22, 2014

EXPRESSION OF INSULIN-LIKE GROWTH FACTOR I ISOFORMS IN WOMEN WITH AND WITHOUT POLYCYSTIC OVARY SYNDROME. M. Brazert, a D. Antoni, a G. Gózdziak-Józefik, a P. Leszek, b Poznan University of Medical Sciences, Poznan, Poland; b University of California, San Diego, La Jolla, CA; d Adam Mickiewicz University, Poznan, Poland.

OBJECTIVE: Altered folliculogenensis and ovarian dysfunction is one of the hallmarks of polycystic ovary syndrome (PCOS). Insulin-like growth factor-I (IGF-1) affects follicular development including growth and steroidogenesis of theca-interstitial compartment; abnormal expression of IGF-1 may contribute to ovarian dysfunction associated with PCOS. Growing evidence in studies of tissues such as muscles and bones indicates that alternative splicing of IGF-1 pre-mRNA produces several isoforms including IGF-1Ea, IGF-1Eb and IGF-1Ec. IGF-1Ec, also named mechnano-growth factor (MGF), is associated with processes such as regeneration and hypertrophy of muscles. This study was designed to evaluate expression of different species of IGF-1 mRNA in ovaries of women with PCOS and healthy controls.

DESIGN: Research study.

MATERIALS AND METHODS: Serum and ovarian tissues were obtained from women with PCOS not taking metformin (PCOS-M, n=37), PCOS women taking metformin (PCOS+M, n=12) and Control subjects (n=21). Expression of mRNA species of IGF-1 in ovarian fragments was determined by quantitative RT-PCR. Serum steroids were measured by specific radioimmunoassays. Comparisons involved Wilcoxon/Kruskal-Wallis test and host hoc tests of pairs by Dunn method for joint ranking.
RESULTS: In all groups, IGF-1 mRNA was the predominant form with no significant differences between the groups. In contrast, individual groups differed with regard to expression of IGF-1Eb and IGF-1Ec mRNA. Expression of IGF-1Eb was the greatest in ovaries from PCOS-M (respectively, by 99% and by 154% higher than in Controls and PCOS+M; p < 0.05). Similarly, the expression of IGF-1Ec was the greatest in PCOS-M (respectively, by 79% and 202% higher than in Controls and PCOS+M; p < 0.05). Relative expression of IGF-1Ec correlated positively with total testosterone, free testosterone, and IGF-1 (p < 0.01).

CONCLUSION: To our knowledge this is the first report identifying different species of IGF-1 mRNA in ovary. Significantly increased expression of isoforms 1Eb and 1Ec is found in ovaries of women with PCOS, but only in those who are not treated with metformin. Furthermore, both ovarian and adrenal androgen levels correlated with mRNA encoding for MGF. We propose that altered expression of IGF-1 isoforms may play a role in pathophysiology of PCOS.

Supported by: N N407 298440: National Science Centre Poland.

P-379 Wednesday, October 22, 2014

HCG-STIMULATED 17OHPR RESPONSES AND BASAL SERUM AMH LEVELS AMONG ADOLESCENT WOMEN WITH POLYCYSTIC OVARY SYNDROME. J. Hou,1 K. H. Maas,2 H. Cook-Andersen,3 C. M. Burt-Solorzano,4 R. Shaya1, A. Kumar,5 R. J. Chang5 Reproductive Medicine, University of California, San Diego, La Jolla, CA; 2Department of Pediatrics, Center for Research in Reproduction, Charlottesville, VA; 3Ansh Labs, Webster, TX.

OBJECTIVE: We have previously shown that some adult women with PCOS have exaggerated 17-hydroxyprogesterone (17-OHP) responses to hCG (HR-PCOS), while the others exhibit responses equivalent to normal women (NR-PCOS)1. Additionally, differences in hormonal profiles were observed among these subgroups. Notably, serum AMH levels were lower in HR-PCOS compared to that of NR-PCOS2. These studies were conducted in adult women. We sought to determine whether disparate responses to hCG exist in adolescents with PCOS. To address this issue, we evaluated 17OHP responses to hCG in normal and PCOS adolescent women. We hypothesized that the subgroup prevalence and hormonal profiles in adolescents with PCOS would be similar to that observed in adults.

DESIGN: A prospective study in an academic center.

MATERIALS AND METHODS: Adolescent women (12-18yo) with PCOS (n = 22) and age-matched normal controls (n = 10) underwent iv administration of recombinant hCG (r-hCG) following dexamethasone suppression. Blood samples were obtained before and 24 hours after r-hCG injection. Serum 17-OHP levels were measured in all samples whereas AMH was measured at baseline. Unpaired Student’s T-test or one-way analysis of variance using Tukey’s post hoc comparisons was used for data analysis.

RESULTS: In adolescent HR-PCOS the mean basal 17-OHP was higher than that observed for NR-PCOS (p < 0.001) or normal controls (p < 0.001). Following r-hCG, 8 adolescent PCOS subjects exhibited exaggerated 17OHP responses while 14 had responses equivalent to those of normal adolescents. Both HR- and NR-PCOS groups had similar BMI values. Serum AMH levels were higher in adolescent PCOS compared to normal controls, but significant differences among groups were not observed.

CONCLUSION: Similar to adult, adolescent women with PCOS may be designated as HR-PCOS or NR-PCOS according to responses to r-hCG. However, unlike their adult counterparts, serum AMH levels are not significantly different between subgroups, possibly reflecting differences in either granulosa cell function or follicle distribution between adolescent and adult women with PCOS. Additional studies will be necessary to confirm these preliminary results in adolescent women with PCOS.

Supported by: Eunice Kennedy Shriver NICHD/NIH (U54 HD012303-29), NIH grant MO1 RR00827and T32 HD07203.

P-381 Wednesday, October 22, 2014

ARE OLIGOMENORRHEA AND HYPERANDROGENISM DURING ADOLESCENCE PREDICTIVE OF A CONFIRMED DIAGNOSIS OF POLYCYSTIC OVARIAN SYNDROME IN ADULTHOOD? M. Khrouf1, F. Douik1, K. H. Maas,2 F. Zhioua3 A. Kumar4, I. Kamoun5, C. M. Burt-Solorzano6, B. Arbeau7, G. Zeffane8, J. Hou1 and E. Veldhuis9. 1Obstetrics, Gynecology and Human Reproduction, University of California, San Diego, La Jolla, CA; 2Department of Pediatrics, Center for Research in Reproduction, Charlottesville, VA; 3Ansh Labs, Webster, TX; 4Obstetrics and Gynecology, Southern California Permanente Medical Group, Irvine, CA; 5Obstetrics and Gynecology, Southern California Permanente Medical Group, El Cajon, CA; 6Obstetrics and Gynecology, Center for Research in Reproduction, Charlottesville, VA; 7Obstetrics and Gynecology, Southern California Permanente Medical Group, El Cajon, CA; 8Obstetrics and Gynecology, University of California, San Diego, La Jolla, CA.

OBJECTIVE: To verify if Oligomenorrhea and hyperandrogenism manifestations during adolescence are predictive of a confirmed diagnosis of polycystic ovarian syndrome(PCOS) in adulthood according Rotterdam Criteria.

DESIGN: Retrospective case-control study.

MATERIALS AND METHODS: Forty (40) PCOS adult women, diagnosed according Rotterdam Criteria, (PCOS group) and 36 normo-ovulatory women matched for age and BMI (control group) were questioned about oligomenorrhea (defined as ten cycles or less per year) and hyperandrogenism manifestations (hirsutism and acne) occurring during two periods of their adolescence : the first two years after menarche (first period) and from 2 years after menarche to age of 18 years(second period). We compared the two groups for the occurrence of each sign (cycle’s regularity and hyperandrogenism manifestations), during the two defined periods using the Chi² test. We also calculated odds ratio for prediction of a confirmed diagnosis of PCOS on adulthood. A p value of 0.05 was considered as significant.

RESULTS: The two groups were comparable regarding age and BMI (respectively 39.9 ± 4.88 versus 39.8 ± 4.86 years and 30.2 ± 8.6 versus 29.5 ± 5.3 Kg/m²). Concerning the first period of adolescence: only oligomenorrhea (defined as ten cycles or less per year) and hyperandrogenism manifestations (hirsutism and acne) were significantly associated with a confirmed diagnosis of adult PCOS (OR = 2.3 CI 95% [1.57-3.37]). Hirsutism and Acne have not been associated with a confirmation of PCOS diagnosis during this period (Hirsutism: OR = 1.34 CI 95% [0.91-1.91]; Acne: OR = 0.72 CI 95% [0.42-1.21]). During the second period of adolescence, both oligomenorrhea and hirsutism were significantly predictive of a confirmed diagnosis of PCOS on adult age (respectively OR=1.68 CI95% [1.14-2.47] and OR=1.77 CI95%
Acne during the second period of adolescence was not predictive of confirmation of PCOS diagnosis in adulthood (OR = 0.86 CI 95% [0.49-1.51]).

CONCLUSION: Oligomenorrhea during adolescence and hirsutism occurring after the two years following menarche increase risk to be diagnosed as PCOS in adulthood. Acne during adolescence is not related to PCOS confirmation in adult age.

P-382 Wednesday, October 22, 2014
CDKN2A-CDKN2B AND IGFBP2 GENE POLYMORPHISMS IN WOMEN WITH POLYCYSTIC OVARY SYNDROME. J. Kim, Y. Choi, a,b K. Hwang, a,b S. Kim, a,b S. Chae, c S. Yoon. aObstetrics and Gynecology, Healthcare System Gangnam Center, Seoul National University Hospital, Seoul, Republic of Korea; bObstetrics and Gynaecology, Seoul National University College of Medicine, Seoul, Republic of Korea; cThe Institute of Reproductive Medicine and Population, Medical Research Centre, Seoul National University College of Medicine, Seoul, Republic of Korea;
Objective: To discuss the clinical features of glucose metabolism - CDKN2A-CDKN2B and IGFBP2 - uncovered.

DESIGN: Case-control study.

MATERIALS AND METHODS: Measurements: DNA samples from 552 PCOS patients and 559 age-matched controls were genotyped. Four SNPs (rs564398, rs1333040, rs10757278 and rs10811661) in CDKN2A-CDKN2B and one SNP (rs4402960) in IGFBP2 were evaluated. To investigate the association between the presence of PCOS and each individual SNP, logistic regression analyses were performed using the homozygote of wild-type allele as the reference category.

RESULTS: Compared with the 559 controls, we found that PCOS was significantly associated with heterozygote of the rs10757278 in CDKN2A-CDKN2B, and their ORs ranged from 1.10 to 1.89. None of the remaining four SNPs was associated with PCOS. For further analysis, the PCOS patients were divided into two or three subgroups according to genotype, and the associations between the genotypes and insulin resistance or insulin secretory capacity were assessed. No SNPs were significantly associated with HOMA-IR, HOMA_b (β), or 2-hour 75-g OGTT insulin levels in the PCOS patients: there were no significant associations with other serum hormonal and metabolic markers, such as androgen or glucose levels.

CONCLUSION: Our results suggest that except rs10757278 in CDKN2A-CDKN2B, most of the CDKN2A-CDKN2B and IGFBP2 polymorphisms are not associated with PCOS.

Supported by: This research was Supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (NFR-2010-0009075).

P-384 Wednesday, October 22, 2014
NUCLEAR MAGNETIC RESONANCE (NMR) METABONOMICS REVEALS ALTERED SERUM METABOLITES AS PREDICTIVE MARKERS FOR POLYCYSTIC OVARY SYNDROME (PCOS).

B. R. Geetha, 1 S. R. Choudhury, 1 R. Chattopadhyay, 1 S. K. Goswami, 1 S. Ghosh, 1 K. Choudhury, 1 B. Chakravarty. 1Reproductive Medicine, Institute of Reproductive Medicine, Kolkata, West Bengal, India; 2School of Medical Science and Technology, Indian Institute of Technology, Kharagpur, West Bengal, India.

OBJECTIVE: To identify differently expressed metabolites and evaluate them as predictive markers of polycystic ovary syndrome (PCOS).

DESIGN: 25 PCOS women (according to Rotterdam criteria 2003; ≥18 yrs to ≤40 years)[1] diagnosed with hyperandrogenism and ovarian dysfunction were recruited from January 2013 to October 2013. Age and parity matched proven fertile women in good health condition and reporting for tubal ligation were considered as the control group (n=25).

MATERIALS AND METHODS: Serum samples were collected from PCOS and controls. During nuclear magnetic resonance (NMR) analysis, samples were mixed with deuterium oxide containing reference and Carr-Purcell-Meiboom-Gill (CPMG) spin-echo spectral[2] obtained, data processed and subjected to statistical analysis. Principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA) and orthogonal-PLS-DA were applied to discriminate between the 2 groups.

RESULTS: Several multivariate analyses, including PCA and supervised higher models, i.e. PLS-DA (R2 0.88 and Q2 0.8) and OPLS-DA (R2 0.92 and Q2 0.82) have shown highly distinct classification based on the differently expressed metabolites in serum from PCOS as compared to controls. The metabolites which contributed most towards this differentiation were identified. The energy metabolites such as citrate, choline and glucose were found to be down-regulated in PCOS as compared to that of controls, whereas in case of amino acids, glycine was down-regulated and histidine, proline up-regulated. These observations suggest that in PCOS, there is a detrimental role of impaired metabolism of amino acid and energy metabolites. These molecules also bring about the hope for development of predictive markers for the disease.

CONCLUSION: Six metabolites, including citrate, choline, glucose, glycine, histidine and proline were found to be significantly altered in PCOS patients as compared to controls. The differences in expression of these metabolites were able to discriminate between PCOS and control groups very efficiently while building predictive statistical models. The observations from these models verify the merit of this set of metabolites as predictive markers for the disease. Though these findings appear to be promising, this study needs to be extended to a larger sample size for accurate predictability.

Supported by: The study was Supported by the Department of Biotechnology, Government of India (BT/PR/063/BRB/10/1058/2012).
ELEVATED LEVELS OF DIABETES ASSOCIATED PEPTIDE HORMONES ARE FOUND IN FOLLICULAR FLUID AND SERUM OF OBESE POLYCYSTIC OVARY SYNDROME PATIENTS. A. E. Batcheller, A. M. Martinez, R. Linderheim, D. Cool, W. Grunwald, Jr., K. B. DiPaolo, J. M. Strogar. UC Center for Reproductive Medicine, University of Cincinnati, West Chester, OH; Obstetrics and Gynecology, Wright State University, Dayton, OH.

OBJECTIVE: To identify the relationship between diabetes associated peptide hormones (DAP) and inflammatory markers in follicular fluid and serum of obese and lean polycystic ovary syndrome (PCOS) patients with in vitro fertilization (IVF) cycle parameters and pregnancy outcomes.

DESIGN: Prospective Descriptive Study.

MATERIALS AND METHODS: An IRB approved study of three groups of IVF patients <35 yrs old: obese PCOS (BMI>30 kg/m2; n=8), lean PCOS (BMI=25 kg/m2; n=12), and donor or tubal factor controls (n=11). PCOS was diagnosed by Rotterdam criteria. Serum was collected prior to IVF cycle initiation. Follicular fluid was collected at retrieval. A Human Diabetes 10-plex Assay was used to quantify DAP. A Human Cytokine 27-plex Assay was used to quantify inflammatory markers. Results were analyzed using one-way ANOVA with Tukey post hoc analysis using SPSS v 21. Significance was defined as p<0.05.

RESULTS: Of the 10 DAP assessed, C-peptide, insulin, and leptin had significant associations. Obese PCOS patients had higher levels of C-peptide (194.4±163.5 pg/mL), insulin (172.6±113.6 pg/mL), and leptin (10,046.1±4920.2 pg/mL) in follicular fluid compared to lean PCOS and controls. A difference in C-peptide (p<0.03), insulin (p<0.01), and leptin (p=0.04) was noted between obese and lean PCOS follicular fluid. A difference in C-peptide (p<0.03) and insulin (p<0.01) was noted between obese PCOS and control follicular fluid. Obese PCOS had higher levels of serum leptin (5575.5±1650.2 pg/mL), with a difference in leptin concentrations noted between obese PCOS and controls (p<0.01), and obese and lean PCOS (p<0.01). Higher levels of C-peptide (p<0.04) and leptin (p=0.01) in the follicular fluid were associated with increased gonadotropin usage. A trend toward fewer oocytes retrieved (p=0.06), and significantly lower number of normally fertilized zygotes (p=0.04) was seen with higher C-peptide levels. A trend toward an increased clinical pregnancy rate was noted with lower serum leptin levels (p=0.08). There was no significant difference in inflammatory marker concentration or pregnancy outcomes between the groups.

CONCLUSION: Elevated levels DAP, which are highly correlated with adverse long-term health outcomes, are present in young obese PCOS patients undergoing IVF. DAP levels may play a role in the need for higher gonadotropin usage. A trend toward fewer oocytes retrieved, and significantly lower number of normally fertilized zygotes was seen with higher C-peptide levels. A trend toward an increased clinical pregnancy rate was noted with lower serum leptin levels. There was no significant difference in inflammatory marker concentration or pregnancy outcomes between the groups.

P-386 Wednesday, October 22, 2014

SITAGLIPTIN AND METFORMIN ALONE OR IN COMBINATION IN PATIENTS WITH POLYCYSTIC OVARIAN SYNDROME (PCOS): A PILOT STUDY. J. C. Paredes-Palma E. López-Bayg hen. *Departamento de Toxicología, Cinvestav-IPN, México, DF, Mexico; **Hospital General Dario Fernández, ISSSTE, México, DF, Mexico; ***Maestría en Endocrinología Ginecológica e Infertilidad, Instituto de Infertilidad y Genética, Ingenes, México, DF, Mexico.

OBJECTIVE: To evaluate the effect of treatment with sitagliptin and metformin alone and in combination on menstrual frequency, hormonal and metabolic profiles in obese and non-obese women with PCOS.

DESIGN: Open-randomized controlled clinical trial.

MATERIALS AND METHODS: IC; age 18-37 yrs; PCOS (Rotterdam criteria); no history of ART (previous 6 mo). EC: diabetes mellitus, smokers, or those under hormone therapy or treatment with drugs affecting intestinal motility, lipid levels, weight, or metformin intake (6 mo prior to study entry). Candidates were interviewed every Friday and explained the reason for the study, advantages and disadvantages (transvaginal USG and quantification of LH, FSH, testosterone, androstenedione, dehydroepiandrosterone-sulfate, prolactin, cortisol, ACTH, TSH, T4, T3). Patients were quoted in follicular phase of menstrual cycle (days 1 to 5 of menstruation); at this time randomization was performed: G1, metformin 850 mg/24 h; G2, Sitagliptin 100 mg/24 h; G3, Sitagliptin plus metformin same doses. Before first dose, a glucose tolerance curve (5 h/75g of glucose); glucose, lipid pro-

e266 ASRM Abstracts Vol. 102, No. 3, Supplement, September 2014

tile and insulin were recorded. Same measurements: 24 weeks after completion of treatment. Anthropometry was recorded. Normal menstrual proliferative periods in 24 weeks. Statistical analysis: paired t tests and chi-square test; P<0.05.

RESULTS: Sitagliptin or metformin improved menstruation index and progesterone levels in women with PCOS. The sitagliptin plus metformin combo was more effective improving the ovulation percentage and the progesterone levels than the other two treatments with no significant change in BMI. While the trend suggests that metformin would have a greater effect on increasing the frequency of menstrual periods, no statistical significant differences were found. Metformin alone produced weight and waist circumference losses but weight was not modified in the group treated with the combo. No statistically significant differences were observed in weight with the combo treatment, indicating that the observed increase in the frequency of menstruation and ovulation could be directly attributed to treatment. A significant decrease in insulin secretion was noticed in the combo and metformin groups with more changes in the metformin group.

CONCLUSION: The sitagliptin plus metformin combo improved menstrual function in PCOS patients, independently of weight loss suggesting a direct effect over ovarian function.

Supported by: Conacyt 212650.

P-387 Wednesday, October 22, 2014

PRENATAL ANDROGEN EXCESS ENHANCES STIMULATION OF THE GnRH PULSE BY NEUROKININ B AND KISSPEPTIN IN PUBERTAL FEMALE RATS. X. Yan, C. Yuan, N. Zhao, Y. Cui, J. Liu. “State Key Laboratory of Reproductive Medicine, Clinical Center of Reproductive Medicine, First Affiliated Hospital, Nanjing Medical University, Jiangsu, China; “Clinical Center of Reproductive Medicine, First Affiliated Hospital, Xuzhou Medical College, Xuzhou, Jiangsu, China.

OBJECTIVE: Adolescent girls with polycystic ovary syndrome (PCOS) manifest neuroendocrine derangements after the onset of puberty, characterized by a rapid LH pulse frequency. The early mechanism underlying the pubertal regulation of the GnRH/LH pulsatile release in adolescents with PCOS remains uncertain. The aim of the study was to determine the effects of prenatal androgen exposure on the GnRH pulse and LH secretion at puberty.

DESIGN: We administrated DHT to pregnant rats and observed serum LH levels and hypothalamic genes expression in female offspring from postnatal 4 to 8 weeks.

MATERIALS AND METHODS: At the age of 4.6 and 8 weeks, we measured the level of serum LH and evaluated Kissl,NKB,0rb mRNA levels in the hypothalamic arcuate nucleus of PCOS rats. Kisspeptin, NK3R agonist and Leptin were intracerebrally injected into the lateral cerebral ventricle of 6-week-old PCOS rats. The serum LH were measured 0,15,30 and 60 min after administration.

RESULTS: The 6-week-old prenatally androgenized (PNA) female rats exhibited significantly elevated LH pulse frequency. The hypothalamic expression of NKB and 0rb mRNA in the PNA rats remarkably increased before puberty and remained high during puberty. However, increased Kissl mRNA levels were detected only after the onset of puberty. Exogenous Kisspeptin, NK3R agonist and Leptin exerted tonic stimulation of GnRH neurons and increased LH secretion in pubertal PNA rats. Moreover, Leptin upregulated Kissl mRNA expression in the hypothalamus of pubertal PNA rats; however, pretreatment with a Kisspeptin antagonist failed to suppress the elevated serum LH levels stimulated by Leptin, indicating that the stimulatory effects of Leptin may be conveyed indirectly to GnRH neurons via other neural components, rather than through the Kisspeptin-GPR54 pathway.

CONCLUSION: These findings showed that NKB and Leptin play an essential role in the activation of GnRH neurons and initiation of increased LH pulse frequency in pubertal PNA rats. Kisspeptin may coordinate their stimulatory effects on LH release.

P-388 Wednesday, October 22, 2014

EVALUATING RISK VARIANTS FOR KOREAN WOMEN WITH POLYCYSTIC OVARY SYNDROME IN WOMEN OF EUROPEAN ETHNICITY. H.-J. Kim, A. Bjonnes, R. Saxena, C. Welt. “Obstetrics and Gynecology, Seoul National University Bundang Hospital, Seongnam, Gyonggi, Korea; “Anesthesia and Critical Care, Massachusetts General Hospital, Boston, MA; “Reproductive Endocrine, Massachusetts General Hospital, Boston, MA.


OBJECTIVE: Recent genome-wide association studies have identified variants that are associated with risk for polycystic ovary syndrome (PCOS) in women of Korean ethnicity. We hypothesized that these genetic variants also result in risk for PCOS in women of European ethnicity.

DESIGN: Subjects were U.S. women of reported European ethnicity, aged 18 to 45 years, with PCOS as defined by NIH criteria (n=522) and controls with regular menstrual cycles and no signs of hyperandrogenism (n=472).

MATERIALS AND METHODS: DNA was genotyped using the OmniExpress (Illumina). Forty-one SNPs were selected with a p value of <10.4 for association in women of Korean ethnicity. X² analysis was used to compare allele frequencies in women with PCOS and controls carrying the variants. A p value ≤0.001 was considered significant to account for multiple testing of 41 independent variants.

RESULTS: Four variants were nominally associated with PCOS (rs10492118, rs7666129, rs10488602 and rs11678092; all p<0.05). However, none remained significant after correction for multiple testing.

CONCLUSION: Risk variants that are associated with PCOS in women of Korean ethnicity are not replicated in women of European ethnicity. Replication in additional subjects will be necessary.

Supported by: R01HD065029.

P-390 Wednesday, October 22, 2014

COMPARISON BETWEEN IVF ANTAGONIST PROTOCOL VERSUS IVM PROTOCOL TO TREAT INFERTILE PCOS PATIENTS. A PROSPECTIVE RANDOMIZED STUDY. PRELIMINARY RESULTS. T. Shavit, E. Shalom-Paz, M. Michaeli, A. Ellenbogen. Obstetrics & Gynecology Department IVF Unit, Hillel Yaffe Medical Center, Hadera, Israel.

OBJECTIVE: Triggering ovulation with GnRH-agonist in GnRH antagonist protocols can prevent Ovarian-HyperStimulation Syndrome (OHSS) in Polycystic Ovarian Syndrome (PCOS) patients. However large amounts of gonadotropins are used. In-Vitro Maturation (IVM) may be a potential alternative for these patients, without exposure to gonadotropins and risk of OHSS. The aim of this study was to compare the outcomes of IVM versus antagonist protocols in PCOS patients.

DESIGN: A prospective randomized controlled trial.

MATERIALS AND METHODS: PCOS patients were enrolled randomly to IVM or antagonist protocol. Patients in IVM group were primed with 150IU fSH for 3 days and hCG. Patients in the antagonist protocol were treated routinely. The two groups were compared regarding the number of oocytes retrieved, maturation rate / mature oocytes, fertilization and cleavage rates, quality of embryos, OHSS and ongoing pregnancy rates.

RESULTS: 11 and 10 patients were recruited so far for antagonist and IVM protocol. No significant differences in number of mature oocytes (11.2±5.7 vs.12.2±8.45), fertilization rate (73% vs. 67%) and top quality of embryos (45.3% vs. 43.9) were observed. The average dose of gonadotropins in the antagonist protocol was 461 IU±560IU per cycle, compared to 382±108IU in the IVM group (p<0.001). Pregnancy rates were comparable - only 9% (1/11) in the antagonist (surprisingly low compared to previous studies) and 40% in the IVM group (4/10) (p=0.102).

No OHSS developed.

CONCLUSION: IVM protocol may be an alternative for infertile women with PCOS who desire to prevent potential adverse effects of gonadotropins treatment and prevent OHSS. These preliminary data demonstrate that IVM has comparable results to standard IVF treatments.

REPRODUCTIVE HORMONES

P-391 Wednesday, October 22, 2014

REGULAR EXERCISE INCREASES REPRODUCTIVE OUTCOMES OF AGED FEMALE IN MOUSE MODEL. B. S. Joo,ª Y. J. Chang,ª J. K. Joo,ª J. S. Koo,ª H. S. Moon,ª K. S. Lee.ª IVF-Busan Center for Reproductive Medicine, Good Moonhwa Hospital, Busan, Korea;ªDepartment of Obstetrics and Gynecology, Medical Research Institute, Pusan National University School of Medicine, Busan, Korea.

OBJECTIVE: Advancing females’ age remains a fastidious problem in infertility treatment. This study was aimed to investigate the effect of regular exercise on the reproductive outcome of aged female using mouse model.

DESIGN: Controlled experimental study.

MATERIALS AND METHODS: Twenty of C57BL inbred female mice of 32 weeks were constituted. Ten 10 mice were forcefully exercised by illuminating incandescent lights (60 Watts, 220V), placed on the top of the cage, starting at 11:100 a.m. for 30 minutes daily. The remaining 10 mice served as control without forceful exercise. After 4 weeks, the female mice were mated with the same strained individual male mice of 12 weeks during 2 weeks maintaining the exercise protocol. Then the outcome of pregnancy was observed for subsequent 2 weeks. Mice that were not pregnant were re-mated during 2 weeks and re-examined for the outcome of pregnancy the following 2 weeks. The expression of vascular endothelial growth factor (VEGF) and endothelial nitric oxide synthase (eNOS) were evaluated in ovaries of both groups by western blot analysis and immunohistochemistry.

RESULTS: The total number of pregnant mice was 8 (80%) in the exercise group and 2 (20%) in the control group (P<0.05). The number of pregnant mice within the first 6 weeks was 5 in the exercise group and one in the control group. The mean number of offspring was significantly higher in the exercise group (8.5) than the control group (6.5) (P<0.05). VEGF expression was increased in the exercise group, but eNOS expression was not different in both groups.
CONCLUSION: These results demonstrate that regular exercise in aged female improves their reproductive outcomes. This effect of exercise may be associated with the stimulation of ovarian function by increasing VEGF expression.

P-392 Wednesday, October 22, 2014

OBESITY ADVERSELY AFFECTS INTERACTION BETWEEN ANTI-MULLERIAN HORMONE (AMH) AND AGE AMONG AFRICAN AMERICAN (AA) BUT NOT CAUCASIAN (C) WOMEN. V. Moy, S. Jindal, H. Lieman, E. Buyuk, Montefiore Institute for Reproductive Medicine and Health, Hartsdale, NY.

OBJECTIVE: To determine the effect of obesity on interaction between AMH and age among women from different racial backgrounds.

DESIGN: Retrospective controlled study at a university-based reproductive endocrinology and infertility (REI) clinic.

MATERIALS AND METHODS: Medical records of reproductive age women (age 22-46) undergoing fertility workup from January 2012 to March 2014 and who had AMH levels measured as part of their evaluation were reviewed. Age, AMH, body mass index (BMI), self-reported race, etiology of infertility, gravidity, parity, smoking history, maximum serum early follicular follicle stimulating hormone (FSH) levels, antral follicle count (AFC), average ovarian volume (AOV), and history of ovarian surgery, chemo- or radio-therapy were recorded. Women with identified medical conditions or iatrogenic interventions that could diminish their ovarian reserve or those whose race were not reported or available were excluded from the study. STATA was used for data analysis. Pearson and Spearman rank correlations, ANOVA, Kruskal-Wallis, Chi-square tests were used. Data are given as mean ± SD and p<0.05 was considered statistically significant.

RESULTS: Of 267 medical records reviewed, 206 were included in the study and comprised of 64 AA, 88 C, 36 Hispanic (H) and 18 Asian (A) women. All 4 groups (AA, C, H, A) showed no differences in mean age, AMH, parity, maximum early FSH, AFC, AOV, smoking history, or etiology of infertility. AA women had greater mean BMI compared to C and A women (30.9±9 vs 26.2±5.9 vs 24.7±4.9, p<0.001) and greater gravidity compared to C women (1.9±1.1 vs 1.1±1.0, p=0.01). There was a strong negative correlation between age and AMH for the entire study population (r=-0.57, p<0.0001) and for each group (AA: r=-0.45, p=0.0002; C: r=-0.6, p<0.0001; H: r=-0.56, p=0.0003; A: r=-0.77, p=0.0002). When categorized by BMI subgroups, the negative correlation between age and AMH became stronger with increasing BMI for AA women (BMI 25-29.9: r=-0.4, p=0.007; BMI 30-34.9: r=-0.55, p=0.01). No such correlation was observed among C women. Numbers of AA women with BMI <25 and H and A subgroups were too few for analysis.

CONCLUSION: The negative correlation between age and AMH becomes stronger among AA women with increasing BMI, suggesting that obesity may affect ovarian reserve more strongly among AA women, compared to C women. However, a larger cohort of women is needed for analysis to support this conclusion and to explore similar associations among H and A women.

P-394 Wednesday, October 22, 2014

DO ANTI-MULLERIAN HORMONE (AMH) LEVELS PREDICT THE OPTIMAL CLOMIPHENE CITRATE (CC) DOSAGE IN POLYCYSTIC OVARIAN SYNDROME (PCOS) AND NON-PCOS PATIENTS UNDERGOING CC STIMULATED IUI (CC/IUI)? C. Mullin, A. V. Libby, A. Ulrici, M. Lessor, A. Hershlag. 1Center for Human Reproduction, North Shore Long Island Jewish Health Systems, Manhasset, NY; 1Biostatistics Unit, Feinstein Institute for Medical Research, Manhasset, NY.

OBJECTIVE: AMH has been used as an important biomarker of ovarian activity. In non-PCOS patients, low AMH levels have been used in IVF to indicate diminished ovarian reserve and, according to some studies, lower pregnancy rates. It is unclear whether AMH can be similarly utilized in patients with PCOS. This study was designed to examine the predictive value of AMH with respect to optimal CC dosing in PCOS versus non-PCOS patients undergoing CC/IUI.

DESIGN: Retrospective cohort study.

Clinical Pregnancy Rate (n) according to CC dosage and AMH in PCOS and non-PCOS patients

<table>
<thead>
<tr>
<th>AMH</th>
<th>0.0-0.9</th>
<th>1.0-1.9</th>
<th>2.0-4.9</th>
<th>≥ 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC Dosage in Non-PCOS</td>
<td>50</td>
<td>100</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>CC Dosage in PCOS</td>
<td>50</td>
<td>100</td>
<td>150</td>
<td></td>
</tr>
</tbody>
</table>

CONCLUSION: The progesterone-to -estradiol ratio measured the day of the agonist administration in an oocyte donation program with good prognosis donors may be useful as a predictor to identify better competent oocytes cohorts, based in improvements in terms of blastocyst formation, implantation and pregnancy rates. More studies are needed to confirm these data, not only in oocyte donor, but in ovarian stimulation patients. Based on this purpose, a multicenter study is ongoing.
AN ANTI-MULLERIAN HORMONE (AMH) ASSOCIATED WITH PRETERM BIRTH? RESULTS FROM THE EFFECTS OF ASPIRIN ON GESTATION AND REPRODUCTION (EAGER) TRIAL. S. M. Zarek, E. F. Schisterman, E. M. Mitchell, A. Lynch, D. Faraggi, S. L. Mumford, N. IH, Rockville, MD; University of Colorado, Aurora, CO; University of Haifa, Haifa, Israel.

OBJECTIVE: Although recent studies suggest that lower AMH is associated with adverse obstetric outcomes like preeclampsia, less is known about an association with preterm birth. The objective was to assess a possible association between preconception AMH levels with preterm birth. DESIGN: Secondary analysis of a multicenter, block-randomized, double-blind, placebo-controlled clinical trial.

MATERIALS AND METHODS: 1228 women attempting pregnancy, aged 18–40 years, with one to two prior pregnancy losses and no history of infertility, pelvic inflammatory disease, tubal occlusion, endometriosis, anovulation, uterine abnormalities, or polycystic ovarian syndrome were included. Women were randomized to preconception-initiated daily low-dose aspirin or placebo. The primary outcome for this analysis was preterm birth (defined as a living infant born before 37 weeks and 0 days gestation). AMH was assayed using the Gen II ELISA assay (Beckman-Coulter) at the baseline visit before randomization. AMH was clinically categorized and verified by data analysis into low (<1.25 ng/mL), normal (1.25 to 4.0 ng/mL), and high (>4.0 ng/mL). The Mann Whitney test was utilized to evaluate AMH levels by preterm birth status. Relative Risk (RR) and 95% confidence intervals (CIs) for preterm birth by AMH categories were estimated using generalized linear models adjusted for age.

RESULTS: Women were followed for up to six menstrual cycles with N=776 (72%) achieving pregnancy and N=51 (9%) with a preterm birth. Women were predominately of white race (95.6%) with a mean age of 28.9 years (standard deviation (SD) 4.7) and body mass index (BMI) of 26.1 (SD 6.5). Mean AMH among women with a preterm birth were 3.4 ng/ml (SD 2.6) compared to women without a preterm birth at 3.6 (2.7) (p=0.81). There were no significant associations between AMH and preterm birth in women with low AMH (<1.25 ng/mL) (RR=0.72, 95% CI 0.30, 1.7) or high AMH (>4.0 ng/mL) (RR=0.78, 95% CI 0.43, 1.4) compared to women with normal AMH levels after adjustment for age.

CONCLUSION: Although AMH has been recently associated with preeclampsia and pregnancy loss, AMH was not associated with preterm birth in women with normal fertility, suggesting that AMH may be a marker of ovarian and reproductive vasculature, thus affecting vascular diseases of pregnancy.

Supported by: Intramural Research Program, DIPHR, PRAE.
population had significant cardiovascular risk factors because of increased cholesterol at baseline, with low HDL levels which did not improve with CC treatment. Prospective trials will be needed to validate these findings and can be used to help counsel patients and guide management.

Supported by: Theresa and Frederick Dow Wallace Fund of NYCT.

P-398 Wednesday, October 22, 2014

ASSOCIATION OF ANTI-MULLERIAN HORMONE (AMH) AND PREGNANCY LOSS. S. M. Zarek, E. F. Schisterman, E. M. Mitchell, R. M. Silver, J. H. Segars, S. L. Mumford. NIH, Bethesda, MD; University of Utah, Salt Lake City, UT.

OBJECTIVE: Developing literature suggests lower AMH is associated with adverse obstetric outcomes like preeclampsia, but less is known about the association with pregnancy loss. The objective was to assess a possible association between preconception AMH levels with pregnancy loss.

DESIGN: Secondary analysis of a multicenter, block-randomized, double-blind, placebo-controlled clinical trial.

MATERIALS AND METHODS: 1228 women attempting pregnancy, aged 18-40 years, with one to two previous pregnancy losses and no history of infertility, pelvic inflammatory disease, tubal occlusion, menorrhagia, anovulation, uterine abnormality, or polycystic ovarian syndrome were included. Women were block-randomized by center in a 1:1 ratio to preconception-initiated daily low-dose aspirin or placebo. The primary outcome was pregnancy loss (defined as chemical or clinical loss, < 20 weeks gestation). AMH was assayed using the Gen II ELISA assay (Beckman-Coulter) at the baseline visit before randomization. AMH was clinically categorized and verified by data analysis into low (<1.25 ng/mL), normal (1.25 to 4.0 ng/mL), and high (>4.0 ng/mL). Relative Risk (RR) and 95% confidence intervals (CIs) for pregnancy loss by AMH categories were estimated using generalized linear models adjusted for age.

RESULTS: Women were followed for up to six menstrual cycles with N=776 (72%) achieving pregnancy and N=193 (18%) having a loss. Women were mostly of white race (95.6%) with a mean age of 28.9 years (standard deviation (SD) 4.7) and body mass index (BMI) of 26.1 (SD 6.5). AMH was significantly associated with age (p=0.0001) but not associated with treatment group (low-dose aspirin or placebo), BMI, number of prior losses, race or tobacco use. After adjusting for age, women with low (<1.25 ng/mL) versus normal AMH levels had an increased risk of pregnancy loss (RR=1.7, 95% CI 1.1, 2.6) whereas women with high AMH (>4.0 ng/mL) had no significant increased risk of pregnancy loss compared to women with normal AMH levels (RR=1.2, 95% CI 0.78, 1.7).

CONCLUSION: Low AMH levels were associated with higher pregnancy loss, suggesting a woman with low AMH who is able to achieve pregnancy may require counseling for increased risk of subsequent loss. The association of AMH to vascular diseases of pregnancy underscores the role of AMH as a marker of ovarian and reproductive vasculature. To our knowledge, this is the first report of a significant association between AMH levels and pregnancy loss among women with normal fertility.

Supported by: Intramural Research, DIPHR, PRAE.

P-399 Wednesday, October 22, 2014

HYPOTHYROIDISM IN Recipients Decreases Live Birth Rate in Donor-Recipient (DR) IN-VITRO FERTILIZATION (IVF) CYCLES. M. D. Johnson,* A. Althouse,* A. N. Wakim. Department of Obstetrics, Gynecology and Reproductive Sciences, Magee-Womens Hospital of University of Pittsburgh Medical Center, Pittsburgh, PA; Department of Obstetrics, Gynecology and Reproductive Sciences, Magee-Womens Research Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA.

OBJECTIVE: To determine if oocyte donors’ or recipients’ TSH levels during DR IVF cycles affect the pregnancy rates in recipients who undergo a fresh embryo transfer.

DESIGN: Retrospective cohort study in a university hospital-based practice.

MATERIALS AND METHODS: We reviewed 358 DR IVF cycles from 141 oocyte donors and 314 recipients between November 2002 and April 2013. We included cycles in which the oocyte donor had a TSH level prior to stimulation and the recipient had a TSH level prior to a fresh transfer. The recipients and donors were divided into 3 groups: Normal (NT) (TSH <2.5), Subclinical Hypothyroidism (SH) (TSH ≥2.5 and <5), and Clinical Hypothyroidism (CH) (TSH ≥5). ANOVA was used to compare implantation (IMP), clinical pregnancy (CP), miscarriage (SAB), and live birth (LB) rates among various groups. A stepwise logistic regression was performed to determine the factors associated with a LB.

RESULTS: Pregnancy occurred in 43.2% (92/213) of DR IVF cycles where a fresh embryo transfer occurred. The average number of embryos transferred was similar for recipients with NT (mean=1.8), SH (1.8), and CH (1.5). Recipients with CH had the lowest IMP rate (46.2% vs. 56.5% for NT and 63.8% for SH), lowest CP rate (38.5% vs. 54.3% for SH and 52.6% for NT), and highest SAB rate (8.3% vs. 2.2% for SH and 5.7% for NT); these differences were not statistically significant. There was a significantly decreased probability of LB in recipients with CH (21.7% vs. 44.0% in NT [p=0.04] and vs. 48.9% in SH [p=0.03]). This was consistent regardless of the donor’s thyroid status. Models adjusting for donor age and recipient lining trended towards an association between CH and likelihood of LB (OR=0.31, 95% CI [0.09, 1.01], p=0.057). There was no significant association between donor thyroid function and LB.

<table>
<thead>
<tr>
<th>Percentage Pregnancy Outcomes, by Recipient Thyroid Status</th>
<th>Implantation</th>
<th>Clinical Pregnancy</th>
<th>Miscarriage</th>
<th>Live Birth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>63.8</td>
<td>52.6</td>
<td>5.7</td>
<td>44.0</td>
</tr>
<tr>
<td>Subclinical</td>
<td>56.5</td>
<td>54.3</td>
<td>2.2</td>
<td>48.9</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>46.2</td>
<td>38.5</td>
<td>8.3</td>
<td>21.7</td>
</tr>
<tr>
<td>Total</td>
<td>60.3</td>
<td>51.3</td>
<td>5.2</td>
<td>43.2</td>
</tr>
</tbody>
</table>

CONCLUSION: Recipient TSH levels but not oocyte donor TSH levels affect pregnancy rates in DR IVF cycles with a fresh embryo transfer. The data presented here demonstrate that TSH levels have more of an effect on the endometrium than on oocyte quality.

P-400 Wednesday, October 22, 2014

LEVONORGESTREL DECREASES TISSUE PLASMINOGEN ACTIVATOR LEVELS THROUGH THE ANDROGEN RECEPTOR IN HUMAN ENDOMETRIAL ENDOTHELIAL CELLS. T. Pakrashi, T. Jacot, S. Godbout, D. F. Archer. Obstetrics and Gynecology, Jones Institute for Reproductive Medicine-Eastern Virginia Medical School, Norfolk, VA.

OBJECTIVE: Heavy menstrual bleeding (HMB) affects almost 18 million women in the United States, leading to a loss of quality of life and decreased productivity. The Levonorgestrel Intra Uterine System is a highly effective first line treatment in reducing blood loss in HMB. However, the exact mechanism by which it affects the coagulation cascade remains unknown. The plasminogen activator system is an important pathway that controls fibrin clot formation and dissolution through regulation of plasmin. Very little is known about the role of this system in HMB. We recently observed a decrease in tissue plasminogen activator (tPA) levels in human endometrial endothelial cells (HEECs) exposed to levonorgestrel (LNG) but not progesterone in vitro. This decrease in tPA shifts the hemostatic balance in favor of coagulation. The aim of this study is to determine whether LNG decreases tPA levels in HEECs through androgen receptors present on endometrial endometrial cells.

DESIGN: In vitro cell culture study.

MATERIALS AND METHODS: Immortalized HEECs were cultured in endothelial cell culture media for 6 days. The cells were steroid starved by culturing them in phenol red free media containing 5% stripped fetal bovine serum, with additives present in regular growth media for 2 days. For androgen receptor western blot experiments, either ethanol (control), 100 nM of progesterone or LNG was added to cells phenol red free media containing 5% stripped fetal bovine serum, with additives present in regular growth media for 2 days. For androgen receptor blockade experiments, ethanol (control), 100 nM of LNG, 100 nM of LNG with 100 nM of Flutamide, an androgen receptor blocker, was added to cells cultured in phenol red free media with 0% FBS and no additives. Cells were lysed and culture media were collected after 24 hours of treatment.

RESULTS: The androgen receptor was detected at 110 kDa from sample lysates by western blot. An ELISA performed on culture media confirmed a statistically significant decrease in tPA levels in cells treated with LNG (71.50±14.80% of control) (p<0.05 vs control). However, this decrease was blocked by the addition of flutamide (101.3±31.05% of control).
CONCLUSION: The androgen receptor is present on HEECs. The abrogation of the decrease in iPAs levels by addition of flutamide suggest that the procoagulatory effect of LNG on HEECs is mediated via the androgen receptor. This is clearly feasible since LNG is a highly androgenic synthetic progestin. This in vitro finding suggests a direct role of the endometrial endothelial cell in response to LNG in women with HMB.

Supported by: Department of OB/GYN-EVMS.


OBJECTIVE: Decisions to cancel, continue or convert to a cryopreservation IVF cycle are made based on progesterone levels. Multiple platforms and assays exist for progesterone testing and their variability affects the test’s utility. We aim to evaluate: 1) variability between fresh and frozen samples 2) variability between platforms and 3) variability between assays.

DESIGN: Retrospective study of 239 consented women undergoing treatment at Boston IVF.

MATERIALS AND METHODS: 8ml of serum was collected from each participant and aliquoted. Each aliquot was tested for estradiol, LH, FSH, hCG and progesterone. A fresh sample was run on the platform at the collection site. Remaining aliquots were frozen and tested on the following assay and platform combinations: A) Immulite 1000 Assay 1 B) Immulite 1000 Assay 2 C) Immulite 2000 Platform 1 and D) Immulite 2000 Platform 2. Spearman correlation coefficients were calculated. Progesterone correlations were stratified by progesterone level: <1.2ng/ml, <2ng/ml and >3ng/ml.

RESULTS: Correlation coefficients for estradiol, LH, FSH and hCG were ≥0.86. Progesterone correlations for fresh and frozen samples using the same assay and platform were ≥0.89 (all p<0.0001). Progesterone intra-assay correlations ranged from 0.67 to 0.97. Stratification by progesterone level highlighted poor correlation in clinically relevant levels: <1.2ng/ml and <2ng/ml. Using progesterone of ≥1.2ng/ml as an indication for cryopreservation, 17% of samples had discrepant results.

OBJECTIVE: To determine the role of the genomic progesterone receptor (PGR) in primate ovulation/luteinization.

DESIGN: Repeated Measures in vitro and in vivo.

MATERIALS AND METHODS: Short-hairpin RNAs (shRNA) complementarity to rhesus macaque PGR (shPGR) or a non-targeting control (shControl) were packaged into an adenoviral vector expression system (ad vectors). Experiment 1: Nonluteinized granulosa cells (NLGCs) were collected from monkeys (n=4) undergoing controlled ovarian stimulation cycles [1] without an ovulatory stimulus and placed into culture [2]. NLGCs were exposed to either shPGR, shControl, or no advector (NAV) for 24 hr, when hCG was added to half of the wells to induce luteinization (LGCs; n=4-6 wells/treatment/mouse). Cells were analyzed for PGR mRNA expression and media progesterone (P4) levels 48, 72 and 120 hr post-advector. Experiment 2: In macaques undergoing controlled ovulation protocols [3], either shPGR or shControl advector was injected into preovulatory follicles (n=4/group) 20 hr before the ovulatory hCG bolus. Ovulation was evaluated 96 hr after advector injection, and ovaries collected for histological and immunohistochemical (PGR) analyses. Serum P4 levels were also assessed. Data were analyzed by Repeated Measures ANOVA.

RESULTS: Experiment 1: Addition of hCG increased levels of PGR mRNA and media P4 in NAV and shControl-exposed LGCs by 120 hr post-advector (P<0.01, 0.001, respectively). In LGCs, shPGR advector induced a time-dependent decline in PGR mRNA (31, 74, 87%) and P4 (12, 50, 78%) when compared to NAV (Interaction Time*Advector P<0.01). There were no significant changes in either PGR mRNA or P4 synthesis induced by shControl vector at any time point when compared to NAV (P>0.1). Experiment 2: Intrafollicular injection of shPGR blocked ovulation of 3/4 follicles. The shPGR advector also prevented the hCG-induced rise in serum P4 levels relative to shControl (P<0.04). In addition, PGR protein was undetectable by IHC in LGCs from within the one ruptured follicle 96 hr post-shPGR injection, when PGR is usually at high levels during luteinization.

CONCLUSION: These data support the concept that PGR mediates ovulation in primates, as suggested by previous macaque studies using a steroid synthesis inhibitor and P4 replacement [4]. In vivo and in vitro effects of PGR knockdown in LGCs support Roddick's hypothesis [5] that P4 drives its own biosynthesis in the primate corpus luteum.

Supported by: RO1HD020869 (RLS) and P51OD011092 (Support for Private Research Center, PI: Robertson, J. E.).

<table>
<thead>
<tr>
<th>Progesterone &lt;1.2ng/ml</th>
<th>Fr 2000 P1</th>
<th>Fr 1000 A1</th>
<th>Fz 2000 P1</th>
<th>Fz 1000 A1</th>
<th>Fz 2000 P2</th>
<th>Fz 1000 A2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fr 2000 P1</td>
<td>r=1</td>
<td>r=0.79 (p&lt;0.0001)</td>
<td>r=0.48 (p&lt;0.0001)</td>
<td>r=0.73 (p&lt;0.0001)</td>
<td>r=0.74 (p&lt;0.0001)</td>
<td></td>
</tr>
<tr>
<td>Fr 1000 A1</td>
<td>r=1</td>
<td>r=0.48 (p=0.03)</td>
<td>r=0.77 (p&lt;0.0001)</td>
<td>r=0.40 (p=0.07)</td>
<td>r=0.52 (p=0.02)</td>
<td></td>
</tr>
<tr>
<td>Fz 2000 P1</td>
<td>r=0.78 (p&lt;0.0001)</td>
<td>r=0.51 (p=0.004)</td>
<td>r=0.52 (p&lt;0.0001)</td>
<td>r=0.83 (p&lt;0.0001)</td>
<td>r=0.84 (p&lt;0.0001)</td>
<td></td>
</tr>
<tr>
<td>Fz 1000 A1</td>
<td>r=0.16 (p=0.07)</td>
<td>r=0.68 (p=0.0005)</td>
<td>r=0.22 (p=0.01)</td>
<td>r=0.83 (p&lt;0.0001)</td>
<td>r=0.84 (p&lt;0.0001)</td>
<td></td>
</tr>
<tr>
<td>Fz 2000 P2</td>
<td>r=0.73 (p&lt;0.0001)</td>
<td>r=0.56 (p&lt;0.0001)</td>
<td>r=0.83 (p&lt;0.0001)</td>
<td>r=0.50 (p&lt;0.0001)</td>
<td>r=0.82 (p&lt;0.0001)</td>
<td></td>
</tr>
<tr>
<td>Fz 1000 A2</td>
<td>r=0.73 (p&lt;0.0001)</td>
<td>r=0.52 (p=0.007)</td>
<td>r=0.84 (p&lt;0.0001)</td>
<td>r=0.65 (p&lt;0.0001)</td>
<td>r=0.82 (p&lt;0.0001)</td>
<td>r=1</td>
</tr>
</tbody>
</table>


CONCLUSION: Poor progesterone level correlation across assay and platform in clinically significant levels impacts its utility for clinical decisions. As much as 17% of cycles may be affected by assay variability. Clinical decisions should be made based on data from the assay and platform used at an individual institution.

OVARIAN FUNCTION

P-402 Wednesday, October 22, 2014

RNAI-KNOCKDOWN OF GENOMIC PROGESTERONE RECEPTOR IN NONHUMAN PRIMATE GRANULOSA CELLS AND PREOVULATORY FOLLICLES. C. V. Bishop,* J. D. Hennebold,* C. Kahil,* R. L. Stouffer,* Division of Reproductive & Developmental Sciences, Oregon National Primate Research Center, Beaverton, OR; +Molecular Virology Support Core, Oregon National Primate Research Center, Beaverton, OR.

OBJECTIVE: FSH drives ovarian folliculogenesis via protein kinase A (PKA). The PKA anchoring protein 13 (AKAP13) is highly expressed in primate ovulation/luteinization.

MATERIALS AND METHODS: To determine the role of the genomic progesterone receptor (PGR) in primate ovulation/luteinization.

DESIGN: Repeated Measures in vitro and in vivo.

CONCLUSION: These data support the concept that PGR mediates ovulation in primates, as suggested by previous macaque studies using a steroid synthesis inhibitor and P4 replacement [4]. In vivo and in vitro effects of PGR knockdown in LGCs support Roddick’s hypothesis [5] that P4 drives its own biosynthesis in the primate corpus luteum.

Supported by: R01HD020869 (RLS) and P51OD011092 (Support for Private Research Center, PI: Robertson, J. E.).

FERTILITY & STERILITY e271
MATERIALS AND METHODS: Quantitative real-time polymerase chain reaction (qRT-PCR) confirmed endogenous expression of akap13 in COV434 cells. To determine time of maximal FSH response, cultured cells were treated with 11uM of FSH for 10 minutes to 72 hours. qRT-PCR was used to quantify aromatase transcripts. The Western Blot was used to assess relative CREB phosphorylation. To determine whether baseline and FSH-induced aromatase and lhr expression were AKAP13 dependent, similar experiments were performed following transfection with control siRNA or siRNA directed against akap13.

RESULTS: FSH treatment resulted in a 2.5-fold induction of aromatase mRNA by 32 hours of FSH treatment, and aromatase levels remained elevated through 72 hours FSH treatment. Relative CREB phosphorylation increased ~2 fold (p<0.03) after 10 minutes FSH treatment, then declined, returning to baseline by 60 minutes. AKAP13 siRNA transfection decreased akap13 mRNA by 40-85% depending on the experiment and duration of treatment. AKAP13 knockdown lowered basal transcripts of aromatase 50-60% (p<0.04). Induction of aromatase and lhr message following ~48 hours FSH treatment was reduced 56% (p<0.001) and 50% (p<0.001) respectively.

CONCLUSION: Optimal lhr and aromatase in COV434 cells required akap13. Our findings suggest that AKAP13 may be required in granulosa cells for normal FSH induction of LHR and aromatase, which are essential for ovulation. Further studies into the molecular impact of AKAP13 deficiency in the ovary has potential to yield significant insight into human ovulatory disorders.

Supported by: ZIA-HD008737 to J.H.S. Program in Adult and Reproductive Endocrinology, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health

P-404 Wednesday, October 22, 2014

THE EFFECTS OF DEHYDROEPIANDROSTERONE (DHEA) ON CULTURED HUMAN GRANULOSA CELLS FROM POSTOVULATORY HUMAN FOLLICLES AFTER IN VITRO FERTILIZATION (IVF), Y.-G. Wu, H.-J. Lee, D. H. Barad, V. A. Kashin, E. Lazzaroni-Tealdi, A. Sen, N. Gleicher, Center for Human Reproduction, New York, NY; ’Foundation for Reproductive Medicine, New York, NY; ’Section of Medical Endocrinology and Metabolism, Department of Medicine, Rochester School of Medicine and Dentistry, Rochester, NY.

OBJECTIVE: Human clinical and animal data suggest that DHEA improves oocyte and embryo yields, leading to improved fertility treatment outcomes. The molecular mechanisms of these effects have, however, not been fully established. Though DHEA, via conversion to testosterone (T), has been demonstrated to synergistically enhance FSH effects on granulosa cells (GCs), we in this study investigated whether DHEA does not also improve estrogen (E2) production, thereby enhancing GC response to FSH.

DESIGN: Prospective case control study.

MATERIALS AND METHODS: We investigated primary GCs from 4 young egg donors, obtained at routine egg retrieval during in vitro fertilization (IVF). They were cultured in defined DMEM/F12 medium with increased concentrations (1, 100, 1000 nM) of DHEA for 48 hours with or without 50ng/ml FSH, P4050Cyp19a1 (aromatase) and FSH receptor (FSHR) expression were examined by real-time PCR. Since DHEA by 3β-hydroxy-steroid dehydrogenase (HSD) is converted to T, into estradiol (E2) by aromatase and by 5α-reductase into 5α-dihydrotestosterone (DHT), but it is unknown whether these metabolites are involved in the stimulatory effects of DHEA, we examined time of maximal FSH response with or without DHEA treatment GCs with 100mM of T, DHT or E2 to test their effects on gene expression.

RESULTS: Though FSH, alone, stimulated aromatase and FSHIR expression, the presence of DHEA strongly enhanced this stimulation in a dose dependent manner. In addition, in absence of FSH, DHEA alone, did not affect aromatase and FSHIR expression. DHT and E2 also demonstrated potentiating effects with FSH, but to significantly lower degrees than T and DHEA (P<0.05). DHEA and T regulated gene expression to similar degrees. Stimulatory effects of DHEA, in addition, were blocked by inhibition of HSD, but not by aromatase and/or 5α-reductase.

CONCLUSION: These results confirm in animals previously reported synergism between FSH and DHEA in regulation of gene expression of aromogenesis in GCs by conversion of DHEA to T but not 5α-DHT or E2. DHEA and T, thus, play a unique and crucial role in female reproduction.

Supported by: Foundation for Reproductive Medicine, Center for Human Reproduction.

P-405 Wednesday, October 22, 2014

TRANSCRIPTION FACTOR KLF12 NEGATIVELY REGULATES ESTRADIOL SYNTHESIS IN OVARIAN GRANULOSA CELLS, Y. Jiang, B. Wang, Y. Hu, H. Sun, G. Yan. Reproductive Medicine Center, Nanjing Drum Tower Hospital, Nanjing, Jiangsu, China.

OBJECTIVE: To explore the mechanism of KLF12 (Krüppel-like factor 12) involved in regulation of estradiol(E2) synthesis in ovarian granulosa cells.

DESIGN: Adenovirus-mediated overexpression of KLF12 in KGN cells to detection of CYP19A1 expression and E2 release.

MATERIALS AND METHODS: Ovarian granulosa cell line (KGN) were cultured in phenol red-free DMEM/F12 medium containing 10% charcoal-dextran-treated FBS, with added 2 μM androstendione. E2 release were measured using Access Immunoprecipasy System. The chromatin immunoprecipitation technique/PCR (ChIP/PCR) and avidin-biotin conjugate DNA precipitation (ABC) assay were used to identify the target gene for KLF12 in KGN cells. Otherwise, the effect of KLF12 in the regulation of CYP19A1 gene expression in KGN was determined by luciferase reporter assay and Q-PCR. The data are expressed as the means ± SEM from at least three independent experiments. Student’s t-test and ANOVA were performed to detect differences between two groups and among more than two groups, respectively. The values were determined to be significant when P < 0.05.

RESULTS: Immunocytochemistry for the zinc-finger-containing transcription factor KLF12 protein was high level expression in the cytoplasm of granulosa cells and oocyte of maturing wild-type follicles. Adenovirus-mediated overexpression of KLF12 significantly suppressed E2 release in KGN cells culture media (3075.0 ± 430.1 vs 4817.5 ± 464.2, P < 0.05, compared to Ad-GFP group). In addition, Q-PCR demonstrated that KLF12 decreased CYP19A1 mRNA expression by 40% in KGN. Moreover, luciferase reporter, ChIP/PCR and ABCD assay further confirmed that KLF12 directly binds to the CAGTGG sequence within the promotor region of CYP19A1 gene. Furthermore, KLF12 mRNA and protein expression in KGN cells were significantly decreased after treatment with FSH. Importantly, overexpression of KLF12 significantly attenuated FSH-induced CYP19A1 mRNA expression and E2 release in KGN (6071.8 ± 974.5 vs 12818.4 ±1116.6, P < 0.01, compared to FSH).

CONCLUSION: Our study provides the first evidence that KLF12 is a novel transcription factor directly binds to CYP19A1 promoter and suppress CYP19A1 expression, and then negatively regulates ovarian granulosa cell estradiol synthesis.

Supported by: The study was sponsored by National Natural Science Foundation (No. 81370683) and University Graduate Research and Innovation Project (Nos. CXZZ13_D587).

ENVIRONMENT AND TOXICOLOGY

P-406 Wednesday, October 22, 2014


OBJECTIVE: Bisphenol-A (BPA) is an environmentally ubiquitous estrogen-like endocrine-disrupting compound. Exposure to BPA in utero has been linked to female reproductive disorders, including breast cancer. In rodents, BPA exposure has been shown to cause malformation of the mammary gland leading to carcinoma. We hypothesize that high urinary BPA concentrations would be positively associated with breast cancer.


OBJECTIVE: Bisphenol-A (BPA) is an environmentally ubiquitous estrogen-like endocrine-disrupting compound. Exposure to BPA in utero has been linked to female reproductive disorders, including breast cancer. In rodents, BPA exposure has been shown to cause malformation of the mammary gland leading to carcinoma. We hypothesize that high urinary BPA concentrations would be positively associated with breast cancer.

MATERIALS AND METHODS: Data from 3 NHANES cycles was appended to create a pooled data set representing survey years 2005-2010. Analysis was restricted to women aged 20 and older with environmental subsample data (n=2644). BPA was categorized into tertiles based on the range of urinary BPA values. Breast cancer was evaluated as reported history of breast cancer diagnosis. Bivariate analysis was performed using \( \chi^2 \) statistic for explanatory variables, BPA and breast cancer. Multivariable analysis of BPA and breast cancer was performed using logistic regression modeling adjusting for confounders with odds ratio (OR) estimates and 95% confidence intervals (95% CI) presented for BPA tertiles. Appropriate sample weights were constructed using original 2-year environmental subsample weights across cycles. Analysis was performed in SAS 9.3 using statistical procedures to account for the NHANES complex, clustered multistage sampling design and environmental subsample data.

RESULTS: We observed a negative association between increasing levels of urinary BPA and breast cancer. Compared to the lowest category of BPA exposure (tertile 1), the breast cancer OR and 95% CI for BPA and breast cancer was OR 0.51 (0.21-1.27) for BPA tertile 2 and 0.39 (0.16-0.96) for BPA tertile 3. After further adjustment for age, race and ethnicity, risk estimates for BPA tertiles 2 and 3 were OR 0.56 (0.24-1.33) and OR 0.43 (0.18-1.02), respectively, compared to the referent group.

CONCLUSION: Higher levels of urinary BPA were negatively associated with breast cancer diagnosis, independent of major risk factors. These findings contrast with experimental data relating BPA exposure with breast cancer risk. Although the cross-sectional survey design of NHANES and concept of reverse causation can explain the observed relationship, these data identify a need to better understand relevance of BPA exposure for human mammary tissue through appropriately designed prospective studies to assess any potential mechanisms between BPA and breast cancer development in humans.

P-407 Wednesday, October 22, 2014
HIGH FAT BUTTER AMELIORATES THE IMPAIRED EMBRYO IMPLANTATION OF FEMALE RATS EXPOSED TO DIETARY BISPHENOL-A. A. M. Martinez, A. Cheong, A. E. Batcheller, M. A. Thomas, S.-M. Ho. Department of Obstetrics & Gynecology, University of Cincinnati, West Chester, OH; Department of Environmental Health, University of Cincinnati, Cincinnati, OH; Cincinnati Veteran Affairs Hospital Medical Center, Cincinnati, OH.

OBJECTIVE: To study the effect of low dose dietary bisphenol-A (BPA) exposure on embryo implantation and to determine whether high fat butter (HFB) could modulate it.

DESIGN: Adult Sprague Dawley female rats exposed to experimental diets were mated and embryo implantation was assessed on gestational day (GD) 4.5 and 8.

MATERIALS AND METHODS: Ninety-two rats were exposed to various diets: (1) AIN control (4% kcal butter), (2) HFB (40% kcal butter), (3) BPA 250 µg/kg body weight (BPA250), (4) HFB+BPA250, (5) HFB+BPA2500 (2.500 µg/kg body weight), and (6) ethinyl estradiol (EE2). Number and proportion of implantation sites were recorded on GD8 uterine sections. Stage of embryos retrieved on GD8 were categorized as eitherGD4.5 orGD8.

RESULTS: There was a trend towards increased implantation sites in females exposed to diet groups 2,3,4,5,6 compared to group 1. The lacto-ovo diet group was significantly different from all diet groups.

CONCLUSION: Low fat diet may increase susceptibility of human to EDCs. The objective was to compare sperm characteristics of male infertility patients on vegetarian and non-vegetarian diets.

DESIGN: Retrospective study.

MATERIALS AND METHODS: Semen analyses were carried out in 474 males from 2009 to 2013. Patients categorized themselves as either lacto-ovo vegetarians (N = 26), vegans (N = 5) or non-vegetarians (N = 443). Exclusion criteria were microepididymal sperm aspiration, testicular extraction and donor cases. Parameters measured were concentration, progression, strict normal morphology, chromatin integrity, hyperactivation and zona-free hamster oocyte/sperm penetration assay (SPA) as described in the W.H.O. 5th Edition manual. A CASA system was used for analyzing motility parameters. Differences in means were tested using ANOVA and the student’s t-test, adjusted for unequal variance. Power for sample size tested was determined to be 74.7%.

RESULTS: Lacto-ovo vegetarians had significantly lower sperm concentration (50.7 ± 7.4 M/mL, mean ± S.E.M.) when compared with non-vegetarians (69.6 ± 3.2 M/mL). Furthermore, total motility was lower in the lacto-ovo group (33.2 ± 3.8% versus non-vegetarian 58.2 ± 1.0%). Similarly, vegans had lower total motility (51.8 ± 13.4%) with a trend towards lower sperm concentration (51.0 ± 13.1 M/mL). Interestingly, hyperactive motility was lowest in the vegan group. The percent strict normal sperm morphology in all groups were within normal range. There were no differences in the remaining parameters: rapid progression, chromatin integrity and SPA capacitation index.

CONCLUSION: The results showed that the vegetarian diet reduced sperm concentration and motility but did not extend into the infertile range. The findings suggested that estrogenic compounds or chemical residues in the diet had a negative effect on sperm parameters. Hyperactive motility indicative of the CatSper calcium selective channel was compromised in the vegan group. Clinical management would include dietary supplements to offset deficiencies. More studies are needed to corroborate the present findings.

P-409 Wednesday, October 22, 2014
PESTICIDES CONCENTRATIONS IN THE FOLLICULAR FLUID AND INTRACYTOPLASMIC SPERM INJECTION (ICSI) OUTCOME. I. Elnashar, T. K. Al-Hussaini, T. A. Farghaly, M. A. H. El-Baz, S. E. M. El-Deek, O. M. Shabaan. Women Health Hospital, Assiut University, Assiut, Egypt; 9Biochemistry, Assiut University, Assiut, Egypt.

FERTILITY & STERILITY® e273
OBJECTIVE: To correlate the levels of different pesticides concentrations in the follicular fluid (FF) collected during Intra cytoplasmic sperm injection (ICSI) to the ovarian response, endometrial thickness, embryological and clinical outcomes.

DESIGN: Cross sectional observational study.

MATERIALS AND METHODS: Couples who presented to a university affiliated fertility center for ICSI secondary to isolated male factor infertility during a one-year period were approached for participation. Women under the age of 38 year who consented and from whom we obtained FF samples for analysis (94) were included in the study. The FF samples were centrifuged, and stored in liquid nitrogen. Two organochlorine pesticides (OCPs) Lindane and DDT, Three Organophosphate (OPs) Chlorpyrifos, Diazinon, Malathion, one Chloroacetonilide (Pretilachlor) and two Pyrethroid (Bioallethrin and beta Cyfluthrin) were examined in the FF using Gas Chromatography/Mass Spectrometry. Correlations between the level of the above pesticides and ovarian response, endometrial thickness, embryological and clinical outcomes were done. Multiple regression analysis was used to correlate the values of the pesticides to the outcomes.

RESULTS: The mean age of study participants was 31.5 ± 6.2 years. Baseline FSH of study participants was 7.6 ± 2.6, and Estradial 59.3 ± 10.5. There were significant negative correlations between FF concentrations of the 8 examined pesticides on the endometrial thickness (Adjusted r²=0.2, P=0.0001). However, Pretilachlor, chloropyrifos, B-cyfluthrin, and Diazinon were the only toxic agents that negatively correlated with the number of the oocytes retrieved (adjusted r=0.07, P=0.0001). Fertilization and early embryos cleavage rates were negatively correlated with Pretilachlor and B-cyfluthrin P < 0.05. Moreover, high concentrations of Lindane, DDT, Diazinon , and chloropyrifos were significantly associated with fewer number of implanted embryos (adjusted r=0.2, P= 0.006, 0.02 , 0.0001, and 0.006, respectively).

CONCLUSION: High FF concentrations of toxic pesticides including OCPs, OPs, Chloroacetonilide, Pyrethroid may adversely affect the different stages of ICSI process and consequently its embryological outcome. Still more data is needed to evaluate the effect of these pesticides on the clinical outcome.

P-410 Wednesday, October 22, 2014

INTRAUTERINE HYPERGLYCEMIA ENVIRONMENT ALTERS IGFL2 DMR METHYLATION STATUS OF PLACENTA IN BOTH FIRST AND SECOND FILIAL GENERATIONS. G.-L. Ding1,2, H.-F. Huang2,3,4,5 The International Peace Maternity And Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China; 2The Key Laboratory of Reproductive Genetics, Ministry of Education, Hangzhou, Zhejiang, China.

OBJECTIVE: Intrauterine hyperglycemia environment of gestational diabetes mellitus (GDM) could lead to abnormal gene expression of placenta in the offspring of GDM. Heritable transmission of differential gene expression may be related to epigenetic regulation. As imprinted genes, Igfl2 expression in mice is regulated by allele-specific methylation at differentially methylated regions (DMRs). Therefore, the aim of our study is to examine the level of Igfl2 expression in the first generation and the second generation offspring of GDM and investigate the potential epigenetic mechanism involved.

DESIGN: Animal model study.

MATERIALS AND METHODS: We established a mouse model of GDM which induced by a single intraperitoneal injection of streptozotocin on the first day of pregnancy. At D18 of pregnancy, we collected the placenta of GDM offspring (F1-GDM) by uterine-incision. In addition, some GDM mice were allowed to deliver spontaneously and the pups were fostered by normal female mice and remained with their foster mothers until they were weaned. We intercrossed male and female adult F1-GDM mice and collected the placenta of F2 offspring (F2-GDM). F2 offspring were obtained from four groups including (1) C57-C5 (2) C57-GDM (3) GDM-C5 and (4) GDM-GDM. Gene expression was detected by real-time quantitative PCR. The methylation status of phosphoguanine (CpGs) of the Igfl2 DMR was analyzed by bisulfite genomic sequencing PCR. T test or Chi-square test was used to compare treatment group with control group. A P<0.05 was considered statistically different.

RESULTS: (1) The relative mRNA level of Igfl2 in placenta was significantly lower in F1-GDM mice. (2) In the four groups of F2-GDM offspring, the relative mRNA levels of Igfl2 in placenta were all significantly lower than that in controls. (3) The CpGs of Igfl2 DMR2 of placenta were moderately methylated in the control group, while the F1-GDM and GDM-2-GDM5 groups showed significantly higher methylation levels.

CONCLUSION: Intrauterine hyperglycemia induced down-regulation of Igfl2 expression of placenta in both first and second filial generations. Abnormal Igfl2 expression related to dysregulation of Igfl2 DMR methylation is one of the mechanisms involved in the effects of intrauterine hyperglycemia on F1 and F2 offspring.

Supported by: The research of the authors is Supported by the National Natural Science Foundation of China (31171444 and 81200485) and the Research Fund for the Doctoral Program of Higher Education (201201120015).

P-411 Wednesday, October 22, 2014

INFERTILE WOMEN WHO CONCEIVED USING ASSISTED REPRODUCTIVE TECHNOLOGY (ART) HAVE LOWER URINARY PHTHALATE CONCENTRATIONS THAN INFERTILE WOMEN WHO CONCEIVED SPONTANEOUSLY. S. Alar1, K. Hoeger1, H. Wang2, S. H. Swan3, E. S. Barrett4, H. Wang5, H. Wang6. 1Obstetrics and Gynecology, University of Rochester, Rochester, NY; 2School of Medicine, University of Rochester, Rochester, NY; 3Icahn School of Medicine, Mount Sinai, New York, NY.

OBJECTIVE: Phthalates, a class of endocrine-disrupting chemicals, are ubiquitous in the environment and have been linked to many adverse health outcomes. We examined the association between phthalate exposure and time > 1 year to conceive, with or without use of fertility treatments for pregnancy.

DESIGN: The Infant Development and Environment Study (TIDES), a large multi-center prospective pregnancy cohort study, measured urinary phthalate metabolites during the first trimester. Subjects completed questionnaires on their reproductive histories including fertility issues related to the index pregnancy.

MATERIALS AND METHODS: Our primary analyses focus on the four metabolites of diethylhexyl phthalate (DEHP): MEHP, MEOHP, MEHHP, and MECCP, and their molar sum (SUM DEHP). In a series of multivariable models, we used analysis of covariance to examine log-transformed, specific gravity adjusted phthalate metabolite levels in relation to fertility. Our analyses compared: (1) women who reported infertility (>1 year to conceive) versus those who did not (controls); and (2) infertile women who used ART to conceive the index pregnancy versus infertile women who did not. All analyses adjusted for covariates including BMI, age, tobacco use, income/socioeconomic status, and study center.

RESULTS: Of 791 subjects, 116 reported >1 year to conceive the current pregnancy and of those, 50 used ART to conceive. There were no differences in phthalate metabolite levels between women who reported >1 year to conceive vs ≤ 1 year. After adjusting for potential confounders, SUM DEHP was significantly lower in infertile women who used ART compared to infertile women who did not (geometric mean ratio= 0.7130; 95% CI 0.5161, 0.9851). Similar significant associations were seen for all DEHP metabolites.

CONCLUSION: In the total population phthalate metabolite concentration did not differ between women who took > and ≤ 12 months to conceive. In fertile women who used ART to conceive had lower first trimester phthalate metabolite concentrations than infertile women who conceived without ART. Women who pursue fertility treatments may be more cognizant of environmental health risks and take precautions to avoid exposure to toxins.

Supported by: NIH R01ES016863-04, UL1 TR000042.
SPERM EXPOSURE TO CYCLOPHOSPHAMIDE REDUCES PREIMPLANTATION EMBRYO DEVELOPMENT AFTER ICSI. M. D. Johnson, C.-C. Lin, M. Sukhwani, S. Malik, K. E. Orwig. Department of Obstetrics, Gynecology and Reproductive Sciences, Magee-Womens Research Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA.

OBJECTIVE: To determine the effect of cyclophosphamide administration on epididymal sperm and subsequent embryo development.

DESIGN: Prospective Laboratory Study.

MATERIALS AND METHODS: Sperm was collected from the cauda epididymis of adult male B6D2 mice. Seven days later intraperitoneal cyclophosphamide (CYC) (n=4) or vehicle (VEH) (n=4) were administered at 300 mg/kg. Mice were sacrificed seven days later to obtain sperm from the contralateral cauda epididymis. Sperm samples were cryopreserved at the time of collection and thawed prior to use for intracytoplasmic sperm injection (ICSI). Oocytes were collected from B6D2 female mice (n=16) after controlled ovarian stimulation and divided equally into two groups. Half the oocytes from each female were fertilized with CYC or VEH exposed sperm using ICSI, the other half were fertilized with pre exposed sperm from the same male. Sperm motility was assessed at the time of ICSI. Embryos were monitored for in-vitro development for two days. The number of oocytes obtained, injected and survived as well as embryo development were compared among the four groups using a student’s t-test or ANOVA.

RESULTS: Five hundred twenty-three embryos from eight in-vitro fertilization cycles with ICSI were monitored for preimplantation development. The mean number of oocytes surviving ICSI was 16.4 ± 1.8 (94.4%) and was similar in all four groups (P>0.50). In the CYC arm, the number of 8-cell embryos was significantly lower for the Post exposure group than the Pre exposure group (7.6% vs 47%, P=0.39). There was no difference between the Pre and Post groups in the VEH arm (56% vs 47%, P=0.39).

MATERIALS AND METHODS: Sperm was collected from the cauda epididymis of adult male C57BL/6 mice prior to CTx. After recovery, the mice were given intraperitoneal busulfan (55mg/kg) and sacrificed seven days later to obtain CTx exposed sperm from the contralateral epididymis. All sperm was cryopreserved at collection and thawed prior to use. Oocytes collected from individual C57BL/6 female mice were divided into two groups and fertilized by ICSI with motile sperm with normal morphology. Half were fertilized with pre-CTx sperm and the other half were fertilized with busulfan-exposed sperm from the same male. This design was repeated for 10 replicate males. Primary outcome measures were semen analyses and preimplantation embryo development after ICSI. Semen parameters were compared using pairwise comparisons and blastocyst development rate was analyzed using the Generalized Estimating Equations approach.

RESULTS: Two hundred-forty embryos from ten cycles were monitored for preimplantation development to the blastocyst stage. CTx had a significant negative effect on sperm motility (38% ± 6.3SEM vs 19% ± 5.06; p=0.03) and morphology (84% ± 1.58 vs 80 ± 1.26; p=0.02). However, overall sperm counts (100.36 ± 8.8 vs 71.8 ± 11.7 million; p=NS) were not significantly affected. Chemotherapy did not have a significant effect on the rate of blastocyst development (p=NS). In the pre-CTx group, blastocyst development was 23.08% compared to 18.67% in the post-CTx group.

CONCLUSION: Alkylating CTx acutely impacts semen motility and morphology. Pre-implantation embryo development was not affected when CTx-exposed sperm was used for ICSI fertilization. Exome sequencing of the resultant blastocysts is in process to assess for genetic variability between the pre-CTx and post-CTx groups.

Supported by: NIH grant HD055475, Magee-Womens Research Institute and Foundation and gifts from Sylvia Bernassoli.

IMPACT OF INTERMENSTRUAL BLEEDING ON NATURAL FERTILITY. N. M. Crawford, K. Chantala, A. Z. Steiner. University of North Carolina, Chapel Hill, NC.

OBJECTIVE: To determine the impact of intermenstrual and luteal phase bleeding on natural fertility.

DESIGN: Prospective, time-to-conceive study.

MATERIALS AND METHODS: Women, 30-44 years old, with no history of infertility, who were trying to conceive for less than 3 months, were enrolled and followed until pregnancy. Each day women recorded bleeding, ovulation test results, intercourse, and pregnancy test results for up to 4 months while attempting to conceive. For each cycle, presence of intermenstrual bleeding or luteal phase bleeding was determined. Intermenstrual bleeding was defined as any bleeding which occurred after completion of menses. Luteal phase bleeding was defined as any bleeding which occurred after ovulation and prior to the start of the next menstrual cycle. Cycles were restricted to 25 to 35 days in length to exclude anovulatory bleeding. We examined both the impact of bleeding on conception, defined as a positive pregnancy test, in the current cycle (current cycle fecundability) as well as the impact of bleeding on conception in the next cycle (future cycle fecundability). Discrete models were used to calculate fecundability ratios (FR) to compare subjects with bleeding to those without adjusting for maternal age.

RESULTS: 1767 cycles from 537 women were included in the analysis. Intermenstrual bleeding occurred in 35% of cycles, and luteal phase bleeding occurred in 33% of cycles. Women, who had bleeding, tended to be younger (<35 years), Caucasian, and of a normal body mass index. Compared to subjects without any bleeding, women with intermenstrual bleeding had 0.29 times the odds of pregnancy (95% CI: 0.2-0.41) in that cycle. Furthermore, if bleeding occurred in the luteal phase of the cycle, women had 0.23 times the odds of pregnancy (95% CI: 0.16-0.35). However, if a woman had intermenstrual bleeding or luteal phase bleeding, her future cycle fecundability was not significantly different from women without bleeding (FR 1.06, 95% CI: 0.77-1.48, and FR: 1.24, 95% CI: 0.88-1.74, respectively).

CONCLUSION: Intermenstrual bleeding significantly decreases the odds of pregnancy detection in that cycle; however, it does not appear to impact a woman’s future reproductive potential.

Supported by: This work was funded by the NIH/NICHD grant R01HD67683.
HEALTH DISPARITIES

P-415 Wednesday, October 22, 2014

INSURANCE COVERAGE FOR IN VITRO FERTILIZATION AND CUMULATIVE PROBABILITY OF LIVE BIRTH. M. Leung,1 B. Hamilton,2 L. Pollack,3 E. S. Jungheim.4 1OB-Gyn, Washington University, St. Louis, MO; 2Olin School of Business, Washington University, St. Louis, MO; 3Population Health Sciences, Washington University, St. Louis, MO.

OBJECTIVE: In vitro fertilization (IVF) is self-funded for many patients, but covered by insurance for some. We determined the impact of IVF insurance coverage on the cumulative probability of having a live birth.

DESIGN: Observational cohort.

MATERIALS AND METHODS: Data were collected for women initiating IVF at our center between 2001 and 2007. Women were observed until 2010. Women using oocyte donors or gestational carriers were excluded. Demographic data included IVF insurance coverage status, income, age and antral follicle count associated with oocyte retrieval, race, infertility diagnosis and obstetrical history. IVF cycle data included use of fresh versus frozen embryos, IVF cycle year, number of IVF cycle, total gonadotropin used, peak estradiol, number of embryos transferred and IVF cycle outcome. Standard univariate statistics were used to determine differences in baseline characteristics between women with and without IVF coverage. Our primary outcome was cumulative live birth (CLB) rate according to IVF insurance coverage status. Logistic regression estimates of the probability of live birth per IVF cycle were calculated controlling for relevant covariates. Probabilities of returning for additional IVF cycles after failing were also calculated. The CLB rate after five cycles of IVF was constructed as a function of live birth and return probability. Confidence intervals for CLB rates were constructed using the 2.5 and 97.5 percentiles of predicted CLB rates. All statistical analyses were performed in STATA 12.0.

RESULTS: 1061 women were included. 57.9% had IVF insurance coverage. Women with coverage were younger (p<0.01) and their estimated income was lower (p=0.01) than women without coverage. They were otherwise similar. Insurance coverage did not independently impact probability of live birth in individual cycles of IVF, but it did have a significant impact on women returning for additional treatment if cycles 1 and 2 failed (p<0.0001, p=0.02 respectively). The CLB rate after five cycles for women with coverage and those without coverage was 0.64 (95% CI 0.56-0.71) and 0.56 (95% CI 0.47-0.64) respectively. The difference in CLB rate was significant at 0.08 (95% CI 0.02-0.15).

CONCLUSION: By increasing the likelihood that individuals will return for additional IVF treatment after a failed IVF cycle, IVF insurance coverage is associated with an increased chance of having a live birth.

Supported by: K12HD063086.

P-416 Wednesday, October 22, 2014

RACIAL DISPARITIES IN IN VITRO FERTILIZATION (IVF). D. B. McQueen,1 B. Van Voo-dert,2 D. A. Haas,3 S. S. Lee,4 A. Schofield,4 M. Uhler,4 Ob/Gyn, University of Chicago, Chicago, IL; 1Fertility Centers of Illinois, Chicago, IL; 2Ob/Gyn, NorthShore University Health System, Evanston, IL; 3Department of Health Studies, The University of Chicago, Chicago, IL.

OBJECTIVE: To evaluate the impact of race on IVF outcomes.

DESIGN: Retrospective analysis.

MATERIALS AND METHODS: All women undergoing first autologous IVF cycle between January 2010 and December 2012 were included. Information was collected on baseline characteristics, IVF cycle parameters and outcomes. Race was self reported. Outcomes in each racial group were described. Overall outcomes were compared between women with and without IVF coverage. Women with coverage were younger (p<0.01) and had lower income (p<0.01) than women without coverage. Women of Asian race were other-wise similar. Logistic regression analysis was performed to control for age, body mass index, day 3 follicle stimulating hormone levels, smoking, fertility diagnosis and number of embryos transferred. The adjusted odds ratio for live birth was 0.26 (95% CI 0.11-0.57) in Black women, 0.41 (95% CI 0.27-0.60) in Asian women and 0.83 (95% CI 0.50-1.35) in Hispanic women compared to White women. The spontaneous abortion rate was also significantly different between races: 14.6 % in White women vs 28.9% in Black women (p = 0.01), 20.6% in Asian Women (p = 0.06) and 15.3% in Hispanic women (p = 0.97).

Group Characteristics (N=4045)

<table>
<thead>
<tr>
<th></th>
<th>White (N=3003)</th>
<th>Black (N=213)</th>
<th>Asian (N=541)</th>
<th>Hispanic (N=288)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Age (SD)</td>
<td>35.2 (4.6)</td>
<td>36.0 (5.0)*</td>
<td>34.9 (4.5)</td>
<td>34.7 (5.0)</td>
</tr>
<tr>
<td>Mean D3 (SD)</td>
<td>10.6 (17.8)</td>
<td>10.2 (7.8)</td>
<td>9.6 (6.3)*</td>
<td>8.8 (4.8)**</td>
</tr>
<tr>
<td>Mean FSH (SD)</td>
<td>2.2 (3.0)</td>
<td>2.0 (2.4)</td>
<td>2.4 (2.8)</td>
<td>2.3 (2.5)</td>
</tr>
<tr>
<td>Mean AMH (SD)</td>
<td>25.1 (5.8)</td>
<td>27.9 (6.0)*</td>
<td>23.3 (4.3)**</td>
<td>27.6 (6.3)**</td>
</tr>
<tr>
<td>% Smoker (SD)</td>
<td>4.9% (1.7)</td>
<td>5.2%*</td>
<td>3.0%***</td>
<td>9.4%*</td>
</tr>
<tr>
<td>Mean # Embryos Transferred (SD)</td>
<td>1.9 (0.7)</td>
<td>2.1 (0.8)</td>
<td>1.9 (0.7)</td>
<td>2.0 (0.8)</td>
</tr>
<tr>
<td>Clinical Pregnancy Rate</td>
<td>36.2 %</td>
<td>24.4%**</td>
<td>31.4%*</td>
<td>34.0%*</td>
</tr>
<tr>
<td>Live Birth Rate</td>
<td>30.7 %</td>
<td>16.9%***</td>
<td>24.0%**</td>
<td>28.5%</td>
</tr>
</tbody>
</table>

*p<0.001, *p<0.01, *p<0.05 calculated by ANOVA, **p<0.001, ***p<0.01

CONCLUSION: Black and Asian women demonstrated lower odds of clinical intrauterine pregnancy and live birth compared to White women despite controlling for confounders. Additionally, there were significant differences in rates of spontaneous abortion between women of different races. Further research is needed to understand the etiology of this disparity.

P-417 Wednesday, October 22, 2014

GEOSPATIAL MODELING OF IN-VITRO FERTILIZATION (IVF) ACCESSIBILITY IN A RURAL MIDWESTERN STATE. K. Summers,1 K. Stewart,2 P. Gharani,2 G. Ryan,2 B. Van Voorhies.3 Obstetrics and Gynecology, University of Iowa Carver College of Medicine, Iowa City, IA; 2Geographical and Sustainability Sciences, University of Iowa, Iowa City, IA.

OBJECTIVE: A recent study estimates that IVF meets only 24% of demand in the U.S.1 More research is needed to understand this discrepancy and how different populations are affected. Our objective was to develop a comprehensive and novel spatial accessibility model for a Midwestern state’s IVF centers in order to identify underserved populations.


MATERIALS AND METHODS: Spatial interaction techniques were applied to a modified gravity model to map spatial accessibility of census tracts to the IVF centers in a Midwestern state. Important sociodemographic (SD) variables were identified and weighted based on a survey of experts. Self-organizing map techniques were used to identify and map clusters of degree of match between census-level SD data and expert-identified SD variables. Spatial accessibility maps were then combined with SD clusters to identify underserved regions. Accessibility and degree of SD match were abstracted to three classes each (high, moderate, and low access, and high, moderate, and low SD match). Patient address data from fresh and frozen cycles (n=1588) were then overlaid on the resulting accessibility map to determine degree of fit of the model.

RESULTS: 39% of patients resided in the 79 census tracts identified as high access and high SD match, while <1% of patients resided in the 10 tracts identified as poor access and low SD match. 61 tracts of high to moderate SD match were identified as moderate access.
Fresh and frozen patient cycles by degree of patient location accessibility and SD match

<table>
<thead>
<tr>
<th>Low SD match</th>
<th>Moderate SD match</th>
<th>High SD match</th>
</tr>
</thead>
<tbody>
<tr>
<td>Access (n) %</td>
<td>Access (n) %</td>
<td>Access (n) %</td>
</tr>
<tr>
<td>(2) 1%</td>
<td>(19) 1%</td>
<td>(617) 39%</td>
</tr>
<tr>
<td>(20) 1%</td>
<td>(179) 11%</td>
<td>(596) 38%</td>
</tr>
<tr>
<td>(24) 2%</td>
<td>(282) 18%</td>
<td>(1282) 81%</td>
</tr>
</tbody>
</table>

CONCLUSION: Combining accessibility results with sociodemographic mapping provide valuable insights into the accessibility of IVF services to a state’s population and the degree to which demand for IVF care is being met. Geospatial techniques are a promising tool for IVF providers considering how best to expand access to care.

Supported by: Funding was provided by an ASRM Research Grant and the University of Iowa Social Sciences Funding Program. Dr. Ryan is a Scholar in the WRHR program at the University of Iowa (K12-NIH-HD063117).

P-418 Wednesday, October 22, 2014


OBJECTIVE: Studies suggest that AA women suffer worse in vitro fertilization (IVF) and pregnancy outcomes compared to Caucasians, with differences often attributed to their increased incidence of uterine pathology. We aimed to control for known confounders by comparing outcomes in donor IVF (dIVF) recipients to determine the specific impact race might have on implantation.

RESULTS: Both groups had similar demographics and histories. AA recipients were matched with Caucasian recipients (controls) with similar fibroid and uterine surgery histories during similar time periods. After chart review, 71 AA women and 81 Caucasian controls were matched more than once after review of 142 control charts. Demographics, history, cycle information, implantation, and pregnancy outcomes were analyzed. Comparisons were made using t-tests and odds ratios where appropriate.

RESULTS: Both groups had similar demographics and histories. AA women had a significantly higher mean body mass index (BMI) (26.4 ± 4 vs. 23.3 ± 3, P < 0.0001), however only 11 AA and 2 Caucasian women met criteria for obesity (BMI > 30). dIVF cycle outcomes were similar, however controls had a significantly higher number of normally fertilized oocytes (14.5 ± 7 vs. 12.6 ± 7, P < 0.008) and fertilization rates (76.1 ± 14% vs. 68.1 ± 19%; P < 0.003) despite similar rates of intracytoplasmic sperm injection. Controls also had significantly higher implantation rates. Live birth rates appeared higher in controls, but failed to reach significance. There was no significant change in outcomes when obese women were excluded.

CONCLUSION: After controlling for history of uterine pathology, AA women appear to have lower implantation rates and may have lower live birth rates compared to Caucasian women undergoing oocyte donation. AA women also had decreased number of donor oocytes and fertilization rates. Further studies matching AA women to controls undergoing autologous IVF cycles would further elucidate these findings.

P-419 Wednesday, October 22, 2014

REPLETE VITAMIN D LEVELS ARE ASSOCIATED WITH HIGHER PREGNANCY RATES AND INCREASED NUMBER OF LIVE BIRTHS IN AUTOLOGOUS IVF CYCLES. K. N. Fru, T. Segal, J. M. Cox, S. L. Mumford, F. I. Sharara, J. H. Segars. *NICHID, NIH, Bethesda, MD; †NS-LIJ HS, Manhasset, NY; ‡VCRM, Reston, VA.

OBJECTIVE: Prior studies described reduced live birth rates in Whites but not Asians with decreased vitamin D levels. Our study examined this relationship in a racially diverse population.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: Autologous IVF cycles at a private US ART center (1/2011-12/2013) were reviewed for pre-cycle Vit D (deficient <20, insufficient 20-30, replete >30 ng/ml), positive hCG, chemical loss, spontaneous and missed abortions (SAB, MAB) and live births. Repeated measures ANOVA, Fisher’s exact test, and generalized linear models were used to evaluate associations.

RESULTS: 124 cycles (n=102 women) were included in this analysis. Baseline characteristics of age, body mass index, parity and stimulation protocol were similar between groups. Women with replete Vit D levels had 67% positive hCG vs 33% in deficient women (p=0.03). Similar proportions of live births were achieved within groups (5/6 or 83% in deficient, 15/24 or 62% in insufficient and 25/37 or 68% in replete). Overall, 28% of deficient women had live births vs 43% of the replete women (p=0.31). No differences by race were found in IVF outcome by Vit D category. However, in all non-replete women (Vit D < 30 ng/ml) risk ratios (RR) for positive hCG were 0.78 (95% Confidence Interval 0.61, 0.99, p=0.04) adjusted for age and race, and among Asian women the unadjusted RR was 0.47 (95% CI 0.25, 0.90, p=0.02). Of note, Vit D deficient women required less days of stimulation (P<0.03), had higher peak E2 levels (P=0.007), and tended to require less gonadotropins (P=0.08) than the insufficient and replete groups. Mean AMH by Vit D category was 2.89 vs 2.24 vs 2.0 in deficient, insufficient and replete women respectively (p=0.31).

*Includes 9 patients with ongoing pregnancy

CONCLUSION: Higher Vit D levels correlated with an increased likelihood of positive hCG in a racially diverse population. Given similar rates of pregnancy loss, the overall proportion of live births was highest in the Vit D replete group. The study is ongoing.

Supported by: Supported, in part, by PRAE, NICHID, NIH, Bethesda, MD.

OXIDATIVE STRESS

P-420 Wednesday, October 22, 2014

SEVERE AND PERITONEAL FLUID LEVELS OF ISCHEMIA MODIFIED ALBUMIN IN ENDOMETRIOSIS. K. Gok, Y. Tasci, G. S. Caglar, B. Dilibaz, S. Demirtas, S. Ozdemir, Z. Caglar, Ufuk University Faculty of Medicine, Ankara, Turkey; ‡Ufuk University Faculty of Medicine, Ankara, Turkey.

OBJECTIVE: Recently, the role of oxidative stress in development and progression of endometriosis has been reported (1). Ischemia modified albumin (IMA) is a marker of protein oxidation and very limited number of studies has evaluated the role of IMA in endometriosis. This study was designed to evaluate the serum and peritoneal IMA levels in moderate/severe endometriosis as a marker of oxidative stress.

DESIGN: Prospective controlled clinical trial.
MATERIALS AND METHODS: The study group consisted of 35 cases with dysmenorrhea, dyspareunia and/or pelvic pain and an ovarian mass compatible with endometrioma in transvaginal ultrasonography. The diagnosis of endometriosis of the study group was confirmed histopathologically by laparoscopy. The control group was cases without endometriosis that underwent laparoscopy for tubal sterilization. The serum levels of IMA were measured spectrophotometrically by colorimetric method with complex of albumin non-binding cobalt and diithioerethitol. The endometriosis support the possible role of oxidative stress in endometriosis. With this study, peritoneal fluid IMA levels are initially documented in endometriosis cases.

P-421 Wednesday, October 22, 2014
FOLLICULAR FLUID TOTAL ANTIOXIDANT CAPACITY AND ISCHEMIA MODIFIED ALBUMIN LEVELS IN POLYCYSTIC OVARY SYNDROME. J. N. Buzuguner,1 Y. Tasci,2 G. S. Caglar,2 B. Dilbaz,2 S. Demirtas,1 I. Kaplanoglu,1 S. Duzguner,1 T. Zubeidey Ha- nim Womens Health Research Hospital, Ankara, Turkey; 1Ufuk University, Faculty of Medicine, Ankara, Turkey.

OBJECTIVE: This study is designed to evaluate ischemia modified albumin (IMA) and total antioxidant capacity (TAC) levels in follicular fluid of polycystic ovary syndrome (PCOS) cases undergoing IVF as a marker of oxidative stress.

DESIGN: Prospective controlled clinical trial.

MATERIALS AND METHODS: The study group consisted of PCOS cases (n = 30) (Rotterdam criteria) undergoing IVF cycles. The control group was age-matched normal ovulator, normalgonadotropin IVF cases (n = 30). The age of the participants ranged from 23 to 39 years. The controlled ovaryan hyperstimulation was performed by long protocol down regulation and recombinant FSH stimulation. Aspirated follicular fluid containing mature oocytes were analyzed for TAC and IMA levels. IMA levels of serum and follicular fluid were measured spectrophotometrically by colorimetric method with complex of albumin non-binding cobalt and diithioerethitol, TAC levels of serum and follicular fluid were measured spectrophotometrically by Randox kit. Fertilization, embryo quality, endometrial assessment and final pregnancy outcome were assessed.

RESULTS: No statistically significant difference was found between the groups when compared for mean age, body mass index, duration of infertility and the mean number of previous IVF cycles (p > 0.05). The cycle outcome parameters were also similar (total gonadotropin dose, duration of induction, number of oocytes retrieved, number of MII oocytes, number of transferred embryos, number of grade 1 embryos, implantation rate and clinical pregnancy rate) (p > 0.05). Although not statistically significant, follicular fluid IMA and TAC levels were higher in PCOS group. Follicular fluid IMA levels were positively correlated with embryo grading (r = 0.328; p < 0.05). The sensitivity, specificity, positive and negative predictive values of the best cutoff value of follicular fluid IMA (1.475 abs/u) for the prediction of grade 1 embryo development were 77%, 54%, 73% and 60%, respectively (AUC: 0.669; p = 0.030). The specificity, positive and negative predictive values of the best cutoff value of follicular fluid IMA (1.475 abs/u) for the prediction of grade 1 embryo development were 77%, 54%, 73% and 60%, respectively (AUC: 0.669; p = 0.032). In PCOS cases with TAC between ≤1.299 mmol/L and ≥1.3 mmol/L, the fertilization rates, the number of MII oocytes, embryo grading and implantation rates were significantly different (p < 0.05).

CONCLUSION: Follicular fluid IMA can be used as a marker in assessing oxidative stress in PCOS cases undergoing IVF. In addition, follicular fluid TAC and IMA seem to be a good predictor for estimating the quality of the oocyte and the embryo.

P-422 Wednesday, October 22, 2014
EMBRYO VIABILITY INDEX: METABOLOMICS ANALYSIS UTILIZING NEAR INFRARED (NIR) SPECTROSCOPY TO ANALYZE EMBRYOS IN THE SAME COHORT DOES NOT PREDICT IMPLANTATION RATE: A PROSPECTIVE COHORT STUDY. J. M. Frana,1 K. H. Hong,2 M. D. Werners,2 R. T. Scott, Jr.2 RWJ Medical School, Rutgers University, New Brunswick, NJ; 1RMA of New Jersey, Basking Ridge, NJ.

OBJECTIVE: NIR metabolomic profiling of culture media has been assessed as a predictive tool for embryonic competence. Early validation studies were able to demonstrate statistical differences in single embryo transfers with known outcomes. These data, while provocative, were greatly limited in that they did not distinguish between embryos in the same cohort—something of paramount importance if NIR is to be used to enhance embryo selection. To address this question we evaluated the range and variability of NIR viability index (VI) results amongst double embryo transfers (DET) with 0%, 50%, and 100% implantation rates.

DESIGN: Prospective cohort study.

MATERIALS AND METHODS: Patient underwent fresh DETs at a single center. Spent culture media was collected and embryos transferred on day 3 and NIR analysis subsequently performed. Data were stratified by implantation rate (0%, 50%, and 100%). NIR analysis yielded a mean VI for each stratification statistically compared with an ANOVA test. The F test compared variance amongst the groups and an ROC curve was created for VIs and implantation rates.

RESULTS: Spent media from 180 patients was evaluated and stratified into 0% (n = 71), 50% (n = 59), and 100% (n = 50) implantation rate groups. No difference existed between the mean VIs indicating that NIR did not discriminate between embryos (p = 0.9). In fact, the range of VIs for the 100% group was entirely within confidence range of the 0% group. ROC analysis for VIs from the 0% and 100% yielded an AUC of 0.53 indicating no value was predictive of outcome (p = 0.26). The greatest variability in VIs was expected in the 50% rate group where one sample came from the embryo which delivered and one from the non-viable embryo. However, the F-test for variance showed that the 0% group had a higher level of variance than the 50% group (p = 0.001), further calling into question the discriminatory power of the test.

The viability index does not allow for enhanced embryo selection.

P-423 Wednesday, October 22, 2014
ROLE OF CUMULUS CELLS IN DEFENSE AGAINST REACTIVE OXYGEN SPECIES INSULT IN METAPHASE II MOUSE OOCYTES. H. M. Abou-Soud, S. N. Khan, F. Shaieb, J. Baranjee, M. Thakur, J. Dai, A. Awonuga, G. M. Saed. Obstetrics and Gynecology, Wayne State University, Detroit, MI.

OBJECTIVE: Chronic inflammation in the female genital tract has been associated with poor reproductive outcomes caused by oxidative stress in the form of enhancement of reactive oxygen species (ROS). Specifically, the damage mediated by ROS targets proteins, lipids, and DNA, thus compromising the function and viability of cells including the cumulus oocyte complex (COC). Recently, we have shown that ROS deteriorate oocyte quality by altering the microtubule morphology (MT) and chromosomal alignment (CH).

DESIGN: Basic Science Cell Study.

MATERIALS AND METHODS: In the current work, we extent these studies to investigate the direct effects of increasing concentrations of ROS such as hydrogen peroxide (H2O2), peroxynitrite (ONOO-), hydroxical radical (-OH), and hypochlorous acid (HOCI) on metaphase II mouse oocytes with cumulus and without cumulus with known outcomes. Cumulus cells show protection against H2O2 and -OH insult at low concentrations, but this protection was lost at higher concentrations (50 μM and above). However, cumulus cells offered no statistically significant protection against
ONOO- and HOCl at any concentration (0-100 μM). In all circumstances in which cumulus cells did not offer protection to the oocyte, both cumulus cell number and viability were decreased as judged by viability staining using the trypan blue dye exclusion method.

CONCLUSION: The deterioration in oocyte quality is caused by a decrease in the antioxidant machinery of the COC by the loss of cumulus cells or the lack of scavengers for specific ROS, and/or the ability of the ROS to overcome these defenses. These results provide a mechanistic link between reactive oxygen species and poor reproductive outcomes.

P-424 Wednesday, October 22, 2014


OBJECTIVE: Myeloperoxidase (MPO) is an abundant heme-containing enzyme present in inflammatory cells such as neutrophils, monocytes, and macrophages. MPO is known to generate hypohchlorous acid (HOCl), a damaging reactive oxygen species utilizing the oxidant hydrogen peroxide (H2O2) and chloride (Cl-). MPO is produced in high levels during inflammation, and chronic inflammation of the female reproductive tract has been associated with poor reproductive outcomes. In this work we investigate the effect of the MPO system on oocyte quality.

DESIGN: Basic Science Cell Study.

MATERIALS AND METHODS: Mouse metaphase II oocytes with (n=648) and without cumulus cells (n=648) were incubated with a catalytic amount of MPO (40 nM) for different incubation periods in the presence of 100 μM Cl- with and without H2O2, at 37 °C. After treatment, oocytes were fixed, stained and scored based on the microtubule morphology (MT) and chromosomal alignment (CH).

RESULTS: MPO, both in the presence and absence of H2O2, was found to negatively affect oocyte quality to a similar degree, in a time dependent fashion. In both cases the presence of cumulus cells offered no protection to the oocyte against MPO activity. To determine whether HOCl is the major cause of deterioration in oocyte quality, direct real-time quantitative intracellular levels of O2- were measured utilizing an H2O2-selective electrode and experiments examining the effect of increasing concentration of exogenous HOCl on oocyte quality were performed. Intra-oocyte H2O2 measurements showed substantial amounts of H2O2 in the oocytes (~10 μM). Treatment with HOCl, the major product of MPO, caused decreased quality as a function of concentration as compared to untreated controls with no protection provided by cumulus cells. Inhibition of MPO or scavenging of HOCl by melatonin shows a protective effect on oocyte quality.

CONCLUSION: HOCl generated from MPO mediates damage to the MT and CH of the metaphase-II mouse oocytes, a process that may be prevented by pretreatment with melatonin. This work provides a direct link between MPO and decreased oocyte quality leading to poor reproductive outcomes.

P-425 Wednesday, October 22, 2014

L-CARNITINE AND N-ACETYL-CYSTEINE SUPPLEMENTATION TO IN VITRO MATURATION MEDIA REDUCES OOCYTE MEIOTIC DAMAGE INDUCED BY INCUBATION IN FOLLICULAR FLUID FROM INFERTILE PATIENTS WITH MILD ENDOMETRIOSIS. V. S. I. Giorgi, a M. G. Da Broi, b T. G. M. Jinani, a C. P. de Paz, a R. A. Ferriani, a P. A. S. Navarro, a b Department of Obstetrics and Gynecology, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil; c Department of Genetics, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil.

OBJECTIVE: To assess the impact of follicular fluid (FF) from infertile women with mild endometriosis (ME) on oocyte spindle and chromatin integrity and to evaluate the potential protective effect of L-carnitine (LC) and/or N-acetyl-cysteine (NAC) against deleterious substances present in the FF utilized during in vitro maturation (IVM) in a bovine model.

DESIGN: Experimental study.

MATERIALS AND METHODS: FF samples were obtained from 22 infertile women undergoing ovarian stimulation for intracytoplasmic sperm injection (11 with ME) (EF) and 11 with tubal and/or male factor of infertility (CFF), pooled, and utilized in 9 IVM experiments with immature bovine oocytes (IBO). IBO were submitted to IVM divided in 9 groups: without FF (NF), FF, CFF, EFF + NAC (ENAC), CFF + NAC (CNAC), EFF + LC (ECLC), CFF + LC (ECLC), EFF + NAC + LC (E2Ao), CFF + NAC + LC (C2Ao). After 22–24 h of IVM, oocytes were denuded, fixed, stained for morphological visualization of both microtubules and chromatin, and analyzed by confocal microscopy. Data were analyzed by Poisson distribution.

RESULTS: A total of 1934 oocytes were fixed and 1572 were analyzed. The percentage of normal MII oocytes was significantly lower in the EFF (51.35%), ENAC (62.22%) and C2Ao (61.40%) groups compared to NF and all CFF groups. The percentage of normal MII oocytes was similar compared to FF (86.36%) with all control groups (CFF (83.52%), CNAC (80.95%), CLC (90.22%), C2Ao (81.03%) and ECLC (80.61%)). In the groups with addition of control FF, only LC increased the percentage of normal MII oocytes. And in the groups with addition of mild endometriosis FF, the addition of NAC, LC, and both of them increased the percentage of normal MII oocytes, but LC was significantly superior to NAC and the 2Ao.

CONCLUSION: FF from infertile women with ME may promote meiotic damage of in vitro matured bovine oocytes, which is minimized or completely prevented by co-administration of antioxidants, especially LC. Our data suggest oxidative stress is involved in the worsening of oocyte quality in ME. We question whether the use of LC as a supplement in patients with ME may be a novel approach to improve fecundity.

Supported by: FAPESP (process number: 2012/19070-1), Brazil.

P-426 Wednesday, October 22, 2014

ALTERED REDOX STATE IN THE ENDOMETRIUM OF PATIENTS UNDERGOING OVARIAN STIMULATION FOR ASSISTED REPRODUCTION TECHNOLOGY. N. M. Fletcher, a L. Detti, a B. R. Neubauer, b M. G. Saed, b M. P. Diamond, 3 M. I. Abzeid, 3 G. M. Saed. 2Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI; 3Obstetrics and Gynecology, Georgia Regents University, Augusta, GA; 4In-Vitro Fertilization Michigna, Flint, MI; 5Obstetrics and Gynecology, University of Tennessee Health Science Center, Memphis, TN.

OBJECTIVE: Oxidative stress is the result of alteration in the cellular balance between oxidant and antioxidants. Oxidative stress has been linked to infertility associated diseases such as postoperative adhesions, fibroids, endometriosis, and polycystic ovary syndrome (PCOS). The sources and nature of oxidative stress associated with assisted reproductive technology (ART) are not yet known. We have previously shown that ovarian stimulation is associated with an increase in superoxide dismutase, a potent antioxidant, as well as myeloperoxidase, a potent oxidant and inflammatory marker. In this study, we sought to determine the effects of ovarian stimulation for ART on the expression of additional antioxidants, glutathione reductase (GSR) and glutathione peroxidase (GPX1), and oxidants, nitric oxide synthase (NOS) isoforms and endothelial (eNOS), in endometrial tissues during the peri-implantation period.

DESIGN: Prospective controlled study.

MATERIALS AND METHODS: Endometrial tissue samples were collected with a flexible pipette from women (n=9) during a natural cycle (control) and oocyte donors (n=9, treated) during an assisted reproductive technology (ART) cycle at hypothetical day 3 of embryo transfer (LH+5 or lCG+5). RNA was extracted, and subjected to quantitative real-time RT-PCR for NOS isoforms, GSR and GPX1.

RESULTS: There was a 2.2 fold increase in GPX1 and no change in GSR mRNA levels in stimulated as compared to control endometrium. There was a 1.2, 2.1, and 4.7 fold increase in eNOS, nNOS, and iNOS mRNA levels, respectively, in stimulated as compared to control endometrium.

CONCLUSION: Ovarian stimulation significantly alters oxidative stress profile favoring an endometrial pro-oxidant state, which may impact the overall success of implantation. This provides the base to future clinical trials testing the effects of antioxidant supplements during stimulation.

REPRODUCTIVE IMMUNOLOGY

P-427 Wednesday, October 22, 2014

EVIDENCE THAT A DEGREE OF MUCOSAL INFLAMMATION IS BENEFICIAL FOR PREGNANCY SUCCESS IN ASSOCIATION WITH IVF. M. Vega, a c D. H. Barad, d A. Weghofer, c V. A. Kushnir, d N. Gleicher, a N. Gleicher, b,c aDepartment of Obstetrics and Gynecology, St. Luke’s - Roosevelt Hospital Center, New York, NY; bCenter for Human
OBJECTIVE: We elsewhere in this meeting report that antiphospholipid antibodies (APAs), among a variety of immune parameters, are most closely associated with the diagnosis of low functional ovarian reserve (LFOR). We recently reported that 0.011 of CD8 cells vs. 0.034 10.39 vs 6 days H. Ahmed, S. H. Bao, Vol. 102, No. 3, Supplement, September 2014 S. Jasti, V. Chennathukuzhi, T. Yankee, B. K. Petroff, R. Heitmann, J. Tisdale, A. Decherney, 0.013 in con- gated antibody (eBiosciences), rinsed and assessed for FOXP3 positivity meabilized overnight. Cells were labeled with human FOXP3-Cy5 conju- and enzymatically digested and passed through a 100 um filter to obtain IRB protocol. Endometrial tissue was collected by Pipelle, mechanically was to characterize Tregs in the blood and endometrium during the prolifer- significantly smaller litter sizes after circulating Treg knockdown, as a result of parous females. S. Jasti, V. Chennathukuzhi, T. Yankee, B. K. Petroff, M. G. Petroff. The University of Kansas Medical Center, Kansas City, KS. OBJECTIVE: Pregnancy is a natural phenomenon in which the semi-allo- genetic fetus is tolerated by the mother’s immune system. During this event, paternally inherited antigens expressed by placenta can elicit an immune response leading to generation of anti-fetal T cells in mothers. However, the long-term implications of these persistent T cells on the mother’s health have not been studied. Shared placentatumor antigens are proteins that are expressed both by placenta and tumors, and are absent or minimally expressed in normal tissues. Using a model shared placentatumor antigen, we tested the hypothesis that T cells elicited against these antigens can alter cancer risk in mothers. DESIGN: Act-mOVA (Ovalbumin) transgenic male mice were bred to wild-type females such that fetuses inherited and expressed OVA. Females were sacrificed during pregnancy and post-partum to detect the presence of anti-OVA T cells. Further, to understand whether an infection during preg- nancy would modify the endogenous response to Ovalbumin, pregnant OVA-bred mice were injected with Polyinosinic:polycytidylic acid (Poly I:C) on day 12.5 and sacrificed after 5 days. Finally, to decipher the possible role of anti-OVA T cells, 8-9 weeks post-partum OVA-bred females were inoculated with E.G7-OVA lymphoma cells, which constitutively express OVA, and tumor growth was monitored. MATERIALS AND METHODS: Anti-OVA T cells were detected by flow cytometry. Tumors were monitored using a digital caliper three times/week until mice were sacrificed due to tumor burden. Kaplan Myer survival analysis, T-test and ANOVA were used for statistical eval- uation. RESULTS: Anti-OVA T cells were elicited during pregnancy and per- sisted post-partum until 15-24 weeks in OVA-bred mice. Interestingly, Poly I:C administration during pregnancy did not modify the endogenous response to OVA (0.05% ± 0.011 of CD8 cells vs. 0.034 ± 0.013 in con- trols; p=0.315). Tumor onset was significantly delayed in OVA-bred mice as compared to virgin mice (24 days ± 10.39 vs 6 days ± 0.293; p< 0.05). Further, the progression of tumors to a size of 15mm was delayed in OVA- bred mice in comparison to wild-type bred and virgin females (67 days ± 2.18 vs. 38 days ± 6.06; p=0.005 and 33 days ± 5.13; p=0.001 respec- tively). CONCLUSION: OVA elicited an immune response during murine pregnan- cy, and the development of OVA-expressing tumors was delayed in OVA-bred mice. These data suggest a possible role of anti-placenta/tumor antigen specific T cells in protecting parous females against tumors bearing the same antigen. Supported by: NIH grant R01HD045611.

P-428 Wednesday, October 22, 2014 IMMUNE RESPONSE TO SHARED PLACENTA/TUMOR ANTIGENS MAY REDUCE CANCER RISK IN PAROUS FEMALES. S. Jasti, V. Chennathukuzhi, T. Yankee, B. K. Petroff, M. G. Petroff. The University of Kansas Medical Center, Kansas City, KS. OBJECTIVE: Pregnancy is a natural phenomenon in which the semi-allo- genetic fetus is tolerated by the mother’s immune system. During this event, paternally inherited antigens expressed by placenta can elicit an immune response leading to generation of anti-fetal T cells in mothers. However, the long-term implications of these persistent T cells on the mother’s health have not been studied. Shared placentatumor antigens are proteins that are expressed both by placenta and tumors, and are absent or minimally expressed in normal tissues. Using a model shared placentatumor antigen, we tested the hypothesis that T cells elicited against these antigens can alter cancer risk in mothers. DESIGN: Act-mOVA (Ovalbumin) transgenic male mice were bred to wild-type females such that fetuses inherited and expressed OVA. Females were sacrificed during pregnancy and post-partum to detect the presence of anti-OVA T cells. Further, to understand whether an infection during preg- nancy would modify the endogenous response to Ovalbumin, pregnant OVA-bred mice were injected with Polyinosinic:polycytidylic acid (Poly I:C) on day 12.5 and sacrificed after 5 days. Finally, to decipher the possible role of anti-OVA T cells, 8-9 weeks post-partum OVA-bred females were inoculated with E.G7-OVA lymphoma cells, which constitutively express OVA, and tumor growth was monitored. MATERIALS AND METHODS: Anti-OVA T cells were detected by flow cytometry. Tumors were monitored using a digital caliper three times/week until mice were sacrificed due to tumor burden. Kaplan Myer survival analysis, T-test and ANOVA were used for statistical eval- uation. RESULTS: Anti-OVA T cells were elicited during pregnancy and per- sisted post-partum until 15-24 weeks in OVA-bred mice. Interestingly, Poly I:C administration during pregnancy did not modify the endogenous response to OVA (0.05% ± 0.011 of CD8 cells vs. 0.034 ± 0.013 in con- trols; p=0.315). Tumor onset was significantly delayed in OVA-bred mice as compared to virgin mice (24 days ± 10.39 vs 6 days ± 0.293; p< 0.05). Further, the progression of tumors to a size of 15mm was delayed in OVA- bred mice in comparison to wild-type bred and virgin females (67 days ± 2.18 vs. 38 days ± 6.06; p=0.005 and 33 days ± 5.13; p=0.001 respec- tively). CONCLUSION: OVA elicited an immune response during murine pregnan- cy, and the development of OVA-expressing tumors was delayed in OVA-bred mice. These data suggest a possible role of anti-placenta/tumor antigen specific T cells in protecting parous females against tumors bearing the same antigen. Supported by: NIH grant R01HD045611.


OBJECTIVE: Increasing evidence demonstrates an important role for T regulatory cells (Tregs); defined by CD4+CD25+FoxP3+ surface antigen expression, in embryo implantation. Our group previously demonstrated significantly smaller litter sizes after circulating Treg knockdown, as a result of defective implantation, in a murine model (1). The objective of this study was to characterize Tregs in the blood and endometrium during the prolifer- ative and luteal phases of the menstrual cycle and on OCPs.

DESIGN: Prospective translational research.

MATERIALS AND METHODS: Reproductive aged volunteers were re- cruited to donate research blood and endometrial tissue under an approved IRB protocol. Endometrial tissue was collected by Pipelle, mechanically and enzymatically digested and passed through a 100 um filter to obtain single cell suspensions. WBCC and endometrial cells were fixed and per- meabilized overnight. Cells were labeled with human FOXP3-Cy5 conjugated antibody (eBiosciences), rinsed and assessed for FOXP3 positivity expression, in embryo implantation. Our group previously demonstrated significantly smaller litter sizes after circulating Treg knockdown, as a result of defective implantation, in a murine model (1). The objective of this study was to characterize Tregs in the blood and endometrium during the prolifer- ative and luteal phases of the menstrual cycle and on OCPs.

RESULTS: There was no significant effect of Fa 1 on Clinical Pregnancy. However, Fa 1, composed of thyrotopin receptor antibody, IgA anti- phosphatidylserine, IgG and IgA anti-cardiolipin, was highly predictive of live birth (P=0.0008), as was IgA anti-phosphatidylserine, alone (P=0.0015), suggesting 1.11-times increased odds after adjusting for pa- tient age. There was no significant effect of any immune/inflammatory factor on log(AMH). However, oocyte yield was negatively affected by IgA anti-cardiolipin with each 1-unit change decreasing the yield by -1.9864 units, (P=0.04).

CONCLUSION: This study for the first time suggests that an IgA-driven inflammatory/immune effect may benefit IVF outcomes, suggesting that mucosal immune responses may play a significant role in IVF success. This finding could also explain the widely reported, so far unexplained, benef- icial effects of endometrial scratching on IVF outcomes.

Supported by: Foundation for Reproductive Medicine, Center for Human Reproduction.
OBJECTIVE: Inflammatory immune response and its role in angiogenesis are important determinants of a successful pregnancy. Ultrasonographic evaluation of blood flow using Doppler technique may reflect progression of angiogenesis at maternal fetal junction. In this study, we aim to investigate the relationship of the uterine radial artery resistance index (U-RI) and immune inflammatory markers in RPL patients during pregnancy, who were under preconception anti-coagulant, prednisone and/or intravenous immunoglobulin (IVlg) treatment.

DESIGN: Total 132 patients delivered a live born infant (delivery group) and 30 patients miscarried again (abortion group).

MATERIALS AND METHODS: A retrospective study was carried out on 162 women with RPL (≥2) who were investigated during pregnancy and enrolled in the Reproductive Medicine program at Chicago Medical School at Rosalind Franklin University of Medicine and Science. U-RI, peripheral blood TH1/TH2 intracellular cytokine ratio, immunophenotype assay and NK cytotoxicity (NKC) were analyzed.

RESULTS: U-RIs were decreased significantly during early pregnancy as compared to non-pregnant U-RIs (P<0.001). Abortion group had significantly higher U-RIs as compared to those of delivery group in gestation weeks 7, 8, and 9 (P=0.008, P=0.001 and P= 0.043 respectively). During the first trimester, CD56+ NK cell levels and NKCs were higher in abortion group than delivery group, however not statistically different. In delivery group, NK cell levels were significantly decreased during early pregnancy between gestational weeks 4 and 5 (P=0.002) and to decrease throughout pregnancy. NKC were significantly decreased during early pregnancy but increased during the third trimester; Th1/Th2 cell ratios of abortion group were increased at gestational weeks 7 and 8 as compared to delivery group, however not statistically different.

CONCLUSION: Increased U-RIs during early pregnancy predict pregnancy outcome in women with RPL who were under anti-coagulation and immune therapy. Suppression of increased NK cell levels and NKC by prednisone and/ or IVlg is correlated with decreased U-RIs.

FEMALE REPRODUCTIVE TRACT

P-432 Wednesday, October 22, 2014

GEL-INSTILLATION VERSUS SALINE INFUSION DURING SONOHYSTEROGRAPHY: ANY ADVANTAGES? Y. El-Faisal, A. El-Liby. Obstetrics and Gynecology Department, Cairo University, Cairo, Egypt.

OBJECTIVE: To evaluate if gel-instillation sonography (GIS) is superior to saline infusion, during Sonohysterography performed for patients to investigate the endometrial cavity.

DESIGN: A prospective randomized controlled observational study.

MATERIALS AND METHODS: Setting: The outpatient clinic, the ultrasound department and the office hysteroscopy clinic of Cairo University medical school hospital, Kasr El Eini. Patients: We included 100 women aged 20-45 years, presenting to the outpatient clinic of our university hospital. Interventions: Two groups of patients were included, in the first (53 patients) saline infusion sonography (SIS) was performed, in the second (47 patients), we did GIS. The patients underwent then office diagnostic hysteroscopy (DH).

RESULTS: Mean procedure time was significantly shorter in the saline group than in gel group (10.23 +/-1.69 and 14.45+/1-62 minutes respectively, P-value=0.0001). The uterine distention time was significantly longer in the second group (21.94 sec+/-2.28), while in the first it was 7.96+/-2.37 (P-value=0.0001). The pain score was higher in the second group (Mean=1.45+/0.72) compared to 1.13+/0.68 in the first (P-value of 0.007). The specificity of testing with saline was 100% and the sensitivity 64.28%, whereas in the gel group they were 97.22%/61.81% respectively.

CONCLUSION: Using GIS increased the procedure time, uterine distension time and patients’ discomfort without effect on diagnostic accuracy. Both tests are highly specific, but the sensitivity of gel is higher.

P-433 Wednesday, October 22, 2014

AIR BUBBLE SALINE INFUSED SONOGRAPHY (SIS) IS EQUIVALENT TO HYSTEROSALPINGOGRAM (HSG) IN ASSESSMENT OF TUBAL PATENCY. I. Robertsaw, A. Martinez, A. Batcheller, K. DiPaola, J. Sroga, S. Lindheim. "Obstetrics and Gynecology, University of Cincinnati, West Chester, OH; Obstetrics and Gynecology, Wright State University, Dayton, OH.

OBJECTIVE: To compare air bubble SIS to HSG in assessing endometrial cavity (EC) and tubal patency.

DESIGN: Prospective descriptive study.

MATERIALS AND METHODS: An IRB approved study of 93 women undergoing HSG were recruited. After an SIS for cavity evaluation, a saline-air device was connected to the balloon-catheter. Slow instillation of air-saline was used to sequentially assess right and left tubal patency. This was followed by an HSG performed the same day. Secondary measures included a Likert Pain Scale and time assessment of procedure. Cohen’s kappa coefficient was used to assess agreement between each procedure and a paired t-test was used to compare differences in pain and time between modalities. Significance as determined by p<0.05.

RESULTS: The patient mean age was 34.0 ± 4.9 yrs. Positive correlation was observed between the air bubble SIS and HSG with regards to EC (K=0.554, p=0.001), right (K=0.322, p=0.001) and left (K=0.373, p=0.001) fallopian tube patency. The EC was found to be concordantly normal between both modalities in 95% (41/43) of cases and abnormal in 67% (27/40). Air bubble SIS and HSG equivalently identified right and left tubal patency in 92.8% (64/69) and 96% (73/76) of cases, respectively.
However, only 40% (6/15) and 44% (4/9) of right and left tubal occlusions were in agreement with HSG. The air bubble SIS procedure time was found to be on average 2 minutes longer and conferred a higher degree of pain compared to HSG (p=0.001).

CONCLUSION: There is a strong correlation between air bubble SIS and HSG when both tubes are patent. However, in the absence of clear patency, further evaluation may be indicated with either HSG or laparoscopy. The duration of air bubble SIS is longer, which could affect the degree of pain experienced. This finding may improve with operator experience. Compared to HSG, the cost effectiveness of air bubble SIS and avoidance of radiation exposure may outweigh any drawbacks.

Supported by: Cooper Surgical and Smith and Nephew.

P-434 Wednesday, October 22, 2014
LUCIFERASE IMAGING OF CHLAMYDIA MURIDURUM ASCENDING INFECTION IN MICE. J. Campbell,* Y. Huang,* Y. Liu,* R. Schenken,* G. Zhong,* *Microbiology, The University of Texas Health Science Center, San Antonio, TX; *Obstetrics and Gynecology, The University of Texas Health Science Center, San Antonio, TX.

OBJECTIVE: To understand Chlamydia pathogenic mechanisms by visualizing chlamydial ascending infection in the genital tract.

DESIGN: Constructing luciferase expressing Chlamydia muridarum (C. muridarum) organisms for monitoring C. muridarum trafficking in the female mouse genital tract using bioluminescence technology.

MATERIALS AND METHODS: A shuttle vector containing the luciferase gene was used to transform C. muridarum organisms. A stable transformant was characterized in HeLa cells by monitoring both luciferase mRNA levels using qRT-PCR and luciferase activity using the substrate D-luciferin. The in vitro characterized transformant was then used to infect 10 Balb/cJ mice intravaginally, and the infection was monitored using the Xenogen IVIS imaging system at the whole animal scale.

RESULTS: Luciferase activity was detectable at 12 hours (h), peaked by 15h, and was significantly reduced by 30h after infection in cell cultures infected with luciferase-expressing C. muridarum transformant. Mice intravaginally infected with the same transformant were monitored for luciferase activity on days 3, 7, 10, 14, 21 & 28 after infection. Six of the 10 inoculated mice displayed luciferase signal in lower with two also in upper genital tracts on day 3 after infection. By day 7, all 10 mice developed luciferase signal with 1 only in lower and the remaining 9 in upper genital tracts. The luciferase signal was maintained in upper genital tract in 6 and 2 mice by days 14 and 21, respectively. No luciferase activity was detected in 9 of 10 by day 28. Whole body images revealed a transient airway co-infection among 5 mice housed in the same cage with 5 mice positive on day 3 and 1 remaining positive on day 7. Its presence did not significantly affect the growth of the genital tract infection course.

CONCLUSION: We have successfully visualized Chlamydia muridarum ascent in mouse genital tract. Our observations suggest that C. muridarum organisms are most active in ascending to the upper genital tract within the first 7 days after intravaginal inoculation. Mice of the same inbred strain display variation in the time course of ascending infection. Unexpectedly, co-infection in the airway is common in some cages, but does not seem to affect the time-course of ascending infection. The luciferase detection-based in vivo imaging (without the need for sacrificing mice) will greatly facilitate our understanding of chlamydial infection. The luciferase detection-based in vivo imaging (without the need for sacrificing mice) was performed on all the embryos and T-test of means for significance testing was completed.

RESULTS: PGS indicated that there were 41 Male and 51 Female embryos analyzed. Multiple TLM parameters were considered (based on current literature). When only euploid embryos were considered, there were no significant differences in morphokinetic parameters between the two genders. When only euploid embryos were considered, there were differences in timing events including the start of cavitation (Male=68.1 vs. Female=73.3; p=0.002) as well as time to full blastocyst (Male=78.6 vs. Female=83.9; p=0.05). All other parameters were not found to be significant.

Table1: Morphokinetic parameters in Aneuploid versus Euploid embryos divided based on gender

<table>
<thead>
<tr>
<th>Groups</th>
<th>syngamy</th>
<th>2 cell/ 4cell</th>
<th>8cell</th>
<th>Compaction start/emb</th>
<th>Duration Compaction</th>
<th>Starts cavitation</th>
<th>Time to full blast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aneuploid:</td>
<td>Male</td>
<td>25.36</td>
<td>2.75/14.7</td>
<td>31.2</td>
<td>46.79/6.2</td>
<td>13.7</td>
<td>73.4</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>25.39</td>
<td>2.69/14.8</td>
<td>33.7</td>
<td>45.2/6.1</td>
<td>16.1</td>
<td>71.5</td>
</tr>
<tr>
<td>p-value</td>
<td>0.97</td>
<td>0.84/0.34</td>
<td></td>
<td>0.34</td>
<td>0.04/0.72</td>
<td>0.29</td>
<td>0.94</td>
</tr>
<tr>
<td>Euploid:</td>
<td>Male</td>
<td>24.2</td>
<td>3.3/14.3</td>
<td>31.5</td>
<td>46.2/5.6</td>
<td>10.7</td>
<td>68.1</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>25.9</td>
<td>2.8/15.2</td>
<td>35.1</td>
<td>49.0/9.3</td>
<td>11.3</td>
<td>72.4</td>
</tr>
<tr>
<td>p-value</td>
<td>0.01</td>
<td>0.33/0.06</td>
<td></td>
<td>0.12</td>
<td>0.39/0.45</td>
<td>0.74</td>
<td>0.002</td>
</tr>
</tbody>
</table>

*P-tests used for significance testing **Timing parameters in hours and normalized to syngamy

CONCLUSION: Gender differences are not detected in aneuploid embryos. However, in euploid embryos, Male embryos progress more rapidly, attaining certain morphological stages earlier than Female euploid embryos.

P-436 Wednesday, October 22, 2014
EFFECTS OF ORAL GINKGO BILOBA EXTRACT ON FETAL WEIGHT IN FETUSES WITH INTRAUTERINE GROWTH RESTRICTION. D. E. M. Abd El Aal, M. S. Abdellah, A. Y. Shahine, M. S. Zakhira. Obstetrics & Gynecology, Women’s Health Hospital, Assiut, Egypt.

OBJECTIVE: To evaluate the effect and safety of adjuvant treatment of oral Ginkgo Biloba extract on fetal weight in fetuses with intrauterine growth restriction not associated with congenital anomalies on the improvement of placental functions, when added to empirical antioxidant treatment, as reflected by increase in Doppler indices, fetal weight and amniotic fluid.

DESIGN: Randomized Controlled Trial.

MATERIALS AND METHODS: Two hundred twenty six patients with intrauterine growth restriction (IUGR) fetuses were included and randomly divided into 2 groups. Group 1 received Vitamin E and Omega 3 combination, taken orally twice daily plus Ginkgo Biloba, two tablets per day, while group 2 (controls) received the antioxidant combination alone. Both groups were followed up every 2 weeks by ultrasonography and Doppler.

RESULTS: Group 1 significantly delivered more frequently at term, had significantly higher fetal weights, amniotic fluid indices and Doppler indices’ values during bi-weekly antenatal visits.

CONCLUSION: Adjuvant treatment with Ginkgo Biloba Extract added to antioxidants could improve placental functions, Doppler indices, fetal weight and amniotic fluid index and hence prolong pregnancies complicated with IUGR fetuses not due to congenital anomalies. This effect is mostly due to an oxygen free radical scavenger action combined with a peripheral vascular...
improvement effect of the herb. Further bigger studies are needed to validate our results.

P-437 Wednesday, October 22, 2014


OBJECTIVE: To assess the risk factors and clinical symptoms related to the complete separation of the cesarean section (CS) scar in the nonpregnant uterus.

DESIGN: Observational study.

MATERIALS AND METHODS: The study group included twenty-five women with a history of low transverse CS in whom a complete separation of the CS scar was diagnosed using saline infusion sonohysterography (SIS) and hysteroscopy (HSC). The diagnosis of complete separation of the CS scar was made when at the site of the scar no myometrial tissue could be visualized.

RESULTS: Fifteen women (60%) had a history of one CS, six (24%) had a history of two CSs and four women (16%) of three CSs. No statistically important difference was found between the number of previous CSs and complete separation of the CS scar (p > 0.05). The type of the last CS before the diagnosis of the scar separation was as follows: in 8 cases (32%) elective CS, in 17 cases (68%) emergency intrapartal CS (p > 0.05). None of the women had a history of puerperal infection. The mean interval between CS and diagnosis of scar separation was 14.6 months (± 10.6). Twenty-two women (88%) complained of spotting or bleeding after menstruation. The mean duration of spotting/bleeding was 6 days (± 2.9). Sixteen women (64%) desired future pregnancies. Among that group at the time of evaluation 10 women (62.5%) met the criteria of secondary infertility i.e. 12 months of contraceptive-free intercourse. None of the women was previously treated due to primary infertility. The male factor and ovulation disorders were excluded. One possible explanation of infertility in those patients might be the fact that in all cases during SIS and HSC procedures a blood clot protruding into the cervical canal was observed at the site of the CS scar defect. This may be considered as a mechanical factor causing infertility. During the follow-up one woman became spontaneously pregnant. In that case a CS scar pregnancy was diagnosed. This patient was successful treated with local methotrexate injections.

CONCLUSION: Emergency CS may be considered as a risk factor for complete separation of the uterine CS scar. Women with secondary infertility and abnormal uterine bleeding that have a history of CS need to undergo ultrasonographic assessment of the CS scar. Further studies are needed to confirm if the complete separation of the CS scar is a risk factor for scar pregnancy. Also adequate treatment options for symptoms related to the complete separation of the CS scar should be developed.

P-438 Wednesday, October 22, 2014


OBJECTIVE: To assess the risk factors and clinical symptoms related to the complete separation of the cesarean section (CS) scar in the nonpregnant uterus.

DESIGN: Observational study.

MATERIALS AND METHODS: The study group included twenty-five women with a history of low transverse CS in whom a complete separation of the CS scar was diagnosed using saline infusion sonohysterography (SIS) and hysteroscopy (HSC). The diagnosis of complete separation of the CS scar was made when at the site of the scar no myometrial tissue could be visualized.

RESULTS: Fifteen women (60%) had a history of one CS, six (24%) had a history of two CSs and four women (16%) of three CSs. No statistically important difference was found between the number of previous CSs and complete separation of the CS scar (p > 0.05). The type of the last CS before the diagnosis of the scar separation was as follows: in 8 cases (32%) elective CS, in 17 cases (68%) emergency intrapartal CS (p > 0.05). None of the women had a history of puerperal infection. The mean interval between CS and diagnosis of scar separation was 14.6 months (± 10.6). Twenty-two women (88%) complained of spotting or bleeding after menstruation. The mean duration of spotting/bleeding was 6 days (± 2.9). Sixteen women (64%) desired future pregnancies. Among that group at the time of evaluation 10 women (62.5%) met the criteria of secondary infertility i.e. 12 months of contraceptive-free intercourse. None of the women was previously treated due to primary infertility. The male factor and ovulation disorders were excluded. One possible explanation of infertility in those patients might be the fact that in all cases during SIS and HSC procedures a blood clot protruding into the cervical canal was observed at the site of the CS scar defect. This may be considered as a mechanical factor causing infertility. During the follow-up one woman became spontaneously pregnant. In that case a CS scar pregnancy was diagnosed. This patient was successful treated with local methotrexate injections.

CONCLUSION: Emergency CS may be considered as a risk factor for complete separation of the uterine CS scar. Women with secondary infertility and abnormal uterine bleeding that have a history of CS need to undergo ultrasonographic assessment of the CS scar. Further studies are needed to confirm if the complete separation of the CS scar is a risk factor for scar pregnancy. Also adequate treatment options for symptoms related to the complete separation of the CS scar should be developed.

P-439 Wednesday, October 22, 2014

DIFFERENCES IN 1ST TRIMESTER PLACENTAL VOLUME BASED ON MODE OF CONCEPTION. S. J. Churchill,* E. T. Wang, M. Akhlaghpour, E. H. Goldstein,* G. M. Barlow,* J. Williams, III,* M. D. Pisarska, R. T. Scott, Jr., M. D. Werner, J. M. Franasiak, M. Pomorski, A. Rosner-Tenerowicz, T. Fuchs, R. Woyton, M. Zimmer. Department of Obstetrics and Gynecology, Cedars-Sinai Medical Center, Los Angeles, CA,* Department of Obstetrics and Gynecology, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, CA.

OBJECTIVE: Assisted Reproductive Technologies (ART), specifically in vitro fertilization (IVF), has been associated with small for gestational age babies, which may be the result of placental insufficiency, either due to the IVF itself or the underlying infertility. To determine if this is a phenomenon that starts early in gestation, we measured placental volumes in the first trimester to detect differences between pregnancies conceived with IVF compared to pregnancies conceived with less invasive fertility treatments (in vivo fertilization) and spontaneous conceptions.

DESIGN: Prospective cohort.

MATERIALS AND METHODS: All women with singleton pregnancies who presented to our institution for CVS and first trimester ultrasound from April 2007 to January 2014 were evaluated. Patient and pregnancy characteristics were obtained from chart review. Estimated placental volumes (EPV) were calculated from 2-D ultrasound images using a well-validated computation [1]. Linear regression models were used to compare EPV based on mode of conception (IVF, in vivo fertilization including clomiphene citrate, gonadotropin injections, and/or intrauterine insemination, and spontaneously conceived pregnancies). Analyses were adjusted for maternal age, body mass index, gravidity, hypertension and smoking status.

RESULTS: A total 1517 patients were included in the final analysis (1273 spontaneous, 117 in-vivo, and 127 IVF conceptions). Maternal age was significantly different among the three groups, with IVF conceptions having the highest maternal age (P < 0.001). Fetal gestational age, fetal gender, maternal race and placental location were similar among the 3 groups. After adjusting for confounding variables, linear regression suggested a trend in lower placental volumes in patients with IVF compared to spontaneous conceptions (β coefficient −0.11, p = 0.07) but no difference was seen in in-vivo vs. spontaneous conceptions (β coefficient = −0.06, p = 0.34).

CONCLUSION: IVF may be associated with a smaller placental volume compared to spontaneous conceptions. Further studies are necessary to determine its potential role in the etiology underlying small-for-gestational age infants conceived through IVF.

Supported by: NIH RO1HD074368 and Helping Hand of Los Angeles, Inc.

FERTILITY & STERILITY®

Usable Blasts Unusable Blasts Predictive Value Age

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Usable</td>
<td>Unusable</td>
<td>Predictive Value</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>98</td>
<td>43</td>
<td>PPV 70%</td>
</tr>
<tr>
<td>Low</td>
<td>351</td>
<td>360</td>
<td>NPV 51%</td>
</tr>
</tbody>
</table>

Supported by: Axogyn; Equipment.
THE USE OF TIME-LAPSE OBSERVATIONS TO CONFIRM PRO-NUCLEI FORMATION AND FERTILIZATION. K. Nakayama, a,b N. Fukunaga, a,b,c H. Kitsakara, a,b T. Yoshimura, a,b F. Tamura, a,b M. Katou, a,b N. Aoyagi, a,b Y. Kida, a,b Y. Hashiba, a,b Y. Asada a,b,c IVF Laboratory, Asada Ladies Nagoya Clinic, Nagoya, Aichi, Japan; a,b,c IVF Laboratory, Asada Ladies Kachigawa Clinic, Nagoya, Aichi, Japan; a Asada Institute for Reproductive Medicine, Asada Ladies Clinic, Nagoya, Aichi, Japan.

OBJECTIVE: In our laboratory, fertilization is routinely determined by manual observation, around 19-20 h after intracytoplasmic sperm injection (ICSI). In the present study, we evaluated time-lapse observations to identify PN appearance. The objective of this study was to evaluate the use of time-lapse observations to objectively identify PN formation and fertilization.

DESIGN: prospective cohort study.

MATERIALS AND METHODS: This prospective cohort study was carried out between February 1 and August 31, 2013 and involved 130 infertile couples. Time-lapse analyses were made at 19-20 h after ICSI, at the same time point as our standard manual observation, to judge fertilization status. Images were recorded automatically every 10 min in seven planes (using the Embryo Scope time lapse system) and analyzed for the PN appearance, PN breakdown. Embryos judged as 2 PN were cryopreserved at the PN stage. RESULTS: In 78.4% (567/723) embryos 2 PN formation was observed with PN appearance occurring at 7.7±2.1 h after the initiation of time lapse recordings. In 9.7% (55/567) embryos 2 PN formation and breakdown was observed with PN appearance occurring at 7.2±1.6 h and PN breakdown at 20.1±0.8 h after the initiation of time lapse recordings.

CONCLUSION: We have observed in our series that in 9.7% embryos, PN breakdown occurs earlier than at the routine time point used in our manual observation. By manually observing a single time point it is difficult to correctly judge PN formation. However, using time-lapse imaging to determine the timing of PN formation provides more accurate information on fertilization. Furthermore, Time-lapse evaluation to assess fertilization is feasible as a tool for all patients due to the short time interval to be analyzed.

P-441 Wednesday, October 22, 2014

IMPACT OF AN AUTOMATED TIME-LAPSE SYSTEM IN AN EGG DONATION PROGRAM. E. Rocafort, M. Guizarro, M. A. Fernandez, S. M. D. Rogel, J. M. D. Aizpurua. IVF Laboratory, Asada Ladies Nagoya Clinic, Nagoya, Aichi, Japan; a,b,c IVF Laboratory, Asada Ladies Kachigawa Clinic, Nagoya, Aichi, Japan; a Asada Institute for Reproductive Medicine, Asada Ladies Clinic, Nagoya, Aichi, Japan.

OBJECTIVE: Embryo selection techniques are crucial to perform eSET with good prognosis avoiding multiple pregnancies, especially in egg donation patients with advanced maternal age. The aim of the study was to evaluate the use of time-lapse observations to objectively identify PN formation and fertilization.

DESIGN: prospective cohort study.

MATERIALS AND METHODS: This prospective cohort study was carried out between February 1 and August 31, 2013 and involved 130 infertile couples. Time-lapse analyses were made at 19-20 h after ICSI, at the same time point as our standard manual observation, to judge fertilization status. Images were recorded automatically every 10 min in seven planes (using the Embryo Scope time lapse system) and analyzed for the PN appearance, PN breakdown. Embryos judged as 2 PN were cryopreserved at the PN stage. RESULTS: In 78.4% (567/723) embryos 2 PN formation was observed with PN appearance occurring at 7.7±2.1 h after the initiation of time lapse recordings. In 9.7% (55/567) embryos 2 PN formation and breakdown was observed with PN appearance occurring at 7.2±1.6 h and PN breakdown at 20.1±0.8 h after the initiation of time lapse recordings.

CONCLUSION: We have observed in our series that in 9.7% embryos, PN breakdown occurs earlier than at the routine time point used in our manual observation. By manually observing a single time point it is difficult to correctly judge PN formation. However, using time-lapse imaging to determine the timing of PN formation provides more accurate information on fertilization. Furthermore, Time-lapse evaluation to assess fertilization is feasible as a tool for all patients due to the short time interval to be analyzed.

P-442 Wednesday, October 22, 2014

RESULTS

<table>
<thead>
<tr>
<th>STUDY GROUP</th>
<th>CONTROL GROUP</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blastocyst rate</td>
<td>52.3 ± 3.31</td>
<td>51.1 ± 4.24</td>
</tr>
<tr>
<td>High prediction</td>
<td>28.6</td>
<td>-</td>
</tr>
<tr>
<td>blastocyst (%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Medium prediction</td>
<td>44.3</td>
<td>-</td>
</tr>
<tr>
<td>blastocyst (%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Low prediction</td>
<td>27.1</td>
<td>-</td>
</tr>
<tr>
<td>blastocyst (%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Clinical pregnancy (%)</td>
<td>85/112 (75.9)</td>
<td>72/122 (63.1)</td>
</tr>
<tr>
<td>Ongoing pregnancy rates (%)</td>
<td>76/112 (67.9)</td>
<td>64/122 (52.5)</td>
</tr>
<tr>
<td>Pregnancy (Only high blastocysts transferred)(%)</td>
<td>42/48 (87.5)</td>
<td>72/122 (63.1)</td>
</tr>
<tr>
<td>Clinical pregnancy (Only medium blastocysts transferred)(%)</td>
<td>13/18 (72.2)</td>
<td>72/122 (63.1)</td>
</tr>
<tr>
<td>Clinical pregnancy (Only low blastocysts transferred)(%)</td>
<td>2/6 (33.3)</td>
<td>72/122 (63.1)</td>
</tr>
</tbody>
</table>

CONCLUSION: The Eeva™ test contributes, as a second criterion, enhancing selection of the most suitable embryos for transfer on Day 5, being preferable the high prediction if possible. The selection of the embryo with the highest developmental potential may improve the pregnancy rate with single embryo transfer (eSET), a preferred practice to reduce obstetrical risks and adverse outcomes.

LEIOMYOMA

P-442 Wednesday, October 22, 2014

A LONGITUDINAL RETROSPECTIVE CLAIMS ANALYSIS OF HEALTHCARE COSTS INCURRED BY UTERINE FIBROIDS PATIENTS. A. M. Soliman,a M. Fuldeore,b H. Yang,b E. X. Du,b E. Q. Wu,b C. Winkel,c E. X. Du,b E. Q. Wu,b C. Winkel,c aGlobal Health Economics and Outcomes Research, Abbvie, Inc., North Chicago, IL; bAnalysis Group, Inc., Boston, MA; bDepartment of Obstetrics and Gynecology, Georgetown University School of Medicine, Washington, DC.

OBJECTIVE: Since uterine fibroids (UF) impose a high economic and societal burden, but UF diagnosis is often delayed and economic information on this pre-diagnosis period is scarce, here we compared healthcare costs for 5 years before and 5 after UF diagnosis with those in women without UF. DESIGN: Case-control study, using 2000-2010 Truven Health MarketScan data.

MATERIALS AND METHODS: Patients aged 18-45 years with an UF diagnosis (International Classification of Diseases codes 218.xx) were matched 1:1 to women without UF (controls) by age, region, and insurance type. For each matched pair, the UF case’s first recorded UF diagnosis date was assigned as the index date. At least 1 year of continuous eligibility in a health plan was required for all subjects. Individuals’ ages and comorbidities during the year before the index date were summarized. Annual health-care costs (in 2010 US dollars) during the 5 years pre- and post-index were compared between UF patients and controls using Wilcoxon signed-rank test.

RESULTS: Among the 84,954 matched pairs identified, the mean age at index date was 39.3 years. Inflammatory disease of gynecological organs (12.5%), urinary tract infection (11.0%), depression (6.4%), infertility (3.2%) and endometriosis (3.1%) were the most common comorbidities among UF patients. For three years prior to the index date, average annual total healthcare costs (medical plus pharmacy costs) were significantly higher for UF patients than for controls: ranging, across the years, from $3,381-$4,217 for UF patients versus $3,237-$3,546 for controls (all p<0.05), producing annual cost differences of $144-$671. In the first year after the index date, UF patients incurred an average of $9,933 in total
healthcare costs, compared to only $3,802 for control patients (p<0.05). In the subsequent four years, UF patients’ total costs exceeded those of the controls by $1,443, $5,560, $1,136, and $920, respectively (all p<0.05). Both the medical and pharmaceutical costs components exhibited the same trends as the total cost estimates for UF patients.

CONCLUSION: Patients with UF incurred significantly higher healthcare costs than those without UF, both pre- and post-diagnosis; the costs associated with UF were highest in the years immediately following diagnosis. UF disease imposes a significant economic burden on women aged 18-45 years living in the US.

Supported by: Financial support for the study was provided by AbbVie.

P-443 Wednesday, October 22, 2014
PRICKLE-1 (PK-1) LINKS ENVIRONMENTAL ESTROGEN EXPOSURE TO THE LOSS OF REST IN UTERINE LEIOMYOMA. M. M. McWilliams, a P. Koohestani, a C. Williams, a S. Ganewardena, a T. R. Kumar, a V. Chennathukuzhi. a Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS; b National Institute of Environmental Health Sciences, Research Triangle Park, NC.

OBJECTIVE: To determine the mechanism ofPk-1 in mediating the down-regulation of REST by environmental estrogens in uterine leiomyoma (UL).

DESIGN: The expression status and the role of PRICKLE-1 in the regulation of REST and its downstream markers were analyzed in normal human myometrium and UL. Additionally, the effect of modulating PRICKLE-1 expression on REST was analyzed in vitro using primary SMCs derived from patient samples and in vivo using animal models treated with estrogentic compounds.

MATERIALS AND METHODS: UL and myometrial tissue samples were obtained from women undergoing hysterectomy. Samples were analyzed for PK-1 and REST expression and used for primary cell cultures treated with PK-1 siRNA and a PK-1 expression vector, followed by gene expression analysis. Uteri from animal models, including Lhb null, Esr null, genistein-treated and DES-treated mice were used to determine PK-1 sensitivity to estrogens in vivo.

RESULTS: UL are hormone-responsive, benign tumors of the myometrium. Prepubertal environmental estrogen exposure is a major risk factor for UL. Extensive evidence has indicated the central role of overactive PI3K/AKT-mTOR signaling in UL. Importantly, the tumor suppressor REST (repressor element silencing transcription factor) is lost in UL, leading to aberrant expression of the PI3K/AKT-mTOR pathway. We report a critical link between environmental estrogens and REST dependent epigenetic modifications, via PK-1, an interacting partner of REST required for its nuclear localization. We found silencing of PK-1 by siRNA results in the loss of REST in normal uterine SMCs and the overexpression of PK-1 leads to increased REST in UL SMCs. Further, we report a novel E2-ERα mediated regulation of PK-1. Lhb null mice lacking endogenous E2 showed increased PK-1 expression, and subsequent E2 treatment rescued PK-1 to wild-type levels. Further, Esr1 null mice expressed high levels of PK-1 in the uterus and mice exposed neonatally to environmental estrogens expressed strikingly low levels of PK-1.

CONCLUSION: Our results identify PK-1 as a novel link between estrogen stimulation and downstream REST-dependent tumorigenic signaling pathways in UL pathogenesis.

Supported by: P20 RR016475, P20 GM103418 and 1RO1HD076450-01A1.

P-444 Wednesday, October 22, 2014
DIAGNOSIS AND MANAGEMENT OF HEREDITARY LEIOMYOMA AND RENAL CELL CANCER (HLRCC)-ASSOCIATED UTERINE LEIOMYOMAS. P. S. Runyan, a A. M. Thomas, a L. Middleton, b M. J. Merino, b J. Segars, a A. M. Venkatesan, a W. M. Linehan, b PRAE, Bethesda, MD; UOB, Bethesda, MD; RIS, Bethesda, MD.

OBJECTIVE: To describe the diagnosis and management of uterine leiomyomatia (fibroids) and their effect on reproductive health in women with Hereditary Leiomyoma and Renal Cell Cancer (HLRCC), a fumarate hydratase (FH) gene defect.

DESIGN: Prospective cohort study of women with HLRCC.

MATERIALS AND METHODS: From 2003 to 2014, women in a clinical study were assessed for HLRCC by clinical phenotype (skin leiomyomas, uterine fibroids, renal tumor) or germline FH mutation status. HLRCC—affected reproductive-aged women with a uterus were assessed for uterine fibroids by imaging, symptoms and reproductive history. Surgery was recommended for those with fibroids > 3 cm in diameter with atypical features on T2-weighted MR (T2 hyperintense (Funaki 2/3) appearance) or fibroids > 5 cm regardless of imaging appearance. Myometectomy was performed for those desiring fertility and hysterectomy if childbearing was completed. Histology was reviewed.

RESULTS: Of 182 HLRCC-affected women, 77 were evaluated by gynecology (median age 34yr; range 16-71yr) and 105 others had prior hysterectomy (median age at hyst 34; 22-56). Of 77 women with a uterus, 24(31%) reported at least 1 prior myomectomy; 7 women had 2 or more. 44(57%) women had at least 1 child with several born after myomectomy. Rapid doubling in uterine size was noted within a year in 4 women taking exogenous hormones and in 2 women subsequent to uterine artery embolization; all underwent surgery. Nearly half of the 77 women assessed were recommended for surgery with most having multiple, 5-7 cm, atypical leiomyoma on MRI. Of those undergoing surgery, 17 had hysterectomy (median age 37; 26-53) and 17 myomectomy (median age 31; 24-41). Prior to surgery, 10 FDG PET scans demonstrated multiple foci of pelvic FDG avidity with standardized uptake values ranging from 10-30 (benign fibroids usually <10). Histology showed novel atypical leiomyomas with increased cellularity, nuclear pleomorphism, and <3 mitoses/HFP; no cases of leiomyosarcoma were observed.

CONCLUSION: Women with HLRCC develop uterine fibroids at a younger age than the general population. Fibroids in these patients demonstrate relative T2 hyperintensity on MRI and a novel atypical, cellular histologic appearance of uncertain malignant potential in the absence of leiomyosarcoma. Over their reproductive life, 3 in 4 develop fibroids and will undergo surgery for symptomatic fibroids. Myometectomy in affected women may be considered in those seeking to preserve fertility.

Supported by: NIH Intramural Program, Clinical Center, UOB/NCI and PRAE/NICHD, NCT00050752.

P-445 Wednesday, October 22, 2014
ABSTRACT MOVED TO OR-416

P-446 Wednesday, October 22, 2014
DISSECTING THE MOLECULAR MECHANISM OF MIFEPRISTONE FUNCTION IN LEIOMYOMAS USING 2- AND 3-DIMENSIONAL MODELS. A. Patel, a M. Malik, b J. Britten Webb, a J. Cox, a,b W. Catherino. a,b bDepartment of Obstetrics and Gynecology, Uniformed Services University of the Health Sciences, Bethesda, MD; aProgram in Reproductive and Adult Endocrinology, Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, MD.

OBJECTIVE: The selective progesterone receptor modulator mifepristone is an effective treatment for reducing leiomyoma size and symptoms, but the mechanism of action remains unknown. Our objective is to characterize the mechanism of mifepristone efficacy using both 2-dimensional (2D) and 3-dimensional (3D) leiomyoma models.

DESIGN: Laboratory setting.

MATERIALS AND METHODS: Immortalized leiomyoma cells were treated with mifepristone and the progesterin R5020 at clinically-relevant concentrations and various time-points. 3D cultures were also treated with mifepristone at various concentrations for assessment of extracellular matrix (ECM) development and dissolution. Gene expression was analyzed using Western blot and immunohistochemistry (IHC) was used to analyze the ECM proteins fibronectin, versican and collagen (COL1A1).

RESULTS: Fibronectin protein concentration increased (2 ± 0.02 fold) at 24 hours with progestin-treated cells at clinically-relevant pharmacologic concentrations, while mifepristone-treated 2D cultures resulted in a reduction in protein concentration (3.1 ± .14), when compared to untreated controls. Progestin-treated cells also demonstrated an increase in versican protein concentration (2 ± .34 fold), while versican protein showed a reduction in concentration in mifepristone-treated 2D cultures (24 ± 5 fold) compared to untreated controls. Leiomyoma cells demonstrated a decrease in COL1A1 protein (76 ± 11 fold) when treated with progestin, and there was an increase in protein concentration (1.6 ± .5 fold) in 2D with mifepristone treatment. In order to evaluate the role of mifepristone on ECM formation and dissolution, 3D cultures were treated. Results were similar to 2D work, showing a reduction in fibronectin (0.38 fold)
and versican (0.53 fold), while increasing COL1A1 protein concentration (1.65 fold). IHC results confirmed reduction of fibronectin protein. COL1A1 and versican IHC analysis is ongoing.

CONCLUSION: Our results demonstrate that mifepristone alters ECM formation, reversing the effect of progesterin on fibronectin, versican and collagen expression. 3D cultures demonstrate a direct effect on ECM fibrosis. Our findings provide insight into mifepristone mechanism of action which would result in improved therapeutic options for women suffering with symptomatic uterine leiomyomata.

Supported by: This research was Supported by Intramural grant from Uniformed Services University of the Health Sciences, QPS5GF13 and R21, R08519713. The research was also supported, in part, by the intramural research Program in Reproductive and Adult Endocrinology, NIH.

P-447 Wednesday, October 22, 2014

RADIOGRAPHIC AND HISTOPATHOLOGIC ENDOMETRIAL CHARACTERISTICS OF PATIENTS WITH LEIOMYOMATA COMPLEX UNDERGOING TREATMENT WITH ULIPRISTAL ACETATE (UPA). T. R. Segal, a S. M. Zareh, b S. L. Mumford, e T. C. Plowden, b L. K. Nieman, b J. H. Segars, a A. Y. Armstrong b OB/Gyn, North Shore-LIJ Health System, Manhasset, NY; b Program in Reproductive and Adult Endocrinology, NICHD, NIH, Bethesda, MD; a Division of Intramural Population Health Research, NICHD, NIH, Bethesda, MD.

OBJECTIVE: UPA is a selective progesterone receptor modulator that has efficacy as a medical treatment for women with symptomatic leiomyoma, and is now being studied as a potential new class of contraceptives. Prior findings of endometrial hyperplasia with a related drug, Asoprisnil, raised concerns for potential endometrial effects for this class of drugs. The objective of this study was to examine sonographic and histologic characteristics of the endometrium in women undergoing UPA treatment compared to placebo.

DESIGN: Secondary analysis of two prospective, randomized, double blind, placebo-controlled trials.

MATERIALS AND METHODS: 54 patients with symptomatic fibroids were enrolled from 2003 to 2008 and randomized to 3 treatment arms for 12 weeks: 10 mg (T1, n = 18), or 20 mg (T2, n = 18) of UPA daily, or placebo (PLC, n = 18). T1 and T2 were combined for analysis since there were no differences between the two groups at baseline and post treatment. The effect of UPA on the endometrium was surveyed using transvaginal sonogram to measure endometrial thickness. Endometrial biopsies were obtained after treatment completion on a subset of subjects p = 0.0187. The biopsies were analyzed using paired t tests to compare endometrial thickness before and after treatment, and ANOVA to compare treatment vs placebo after treatment.

RESULTS: There was no difference between the treatment groups in terms of age (mean 43.1 years), body mass index (BMI) (mean 27.7 kg/m2), or race (78% African American). Treatment with UPA resulted in a significantly thicker endometrium than placebo (8.1 vs 5.5 mm; p = 0.0012). There was no significant change in endometrial thickness before vs after UPA treatment (6.7 vs 8.1 mm; p = 0.3196). In the treatment arms, 2/30 developed hyperplasia without atypia and 2/30 developed cystic dilation. The other diagnoses included proliferative (2/30), secretory endometrium (14/36), and no cases of malignancy.

CONCLUSION: There was no difference between the treatment groups in terms of age, BMI, race or race within treatment groups. Treatment with UPA resulted in a significantly thicker endometrium than placebo. Larger studies and longer administration are needed to further elucidate the mechanisms of ulipristal activity, but highlight the value of in vitro 3-dimensional models for rapid assessment of clinically-promising treatments.

Supported by: This research was Supported by Intramural grant from Uniformed Services University of the Health Sciences, QPS5GF13 and NICHD, NIH R21, HD070152-01A1. The research was also supported, in part, by the intramural research Program in Reproductive and Adult Endocrinology, NIH.

P-448 Wednesday, October 22, 2014

ULIPRISTAL ACETATE DECREASES EXTRACELLULAR MATRIX PROTEINS IN PATIENT LEIOMYOMA SPECIMENS AND IN 3-DIMENSIONAL CULTURES. M. Malik, a J. L. Britten, a W. H. Catherino, a,b Obstetrics and Gynecology, Uniformed Services University of the Health Sciences, Bethesda, MD; a Program in Reproductive and Adult Endocrinology, Eunice Kennedy Shriver NICHD, Bethesda, MD.

OBJECTIVE: To optimize the leiomyoma 3-dimensional (3-D) culture model by increasing the stiffness of collagen scaffolds to resemble the in-vivo tissue.

DESIGN: Uterine leiomyomas are characterized by increased stiffness as a result of excessive and disordered extracellular matrix (ECM) which contributes to the total bulk of the tumor. We have developed a 3-D model system to better simulate in-vivo conditions in the laboratory. We altered the matrix stiffness by varying the concentration of ECM proteins on treatment with ulipristal acetate at all concentrations of the compound. These findings not only elucidate the mechanism of ulipristal activity, but highlight the value of in vivo 3-dimensional models for rapid assessment of clinically-promising treatments.

Supported by: This research was Supported by Intramural grant from Uniformed Services University of the Health Sciences, QPS5GF13 and NICHD, NIH R21, HD070152-01A1. The research was also supported, in part, by the intramural research Program in Reproductive and Adult Endocrinology, NIH.

P-449 Wednesday, October 22, 2014

VALIDATION OF EXTRACELLULAR MATRIX COMPOSITION THAT ALTERS THE MORPHOLOGY OF LEIOMYOMA CELLS IN 3-DIMENSIONAL CULTURE TO CLOSELY RESEMBLE SURGICAL TISSUE. M. Malik, a J. L. Britten, a W. H. Catherino, a,b Obstetrics and Gynecology, Uniformed Services University of the Health Sciences, Bethesda, MD; a Program in Reproductive and Adult Endocrinology, Eunice Kennedy Shriver NICHD, Bethesda, MD.

OBJECTIVE: Leiomyoma cells in 3D culture demonstrated a decreased amount of ECM proteins on treatment with ulipristal acetate at all concentrations studied. Fibronectin (FN1) expression decreased, with a maximum reduction of 2.58±0.22-fold observed at 10^{-8} M. Reduction in FN1 protein was Supported by IHC results in 3D cultures. In addition, there was a reduction in ECM proteins, collagen-1A1 (2.75±0.51-fold) and versican (V0/V1; 1.62±0.13-fold) with ulipristal treatment. The cytokine TGFβ3 also demonstrated a 1.77±0.63-fold reduction at 10^{-8} M ulipristal concentration. These findings were confirmed in patient tissue samples.

CONCLUSION: Ulipristal acetate clinically reduced the leiomyomata size, potentially by reducing the total amount of ECM proteins produced, as demonstrated in 3D leiomyoma cultures using clinically-relevant concentrations of the compound. These findings not only elucidate the mechanism of ulipristal activity, but highlight the value of in vitro 3-dimensional models for rapid assessment of clinically-promising treatments.

Supported by: This research was Supported by Intramural grant from Uniformed Services University of the Health Sciences, QPS5GF13 and NICHD, NIH R21, HD070152-01A1. The research was also supported, in part, by the intramural research Program in Reproductive and Adult Endocrinology, NIH.
such as fibronectin (2.51+/−0.64-fold) and collagen (4.03+/−1.02-fold) as compared to myometrial cultures.

CONCLUSION: The results demonstrated that increased stiffness of the 3-D collagen scaffolds provide superior environments for the leiomyoma cells to maintain their morphology as well as exhibit cell-cell contact that is more representative of the surgical tissue.

Supported by: This research was supported by Intramural grant from Uniformed Services University of the Health Sciences, QP85GF13 and the NICHD. NIH R21, HD070152-01A1.

P-450 Wednesday, October 22, 2014

GREAT EXPECTATIONS: A QUALITATIVE ASSESSMENT OF RACIAL/ETHNIC DIFFERENCES IN WOMEN’S TREATMENT EXPERIENCES WITH SYMPTOMATIC UTERINE FIBROIDS. M. S. Ghant, a K. S. Sengoba, a G. Mendoza, a A. Chaudhari, a M. Simon, a E. E. Marsh. a Obstetrics and Gynecology - REI Division, Northwestern University Feinberg School of Medicine, Chicago, IL; a Obstetrics and Gynecology - General, Northwestern University Feinberg School of Medicine, Chicago, IL; a Obstetrics and Gynecology - General/Preventive Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL.

OBJECTIVE: To qualitatively determine the role of race/ethnicity in women’s treatment experiences for uterine fibroids.

DESIGN: Qualitative semi-structured interviews and demographic surveys.

MATERIALS AND METHODS: Forty-eight women with symptomatic uterine fibroids completed interviews, surveys and a health literacy assessment. Participants were recruited from community-based organizations and an urban academic medical center. Interviews were recorded and transcribed verbatim. Data was analyzed using a grounded theory approach and through consensus, three coders identified major themes and subthemes.

RESULTS: The x amongst coders was 0.94. The mean age of participants was 42.8 ± 7.4 (mean ± SD). 62.5% of subjects were African-American (AAW). 20.8% were Non-Hispanic White (WW), 10.4% were Hispanic (HW) and 6.3% were Asian (ASW). There were no significant differences in annual household income or education level across races. 90% of women expressed that they had concerns about the fibroid treatment options with which they were presented. They also, however, were concerned that they were not given all the options. When considering treatment options, AAW were more likely than WW, HW and ASW to want intervention that was permanent and guaranteed to be successful. AAW were much more likely to demonstrate aversions toward surgery in general, toward hysterectomy specifically and toward medication than women of other racial/ethnic groups. Of the women who received a surgical intervention, AAW were also more likely to have had a difficult recovery and to be dissatisfied with their treatment.

CONCLUSION: Although most women had concerns about fibroid treatment options and felt that they weren’t given all of the options, AAW were more likely to report high treatment expectations, multiple knowledge barriers that impede obtaining treatment and unsatisfactory treatment outcomes than women from other racial/ethnic groups. These data suggest that targeted patient counseling and support are critical in a diverse population. It is vital that clinicians provide accurate and comprehensive education regarding treatment options and potential outcomes for women considering interventions for their symptomatic uterine fibroids.

Supported by: NIH WRHR Program K12HD050121; RWJ Foundation; NMH; Evergreen Foundation (EEM).

P-451 Wednesday, October 22, 2014

CLINICAL OUTCOMES OF ROBOTIC VS OPEN MYO- TOMY PERFORMED BY ONE SURGEON. K. Van Heertum, a E. Murphy, b L. Dean, a E. Parent, a B. Marks, a S. Somkuti, a J. Nichols, a J. Schinfeld, a M. Sobel, a L. Barmat. a Obstetrics and Gynecology, Abington Memorial Hospital, Abington, PA; a Reproductive Endocrinology and Infertility, Weill Cornell Medical College, New York, NY; a Trinity School of Medicine, Ratho Mill, Kingstown, Saint Vincent and the Grenadines; a Abington Reproductive Medicine, Abington, PA.

OBJECTIVE: To compare clinical outcomes of robotic versus open myomectomy.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: Data was gathered from hospital records. A total of 235 patients who underwent myomectomy were identified: 134 robotic and 101 open. Operating room (OR) time, estimated blood loss (EBL), body mass index (BMI), fibroid number/weight, surgical complications, and length of stay (LOS) were compared. SPSS was used for statistical analysis.

RESULTS: BMI was similar between the two groups. OR time was significantly longer for robotic myomectomy despite fibroid number and weight being significantly lower in the robotic group. EBL was significantly lower and LOS was significantly shorter in the robotic group. In addition, there were fewer complications in the robotic group. Greater fibroid weight was associated with longer OR time in both groups (p=0.009 for open; p=0.000 for robotic). Higher BMI was associated with a longer OR time in both groups (p=0.024 for open; p=0.024 for robotic). Greater fibroid weight was associated with a longer LOS in the robotic group only (p=0.005). Data are reported as mean ± standard deviation where appropriate and summarized in Table 1.

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
</tr>
<tr>
<td>OR time (hours)</td>
</tr>
<tr>
<td>Fibroid number</td>
</tr>
<tr>
<td>Fibroid weight (grams)</td>
</tr>
<tr>
<td>EBL (mL)</td>
</tr>
<tr>
<td>Complications</td>
</tr>
<tr>
<td>LOS (days)</td>
</tr>
</tbody>
</table>

Statistical significance defined as p<0.05

CONCLUSION: The use of robotic-assisted laparoscopy for myomectomy enables patients to undergo an outpatient procedure in place of a major abdominal surgery. While there was an increase in OR time when compared to open myomectomy, there was also significantly less blood loss and a shorter LOS in the robotic group. Additionally, there were fewer complications in the robotic group. This method appears to be an excellent alternative to traditional open myomectomy; further study is needed to assess the cost to benefit ratio of longer OR times and shorter recovery.

P-452 Wednesday, October 22, 2014

PREGNANCY AFTER ABDOMINAL versus ROBOTICALLY AS- SISTED LAPAROSCOPIC MYOMECTOMY. C. Celestine, a W. Ziegler, a V. Johnson, b Y.-H. Kao, b J. Mann. b Jersey Shore University Medical Center, Neptune, NJ; b Reproductive Science Center of NJ, Eatontown, NJ.

OBJECTIVE: Leiomyomata are the most common benign tumor in females of reproductive age. Though most of these are asymptomatic, some myomas may impair fertility. Robotic myomectomy is a viable alternative to laparotomy. The aim of this study is to compare time to clinical pregnancy after abdominal myomectomy (Group 1) versus robotically assisted laparoscopic myomectomy (Group 2).

DESIGN: Retrospective chart review with IRB approval.

MATERIALS AND METHODS: Women who underwent a myomectomy for infertility from 2004 to 2013 were included. Baseline characteristics analyzed were age, gravidity, parity, maternal BMI, and history of cesarean delivery. Primary outcome was time to clinical pregnancy from time of surgical intervention. Clinical pregnancy was defined by the presence of fetal heart on ultrasound. Secondary outcomes were estimated blood loss (EBL), number of leiomyomata resected, uterine size, mode of conception, first trimester bleeding and subchorionic hematoma. Two-sample t-test or Wilcoxon rank sum test was used to compare continuous variables. Chi-squared test was used to compare discrete variables. Statistical significance was set as p < 0.05.

RESULTS: There were 154 myomectomies included in this study. Group 1 had 90 (58.4%) and group 2 had 64 (41.6%) cases. There was no difference in baseline characteristics. Outcomes are listed in Table 1. No malignancies were identified on pathology.
CONCLUSION: No difference exists in time to conception following abdominal versus robotic myomectomy. Median myoma size of 9 cm and weight of 58 g were safely resected robotically with outcomes comparable to abdominal myomectomy. No statistical difference was found in mode of conception between the two surgical modalities. However, the rate of spontaneous conception was higher following robotic myomectomy with a trend toward significance. This finding may be explained by a lower chance of adhesion formation. Future directions include evaluation of delivery outcomes.

ENDOMETRIOSIS

P-453 Wednesday, October 22, 2014

HIGH MOBILITY GROUP BOX 1 PROMOTES CELL PROLIFERATION THROUGH TOLL-LIKE RECEPTOR 4 AND NF-κB PATHWAY IN ENDOMETRIAL STROMAL CELLS. Y. J. Lee, a,b,c, E.-J. Han a, b, H. Yun a, b, S. I. Chon, a,b, S. Cho a, c, Y. S. Choi a,b, c, B. S. Lee a,b,c Department of Obstetrics and Gynecology, Severance Hospital, Seoul, Republic of Korea; a Department of Obstetrics and Gynecology, Gangnam Severance Hospital, Seoul, Republic of Korea; b Institute of Women’s Life Medical Science, Yonsei University College of Medicine, Seoul, Republic of Korea.

OBJECTIVE: Cell death through necrosis activates the innate immune system and induces sterile inflammation. High mobility group box 1 (HMGB-1) is a DNA-binding nuclear protein, however, mediates inflammatory reaction in extracellular condition whether actively released by endotoxin or passively by cell injury. The aim of the study was to examine the HMGB-1 existence, and HMGB-1 signaling through toll like receptor (TLR) 4 in endometrium, which activates NF-κB pathway that might play a pathogenic role in endometriosis.

DESIGN: Laboratory study using endometrial cell culture.

MATERIALS AND METHODS: From March 2012 to March 2014, 69 patients who had undergone hysterectomy were included as the case group, 50 patients without endometriosis were enrolled as the control group. Endometrial tissue was obtained from premenopausal women undergoing laparoscopy because dometriotic lesions (endometriotic cysts and rectovaginal nodules) were obtained from premenopausal women undergoing laparoscopy because of endometriosis. Criteria for exclusion from the study were: menstrual bleeding on the day of surgery, signs of pelvic inflammatory disease, use of hormonal therapies in the 3 months prior to surgery, use of intrauterine device in the 3 months prior to surgery, pregnancy or breastfeeding in the 6 months prior to surgery. All patients included in the study had histological diagnosis of endometriosis. The expression of peristin and syndecan-1 were evaluated by immunofluorescence techniques. The expression of peristin was assessed by using the murine monoclonal antibody OC-20, a function-blocking anti-peristin antibody (1). The expression of syndecan-1 was assessed by using the human recombinant OC-46F2 antibody that is specific for the extracellular domain of syndecan-1 (2).

RESULTS: Ten patients with rASRM stage III-IV disease were included in the study. Peristin and syndecan-1 were highly expressed in the stroma of eutopic and ectopic endometrium of patients with endometriosis. Both OC-20 and OC-46F2 antibodies were able to recognize very well vascular structures, as shown by the colocalization with antibodies specific to several endothelial (CD31, CD34, VE-Cadherin) and pericytic (smooth muscle actin) markers.

CONCLUSION: Eutopic and ectopic endometrium of patients with endometriosis highly express peristin and syndecan-1. Previous studies showed that OC-20 and OC-46F2 antibodies are able to inhibit angiogenesis during tumor growth in pre-clinical in vivo models (1,2). These observations may contribute to the employment of novel anti-angiogenesis biological drugs in endometriosis.

Supported by: PRA 2012, University of Genova, Italy.
Animal model: male nude mice  
Clinical sample: normal and ectopic endometrium  
Methods:  
- Western-blot  
- Rotary confocal microscopy  
- Transwell assay  
- Wound-healing assay  
- Immunofluorescence  
- Tube formation  
- Electron microscopy  
- Immunohistochemical staining.

RESULTS: (1) Clinical sample: AQP1 was expressed only in the endometrium of small vessels, whereas other cells, CD31 as a endothelial cell specific marker was co-localized. The expression level of AQP1 in the endothelial cells of ectopic endometrium was much higher than the endometrium of normal endometrium. (2) cell experiments: Knocking down AQP1 in HUVECs using specific siRNA-AQP1 lentivirus decreased the proliferation, migration, invasion and tubule formation of HUVECs, and broken the organization of cell skeleton into looping around the nuclei of the cell. Over expression of AQP1 in HUVECs using the flag-AQP1 lentivirus increased the proliferation, migration, invasion and tubule formation of HUVECs, and the formation of transcytoplasmatic F-actin stress fibers. (3) animal model: Down-regulation of AQP1 in the endothelial cells can slow down the development of graft implanted in the nude mice through decreasing the angiogenesis.  
CONCLUSION: Increased angiogenesis induced by Up-regulation of AQP1 in the endothelial cells of ectopic endometrium plays a crucial role in the development of endometriosis.

Supported by: In this part, we want to show the animal model results in detail: Immortal HUVECs were made by transfected with SV40LT lentivirus into normal HUVECs. Immortal HUVECs were tripped, counted, and re-suspended in Matrigel, and then the cell/martigel mixtures were injected subcutaneously into the 5-week-old nude mice; 5 days later, the grafts (tumor) were grown and these nude mice were divided into two groups: one group were injected with the siRNA-AQP1 lentivirus into the grafts every 5 days, and the other group were injected with the control lentivirus; Four weeks later, these grafts in the siRNA-AQP1 lentivirus group showed significant smaller size than the control group; Immunohistological Histology (IHC) with the CD31 antibody indicated that the microvessel density (MVD) was much higher in control lenti-virus group than the siRNA AQP1 lenti-virus group; IHC with the AQP1 antibody showed that the expression of AQP1 in the endometrium was much higher in control lenti-virus group than the SiRNA AQP1 lenti-virus group.

P-456 Wednesday, October 22, 2014  
ANTI-MULLERIAN HORMONE AS A PREOPERATIVE PREDICTOR OF ENDOMETRIOSIS. C. Lipari, a M. Fox, a R. Y. Knowlton, b  
aJacksonville Center for Reproductive Medicine, Jacksonville, FL; b‘Oh Gyn, University of Florida COM-Jacksonville, Jacksonville, FL.

OBJECTIVE: The purpose of this study is to evaluate the correlation of preoperative Anti-Mullerian hormone (AMH) level with the diagnosis and stage of endometriosis in women undergoing laparoscopy for pelvic pain or infertility.

DESIGN: This was a retrospective cross sectional study using analysis of covariance (ANCOVA) to compare AMH values with stages of endometriosis and to identify variables affecting age corrected AMH levels.

MATERIALS AND METHODS: A retrospective chart review was performed. Patients who received a diagnostic laparoscopy for pelvic pain or infertility and had an AMH level obtained within one year prior to surgery at Jacksonville Center for Reproductive Medicine were evaluated. 135 total infertility and had an AMH level obtained within one year prior to surgery.

RESULTS: Patients with endometriosis had significantly lower AMH levels than aged matched patients with no endometriosis (p < 0.001). No significant change in AMH level was seen with advancing stage of endometriosis (p = 0.06). Of the other variables added to the model, only PCOS had a statistically significant effect on AMH values. Mean AMH values with 95% confidence intervals were calculated based upon age, polycystic ovarian disease status, and endometriosis status, with select values shown in Table 1.

Table 1: Predicted AMH values for selected ages based upon presence or absence of endometriosis and PCOS

<table>
<thead>
<tr>
<th>Age</th>
<th>Endo Stage &gt;0</th>
<th>Endo Stage =0</th>
<th>PCOS Present</th>
<th>PCOS Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>4.91 (3.39, 7.12)</td>
<td>8.56 (4.79, 15.31)</td>
<td>2.95 (2.14, 4.07)</td>
<td>5.14 (2.90, 9.12)</td>
</tr>
<tr>
<td>30</td>
<td>3.14 (2.33, 4.21)</td>
<td>5.47 (3.27, 9.13)</td>
<td>1.88 (1.52, 2.33)</td>
<td>3.28 (2.00, 5.39)</td>
</tr>
<tr>
<td>35</td>
<td>2.00 (1.51, 2.66)</td>
<td>3.49 (2.16, 5.65)</td>
<td>1.20 (1.01, 1.43)</td>
<td>2.10 (1.33, 3.30)</td>
</tr>
<tr>
<td>40</td>
<td>1.28 (0.91, 1.80)</td>
<td>2.23 (1.36, 3.66)</td>
<td>0.77 (0.60, 0.98)</td>
<td>1.34 (0.85, 2.12)</td>
</tr>
<tr>
<td>45</td>
<td>0.82 (0.52, 1.28)</td>
<td>1.42 (0.82, 2.47)</td>
<td>0.49 (0.34, 0.70)</td>
<td>0.86 (0.51, 1.42)</td>
</tr>
</tbody>
</table>

(95% Confidence Interval)

CONCLUSION: Endometriosis is associated with decreased AMH levels suggesting a decline in ovarian reserve associated with the disease process.

P-457 Wednesday, October 22, 2014  

OBJECTIVE: Endometriosis is a chronic disease primarily affecting women of childbearing age, typically leading to painful symptoms, fatigue, and infertility. Quality of life studies show that symptoms of endometriosis impact on many aspects of a woman’s life, including work and education, relationships, and social functioning. (1) Dienogest is a progestin investigated for the treatment of endometriosis in several clinical studies in Europe and Japan. (2) The aim of this study is to assess the safety and efficacy of dienogest 2mg/day for long-term treatment of endometriosis associated pain, and to evaluate adverse effects and improvement in quality life for long-term endometriosis treatment.  
DESIGN: prospective, open-label extension study.

MATERIALS AND METHODS: Ninety three patients with chronic pelvic pain entered the study and were divided into three groups: 1) Suspicious of endometriosis without laparoscopic confirmation, 2) Confirmed laparoscopic diagnosis without immediate fertility desire, 3) Confirmed laparoscopic diagnosis without medical treatment after the surgery. Efficacy was assessed by changes in endometriosis-associated pelvic pain.

RESULTS: Fifty three patients completed the trial. Main reasons for discontinuation were adverse effects (5), economic reasons (6), protocol deviations (4), not completing at least six months of treatment (16). Results at 6 months of treatment: Group 1 (n=11): no pain 72%, mild pain 28%. Patients without adverse effects 63%. Normal bleeding 9%, infrequent bleeding 27%, amenorrhea 64%. Group 2 (n=26): no pain 85%, mild pain 14.2%. Patients without adverse effects 69.21%. Normal bleeding 3.84%, infrequent bleeding 19%, amenorrhea 73%. Group 3 (n=21): no pain 80.95%, mild pain 13.80%, severe pain 4.76%. Patients without adverse effects 61.90%. Normal bleeding 6.52%, infrequent bleeding 13.28%, amenorrhea 80.95%.

CONCLUSION: Patients treated with dienogest 2 mg/day for up to 6 months showed sustained decrease in endometriosis-associated pelvic pain and a favorable safety profile. Irregularities in bleeding pattern and other adverse effects were well tolerated by patients in light of accompanying pelvic pain relief. Treatment with dienogest may offer an effective and long-term treatment option for endometriosis associated pain.

P-458 Wednesday, October 22, 2014  
BIOMARKERS FOR OVARIAN ENDOMETRIOSIS IN FOLLICULAR FLUID. E. Shaeffer,a,b,c J. Pedrara, a F. Camargo, a,b E. López-Bayghan, a,b Departamentos de Toxicología y de Genética y Biología Molecular, Cinevatx-IPN, México, DF, Mexico;Laboratorio de Investigación y Diagnóstico Molecular, Instituto de Infertilidad y Genética, Ingenes Mexico, México, DF, Mexico.

OBJECTIVE: To generate and compare the transcriptional expression profile in follicular fluid samples in patients with ovarian endometriosis and healthy women.
MATERIALS AND METHODS: Infertile women with different degrees of endometriosis, diagnosed by ultrasonography and healthy egg donors were submitted to a standard ovarian stimulation protocol for IVF. Follicular fluid samples were collected during the ovarian puncture, then centrifuged and the cell pellet was used for RNA extraction with TRizol® Reagent. Expression profiles were analyzed using one step qRT-PCR for eight different markers: FST (Follistatin), CA-125 (Cancer Antigen 125), HOXA-10 (Homeobox A-10), OCT-3 (Octamer-binding Transcription Factor 4), SOX-2 (Sex Determining Region Y-box 2), PTGS2 (Prostaglandin-endoperoxide Synthase 2), VCAN (Versican) and KCNA10 (Potassium Voltage-gated Channel, Shaker-related Subfamily, Member 10).

RESULTS: Samples of follicular fluid from healthy and infertile women with different degrees of ovarian endometriosis were obtained after classification by ultrasound and submitted to the following characteristics such as percentage of ovarian involvement, presence of endometriomas and adhesions. Other features considered in the study groups were age, BMI and follicular reserve. From the follicular fluid pellet was possible to obtain RNA and generate a transcriptional profile for eight genes. A statistically significant difference in the expression of HOXA-10 and FST markers was found when endometriosis samples were compared with samples from healthy women (egg donors). Analysis is still in progress to confirm these differences and to establish a wider transcriptional profile in follicular fluid pellets associated with different degrees of ovarian endometriosis.

CONCLUSION: It is possible to generate a system of transcriptional analysis in follicular fluid samples that can be correlated with the degree of endometriosis ranked based on the ultrasound findings.

Supported by: Conacyt 194477 and 219073.
inhibited the estrogen increased attachment to the PMCs (ICI: p = 0.006, THC: p = 0.006). The ERα/β ratio was inversely correlated with the α-antagonist inhibition of attachment (R² = 0.91) and linearly correlated with the α-antagonist (R² = 0.95).

CONCLUSION: EECs primarily express ER α and there is no significant difference in their ER α/β expression or ratio when comparing EECs from women with and without endometriosis. Both ICI and THC inhibit estrogen induced attachment of EEC to PMCs and this effect is correlated with the ER α/β ratio. These findings may contribute to future preventative treatment for endometriosis.

Supported by: Departmental support.

P-462 Wednesday, October 22, 2014


OBJECTIVE: Determination of changes in cellular bioregulators in the development of external genital endometriosis (EEG) in patients of reproductive age.

DESIGN: Laparoscopy by means of Storz apparatus was performed in 96 patients (group 1 – 28 patients with the stages I and II of EEG, group 2 – 46 patients with the stages III and IV of EEG, group 3 – 22 patients without endometriosis) and their blood serum and peritoneal fluid (PF) were analysed.

MATERIALS AND METHODS: High-density lipoproteins (HDLp) and low-density lipoproteins (LDLp) were determined by Randox kits (Germany). The study of paraoxonase (PON1) and endothelial NO-synthase (eNOS) gene alleles was performed by PCR method with the help of DNA-Technology (Moscow) and PTC-220 (MI Research, USA) kits. Nitric oxide (NO) was determined by methods of Griss reagent. NOs was measured according to the increase of NO production from L-arginine with NADPH. NF-kb in PF was determined by Ebioscience Company kits (USA).

RESULTS: Endometriosis is associated with the general inflammatory response, in which oxidative stress takes part. In the blood serum of the patients with EEG HDLP appeared to be increased in 1.1 time (p < 0.013). This leads to higher content of PON1 connected with HDLP that prevents oxidative modification of LDLp. At that, we did not receive significant differences in the distribution of Ghn192Arg polymorphism of PON1 gene among women with endometriosis and a control group. DNA-binding activity of the p65 subunit of NF-kB determined in PF of the patients with EEG corresponded to the indices of the control group. Thus, its protective function is still remained and there is no activation of the genes responsible for the progression of the disease. The performed genetic typing of T-786C polymorphism of eNOS gene promoter in the patients with endometriosis and in the control group did not reveal significant differences in the distribution of alleles.

CONCLUSION: 1. We did not receive any reliable data concerning the changes in p65 activity of NF-kB in PF. 2. According to our data PON1 and eNOS genes are not associated with EEG. 3. It was revealed that at the system level in the patients with EEG there is a metabolic correlation of the disturbed metabolism of cholesterol and its products participating in post-translational modification of G-proteins. The complex of the presented metabolic peculiarities stimulates cell proliferation typical for EEG.

P-464 Wednesday, October 22, 2014

BENZYL BUTYL PHthalate DECREASES THE DIFFERENTIATION ABILITY OF ENDOMETRIAL MESENCHYMAL STEM CELLS THROUGH MIR-137 BY REGULATING SRC AND PITX2. E.-M. Tsai<sup>a,b</sup> Y.-C. Chang.<sup>c</sup> Graduate Institute of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan. <sup>b</sup>Department of Obstetrics and Gynecology, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan.

OBJECTIVE: To study the influence of BBP on differentiation ability and find the novel signaling pathway in endometrial mesenchymal stem cells (EN-MSCs).

DESIGN: To observe the change of EN-MSCs’ differentiation ability with BBP treatment by in vitro and in vivo experiment.

MATERIALS AND METHODS: We isolated EN-MSCs from the eutopic endometrium of endometriosis patients and identified the ability to differentiate into osteogenic and myogenic lineages. To understand the global impact of BBP on differentiation in EN-MSCs, we used whole-genome screening with a high-density microarray assay and explored the significant candidate genes. We further used a web-based microRNA target prediction program (TargetScan 6.2) to search for potential targets. We confirmed the significant targets with real-time qPCR by overexpression or knockdown of microRNA. We further used the immunocytochemistry to observe the differentiation ability of EN-MSCs. Then we validated the in vitro results by immunohistochemistry and immunofluorescence stain in the tissue specimen obtained from the animal model.

RESULTS: In this study, we found that BBP decreased the differentiation ability of osteogenesis and myogenesis in EN-MSCs. To further examine the role of BBP in EN-MSCs, we used a CCK-8 microarray to screen BBP regulation of gene expression and analyzed the biological function network with Ingenuity Pathways Analysis (IPA) software in EN-MSCs. We demonstrated that the candidate genes, SRC and PITX2 regulate concomitantly the Skeletal and Muscular Disorders, the Cell Morphology, Tissue Development from the Top Bio Functions group. In addition, we explored the novel signaling pathway that mir-137 can target SRC and PITX2 when BBP treatment in EN-MSCs. Finally, we confirmed that BBP reduced the differentiation ability of myogenesis of EN-MSCs in muscle regeneration animal model.

CONCLUSION: Our study shows that BBP decreases the differentiation ability of EN-MSCs through activation of mir-137 expression. Subsequently, mir-137 targets SRC and PITX2 to affect osteogenesis and myogenesis. These findings contribute to our understanding of the differentiation ability of EN-MSCs in human, and the hazard potential of environmental hormone.

Supported by: This work is supported by National Science Council, Taiwan [grant number 102-2628-B-037-011-MY3 and 102-2628-B-037-001-MY3].
RATIO OF PROGESTERONE TO NUMBER OF FOLLICLES ON THE DAY OF FINAL OOCYTE MATURATION AS A PROGNOSTIC TOOL IN IN VITRO FERTILIZATION CYCLES. M. Roque, S. Geber, M. Sampaio, E. Guimaraes, M. Valle, M. A. Checa. Origien - Center for Reproductive Medicine, RJ, RJ, Brazil; 3Department of Obstetrics and Gynecology, Hospital Del Mar, Universitat Autonoma de Barcelona, Barcelona, Catalonia, Spain.

OBJECTIVE: The main objective of this study was to establish a ratio of progesterone (P) levels to the number of follicles (P/F ratio) on the day of human chorionic gonadotropin (hCG) administration and to evaluate whether this ratio was associated with in vitro fertilization (IVF) outcomes.

DESIGN: Prospective observational cohort study.

MATERIALS AND METHODS: The study was conducted between January 2012 and June 2013. Accepting an alpha risk of 0.05 and a beta risk of 0.2 in a two-sided test, 145 subjects in each group were necessary to recognize a relative risk (RR) ≥ 0.6 as statistically significant. A total of 337 patients submitted to controlled ovarian stimulation with gonadotropin-releasing hormone antagonist protocol and day 3 fresh embryo transfer were included; all had P levels ≤ 1.5 ng/mL on hCG day. The P/F ratio was calculated as [P[ng/ml]/number of follicles], measured on the day of final oocyte maturation. The statistical analysis was performed using Student’s t test, the chi-square test, and linear regression models. Receiver operating characteristics (ROC) analysis was conducted to establish the most efficient cut-off value for the P/F ratio to discriminate between successful and unsuccessful IVF outcomes. This value was determined based on an equivalent sensitivity and specificity level, and the highest value of the area under the curve (AUC) was determined. A p value of <0.05 was considered statistically significant. The main outcome measure was ongoing pregnancy rate.

RESULTS: Using ROC, we established a cut-off level of 0.075 for the P/F ratio. The sensitivity (71%), specificity (71.1%), and AUC (0.756; 95% CI 0.704-0.807) of the test showed that it was a good prognostic test. Overall results are shown in the table below.

<table>
<thead>
<tr>
<th>P/F ≤ 0.075 (n=157)</th>
<th>P/F &gt; 0.075 (n=178)</th>
<th>RR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IR, %</td>
<td>32.8</td>
<td>12</td>
<td>0.37 (0.27-0.49)</td>
</tr>
<tr>
<td>CPR, n (%)</td>
<td>96 (61)</td>
<td>34 (19)</td>
<td>0.31 (0.23-0.43)</td>
</tr>
<tr>
<td>OPR, n (%)</td>
<td>86 (55)</td>
<td>32 (18)</td>
<td>0.33 (0.23-0.46)</td>
</tr>
</tbody>
</table>

CPR: clinical pregnancy rate; IR: implantation rate; OPR: ongoing pregnancy rate; P/F: ratio of progesterone levels to number of follicles

CONCLUSION: Even in a selected group of patients without progesterone elevation (P levels ≤ 1.5 ng/mL), the P/F ratio is a good prognostic test for IVF outcomes that can correlate the P levels with the ovarian response. It would be better to define a ratio between P levels and ovarian response instead of using a single P level as prognostic tool in IVF cycles.

P-466 Wednesday, October 22, 2014

CLINICAL EFFICIENCY OF EMBRYO TRANSFER PERFORMED IN RECEPTIVE VS NON-RECEPTIVE ENDOMETRIUM DIAGNOSED BY THE ENDOMETRIAL RECEPTIVITY ARRAY (ERA) TEST. M. Ruiz Alonso, P. Diaz-Gimeno, E. Gomez, A. Rincón-Bertolín, Y. Vlaminor, N. Garrido, C. Simón, IVIOMICS, Valencia, Spain; Fundación Instituto Valenciano de Infertilidad, Valencia University and Instituto Universitario IVI/INCLIVA, Valencia, Spain; 3Sophia Hospital of Reproductive Medicine, Sofia, Bulgaria; 4Department of Ob/Gyn, Stanford University School of Medicine, Stanford, CA.

OBJECTIVE: To evaluate the clinical efficiency of embryo transfer (ET) performed in receptive (R) vs non-receptive (NR) days by the ERA test [1-3].

DESIGN: Multicenter prospective and retrospective study analyzing the clinical outcome in patients with ET in R vs NR days according to ERA.

MATERIALS AND METHODS: We analyzed 2,445 patients with ERA. Embryos were not chromosomally analyzed in these cycles. Clinical outcome was reviewed retrospectively for ET at NR day in the NR group, and prospectively for ET at R day in the R group. We measured implantation rate (IR), pregnancy rate (PR), and ongoing pregnancy rate (OPR). Sensitivity was calculated as the proportion of non-pregnant with ET at NR day, and specificity as pregnancies obtained after ET in R day. Positive and negative predictive values (PPV and NPV) were the ratio of true positives and true negatives respectively.

RESULTS: From a total of 2,445 patients analyzed, 1,877 were R (77%), and 568 were NR (23%). Clinical documented follow-up was possible only in 257 patients (205 R (80%) and 52 NR (20%)). In the R group, PR, OPR and IR were 45%, 60%, and 74% respectively, whereas in the NR group were 13%, 23%, and 0%. Specificity of ERA was 91%, although sensitivity due to the multifactorial condition of the implantation process was 33%. PPV was 0.77 and NPV was 0.60.

Clinical outcome and efficiency of ET according ERA diagnosis

<table>
<thead>
<tr>
<th>Clinical Outcome</th>
<th>NR (52)</th>
<th>R (205)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IR First attempt</td>
<td>13% (12/90)</td>
<td>45% (161/355)</td>
</tr>
<tr>
<td>Total attempts</td>
<td>10% (17/174)</td>
<td>41% (182/441)</td>
</tr>
<tr>
<td>PR First attempt</td>
<td>23% (12/52)</td>
<td>60% (123/205)</td>
</tr>
<tr>
<td>Total attempts</td>
<td>17% (17/100)</td>
<td>55% (140/253)</td>
</tr>
<tr>
<td>OPR First attempt</td>
<td>0% (0/12)</td>
<td>74% (91/123)</td>
</tr>
<tr>
<td>OPR Total attempts</td>
<td>0% (0/100)</td>
<td>74% (103/140)</td>
</tr>
<tr>
<td>Clinical efficiency</td>
<td>Positive (52)</td>
<td>Negative (205)</td>
</tr>
<tr>
<td>True</td>
<td>40</td>
<td>123</td>
</tr>
<tr>
<td>False</td>
<td>12</td>
<td>82</td>
</tr>
<tr>
<td>Sensitivity (TP/TP+FN)</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>Specificity (TN/TN+FP)</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td>PPV (TP/TP+FP)</td>
<td>0.77</td>
<td></td>
</tr>
<tr>
<td>NPV (TN/TN+FN)</td>
<td>0.60</td>
<td></td>
</tr>
</tbody>
</table>

CONCLUSION: Our data demonstrate that embryos transferred in a NR endometrium diagnosed by ERA have lower IR and PR and in this retrospective series never produced a live birth, whereas when a personalized ET is performed in the R endometrium, clinical results were above the standard (45% IR, 60% PR, and 74% OPR). These results highlight the relevance of the endometrial factor and its personalized diagnosis in ART.

Supported by: FIVI & IVIOMICS

P-467 Wednesday, October 22, 2014

PROGNOSTIC VALUE OF ENDOMETRIAL THICKNESS ON PREGNANCY OUTCOMES OF SUBGROUPS OF INFERTILE WOMEN UNDERGOING LETROZOLE/INTRAUTERINE INSEMINATION (IUI/IVF) THERAPY. A. N. Blevins, H. C. L. Bohler, R. K. Hunter. Obstetrics, Gynecology and Women’s Health, University of Louisville School of Medicine, Louisville, KY.

OBJECTIVE: To determine whether the incidence and prognostic value of thin endometrium differs among women with different causes of infertility who are undergoing letrozole/IUI therapy.

DESIGN: Retrospective cohort.

MATERIALS AND METHODS: Infertile women undergoing letrozole/ IUI therapy at our academic medical center between January 01, 2012 and January 31, 2014 were eligible for analysis. Inclusion criteria required performance of a mid-cycle transvaginal ultrasound to assess follicular and endometrial development prior to administration of human chorionic gonadotropin as an ovulation trigger. Exclusion criteria included active smoking status, body mass index greater than 40 kg/m², use of frozen or donor sperm, and use of in-cycle estrogen supplementation. Charts were retrospectively reviewed, and data regarding patient demographics, medical history, cycle characteristics, and pregnancy outcomes were recorded. Data were analyzed using chi-square, one-sample t-tests, one-way ANOVA, and Mann-Whitney U tests as appropriate, with p values of <0.05 being considered statistically significant.

RESULTS: 215 women met criteria for inclusion and were divided into subgroups based on primary diagnoses of polycystic ovarian syndrome (PCOS, 31.1%), male factor (30.2%), unexplained (19.0%), endometriosis (9.3%), diminished ovarian reserve (DOR, 7.4%), or recurrent pregnancy loss (2.8%). The incidence of thin endometrium, defined as less than 7.0mm, was significantly higher in the unexplained group (39.0%) compared to the PCOS (20.8%) and endometriosis (15.0%) groups. Overall pregnancy rates were significantly higher in the PCOS group (25.4%) and lower in the
IS ENDOMETRIAL THICKNESS ON DAY OF TRANSFER A PREDICTOR OF PREGNANCY RATE IN IVF?1, 2, 3  D. Chavkin,4  for 48 hr. Another cohort
6.0 vs 10.4   /C6  P. J. Buzzi,4  0.05  3.9 (51.8%) 5.8/C6  J. M. Bolnick,3  M. P. Diamond,4  A. D. Bolnick,4  4.1 (56.3%).A similar number of good
J. Makarov,3  J. Dai,2  S. K. Dey,2  retrieved: 11.0 with prior IF. tors have suggested that patients with implantation failure (IF) may benefit
WITH RECURRENT IMPLANTATION FAILURE. IN INTRACYTOPLASMIC SPERM INJECTION (ICSI) PATIENTS
BENEFICIAL EFFECT OF LOCAL INJURY TO THE ENDOMETRIUM
P-469 Wednesday, October 22, 2014
BENEFICIAL EFFECT OF LOCAL INJURY TO THE ENDOMETRIUM IN INTRACYTOPLASMIC SPERM INJECTION (ICSI) PATIENTS
MATERIALS AND METHODS: EMT-hCG and EMT-ET were measured by transvaginal sonogram in 101 women (22-48 years old) undergoing IVF
between September 2013 and February 2014. Chemical pregnancy, clinical pregnancy, and miscarriage rates were evaluated. T-test and Pearson correla-
tions were used as appropriate.
RESULTS: Age was a significant predictor of IVF success rate (p=0.037). After adjusting for age, fresh cycles were associated with significantly higher clinical pregnancy rates compared to frozen cycles (64.2% versus 41.7%; p=0.024). The overall rate of miscarriage was 24.3%. There was a trend to-
ward a negative correlation between the rate of miscarriage and EMT-ET (r=−0.41; p=0.08) but not with EMT-hCG (p=0.17). For women younger than 35 years, there was a trend toward a positive correlation between EMT-ET and clinical pregnancy rate (r=0.71; p=0.055). In the same age
group, EMT-hCG was significantly higher in women who achieved clinical pregnancy (11.1mm+/-0.4 [SEM]) when compared to women who did not
(9.5mm+/-0.4; p=0.02).
CONCLUSION: EMT-hCG is a better predictor of clinical pregnancy rate than EMT-ET; however, EMT-ET may be more informative of potential
miscarriage than EMT-hCG.

BENEFICIAL EFFECT OF LOCAL INJURY TO THE ENDOMETRIUM IN INTRACYTOPLASMIC SPERM INJECTION (ICSI) PATIENTS
WITH RECURRENT IMPLANTATION FAILURE. P. J. Buzzi,4  M. P. Diamond,4  A. D. Bolnick,4  J. M. Bolnick,3  D. Chavkin,3  R. V. Geazi,3  OB/Gyn, Maimonides Medical Center, Brooklyn, NY;
OBJECTIVE: Successful embryo implantation depends on a well-func-
tioning endometrium as well as a normally healthy embryo. Several investiga-
tors have suggested that patients with implantation failure (IF) may benefit from mechanical endometrial stimulation performed in the cycle preceding the actual treatment cycle. The aim of this study is to assess the influence of local injury to the endometrium in a selected group of ICSI patients with prior IF.

MATERIALS AND METHODS: Patients ≤ 36 years old undergoing IVF between October 2012 –December 2013 with ≥ 2 IVF failures and with at
least 2 good quality embryos transferred were considered for the analysis. A study group (n=21) included women who underwent hysterectomy–biopsy in the cycle preceding the current IVF treatment. Control group (n=20) underwent a repeat cycle with no intervention. Baseline characteris-
tics of both groups confirmed no different history of IVF-ET failures and a similar performance in the present IVF-ET treatment: mean number of IVF trials 2.2 vs. 2.1 cycles, age 31.8 vs. 31.9 yrs Number of oocytes retrieved: 11.0 ± 6.0 vs. 10.4 ± 5.3 Number of good quality Day 3 embryos obtained 5.7 ± 3.9 (51.8%) 5.8 ± 4.1 (56.3%).A similar number of good
P-470 Wednesday, October 22, 2014
THE MIR-16 FAMILY IS HORMONALLY REGULATED IN ENDO-
METRIAL STROMAL CELLS AND ALTERED BY SUPEROVULA-
TION IN A MURINE MODEL. S. White P. T. Jimenez. Obstetrics & Gynecology, University of Texas Southwestern Medical Center, Dallas, TX.

OBJECTIVE: The purpose of this study was to determine the expression and hormonal regulation of the microRNA (miRNA, miR -16) family in endometrial stromal cells. Local injury to the endometrium prior to controlled ovarian stimulation may considerably improve implantation rates and preg-

RESULTS: In the miRNA array, miR-16 was significantly downregulated at 4.5 dpc versus 0.5 dpc; this was confirmed by RT-qPCR. The miR-16 fam-
ily members, miR-15a and -15b were also decreased at 4.5 dpc. The putative targets of the miR-16 family, IHH, HOXA10 and PTCH1, were significantly,

CONCLUSION: The miR-16 family is gradually downregulated following ovulation with a maximal decrease at implantation (4.5 dpc). The coordinate increase in the miR-16 targets may serve an important role in embryo implantation. By contrast, superovulation or estradiol treatment caused rapid and profound upregulation of miR-16 family expression. Our findings suggest that aberrant induction of the miR-16 family by estradiol treatment or superovulation may alter the expression of its targets necessary for successful implantation.

Supported by: K12 HD000849-25 (FTJ).

HOMEBOX TRANSCRIPTION FACTOR MSX1 IS REDUCED IN HUMAN ENDOMETRIAL BIOPSY SAMPLES OF WOMEN FROM INFERTILE COUPLES. A. D. Bolnick,5  J. M. Bolnick,4  B. A. Kilburn,1  J. Oakes,1  A. J. Dui,3  M. P. Diamond,5  S. K. Dey,1  D. R. Armant,5  Obstetrics and Gynecology, Wayne State University, Detroit, MI; 5Obstetrics and Gynecology, Georgia Regents University, Augusta, GA; Division of Reproductive Sciences, Cincinnati Children’s Hospital, Cincinnati, OH.

OBJECTIVE: To evaluate MSX1 protein expression in human endome-

P-471 Wednesday, October 22, 2014
HOMEBOX TRANSCRIPTION FACTOR MSX1 IS REDUCED IN HUMAN ENDOMETRIAL BIOPSY SAMPLES OF WOMEN FROM INFERTILE COUPLES. A. D. Bolnick,5  J. M. Bolnick,4  B. A. Kilburn,1  J. Oakes,1  A. J. Dui,3  M. P. Diamond,5  S. K. Dey,1  D. R. Armant,5  Obstetrics and Gynecology, Wayne State University, Detroit, MI; 5Obstetrics and Gynecology, Georgia Regents University, Augusta, GA; Division of Reproductive Sciences, Cincinnati Children’s Hospital, Cincinnati, OH.

OBJECTIVE: To evaluate MSX1 protein expression in human endome-

supported by: K12 HD000849-25 ( FTJ ).
P-472 Wednesday, October 22, 2014
MICRONAS AND THEIR TARGET GENES RELATED TO ENDOMETRIAL DECIDUALIZATION. H. Toguchi, a,b T. Kajihara, a Y. Mizzo, b S. Tamaru, a,b Y. Kamei, a Y. Okazaki, a,b,c O. Ishihara, a,b Obsterics and Gynecology, Saitama Medical University, Moroyama-machi Iruma-gun, Saitama, Japan; bDivision of Functional Genomics and Systems Medicine, Research Center for Genomic Medicine, Saitama Medical University, Saitama, Hitada City, Saitama, Japan; cDivision of Translational Research, Research Center for Genomic Medicine, Saitama Medical University, Hitada City, Saitama, Japan.
OBJECTIVE: Endometrial decidualization is the essential step for successful implantation of embryo, however, molecular mechanism of the change is still under debate. The aim of this study is to investigate the role of microRNAs and their target genes at decidualization in human endometrial stromal cells (HESCs).

DESIGN: In vitro experiment on primary culture of HESCs.

MATERIALS AND METHODS: Human endometrial tissues were obtained at the time of hysterectomy for uterine fibroids from normally cycling premenopausal women. The patients were not on hormonal treatment at the time of surgery. All the samples were collected during the proliferative phase of the cycle. Written informed consent for the study was obtained before the operation and the study protocol was approved by Institutional Review Board of Saitama Medical University Hospital. Human endometrial stromal cells (HESCs) were isolated and cultured as previously described. The HESCs was treated with or without 0.5 mM 8-bromo-cAMP (cAMP) and 10-6 M medroxyprogesterone acetate (MPA) for 6 days. The significantly altered expression of miRNAs target genes were identified with using miRNA microarray, mRNA microarray and RT-qPCR assay. We then investigated the roles of the miRNAs and the predicted target gene IGBP1 known as a decidualization marker using gene overexpression experiments. Luciferase reporter assay was conducted to confirm whether the miRNA directly regulates the expression of IGBP1.

RESULTS: Nine miRNAs significantly altered at decidualization were identified by microarray experiments. Among them, one miRNA was up-regulated and eight were down-regulated including 0.27 times expression of miR-542-3p that predicted target gene as IGBP1. The level of IGBP1 mRNA expression was significantly reduced in decidualized HESCs by overexpression of the miR-542-3p. In addition, overexpression of miR-542-3p in HESCs inhibited morphologica decidualization by the stimulation with cAMP and MPA. Luciferase reporter assay confirmed that the 3’-UTR of IGBP1 mRNA is directly targeted by the miR-542-3p.

CONCLUSION: These results suggest that miR-542-3p play an important role in endometrial decidualization through regulating IGBP1 expression.

P-473 Wednesday, October 22, 2014
EFFECTS OF LEVONORGESTREL-RELEASING INTRARINE SYSTEM ON THE EXPRESSION OF STEROID RECEPTOR COREGU- LATORS TIF-2, AIB-1 AND NCOR IN ADENOMYOSIS. M. K. Kim, B. H. Yun, Y. S. Choi, S. K. Seo. Obstetrics and Gynecology, Severance Hospital, Yonsei University College of Medicine, Seoul, Korea.

OBJECTIVE: In the present study, the expressions of steroid receptor coregulator transcriptional intermediary factor-2 (TIF-2), amplified in breast cancer-1 (AIB-1) and nuclear receptor corepressor (NCoR) in endometrial tissues from control group, untreated adenomyosis group and levonorges trel-releasing intrauterine system (LNG-IUS)-treated adenomyosis group are immunohistochemically evaluated.

DESIGN: Clinical study.

MATERIALS AND METHODS: Tissue samples of endometrium were obtained from 38 premenopausal women with symptomatic adenomyosis, and 23 normal ovulatory women who had carcinoma in situ of uterine cervix without any other significant uterine abnormalities after hysterectomy. Seventeen women with adenomyosis were treated with LNG-IUS. Immunostaining for TIF-2, AIB-1 and NCoR was performed and assessed semiquanitative.

RESULTS: TIF-2 was distributed diffusely in endometrial glandular cytoplasm and stromal cell irrespective of menstrual phase. The expression of TIF-2 in glandular and stromal cell showed strong intensity in the control group and untreated adenomyosis group. The endometrium from LNG-IUS-treated adenomyosis group demonstrates significantly decreased expression of TIF-2 compared with those from control group and untreated adenomyosis group. The expression of AIB-1 was observed diffusely in endometrial stromal cells and negligible in glandular cells. AIB-1 expression in stromal cell was greatest in untreated adenomyosis group. The endometrium from untreated adenomyosis group demonstrated significantly higher expression of AIB-1 compared with those from control group and LNG-IUS treated adenomyosis. The immunoreactivity of NCoR was observed in both endometrial glandular and stromal cell throughout the menstrual cycle. NCoR expressions in endometrial glandular nucleus were significantly decreased in untreated adenomyosis group compared with normal control group, and significantly increased in LNG-IUS-treated group compared with untreated adenomyosis group during the proliferative phase.

CONCLUSION: The alteration of expression of TIF-2, AIB-1 and NCoR in adenomyosis may be a possible underlying pathogenetic mechanism of adenomyosis and these coregulators may also be associated with the treatment mechanism of LNG-IUS in adenomyosis.

P-474 Wednesday, October 22, 2014
LIPID PROFILE AS A NON-INVASIVE TOOL TO PREDICT ENDOMETRIAL RECEPTIVITY – A PILOT STUDY. D. P. A. F. Braga, a,b A. S. Setti, a,b M. N. Eberlin, a,b E. Cabral, a,b E. Lo Turco, a,b A. Iaconelli, a,b E. Borges, Jr. a,b Fertility - Centro de Fertilização Assistida, São Paulo, SP, Brazil; aInstituto SapienTia, São Paulo, SP, Brazil; bThoMson Mass Spectrometry Laboratory, Barão Geraldo, SP, Brazil; cUniversidade Federal de São Paulo - UNIFESP, São Paulo, SP, Brazil.

OBJECTIVE: To make use of the analytical power of mass spectrometry (MS) to characterize the lipid profile of the receptive endometrial fluid of patients undergoing frozen-thawed embryo transfer cycles.

DESIGN: Prospective study.

MATERIALS AND METHODS: For this study 22 samples of endometrial fluid were collected from patients undergoing hormonal replacement therapy for frozen-thawed embryo transfers. Samples were split into groups according to the pregnancy result: Positive, the Receptive-Endometrium Group (RE-Group, n=13) and negative, the Non-Receptive-Endometrium Group (NRE-Group, n=9). To avoid the bias of the embryo quality to the implantation success, the study included exclusively frozen-thawed cycles with blastocyst embryo transfer in which one or two top quality embryos were transferred. The lipid profiles of samples from the RE-Group were compared with the lipid profiles of samples from the NRE-Group, aiming to identify a possible biomarker of the receptive endometrium. Mass spectra fingerprinting were acquired using a 7.2T LTQ FT Ultra-MS, equipped with
a chip-based direct infusion nanoelectrospray ionization source. Data were analyzed using the Partial Least Square Discrimination Analysis (PLS-DA) combined with variance influence on projection (VIP) scores and a ROC Curve was constructed.

RESULTS: Following the VIP analysis, the most important lipid species were identified. The model detected a significant increase of 14 lipids in the RE-Group including glycerolipids, glycerophospholipids and polyketides. Samples of RE-Group were correctly identified with an 81% average probability and an area under the curve of 98.7% was observed.

CONCLUSION: The MS with ultra-high resolution enables the identification of specific lipids that are differentially represented depending on the endometrial status, therefore, the MS fingerprinting is a valuable, non-invasive tool to predict the endometrial receptivity. However, the study must be performed in a higher number of samples to validate the results.

P-475 Wednesday, October 22, 2014

ANTIPROLIFERATIVE ACTION OF METFORMIN IN ENDOMETRIAL STROMAL CELL AND ENDOMETRIAL CANCER CELL. Y. J. Lee,° E.-J. Han,° B. H. Yun,° S. J. Chon,° S. Cho,° Y. S. Choi,° B. S. Lee,° S. K. Seo,° Department of Obstetrics and Gynecology, Severance Hospital, Seoul, Republic of Korea; Department of Obstetrics and Gynecology, Gwangmann Severance Hospital, Seoul, Republic of Korea; Institute of Women’s Life Medical Science, Yonsei University College of Medicine, Seoul, Republic of Korea.

OBJECTIVE: Metformin could block the insulin-like growth factor -1 (IGF–1)/mamalian target of rapamycin (mTOR) signaling pathway and inhibit cell growth through an activation of adenosine monophosphate-activated protein kinase (AMPK) pathway. Also, metformin had been suggested to inhibit angiogenic pathways and inflammatory response. The aim of this study was to investigate the effects of metformin on inflammatory response and proliferation in endometrial stromal cells (ESCs) and endometrial cancer cell lines and demonstrate a possible role of nonsteroidal anti-inflammatory drug-activated gene-1 (NAG-1) expression in anti-tumor effect of metformin.

DESIGN: Laboratory study using endometrial cell culture.

MATERIALS AND METHODS: From March 2012 to March 2014, ESCs were isolated and cultured from endometrial tissue of 10 patients with fibroids undergoing hysterectomy. ECC-1 and Ishikawa cells were used as endometrial cancer cell lines. Cell proliferation was assessed after exposure to different concentrations of metformin. ESCs were treated for 24 hours with various concentrations of metformin before incubation in medium containing 1 ng/mL interleukin-1 beta (IL-1b). ECC-1 cells were treated for 48 hours with various concentrations of metformin. Apoptosis was assessed by Annexin V and propidium iodide staining and real-time polymerase chain reaction (PCR) was used to quantify NAG-1 mRNA levels.

RESULTS: Metformin decreased IL-1b-induced IL-8 production and inhibited growth in ESCs in a dose-dependent manner. It also inhibited growth in both ECC-1 and Ishikawa cells in a dose-dependent manner, but induced apoptosis only at high doses of treatment in both ECC-1 and Ishikawa cells. And metformin increased the levels of NAG-1 mRNA in a dose-dependent manner.

CONCLUSION: This study showed that metformin is a potent inhibitor of cell proliferation in ESCs and endometrial cancer cell lines. NAG-1 might be a therapeutic target for metformin’s anti-tumor action. These results suggest that further studies are needed to investigate metformin as a strategy for endometrial cancer prevention in patients with polycystic ovary syndrome.

Supported by: This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (4-2012-0458).

P-476 Wednesday, October 22, 2014

IMPACT OF S100 PROTEIN EXTINCTION IN THE GRADUAL ACQUISITION OF THE RECEPTIVE ENDOMETRIAL PHENOTYPE. L. Bissounette,a,c,d H. Dechaud,a S. Traver,a M. Montfort,a H. Dechaud,a S. Hamanah,a,b,c CHU Montpellier Hôpital St Eloi, IRB Inserm U1040, Montpellier, France; CHU Montpellier Hôpital Arnaud de Villeneuve, Département de Biologie de la Reproduction, Montpellier, France; UFR Médecine Laboratoire ‘Développement Embryonnaire Précocé et Cellules Souches Embryonnaires Humaines’, Université Montpellier, Montpellier, France; ‘Clinic OVO, Montréal, Canada; ‘Gynéco, Montpellier, France.

OBJECTIVE: We previously identified and validated the over-expression of S100 protein family member during the receptive phase as biomarker of endometrial receptivity, using omics (transcriptome and proteome). This study aims to identify this candidate roles in receptive endometrial phenotype acquisition of fertile woman.

DESIGN: Primary stromal and epithelial cells cultures were purified from endometrial biopsies of fertile woman. Protein extraction was completed by loss of function (shRNA) for stable gene silencing. We investigated the impact of candidate knockdown on transcriptome, migration and decidualization.

MATERIALS AND METHODS: Extinction of candidate was performed using 3-shRNAs (pLKO.1-puro-CMV-GFP) in purified primary endometrial cells. Transcriptome of infected and control cells were studied using DNA microarray. We performed migration assay by wound healing and decidualization assay using 8-Br-cAMP with quantification by qRT-PCR of decidualization biomarkers.

RESULTS: Transcriptome analysis demonstrated, in stromal cells, that 256 genes were differentially expressed in infected cells compared to control cells (174 up-regulated, 82 down-regulated). Functional annotation revealed alteration of leukocyte transendothelial migration (VAV3, MMP16) and TGFβ signaling (ACVR1C, BMPER). In epithelial cells, 34 genes were up-regulated in shRNA cells compared with controls. Several of these genes were components of the extracellular matrix or intercellular connections (COL4A5, Gajas). Candidate extinction significantly reduced cells migration over 24hrs. As well, 9 days after the start of cAMP treatment, we observed in infected cells, a reduction of cellular differentiation/decidualization that was confirmed by the down-regulation of connexin 43. Interestingly, candidate extinction induced a significant reduction of ARNm expression of the decidualization biomarker prolactin in stromal cells (44%) while a strong increase (61%) was observed in epithelial cells compared to controls.

CONCLUSION: Extinction of our candidate affected decidualization, migration and transcription of endometrial cells demonstrating its essential role in the gradual acquisition of the receptive endometrial phenotype.

Supported by: This work was partially Supported by a grant from the Ferring Pharmaceutical Company.

P-477 Wednesday, October 22, 2014

SIGNALLING THRU MILK FAT GLOBULE EGF FACTOR 8 ON HUMAN ENDOMETRIAL ENDODHELIAL CELLS PROMOTES ANGIgenesIS. L. Yu, S. Bocca, S. Oehninger. The Jones Institute for Reproductive Medicine, Department of Obstetrics and Gynecology, Eastern Virginia Medical School, Norfolk, VA.

OBJECTIVE: Endometrial milk fat globule EGF factor 8 (MFG-E8) has been discovered and studied by our group with predominant localization in the epithelium during the window of implantation. Our recent findings have shown that dysregulated MFG-E8 impairs endometrial receptivity and embryo attachment. This protein is traditionally thought to play a role in promoting tumor cell invasion, metastasis and angiogenesis. However, it is not known whether MFG-E8 regulates angiogenesis in the endometrium. Thus, we sought to determine the function of MFG-E8 on human endometrial endothelial cells (HEEC), hypothesizing that activation of the MFG-E8 production would stimulate angiogenesis.

DESIGN: In this study, we used in vitro culture of HEEC to investigate the ability of MFG-E8 to stimulate angiogenic processes.

MATERIALS AND METHODS: HEEC were treated with MFG-E8 at different doses and cell proliferation was measured with a sprouting assay. Cells were plated on a 3D gel matrix containing extracellular matrix components, and their formation of branches and tube-like structures were studied. A well-characterized angiogenic factor, VEGF was used as positive control and plain culture medium was used as negative control. The number of sprouts (in vitro angiogenesis) was recorded and compared between treatment groups. The level of phosphorylated focal adhesion kinase (p-FAK) was measured by western blot.

RESULTS: The maximally stimulatory concentration of MFG-E8 was determined. Quantitative comparison among treatment groups revealed that MFG-E8 is as effective as VEGF in promoting angiogenic processes as compared to control (p<0.05). Changes of p-FAK/FAK protein level were observed in response to MFG-E8, and with additional FAK inhibitor, suggesting that MFG-E8 stimulation of HEEC via FAK signaling pathway.

CONCLUSION: This study reveals the ability of MFG-E8 to promote angiogenesis in endometrial endothelial cells, suggesting that it may have
OBJECTIVE: Investigate whether high serum follicle-stimulating hormone (FSH) in post-menopause women might promote endometrial atrophy by crosstalk with TGF beta signal transduction pathway.

DESIGN: Observe effects of FSH on post-OVX endometrial atrophy in mice and effects of FSH on proliferation and apoptosis in human primary cultured endometrial cell.

MATERIALS AND METHODS: Endometrium was obtained from childbearing age women for primary culture. After digestion, endometrial cell was cultured in medium with 0, 50, 100, 150 IU/L FSH for 72hrs. Proliferation was measured via BrDU assay and markers for apoptosis and proliferation were measured via western-blot. Endometrial cell were cultured in medium with 100 IU/L FSH for 0, 30, 60,120mins. Activation of TGF beta signal transduction pathway was measured by western-blot. We made four mouse models: SHAM, OVX, OVX+GnRH, OVX+GnRH+FSH. Serum levels of FSH and estradiol were measured. The weight and morphology of uterus from those animal models were analyzed.

RESULTS: [1] FSH receptor was expressed in human endometrial cell using western blot, RT-PCR and IF. Its expression levels did not varied significantly in pre- and peri-menopausal women. [2] FSH inhibited the proliferation and promoted the apoptosis of primary cultured endometrial cell in a dose-dependent way, which reached a peak at 100IU/L (P<0.05).

Up-regulation of cell apoptosis markers (capsase3, caspase8 and caspase9) and down-regulation of proliferation relative gene c-Jun were also shown. [3] FSH can promote the phosphorylation and nucleus translocation of Smad2/Smad3 in a time-dependent way which reached a peak at 60mins (p<0.05) which can be partly reversed by pretreatment with antibody against TGF beta receptor II (TβRII, 100ng/ml). [4]Serum FSH of OVX and OVX+GnRH+FSH groups were higher than SHAM and OVX+GnRH groups(P<0.01). In groups with high FSH, more significant changes involved with apoptosis after OVX were observed. Those changes include the smaller size of glandular tube, the pyknotic nuclei, the vacuolization in mitochondria and the hollowed rough endoplasmic reticulum.

CONCLUSION: High FSH in post-menopause women inhibited proliferation of endometrial adenocyte by cross-talking with TGF beta signaling, which promoted endometrial atrophy.

Supported by: the National Basic Research Program of China (No. 2013CB967404), the National Natural Science Foundation of China (No.81270664, 81170310), the Public Welfare Technology Application Research Project of Zhejiang Province (No.2010C33167), the Talent Project of Zhejiang Province (No.2011RCA028), and the Program for Changjiang Scholars and Innovative Research Team in University(No.IRT1184).

Table 1

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Control Cycles (n=105)</th>
<th>Gonadotropin Cycles (n=63)</th>
<th>OR (95%CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak Estradiol</td>
<td>2333</td>
<td>930</td>
<td>-1403 (1036-1770)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mean Endometrial Thickness</td>
<td>7.1 ± 1.8</td>
<td>8.3 ± 2.7</td>
<td>1.2 (0.85-1.58)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>% Thickness of EMT</td>
<td>43.8</td>
<td>17.4</td>
<td>0.76 (0.65-0.89)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>% Live Birth</td>
<td>6.7</td>
<td>33.3</td>
<td>1.31 (1.41-1.50)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

CONCLUSION: This retrospective analysis suggests that some patients who have poor endometrial development from exogenous estrogen alone may benefit from exogenous gonadotropins in FET and DRC. This effect was not associated with estradiol concentration, suggesting potential benefit of endogenous estrogen or gonadotropin effect. The study design has potential bias due to regression to the mean, however, these data suggest this treatment should be evaluated in a prospective controlled fashion.

Supported by: In part by PRAE, NICHD, NIH.
endometrial thickness and prevent cycle cancellation and improve pregnancy rate. Further studies are needed to clarify whether this effect only associated with flare-up effect of long-acting GnRH or other mechanisms are involved.

Supported by: Partial grant by Ferring pharmaceutical.

P-481 Wednesday, October 22, 2014

ENDOMETRIAL RECEPTIVITY ASSESSMENT AND PERSONALIZED PATIENT CARE MANAGEMENT: A MEAN TO OPTIMIZE PREGNANCY RATE. D. Haouzi,1,2, F. Entezami,1 M. Monforte,2 S. Hamamah,1 S. L. Young,1 C. Vincens,3 D. Peterse,4 E. Evans-Hoeker,2 A. Fassbender,2 D. Haouzi,2 Obstetrics and Gynecology, Virginia Tech - Carilion School of Medicine, Greenville, Greenville, SC; 1Department of Biologie de la Reproduction, CHU Montpellier, IN-SERM U 1040, Institut de Recherche en Biothérapie, Montpellier, Languedoc-Roussillon, France; 2Centre FIV La Muette, ELYLU UNILABS, Paris, France; 3Département de Biologie de la Reproduction, CHU Montpellier, ART/PGD Division, Montpellier, Languedoc-Roussillon, France.

OBJECTIVE: The aim of this study was to optimize pregnancy outcome using personalized care management by combining embryo replacement according to the evaluation of endometrial receptivity in patients with multiple implantation failure after several unsuccessful IVF cycles awaiting for transfer of cryopreserved embryos under hormonal substitution treatment (HST) or natural cycle.

DESIGN: Endometrial biopsies from patients (n=26) under HST (5/6 days after progesterone administration) or natural cycle (LH+7/LH+9) were performed during the peri-implantation period. RNAs were extracted and biomarkers of endometrial receptivity were assessed by RT-qPCR. According to the endometrial status, embryo transfer (day 3 embryos or blastocysts day 5/6) was performed either during the same cycle in which endometrial biopsy has been performed or the following cycle.

MATERIALS AND METHODS: The test consists of measuring the expression level of genes predictive of endometrial receptivity in endometrial biopsies (Patent EP1030556/12; PCT/EP2011/058757). Determination of the mRNA expression levels are performed by qRT-PCR and data are converted into a score by using algorithms.

RESULTS: For patients under HST, if endometrium is diagnosed as ‘receptive’, transfer of cryopreserved blastocysts have been performed after 8 days of progesterone treatment during the same cycle in which endometrial biopsy (Pg+5/6) has been performed or during the next cycle of day 3 embryos at Pg+5/6 precisely. For patients under natural cycle, blastocyst transfer was performed the following cycle at the same cycle moment of the endometrial receptivity appreciation (LH+7; LH+8 or LH+9). Using this personalized patient care management, the clinical pregnancy rate in this group of patients with previous multiple implantation failure (3-7 failures) were 56%.

CONCLUSION: After multiple implantation failure, the endometrial receptivity requires detection by transcriptomic approach. Individual evaluation of endometrial receptivity allows a personalized patient care management and improves pregnancy outcome.

Supported by: This work was partially Supported by a grant from the GFI Merck Serono.

P-482 Wednesday, October 22, 2014

A MORE TRAUMATIC DECIDUALIZATION STIMULUS IN THE MENSTRUATING MOUSE MODEL RESULTS IN AN INCREASED AMOUNT OF DECIDUALIZED ENDOMETRIUM. D. Peterse,1,2, Dorien O.,1,2 A. Fassbender,1,2 T. D’hooghe,1,2 Development and Regeneration, KU Leuven, Leuven, Vlaams-Brabant, Belgium; 1Obstetrics and Gynaecology, UZ Leuven, Leuven, Vlaams-Brabant, Belgium.

OBJECTIVE: Retrograde menstruation is accepted to be important in the pathogenesis of endometriosis. Although a major limitation of rodent models for endometriosis is the lack of menstruation, it is relevant for preclinical research to optimize and standardize methods to induce endometrial decidualization and menstruation in donor mice, before this menstrual endometrium can be used reproducibly for intra-abdominal inoculation in syngeneic recipient mice. In this study, we hypothesized that bicornuate menstrual decidualization can be achieved in 100% of mice after combined intra-uterine oil injection and mechanical stimulus.

DESIGN: Randomized controlled trial in mice.

MATERIALS AND METHODS: 60 ovariectomized C57Bl/6J mice were injected with E2 (100ng/day s.c. for 3 days), s.c. implanted with a P4-pellet (implanted at day 6; serum levels: 22ng/ml), followed by E2 injections (5ng/3-day/3 days). Decidualization was induced without mechanical stimulus and only by intra-uterine injection of 20µl oil per horn (group 1; n=10); or 100µl oil per horn (group 2; n=10); or with mechanical stimulus combined with intra-uterine injection of 20µl oil per horn (group 3; n=10); or 100µl oil per horn (group 4, n=10); or with only a mechanical stimulus (group 5, n=10); or without any stimulus, by only touching the vagina with the needle (group 6, n=10). Pellets were removed 4 days later, followed by hysterectomy after 4-6 hours. Endometrial decidualization was quantified macroscopically uterine weight/ body weight.

RESULTS: We could observe macroscopical bicornuate decidualization in 78% (79) of the animals after inducing decidualization with 100 µl of oil (group 2). This number was increased to 100% (8/8) after giving an additional mechanical stimulus (group 4). In all other groups, bicornuate decidualization could be found in 70%-80% (group 1: 8/10; group 3: 7/10; group 5: 6/8) of mice. One mouse from group 3 showed a total lack of decidualization. Compared to the sham group (group 6; 41 ± 16mg), relative uterine weight was significant higher in all in groups in which oil was administered (group 1: 290 ± 141mg (p<0.001); group 2: 271 ± 108mg (p<0.001); group 3: 223 ± 122mg (p<0.05); group 4: 313 ± 118 (p<0.001)).

CONCLUSION: We confirmed our hypothesis that giving a decidualization stimulus of 100µl of oil + a mechanical stimulus would result in 100% decidualization. For future research, this will be the preferable method to obtain menstrual tissue for our endometriosis mouse model.

Supported by: This project is Supported by the Flemish government via a FWO-grant.

P-483 Wednesday, October 22, 2014

DOES THE ENDOMETRIUM SYNTHESIZE PROGESTERONE? A. L. Gentry,1,3 E. Evans-Hoeker,1,2 B. A. Lessey,1,2 L. Yuan,1,2 S. L. Young,1,2 Obstetrics and Gynecology, Virginia Tech - Carilion School of Medicine, Roanoke, VA; 1Obstetrics and Gynecology, University of North Carolina, Chapel Hill, NC; 3Obstetrics and Gynecology, University of South Carolina School of Medicine, Greenville, Greenville, SC.

OBJECTIVE: Recent studies in murine and human endometrium have suggested the possibility of a steroidogenic capacity, but details of steroid biosynthetic enzyme expression and their potential regulation by cyclic hormones and/or endometriosis remain unclear. The objective of this study was to characterize the cycle and disease regulation of eutopic endometrial mRNA coding for the 3 essential components of progesterone (P) biosynthesis: steroid acute regulatory protein (STAR, mediates cholesterol transport), Cholesterol 20-22 Desmolase (CYP11A1, catalyzes cleavage of the cholesterol side chain to form pregnenolone), and 3-Beta-HSD, type I (HSD3B1, catalyzes conversion of pregnenolone to progesterone).

DESIGN: Laboratory-based cohort study.

MATERIALS AND METHODS: Normal subjects (n=18) and endometriosis subjects (n=17) were randomized to endometrial biopsy during late proliferative (Prol), early (ES), mid (MS), or late secretory (LS) phase. Coding mRNA expression was assessed by TaqMan®, quantitative, real time RT-PCR (qRT-PCR). An additional 36 samples from across the cycle were used to confirm STAR findings. All samples were collected under UNC IRB approval. Cell types from normal controls were separated enzymatically to allow discrete assessment of epithelium and stroma. Differences between groups were assessed using Student’s t-test or ANOVA with Tukey’s post-hoc test.

RESULTS: In normal control samples, STAR demonstrated cyclic expression with maximal expression in the MS phase (5-fold increase over Prol, p<0.04). Both CYP11A1 and HSD3B1 were expressed at variable levels within each cycle phase, but were easily detected in all phases. qRT-PCR of cyclic STAR mRNA expression confirmed the findings in the initial sample in a separate set of samples (n=36, p<0.001). Expression of all three mRNA species localized largely to the stromal cells. Samples from women with endometriosis, as compared to cycle stage matched controls, STAR and CYP11A1 expression showed a trend toward increase in the ES phase (p=0.053 and 0.1 respectively).

CONCLUSION: This is the first demonstration of cyclic endometrial STAR mRNA expression and suggests that mid-secretory endometrial stromal cells can synthesize P from cholesterol. We speculate that local P synthesis can amplify mid-secretory P actions on endometrium and embryo.

Supported by: This work was Supported by NIH R01 HD067721 to S.L.Y and B.A.L.
PROTEIN INHIBITOR OF ACTIVATED STAT-3 (PIAS-3) IS DOWN-REGULATED IN EUTOPIC ENDOMETRIUM OF INFERTILE WOMEN WITH ENDOMETRIOSIS BY STROMAL-DERIVED CXCL10 (IP-10). B. A. Lessey,\textsuperscript{a} J.-W. Jeong,\textsuperscript{b} J.-Y. Yoo,\textsuperscript{c} J. F. Langenhan,\textsuperscript{a} A. T. Fazleabas,\textsuperscript{b} S. L. Young,\textsuperscript{a} C. Tayade,\textsuperscript{a} Department of Obstetrics and Gynecology, Greenville Health System, Greenville, SC; \textsuperscript{b}Department of Obstetrics and Gynecology and Reproductive Biology, Michigan State University College of Human Medicine, Grand Rapids, MI; \textsuperscript{c}Department of Obstetrics and Gynecology, University of North Carolina Chapel Hill, Chapel Hill, NC; \textsuperscript{d}Department of Biomedical and Molecular Sciences, Queens University, Kingston, ON, Canada.

OBJECTIVE: Activation of STAT-3 appears central to the inflammatory phenotype of eutopic endometrium in women with endometriosis. Our objective was to determine how changes in the eutopic endometrium contribute to infertility.

DESIGN: Case-control study of protein levels of Protein Inhibitor of Activated STAT-3 (PIAS-3) and p-STAT3 in eutopic endometrium from women with endometriosis. Cytokine analysis was performed on media from cultured endometrial stromal cells (ECS) from normal and endometriosis subjects. Ishikawa cells were used to study cytokine/chemokine effect on PIAS-3.

MATERIALS AND METHODS: The activation of STAT3 and levels of PIAS-3 were compared in endometrium from women with and without endometriosis using Western blot and immunohistochemical analysis (IHC). ESCs were cultured from similar patients and cytokines measured using Bioplex assay. Ishikawa cells were treated with the interferon-γ induced CXCL10 (IP-10) and PIAS-3 mRNA assessed by qRT-PCR.

RESULTS: PIAS-3 is a negative regulator of pSTAT3 activity. In our samples pSTAT3 was elevated in eutopic endometrium of infertile women with endometriosis. CXCL10 was significantly increased (p < 0.05) in ESC cells derived from endometriosis patients compared to normal controls. Activation of pSTAT3 was significantly increased and expression of PIAS-3 was significantly decreased in epithelial compartment in eutopic endometrium from women with endometriosis compared to controls by western blot and IHC. Ishikawa cells treated with CXCL10 had an 8-fold decrease in PIAS-3 mRNA expression within 1 hr.

CONCLUSION: These results demonstrate for the first time that PIAS-3 is reduced in women with endometriosis and that CXCL10 down-regulates endometrial PIAS-3 expression. The activation of pSTAT3 associated with inflammation and infertility appears to be due, in part, to aberrant regulatory pathways involving over-expression of stromal CXCL10. Activation of pSTAT3 and down-stream events affecting endometrial receptivity appears to be a central feature of endometriosis-related infertility. STAT3 and PIAS-3 represent new therapeutic targets for the treatment of this disease.

Supported by: We would like to thank the Human Female Reproductive Tract Biorepository and the Spectrum Health Medical Group, Department of Obstetrics, Gynecology and Reproductive Biology and Angela Houwing at GHS. This work was supported by NIH R01 HD077210 to S.L.Y and B.A.L and NIH R01 HD057873 to J.W.J.

FEMALE REPRODUCTIVE SURGERY

P-485 Wednesday, October 22, 2014

HYSTEROSCOPY ADHESIOLOGY FOR ASHERMAN’S SYNDROME: LIVE-BIRTH RATE AND FACTORS AFFECTING REPRODUCTIVE OUTCOME AFTER SURGERY. W.-S. Han. Obstetrics and Gynecology, University of Ulsan College of Medicine, Asan Medical Center, Seoul, Republic of Korea.

OBJECTIVE: The objective of this study is to investigate the impact of various factors of hysteroscopic adhesiolysis of Asherman’s syndrome on live-birth rate after surgery.

DESIGN: It is a retrospective study.

MATERIALS AND METHODS: Fifty four women who were suffered from infertility or recurrent spontaneous abortion were selected. Medical records were retrospectively analyzed. The independent variables were degree of intrauterine adhesion, surgical technique, use of intrauterine Foley catheter after adhesiolysis, insertion of intrauterine device after adhesiolysis, and dose of postoperative estradiol after adhesiolysis. Multivariate analysis as well as univariate analysis was performed.

CONCLUSION: Among the fifty four women, seventeen women gave live-birth. The degree of intrauterine adhesion was found to be a sole factor affecting the live-birth rate. By using univariate and multivariate analysis. The mechanical dissection with scissors had been resulted a higher live-birth rate against resectoscopic electrosurgery, but the difference was not statistically significant. Also use of intrauterine Foley catheter, insertion of intrauterine device, and dose of postoperative estradiol were not significantly affecting to reproductive outcome after hysteroscopic adhesiolysis.

P-486 Wednesday, October 22, 2014

THE EFFECT OF PRESENCE AND MANAGEMENT OF HYDROSPALPINX ON MISCARRIAGE IN IVF. H. Harb, F. Al-rahoud, A. Coomarasamy. School of Clinical and Experimental Medicine, University of Birmingham, Birmingham, West Midlands, United Kingdom.

OBJECTIVE: To investigate the effect of hydrospalpinx on miscarriage.

DESIGN: Systematic review and meta-analysis of randomised controlled trials and observational studies.

MATERIALS AND METHODS: Searches were conducted on MEDLINE, EMBASE, Cochrane Library and Web of Science (inception-March2014) in all languages, together with reference lists of retrieved papers. Studies comparing miscarriage rate in women with hydrospalpinx with women without hydrospalpinx were included. Moreover, studies in which women underwent treatment for hydrospalpinx were identified. The outcome of interest was miscarriage. Study selection was conducted independently by two reviewers. The Cochrane scale for the randomised trials, and the Newcastle-Ottawa Quality Assessment Scale were used for quality assessment. Data extraction was conducted independently by two reviewers. Relative risks from individual studies were meta-analysed using RevMan.

RESULTS: This review includes 22 studies. Pooling of results from 11 studies that reported miscarriage as an outcome showed a 2-fold increase in the risk of miscarriage in women with hydrospalpinx compared to women without hydrospalpinx (OR=2.25, 95% CI 1.67, 3.03, p=0.00001). There was moderate variation across studies as indicated by an I2 value of 33% (p=0.13). Pooling of results from 6 randomised controlled trials that reported miscarriage as an outcome showed a reduction in miscarriage in women who had treatment for hydrospalpinx in comparison with women with untreated hydrospalpinx ( OR=0.39, 95% CI 0.17-0.88, p=0.02). There was little variation across studies as indicated by an I2 value of 0% (p=0.58). Pooling of results from 5 observational studies that reported miscarriage as an outcome showed a reduction in miscarriage in women who had treatment for hydrospalpinx ( OR=0.34, 95% CI 0.17-0.88, p=0.0003). There was little variation across studies as indicated by an I2 value of 0% (p=0.68).

CONCLUSION: There is evidence to suggest that the presence of hydrospalpinx increases the risk of miscarriage in IVF/ICSI pregnancies. Treatment for hydrospalpinx may reduce the risk of miscarriage.

Multivariate analysis of factors affecting live-birth rate

<table>
<thead>
<tr>
<th>Factors</th>
<th>Coefficient estimate</th>
<th>Odd ratio</th>
<th>95% confidence interval</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade</td>
<td>-0.566</td>
<td>0.271</td>
<td>-0.709-1.251</td>
<td>0.009</td>
</tr>
<tr>
<td>Surgical technique</td>
<td>-0.295</td>
<td>0.507</td>
<td>-1.350-2.364</td>
<td>0.473</td>
</tr>
<tr>
<td>Foley</td>
<td>0.522</td>
<td>3.332</td>
<td>1.664-4.999</td>
<td>0.157</td>
</tr>
<tr>
<td>Intrauterine device</td>
<td>-9.115</td>
<td>0.0000</td>
<td>-35214.1-35214.1</td>
<td>0.999</td>
</tr>
<tr>
<td>Estrogen</td>
<td>-0.264</td>
<td>0.519</td>
<td>-1.086-2.214</td>
<td>0.423</td>
</tr>
</tbody>
</table>

e298 ASRM Abstracts Vol. 102, No. 3, Supplement, September 2014
P-487 Wednesday, October 22, 2014

A COST-EFFECTIVENESS ANALYSIS OF MORCELLATION HYSTERECTOMY FOR FIBROIDS. P. Bortoletto, B. Einerson, E. Miller, M. Milad. Northwestern University Feinberg School of Medicine, Chicago, IL.

OBJECTIVE: To estimate the cost-effectiveness of eliminating morcellation in the surgical treatment of leiomyomas, from a societal perspective that includes direct health care and patient costs.

DESIGN: Cost effectiveness & decision analysis.

MATERIALS AND METHODS: A decision analysis model was constructed using costs, outcomes, and utility data from published sources. A cost-effectiveness analysis based on quality-adjusted life years (QALYs) was performed to determine the incremental cost-effectiveness ratio (ICER) of eliminating morcellation in the surgical treatment of leiomyomas.

RESULTS: A strategy of non-morcellation hysterectomy via laparotomy was more expensive and produced more QALYs relative to morcellation hysterectomy.

<table>
<thead>
<tr>
<th>Total Cost per Strategy</th>
<th>Total QALY per Strategy</th>
<th>Incremental cost effectiveness ratio (ICER)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Base case results, societal perspective</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morcellation $20,764 21.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-morcellation $29,833 21.26</td>
<td>$983,897/QALY</td>
<td></td>
</tr>
<tr>
<td><strong>Base case, health care system costs only</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morcellation $11,614 21.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-morcellation $15,625 21.26</td>
<td>$435,225/QALY</td>
<td></td>
</tr>
<tr>
<td><strong>Sensitivity analysis: assuming high incidence of occult sarcoma (1.7%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morcellation $20,904 21.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-morcellation $29,925 21.07</td>
<td>$282,143/QALY</td>
<td></td>
</tr>
<tr>
<td><strong>Sensitivity analysis: assuming low incidence of occult sarcoma (0.08%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morcellation $20,904 21.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-morcellation $29,925 21.07</td>
<td>$6,036,811/QALY</td>
<td></td>
</tr>
</tbody>
</table>

The ICER for non-morcellation compared to morcellation hysterectomy was $983,897/QALY. The cost to prevent one case of disseminated cancer attributable to morcellation was $5,950,534. Health care system costs (prolonged hospitalizations) and costs to patients of time away from work were the primary drivers of cost differences between the two strategies. When the incidence of occult sarcoma in leiomyoma surgery was assumed to be higher than traditionally reported (1.7%), the ICER for non-morcellation hysterectomy was $282,143.

CONCLUSION: Eliminating morcellation hysterectomy as a treatment for fibroids is not cost-effective under a wide variety of cost and outcome assumptions. Performing laparotomy for all patients who might otherwise be candidates for morcellation hysterectomy is a costly policy from both societal and health care system perspectives.

P-488 Wednesday, October 22, 2014

RE-DO SURGERY OF THE PATIENTS WITH FEMINIZING GENITOPLASTY IN CONGENITAL ADRENAL HYPERPLASIA. I. Suruc, Pediatric Surgery, Gulhane Military Medical Academy, Ankara, Turkey.

OBJECTIVE: A successful feminizing genitoplasty depends on creating morphologically and functionally near normal genitalia. But it’s still a challenging problem in the pediatric age group to perform a fully satisfactory genitalia. Our aim is to establish a guideline for redo genitoplasty procedures in patients with congenital adrenal hyperplasia that previously performed genitoplasty.

DESIGN: The study was designed as a retrospective analysis of the data which obtained from the patients’ charts.

MATERIALS AND METHODS: 10 patients who underwent Passerini-Glazet genitoplasty and followed-up for postoperative problems between the years 1998 and 2014. These patients were evaluated retrospectively for the complications and problems which required redo surgery.

RESULTS: Six patients were underwent redo surgery 7-11 years (mean 9 years) after initial surgical procedure. In all the patients with stenotic vaginal introitus (4) primary surgical intervention time was around 1 year of age. Clitoromegaly (2 pts), stenotic vaginal introitus due to inadequate Fortunoff flap (3 pts) and clitoromegaly-urethral retraction +stenotic vaginal introitus (1 pts) were the major problems. Complete clitoral body resection with neurovascular bundle and hood preservation were performed in clitoromegaly. Re-do vaginoplasty with adequate Fortunoff flap was performed for all the vaginal introitus and vaginoplasty by urogenital sinus pubiculation plus urethroplasty using common channel roof for re-do surgery due to clitoromegaly +vaginal stenosis+ short urethra. All vaginal stenosis cases were underwent vaginal dilatation program with Hegar dilators for the next 6 months. No problems were determined during the follow-up period up to date.

CONCLUSION: Appearance and the function of the genitalia gain much more importance in adolescence who had feminizing genitoplasty, Therefore long term follow-up has utmost importance to diagnose and solve the problems in time. Although most common determined problems are vaginal stenosis and clitoromegaly, problems may be more complicated both anatomical and psychological. Hence we recommend vaginoplasty at older age (delaying until adolescence) due to high incidence of vaginal stenosis in children with early vaginoplasty.

P-489 Wednesday, October 22, 2014


OBJECTIVE: Our primary objective was to determine the impact of myomectomy by laparotomy on AMH level, a reliable marker of ovarian reserve. Our secondary objective was to determine whether the myomectomy technique using vasopressin or tourniquet or both influences the variation of AMH, considering that a tourniquet causes a temporary ovarian ischemia.

DESIGN: This was a prospective study in women undergoing myomectomy by laparotomy for fertility purposes.

MATERIALS AND METHODS: Women 18 to 42 years of age who underwent myomectomy by laparotomy for fertility purposes at the Centre Hospitalier de l’Universite de Montreal (CHUM) from 2011-2014 were recruited. AMH levels were measured preoperatively, 1 day and 6 weeks postoperatively. Patients with concomitant ovarian surgery, low ovarian reserve (AMH ≤0.3 ng/mL) preoperatively as well as those taking hormonal contraception or GnRH agonist in the 3 months prior to the surgery were excluded. 21 patients were needed to obtain a power level of 90% with alpha error of 5%.

RESULTS: Our preliminary results after recruitment of 18 patients, showed a significant decrease in the AMH value 1 day postoperatively (p = 0.001). However, a full recovery was noted 6 weeks after (p = 0.54). Moreover, there were no differences shown between the different bleeding control techniques. The amount of vasopressin used did not affect AMH value (p = 0.70), nor did the duration of use of tourniquet (p = 0.29) or the duration of surgery (p = 0.095).

CONCLUSION: There is a prominent decrease in the value of the AMH levels following a myomectomy with recovery of the AMH level at 6 weeks postoperatively. Therefore, myomectomy does not seem to have a deleterious effect on ovarian reserve. This study is still ongoing until the desired power level is reached.

P-490 Wednesday, October 22, 2014

SUPERAMIBILIAL PRIMARY LAPAROSCOPIC ACCESS: RELATIONSHIP BETWEEN POINT OF ENTRY AND RETROPERITONEAL VITAL STRUCTURES BY IMAGING. E. Soto, I. Al-Aref, J. Wu, A. Gojayev, L. Goodman, K. Holoch, J. Goldberg, T. Falcone. Obstetrics, Gynecology and Women’s Health Institute, Cleveland Clinic, Cleveland, OH.

OBJECTIVE: Complex laparoscopic and robotic gynecologic surgery may require the placement of supraumbilical trocars for which knowledge
of the distances between this location and retroperitoneal structures is of clinical significance. The objective of this study was to describe the anatomic relationship between commonly described supraumbilical points of entry for trocar placement and vital retroperitoneal structures.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: Random samples of CT imaging studies were obtained until 100 patients satisfied the inclusion criteria. These were: 1) female patient, 2) benign conditions, 3) ages 18-50 and 4) no major retroperitoneal defects, masses or other factors that would affect the measurements obtained. Baseline characteristics were recorded. CT images were reconstructed into 3D models using the IMPAX software. The distances from points of entry (umbilicus and two supraumbilical places -3cm and 5cm cephalad to the umbilicus) and retroperitoneal structures were measured at 90 and 45-degree angles. All measurements were obtained by 3 investigators (including one radiologist). Student t-test, Fisher’s exact test and one-way ANOVA were used as appropriate.

RESULTS: One hundred subjects were included, mean age 36±9.7 and BMI 27.8±8.1. The distance from the skin to the aorta at a 90-degree angle was greater at the point 3cm cephalad from the umbilicus in comparison to the distance from the umbilicus and at 3cm (10.1cm:±0.3, 8.3cm:±0.3 and 9cm:±0.4 respectively, p=0.0004). At a 45-degree angle, the aorta was encountered at 0%, 3% and 4% of cases at the umbilicus, 3cm and 5 cm points respectively. The distances between the different points of entry and other major retroperitoneal vessels were comparable at the different points. A vascular structure other than the aorta was the most anterior retroperitoneal structure in one-third of all cases at the different points (30% umbilicus, 33% at 3cm and 36% at 5cm). The most anterior retroperitoneal vascular structures were the right common iliac vessels at the umbilicus (13%) and the superior mesenteric vein supraumbilically (22% at 3cm and 29% at 5cm).

CONCLUSION: At a 90-degree angle, the distance between the umbilicus and aorta was greater at 3cm. Importantly, the 45-degree path at this level encountered the aorta in only 3% of cases. Retropitoneal vessels other than the aorta are the most anterior structures in one-third of cases at the different points of entry.

P-491 Wednesday, October 22, 2014

A COST BENEFIT ANALYSIS OF HYSTEROSCOPIC POLYPECTOMY BEFORE NATURAL CYCLE OR ClOMIPHINE STIMULATED INTRAUTERINE INSEMINATION IN INFERTILE WOMEN. L. W. Sundheimer,a A. Kathiresan,ab D. Dumesic,a,b R. Parvataneni,a M. Shamonki.a,b “Department of Obstetrics and Gynecology, University of California, Los Angeles, Los Angeles, CA; bDivision of Reproductive Endocrinology and Infertility, University of California, Los Angeles, Los Angeles, CA

OBJECTIVE: Studies on reproductive performance after hysteroscopic polypectomy (HP) for endometrial polyps are limited. Our group has performed a study on HP prior to gonadotropin controlled ovarian hyperstimulation (COH) prior to intrauterine insemination (IUI), but no studies exist on the less costly methods of IUI such as natural cycle (NC) or clomiphene citrate (CC) stimulated cycles. This study examined the cost benefit of performing HP in infertile women preparing for NC/IUI or CC/IUI and identified the threshold cutoff at which HP prior to fertility treatment is cost beneficial over immediate fertility treatment.

DESIGN: Decision-analytic model.

MATERIALS AND METHODS: A decision model was developed to compare cost of HP versus no HP before three cycles of expectant management followed by either NC/IUI or CC/IUI (≤ 4 cycles). Using TreeAge Pro 2012 software, this model was constructed from the only prospective randomized controlled ovarian hyperstimulation/pregnancy outcome following HP in infertile women. With the protocol described above, this study demonstrated a twofold increase in pregnancy rates (relative risk 2.1, 95% confidence interval 1.5-2.9) if HP was performed (1). Costs of HP and NC/IUI or COH/IUI in our study were based on a survey of United States (US) fertility centers, a literature review, and expert opinion. All costs were converted to 2014 US dollars and adjusted for inflation. The primary outcome was cost savings per clinical pregnancy (gestational sac by transvaginal ultrasound) and sensitivity analyses were performed across all ranges.

RESULTS: HP cost ranged from $537-12,530 and a mean-median cost of an office based HP was assigned at $1,800. NC/IUI and CC/IUI costs averaged $550 and $1,250, respectively. When HP was performed prior to NC/IUI, there was no cost benefit. However, when HP was performed prior to CC/IUI there was a lowered fertility cost by $1,393 per clinical pregnancy. Sensitivity analysis across a range of potential NC/IUI and CC/IUI costs identified the threshold value of $704 as the cost at which above this point, HP prior to fertility intervention was more cost beneficial.

CONCLUSION: When IUI is planned in infertile women with endometrial polyp(s), there is a cost-benefit to performing a HP when anticipated IUI costs are greater than $704.

P-492 Wednesday, October 22, 2014

NEW COMPLEMENT FOR AFS SCORE SYSTEM IN PREDICTING THE PREGNANCY OUTCOME IN INFERTILITY WOMEN—A PROSPECTIVE STUDY WITH LONG-TERM FOLLOW-UP. H. Wang,a X. L. Tao,a X. J. Li,a “Hangzhou First People’s Hospital, Hangzhou, Zhejiang, China; bEnze Maternity Hospital of Taizhou Hospital, Jiaojing, Zhejiang, China.

OBJECTIVE: The purpose of this study was to investigate the relationship between American Fertility Society (AFS) scores and pregnancy rate in infertility women who were treated by laparoscopic surgeons, and to compare the pregnancy outcomes in different AFS score groups.

DESIGN: prospective study.

MATERIALS AND METHODS: 129 infertility women underwent laparoscopic surgery were recruited randomly to follow up continuously for 6 months and 122 patients were followed up long for 12 months. Pelvic adhesion was classified according to AFS score system of adnexal adhesions (AFS, 1988). AFS scores were assessed for every patient during operation by assigned doctor. The management of patients after surgery was according to the Zhejiang standard treatment strategy for infertility women.

RESULTS: After 6 months follow up, the total spontaneous pregnancy rate was 17.05% (22/129). Spontaneous pregnancy rate decreased correspondingly with ascending of AFS. AFS score is negative correlation with pregnancy rate (r=−0.224, p<0.05). When AFS score more than or equal to 32, the pregnancy rate was only 3.8%, while when AFS score less than 32, the pregnancy rate is 20.39%, and odds ratio was 1.12 (95%CI:0.015-0.993). The clinical investigation after 12 months follow up showed the total spontaneous pregnancy rate was 24.59% (30/122). The AFS score was lower in pregnancy group (18.7±10.53 vs. 26.3±12.87, P<0.05) and it was also negative correlation with pregnancy rate (r=−0.216, p<0.05). When AFS score more than or equal to 32, the pregnancy rate was only 3.85%, while when AFS score less than 32, the pregnancy rate is 30.21%, and odds ratio was 0.092 (95%CI:0.012-0.715).

CONCLUSION: Long-term spontaneous pregnancy rate of infertility patients with severe pelvic adhesions is very low. People with AFS score more than 32, should be advised to resort to IVF earlier after laparoscopy.

P-493 Wednesday, October 22, 2014

AWARENESS SURVEY ON CLINICAL APPLICATION OF UTERINE TRANSPLANTATION AMONG GENERAL PUBLIC. A. Hayashi,a S. Hirai,a Y. Tsubumishita,a I. Kisu,a M. Mihara;b aHuman Health Sciences, Kyoto University Graduate School of Medicine, Kyoto, Japan; bObstetrics and Gynecology, Keio-Gijuku University School of Medicine, Tokyo, Japan; cVascular Surgery, Saiseikai Kagawuchi General Hospital, Kagawuchi, Saitama, Japan.

OBJECTIVE: Uterine transplantation (UTx) is a potential option for child-bearing in women with uterine factor infertility. However, the uterus is not a vital organ, and therefore the procedure remains controversial in humans. Although the UTx has been already applied clinically in some countries, social consensus is not evaluated enough yet. To address this point, awareness survey was performed among Japanese citizen by gathering public comments for the new technology.

DESIGN: A cross-sectional survey was conducted on Japanese general population aged from 20 to 39 years except fertile persons. Self-reported questionnaire was used through Internet.

MATERIALS AND METHODS: Answers were obtained from 647 people (326 females of 29±6.5±9.9 (mean±SD) years old and 321 males of 29.8±6.0 years old). The questionnaire was consisted of ethical, social and clinical aspects on UTx and gestational surrogacy.

RESULTS: Around 3/4 (78.1% of the females and 73.8% of the males) of the peoples permitted the clinical UTx application morally, and about 40% of peoples (42.9% in females and 36.5% in males) showed interest in the UT. Both 22.4% of the females and 22.8% of the males hoped for UTx treatment
UTx: uterine transplantation

As uterine donor candidates, physical woman with gender identity disorder who would receive sex reassignment surgery was selected equally to cadaver and her or his partner’s mother/sister.

CONCLUSION: Although UTx seems to be acceptable for general peoples, more information concerning UTx procedure should be opened and spread to public.

P-494 Wednesday, October 22, 2014


OBJECTIVE: To determine the effect of hysteroscopic uterine septum resection on reproductive outcomes after ART compared to patients with a normal uterine cavity.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: A retrospective chart review of all patients with infertility who underwent hysteroscopic septum resection from January 2000 to August 2013 and then underwent an ART cycle at our center was performed. The primary outcome was implantation rate. Clinical pregnancy and live birth rates following septum resection were also evaluated. Statistical analysis included chi square and t-test. P<0.05 was deemed statistically significant.

RESULTS: 48 patients (104 cycles) underwent uterine septum resection between January 2000 and August 2013. 47 age matched patients (controls) with a normal uterine cavity by HSG or saline sonogram underwent 142 subsequent cycles. The two groups were similar in age, BMI, average number of cycles per patient, and infertility diagnosis. There were no significant differences in patients who underwent day 3 or day 5 transfer, implantation (IR), clinical pregnancy or live birth rates. The patients who underwent uterine septum resection were found to have higher birth weights and fewer preterm deliveries (20-36 67 completed weeks).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sex</th>
<th>hope</th>
<th>unknown</th>
<th>not hope</th>
<th>others</th>
</tr>
</thead>
<tbody>
<tr>
<td>UTx</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>female</td>
<td>10</td>
<td>3.1%</td>
<td>63 (19.3%)</td>
<td>141 (43.3%)</td>
<td>106 (32.5%)</td>
</tr>
<tr>
<td>male</td>
<td>12</td>
<td>3.7%</td>
<td>61 (19.0%)</td>
<td>177 (55.5%)</td>
<td>68 (21.3%)</td>
</tr>
<tr>
<td>surrogacy female</td>
<td>12</td>
<td>3.7%</td>
<td>52 (16.0%)</td>
<td>149 (45.7%)</td>
<td>110 (34.0%)</td>
</tr>
<tr>
<td>male</td>
<td>14</td>
<td>4.4%</td>
<td>57 (17.8%)</td>
<td>162 (50.5%)</td>
<td>85 (26.5%)</td>
</tr>
</tbody>
</table>

CONCLUSION: Infertility patients undergoing ART treatment who have a uterine septum resection have similar pregnancy and live birth rates compared to patients who are found to have a normal uterine cavity prior to starting ART. Patients with a uterine septum who require ART should be appropriately counseled that normalization of the uterine cavity enhances reproductive outcomes.

P-495 Wednesday, October 22, 2014

SERUM ANTI-MULLERIAN HORMONE LEVEL GUIDING LAPAROSCOPIC MANAGEMENT OF OVARIAN ENDOMETRIOMA IN INFERTILE WOMEN. T. M. Elhawary. Gynecology & Obstetrics, Faculty of Medicine-tantauniversity, Tanta, Elgharbia, Egypt.

OBJECTIVE: As endometrioma affects female ovarian function by itself and by its management, treatment of endometrioma should be tailored according to ovarian reserve. Aim of the study: to evaluate the role of AMH in guiding the management of endometrioma.

DESIGN: Prospective comparative study; Gynecology department-Tanta University and private clinic.

MATERIALS AND METHODS: 330 women with ovarian endometrioma, complaining of infertility were classified according to AMH level and laterality of endometrioma into 4 groups: group I (120 women) without unilateral endometrioma and AMH above 3ng/ml, group II (80 women) with unilateral endometrioma and AMH below 3ng/ml, group III (50 women) with bilateral endometrioma and AMH above 2.7ng/ml and group IV (80 women) with bilateral endometrioma and AMH below 2.7ng/ml. In groups I and III, laparoscopic ovarian endometrioma resection was done while in groups II and IV, laparoscopic endometrioma drainage with bipolar coagulation was done.

RESULTS: AMH did not decrease significantly in all groups after 3 months while, it decreased significantly after 6 months in all groups. Endometrioma recurrence occurred in groups (I&IV), while no recurrence occurred in groups (I&III).

CONCLUSION: laparoscopic management of endometrioma whether resection or drainage with bipolar coagulation should be guided by serum AMH level.

Supported by: I hope to accept this abstract in the next ASRM meeting.

P-496 Wednesday, October 22, 2014

THE APPLICATION VALUE OF HYSTEROSCOPY IN WOMEN WITH ENDOMETRIAL POLYP OR UTERINE MALFORMATIONS SEEKING FOR INFERTILITY TREATMENT. L. Hu, Z. Bu, Y. Wang, Y. Sun. Reproductive Medicine Center, First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China.

OBJECTIVE: To explore the application value of hysteroscopy in women with endometrial polyp (EP) or uterine malformations (uterus unicornis, uterus bicornis, or uterus duplex) undergoing intrauterine insemination (IUI) or in vitro fertilization and embryo transfer (IVF-ET) treatment.

DESIGN: Retrospective data analysis.

MATERIALS AND METHODS: Women undergoing IUI or IVF-ET have hysteroscopy examination as a routine procedure in our center. For patients with endometrial polyp, transcervical resection of polyp (TCRP) will be performed. However, for patients diagnosed as uterine malformation with hysteroscopy examination, we give no future treatment. We observed the clinical pregnancy rate in patients (from January 2011 to December 2013) with normal uterine, endometrial polyp, or uterine malformation.

RESULTS: In the 3291 first IUI cycles, the clinical pregnancy rates for women with normal uterine, with endometrial polyp, and with uterine malformation were 16.35% (473/2893), 19.14% (71/371), and 14.81% (4/27), respectively. However, the difference was not statistically significant (P>0.05, by Chi-square test). In the 6564 first IVF cycles, the clinical pregnancy rates for women with normal uterine, with endometrial polyp, and with uterine malformation were 50.95% (2865/5623), 50.46% (443/878), and 47.62% (30/63), respectively. There were also no significant difference among these three groups (P>0.05).

CONCLUSION: For patients undergoing IUI or IVF with endometrial polyp after TCRP treatment, clinical pregnancy rate is comparable with that in patients with normal uterine. However, the clinical pregnancy rate is slightly lower in patients with uterine malformation. The benefit of TCRP for patients with endometrial polyp should be confirmed by larger prospective studies.

Supported by: This work was Supported by the National Natural Science Foundation of China (Grant NO. U1304315).

OBJECTIVE: Before conducting our study, we did not have a definitive answer regarding whether women with unilateral fallopian tube blockage could achieve a satisfactory pregnancy rate without ART. Therefore, treatment of unilateral fallopian tube blockage was a topic in need of evaluation regarding whether ART should be the primary treatment option.

DESIGN: In 2011, the outpatient’s in our ART clinic underwent HSG for infertility screening. The patients were divided to two groups, normal and abnormal (hydrosalpinx, adhesions, and blockage) by HSG; retrospective pregnancy rates of the two groups were then compared.

MATERIALS AND METHODS: The study group comprised 515 women desirous of pregnancy. HSG was conducted using an oil contrast agent. We divided the two groups in regard to whether the HSG was normal or abnormal. Subsequently, we evaluated the pregnancy rates of two groups: those with and those without ART.

RESULTS: The 515 women were diagnosed with either primary infertility (381 of 515; 74.0%) or secondary infertility (134 of 515; 26.0%). Normal HSG women comprised 418 of 515 (81.2%), and abnormal HSG women comprised 97 of 515 (18.8%). The pregnancy rate of the normal HSG group was 177/418 (42.3%). The rate of the unilateral fallopian blockage group was 26/67 (38.8%); this difference was not statistically significant. Therefore, when a patient was diagnosed with unilateral fallopian tube blockage, we did not employ ART as the first treatment option. This was because we found no benefit to using ART as the primary treatment option. For 3 of the 26 pregnant patients with unilateral fallopian tube closure, ultrasonography suggested that ovulation occurred from the contralateral ovary. Among the 26 pregnancies, 2 multiple pregnancies, 3 spontaneous abortions, and 3 ectopic pregnancies occurred.

CONCLUSION: For patients with unilateral fallopian tube blockage, ART is not indicated as a primary treatment option because the pregnancy rate is not significantly different between hysterosalpingography (HSG) normal patients and unilateral fallopian tube blockage patients.

CLOMIPHENE CITRATE (CC) STARTING DOSE IN INTRAUTERINE INSEMINATION (IUI) CYCLES FOR WOMEN UNDER AGE 35 WITH UNEXPLAINED INFERTILITY: IS HIGHER BETTER? A. L. Park, T. Q. Pham, L. Craig, K. Hansen, R. A. Wild, A. M. Quas, Obestetrics and Gynecology, University of Oklahoma Health Science Center, Oklahoma City, OK.

OBJECTIVE: To compare the effectiveness of 50 vs. 100 mg of CC in CC/IUI cycles in women <35 with unexplained infertility with respect to pregnancy outcomes in the first treatment cycle.

DESIGN: Case series.

MATERIALS AND METHODS: All consecutive couples with female age <35 and a diagnosis of unexplained infertility treated with CC/IUI at a dose of 50 or 100 mg at the physician’s discretion from July 2012 to April 2014 were included. Unexplained infertility was defined as at least unilateral tubal patency, normal ovarian reserve, ovulatory female partner and total motile sperm count of >10 million. Patients with multiple inseminations during the same treatment cycle and those using frozen sperm were excluded. The primary outcome was clinical pregnancy rate (heartbeat on ultrasound at 6-9 weeks gestation) in the first treatment cycle. Secondary outcomes were biochemical pregnancy (positive serum pregnancy test with no heartbeat on early ultrasound), multiple pregnancy, and ectopic pregnancy rates. Statistical analysis was performed using Chi Square, Fisher’s exact test and Student’s t-test.

RESULTS: Of the 100 patients meeting inclusion criteria, 48 were treated with 50 mg, and 52 with 100 mg of CC. Demographic and clinical characteristics are listed in Table 1. Clinical pregnancies occurred in 6 of 48 women (12.5%) in the 50 mg group and 7 of 52 women (13.5%) in the 100 mg group (NS). Rates of biochemical pregnancies were 8/48 (16.7%) and 1/52 (1.9%) in the 50 mg and 100 mg group, respectively (p=0.01). There were no multiple or ectopic pregnancies in the study population.

Table 1: Patient characteristics and clinical outcomes by dose

<table>
<thead>
<tr>
<th>Patients treated with 50 mg</th>
<th>Patients treated with 100 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean)</td>
<td>29.4</td>
</tr>
<tr>
<td>BMI (mean)</td>
<td>25.6</td>
</tr>
<tr>
<td>Antral follicle count (mean)</td>
<td>22.9</td>
</tr>
<tr>
<td>Clinical pregnancy rate</td>
<td>12.5</td>
</tr>
</tbody>
</table>

- Biochemical pregnancy rate (%, first cycle)
  - 16.7
  - 1.9
  - P=0.01

- Multiple pregnancy rate (%, first cycle)
  - 0
  - 0
  - n/a

- Ectopic pregnancy rate (%, first cycle)
  - 0
  - 0
  - n/a

P-498 Wednesday, October 22, 2014

HOW CAN WE INCREASE THE PREGNANCY RATES IN PATIENTS TREATED WITH INTRAUTERINE INSEMINATION (IUI)? P.-C. Ma, C.-W. Wang, C.-R. Tseng, Obstetrics and Gynecology, Taipei Medical University Hospital, Taipei, Taiwan; Obstetrics and Gynecology, TMU-Wan Fang, Taipei, Taiwan; Obstetrics and Gynecology, Taipei Medical University, Taipei, Taiwan.

OBJECTIVE: IUI has been implicated in the treatment of infertile female patient. The objective of this study was to access the efficacy of IUI and identify the predictive factors led to higher pregnancy rates by single variable analysis.

DESIGN: Retrospective analysis of patients with controlled ovarian stimulation (COS) and IUI treatment in a university hospital.

MATERIALS AND METHODS: From 2011 to 2013, a total of 2140 cycles of COS and IUI treatment were included. The analysis of the pregnancy rates were based on age of patient, years of infertility, serum Anti-Mullerian Hormone (AMH) level, indication, medication of COS, E2, progesterone levels and endometrial thickness at time of HCG administration, timing of insemination after HCG, and sperm count for insemination.

RESULTS: The overall pregnancy rate was 24.9% (533/2140), abortion rate was 25.0%. For single variable analysis the pregnancy rate was respectively 13.0%, 27.6%, 33.1%, 29.9% for AMH≤2, 2.4-4.6, and ≥6 (p<0.001). For indication analysis the pregnancy rate was 27.5% for endometriosis, 25.0% for male factor, 33.5% for PCOS and 9.9% for advanced patient’s age, respectively (p<0.001). The pregnancy rate was 33.5%, 30.0%, 20.4% and 9.6% for patient’s age ≤30, 31-35, 36-40 and ≥40, respectively. The pregnancy rate was significantly higher in ultra-long protocol (29.0%) and flare up protocol (37.8%) than other protocol (19.1%) (p<0.001). The pregnancy rate of 20.7% was significantly lower for E2 levels ≤500 pg/mL compared with 29.2% for E2 levels ≥500 pg/mL, respectively (p<0.001). The insemination time of 36-40 hours post HCG trigger also achieved better pregnancy outcome of 27.3%, compared with 13.4% of other time frame (<5hrs or >48hrs, p=0.05). Sperm count ≥10 x 10^6/mL compared with <10 x 10^6/mL also showed significantly higher pregnancy rate (26.2% vs.11.6%), respectively (p<0.001). Higher pregnancy rate was observed when endometrial thickness ≥8mm (25.9%).

CONCLUSION: This data examine the possible role of stratified every variable related to higher pregnancy rate in IUI patients. We confirm that AMH ≥2 ng/mL, ultra-long protocol, in patients with endometriosis and PCOS, age ≤35, insemination after 36-40 hours post HCG, sperm count ≥10 x 10^6/mL, endometrial thickness ≥8 mm and E2 ≥500 pg/mL at time of HCG, all are parameters of important contributing factors and significantly related to achieve higher likelihood of pregnancy.
CONCLUSION: In our study population, clinical pregnancy rates in women <35 with unexplained infertility undergoing their first CC/IUI cycle were not different with the higher initial CC dose. Contrary to the traditional practice of using 100 mg of CC in all couples, a starting dose of 50 mg in couples with female age <35 may be considered. The finding of statistically significantly increased rates of biochemical pregnancies on the lower CC starting dose warrants further prospective investigation.

P-502 Wednesday, October 22, 2014


OBJECTIVE: We have conventionally cultured thawed blastocysts for over 3 hours until embryo transfer. However, some reports showed that pregnancy rate would be improved by shortening culturing period until embryo transfer after thawing.

DESIGN: In this study, we tried to determine whether pregnancy rate would be affected by culturing period after thawing frozen blastocysts.

MATERIALS AND METHODS: Between Dec. 2008 and Dec. 2013, 293 patients received frozen blastocysts transfer in our hospital (570 cycles: c-IVF 356 cycles, ICSI 214 cycles). Among them, clinical pregnancy rates were compared by different culturing periods (culture of thawing blastocysts before transfer; over 3 hours (≥3h) vs. within 3 hours (<3h)) in each c-IVF and ICSI cycles. In addition, the influence of quality of transferred blastocysts (Gardner classification: over BB or under BB) and the day of frozen (D5 or D6) were also compared.

RESULTS: In the c-IVF cycles, we found no significant differences in pregnancy rate between ≥3h culture and <3h culture [36.0% (72/200) (≥3h) vs. 35.9% (56/156) (<3h)]. Pregnancy rates in each quality of blastocysts (BB or under BB) were not significantly different by different culture period (BB: 39.7% (56/141) (≥3h) vs. 42.4% (42/99) (<3h); under BB: 27.1% (16/59) (≥3h) vs. 24.6% (14/57) (<3h)). The day of blastocyst frozen was not influenced to the pregnancy rates [Day5: 40.0% (67/164) (≥3h) vs. 39.3% (48/122) (<3h); Day6: 13.9% (5/36) (≥3h) vs. 23.5% (8/34) (<3h)]. In the ICSI cycles, no significant differences were observed in pregnancy rate between ≥3h culture and <3h culture after blastocysts thawing [41.7% (45/108) (≥3h) vs. 34.9% (37/106) (<3h)]. Quality of blastocysts was not correlated to the clinical pregnancy rates [BB: 47.4% (37/78) (≥3h) vs. 44.3% (27/61) (<3h); under BB: 26.7% (8/30) (≥3h) vs. 22.2% (10/45) (<3h)]. Although the outcomes of D5 frozen blastocysts were not changed by the periods of culture after thawing in ICSI cycles [41.6% (32/77) (≥3h) vs. 42.6% (29/68) (<3h)], clinical pregnancy rate in D6 frozen blastocysts which were cultured over 3 h after thawing were significantly higher than the blastocysts which were cultured within 3 h after thawing [41.9% (13/31) (≥3h) vs. 21.1% (8/38) (<3h), p<0.05]. Because average age of patient in this group (D6 frozen, culture over 3h) was lower than the average age in another group (D6 frozen, culture within 3h), this difference might be the cause of patient age.

CONCLUSION: Our study showed that the pregnancy rate was not altered by the periods of culture after blastocyst thawing. Our study is ongoing.

P-503 Wednesday, October 22, 2014

ABNORMAL VILLOUS MORPHOLOGY IN ABORTUS SPECIMENS IS NOT INCREASED IN ART PREGNANCIES. G. Eko,1 J. Rabban,2 P. Rinaudo,2 1Center for Reproductive Health, University of California San Francisco, San Francisco, CA; 2Pathology Department, University of California San Francisco, San Francisco, CA.

OBJECTIVE: Increased incidences of placenta previa, abnormal cord insertion and abruption have been described following ART. Anecdotal experience suggests that abnormal villous morphology (AVM) is diagnosed more frequently in abortuses following ART. AVM specimens have a partial mole-like morphology and equivalent staining, but do not share the fully developed features of a partial mole. Further, AVM seems more frequent in aneuploid pregnancies. The objectives of this study were to determine the prevalence of AVM in ART pregnancies compared to non-ART pregnancies, and the ability of AVM to predict chromosomal abnormalities.

DESIGN: Retrospective Cohort Study.

MATERIALS AND METHODS: We identified 362 failed 1st trimester intrauterine pregnancy that were managed with manual uterine evacuation. The study was compared to 655 consecutive non-ART pregnancies.

RESULTS: AVM was seen in 4% of ART and 2.5% of non-ART pregnancies. The rate of AVM was not significantly different between ART and non-ART pregnancies. AVM share the fully developed features of a partial mole. Further, AVM seems more frequent in aneuploid pregnancies. The objectives of this study were to determine the prevalence of AVM in ART pregnancies compared to non-ART pregnancies, and the ability of AVM to predict chromosomal abnormalities.
aspiration (MUA) at the UCSF Center for Reproductive Health from 2008 to 2012. To be included, products of conception (POC) had to be evaluated by a pathologist and have identifiable villi. Chi-square analysis (PASW Statistics 18) was used to compare prevalence of AVM in spontaneous, IUI, IVF and ICSI pregnancies. A subgroup of 100 patients with valid cytogenetic results had slides further reviewed by a blinded expert pathologist, and sensitivity of AVM in predicting aneuploidy was determined.

RESULTS: The prevalence of AVM was not statistically different among groups (Table 1, p=0.09). Of the 100 patients with valid cytogenetic testing, 63% were aneuploid. Prevalence of aneuploidy did not differ by treatment type (p=0.13). The sensitivity of AVM diagnosis in detecting chromosomal abnormalities was 11% by general pathologists and 48% by an expert pathologist, with a positive predictive value of 70% and 79%, and an overall of 8% and 22% respectively.

Table 1

<table>
<thead>
<tr>
<th>Age (mean±SD)</th>
<th>GA at MUA (mean±SD)</th>
<th>AVM prevalence (%)</th>
<th>Aneuploidy prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous</td>
<td>37.0±3.9</td>
<td>8.1±1.3</td>
<td>18/77 (23)</td>
</tr>
<tr>
<td>IUI</td>
<td>38.1±3.4</td>
<td>7.9±1.1</td>
<td>15/93 (16)</td>
</tr>
<tr>
<td>IVF</td>
<td>38.4±3.4</td>
<td>7.5±1.1</td>
<td>15/60 (25)</td>
</tr>
<tr>
<td>ICSI</td>
<td>39.1±3.9</td>
<td>7.8±1.4</td>
<td>16/130 (12)</td>
</tr>
<tr>
<td>Total</td>
<td>38.2±3.8</td>
<td>7.8±1.3</td>
<td>64/560 (18)</td>
</tr>
</tbody>
</table>

CONCLUSION: Reassuringly, the prevalence of AVM is not increased in ART pregnancies. An expert pathologic diagnosis of AVM may alert clinicians about potential chromosomal abnormalities, however improvement in diagnostic sensitivity is needed.

P-504 Wednesday, October 22, 2014

OCYTE DONOR GENETIC SCREENING PRACTICES. E. Moyle, A. Mathiesen, E. Johnstone, R. Hulinsky. University of Utah, Salt Lake City, UT.

OBJECTIVE: To assess current genetic screening practices of oocyte donor applicants in the United States.

DESIGN: An electronic questionnaire was distributed to 202 oocyte donation facilities (159 fertility clinics & 43 oocyte donation agencies) to evaluate genetic screening practices and consistency with guidelines.

MATERIALS AND METHODS: Contact e-mail addresses for one hundred eighty fertility clinics and seventy-three egg donor agencies were collected (a total of 253). These facilities were sent an e-mail invitation to participate in the current study regarding genetic screening practices for oocyte donors. Twenty-one clinics and thirty oocyte donation agencies were excluded based on invalid e-mail addresses or a request to be removed from the e-mail list. A reminder survey invitation was redistributed three additional times to the remaining two hundred and two facilities (159 fertility clinics & 43 egg donation agencies). The questionnaire inquired about type of facility, donor family history risk assessment, genetic testing performed on donors, genetic testing consent process, and factors influencing facility practice. The questionnaire was distributed through the online survey tool, REDCap.

RESULTS: Seventy-seven responses were received. Ninety-six percent (74/77) of facilities assessed donors' family histories and 52% (40/77) of these facilities routinely inform donors about the results of these evaluations. Eighty-eight percent (68/77) of facilities reported screening all donors for the same conditions regardless of ethnicity or family history. Sixty-four percent (49/77) of facilities provide a consultation to donors regarding the genetic screening performed. Professional organization guidelines were identified as the most influential factor on genetic screening policies at an average of 62% of facilities.

CONCLUSION: The majority of facilities report professional organization guidelines are the most influential factor with regards to development of institutional policies regarding genetic screening; however, when reporting what screening is being performed it became clear that most of the facilities are not actually following current guidelines. These data illustrate that there is room for improvement in the implementation of genetic screening practices at oocyte donation facilities. The authors propose development of a consensus guideline with representatives from genetic and reproductive care professionals, which will clarify conflicting guidelines, ensuring high quality reproductive care for all parties involved.

Supported by: University of Utah Graduate Program of Genetic Counseling Research Stipend; National Society of Genetic Counselors Assisted Reproductive Technology Special Interest Group Research Grant Award.

P-505 Wednesday, October 22, 2014


OBJECTIVE: Despite a growing number of patients utilizing third-party reproductive options, there is little data describing the information available to these patients on the Internet. Our objective was to evaluate SART-member fertility clinic websites for the availability and quality of information on third-party reproduction.

DESIGN: Cross-sectional evaluation.

MATERIALS AND METHODS: Between 2/2014-4/2014, 396 SART-member fertility clinic websites were evaluated by two independent researchers. Websites were surveyed for the following characteristics: practice location, type and size, presence of donor egg and donor embryo programs, information for donors, recipients, gestational carriers (GC) and family building for Lesbian Gay Bisexual Transgender (LGBT) couples and individuals. Chi-square tests were used to assess for differences between groups.

RESULTS: 98% (387/396) of clinics had a website, of which 80% were private and 20% academic. 26% of practices performed ≥500 cycles/year while 74% performed <500 cycles/year. 85% of clinics offered information for egg donor recipients and 56% offered information for potential egg donors. 67% had egg donor programs, 27% had donor embryo programs, and 8% had GC availability. Larger practices were more likely to advertise donor egg programs (77% vs. 66%, p=0.04) and were more likely to provide information on GC (64% vs. 47%, p=.005). Compared with academic clinics, private practices were more likely to have donor embryo programs (30% vs. 17%, p=.02), but there was no difference in donor egg availability (69 vs. 60%, p=.12). Of practices that have egg donor availability, 14% provided public information on donor traits. 33% of websites provided LGBT reproductive options with private more likely than academic clinics to advertise these services (35% vs. 22%, p=.02). Large volume practices were also more likely to advertise LGBT services (44 vs. 29%, p=.007). Percentage of practices in the West (49%) and Northeast (44%) providing LGBT options were the highest, as compared with the Midwest (19%) and South (20%), p<.001.

CONCLUSION: There is considerable heterogeneity regarding information on third-party reproduction on patient-oriented websites. While many fertility clinic websites provide general information about third party reproduction and egg donation, there is still a significant lack of information describing family building options for LGBT individuals. There is also a significant difference in the regional distribution of practices providing this information.

P-506 Wednesday, October 22, 2014


OBJECTIVE: Class I data demonstrate that selection of euploid blastocysts results in enhanced implantation and delivery rates, approaching 40% for single embryo transfers. While reassuring, there are a substantial number of "optimally selected" embryos which fail to implant. Time-lapse imaging parameters through day 3 of development correlate with blastulation rate, but do these same parameters extend to selection between euploid blastocysts? The purpose of this study is to determine if time lapse imaging can provide meaningful data which might aid in distinguishing euploid
blastocysts which will implant and progress to delivery from those destined to fail.

DESIGN: Prospective blinded observational.

MATERIALS AND METHODS: Patients with normal ovarian reserve were recruited for participation. Time-lapse imaging utilizing the Eeva® System (Auxogyn, Menlo Park, CA) was recorded on all embryos through the blastocyst stage. Each blastocyst underwent trophoderm biopsy for comprehensive chromosomal screening. Euploid blastocysts were transferred and outcome data collected. In the case of two embryo transfers where only one progressed in development, DNA fingerprinting was used to correctly establish the identity of the competent embryo and link this data with time-lapse imaging.

RESULTS: 100 transferred embryos were included in the analysis, 52 embryos implanted and progressed through development while 48 did not. The time-lapse imaging cleavage parameters were compared with implantation outcome and no significant difference was noted in any of the following parameters: time from IC toini (p=0.64), syngamy (p=0.78), duration of 1st cytokinesis (p=0.82), duration of 2-cell stage (p=0.19), duration of 3-cell stage (p=0.82), duration of 4-cell stage (p=0.32), time from start of 1st cytokinesis to start of cavitation (p=0.65).

CONCLUSION: Cleavage stage parameters generated from time-lapse imaging do not distinguish outcomes between euploid blastocysts. These parameters do not provide a selection advantage to assist the embryologist in choosing which euploid blastocyst is more likely to implant or deliver. Further investigation is needed to determine if there are additional morphokinetic parameters evaluable through the blastocyst stage that could meaningfully improve embryo selection. At the current time, there are no adjuncts, including time-lapse imaging, which improve selection beyond the combination of traditional morphologic selection at the blastocyst stage with CCS.

Supported by: Auxogyn; Equipment.

P-507 Wednesday, October 22, 2014

EMBRYOLOGIST INTERPRETATION OF TIME-LAPSE IMAGING PARAMETERS AT THE BLASTOCYST STAGE DO NOT ALTER SELECTION AMONG TRANSFERRED EUPLOID BLASTOCYSTS. K. H. Hong, a M. D. Werner, a J. M. Fransiak, a E. J. Forman, a,b A. Prodoehl, a K. Upham, a K. Scott, b R. T. Scott, Jr., c,d RWJ, Rutgers, Basking Ridge, NJ, a RMA NJ, Basking Ridge, NJ, a,b RMACT, Norwalk, CT.

OBJECTIVE: Time-lapse imaging software has been successfully utilized at the cleavage stage to predict which embryos will blastulate. However, the literature does not contain an analysis of time-lapse imaging parameters during extended culture to the blastocyst stage and clinical outcomes. The goal of the present analysis was to determine if the interpretation of time-lapse images by experienced embryologists offered a selection advantage between high quality euploid blastocysts.

DESIGN: Prospective blinded observational.

MATERIALS AND METHODS: Patients undergoing IVF < age 42, with normal ovarian reserve, were recruited for participation. Time-lapse imaging with the Eeva® System (Auxogyn, Menlo Park, CA) was recorded on all embryos through the morning of day 5. All embryos underwent trophoderm biopsy for comprehensive chromosomal screening. Euploid blastocysts were transferred and implantation and pregnancy data was collected. DNA fingerprinting was used to establish the identity of the competent embryo and link this data with time-lapse imaging. In the case of two embryo transfers where only one progressed in development, DNA fingerprinting was used to correctly establish the identity of the competent embryo and link this data with time-lapse imaging.

RESULTS: 5 embryologists individually viewed 100 time lapse videos and recorded parameters as outlined above. Of the 100 embryos included in this analysis, 52 embryos implanted and progressed through development while 48 did not. The time-lapse imaging cleavage parameters were compared with implantation outcome and no significant difference was noted in any of the following parameters: time from IC toini (p=0.64), syngamy (p=0.78), duration of 1st cytokinesis (p=0.82), duration of 2-cell stage (p=0.19), duration of 3-cell stage (p=0.82), duration of 4-cell stage (p=0.32), time from start of 1st cytokinesis to start of cavitation (p=0.65).

CONCLUSION: Cleavage stage parameters generated from time-lapse imaging do not distinguish outcomes between euploid blastocysts. These parameters do not provide a selection advantage to assist the embryologist in choosing which euploid blastocyst is more likely to implant or deliver. Further investigation is needed to determine if there are additional morphokinetic parameters evaluable through the blastocyst stage that could meaningfully improve embryo selection. At the current time, there are no adjuncts, including time-lapse imaging, which improve selection beyond the combination of traditional morphologic selection at the blastocyst stage with CCS.

Supported by: Auxogyn; Equipment.

P-508 Wednesday, October 22, 2014

LIVE BIRTH RATE FOLLOWING IVF/ICSI IN PATIENTS WITH SPERM DNA FRAGMENTATION: A SYSTEMATIC REVIEW AND META-ANALYSIS. G. M. AlKusayer, a,b N. Amily, a A. M. Abou-Setta, a M. A. Bedaiwy, a Division of Reproductive Endocrinology and Infertility, University of British Columbia, Vancouver, BC, Canada; a,b Department of Clinical Sciences, Faculty of Medicine, Princess Nora Bint Abdulrahman University, Riyadh, Saudi Arabia; a Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada; a George & Fay Yee Centre for Healthcare Innovation, University of Manitoba, Winnipeg, MB, Canada.

OBJECTIVE: Our aim was to examine the association between DNA fragmentation index (DFI) levels and clinical outcomes in couples undergoing in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI).

DESIGN: Systematic review and meta-analysis.

MATERIALS AND METHODS: We searched MEDLINE, EMBASE and the Cochrane Library (inception to April 2014) using the following search terms: DNA Fragmentation or DNA damage combined with IVF, ICSI, pregnancy, pregnancy loss, miscarriage or live birth. We included studies investigating the association between sperm DNA fragmentation and clinical outcomes in couples undergoing IVF or ICSI. The primary outcome was live birth rate. Secondary outcomes were clinical pregnancy and miscarriage rates. DFI cut-off values varied in the assays conditions and threshold values. In three studies the threshold value of high DFI was more than 30%. Both Sperm Chromatin Structure Assay and Sperm Chromatin Dispersion. Two studies used Comet assay with a threshold of more than 50%. One study only reported a threshold of more than 45% or equal to >50% using tUNE Lassay. Study selection, data extraction and quality assessment was conducted independently by two reviewers. Meta-analysis was conducted using a random-effects model.

RESULTS: Six cohort studies (2460 couples) meeting the inclusion criteria were identified. Across the studies, there were more live births and clinical pregnancies in couples with low DFI index undergoing IVF/ICSI compared to couples with high DFI index (RR 1.55 [1.01 to 2.39] and RR 1.20 [0.86 to 1.68], respectively). Miscarriage rates were also lower in patients with low DFI (RR 0.56 [0.50 to 1.03]). Overall there was evidence of moderate to high heterogeneity in most analyses not explained by subgroup analyses of the method of fertilization (IVF vs. ICSI), timing of transfer (fresh vs. frozen embryo transfer), or methodological concerns (e.g. unit of analysis). These results were limited by different assays thresholds for the DNA fragmentation levels and the methodological quality of the included studies.

CONCLUSION: Our systematic review suggests that couples with low DNA fragmentation index levels are more likely to have better clinical outcomes compared to those with higher DNA fragmentation following IVF/ICSI.

FERTILITY & STERILITY® e305
MATERIALS AND METHODS: We have developed a novel computational system that simulates the genetics of gamete production and zygote formation to create “virtual progeny” from the DNA sequences of any two people. This process is applied to reproducibly pair male and female participants in the “1000 Genomes Project” based on publicly available exome sequences. The output of a single pairing is a 1,000 unique virtual progeny genomes. Each digital genome is analyzed for DNA sequence combinations that could cause one or more recessive Mendelian diseases based on clinical annotations and the total expected outcome of functional gene products from the two copies of each gene. The data are integrated to produce a detailed assessment of reproductive disease risk.

RESULTS: Genetic screening of simulated genomes from over 50,000 pairings uncovered reproductive risk far beyond that exposed by carrier screening of individual persons. Based on coding regions interrogated in over 400 genes, all “donors” for the purposes of this investigation were considered healthy reproductive matches for multiple “clients.”

CONCLUSION: Virtual progeny analysis can provide a comprehensive approach to the genetic screening of sperm donors in two distinct ways: 1) by incorporating client DNA information as a necessary input into risk analysis and 2) by selectively avoiding sperm donors based on the analysis of recessive genetic disease risk in the virtual offspring of qualified sperm bank donors and clients. 

Supported by: GenePeeks, Inc.

P-510 Wednesday, October 22, 2014

CLOMIPHENE CITRATE (CC) ADMINISTRATION CHANGES CERVICAL MUCUS PERMEABILITY IN PATIENTS UNDERGOING FERTILITY TREATMENTS. V. V. Snegovskikh.1,2 a, K. B. Smith-Dupont.3 M. House.4 K. Ribbeck.5 K. Pagidas.6,7 1OB/Gyn, Women and Infants Hospital, Providence, RI; 2Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA; 3OB/Gyn, Tufts Medical Center, Boston, MA.

OBJECTIVE: The properties of cervical mucus (CM) have been investigated for many years, but limited data is available regarding its changes in patients undergoing different types of fertility treatments. In this study we use microfluidics technology to investigate the permeability properties of CM from ovulation induction (OI) with CC and natural cycle patients.

DESIGN: Pilot case control study.

MATERIALS AND METHODS: Patients presented to our clinic on the day of ovulation for intrauterine insemination (IUI). After informed consent was obtained CM samples were collected via speculum exam using insulin syringe directly from the cervix. Specimens were snap frozen in liquid nitrogen and stored at -80°C until use. They did not contain any blood or tissue.

Microfluidic devices were fabricated from polydimethylsiloxane (PDMS) as described previously (1), 2–5. µL of whole mucus sample was pumped into the channel. Fluorescently labeled positively (PCP) and negatively charged peptides (NCP) were used as reporters for permeability. The transport peptides were flowed by gravity orthogonally to the mucus channel and the mucus-water interface. Images of fluorescent peptide transport were acquired every 10 sec for at least 15 min using an inverted epifluorescence microscope (IX-71, Olympus, Central Valley, PA) equipped with a 10x objective, a light-emitting diode excitation source and a cooled camera.

RESULTS: NCPs showed a similar transport behavior in OI and natural cycle mucus. In both cases the average peptide concentration as a function of distance along the length of the 300 µm microfluidic channel follows a similar trend as observed with phosphate buffer (H7), indicating that peptides diffuse largely unhindered through both types of CM samples. However, the mucus differed in permeability toward PCPs. In a group of OI patients (N = 14) receiving CC100 mg from days 3 to 7 of the cycle we observed a greater concentration of PCPs within 100 µm from the mucus barrier compared to natural cycle ovulatory patients (N = 7). This indicates that CM from OI patients may be more adhesive toward positively charged epitopes.

CONCLUSION: CM from women undergoing OI with CC has altered permeability properties compared to CM from natural cycle patients. This difference in mucus permeability could be clinically significant, as it may decrease the number of available sperm at the time of ovulation and prove the need of such procedure as IUI.

Supported by: Burroughs Wellcome Fund Preterm Birth Initiative.

P-511 Wednesday, October 22, 2014

DHEA SUPPLEMENTATION RESULTS IN SUPRAPHYSIOLOGIC DHEA-S SERUM LEVELS THAT INTERFERE WITH PROGESTERONE (P) IMMUNOASSAYS, RESULTING IN SPURIOUS P ELEVATIONS THAT MAY ALTER CLINICAL MANAGEMENT IN IVF. E. J. Forman, a,b J. M. Franskaia, a,b K. Scott, c E. K. Dubell, c M. Fano, a S. Ng, a R. T. Scott, Jr., a,b RMANJ, Basking Ridge, NJ; cRWJ, Rutgers, Basking Ridge, NJ; bRMACT, Norwalk, CT.

OBJECTIVE: Despite a lack of prospective evidence of benefit, DHEA is being increasingly used for poor responders in an attempt to improve response to stimulation. The impact of the resulting supraphysiologic DHEA-S serum levels on sex steroid assays has not been evaluated. Since late follicular rises in P have been shown to adversely affect outcomes from fresh embryo transfers, even modest alterations in this assay may impact clinical management. This study seeks to determine the relationship between DHEA supplementation and P measurements to characterize the degree of interference with particular immunoassays.

DESIGN: Prospective, endocrine.

MATERIALS AND METHODS: DHEA-S control reagents with no P present (Advia Centaur, Siemens) were assayed for DHEA-S and P levels. Serum pools were created from IVF patients who were not on DHEA supplementation. Baseline DHEA-S and P was measured in these pools. Increasing amounts of DHEA-S was then added to the pooled serum and P was measured in quadruplicate on 3 different immunoassay instruments (Advia Centaur, Immulite 2000 – Siemens; eCobas 411 – Roche). The mean values were compared using Pearson’s correlation.

RESULTS: When measuring the manufacturer’s DHEA-S controls (Centaur), there was a linear increase in the P detected, ranging from 0 ng/mL in the blank control (no DHEA-S) to 1.5 ng/mL in the high control (DHEA-S >1500 pg/mL). The mean DHEA-S of the pooled serum was 142 pg/mL. The Centaur and eCobas assays had a linear relationship between DHEA-S added and the measured P value (P<0.05), with the eCobas having the steepest slope.

P-512 Wednesday, October 22, 2014


OBJECTIVE: Newborns after assisted reproductive techniques (ART) usually have poorer perinatal outcome when compared to newborns after spontaneous conception. Low birthweight, preterm birth and proportion of large for gestational age have been attributed to in vitro culture negative effects. The purpose of the study was to analyse if in vitro culture of embryos can influence perinatal outcome of infants born after intracytoplasmic sperm injection and from ART pregnancies.

CONCLUSION: DHEA-S can interfere with standard P immunoassays used in clinical ART programs. These spurious P elevations may reach clinical ranges shown to be detrimental in fresh IVF cycles, potentially altering clinical management. As many programs incorporate late follicular P levels when making clinical decisions (timing of hCG administration, whether to cryopreserve all embryos), it is essential that each program identify the effect of DHEA supplementation on steroid assays. Further research is needed regarding the pharmacokinetics of DHEA supplementation to determine when it can be discontinued prior to an IVF stimulation so as not to interfere with assays.

Concentrations of Progesterone on different assays (ng/mL) 

<table>
<thead>
<tr>
<th>DHEA-S added (ug/mL)</th>
<th>Centaur</th>
<th>eCobas</th>
<th>Immulite</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.63</td>
<td>0.56</td>
<td>0.75</td>
</tr>
<tr>
<td>307</td>
<td>0.83</td>
<td>1.24</td>
<td>0.66</td>
</tr>
<tr>
<td>722</td>
<td>1.19</td>
<td>2.00</td>
<td>0.83</td>
</tr>
</tbody>
</table>

There was no correlation between measured P and increasing DHEA-S on the Immulite 2000.
injection (ICSI) compared to infants born after timed intercourse or intrater-
inine insemination (UI).

DESIGN: A retrospective cohort of patients attending a fertility clinic in
Germany between January 2011 and March 2013 were included in the study.
Singletons and twins born after fresh ICSI cycles (n=206 and 87, respect-
tively) were compared to singletons and twins born after UI and timed inter-
course (n=63 and 10, respectively).

MATERIALS AND METHODS: Patients having in vitro culture (5.5%
OC2, 5.2%) were divided into two groups, SI (n=204) and TLI (Embryo-
ScopeTM, n=89). Perinatal outcomes were compared among newborns in
the groups SI, TLI and ‘in vivo’ incubation (IVI). Continuous variables
were tested by ANOVA and binary variables by Fischer’s exact test.

RESULTS: We could not observe any significant difference in singletons
among the groups IVI, TLI and SI, respectively, regarding gestational age at
birth (37.89 ± 0.4 weeks, 37.80 ± 0.21, 35.10 ± 0.16 and 37.66 ± 0.15), birthweight
(3416 ± 69.8, 3362 ± 65.3 and 3280 ± 46.3), size (51.6 cm ± 0.3, 51.1 ±
0.4 and 50.8 ± 0.3), gender (66.0%, 49% and 47% boys), Z-score (0.70 ±
0.13, 0.45 ± 0.11 and 0.52 ± 0.07) and malformations (0, 1 and 2 infants).
Interestingly, there were less preterm birth (GA < 37 weeks) in the TLI group
than in the SI group (4 versus 30, respectively, P=0.02, relative risk 2.93, CI
1.09 to 8.030) after singleton pregnancy. Data are presented as mean ± stan-
dard error.

CONCLUSION: In vitro culture of embryos seems not to affect primary
perinatal outcome of newborns after ICSI compared to infants after timed
intercourse and UI. However, use of time-lapse incubation (TLI) could signif-
ically reduce number of preterm birth in singletons. Improving current
culture system in ART laboratories may be a valuable step towards more
physiological and embryo-friendly in vitro culture. Optimizing culture media
and incubation systems is thus urgently needed in order to minimize poorer
neonatal outcomes in ART newborns.

P-513 Wednesday, October 22, 2014
CARRIER SCREENING OF 22,296 PATIENTS IN THE IVF SETTING
UTILIZING NEXT GENERATION DNA SEQUENCING DETECTS
COMMON, UNCOMMON & OTHERWISE UNDETECTABLE
MUTATIONS IN PREVALENT, SOCIETY-RECOMMENDED
DISORDERS. S. Hallam, D. Neitzel, J. Brennan, V. Greger. Good Start
Genetics, Inc., Cambridge, MA.

OBJECTIVE: Carrier screening for specific genetic disorders is recom-
manded by the American Congress of Obstetricians and Gynecologists
(ACOG), the American College of Medical Genetics (ACMG), and soci-
eties representing the Ashkenazi Jewish population. Due to cost consider-
ations and technological limitations, traditional carrier screening assays
are designed to detect only the most common mutations in a gene. This
older approach can yield high detection rates in specific populations
(e.g., Ashkenazi Jewish); however, it can be suboptimal for other ethnic-
ities and for patients of mixed or unknown ethnic background. Next-gen-
eration DNA sequencing (NGS) is able to test for five- to ten-fold more
pathogenic mutations and hence has the potential to yield higher detection
rates across ethnicities compared to traditional carrier tests. Consequently,
NGS is expected to provide a more comprehensive deter-
mination of carrier status. Our objective was to evaluate the clinical effect-
niveness of NGS-based screening for carriers of society-recommended disorders.

DESIGN: Using NGS, we evaluated carrier status for up to 14 disorders (as
ordered by physicians in IVF centers) for 22,296 patients.

MATERIALS AND METHODS: A high-throughput and proprietary
methodology (comprised of multiplex gene capture, NGS and computational
analysis) was used to test samples from patients representing a broad spec-
trum of ethnicities. Clinical reports were issued on the presence or absence
of disease-causing mutations in genes associated with society-recommended disorders.

RESULTS: Among the 22,296 clinical samples evaluated, our NGS-based
tests routinely detected common mutations among 14 disorders, as well as
numerous less common mutations that would not be detected by traditional
screening assays routinely used in IVF centers. 1,463 (6.6%) patients were
found to be carriers of 225 distinct pathogenic mutations among the 14 dis-
orders. 149 (63.4%) of those distinct pathogenic mutations were either un-
common or never-before reported, i.e., unique to Good Start Genetics’ set
of mutations. Of the 1,463 carriers detected, 12.3% - 17.9% would have
been missed by other major laboratories using traditional carrier tests, putting
the patients at risk of having a child with a genetic disorder.

CONCLUSION: Due to the more extensive set of pathogenic mutations
detectable for the genes assessed, NGS enables more comprehensive exam-
ination of carrier status, and is, therefore, able to yield higher detection rates
resulting in fewer missed carriers than if traditional carrier tests were used.
Supported by: Good Start Genetics, Inc., Cambridge, MA. All authors are
employees of Good Start Genetics.

P-514 Wednesday, October 22, 2014
VALIDATION OF ANTI-MULLERIAN HORMONE FOR PREDICT-
NING OVARIAN RESPONSE: DATA FROM THE PURSUE
TRIAL. V. Schnell, A. Dokras, C. Slater, B. Stegmann, K. Gordon, P.
Vercei. Center of Reproductive Medicine, Webster, TX; Univ of Penn
Medical Center, Philadelphia, PA; Idaho Center for Reproductive Medicine,
Boise, ID; Merck, Whitehouse Station, NJ; MSD Oss, Oss, Netherlands.

OBJECTIVE: To assess the value of Anti-Mullerian hormone (AMH) in
predicting ovarian response during ovarian stimulation in women aged 35-
42 with comorilfolitropin alfa (CFA) or recombinant follicle-stimulating
hormone (rFSH).

DESIGN: Retrospective analysis of data from the PURSUE trial.

MATERIALS AND METHODS: Women were randomized to a single in-
jection of 150 µg CFA (n=694) or daily 300 UI rFSH (n=696) for the first 7
days of ovarian stimulation in a gonadotropin-releasing hormone (GnRH)
antagonist protocol. Antral follicle count ( AFC ) and serum AMH, follicle
stimulating hormone (FSH), luteinizing hormone ( LH ), estradiol ( E2 ) and pro-
gesterone ( P ) were measured at baseline. Ovarian response was categorized as
low (<16 oocytes), normal (6-18 oocytes) or high (>18 oocytes). Multivariable
logistic regression models were constructed for high and low ovarian response
separately, treatment (CFA or rFSH) was included in all models.

RESULTS: AMH was the strongest predictor of ovarian response
(P<0.0001), followed by AFC (P<0.0001) and age (0.0007); other hormones were not significantly associated. Increasing AMH
and AFC were associated with a higher probability of high ovarian response;
increasing FSH and age were associated with a lower probability of high
ovarian response.

Oocytes Retrieved and Response Category by Treatment Group

<table>
<thead>
<tr>
<th>CFA</th>
<th>rFSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.7</td>
<td>10.3</td>
</tr>
<tr>
<td>24.1%</td>
<td>25.1%</td>
</tr>
<tr>
<td>62.0%</td>
<td>63.2%</td>
</tr>
<tr>
<td>14.0%</td>
<td>11.6%</td>
</tr>
</tbody>
</table>

**MULTIVARIATE REGRESSION**

<table>
<thead>
<tr>
<th>OR 95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMH [ng/mL]</td>
<td>1.93 1.68-2.21</td>
</tr>
<tr>
<td>AFC [count]</td>
<td>1.15 1.09-1.22</td>
</tr>
<tr>
<td>FSH [IU/L]</td>
<td>0.83 0.73-0.94</td>
</tr>
<tr>
<td>Age [years]</td>
<td>0.88 0.80-0.96</td>
</tr>
<tr>
<td>Treatment Effect</td>
<td>1.42 0.97-2.07</td>
</tr>
<tr>
<td>(CFA vs. rFSH)</td>
<td>0.95 0.71-1.11</td>
</tr>
</tbody>
</table>

*15 subjects (1.1%) were excluded due to missing values. Odds ratio is per
unit increase. (For) adjustment for AMH, AFC, FSH, and age.

After adjusting for these factors, subjects receiving CFA tended to have a
higher probability for high response, but there was no difference between
treatment groups for low response (table 1).

CONCLUSION: AMH was the strongest predictor of ovarian response in
women ages 35 to 42. A trend towards higher ovarian response with CFA
was observed in the high response group.

Supported by: Merck & Co., Inc., Whitehouse Station, NJ, USA.

P-515 Wednesday, October 22, 2014
A SIMPLE SPERM HYPERACTIVATION ASSAY - FOR SELECTING
THE OPTIMAL BLASTOCYST FOR TRANSFER BASED ON SPENT
MEDIA SECRETOME. J. D. Jacobson. Center for Fertility and IVF, Gyn/Ob Dept.
Loma Linda University School of Medicine, Loma Linda, CA.

OBJECTIVE: Different signaling molecules or metabolic secretomes have
been detected from healthy and defective human embryos in culture. A
preliminary study showed that healthy embryos linked to subsequent pregnancies could be distinguished from abortous embryos by differences in extended and affected sperm behavior. The objectives were: (a) to verify observed differences in motility parameters after incubating the sperm in spent media of embryos from pregnant and non-pregnant cases and (b) to differentiate between stimulatory or inhibitory action of the secretomes.

**DESIGN:** Retrospective study.

**MATERIALS AND METHODS:** Cryopreserved-thawed sperm were washed by 2-centrifugation and resuspended in modified HTF medium. Twenty-four-hour spent media (G-2 ver 5) were collected from IVF-derived individual blastocyst on Day 5. A single blastocyst was transferred to each recipient that either became pregnant (N=5) or nonpregnant (N=5). An aliquot (100 µL, 50 M/mL) of sperm was added to each spent medium from either pregnant cycles, non-pregnant cycles or fresh control medium. Sperm were also added to unused droplets of spent media in each culture dish (matching spent controls). Computer-aided sperm analyses were performed at 0 and 4 hours of incubation (heated 40°C). Data were expressed as percent of matching controls (5 experiments) and analyzed (ANOVA, Student’s t-test).

**RESULTS:** Sperm hyperactive motility was 4.3 fold higher (P<0.05) in spent media in the pregnant group (160.0 ± 1.4% of control; mean ± SEM) when compared with the non-pregnant group (36.8 ± 0.4% of control).

Furthermore, sperm progressive motility was greater in the pregnant (143.3 ± 5.2% of control) versus non-pregnant group (112.2 ± 5.6% of control). Additionally, straight line velocity (VSL) of sperm was greater in the pregnant group. However, there were no differences in the remaining parameters. A comparison of sperm hyperactivity indicated an inhibitory effect of the secretomes.

**CONCLUSION:** The results demonstrated embryo-derived secretomes in the spent media that affected specific sperm motility parameters, particularly hyperactivation linked to Catserp Cal2+ ion channels. The findings suggested that the secretomes from blastocysts in the non-pregnant group were inhibitory to membrane ion transport. The significance here was the novel use of a simple sperm hyperactive motility assay to identify healthy embryos for transfer and the finding of motility-inhibiting molecules from damaged but normal appearing embryos.

---

**P-516 Wednesday, October 22, 2014**

**PREDICTING IMPLANTATION POTENTIAL USING MORPHOMETRIC ANALYSIS OF BLASTOCYSTS AND CALCULATING FOR PHI TO DETERMINE A REFERENCE POINT.** W. E. Routdebush,a S. E. Williams,a D. A. Forstein,a B. A. Lessey,a C. E. Likes, III,a P. B. Miller,a Obestetrics & Gynecology, Greenville Health System University Medical Center, Greenville, SC; aBiomedical Sciences, University of South Carolina School of Medicine Greenville, Greenville, SC.

**OBJECTIVE:** Embryo grading is a mixture of subjective/objective assessments (e.g. cell stage/number; percent fragmentation) that has been minimally linked with implantation potential. Previously, we found that blastocyst ratios (length-width across the inner cell mass (ICM) axis) closely approximate phi (P<1.618, AKA the Golden Mean or Ratio) in successful implantations (Routdebush et al., 2014). To further explore phi’s potential in ART, this study calculated and compared ratios derived from ICM and total blastocyst areas of high quality classified embryos reported to have either successfully implanted or failed to implant.

**DESIGN:** Retrospective post-transfer analysis of day-5 blastocyst (graded as high quality at time of embryo transfer) ICM and total area ratios were calculated for phi, and analyzed with pregnancy outcomes.

**MATERIALS AND METHODS:** Day-5 blastocyst stage embryo images, graded as being of high quality at the time of embryo transfer, were measured for ICM and total areas using tpsDIG2 software (life.bio.sunysb.edu/morph/). Area ratios were calculated. Receiver operator curve (ROC) and Student’s-t test were used to assess to pregnancy outcomes.

**RESULTS:** A total of 21 highly graded day-5 blastocysts were evaluated. Ratios ranged from a low of 0.822 (highly expanded) to a high of 2.086 (minimal expansion) with a mean of 1.272 (78.6% of the Golden Ratio). An ROC demonstrated a definitive phi cut-off value (i.e. criterion) for a viable pregnancy of 1.31: criterion >1.31, specificity 100.0, and sensitivity 71.4. With this criterion, pregnancy rates between the “implanted” phi group (>1.31 ng/mL; 90.9% pregnancy rate) and the “failed-to-implant” phi group (<1.31 ng/mL; 40.0% pregnancy rate) were significantly different (P < 0.001).

**CONCLUSION:** Blastocyst stage embryo ratios (measured for ICM/total area) approximating phi were found to provide a more objective assessment of morphology than traditional approaches. Moreover, degree of blastocyst expansion appears to affect comparisons. While our initial data permit a calculated “cut-off value” or reference point, to achieve a viable pregnancy, this value does not include poor or low blastocyst grades. However, these results suggest calculating blastocyst area ratios relative to phi may assist in predicting pregnancy potential by offering a more objective means of selecting embryos at the time of transfer.

---

**P-517 Wednesday, October 22, 2014**

**EARLY ONSET OF CABERGOLINE THERAPY FOR PROPHYLAXIS AGAINST OVARIAN HYPERSTIMULATION SYNDROME: A POTENTIALLY SAFER AND MORE EFFECTIVE PROTOCOL.** S. S. Gaafar,a D. A. El-Gezary,a H. A. El Maghraby,a Obestetrics, Gynecology and Reproductive Medicine, El Shathy University Hospital, Alexandria University, Alexandria, Egypt; aClinical Pathology, Faculty of Medicine, Alexandria University, Alexandria, Egypt.

**OBJECTIVE:** The objective of this study is to investigate the effectiveness of early onset of cabergoline therapy as a prophylactic measure against ovarian hyperstimulation syndrome (OHSS) in high risk patients prepared for ICSI using the antagonist protocol.

**DESIGN:** A case series study.

**MATERIALS AND METHODS:** The study was conducted on 126 high risk patients prepared for ICSI using the fixed antagonist protocol. High risk patients for OHSS was defined as those having more than 20 follicles more than 13 mm in diameters and/or having a serum estradiol more than 3000 pg/ml when the size of the leading follicle reaches 15 mm. When the size of the leading follicle reaches 15 mm 0.5 mg/day cabergoline therapy is initiated and is continued for 8 days. Gonadotropins and antagonists were continued till the leading follicles reach 19 mm. Ovulation was triggered using both gonadotropin releasing hormone agonist and 2500 IU of human chorionic gonadotropins. Patients were followed up clinically and hematologically. Cases who developed OHSS were classified according to Navot classification (1). Day 5 blastocyst transfer was done to all cases.

**RESULTS:** The average stimulation days in the studied group was 10.38 ± 1.7 days and the final estradiol level was 6099.5 ± 2730.4 pg/ml. The mean number of retrieved oocytes was 19.7 ± 7.8. There was no significant change in hematological (hemoglobin, WBCs, RBCs, platelets and hematocrit), renal function tests (urea, creatinine) liver function tests (ALT, AST, serum albumin) (P>0.05) in the day of embryo transfer (ET) compared with pre-treatment values. The incidence of severe OHSS was 1/126 (0.8%), while moderate OHSS was 12/126 (9.5%). There was no cases of critical OHSS. Clinical pregnancy rate among the studied group was 49.2%.

**CONCLUSION:** To the best our knowledge this is the first study to demonstrate that early administration of cabergoline is a safe and potentially more effective approach in the prophylaxis against OHSS in high risk cases. Application of this protocol in cases that are planned for blastocyst transfer will enable completion of the course and hence assessment of success of cabergoline before transfer, so resistant cases can be candidate for cryopreservation of all embryos and cancellation of transfer.

---

**P-518 Wednesday, October 22, 2014**

**THE ACCURACY OF BLASTOCOEFLUID COMPREHENSIVE CHROMOSOMAL SCREENING (CCS) IS DEPENDENT ON AMPLIFICATION YIELD AND SEQUENCING DEPTH WHEN USING NEXTGEN SEQUENCING.** M. D. Werner,a K. Scott,a C. Bohrer,a D. Gabriele,a X. Tao,a K. H. Hong,a D. Taylor,b N. R. Treff,b R. T. Scott, Jr.,a REI, RWJ, Rutgers, Basking Ridge, NJ; bRMANI, Basking Ridge, NJ; cRMACT, Norwalk, CT.

**OBJECTIVE:** The presence of DNA in the blastocoele provides an attractive target for less invasive aneuploidy screening. However, blastocoele fluid provides many challenges including DNA quantity and integrity. To date, a functional screening algorithm that overcomes these challenges and accurately identifies the karyotype of the blastocyst has not been validated.
This study seeks to determine consistency of diagnosis between DNA isolated from trophectoderm and blastocoeel fluid using NextGen based CCS.

MATERIALS AND METHODS: Embryos diagnosed as aneuploid by CCS following trophectoderm biopsy (TE Bx) had their blastocoeel fluid aspirated using a standard microinjection pipette. Whole genome amplification (WGA) and NextGen sequencing was performed using a protocol validated on TE Bx’s. Aneuploidy screening results from the blastocoeel were then compared to results obtained from TE Bx’s to determine concordance levels. The amplification efficiency of the blastocoeel sample and sequencing depth were also compared to see if they prognosticated the accuracy of the result.

RESULTS: Forty nine embryos with a wide variety of chromosomal aneuploidies were studied. WGA was successful in 47/49 (96%) samples. Of those, 13 (26%) gave a consistent diagnosis, which exactly matched the TE Bx result. An additional 4 (9%) samples predicted at least one aneuploidy chromosome correctly, but did not completely match the original result. WGA yield (p=0.003) and sequencing depth (p=0.008) were higher in samples with results identical to those attained in the TE Bx’s. Samples with depths below 4400 were consistently inaccurate. Those with >36,000 reads were correct in 12 of 13 samples.

CONCLUSION: This study demonstrates that the accuracy of CCS from blastocoeel fluid is dependent on the amplification efficiency and the total reads attained through NextGen sequencing. This algorithm is not reliable at this time. Can the efficiency be improved by enhancing the analytical algorithm? Or do low yield samples represent a biologic limit reflecting the amount of intact DNA in the fluid? Progress is being made but further investigation is required. Direct testing through trophectoderm biopsy remains the optimum methodology for safely and effectively applying CCS technology at this time.

P-519 Wednesday, October 22, 2014

SAMPLING BIAS IN MICROARRAY EXPERIMENTS AFFECTS DIFFERENTIAL GENE EXPRESSION ANALYSIS IN OOCYTES AND CUMULUS CELLS. A. Uyar,* S. Manafi,* S. Manafi,* A. Benet,* E. Seli,* Yale School of Medicine, New Haven, CT; Ryerson University, Toronto, ON, Canada.

OBJECTIVE: Microarrays are widely used for whole-genome transcriptomic analysis of oocytes and cumulus cells to identify biomarkers of viability. Sample size estimation is critical for microarray experiments to achieve power and consistency. However, by convention, three biological replicates per condition are assumed to be sufficient and researchers commonly rely on this assumption since sample collection is complex and costly. Here we investigate the effect of sampling bias on consistency of differential expression results derived from oocyte and cumulus microarray studies.

DESIGN: Experimental study.

MATERIALS AND METHODS: (1) ArrayExpress functional genomics database was mined using ‘oocyte’ and ‘cumulus’ keywords to identify microarray datasets. The number of replicates per condition in these datasets was determined. (2) Four datasets (2 human cumulus [C1, C2] and 2 mouse oocyte [O1, O2]) with 5 samples in both experimental and control groups were selected. For each dataset, differential expression analyses were conducted for all possible 3-sample subsets. Background adjustment, quantile normalization and probe set summarization were performed according to the Robust Multi-Chip Average algorithm. Genes with a false discovery rate-corrected p value of <0.05 and |Fold Change| > 2 were considered as differentially expressed. The number of differentially expressed genes (DEGS) was determined for each 3-sample subgroup and compared to determine variation in each dataset.

RESULTS: (1) We identified 167 oocyte and cumulus cell microarray datasets. Of these, 31.7% had <3 replicates for at least one condition; 22.2% had 3 replicates in all conditions; and 46.1% with >3 samples in all conditions. (2) A total of 10 (combination 3 out of 5) differential expression analyses were performed for each dataset. The number of DEGs across the 10 comparisons within each dataset was [min, max, mean±std]: C1[90, 2242, 778±923.9]; C2[3, 72, 30.7±22.5]; O1 [3556, 5022, 4225±1526.3]; O2[1230, 4053, 2241±1026.3]; and varied 2- to 30-fold depending on which 3 samples were chosen. Importantly, DEGs consistently detected in all subgroups of each dataset was only 1.2%.

CONCLUSION: We found that low number of replicates is likely to result in sampling bias associated with high variability in the number of DEGs identified. Our findings suggest that three biological replicates may not be sufficient for robust transcriptomic analysis, and sample size estimation and assessment of inter-sample variation is crucial for consistency of the microarray experiments in IVF research.

P-520 Wednesday, October 22, 2014

EFFECT OF 8-CELL STAGE BIOPSY ON CELL LINEAGE ALLOCATION IN MAMMALIAN EMBRYOS. L. P. Sepulveda-Rincon,* B. K. Campbell,* N. Beaujouan,* W. E. Maalouf,* “Child Health, Obstetrics and Gynecology, University of Nottingham, Nottingham, Nottinghamshire, United Kingdom; Developmental Biology and Reproduction, INRA, Jouy-en-Josas, Ile de France, France.

OBJECTIVE: It has been suggested that embryonic-embryonic (Em-Ab) axis in mammalian embryos can be predicted according to the first cleavage plane of the zygote [1]. In turn, this has been related with developmental potential [2]. The objective of the present study is to evaluate the effect of single blastomere biopsy at 8-cell stage on cell allocation during pre-implantation development.

DESIGN: Blastocysts from two treatment groups: biopsied (n=144), injected at 2-cell stage plus biopsied at 8-cell stage and injected group without biopsy (n=92).

MATERIALS AND METHODS: Zygotes were obtained from superfertilized B6CBA/F1 mice females mated naturally. Embryos were cultured in M16 medium at 37°C and 5% CO2 in a time-lapse incubator (EmbryoScope). Then at two-cell stage one blastomere was injected with a lipophilic tracer. At eight-cell stage the zona pellucida was reached by laser (Hamilton Thorne) and one blastomere was removed by aspiration. Embryos were cultured up to blastocyst stage and were classified according to their cell distribution: orthogonal, if the borderline between labelled and non-labelled cells was orthogonal to the Em-Ab axis; deviant, if it was overlapping or mixed, if labelled and non-labelled cells were intermingled. Total cell count was performed on blastocysts. Chi Square test or two-way ANOVA were performed to determine any difference between the treatment groups and the pre-patterns using IBM SPSS 21. P values <0.05 were considered significant.

RESULTS: No significant difference (p=0.356) was found in the incidence of the three different pre-patterns between biopsied and non-biopsied groups. Where, for the biopsied group 56.3% presented mixed pattern, 18.8% orthogonal and 25% deviant; for the non-biopsied group 64%, 18.5% and 17.4% correspondingly. Significant difference between biopsied and non-biopsied group on TCC was found in pre-hatching embryos (p<0.05) but not in hatching embryos (p=0.386). Nevertheless, significant higher number of hatching embryos (p<0.001) were found in the biopsied group.

CONCLUSION: Cell lineage allocation patterns during pre-implantation development seemed not affected by blastomere biopsy, suggesting that it might be determined before the 8-cell stage. This study sheds some light on the safety of the blastomere biopsy at 8-cell stage in terms of cell lineage allocation pattern and morphokinetics in mammalian embryos, yet epigenetic modifications are still unknown.

Supported by: PhD studies sponsored by CONACyT Mexico.

P-521 Wednesday, October 22, 2014

HOW TO RESCUE CRYOPRESERVED EJACULATED SPECIMEN WITH BACTERIAL CONTAMINATION. Q. V. Neri, Z. Rosenwaks, G. D. Palermo. Reproductive Medicine, Weill Cornell Medical College, New York, NY.

OBJECTIVE: To evaluate for eventual persistence of bacteria in cryopreserved semen in relation to virulence and sperm selection method. To assess the ability of a sperm selection method on curbing bacterial growth.

DESIGN: Donated semen samples were aliquoted and processed by centrifugation (C) and density gradient (DG) prior to cryopreservation. Bacterial cultures were assessed in the raw sample and after thawing for both selection methods. IUI outcomes were compared.

MATERIALS AND METHODS: Ejaculated samples were screened for aerobic and anaerobic bacteria then cryopreserved following C and DG.
Bacterial strains and growth patterns defined as sparse, few, moderate, or many were categorized into saprophyte or foreign to the urethra. In IUI procedures, the utilization of fresh semen in sexually intimate couples does not require screening in NYS. We therefore assessed whether the selection method was capable of minimizing bacterial growth with the aim of yielding a superior outcome.

RESULTS: There were a total of 29 male patients who ranged in age from 24 years to 73 (39.3±8.4y). This cohort provided ejaculated samples for 41 observations yielding bacterial growth. DG provided a contamination at 28.6 vs 70.0% for C (P<0.01). DG was capable of removing pathogens in 25.0% of the specimens vs 60.0% for C. This was particularly evident for the saprophytic bacteria at 27.3% for DG vs. 76.9% for C (Ps<0.01). Subsequently, a total of 1291 cycles (maternal age 39.3±5y) were identified where 447 had DG and 844 had their specimens C. The initial concentration for DG was 42.0±14 million, motility of 40.5±5% with post-selection being lower concentration 38.2 million (P<0.01) but with higher progressive motility 80.7% DG (P<0.0001). The initial concentration for C was 39.2±17 million and motility of 41.5±5%. The post-selection concentration was 47.5 million with a progressive motility of 46.3%. This yielded a pregnancy rate of 13.5% DG and 16.5% C. Once we controlled for the influence of a female factor (≤35y), pregnancy rate was 22.2% for DG and 19.4% for C.

CONCLUSION: It remains paramount to inform patients how to properly collect specimens for IVF. The process of cryopreservation in combination with DG selection is capable of reducing the bacterial growth particularly of saprophytic and avoiding the need to discard precious specimens. The utilization of a DG selection utilizing specimens with unknown bacterial presence may abate risks of exposing the female genital tract to seminal bacteria and most importantly avoiding undesired immunological responses and decreased risk of infections.

Supported by: Reproductive Medicine, Weill Cornell Medical College.

P-522 Wednesday, October 22, 2014

USE OF A SEMI-QUANTITATIVE PREGNANCY TEST (SQPT) TO MONITOR hCG LEVELS AFTER ASSISTED REPRODUCTION. I. Comstock, A. Gonzalez, W. Sheldon, J. Blum, B. Winikoff, P. Blumenhal, L. Westphal. Obstetrics and Gynecology, Stanford University Medical Center, Palo Alto, CA; Gynuity Health Projects, New York, NY.

OBJECTIVE: To examine the feasibility and acceptability of a semi-quantitative urine pregnancy test for at-home monitoring of pregnancy after assisted reproductive technologies (ART).

DESIGN: 50 women undergoing ART participated in a pilot study and used a SQPT to ascertain pregnancy status along with standard serum hCG levels.

MATERIALS AND METHODS: Patients undergoing fertility treatment were invited to participate in the clinical study. Each participant was given a SQPT to be used 14 days after an intrauterine insemination or oocyte retrieval when they would routinely have a serum beta hCG drawn. Women interpreted the test result at home and completed a short questionnaire to document their results. The patient returned the completed form to her provider when she presented for a serum hCG level that same day. The results of the SQPT and serum hCG were recorded by the researcher at each visit. If the patient was pregnant, she was asked to complete another SQPT in 48 hours and on the day of her first ultrasound to monitor the progress of her pregnancy.

RESULTS: The mean age of the patients was 36 years. 17/50 patients (34%) achieved a pregnancy, as documented by a positive beta hCG. Of these patients, 13/17 (76.5%) had a positive SQPT on initial testing. Two pregnant patients had initial beta hCG levels that were below the minimum threshold for detection by the SQPT (at least 25 mIU/mL). The remaining two interpreted their SQPT as negative despite having a positive beta hCG. There were no false positive results. Each participant was asked to complete a short questionnaire to respond to a series of SQPT acceptability and satisfaction questions. 43 patients completed an exit interview. Their responses are shown in Table 1.

CONCLUSION: SQPTs offer an inexpensive and well-accepted method of at-home monitoring of pregnancy after ART. These urine pregnancy tests may serve as a feasible alternative or adjunct to current monitoring protocols. This option offers women a patient-friendly diagnostic tool to monitor early pregnancy.

Supported by: Gynuity Health Projects.

P-523 Wednesday, October 22, 2014

NEW APPROACH TO INFERTILITY ASSOCIATED WITH PREVIOUS CESAREAN SECTION. A. Kuwahata, M. Ochi, T. Douchi. Ochi Yume Clinic Nagoya, Nagoya, Aichi, Japan; Faculty of Medicine Kagoshima University, Kagoshima, Japan; Faculty of Medicine, Kagoshima University, Kagoshima, Japan.

OBJECTIVE: It has long been reported that there are cases that show thinning of the myometrium or irregular bleeding after cesarean section, which has been referred to as post-cesarean syndrome. Although varied in reports, such symptoms occur in approximately 7% of cases after cesarean section. In this study, we examined the possibility that the accumulation of exudate from the wound of a previous cesarean section disturbed implantation of the embryo in the next pregnancy and whether it is possible to get pregnant again and safe birth can be attained with conservative treatment.

DESIGN: Surgicel was applied to the cesarean section wound to control the exudate in the pre-implantation cycle.

MATERIALS AND METHODS: From October 2011 to August 2013, in 26 cycles of 23 women (mean age 37.6 years old) who had a history of a previous cesarean section and showed exudate accumulation in the uterine cavity in the proliferative phase of the endometrium that was considered to disturb implantation of the embryo by ART, Surgicel was applied to the wound to control the exudate in the pre-implantation cycle. This was done with the approval of the ethics committee of our hospital and the patient’s consent. During the following cycle, the endometrium was thickened by hormone replacement therapy and, after confirming that exudate accumulation was not observed, the endometrium was brought into the secretory phase and the embryo was transferred.

RESULTS: The pregnancy test was positive in 19 of 26 cycles (73.1%) in which the embryo transfer was carried out. Clinical pregnancy was obtained in 15 cases (57.7%). 5 cases were aborted halfway through and 10 cases gave birth by cesarean section (there were no complications, such as uterine rupture). Chemical abortion occurred in 4 cases. 7 cases were negative for the pregnancy test.

CONCLUSION: It is not widely recognized that infertility is one of the symptoms of post-cesarean syndrome. One of the causes of the pathology is that exudate accumulation in the uterine cavity disturbs implantation of the embryo. This study suggested the presence of chronic inflammation occurred at the cesarean section wound. In addition, a method of controlling exudate in the uterine cavity was developed which was shown to enable patients to become pregnant and give live birth. It is necessary to elucidate the pathogenesis of secondary infertility due to a previous cesarean section in order to improve treatment as well as to evaluate the safety.

Supported by: Surgicel absorbable hemostat is made of acidic polysaccharide fibers obtained by oxidizing cellulose originating from plants. It is adjusted to the form of gauze or fibers. It is often used for hemostasis in surgery and in obstetrics and gynecology. The hemostatic effect lasts for approximately 14 days, and then it is absorbed.
ART - IN VITRO FERTILIZATION

P-524 Wednesday, October 22, 2014


OBJECTIVE: Elective single embryo transfer (eSET) has been used to limit multiple pregnancy rates (MPR) while maintaining excellent live birth rates (LBR) in women up to 37 years (yrs), above which ASRM criteria suggest a 2 blastocyst transfer. We herein investigate whether identification of a high-quality blastocyst can be used to appropriately select older patients for eSET.

DESIGN: Retrospective review.

MATERIALS AND METHODS: All autologous IVF embryo transfers of either a single blastocyst (SBT) or two blastocysts (DBT) to women up to and including 40 yrs of age conducted during 2010 to 2012 at a single ART center were analyzed. The inner cell mass and trophectoderm of each transferred blastocyst were graded A, B, or C according to Gardner’s system. Single blastocyst transfers of embryos graded either AA or BA had the highest LBR, and were therefore considered “top quality.” LBR and MPR were compared between SBT and DBT for all transfers including at least one of these top quality embryos. Multiple pregnancy was defined as a minimum of two gestational sacs and/or fetal hearts among sonographically-confirmed pregnancies.

RESULTS: A total of 3256 transfers (1689 SBT and 1567 DBT) met the inclusion criteria. Chi square analysis indicated no statistically significant differences in LBR/transfer for SBT vs. DBT in the <30 and 38-40 year old age groups, and only slight differences in the 30-37 year old age groups. However, in all age groups, LBR were excellent for SBT with very low corresponding MPR, while approximately half of DBT pregnancies were multiples.

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Multiple Pregnancy</th>
<th>Live Birth</th>
<th>P-value for Live Birth</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBT DBT</td>
<td>SBT DBT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;30</td>
<td>5/270 (1.8%) 104/186 (55.9%) 239/387 (61%) 162/246 (66%)</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>30-37</td>
<td>14/438 (2.5%) 210/385 (54.5%) 463/854 (54%) 339/524 (66%)</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>35-37</td>
<td>4/234 (1.7%) 153/287 (53.3%) 195/387 (50%) 256/424 (60%)</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>38-40</td>
<td>1/40 (2.5%) 122/253 (48.2%) 31/61 (51%) 203/373 (54%)</td>
<td>0.60</td>
<td></td>
</tr>
</tbody>
</table>

CONCLUSION: Blastocyst eSET of top quality embryos can be utilized with very favorable LBR that are comparable to DBT, while virtually eliminating the potential for multiples in patients up to and including 40 years of age. A LBR of 51% among patients aged 38-40 years resulted from transfers of single top quality blastocysts. Use of eSET may be of particular benefit in well-selected older patients given their increased maternal and fetal morbidity.

P-525 Wednesday, October 22, 2014

DEFINE THE PARAMETERS FOR PATIENT SELECTION IN ELECTIVE SINGLE-EMBRYO TRANSFER: A FOCUS ON MAINTAINING IMPLANTATION RATES. S.-Y. Lin, R. K. -K. Lee, Y.-M. Hwu, M.-H. Lin. Division of Infertility and Reproductive Endocrinology, Department of Obstetrics and Gynecology, Mackay Memorial Hospital, Taipei, Taiwan.

OBJECTIVE: In an endeavor to reduce the twin pregnancy rate of the patients receiving two-blastocyst transfer (DET) in IVF/ICSI, we are trying elective single-embryo transfer (eSET) for the patients. However, maintaining the success rates may be a concern. Is there a simple method to help clinicians in the patient selection for eSET (elective single-embryo transfer) while still maintaining implantation rates?

DESIGN: A retrospective study for the database of the patients who received DET in fresh IVF/ICSI cycles from 2009 January to 2013 May in Mackay Memorial Hospital.

MATERIALS AND METHODS: Totally, 273 patients receiving DET without uterine factors were included for analysis. Among these cases, there are 78 cases with two implantation sites, 85 cases with single implantation site, and the remaining 110 cases with no successful implantation. The patient parameters include age, blastocyst score, collected oocyte number, estrogen and progesterone levels at triggering day, AMH, endometrial thickness. All statistical comparisons were done between the group with two implantation sites (78 cases) and the group with no more than 1 implantation site (195 cases).

RESULTS: There are only age and blastocyst score showing the statistical differences between the groups. However, the age difference is too narrow to be useful for differentiating between the groups. We then screened all embryo database, and define the blastocyst scores as follows: 50 for grade 6, 4A; 42 for 4AB, 4BA and 4BB; 30 for 4AC, 4BC and 4CB; 35 for 3AA, 3AB, 3BA and 3BB; 30 for 3BC and 3CB; 10 for 4CC and 3CC; 16 for grade 2 or 1. We found that if we change the cases with the blastocyst score more than 90 from DET to eSET, we can decrease 14.3% of twin pregnancy rate (this reduces the original twin pregnancy rate by half), at the expense of 3.9% of the success rate.

CONCLUSION: Simply using the blastocyst scoring system we defined, we probably can make the right decision for eSET. That is, reducing the twin pregnancy rate while still maintaining the success rate.

P-526 Wednesday, October 22, 2014

IN VITRO FERTILIZATION (IVF) SUCCESS RATES IN PATIENTS WITH SURGICALLY DIAGNOSED ENDOMETRIOSIS AND EFFECT OF TIME INTERVAL FROM SURGERY TO IVF CYCLE. B. Alkudmani, D. Buell, A. J. Salman, C. Librah, P. A. Sharma, Obstetrics and Gynecology, University of Toronto, Toronto, ON, Canada; Reproductive Endocrinology and Infertility, Create Fertility Centre, Toronto, ON, Canada.

OBJECTIVE: Endometriosis is a condition that affects a significant proportion of the fertile population. Natural fecundity as well as IVF success decreases with increasing severity of disease. Our objective was to determine whether IVF outcomes after laparoscopic treatment of endometriosis are affected by surgical stage at diagnosis and to determine whether there is a relationship between time interval to start of first IVF cycle and pregnancy rate.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: Retrospective chart review of 197 patients with surgically diagnosed and treated stages 1-4 endometriosis (ages 18-45, between Jan 2009 and Dec 2012) who underwent IVF for infertility compared to 104 control patients (tubal or unexplained infertility, ages 18-45, between Jan 2009 and Dec 2012) who also underwent IVF. Patients were divided according to their stage of endometriosis. Outcome data included: IVF outcomes (time interval between surgery and IVF, days of stimulation (DOS), total gonadotropin dosage, peak E2 level, #oocytes retrieved, fertilization rate, implantation rate (IR), clinical and ongoing pregnancy rate (CPR/OPR), and miscarriage rate). Multivariable logistic regression analysis was used to control for confounding factors. For time interval to IVF and pregnancy data, Pearson’s Chi-squared test and logistic regression were utilized.

RESULTS: 197 endometriosis patients were classified by stage after surgery (52 Stage 1, 58 Stage 2, 59 Stage 3, 26 Stage 4). Progressive stages of endometriosis (from Stage 1 to 3) were associated with lower CPR after IVF (Stage 1–4 vs control, p=0.07, 0.014, 0.003, 0.64 respectively) despite no differences in DOS, peak E2 levels, and # of dominant follicles. Stage 4 did not reach statistically significance. There was a trend towards higher ongoing pregnancy rates in patients who underwent IVF between six and twenty-five months post surgery versus those who underwent IVF less than six months or greater than twenty-five months post surgery (0.36mos vs 2.4mos, 22%, 6 mos, 44%, 12-25mos 44%, >25mos 30%), particularly in patients with Stage 2 endometriosis (p=0.005).

CONCLUSION: Our data suggests that IVF outcome decreases with increasing severity of endometriosis in those women who underwent surgical treatment prior to IVF and that time interval from surgery to IVF may affect pregnancy outcome.
P-527 Wednesday, October 22, 2014

3-DIMENSIONAL ENDOMETRIAL VOLUME AS A PREDICTOR OF PREGNANCY IN IN-VITRO FERTILISATION CYCLES OVER 2-DIMENSIONAL ENDOMETRIAL THICKNESS. M. Gupta, N. Singh, N. Malhotra, R. Malhey, P. Vanamal, S. Pant. All India Institute of Medical Sciences, New Delhi, India.

OBJECTIVE: To evaluate the role of endometrial volume as predictor of pregnancy in In Vitro Fertilisation Cycles (IVF) over endometrial thickness.

DESIGN: Prospective, non randomised clinical study.

MATERIALS AND METHODS: This was a prospective observational study. A total of 100 infertile women were recruited from our IVF-Embryo transfer program from Feb to March 2014. Endometrial volume was measured on the day of hCG using the VOCAL (Virtual Organ Computer-aided Analysis) imaging program integrated into Voluson E8 ultrasound system.

RESULTS: The mean age was 31.5 years and the mean duration of infertility was 5 years. The mean endometrial thickness was 9.7 mm and the mean endometrial volume was 4.6 cm3. Overall 31(31%) patients conceived and in these women the endometrial volume was between 3-8 cm3. All patients who conceived had thickness more than 7 mm. 38 (38%) patients had thickness >7 mm but endometrial volume < 4 cm3 and did not conceive. The positive predictive value of endometrial volume for conception was 40% with sensitivity as high as 93.5%. Endometrial thickness had a high negative predictive value of 98% and specificity. Among those who conceived 16 (51%) patients had blood flow in Zone 3, 14 (45%) patients had flow in Zone 2 and only one had flow in Zone 1.

CONCLUSION: With a thin endometrium and low endometrial volume, the probability of conception is very low in an in-vitro fertilisation/embryo transfer cycle and cryopreservation should be recommended. Endometrial thickness more than 7 mm and volume more than 3cm3 together increase the probability of pregnancy more than thickness alone as volume is an objective measure of implantation potential of endometrium. However further study is needed for definitive conclusion.

P-528 Wednesday, October 22, 2014

EVALUATION OF SPERM DNA INTEGRITY AND ITS EFFECT ON EMBRYO DEVELOPMENT USING TIME-LAPSE MICROSCOPY. T. Lundberg, F. Hambiliki, F. Sondén, E. Akerlund, M. Bungum. Reproductive Medicine Centre, Skanes University Hospital, Malmö, Sweden.

OBJECTIVE: To examine whether there are any correlation between the degree of sperm DNA fragmentation as measured with Sperm Chromatin Structure assay (SCSA) and early embryo development assessed by time-lapse microscopy.

DESIGN: The study was performed in a retrospective manner in the setting of a university hospital clinic.

MATERIALS AND METHODS: The study included 642 oocytes from 107 couples treated with Intracytoplasmic Sperm Injection (ICSI). Embryos were cultured in an Embryoscope (time-lapse system), with 6% CO2 and 5% O2 for a maximum of six days. All annotations for each zygote/embryo were performed in regard to predefined morphokinetic events. The semen sample was collected at the day of oocyte aspiration and the degree of DNA fragmentation was assessed using the SCSA. The percentage of denatured, damaged DNA was expressed as DFI (DNA Fragmentation Index). Oocytes/embryos were categorized into four different groups according to DFI value (0-10%; 10.1-20%; 20.1-30% and ≥30%). The endpoints were fertilization rate as well as time for: i) extrusion of second polar body (2PBe); ii) pronuclei appearance (PNa); iii) pronuclei fusing (PNI); iv) early cleavage (12); v) blastocyst development (BB) and vi) blastocyst rate. Mean values for all parameters were tested in a linear regression analysis model with the respective parameters as dependant and the four DFI categories as independent factors. Statistical analysis was performed using statistical software (SPSS 17.0 for Windows; SPSS Inc., Chicago,IL). A p-value of less than 0.05 was considered statistically significant.

RESULTS: For endpoints; rate of 2PN, 2PBe, PNa, PNI, 12, BB and blastocyst rate the means were equal between all four DFI groups. No statistical differences between groups were detected.

CONCLUSION: Neither rate of 2PN, 2PBe, PNI nor embryo development as assessed by time-lapse microscopy are related to degree of sperm DNA fragmentation as measured by SCSA.

P-529 Wednesday, October 22, 2014

THE EFFECT OF PERCEIVED QUALITY OF LIFE ON PHYSICAL, EMOTIONAL, SOCIAL, RELATIONAL SITUATIONS IN WOMEN WITH FERTILITY PROBLEMS. A. S. Ugur, H. Ertan Yaman, M. Aygun. 4 Department of Infertility and In vitro Fertilization, Florence Nightingale Hospital, Kadikoy, Istanbul, Turkey; 5 Health School, Department of Nursey, Istanbul Bilim University, issi, Istanbul, Turkey.

OBJECTIVE: The aim of this study was to investigate the effect of reproductive problems of women who were under the treatment of infertility on their physical, emotional, social, relational life by using a relational screen model.

DESIGN: The study was done at Florence Nightingale Hospital IVF Center between the dates of 21st March-31st July 2013. Ninety women who were diagnosed as infertility voluntarily joined to our study. All of the women were married and they have a basal education level as a graduate of primary school. Therefore, there were capable of participating the study and achieving a good communication.

MATERIALS AND METHODS: The data was collected by using a Statement Form that was prepared according to the literature and clinical experiences and FertiQol. Quality of Life Questionnaire (FERTIQOL) 2008, approved by an availability-safety test. Statistical evaluation was done by using SPSS 17.0 package program.

RESULTS: Mean age of the study group was 33.96 ± 5.38. Demographic evaluation also revealed that 65.6% of these patients were living in a city. 89.8% of them were working and 23.3% have no previous infertility treatment. It was the first attempt of IVF treatment for 48.9% of those women. Almost half of these patients (51.1%) described a kind of pressure. They claim that the main part of pressure they feel was self-dependent. The highest quality of life score in core module, relational sub-scale was 79.21 and in relational module, environment sub-scale was 80.23. The lowest life quality scores were recorded for emotional sub-scale as 62.96. The relation between the individual FertiQol sub-scores and age was not statistically significant. However, relations between the consistency of pressure feeling caused by a fertility problem and emotional, mind-body and social sub-scale were statistically significant.

CONCLUSION: Our results revealed that individuals with fertility problems and under IVF treatment need professional counseling to having a better quality of life. Supported by: Self Support.

P-530 Wednesday, October 22, 2014

DAIRY INTAKE IN WOMEN AND IN VITRO FERTILIZATION OUTCOMES. M. C. Aeife, A. J. Gaskins, P. L. Williams, T. L. Toth, D. L. Wright, R. Hauser, J. E. Chavarro. 4 Department of Nutrition, Harvard School of Public Health, Boston, MA; 3 Department of Biostatistics, Harvard School of Public Health, Boston, MA; 2 Vincent Obsterics and Gynecology, Massachusetts General Hospital and Harvard Medical School, Boston, MA; 1 Department of Environmental Health, Harvard School of Public Health, Boston, MA.

OBJECTIVE: To examine the relation between dairy intake in women and assisted reproductive technology (ART) outcomes.

DESIGN: Prospective cohort study.

MATERIALS AND METHODS: Women in subfertile couples presenting for evaluation at the Massachusetts General Hospital Fertility Center were invited to participate in an ongoing study. Diet was assessed before ART treatment using a validated food frequency questionnaire. ART outcomes included controlled ovarian hyper-stimulation outcomes (estradiol levels, overall and mature oocyte yields), fertilization rates, embryo quality, and clinical outcomes (implantation, clinical pregnancy, and live birth). We used generalized linear mixed models with random intercepts to account for multiple ART cycles per woman. Crude models were adjusted for age and calorie intake. Full models were adjusted for age, calorie intake, BMI, race, smoking status, infertility diagnosis, protocol type, and dietary patterns. We conducted a sensitivity analysis restricting to ART cycles started within 18 months of completing the FFQ.

RESULTS: Dairy intake was not related to peak estradiol, total or mature oocyte yields, fertilization rates, or embryo quality. Dairy intake was associated with higher live birth rates in crude models. The adjusted difference (95% confidence interval (CI)) in live birth rate between women in the highest (>3.0 servings/day) and lowest (<1.34 servings/day) quartile of dairy intake was 21% (9-32%). This association was attenuated after adjustment for other potential confounders. In the fully adjusted model, the adjusted...
percentage (95% CI) of initiated ART cycles resulting in live birth for women in increasing quartiles of total dairy intake were 38% (26-52%), 41% (30-54%), 39% (28-52%), and 54% (42-66%) (p, trend=0.09). In the sensitivity analysis, the results were similar with adjusted percentages (95% CI) of 32% (20-47%), 39% (26-53%), 38% (25-52%), and 57% (43-71%) (p, trend<0.02).

CONCLUSION: There was a suggestion of a positive association between total dairy food intake and live birth rate among women undergoing ART.

Supported by: NIH grants R01ES009718, R01ES000002, P30DK46200, T32DK07703, and T32-HD06454.

P-531 Wednesday, October 22, 2014

EFFECT OF EMBRYO GLUE TRANSFER MEDIUM DURING FRESH AND FROZEN-THAWED EMBRYO TRANSFER. H. Tomari, K. Honjou, K. Kunitake, K. Nishimura, N. Hidaka, Y. Nagata. Center for Reproductive Medicine, IVF Nagata Clinic, Fukuoka, Japan.

OBJECTIVE: Culture medium best suited to human embryonic development has been developed recently. The medium used for embryo transfer is known as embryo glue (EG) and contains hyaluronic acid (HA). HA, an important glycosaminoglycan, exists in the ovarian follicle, oviduct, and uterus in humans. HA is an adhesion factor between cells and the adhesion facilitatory effect of an embryo and endometrium is expected. Here, we examine the effect of EG on the cleavage stage in fresh and frozen-thawed embryo transfer.

DESIGN: Randomized controlled study.

MATERIALS AND METHODS: We examined 186 cycles of fresh embryo transfer (Day 2) and 550 cycles of frozen-thawed embryo transfer (Day 3). Assisted hatching was performed in frozen-thawed cycles. Patients were randomly allocated to two groups: group A, embryos were transferred to control medium containing no HA; group B, embryos were transferred to EG for more than 30 min before intrauterine transfer. Clinical pregnancy and implantation rates after embryo transfer were compared among the two groups. Chi square analysis was used to analyze data.

RESULTS: There were no significant differences in patient characteristics between the two groups. Among the 186 fresh embryo transfer cycles, 120 were performed as group A and 66 as group B. Clinical pregnancy rate in group B was higher than in group A (33% vs. 23%, respectively). Implantation rate in group B was higher than in group A (22% vs. 18%, respectively). Among the 550 frozen-thawed embryo transfer cycles, 320 were performed as group A and 230 as group B. Clinical pregnancy rate in group B was significantly higher (p<0.01) than in group A (37% vs. 24%, respectively). Implantation rate in group B was significantly higher (p<0.01) than in group A (29% vs. 19%, respectively).

CONCLUSION: The use of EG as embryo transfer medium produced high clinical pregnancy and implantation rates during fresh and frozen-thawed embryo transfer. Our results suggest that EG improves outcomes in human in vitro fertilization programs. In addition, the clinical pregnancy and implantation rates were highest in the frozen-thawed cycles, suggesting that the high viscosity of EG may physically protect the embryos treated with assisted hatching.

P-532 Wednesday, October 22, 2014

PREVALENCE OF VELAMENTOUS AND MARGINAL UMBILICAL CORD INSERTIONS; A COMPARISON OF TERM SINGLETON ART AND NON-ART PREGNANCIES. S. Furuya, K. Kubonoya, K. Kubonoya. Obstetrics & Gynecology, Kubonoya Oh Gyn Clinic, Kashiwa City, Chiba Prefecture, Japan.

OBJECTIVE: Abnormal umbilical cord insertion (i.e., velamentous or marginal insertion) sometimes results in serious obstetric complications. The reported adverse outcomes include placenta previa, fetal growth restriction, non-reassuring fetal status requiring an emergency Cesarean section, and fetal exsanguination due to the rupture of vasa previa. This study was conducted to investigate whether pregnancies obtained by assisted reproductive technologies (ART) influence the prevalence of anomalous cord insertions.

DESIGN: Observational study and comparative analysis.

MATERIALS AND METHODS: We reviewed the records of 6604 consecutive singleton, term labor and delivery cases in our clinic between the study period of January 2010 to April 2014, including personal details, obstetric history, details of infertility treatment, and insertion site of the umbilical cord. They were categorized according to their conception method (Group A: ART pregnancies, n=253; Group B: non-ART pregnancies, n=6351). Abnormal cord insertion was divided into two categories: velamentous insertion(VI) and marginal insertion(MI). Odds ratio, 95% confidence intervals(CI), and significance of the odds ratio were calculated for the conditions of interest.

RESULTS: As for maternal age and their parity both in Group A and Group B, there were no statistically significant differences between the presence and the absence of abnormal cord insertion. The prevalence of abnormal cord insertion was 19.3% (VI: 4.3%, MI: 15.0%) in Group A, and 6.4% (VI: 0.6%, MI: 5.8%) in Group B. The odds ratio for delivery with VI in Group A as opposed to Group B was calculated as 7.6 (95% CI: 3.8 - 15.0), P<0.001 (X²=46.4, 1 df). The odds ratio for delivery with MI in Group A as opposed to Group B was 2.9 (95% CI: 2.0 - 4.1), P<0.001 (X²=35.7, 1 df).

CONCLUSION: 1) These findings suggest that the ART procedures have a positive correlation on the incidence of abnormal cord insertion (esp. velamentous insertion). 2) ART-conceived cases should be more proactively screened and assessed for insertion site of the umbilical cord during routine obstetric ultrasound examinations, in addition to other already-known ART-associated risk factor assessments, to improve their perinatal outcomes.

P-533 Wednesday, October 22, 2014


OBJECTIVE: Recently, many IVM (in vitro maturation) media have been used in IVF(In Vitro Fertilization)-IVM(In Vitro Maturation) program. Nevertheless, the optimal culture conditions for the fertilization and cleavage were not clarified yet. Therefore, we investigated the role of GM-CSF in IVM program.

MATERIALS AND METHODS: We investigated the role of GM-CSF in IVM program. Our results showed that GM-CSF increased the cleavage rate and the number of mature oocytes. However, we could not find a significant effect of GM-CSF on the number of 2PN, the number of 2PN arrest, and the number of mature oocytes. These results suggest that GM-CSF may accelerate the cleavage rate and the number of mature oocytes.

CONCLUSION: GM-CSF may be useful for improving the fertilization rate and the number of mature oocytes in IVM program.

Table 1. Clinical outcomes.

<table>
<thead>
<tr>
<th>M ±SD(%)</th>
<th>Control</th>
<th>GM-CSF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conventional IVF</td>
<td>ICSI</td>
</tr>
<tr>
<td>No. of cycles</td>
<td>64</td>
<td>74</td>
</tr>
<tr>
<td>Age</td>
<td>33.5±2.7</td>
<td>34±1.3</td>
</tr>
<tr>
<td>No of immature oocytes</td>
<td>218(3.4±2.3)</td>
<td>259(3.8±2.4)</td>
</tr>
<tr>
<td>No. of 2PN</td>
<td>94(43.1)</td>
<td>142(70)</td>
</tr>
<tr>
<td>No. of 2PN arrest</td>
<td>5(5.3)</td>
<td>11(7.7)</td>
</tr>
<tr>
<td>~8Cell</td>
<td>64(68)</td>
<td>81(57)</td>
</tr>
<tr>
<td>8Cell~12Cell</td>
<td>15(16)</td>
<td>36(25.4)</td>
</tr>
<tr>
<td>No. of morula</td>
<td>6(6.4)</td>
<td>0</td>
</tr>
<tr>
<td>No. of blastocyst</td>
<td>6(6.4)</td>
<td>13(9.2)</td>
</tr>
</tbody>
</table>

P: NS
P-335 Wednesday, October 22, 2014


OBJECTIVE: Frozen donor eggs offer recipients a new option to become pregnant and provides advantages for both the recipient and IVF program avoiding the delays, uncertainties, and administrative challenges associated with matching/synchronization of donors and recipients. However, many fertility programs don’t have extensive experience in oocyte vitrification/warming techniques and so are hesitant to move from traditional fresh cycles.

DESIGN: Retrospective review.

MATERIALS AND METHODS: All anonymous DER cycles using vitrified-warmed or fresh donor eggs over a 5-year period at a university based IVF program were reviewed. Outcome parameters of: number of oocytes, survival, fertilization, clinical pregnancy and cryopreservation rates were compared. Statistical analysis was performed using T tests or Chi Squared as appropriate.

RESULTS: Table 1 shows outcome parameters for recipients using either fresh or vitrified-warmed (V-W) donor eggs. Fertilization rates are similar in both groups although it should be noted ICSI must be performed with V-W eggs. Blastocyst formation rates are lower with warmed eggs although Clinical Pregnancy rates (+FH) and Implantation Rates (IR; sac/embryo transfer) are comparable in both cycle types despite recipients of V-W oocytes having a lower average number of mature eggs and fewer embryos transferred. The difference in average number ET may also reflect our recent trend toward single embryo transfer since a large portion of V-W cycles occurred recently.

CONCLUSION: When compared to fresh cycles, frozen donor eggs offer valuable logistical advantages, providing that success rates are maintained. Recipients may choose from a frozen inventory and are saved the uncertainties involved with donor rejection/withdrawal, poor response to gonadotropins and timing issues. V-W cycles resulted in lower blastocyst formation rates which may account for less patients having surplus embryos to freeze. In some instances this may be advantageous since recipients often opt not to return if successful. The results show that frozen donor eggs offer viable alternatives to fresh DER and will most likely be the treatment of choice for patients and programs going forward.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Fresh DER</th>
<th>V-W DER</th>
<th>Signif</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Blast form</td>
<td>54</td>
<td>66</td>
<td>S</td>
</tr>
<tr>
<td>% Fert</td>
<td>80</td>
<td>80</td>
<td>NS</td>
</tr>
<tr>
<td>% Surv</td>
<td>88</td>
<td>88</td>
<td>NA</td>
</tr>
<tr>
<td>% M2 Eggs</td>
<td>10.6</td>
<td>14.6</td>
<td>S</td>
</tr>
<tr>
<td>% pt w/cryo</td>
<td>50</td>
<td>70</td>
<td>S</td>
</tr>
<tr>
<td>% Cl Preg/ET</td>
<td>62</td>
<td>70</td>
<td>NS</td>
</tr>
<tr>
<td>No. ET</td>
<td>1.5</td>
<td>1.8</td>
<td>S</td>
</tr>
<tr>
<td>No. embry</td>
<td>3.6</td>
<td>5.6</td>
<td>S</td>
</tr>
<tr>
<td>% live birth+on preg</td>
<td>61</td>
<td>59</td>
<td>NS</td>
</tr>
<tr>
<td>IR</td>
<td>62</td>
<td>59</td>
<td>NS</td>
</tr>
</tbody>
</table>

** Table 1**

<table>
<thead>
<tr>
<th></th>
<th>Fresh DER</th>
<th>V-W DER</th>
<th>Signif</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Blast form</td>
<td>54</td>
<td>66</td>
<td>S</td>
</tr>
<tr>
<td>% Fert</td>
<td>80</td>
<td>80</td>
<td>NS</td>
</tr>
<tr>
<td>% Surv</td>
<td>88</td>
<td>88</td>
<td>NA</td>
</tr>
<tr>
<td>% M2 Eggs</td>
<td>10.6</td>
<td>14.6</td>
<td>S</td>
</tr>
<tr>
<td>% pt w/cryo</td>
<td>50</td>
<td>70</td>
<td>S</td>
</tr>
<tr>
<td>% Cl Preg/ET</td>
<td>62</td>
<td>70</td>
<td>NS</td>
</tr>
<tr>
<td>No. ET</td>
<td>1.5</td>
<td>1.8</td>
<td>S</td>
</tr>
<tr>
<td>No. embry</td>
<td>3.6</td>
<td>5.6</td>
<td>S</td>
</tr>
<tr>
<td>% live birth+on preg</td>
<td>61</td>
<td>59</td>
<td>NS</td>
</tr>
<tr>
<td>IR</td>
<td>62</td>
<td>59</td>
<td>NS</td>
</tr>
</tbody>
</table>

** = statistically significant where P<0.001

E314 ASRM Abstracts

Vol. 102, No. 3, Supplement, September 2014

OBJECTIVE: To compare human chorionic gonadotropin (HCG)-administered natural cycle with spontaneous ovulatory cycle in patients undergoing frozen-thawed embryo transfer (FET) in natural cycles.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: A total of 166 consecutive FTET cycles that were performed in either the natural cycle after ovulation triggering with HCG (HCG group, n=110) or the natural cycle after the detection of spontaneous ovulation (control group, n=56) in 166 patients between January 2009 and November 2012 were included.

RESULTS: There were no differences in patients’ characteristics between the two groups. The two groups were comparable with respect to the characteristics of previous IVF cycle in which embryos were cryopreserved including the numbers of oocytes retrieved, mature oocytes, fertilized oocytes, grade I or II embryos and frozen embryos. Significant differences were not also observed between the HCG and control groups in clinical pregnancy rate (CPR), embryo implantation rate, miscarriage rate, live birth rate and multiple CPR. However, the number of hospital visits for follicular monitoring was significantly fewer in the HCG group than in the control group (P<0.001).

CONCLUSION: HCG administration for ovulation triggering reduces the number of hospital visits for follicular monitoring without any detrimental effect on FTET outcome when compared with spontaneous ovulatory cycles in infertile patients undergoing FTET in natural cycles.

P-538 Wednesday, October 22, 2014


OBJECTIVE: To determine the optimal time for oocyte retrieval in long protocol, flare up and antagonist protocols in terms of egg maturation, fertilization, implantation and clinical pregnancy rates.

DESIGN: A prospective study was conducted at Vietnam National Center for Reproductive Medicine.

MATERIALS AND METHODS: The study recruited 1049 patients undergoing ICSI/IVF. Inclusion criteria included maternal age ≤38; FSH ≤10 IU/L; IVF cycle attempt ≤2; AFC>4; ovarian stimulated with fRSH. uICG of 10,000 IU was used for trigger. The time interval between hCG injection and egg retrieval (T) was divided into four groups: T1 ≤35 h; 35h<T2 ≤36h; 36h<T3 ≤37h; T4 >37h. The primary outcomes were oocyte maturation, fertilization, implantation and clinical pregnancy rates.

RESULTS: There were 663 patients undergoing long protocol, 208 patients undergoing flare-up protocol, and 178 undergoing antagonist protocol. The characteristics of patients between time intervals in terms of age, FSH, AFC, infertility duration were comparable. In long protocol, oocyte maturation rate was significantly lowest in group of T1 ≤35 h (86.6%) compared to groups of T2, T3, and T4 (97.7%, 97.7% and 92.6% respectively). Fertilization rate was significantly highest in group of 35h<T2 ≤36h (82.9%). The implantation and clinical pregnancy rates were lowest in group of T1 ≤35 h. The optimal time for oocyte retrieval in long protocol was from 35h to 36h. In flare-up protocol, oocyte maturation rate was significantly lower in group of T1 and T4 (92.2% and 81.2%) compared to groups of T2 and T3 (95.4% and 95.4%). Fertilization rate was also significantly lower in groups of T1 and T4 (70.9% and 69.2%) compared to groups of T2 and T3 (85.9% and 85.2%). The implantation and clinical pregnancy rates did not differ among the four groups. The optimal time for oocyte retrieval in flare-up protocol was from 35h to 36h. In antagonist protocol, oocyte maturation rate was significant lowest in group of T1 ≤35 h (86.6%) compared to groups of T2, T3, and T4 (97.7%, 97.7% and 92.6% respectively). Fertilization rate was significantly highest in group of 36h<T3 ≤37h (84.7%) compared to groups of T1 and T2 (67.9% and 79.9%). The implantation and clinical pregnancy rates did not differ among the four groups. The optimal time for oocyte retrieval in antagonist protocol was from 36h to 37h.

CONCLUSION: Oocyte retrieval should not be performed before 35h after hCG administration. The optimal time for oocyte retrieval should be from 35h to 36h in long protocol; from 35h to 37h in flare-up protocol, from 36h to 37h in antagonist protocol.

Supported by: The authors would like to thank Dr Le Hoang, Dr Le Toan Anh, Mrs Nguyen Hong Hanh, Ms Nguyen Lien Haung, Ms Nguyen Thanh Phuong, and other staff in Vietnam National Center of Reproductive Medicine for their kind support to this research.

P-539 Wednesday, October 22, 2014


OBJECTIVE: It is important to evaluate the fertilization at the time of conventional IVF to avoid the fertilization failure. It is widespread to use rescue intracytoplasmic sperm injection (ICSI) for unfertilized oocytes. So we need to evaluate the fertilization 4 to 6 hours after insemination using second polar body. The purpose of this study is to evaluate the observation of second polar body and the spindle for judgment of rescue ICSI in fertilization failure of the conventional IVF.

DESIGN: Retrospective study.

MATERIALS AND METHODS: The study consisted of couples that underwent IVF-ET. In the study group, we observed second polar body and the spindle 4-6h after insemination to assess fertilization in 70 cycles (female age 37.9±4.4 years) between August 2013 and March 2014. In the control group,
we observed only second polar body 4-6h after insemination to assess fertilization in 147 cycles (female age 38.0±4.5) between July 2012 and July 2013. Rescue ICSI was performed on the unfertilized oocytes that we judged. We evaluated the fertilization rate. The X2-test was used and differences were considered significant at p<0.05.

RESULTS: The fertilization rate of the conventional IVF (2PN or 1PN) was 65.8% (300/456) in the study group and 62.9% (556/884) in the control group. The final fertilization rate with rescue ICSI (2PN or 1PN) was 72.1% (329/456) in the study group and 68.1% (602/884) in the control group. There was no significant difference in fertilization rate among both groups.

In the study group, total 456 oocytes were observed, oocytes with second polar body and the spindle was 71.3% (325/456), oocytes with only second polar body was 7.2% (33/456), oocytes without second polar body and the spindle was 19.1% (87/456) and 11 oocytes had difficulty in judgment. Fertilization rate in each group was 92.6% (301/325), 84.8% (28/33), 45.9% (17/37) and 54.5% (6/11).

CONCLUSION: Our results suggested that the observation of second polar body only was useful for adaptation judgment of rescue ICSI. The process to observe the spindle is complicated. If it is difficult to identify the second polar body, the observation of the spindle is helpful to assess fertilization.

**P-540 Wednesday, October 22, 2014**


OBJECTIVE: Aneuploidy is one of the main reasons for implantation failure and spontaneous abortion, techniques for genetic analysis are invasive and expensive. It is well accepted that embryo morphology correlates with implantation rates, implying that embryo morphology may be a predictor of chromosomal health. Recent publications indicate that kinetics in embryo development are predictive for chromosomal status (1-3). We analysed the predictive value of static morphological and cinematographic analysis for genetic status in human embryos.

DESIGN: In a retrospective analyses we correlated the static morphological appearance and morphokinetic parameters with the genetic results of embryos after PGD/PGS.

MATERIALS AND METHODS: We included 235 embryos from 44 cycles allocated for PGD/PGS either due to parental translocations (82), or recurrent implantation failure (153). Morphokinetics were monitored and embryos were biopsied on day 5. Genetic analysis was performed by aCGH or FISH. Retrospectively, maternal age, kinetics, and morphology of embryos, both static and dynamic, were correlated with PGD/PGS results.

RESULTS: Early cell deviations were delayed in aneuploid embryos, but cutoff limits for 1c, 2c, and 1c-2c (1-3) or static morphological evaluation on day 2/3 were not predictive for euploidy. Embryos with blastulation after 96.2h were 21.5% more likely to be aneuploid. However, static morphological evaluation of blastocysts had comparable and also statistically significant predictive value, suboptimal quality blastocysts were 19.9% more likely to be genetically abnormal. For unbalanced translocations a trend towards a lower rate of embryos with unbalanced translocations was found when they were classified as high quality blastocysts.

CONCLUSION: Kinetic of blastocyst formation and static morphological evaluation of the blastocyst quality on day 5 were found to have a comparable and statistically significant predictive value for aneuploidy. Combining both parameters increased the predictive value, but still only 45% of all blastocysts fulfilling both criteria were euploid.

P-542 Wednesday, October 22, 2014

**LIVE BIRTH RATES IN IVF HIGH RESPONDERS ARE HIGH WHETHER USING AGONIST TRIGGER ALONE OR USING DUAL TRIGGER IF INTENSIVE LUTEAL SUPPORT IS GIVEN.** R. Sherbahn, M. Catenacci. Advanced Fertility Center of Chicago, Gurnee, IL.

OBJECTIVE: Investigate IVF live birth rates, pregnancy loss rates and OHSS occurrence rates in high responders that had a pure agonist trigger vs. a dual trigger.

DESIGN: Retrospective chart review.

MATERIALS AND METHODS: All 135 women under age 38 who used their own eggs for IVF and who received either a pure agonist trigger or an agonist trigger plus 1500-2000 units of HCG between November 2011 and July 2013 were included. Patients were put in 2 groups based on their trigger injections (pure agonist trigger or dual trigger). Cycle outcomes were compared. Chi-square, Fisher’s exact test and T-tests were used for statistical analysis.

RESULTS: The 2 groups had similar characteristics other than significantly higher AMH levels and resting antral follicle counts in the pure agonist trigger group. The mean number of eggs retrieved, pregnancy loss rates, live birth rates and the rate of OHSS development were not significantly different between the pure agonist trigger group and the dual trigger group. The live birth rate per egg retrieval was 52.6% in the pure agonist trigger group and 64.4% in the dual trigger group. The difference was not statistically significant. There was a trend for higher pregnancy loss rates in the pure agonist group (not significant). When the pure agonist trigger group was split into 2 groups based on peak estradiol < 4000 pg/ml vs. > 4000 pg/ml the live birth rate was significantly higher (p < 0.05) in the group with estradiol > 4000 pg/ml (19/45, 42.2% vs. 21/31, 67.7%).

CONCLUSION: Contrary to what has been reported, live birth rates with IVF using a pure agonist trigger can be excellent. Live birth rates are higher when using a pure agonist trigger if the peak estradiol is > 4000 pg/ml.
P-543 Wednesday, October 22, 2014

THE TRIGGER OF OOCYTE MATURATION WITH HIGH DOSAGE OF HCG DURING IVF STIMULATION NEGATIVELY AFFECT OOCYTE/EMBRYO QUALITY IN PATIENTS WITH PEAK SERUM ESTRADIOL LEVEL > 4,000 PG/ML. Y. Ying, L. Lam, J. Mayer, S. Plosser. Obstetrics & Gynecology, University of South Florida, Tampa, FL.

OBJECTIVE: 10,000 IU hCG (10K) has been routinely used for the induction of oocyte maturation during IVF cycle, but 5,000 IU (5K) may be a safer dose in high responding patients. We compared embryo development, implantation rate, and ongoing pregnancy rate/live birth rate in high responding patients who received hCG 5K vs hCG 10K in donor and non-donor IVF cycles.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: All high responders defined as patients with peak serum estradiol > 4,000 pg/ml during IVF stimulation and < age 40 who underwent IVF from September of 2006 to March of 2014 were included in the study. Patients who received 5K hCG (n=101) were compared with those who received 10K hCG (n=104). Patient age, number of oocytes retrieved, percentage of matured oocytes, fertilization rate, number of embryos transferred, percentage of embryos reaching blastocyst stage, implantation rate and percentage of ongoing pregnancy/live birth were compared between the two groups.

RESULTS: There were no significantly different in terms of patient’s age, number of oocytes retrieved, percentage of matured oocytes, fertilization rate and number of embryos transferred between the two groups (P > 0.05). 42.7% of embryos in the hCG 5K group reached blastocyst stage, compared with 36.1% in the hCG 10K group (P < 0.05). Implantation rate in the hCG 5K was 38.1%, vs 25.2% in the hCG 10K group (P < 0.01). The ongoing pregnancy/live birth rate in the hCG 5K group was 57.4%, vs 39.4% in the hCG 10K group (P < 0.05). When the non-donor cycles were separated from the donor cycles, the implantation and blastocyst rates in the non-donor cycles were 35.6% and 39.7% in the hCG 5K group (n=86), vs 23.3% (P<0.01) and 32.1% (P<0.05) in the hCG 10K group (n=71). In IVF cycles with donor eggs, the implantation and blastocyst rates in the hCG 5K group (n=15) were 56.7% and 59.9%, significantly higher than 30.3% and 44.4% in the hCG 10K group (n=34, P<0.05).

CONCLUSION: In high responders, the trigger of final oocyte maturation with hCG 5K resulted in more embryos developing to blastocyst, higher implantation rate, and higher ongoing pregnancy/live birth rate than did trigger with hCG 10K. Given that higher blastocyst development and implantation rates were seen in both donor and non-donor cycles, oocyte/embryo quality may play a greater role in our observations than endometrial receptivity.

P-544 Wednesday, October 22, 2014


OBJECTIVE: To investigate the effect of dehydroepiandrosterone (DHEA) supplementation on ovarian reserve markers and in-vitro Fertilization (IVF) cycle outcomes in poor responders.

DESIGN: Retrospective case controlled cohort study.

MATERIALS AND METHODS: Totally 148 poor responders (<40 years-old) fulfilling Bologna criteria were enrolled to the study. Of these 68 patients were given DHEA supplementation (25 mg t.i.d.) for at least 12 weeks (max. 36 weeks) prior to their 2nd IVF cycle. The remaining 80 patients were selected as BMI and age match control group from patients underwent IVF in the same period. In all enrolled COS cycles, a flexible GnRH antagonist with rFSH + hMG (225-300 IU/day) protocol was used. All data concerning COS and laboratory outcomes were then compared, wherein the 1st and 2nd cycle data was included in DHEA group.

RESULTS: Patient’s characteristics, day 3 features, COS and laboratory outcomes in study and control population were all given in table 1.

Cycle and Laboratory Outcomes in all Groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before DHEA</th>
<th>After DHEA</th>
<th>Control</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>35.8± 3.2</td>
<td>36.4± 3.1</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.1± 4.2</td>
<td>24.9± 3.9</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>16.4± 7.5</td>
<td>14.9± 7.2</td>
<td>0.05 a</td>
<td></td>
</tr>
<tr>
<td>LH (mIU/ml)</td>
<td>11.7± 7.2</td>
<td>10.7± 9.2</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>E2 (pg/ml)</td>
<td>68± 48.1</td>
<td>62± 27</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>AMH (ng/ml)</td>
<td>1.18± 0.9</td>
<td>1.07± 0.8</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Antral Follicle Count</td>
<td>1.9± 2.0</td>
<td>2.3± 1.6</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Serum oestradiol concentrations on HCG day (pg/ml)</td>
<td>965± 484</td>
<td>1056± 1136</td>
<td>1005 NS</td>
<td></td>
</tr>
</tbody>
</table>

P value was set at <0.05. a, statistical importance between DHEA before and after groups.

CONCLUSION: DHEA supplementation might only reduce basal FSH levels and cycle cancellation rates in poor responders. However, it may not realistic to expect increased cycle outcomes regarding retrieved oocyte numbers and clinical pregnancy rates with DHEA supplementation.

P-545 Wednesday, October 22, 2014


OBJECTIVE: Poor responders are one of the most challenging patients to treat with IVF. Moreover, experiences in the treatment of extreme POs are limited so far. The aim of this study was to evaluate the clinical outcomes of IVF in extreme POs with very low AMH level less than 0.01ng/mL.

FERTILITY & STERILITY®
ASRM Abstracts

P-546 Wednesday, October 22, 2014

OVARIAN STIMULATION DOES NOT DECREASE ENDOMICRONAL RECEPTIVITY IN LOW/AVGERAGE RESPONDERS: A COMPARISON OF 6,348 FRESH AUTOLOGOUS VS. DONOR OOCYTE IVF CYCLES FROM THE SART REGISTRY. J. S. Yeh, R. G. Steward, M. P. Provost, A. M. Dude, K. S. Acharya, J. L. Eaton, J. M. Goydolfarb, S. J. Muasher. Division of Reproductive Endocrinology and Infertility, Duke University Medical Center, Durham, NC; Department of Obstetrics and Gynecology, University of Illinois at Chicago, Chicago, IL; University Hospitals Fertility Center, Beachwood, OH.

OBJECTIVE: Much of the existing evidence describing the potential detrimental effect of controlled ovarian hyperstimulation (COH) on fresh IVF success rates is derived from patients with abundant ovarian reserve (1,2). The impact of COH on low/average responders, however, is unclear. Therefore, in order to isolate ovarian stimulation as a potential modifier of receptivity, we used a large national database to compare pregnancy outcomes in fresh autologous vs. donor-recipient IVF cycles in young women with low/average ovarian response.

RESULTS: From 170 cycles initiated, cycle cancellation rate was 4.7%/170) and oocyte retrieval rate was 63.0%/102/162). In all retrieved cases, total collected oocytes were 143 and mean number of oocytes per retrieved cycle was 1.4±0.8. The mature oocyte rate was 50.3%/72/143 and mean number of mature oocytes per retrieved cycle was 1.2±0.7. After exclusion of degenerated oocytes, we performed the insemination procedure in 129 oocytes and FR was 79.7%/104/128). Finally we performed ET in 34-cycles with a total of 61 embryos(mean number of embryo transfer was 1.79±0.86). IR was 11.3%/8/61) and CPR was 23.5%/8/34). After exclusion of patient whose age is over 40, the IR was 25.9%/7/27) and CPR was 41.1%/7/17).

CONCLUSION: Extreme PORs with very low AMH level can be the candidates for IVF-ET treatment, if clinicians and patients both accepting the high risk of cycle cancellation with lower rate of oocyte retrieval. Especially in the group whose age is less than 40 years, IVF is an effective treatment option.

P-547 Wednesday, October 22, 2014

THE TRUE IMPACT OF ELEVATED PROGESTERONE ON THE DAY OF HCG ON PREGNANCY RATES AFTER IVF: A COHORT ANALYSIS OF MORE THAN 3,600 CYCLES. C. A. Venetis, E. M. Kolbiamakis, G. T. Lainas, I. A. Sfontouris, J. S. Yeh, M. P. Provost, Women’s & Children’s Health, St. George Hospital, University of New South Wales, Kogarah, New South Wales, Australia; 3Unit for Human Reproduction, 1st Dept. Of OB/Gyn, Medical School, Aristotle University of Thessaloniki, N. Elkaria, Thessaloniki, Greece; 4Eugonia Unit of Assisted Reproduction, Athens, Attica, Greece.

OBJECTIVE: The detrimental role of progesterone elevation (PE) on the day of hCG for pregnancy achievement in fresh IVF cycles has been suggested by many studies, although some clinicians still doubt the presence of such an effect. Most of the studies have assessed the impact of progesterone (P) by univariate analysis of observational data, thus ignoring any potential confounding effect by other known predictors of pregnancy after IVF. The aim of this study is to estimate the true effect of PE on the day of hCG on ongoing pregnancy rates after removing any potential confounding effect.

DESIGN: Retrospective study.

MATERIALS AND METHODS: All fresh IVF cycles in which stimulation was performed with gonadotrophins and GnRH analogues were analysed (N=3,617). Multivariate regression analyses were performed in order to evaluate the predictive performance of P concentration on the day of hCG on the probability of ongoing pregnancy with and without taking into account female age, number of oocytes retrieved, the number of embryos transferred and the developmental stage of embryos at transfer (cleavage vs. blastocyst) (“known predictors”). Additional analyses were performed in order to estimate the impact of PE (>1.5 ng/mL) with and without taking into account the aforementioned known predictors.

RESULTS: Progesterone elevation was observed in 247 cycles (6.8%, 95% CI: 6.1-7.7). Logistic regression analysis indicated that P concentration was positively associated with the probability of ongoing pregnancy (OR: 1.29, 95% CI: 1.10-1.51), while PE was not associated with probability of ongoing pregnancy (OR: 0.94, 95% CI: 0.70-1.25). However, in a multivariate logistic regression analysis model in which the confounding effect of the known predictors was removed, P concentration (OR: 0.68, 95% CI: 0.55-0.82) and PE on the day of hCG (OR: 0.60, 95% CI: 0.43-0.82) were both negatively associated with the probability of ongoing pregnancy.

CONCLUSION: Elevated P is independently associated with lower ongoing pregnancy rates after fresh IVF cycles. Assessing the impact of P on the day of hCG on pregnancy rates without accounting for potential confounders can lead to spurious conclusions and this might be a major source of the discrepancy present in the published literature.

P-548 Wednesday, October 22, 2014

STATE-SPECIFIC OVERVIEW OF 2011 U.S. ASSISTED REPRODUCTIVE TECHNOLOGY (ART) TREATMENT OUTCOMES AND CONTRIBUTION TO MULTIPLE-BIRTH, LOW BIRTH WEIGHT, AND PRETERM INFANTS. S. Sundaram, D. M. Kissin, S. Crawford, D. J. Jamieson, W. D. Barfield. Division of Reproductive Health, Centers for Disease Control and Prevention, Chamblee, GA.

OBJECTIVE: To report state-specific ART procedures and compare ART infant outcomes to all U.S. infant outcomes.

DESIGN: Population-based retrospective analysis.

MATERIALS AND METHODS: Data were obtained from CDC’s National ART Surveillance System (NASS) and U.S. National Vital Statistics System. The number of ART interventions per million women of reproductive age (ART utilization) and the average number of embryos transferred are reported for each state. Rates of elective single embryo transfers (cSET) and ART-conceived multiple-birth, low birth weight, and preterm...
infants are reported by state and plurality. The proportion of ART infants among all infants, multiple-birth, low birth weight, and preterm infants was calculated.

RESULTS: Among 3,994,670 infants born in 2011 in the U.S. and Puerto Rico, 1.5% (59,631) were conceived with ART (range: 0.2% in Puerto Rico to 5% in Massachusetts). ART utilization among states ranged from 313 to 7,502 procedures per million women aged 15-44 years, and was higher than the national rate of 2,401 in 11 of 50 states, many of which were located in the northeast. The national eSET rate among women <35 years was 12.2% (range: 1% in Idaho to 53% in Delaware). Approximately 46%, 31%, and 36% of ART infants were multiple-birth, low birth weight, or preterm infants versus 3%, 8%, and 12% of all infants, respectively. The percentage of preterm ART infants varied from 13% among singleton to 62% among twins and 97% among triplets or higher-order multiples; comparable percentages for all infants were 10%, 57%, and 93%, respectively. Nationally, ART contributed to 20% of multiple-birth (range: 5% in Mississippi to 41% in New York State), 6% of low birth weight (range: 1% in Puerto Rico to 15% in Massachusetts), and 5% of preterm (range: 1% in Puerto Rico to 13% in Massachusetts) infants.

CONCLUSION: Wide variations among states were observed in the rates of ART births, utilization, eSET rates, and the contribution of ART to multiple-birth, low birth weight, and preterm infants. Twins and higher order infants were approximately five and seven times more likely to be preterm than were singletons for both ART and all infants. Among ART-conceived infants, greater utilization of eSET, where appropriate, could reduce the contribution of ART to multiple births and preterm delivery.

P-549 Wednesday, October 22, 2014

EFFECT OF SPERM HEAD FIRST OR SPERM TAIL FIRST INJECTION INTO OOPLM ON OOCYTE SURVIVAL, FERTILIZATION AND EMBRYO DEVELOPMENT WITH PIEZO-ICSI IN HUMAN OOCYTES. K. Hirao, a S. Kitamura, b M. Kuwayama, c a Research and Development Division, Repro-Support Medical Research Center, Tokyo, Japan; b ART Section, Niji Clinic, Tokyo, Japan.

OBJECTIVE: In human oocytes ICSI, it is believed that the sperm should be injected from sperm head first into the ooplasm for fertilization. This is because internalization of sperm into the ooplasm is initiated from sperm head in natural fertilization.

The ICSP procedure directly injects the sperm into the ooplasm and bypasses the internalization of sperm head into the ooplasm, so the oocytes could be fertilized if the sperm is injected from sperm tail first into the ooplasm. However, there is no report regarding ICSP results and embryo development of sperm tail first into the ooplasm in human oocytes. Therefore, we investigated the effect of sperm head first injection or sperm tail first into the ooplasm on oocyte survival, fertilization, embryo development with Piezo-ICSI in human oocytes.

DESIGN: A prospective randomized study.

MATERIALS AND METHODS: Between June 2012 and June 2013, a total of 152 patients were randomly assigned to either sperm head first injection group or sperm tail first injection group with Piezo-ICSI (PM-I-150HJ, PRIME TECH). Three hundred and forty two mature oocytes (75 patients; mean age 38.3 ± 4.3) were microinjected by sperm head first into ooplasm and 290 mature oocytes (77 patients, mean age 39.5 ± 4.6) were microinjected by sperm tail first into ooplasm. The rates of oocyte survival, fertilization and good quality day-3 embryo were compared. Good quality day-3 embryos were defined as those having regular blastomeres, <20% fragments and those containing at least 7 cells on day 3. Data were analyzed using Fisher’s exact test.

RESULTS: The rates of oocyte survival, fertilization, good quality day-3 embryo per fertilized oocytes of sperm head first injection group and sperm tail first injection group were 99% (339/342) and 99% (288/290), 86% (294/342) and 90% (262/290), 69% (202/294) and 68% (179/262), respectively. There were no significant differences in the rates of oocyte survival, fertilization and good quality day-3 embryo between the groups.

CONCLUSION: Sperm head first or sperm tail first injection into the ooplasm does not affect oocyte survival, fertilization and subsequent embryo development with Piezo-ICSI in human oocytes.

P-550 Wednesday, October 22, 2014

ENHANCEMENT OF MOUSE EMBRYO DEVELOPMENT AND IMPLANTATION BY A NOVEL PROSTACYCLIN-PRODUCING CO-CULTURE SYSTEM – IMPLICATION TO HUMAN IVF. J.-C. Huang, a W.-S. A. Wun, a K.-H. Ruan, a b Obstetrics and Gynecology, Texas Tech University Health Science Center, Lubbock, TX; b Fertility Specialists of Houston, Houston, TX; c Pharmacology, University of Houston, Houston, TX.

OBJECTIVE: Iloprost, a stable analog of prostacyclin has been reported to enhance human IVF success (1). Natural prostacyclin has short half life (3-8 minutes), which renders experiments with naturally occurring prostacyclin extremely difficult. Our objective was to test a novel de-novo prostacyclin-producing co-culture system and to determine its impact on embryo development and implantation.

DESIGN: Prospective animal trial.

MATERIALS AND METHODS: (A) Three to five week old female mice of B6C3H mixed genetic backgrounds were used to generated 2-cell embryos (5 units PMSG IP injection initially, 48 hours later 5 units of hCG and mate with male; another 48 hours later, collect 2-cell embryos). Embryos were randomly assigned to method contraol (no plasmid transfection), experimental control (feeder layer transfected with vector) and experimental group (feeder layer transfected with plasmid containing COX-2/PGIS fusion nucleotide).

The percentages of completely hatched embryos were compared 96 hour later. (B) Two-cell embryos prepared as above were cultured for 48 hours with feeder layer ransfected with plasmid with (experimental) and without (control) COX-2/PGIS fusion nucleotide (2) before transferring to gestational carriers. The number of gestation sacs were compared 72 hours later. Student’s t-test was used for statistical analysis.

RESULTS: In embryo hatching experiment, 50 of the 109 method control embryos hatched completely, 51 of the 110 experimental control embryo hatched completely and 72 of the 108 experimental embryos hatched completely (p=0.0028, HR: 1.44, 95% CI: 1.13-1.83). In co-culture/embryo transfer experiment, there were 96 and 94 embryos in control and experimental groups, respectively. Experimental group had more late blastocyst (43 vs. 28 p=0.024) and fewer morula staged embryos (30 vs. 54, p<0.001). After transferring togestational carriers there were significantly more gestation sacs in the experimental group.

CONCLUSION: De-novo synthesized prostacyclin from feeder layer cells enhances embryo development and implantation. This serves as a model to provide de-novo synthesized prostacyclin in human IVF culture system.

Prostacyclin producing feeder layer enhances embryo implantation

<table>
<thead>
<tr>
<th>Control</th>
<th>Experimental</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. embryos transferred</td>
<td>96</td>
<td>94</td>
</tr>
<tr>
<td>No. gestation sacs</td>
<td>32</td>
<td>50</td>
</tr>
</tbody>
</table>

* HR 1.60, 95% CI 1.14-2.24

P-551 Wednesday, October 22, 2014

THE EFFECT OF EMBRYO TRANSFER DAY OXYTOCIN ANTAGONIST INFUSION ON IVF OUTCOMES: A SYSTEMATIC REVIEW AND META-ANALYSIS. M. S. Kim, a S. K. Kim, b J. R. Lee, b B. C. Jee, a,b C. S. Suh, a,b H. Choi, a S. H. Kim, b Obstetrics and Gynecology, Seoul National University Bundang Hospital, Seongnam, Gyeonggi-do, Republic of Korea; b Obstetrics and Gynecology, Seoul National University College of Medicine, Seoul, Republic of Korea; c Obstetrics and Gynecology, Inje University Sanggye Paik Hospital, Seoul, Republic of Korea.

OBJECTIVE: Several studies demonstrated that transferred embryos could be expelled due to uterine peristalsis and uterine contraction on embryo transfer day was inversely related to the pregnancy rates. Recently there have been increasing attempts of application of oxytocin antagonist (OA, Atosiban) to enhance the pregnancy rate, especially in patients with recurrent implantation failure. The objective of this study was to evaluate the effect of embryo transfer day OA supplementation on IVF outcomes through a systematic review and meta-analysis.

DESIGN: Systematic review and meta-analysis.

MATERIALS AND METHODS: A computerized search was conducted using three online databases (Cochrane Central Register of Controlled Trials (CENTRAL) in the Cochrane Library, Medline, and Embase). Databases were searched to April 2014 without restriction by language or publication status. A combination of the following key words was used in the search: (atosiban or oxytocin antagonist or oxytocin receptor antagonist or oxytocin receptor) and (embryo transfer or IVF or ICSI or implantation). Studies were selected according to predefined inclusion criteria and meta-analyzed using Review Manager 5.2. Only randomized controlled trials (RCTs) were included in this study.
RESULTS: A total of 156 studies were reviewed and assessed for eligibility. Three RCTs including 340 patients met the selection criteria. OA infusions in embryo transfer day was associated with an increased pregnancy rate (odds ratio (OR) = 2.18; 95% confidential interval (CI) 1.40 to 3.41, p = 0.0006) and an increased implantation rate (OR = 1.83; 95% CI 1.31 to 2.55, p = 0.0003).

CONCLUSION: The results of this meta-analysis using the current literature available suggest that use of OA on embryo transfer day improves clinical pregnancy rate and implantation rate. Further large scaled prospective randomized studies are needed to confirm these results.

P-552 Wednesday, October 22, 2014
DONOR OOCYTE PREIMPLANTATION GENETIC SCREENING FOR SEX SELECTION: FEWER EMBRYOS TRANSFERRED WITHOUT DECREASING PREGNANCY RATES. J. Barritt, a,b A. S. Q. Kathiresan, a M. Shamoni, c H. Danzer, a,b M. Surrey, a,b S. Ghadir, a,b W. Chang, a,c C. Wambach, a,b J. Crofoot, a,b D. Johnson, a,b D. Hill, a,b ART Reproductive Center, Beverly Hills, CA; a Southern California Reproductive Center, Beverly Hills, CA; a UCLA Dept of OB/GYN, Los Angeles, CA.

OBJECTIVE: The objective of this study was to compare pregnancy outcomes in oocyte donor IVF cycles that did and did not involve preimplantation genetic screening (PGS) for sex selection.

DESIGN: Retrospective data analysis from a single large private fertility clinic.

MATERIALS AND METHODS: Analysis of 318 donor oocyte IVF cycles between 2010 and 2013 was performed. PGS using array comparative genetic hybridization was performed on day 5 blastocyst. Embryo transfers were performed on day 5 without PGS (n = 245) or on day 6 if PGS was performed (n = 73). Parametric and nonparametric statistical analyses were used to evaluate differences.

RESULTS: There was no difference in mean donor age, number of oocytes or mature oocytes retrieved, fertilization, or number of embryos cryopreserved. Twenty-three percent of cycles involved PGS, in which, the euploid rate was 57.6%. There was no significant difference in pregnancy rates (no PGS vs. PGS: 59.2% vs. 56.2%; p = 0.65). Interestingly, there were significantly fewer embryos transferred in cycles that involved PGS (1.7 vs. 1.4 ± 0.6; p < 0.001).

CONCLUSION: Donor oocyte PGS performed for sex selection did not significantly alter pregnancy rates. However, performing fresh embryo transfer on day 5 without PGS vs. day 6 with PGS could have significantly impacted these findings due to uterine receptivity differences. The significantly reduced number of embryos transferred after PGS may be associated with decreased chances of multiple pregnancies. Additionally, by only transferring genetically normal embryos we decreased possible pregnancy complications from the transfer of undiagnosed embryos. Larger studies are needed to determine if sex selection PGS in donor cycles, with freezing of fewer embryos, can significantly reduce number of embryos transferred after PGS may be associated with decreased chance of multiple pregnancies. However, the delayed ICSI cycles did have significantly fewer mature oocytes at retrieval (61% vs. 81%, P < 0.0001) and lower blastocyst development overall (13.3% vs. 46.9%, P < 0.001).

CONCLUSION: Day-1 ICSI performed on delayed maturation oocytes resulted in a very low 2.7% of embryos of sufficient quality to transfer or cryopreserve at the blastocyst stage. Cycles in which delayed ICSI was performed had fewer mature oocytes, and a second round of ICSI on day-1 did not improve outcomes in these highly compromised cycles. These poor day-1 ICSI outcomes do not justify the laboratory time, expense or increased embryo time outside the incubator required for day-1 ICSI.

Supported by: NIH/National Center for Advancing Translational Science (NCATS) UCLA CTSI Grant Number UL1TR000124.

P-554 Wednesday, October 22, 2014
ECTOPIC PREGNANCY RISK AFTER IN-VITRO FERTILIZATION: IS THERE ANY DIFFERENCE BETWEEN FRESH AND FROZEN-THAWED EMBRYO TRANSFERS? L. Londra, a J. Garcia, a C. Moreau, a C. Alexander, b D. Strobino, b Y. Zhao, c Johns Hopkins Medical Institutions, Baltimore, MD; a, b, c UCLA Cedars-Sinai Medical Center, Los Angeles, CA.

OBJECTIVE: In vitro fertilization (IVF) is associated with an increased risk of ectopic pregnancy (EP) compared to spontaneous pregnancies. As a result of controlled ovarian hyperstimulation (COH), fresh embryo transfers (ET) occur in the context of supraphysiologic estradiol environment, whereas frozen-thawed embryo transfers (FET) occur in the context of a hormonal environment that is closer to a natural cycle. The aim of this analysis was to compare the risk of EP after fresh ET with the risk of EP after FET.

DESIGN: Historical cohort study.

MATERIALS AND METHODS: The Society for Assisted Reproductive Technologies (SART) national database was used to identify all reported pregnancies that resulted from fresh and FET between years 2008-2011 in the US. The primary outcome measure was the occurrence of EP in fresh ET and in FET at the blastocyst stage. Multivariate logistic regression analysis was used to adjust for confounders and previously described EP risk factors, including women’s demographics, type of infertility diagnosis, COH characteristics, insemination and embryo micromanipulation techniques, number of embryos per transfer and ultrasound sound for ET.

RESULTS: A total of 126,041 IVF cycles with at least 1 blastocyst transferred were recorded, of which 40,394 (32%) were FET and 85,647 (67.95%) were fresh ET. Fifteen percent of pregnancies (12% of fresh ET and 20% of FET, p < 0.0001) were only biochemically detected and 0.06% pregnancies had unknown outcome and were not considered in the analysis. Of the 107,522 pregnancies, 98.6% were intrauterine (n = 105,549). 1.3% were EP (n = 1,402) and 0.07% were heterotopic (n = 71). The proportion of EP and heterotopic pregnancies combined was lower in FET cycles than in fresh ET cycles (0.95% versus 1.56%, OR = 0.65 [0.60-0.72], p < 0.001). After controlling for age, race, gravidity, obesity, reasons for infertility including tubal pathology, donor oocyte status and COH characteristics, the odds of EP remained lower in FET cycles than in fresh ET cycles (OR = 0.58 [0.51-0.67], p < 0.001).

CONCLUSION: Compared to fresh ET, FET resulted in lower risk of EP. Despite intrinsic limitations that analyses of a national database with different practice modalities might have, it’s important to identify segments in the IVF treatment that could be modified in order to minimize the risk of EP in infertile couples. Additionally, the analysis of IVF cycles provides an opportunity to elucidate the understanding of the physiopathology of EP.
ECTOPIC PREGNANCY RATE INCREASES WITH THE NUMBER OF RETRIEVED OOCYTES IN AUTOLOGOUS IVF BUT NOT IN DONOR/RECIPIENT CYCLES: AN ANALYSIS OF 109,140 CLINICAL PREGNANcies FROM THE SART REGISTRY. K. S. Acharya, C. R. Acharya, M. P. Provost, J. S. Yeh, R. G. Steward, J. L. Eaton, S. J. Muasher. Division of Reproductive Endocrinology and Infertility, Duke University Medical Center, Durham, NC; Department of Biostatistics and Bioinformatics, Duke University Medical Center, Durham, NC.

OBJECTIVE: The impact of supraphysiologic hormone levels resulting from controlled ovarian stimulation on ectopic pregnancy (EP) risk is unclear. In an attempt to isolate ovarian stimulation as a potential modifier of EP risk, we used a large and recent national registry to compare EP rates between fresh autologous and donor oocyte IVF cycles.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: We used data from the 2008-2010 SART registry. Only fresh autologous and donor oocyte cycles achieving clinical pregnancy (CP) were used in our analysis. Cycles with a diagnosis of tubal infertility were excluded. Cycles were divided into 6 cohorts based on the number of oocytes retrieved, and the percentage of ectopic pregnancies (defined as ectopic or heterotopic pregnancies) were calculated (Table). A linear model was constructed to discern the relationship between the average number of oocytes per category and the EP risk per clinical pregnancy.

RESULTS: There were 91,504 and 17,636 CPs reported among all autologous and donor oocyte cycles, respectively. In autologous cycles, EP rate significantly increased as oocyte yield increased (P=0.04). This association was not found in oocyte recipients (P=0.18).

<table>
<thead>
<tr>
<th>Oocyte yield per cycle</th>
<th>0-5</th>
<th>6-10</th>
<th>11-15</th>
<th>16-20</th>
<th>21-25</th>
<th>&gt;25</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>% EP - Autologous cycles</td>
<td>1.65</td>
<td>1.77</td>
<td>1.89</td>
<td>1.82</td>
<td>1.89</td>
<td>1.92</td>
<td>0.04</td>
</tr>
<tr>
<td>% EP - Donor oocyte cycles</td>
<td>2.64</td>
<td>1.17</td>
<td>0.73</td>
<td>0.77</td>
<td>0.76</td>
<td>0.88</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Note: EP - ectopic pregnancy; rates of EP are reported per 100 clinical pregnancies. P-values reflect the statistical significance of the predicted ability of the number of oocytes per category.

CONCLUSION: Recent national data suggest that the rate of EP increases with higher oocyte yield in autologous but not donor oocyte IVF cycles. Further research is needed to determine if these differences can be attributed to autologous ovarian stimulation.

P-556 Wednesday, October 22, 2014


OBJECTIVE: To determine if IVF success is reduced in young women with a low number of eggs retrieved.

DESIGN: Retrospective chart review.

MATERIALS AND METHODS: Clinical records were examined for all patients under 35 years of age undergoing IVF/ICSI with a fresh, cleavage-stage embryo transfer between January 2007 and December 2013 at our university based practice. Patients who underwent a minimal stimulation protocol were excluded. Data were collected on cycle characteristics and implantation and pregnancy outcomes. SAS was used for statistical analysis. T-tests and logistic regression were used for comparisons.

RESULTS: 519 cycles were included in the analysis, 28 with less than 5 oocytes retrieved and 491 with 5 or more oocytes retrieved. Implantation rates (IR), pregnancy rates (PR), clinical pregnancy rates (CPR) and live birth rates (LBR) were calculated, all controlled for number of embryos transferred. Women with fewer than 5 oocytes retrieved had significantly fewer embryos transferred (ET) than those with 5 or more oocytes retrieved (1.7 vs 2.0, p<0.01). The implantation rates were not significantly different (34% vs. 36%, p=0.95). After adjusting for the number of embryos transferred, there was no difference in the pregnancy rate, clinical pregnancy rate or live birth rate.

CONCLUSION: Young DOR patients who make it to retrieval have a good prognosis with IVF. While declining follicle number is associated with lower implantation and higher miscarriage rates among older women undergoing IVF, the same is not true for young women with DOR. With fewer oocytes retrieved, young DOR patients are likely to have fewer embryos for transfer, but the embryos they have, appear to have the same probability of implanting and resulting in a healthy pregnancy. Additionally, pregnancy and live birth rates following cleavage-stage transfers in this group are very good, indicating that there is still a role for day 3 transfers in an era of increasing use of extended culture.

P-557 Wednesday, October 22, 2014

ESTRADIOL LEVEL AS A PARAMETER FOR OPTIMAL OVAULATION TRIGGER DAY IN IVF/ICSI CYCLES. A. P. Melnick, E. M. Murphy, A. Khalifa, R. Elias, Z. Rosenwaks. The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medical College, New York, NY; Faculty of Medicine, Alexandria University, Elazara, Alexandria, Egypt.

OBJECTIVE: To determine whether the time interval from E2 of 400 pg/mL to trigger has an effect on IVF cycle and pregnancy outcomes.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: IVF cycles in women younger than 35 years old with peak E2 of 1500-2500 pg/mL were analyzed. For accurate calculation of oocyte maturity, only ICSI cycles were included. hCG was used to trigger ovulation according to our center’s sliding scale. Cycles were stratified into two groups: hCG less than four days from an E2 of 400 pg/mL and hCG at least four days from an E2 of 400 pg/mL. Outcomes measured included total and mature oocyte yield, fertilization rate, implantation rate, embryo grade, blastocyst rate, and clinical pregnancy/live birth rates. Statistical analysis included χ² and t-tests. P<0.05 was deemed statistically significant.

RESULTS: We analyzed 965 IVF cycles from January 2006 to September 2011. Trigger occurred at less than four days from E2 of 400 pg/mL in 94 cycles and at least 4 days from E2 of 400 pg/mL in 871 cycles. The two groups were similar in terms of age, BMI, and lead follicle size at trigger. Peak E2 was significantly higher in the <4 days group. There were no differences in number of oocytes retrieved, number of mature oocytes retrieved, embryo grade, and fertilization rate. Blastocyst and implantation rates were significantly higher in the <4 days group. There was a trend toward higher clinical pregnancy and live birth rates in the <4 days group, though this did not reach statistical significance.

<table>
<thead>
<tr>
<th>&lt;4 Days</th>
<th>≥4 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>94</td>
</tr>
<tr>
<td>Age</td>
<td>30.9±2.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.6±5.4</td>
</tr>
<tr>
<td>Lead Follicle at HCG (mm)</td>
<td>18.7±1.5</td>
</tr>
<tr>
<td>Peak E2 (pg/mL)</td>
<td>1822±1.5</td>
</tr>
<tr>
<td>#Oocytes Retrieved</td>
<td>14.3±5.9</td>
</tr>
<tr>
<td>#Mature Oocytes Retrieved</td>
<td>11.4±8.8</td>
</tr>
<tr>
<td>Fertilization Rate</td>
<td>0.74±0.23</td>
</tr>
<tr>
<td>Best Embryo Grade (Day 3)</td>
<td>1.84±0.76</td>
</tr>
<tr>
<td>Blastocysts/Cycle (%)</td>
<td>*36.2</td>
</tr>
<tr>
<td>Implantation Rate</td>
<td>*0.46±0.39</td>
</tr>
<tr>
<td>#Transferred</td>
<td>2.2±0.69</td>
</tr>
<tr>
<td>Clinical Pregnancy/Cycle (%)</td>
<td>70.3</td>
</tr>
<tr>
<td>Live Birth/Cycle (%)</td>
<td>57.4</td>
</tr>
</tbody>
</table>

*P<0.05
CONCLUSION: In good responders, triggering ovulation less than 4 days from an estradiol level of 400 pg/mL leads to higher blastulation and implantation rates and likely higher clinical pregnancy and live birth rates. Further studies are needed to determine whether these findings hold true in other patient cohorts.

P-558 Wednesday, October 22, 2014


OBJECTIVE: To validate a published morphokinetic algorithm (Meseguer et al., 2011) for embryo selection by time-lapse technology.

DESIGN: Prospective, randomized, triple blinded, controlled study. This study includes 930 patients undergoing IVF.

MATERIALS AND METHODS: Patients were randomly divided into a control group (patients whose embryos developed in a conventional incubator (SI) and were assessed only by conventional morphological criteria) and a study group (in which embryos were cultured in the time-lapse monitoring system (TMS) EmbryoScope (Fertilitech, Aarhus, DK) and were evaluated using a hierarchical morphokinetic model). The morphokinetic model establishes five embryo categories based on precise timing of cell division. Embryo implantation rate (IR) was evaluated in each morphokinetic category in the TMS group or morphological criteria in the SI group.

RESULTS: We observed a direct relationship between morphokinetic categories and implantation potential in TMS group. Also a direct relationship was observed between morphology categories and implantation rates in SI.

CONCLUSION: In good responders, triggering ovulation less than 4 days from an estradiol level of 400 pg/mL leads to higher blastulation and implantation rates and likely higher clinical pregnancy and live birth rates. Further studies are needed to determine whether these findings hold true in other patient cohorts.

P-558 Wednesday, October 22, 2014


OBJECTIVE: To validate a published morphokinetic algorithm (Meseguer et al., 2011) for embryo selection by time-lapse technology.

DESIGN: Prospective, randomized, triple blinded, controlled study. This study includes 930 patients undergoing IVF.

MATERIALS AND METHODS: Patients were randomly divided into a control group (patients whose embryos developed in a conventional incubator (SI) and were assessed only by conventional morphological criteria) and a study group (in which embryos were cultured in the time-lapse monitoring system (TMS) EmbryoScope (Fertilitech, Aarhus, DK) and were evaluated using a hierarchical morphokinetic model). The morphokinetic model establishes five embryo categories based on precise timing of cell division. Embryo implantation rate (IR) was evaluated in each morphokinetic category in the TMS group or morphological criteria in the SI group.

RESULTS: We observed a direct relationship between morphokinetic categories and implantation potential in TMS group. Also a direct relationship was observed between morphology categories and implantation rates in SI.

CONCLUSION: In good responders, triggering ovulation less than 4 days from an estradiol level of 400 pg/mL leads to higher blastulation and implantation rates and likely higher clinical pregnancy and live birth rates. Further studies are needed to determine whether these findings hold true in other patient cohorts.

P-558 Wednesday, October 22, 2014


OBJECTIVE: To validate a published morphokinetic algorithm (Meseguer et al., 2011) for embryo selection by time-lapse technology.

DESIGN: Prospective, randomized, triple blinded, controlled study. This study includes 930 patients undergoing IVF.

MATERIALS AND METHODS: Patients were randomly divided into a control group (patients whose embryos developed in a conventional incubator (SI) and were assessed only by conventional morphological criteria) and a study group (in which embryos were cultured in the time-lapse monitoring system (TMS) EmbryoScope (Fertilitech, Aarhus, DK) and were evaluated using a hierarchical morphokinetic model). The morphokinetic model establishes five embryo categories based on precise timing of cell division. Embryo implantation rate (IR) was evaluated in each morphokinetic category in the TMS group or morphological criteria in the SI group.

RESULTS: We observed a direct relationship between morphokinetic categories and implantation potential in TMS group. Also a direct relationship was observed between morphology categories and implantation rates in SI.

CONCLUSION: In good responders, triggering ovulation less than 4 days from an estradiol level of 400 pg/mL leads to higher blastulation and implantation rates and likely higher clinical pregnancy and live birth rates. Further studies are needed to determine whether these findings hold true in other patient cohorts.

P-558 Wednesday, October 22, 2014


OBJECTIVE: To validate a published morphokinetic algorithm (Meseguer et al., 2011) for embryo selection by time-lapse technology.

DESIGN: Prospective, randomized, triple blinded, controlled study. This study includes 930 patients undergoing IVF.

MATERIALS AND METHODS: Patients were randomly divided into a control group (patients whose embryos developed in a conventional incubator (SI) and were assessed only by conventional morphological criteria) and a study group (in which embryos were cultured in the time-lapse monitoring system (TMS) EmbryoScope (Fertilitech, Aarhus, DK) and were evaluated using a hierarchical morphokinetic model). The morphokinetic model establishes five embryo categories based on precise timing of cell division. Embryo implantation rate (IR) was evaluated in each morphokinetic category in the TMS group or morphological criteria in the SI group.

RESULTS: We observed a direct relationship between morphokinetic categories and implantation potential in TMS group. Also a direct relationship was observed between morphology categories and implantation rates in SI.

CONCLUSION: In good responders, triggering ovulation less than 4 days from an estradiol level of 400 pg/mL leads to higher blastulation and implantation rates and likely higher clinical pregnancy and live birth rates. Further studies are needed to determine whether these findings hold true in other patient cohorts.

P-558 Wednesday, October 22, 2014


OBJECTIVE: To validate a published morphokinetic algorithm (Meseguer et al., 2011) for embryo selection by time-lapse technology.

DESIGN: Prospective, randomized, triple blinded, controlled study. This study includes 930 patients undergoing IVF.

MATERIALS AND METHODS: Patients were randomly divided into a control group (patients whose embryos developed in a conventional incubator (SI) and were assessed only by conventional morphological criteria) and a study group (in which embryos were cultured in the time-lapse monitoring system (TMS) EmbryoScope (Fertilitech, Aarhus, DK) and were evaluated using a hierarchical morphokinetic model). The morphokinetic model establishes five embryo categories based on precise timing of cell division. Embryo implantation rate (IR) was evaluated in each morphokinetic category in the TMS group or morphological criteria in the SI group.

RESULTS: We observed a direct relationship between morphokinetic categories and implantation potential in TMS group. Also a direct relationship was observed between morphology categories and implantation rates in SI.

CONCLUSION: In good responders, triggering ovulation less than 4 days from an estradiol level of 400 pg/mL leads to higher blastulation and implantation rates and likely higher clinical pregnancy and live birth rates. Further studies are needed to determine whether these findings hold true in other patient cohorts.
semen analysis parameters in an ART setting, our data does not reveal any statistically significant difference when compared to age matched control in an Irish ART setting.

CRYOPRESERVATION AND FROZEN EMBRYO TRANSFER

P-561 Wednesday, October 22, 2014

COMPARING PREGNANCY OUTCOMES AND LIVE BIRTH RATES OF FRESH AND FROZEN-THAWED SINGLE BLASTOCYST TRANSFERS, ASSESSING ENDOMETRIAL RECEPTIVITY. A. Ershahin, a, b L. Kupeloglou, b J. Ozcan, a A. I. Gonenc, a S. S. Ershahin, a O. Dulger, a, b, c G. A. Obel, a, b S. D. Paris, a, b, c C.-C. Chang, a, b

OBJECTIVE: To compare success rates between fresh elective single blastocyst transfers (eSBT) and frozen-thawed SBT (fSBT) in the same cohort of young normoresponder patients to assess endometrial receptivity. We aimed to investigate the potential effects of controlled ovarian hyperstimulation (COH) on endometrial receptivity by comparing outcomes between fresh eSBT and fSBT.

DESIGN: Prospective cohort study in a private fertility center.

MATERIALS AND METHODS: Of 255 patients receiving a fresh eSBT, 92 came for a SBT in our center from January 2011 to April 2013. Fresh and, when required, frozen ET were performed subsequently in the same cohort of patients. Clinical and ongoing pregnancy rates, implantation and live birth rates were the main outcome measures. Normality of distributions was checked with Kolmogorov-Smirnov test. Statistically significant differences in continuous variables were determined using Student’s t test and Mann-Whitney U test, as appropriate. Chi-squared and Fisher’s exact tests were used to analyze categorical data. P values of <0.05 were considered statistically significant.

RESULTS: Of the 255 normoresponder young patients receiving an eSBT, 147 had a positive bhCG result (57.65%) and 142 a clinical pregnancy (55.69%). Among the 108 patients with a negative bhCG test, 92 came for a SBT and 67 had a positive bhCG result (72.83%) and 65 a clinical pregnancy (70.65%). Both biochemical and clinical pregnancy rates were significantly increased (p<0.001, respectively) in the fSBT. Although not statistically significant ongoing pregnancy (60.87% vs. 52.94%) and live birth rates (59.87% vs. 48.63%) were increased in fSBT compared to fresh cycles.

CONCLUSION: The clinical pregnancy rate was significantly higher for the fSBT than in the fresh group. Although not statistically significant, due to the small sample size, the ongoing pregnancy rate is 7% higher in the fSBT than the fresh eSBT group, even though fSBT were performed with the “second best” blastocyst, possibly pointing out that the endometrial receptivity is altered by the COH regimen.

P-562 Wednesday, October 22, 2014

THE EFFECT OF HEPATITIS B AND C ON OVARIAN RESERVE. D. A. Vaughan, a, b C. Harrit, a E. Mocanu, a Harri Unit, The Rotunda Hospital, Dublin, Ireland, aOb-Gyn, Tufts Medical Center, Boston, MA

OBJECTIVE: Data are conflicting regarding whether assisted reproductive outcomes are impaired by viral infections such as hepatitis B and C. We sought to ascertain whether ovarian reserve was diminished in patients with active or chronic hepatitis B, a past history of hepatitis B, or active or chronic hepatitis C undergoing ART.

DESIGN: A retrospective electronic database and single chart review of all ART cycles undertaken from January 2000 through December 2012 in a single, hospital based academic ART unit in Ireland.

MATERIALS AND METHODS: All female patients who were seropositive for active HBV (HBsAg +, anti-HBe +, anti-HBc +), chronic HBV (HBsAg +, anti-HBe +, IgM anti-HBc -), a past HBV (anti-HBc +, anti-HBs -) or active or chronic HCV (anti-HCV +/- HCV RNA) were identified. These were age matched against controls ratio of 1:5. Ovarian reserve was assessed using serum AMH, FSH and AFC. SPSS v21.0 was used for statistical analysis with test used to compare mean with chi-squared analysis used to interpret proportions. P <0.05 was significant.

FERTILITY & STERILITY® e323

RESULTS: Of the 93 women identified as having active or chronic hepatitis B, 56 women were identified as having evidence of persistent HBV infection and 115 women were identified as having evidence of active or chronic hepatitis C infection. The mean ages among or cohort were 35.5, 35.8 and 35.7 years respectively. These were aged matched against a control group whose mean age was 35.7 years and comprised 1320 women. Mean AMH levels of our cohort were noted to be 15.5, 16.7 and 18.3 pmol/L for patients with either active/chronic HBV, past HBV or active/chronic HCV respectively. These were comparable to our control group (mean AMH 15.1 pmol/L). Mean FSH levels among our cohort were 6.2, 7.9, 8.2 iU/ml for patients with either active/chronic HBV, past HBV or active/chronic HCV respectively. These were comparable to our control group (mean FSH 8.1 iU/ml). Mean AFC among our cohort were 7.6, 8.3, 9.2 for patients with either active/chronic HBV, past HBV or active/chronic HCV respectively. Again, this was similar to our control group (mean AFC 8.5). None of the above difference reached statistical significance.

CONCLUSION: Although there is some data to suggest viral infections such as HBV and HCV may affect ART success rates, our study demonstrates that there is no evidence to suggest diminished ovarian reserve in patients with past/current evidence of HBV or HCV infection among an Irish cohort.

P-563 Wednesday, October 22, 2014

AN OUTCOME EVALUATION OF VITRIFIED AND DRY-SHIPPED BLASTOCYSTS CREATED AT A CRYO DONOR EGG BANK. C. M. Larsen, a C.-C. Chang, a J. Linn, a S. Hamilton, b D. Shapiro, a Z. P. Nagy, a Reproductive Biology Associates, Atlanta, GA; aISIS Regional Fertility Centre, Mississauga, ON, Canada.

OBJECTIVE: Cryo donor egg-banks are becoming an increasingly popular treatment option instead of fresh oocyte donation. Recipients have vitrified donor oocytes shipped to their “home IVF center”, where they will be used for treatment. Alternatively, recipients can send frozen partner sperm to the donor egg-bank for oocyte insemination. High quality embryos are cryopreserved and then shipped to the recipient’s “home IVF Center”, where a “frozen embryo transfer” (FET) is performed. Because this treatment option is relatively new, there is not much data available. Therefore, the objective of the study was to analyze FET outcomes of vitrified and shipped embryos from a cryo donor egg-bank.

DESIGN: Retrospective data analysis.

MATERIALS AND METHODS: From November 2010 to January 2014, embryos were created using vitrified donor oocytes (from 85 donors, younger than 30 years) and frozen-thawed sperm obtained from the recipient’s partner. After warming the donor oocytes, insemination was performed 3-4 hours later by ICSI. Blastocysts were vitrified on Day 5 and/or Day 6, and then transported via dry shipper to the designated center. Clinical outcomes were collected and analyzed.

RESULTS: 103 egg warming cycles were performed (7.72 ± 3.11 eggs/cycle) at the donor egg-bank center. 88.9% of eggs survived, 82.9% fertilized, and 60.2% developed to the blastocyst stage (353 blastocysts were vitrified and shipped). FET cycles were performed at the “home IVF center”. In FET cycle #1 there were a total of 118 embryos transferred, and in FET Cycle #2 there were a total of 43 embryos transferred. Not all 103 recipients returned for a first or second FET cycle. Outcomes are shown in the table. N= # of patients that had a transfer.

FET Cycle Outcomes

<table>
<thead>
<tr>
<th></th>
<th>FET Cycle #1</th>
<th>FET Cycle #2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avg. # Embryos</td>
<td>1.28 ± 0.45</td>
<td>1.02 ± 0.15</td>
</tr>
<tr>
<td>Warmed ± S.D.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avg. # of Embryos Transferred ± S.D.</td>
<td>1.28 ± 0.45</td>
<td>1.02 ± 0.15</td>
</tr>
<tr>
<td>Implantation Rate</td>
<td>49.15%</td>
<td>46.51%</td>
</tr>
<tr>
<td>Clinical Pregnancy Rate</td>
<td>63.04%</td>
<td>47.62%</td>
</tr>
</tbody>
</table>

CONCLUSION: Embryo creation with donor oocytes at a cryo donor egg-bank and subsequent FET cycles at the “home IVF Center” of the recipient demonstrate favorable outcomes. Thus, embryo creation is a promising and practical treatment that can be considered as an alternative option to vitrified donor oocyte shipping.

OBJECTIVE: Advances in cryopreservation have led to comparable ongoing pregnancy rates in oocyte donor in vitro fertilization (IVF) cycles utilizing either cryo-bank oocytes or fresh oocyte donation. We evaluated the cost effectiveness of utilizing cryo-bank donor oocytes compared to that of fresh donors used in an anonymous oocyte donation program.

DESIGN: Calculation of cost effectiveness ratios using retrospective data.

MATERIALS AND METHODS: Cycles performed at a large university-based center during the time period of February 2013 through March 2014 were evaluated. 31 cryo-bank donation cycles and 23 fresh oocyte donation cycles (including 7 shared cycles) were included. Patients undergoing a cryo-bank cycle received on average (4-8) oocytes per cycle. Patients undergoing a fresh donor cycle received on average 15 (8-39) oocytes per cycle. An ongoing pregnancy was considered a viable pregnancy past 12 weeks of gestational age. Patients unable to achieve an ongoing pregnancy in their initial cycle and had frozen embryos available were assumed to undergo a subsequent frozen embryo transfer cycle. The ongoing pregnancy rate per frozen embryo transfer for our model was 60%. Cumulative pregnancy rates were calculated as the percentage of patients in each group who achieved an ongoing pregnancy in their initial cycle or subsequent frozen embryo transfer. Average cost without insurance coverage of a fresh oocyte donor cycle, shared fresh cycle, cryo-bank cycle, and frozen embryo transfer at our center is $32,187.50, $21,673.75, $19,000.00, and $4,500.00, respectively. Cost per ongoing pregnancy was calculated as total cost in each group divided by the cumulative number of ongoing pregnancies. Chi square test was used to evaluate categorical data. A p-value < 0.05 was considered statistically significant.

RESULTS: The two groups had equivalent ongoing pregnancy rates (61% vs. 61%; p=0.98) in their initial cycle. Fresh cycles resulted in more patients having frozen embryos compared to cryo-bank cycles (88% vs. 27%; p<.01). There was no significant difference noted in cumulative ongoing pregnancy rates between the two groups (82% vs. 63%; p=0.13). Average cost per cumulative ongoing pregnancy was $30,280.61 in the cryo-bank cycle and $37,378.52 in the fresh cycle.

CONCLUSION: Although fewer oocytes are available and fewer embryos are frozen in cryo-bank cycles, it still may offer a more affordable option of having a baby compared to a fresh oocyte donation cycle.

THAWING CRYOPRESERVED OOCYTES: GENETIC SCREENING OF THAWED OOCYTES AND ONGOING PREGNANCY SUCCESS RATE. Y. G. Kramer, K. N. Goldman, B. Hodes-Wertz, J. Buldo-Liciardi, D. H. McCulloh, J. A. Grifo. NYU Fertility Center, NYU Langone School of Medicine, New York, NY.

OBJECTIVE: To compare clinical outcomes after Oocyte thaw (OUT) with and without preimplantation genetic screening (PGS), and to compare those outcomes with those of In Vitro Fertilization (IVF) with PGS.

DESIGN: Retrospective cohort study.

MATERIALS AND METHODS: 270 previously cryopreserved oocytes from 15 patients (27-42 years) were thawed/warmed, fertilized, cultured to blastocyst, and underwent trophectoderm biopsy on days 5 or 6 for analysis of ploidy by array comparative genomic hybridization (a-CGH) prior to transfer (OUT/PGS). 96 blastocysts were analyzed for ploidy. Only normal euploid embryos were transferred of embryos biopsied on days 5 or 6. OUT/PGS cycle outcomes were compared with 646 previously cryopreserved oocytes (OUT/No PGS) from 67 patients as well as 1416 blastocysts analyzed by a-CGH from 233 IVF/PGS cycles. Only patients’ first OOF thaw (or warming) /PGS or IVF/PGS cycles were included. Donor egg cases were excluded from analysis. Cryopreservation and IVF/PGS cycles occurred between February 2007 and January 2014, thaw or warming cycles between June 2010 and March 2014. Outcomes included implantation rate (IR), clinical pregnancy rate (CPR) and ongoing pregnancy rate (OPR).

RESULTS: Overall data, between two groups (ICM-LAH, TE-LAH), there were no significant differences in clinical pregnancy rate (CPR: ICM; 41.9% (29/59) vs. TE; 41.2% (30/72), P=0.2546) and implantation rate (IR: ICM; 37.5% (36/96) vs. TE; 32.2% (39/121), P=0.1181). However, in OOF (ICM; 3.8±1.2, TE; 4.2±1.9) patients, the ICM-LAH group was presented significant higher clinical outcomes (CPR: ICM; 61.5% (8/13) vs. TE; 27.3% (7/24), P=0.0314, IR: ICM; 47.7% (9/19) vs. TE; 22.0% (9/41), P=0.0457). Also, female and male factor group were not showed significant differences between two groups. But ICM-LAH group was showed significant differences in unexplained patients (CPR: ICM; 77.8% (8/10) vs. TE; 22.2% (2/10), P=0.073, IR: ICM; 64.7% (11/17) vs. TE; 17.6% (3/17), P=0.0053).

CONCLUSION: Assisted hatching close to ICM of frozen-thawed blastocyst presented significantly higher clinical outcomes than assisted hatching close to TE in patients who were underwent ≥ 2 RIF or who were diagnosed as unexplained. Further evaluation of detailed hatching process will be necessary according to the site of AH.

Clinical outcomes from OOF cycles with and without PGS in comparison to IVF cycles with PGS

<table>
<thead>
<tr>
<th>OOT with PGS</th>
<th>OOT without PGS</th>
<th>IVF with PGS</th>
</tr>
</thead>
<tbody>
<tr>
<td>OOT cycle outcomes</td>
<td>P-value</td>
<td>OOT cycle outcomes</td>
</tr>
<tr>
<td>Implantation Rate</td>
<td>50%</td>
<td>25%</td>
</tr>
<tr>
<td>Clinical pregnancy rate</td>
<td>53%</td>
<td>45%</td>
</tr>
<tr>
<td>Live birth and ongoing pregnancy rate</td>
<td>60%</td>
<td>43%</td>
</tr>
</tbody>
</table>

CONCLUSION: PGS in conjunction with OOT yields IR, CPR and OPR comparable to those observed for IVF/PGS. Due to the preliminary nature of this work, we expect ongoing data will reinforce conclusive significance for OOT/PGS application.

OBJECTIVE: To compare safety and efficiency in vitrification of mouse and human embryos between the use of closed type carrier (Rapid-i) and open type carrier (Cryoloop).

DESIGN: A prospective study.

MATERIALS AND METHODS: Eight-cell and blastocyst stages mouse embryos were vitrified-warmed using Cryoloop and Rapid-i carriers, and then cultured to compare the safety of the carriers with the survival and developmental rates of the mouse embryos. TUNEL assay was performed to assess DNA damage of the mouse embryos vitrified-warmed. Of the 267 human embryos vitrified-warmed on day 3 using Cryoloop and Rapid-i, 179 embryos were transferred. The efficiency of the carriers was investigated by comparison of the survival and pregnancy rates of the human embryos.

RESULTS: The survival rates of 8-cell and blastocyst stage embryos vitrified-warmed by using the Rapid-i (99.8% and 96.7%, respectively) were comparable to those obtained by using the Cryoloop (100% and 97.4%, respectively). Blastulation rates of the 8-cell stage embryos vitrified-warmed by Cryoloop and Rapid-i were 89.9 and 93.2%, respectively. Hatched out rates of the mouse blastocysts vitrified-warmed by Cryoloop and Rapid-i were 68.9 and 70.2%, respectively. Quantitative analysis for DNA damage of the mouse embryos showed that there was no difference in the percentage of TUNEL positive cells (DNA damaged cells) between the CryoLoop and Rapid-i groups. Although the intact survival rate of human embryos vitrified-warmed by CryoLoop (79.7%) was significantly higher than the rate of Rapid-i (64.3%, p<0.05), there was no difference in the clinical pregnancy rate between the CryoLoop (50%) and Rapid-i (54%) groups.

CONCLUSION: We confirmed the safety of the Rapid-i carriers with the high survival and developmental rates of the vitrified-warmed mouse embryos. Moreover the efficiency of the Rapid-i in the vitrification of human embryos was confirmed by the stable clinical pregnancy rate. Therefore, we suggest that use of the closed type carrier could maintain the proper pregnancy rate without the potential risk of cross contamination.

EMBRYO RESCUE: OUTCOME FROM VITRIFIED BLASTOCYSTS ARISING FROM EMBRYOS UNSUITABLE FOR CRYOPRESERVATION ON DAY 3. M. Solé, C. Rosello, C. González, M. Boada, F. Martínez, C. Buenaventura, A. Veiga. Department of Obstetrics, Gynecology and Reproduction, Hospital Universitari Quirón Dexeus, Barcelona, Spain; Center of Regenerative Medicine (CMRB), Barcelona, Spain.

OBJECTIVE: To establish the developmental potential and implantation rate of embryos unsuitable for cryopreservation on day 3.

DESIGN: Retrospective study between 2010-2013. We included all embryos that were not considered suitable for cryopreservation on day 3 and were cultured to the blastocyst stage for cryopreservation. Blastocyst rate and implantation rate were analysed to establish the developmental potential of these embryos.

MATERIALS AND METHODS: A total of 2615 embryos from 1027 patients were cultured to the blastocyst stage for cryopreservation. This group is constituted of embryos with < 6 blastomeres, and/or > 30% fragmentation, and/or > 13 blastomeres, and/or multinucleation. Cleaving embryos that did not show fertilisation signs (2 pronuclei) on day 1 were also included. These embryos were classified in 5 categories: slow rate developing embryos, fast developing embryos, fast developing embryos, multinucleated embryos and embryos with no signs of fertilization on day 1. Chi-Square Test was used to compare proportions between groups. We analysed the results of blastocyst vitrified/warming cycles performed with such blastocysts between 2010-2013. Clinical pregnancy was determined by the observation of positive foetal cardiac activity at 6 weeks of gestation.

RESULTS: There were 520 patients from IVF and 507 from oocyte donation. A total of 2615 embryos were cultured in sequential media (Vitrolife, Sweden) of which 571 reached the blastocyst stage (21.8%). Fast rate developing embryos (n=642; 23.5%) and those with no signs of fertilization (n=117; 25.6%) obtained similar blastocyst rate than multinucleated embryos (n=1135; 28.3%). A significantly lower blastocyst rate was obtained with slow developing embryos (n=575; 7.8%) and highly fragmented embryos (n=146; 16.4%) (p<0.05). Out of 67 vitrified blastocysts, 61 (91.0%) survived. Fifty vitrified-warmed blastocyst transfers were performed. Twenty pregnancies were achieved. The overall implantation rate was 41% (25/61), reaching 46.8% in the group of blastocysts coming from multinucleated embryos on day 3 (22/47).

CONCLUSION: Embryos with slow rate developing embryos, fragmented embryos, fast developing embryos, multinucleated embryos and embryos with no signs of fertilization on day 1 can be rescued for cryopreservation by culture until blastocyst stage and subsequent vitrification with good results in terms of blastocyst, survival and implantation rate after warming and transfer.


OBJECTIVE: We evaluated the clinical efficacy of vitrified oocytes and fresh testicular sperm in TESE-ICSI forazoospermic patients.

DESIGN: Retrospective study.

MATERIALS AND METHODS: The subjects were 284 couples with 320 oocyte-retrieval cycles for TESE-ICSI at Kyono ART Clinic from January, 1996 to December, 2013. These were divided into 2 groups: group I (vitrified oocytes + fresh testicular sperm) in 73 cycles and group II (fresh oocytes + fresh testicular sperm) in 250 cycles. We assessed the fertilization rate (FR), embryo development, and pregnancy rate (PR) in the two groups including cases of obstructive azoospermia (OA), non-obstructive azoospermia (NOA) and Klinefelter’s syndrome (KS).

RESULTS: In group I, the survival rate of oocytes after warming was 88.2% (412/467). There were no significant differences in fertilization rate (57.9% vs. 64.9%), good quality embryo rate (37.9% vs. 38.6%), blastocyst formation rate (45.3% vs. 53.2%), good quality blastocyst rate (19.5% vs. 20.8%), pregnancy rate (33.3% vs. 42.2%) or miscarriage rate (24.3% vs. 22.4%) between group I and group II under OA, NOA and KS.

CONCLUSION: Many studies have shown more positive results after oocyte vitrification than after slow freezing procedures. In this study, there are no significant differences between fresh and vitrified oocytes with TESE-ICSI. The use of vitrified unfertilized oocytes should be recommended for wide medical treatment.

THE DECREASE OF E2 (SERUM Estradiol) LEVEL AT DAY FOUR AFTER ET (EMBRYO TRANSFER) MEANS SOMETHING IN FTET (FROZEN-THAWED ET) CYCLES. C. Yue, C. Fang, W. Li, T. Li, R. Huang, X. Liang. The Reproductive Center, The Sixth Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China.

OBJECTIVE: To determine the chemical pregnancy rate(ChPR), clinical pregnancy rate(CIPR) and miscarriage rate within 12 weeks(MisR) of FTET (Frozen-thawed ET) cycles with or without E2 decrease at day 4 after ET.

DESIGN: Retrospective study.

MATERIALS AND METHODS: A total of 2882 FTET cycles with E2 tested within 5 days before and after ET, among them 12.88% (156/1211) of natural cycles(NC) with an E2 decrease and almost half of the hormone replacement treatment(HRT) cycles(42.67%, 713/1671) with an E2 decrease. The ChPR, CIPR and MisR of the E2 increase group and E2 decrease group were analyzed, then multivariate logistic regressions were performed to determine the factors that influenced the outcome.

RESULTS: In NC cycles, the E2 decrease group had slightly less embroy transfer (1.81±0.68 vs. 1.94±0.66, p=0.030), decreased ChPR (48.08 vs. 59.34%, p=0.009), CIPR (34.44 vs. 51.07%, p=0.001), E2 at
This study was funded by the National Natural Science Foundation of China (81070495) and Guangdong Natural Science Foundation (2013010013404).

**P-571 Wednesday, October 22, 2014**


**OBJECTIVE:** Progesterone (P4) levels on day of transfer have been shown to be a possible predictor of live birth outcome. Our objective was to determine if a change in serum progesterone from day 19 (day of transfer) to day 28 predicts birth outcomes in fresh IVF cycles.

**DESIGN:** Retrospective chart review.

**MATERIALS AND METHODS:** We performed a retrospective analysis of fresh IVF cycles from 2010 to 2013 at the NYU Fertility Center. Patients who used crinone or whose progesterone dose was increased were excluded, resulting in a total of 332 patients analyzed. Primary outcomes were percent change in serum progesterone (%P4), live birth rate, missed abortion, and biochemical pregnancies. Statistical analyses included receiver operator curves and chi-squared tests as well as t-tests of means.

**RESULTS:** The incidence of live birth or ongoing pregnancy (LBOPR) was associated with the percent change in P4 between day 19 and day 28. An ROC curve assessing the ability of %P4 to predict LBOPR had an area under the curve of 0.704. A cutoff for %P4 of 10% drop from day 19 to 28 was used to segregate patients into two groups. Group A (with %P4 of 10% or more drop) had significantly poorer outcome than Group B (with %P4 that rose or with a drop of less than 10%) 26% versus 63%, respectively, (p < 0.001, RR 0.42 [0.31-0.56]).

**CONCLUSION:** A 10% or greater drop in serum progesterone between day 19 and day 28 is associated with decreased rates of live birth and ongoing pregnancy in fresh IVF cycles, despite a "normal" serum progesterone level on day of transfer.

**P-572 Wednesday, October 22, 2014**


**OBJECTIVE:** Oocyte donation is a frequently used treatment option for IVF patients for different reasons including diminished ovarian reserve, diminished oocyte quality, a history of repeated pregnancy loss, and the risk of transmitting genetic diseases. Traditionally, oocyte donation has been performed "fresh", matching donor and recipient at the same time. However, recipients can now also opt to use donors, whose oocytes were cryopreserved at different "donor egg banks". It is still to be established if there may be differences in outcomes depending if "in house" vitrified or "outside location" vitrified, and shipped, donor eggs are used. Therefore, the purpose of this study was to compare recipient outcomes from patients that used vitrified eggs from "in-house" oocyte donors vs "outside location" center donors.

**DESIGN:** Retrospective data analysis.

**MATERIALS AND METHODS:** This study included 1238 cycles of our in-house recipients, of which 1175 were with in-house donors and 63 with donors from an outside location. Data on both groups of donors and recipients was recorded from September 2006 to January 2014. The donor/recipient outcomes were compared using unpaired t-test and chi-square tests (at the level of P<0.05) whenever appropriate to determine any significant difference between the in-house center and outside location donors.

**RESULTS:** See table.

<table>
<thead>
<tr>
<th>In-House Donors vs Outside Location Donors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IN HOUSE</strong></td>
</tr>
<tr>
<td><strong>DONORS-IN HOUSE RECIPIENT</strong></td>
</tr>
<tr>
<td>#CYCLES</td>
</tr>
<tr>
<td>#DONOR CYCLES</td>
</tr>
<tr>
<td>DONOR AVG. AGE ±SD</td>
</tr>
<tr>
<td>PATIENT AVG. AGE ±SD</td>
</tr>
<tr>
<td>AVG. # MII WARMED ±SD*</td>
</tr>
<tr>
<td>SURVIVAL RATE</td>
</tr>
<tr>
<td>FERTILIZATION RATE</td>
</tr>
<tr>
<td>BLASTOCYSTS RATE</td>
</tr>
<tr>
<td>AVG. # EMBRYOS</td>
</tr>
<tr>
<td>TRANSFERRED*</td>
</tr>
<tr>
<td>IMPLANTATION RATE</td>
</tr>
<tr>
<td>CLINICAL PREGNANCY RATE</td>
</tr>
</tbody>
</table>

*P<0.5

**CONCLUSION:** Patients that used in-house vitrified donor oocytes had similar survival, blastocyst development, and implantation and clinical pregnancy rates then patients who use “outside location” vitrified donor eggs. It...
may be concluded that “in-house” and “outside location” vitrified donor oocytes provide comparable outcomes overall.

P-573 Wednesday, October 22, 2014

PI3K/PKC PATHWAY MEDIATES THE ALTERATION OF AQ7 EXPRESSION AND LOCATION IN OOCYTE INDUCED BY CRYOPROTECTANTS AND HYPEROSMOLAR STIMULATION. Y.-J. Tan, G.-L. Ding, J. Sheng, H.-F. Huang. Reproductive Medicine, International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China; 3Pathology & Pathophysiology, School of Medicine, Zhejiang University, Hangzhou, Zhejiang, China.

OBJECTIVE: To investigate molecular mechanisms of cryoprotectants and hyperosmolar stimulating oocyte AQ7 expression and localization changes.

DESIGN: Detect the expression of AQ7, 3 and 7 in human and mouse oocytes. Detect AQ7, 3 and 9 protein level after exposed in cryoprotectants. Detect AQ7 location in hyperosmolar mediums. Investigate signaling pathways of kinase and translation associated protein phosphorylation process of cryoprotectants regulating AQ7 expression and localization.

MATERIALS AND METHODS: 1. AQ7, 3 and 9 mRNA and protein were examined in oocytes. 2. AQ7, 3 and 9 protein level was detected after treated with ethylene glycol (EG) and DMSO respectively. Phosphorylation of CPEB and Aurora A were detected as above. 3. AQ7 was detected in oocytes treatment with different hyperosmolar mediums. 4. Oocytes were pretreated with or without staurosporine, LY294002, U0126, SP600125, respectively. AQ7, phosphorylation of CPEB and Aurora A were detected in EG medium. 293FT cells which expressed GFP-hAQ7 or GFP were treated as above. 5. Co-localization of AQ7 and F-actin was observed in oocytes. Their binding was examined. 6. Survival rates of vitrified oocytes were calculated after knockdown of AQ7. 7. In vitro fertilization rates of thawed oocytes were compared between vitrified with EG and DMSO.

RESULTS: 1. AQ7, 3, 7 were expressed in human and mouse oocytes. 2. EG and DMSO upregulated AQ7 protein level in oocytes, not AQ7 and 3. Phosphorylated CPEB and Aurora A were increased in oocytes treatment with EG, DMSO and sucrose, respectively. 3. Increased osmotic pressure induced an increased AQ7 expression level and distribution at membrane. 4. Staurosporine and LY294002 inhibited the effects of EG up regulating AQ7, phosphorylation CPEB and Aurora A expression in oocytes. It was confirmed in GFP-hAQ7-expressed cells. 5. AQ7 binds with F-actin in oocyte. 6. Survival rate of oocytes with knockdown of AQ7 was significantly lower than that of control. 7. There was no difference in in vitro fertilization capacity between oocytes vitrified with EG and DMSO.

CONCLUSION: Cryoprotectants upregulate the expression of AQ7, not AQ3 and 9. Hyperosmolar stimulation increases AQ7 expression and alters its distribution. PI3K/PKC pathway mediates the upregulation through upregulating phosphorylation CPEB and Aurora A level. AQ7 is a necessary protein in oocyte cryopreservation.

Supported by: National Basic Foundation of China (NO. 2011CB944502)

P-575 Wednesday, October 22, 2014


OBJECTIVE: In patients younger than 37 years increasing the number of embryos being transferred will increase their chance of getting pregnant; however, it will also increase their risk to conceive multiple pregnancies. Recently, in fresh cycles an increasing number of elective single embryo transfers has been observed. However, this trend does not exist yet with cycles using cryopreserved embryos. Therefore, this study looked at autologous frozen embryo transfers with 1 or 2 embryos being transferred, and evaluated their efficiency in regards to clinical pregnancy rate (cPR), ongoing pregnancy rate (oPR), implantation rate (IR), and single vs. multiple pregnancies.

DESIGN: A retrospective analysis.

MATERIALS AND METHODS: A total of 1348 autologous FET transferred vitrified/warmed day 5 blastocysts between 2007 and 2013 were reviewed. In 335 FETs an elective single embryo (eSET) was transferred (mean age 33.7±3.4), whereas in 1013 FETs two embryos (DFET) were transferred (mean age 32.1±3.3) and 320 consecutive VET’s (mean age 33.7±2.8) decided to transfer 2 embryos. ESET population was less than 3% (16/525). In contrast, a twin rate of 36.3% was noted in the VET population because 77% of these patients (247/320) decided to transfer 2 embryos.

CONCLUSION: Our data indicates that ESET in selected patients provides excellent delivery rates with one healthy baby in more than 97% of all pregnant patients after fresh eSET. Furthermore, cryopreservation at the blastocyst stage can increase the reproductive potential of one stimulated cycle by up to 20%. ESET combined with cryopreservation can help to reduce the occurrence of multiple pregnancies, which overall will be beneficial for ART and our patients.

P-576 Wednesday, October 22, 2014


OBJECTIVE: Throughout the three-decade history of in-vitro-fertilization (IVF) treatment, elective single embryo transfer (eSET) has been the exception rather than norm. To compensate the low rate of implantation for individual embryos and to achieve pregnancy rates higher than 50%, the general practice is to systematically transfer multiple embryos in the vast majority of patients. Because eSET is the only truly effective means by which to avoid multiple pregnancy in IVF cycles, we looked at our eSET program implemented in 2007 and the cumulative pregnancy rate after transfer of fresh and vitrified-warmed blastocysts (VET) in patients treated with eSET, to evaluate the total potential of a stimulated cycle. In additional, the augmentation potential of blastocyst vitrification was reviewed.

DESIGN: A retrospective analysis.

MATERIALS AND METHODS: A total of 1017 autologous eSET (mean age 32.0±3.3) and 320 consecutive VET’s (mean age 32.1±3.1) after failed fresh eSET on day 5 between 2007 and 2013 were reviewed. Oocytes had undergone ICSI and then were cultured under low oxygen tension (5%) using a single step media (Global (IVF Online) for extended culture. For cryopreservation, laboratory protocols were followed including vitrification and warming of the blastocysts. To prepare patients for VET, both natural and hormone replacement cycles were used to increase the receptivity of the endometrium. Statistical significance was evaluated by Chi-Square. A probability of <0.05 was considered significant.

RESULTS: Both cPR and oPR were not different between eSET compared to VET (61.7%; 625/1017 vs. 62.8%; 201/320, p>0.05; and 55.9%; 568/1017) vs. 59.0%; 189/320, p>0.05). This leads to a cumulative ongoing PR per oocyte retrieval of 74.4% (757/1017), an added value of 18.5% through the cryopreservation. The occurrence of twin pregnancy in the eSET population was less than 3% (16/525). In contrast, a twin rate of 36.3% was noted in the VET population because 77% of these patients (247/320) decided to transfer 2 embryos.

CONCLUSION: Our data indicates that eSET in selected patients provides excellent delivery rates with one healthy baby in more than 97% of all pregnant patients after fresh eSET. Furthermore, cryopreservation at the blastocyst stage can increase the reproductive potential of one stimulated cycle by up to 20%. ESET combined with cryopreservation can help to reduce the occurrence of multiple pregnancies, which overall will be beneficial for ART and our patients.
A PROSPECTIVE COMPARISON OF OUTCOME FOLLOWING CRYOPRESERVATION USING VITRIFICATION VS. A MODIFIED SLOW-FREEZE PROTOCOL OF 2 PRONUCLEAR (2PN) AND DAY 3 MULTI-CELL EMBRYOS. D. Summers, J. H. Check, J. K. Choe. Cooper Medical School of Rowan University, Camden, NJ.

OBJECTIVE: To determine if vitrification (Vit) can provide improved survival and pregnancy rates compared to a modified slow freeze protocol that had proven very successful for 2PN and multi-cell embryos over the years.

DESIGN: Prospective comparison with Vit performed 3 set days each week vs. 4 for slow freeze.

MATERIALS AND METHODS: Vit: protocol: Irvine media, 6-10 minutes in equilibration solution and 90 seconds in vitrification solution at room temperature then loaded onto High security vitrification (HSV) high-security straw <1ul of medium, sealed and plunged. Modified slow freeze - equilibrate in modified human tubal fluid + 10% Serum Protein Substitute for 10 minutes, then 1.5M propanediol for up to 20 minutes, then loaded in straw, seeded, and cooled from -6°C to -40°C at ramp rate 0.4°C/min in alcohol bath freezer, then plunged. A comparison of survival and cleavage rates were performed and 2 embryo stages were also compared – 2 pronuclear and day 3 cleavage stage.

RESULTS: Vit. vs. slow freeze 2PNs – Vit. 35/36 (97.2%) survived and 33/35 (94.3%) cleaved to more than one cell. Slow freeze - 92/96 (95.8%) survived and 87/92 (94.6%) cleaved. Vit. vs. slow freeze for day 3 cleaved embryos – (50% blastomere survival) – Vit. 65/71 (91.5%) vs. slow - 40/45 (72.7%) survived (p < .05, chi-square). Cleaving – Vit. 59/65 (90.7%) vs. slow 38/40 (95%). Clinical pregnancy rate – 2PN – Vit. – 4/4 (75%) with implantation rate of 50% (4/8), slow freeze – 8/15 (53.3%) with 30% (10/30) implantation rate. Clinical pregnancy rate – multi-cell Vit. – 8/17 (47.1%) with implantation rate of 12/16 (75%). The only comparison with a statistically significant difference was implantation rate of multi-cell embryos with p < .05, Fisher’s exact test in favor of Vit. Slow – 6/13 (46.2%) with implantation rate of 7/23 (30.4%).

CONCLUSION: The results seem comparable between the two techniques with a possible advantage of Vit. Over slow for survival of day 3 multi-cell embryos. The slow freeze process is less expensive. There is not a great deal of studies on the efficacy of Vit. at the 2PN stage. The modified slow freeze is different from the standard Lassalle-Testart slow freeze protocol.

IS ONE CYCLE OF PGS PREDICTIVE OF THE NEXT? EVIDENCE FROM EMBRYO BANKING. S. Munne, X. Zheng, C. Wagner Coughlin, M. Crichton, R. Zody, M. Glassner, J. Zhang. Reprogenetics, Livingston, NJ; New Hope Fertility, New York, NY; Global Genomics Institute, H.C. Highland Park, IL; Southern California Reproductive Center, Beverly Hills, CA; SHER Institute for Reproductive Medicine, New York, NY; Main Line Fertility and Reproductive Medicine, Bryn Mawr, PA.

OBJECTIVE: To determine if PGS results are predictable of future cycles.

DESIGN: Comparison of aneuploidy rates in successive cycles of embryo banking. Aneuploidy rates and pregnancy outcome per cycle were unknown to the low rate of euploidy in this group of patients, the lack of euploid embryos in a first PGS cycle should not preclude finding euploid embryos in successive ones. The study was not designed to determine if embryo banking with PGS is a better alternative to sequential fresh cycles poor prognosis patients (average age was 40 years). However, implantation rates were high (57%) showing that if euploid embryos are found, they implant as well as those of young patients.

P-579 Wednesday, October 22, 2014

COMPARISON OF PREGNANCY OUTCOMES OF FROZEN EMBRYOS THAWED ON THE SAME OR THE DAY BEFORE. T. H. Wong, T. S. Lam, K. A. Rocha, L. Xue, P. Xia. The IVF Center, Dept. of Women’s Health and Obstetrics, Hong Kong Sanatorium & Hospital, Happy Valley, Hong Kong.

OBJECTIVE: The published data is still limited regarding the time to thaw the embryos prior to the frozen embryo transfer (FET) in order to target the right time of implantation window. This project was designed to compare the pregnancy outcomes and live birth rates during the different times of thawing. The primary goal of assisted reproductive technology, and single embryo transfer has become a mainstay in achieving that goal. Although the ongoing pregnancy rate after a single blastocyst transfer on day 6 may be slightly lower than day 5, the recent widespread use of vitrification may improve pregnancy rates to allow elective SET after day 6 blastocyst cryopreservation.

RESULTS: Fisher exact test showed no statistical difference between the different time of thawing – (a)on the same day or (b) the day before including Day 3 and Day 5 frozen embryos.

DESIGN: A retrospective study, approved by the Human Subject Committee of the Hong Kong Sanatorium & Hospital, was conducted to evaluate the pregnancy outcomes and live birth rates of 1431 FET cycles. The data during the years of 2012-2013 was compared between the short-term culture (2-3h) and longer-term culture (18-24h) post thawing the embryos frozen on Day 3 and Day 5.

MATERIALS AND METHODS: Data analysis of FET cycles performed during the years of 2012-2013 including natural cycles and programmed cycles.

RESULTS: Fisher exact test showed no statistical difference between the different time of thawing – same day or day before (Table). Overall the live birth rates from Day 5 frozen embryos per cycle was significantly higher that from Day 3 frozen embryos (46.3% vs. 29.6%, *p<0.05).
P-858 Wednesday, October 22, 2014

MICROARRAY ANALYSIS OF EPAB KNOCKOUT MOUSE OOCYTES REVEAL DIFFERENCES IN THE EXPRESSION OF GENES REGULATING TRANSCRIPTION AND CHROMATIN REMODELING

A. Uyar, K. Lowther, E. Seli. Yale School of Medicine, New Haven, CT.

OBJECTIVE: Embryonic poly(A) binding protein (EPAB) is expressed exclusively in mouse oocytes and early embryos, and regulates translational activation of maternally stored mRNAs. It has been shown that EPAB deficient (Epab−/−) female mice are infertile and do not generate mature oocytes. In the current study, we analyzed the transcriptome of Epab−/− and wild type (WT; Epab+/+) mouse oocytes, to identify differentially expressed genes and affected functional pathways in the absence of EPAB.

DESIGN: Experimental study.

MATERIALS AND METHODS: Oocytes were collected from pregnant mare serum gonadotropin (PMSG)-primed WT and Epab−/− mice at 12-weeks of age. Total RNA was isolated from 300 germinal vesicle (GV)-stage oocytes per replicate and four pooled biological replicates were generated for each group. Microarrays were performed for each of the eight samples using Affymetrix GeneChip Mouse Gene 1.0 ST Array platform. Probe level expression values were extracted and background adjustment, inter-array quantile normalization and probe set summarization were performed according to the Robust Multi-Chip Average algorithm. Genes with a false discovery rate-corrected p value of <0.05 and Fold Change >1.5 were considered as differentially expressed. The biological processes affected by EPAB-deficiency were further explored using DAVID functional annotation tool. Differentially expressed genes in key pathways were confirmed by quantitative reverse transcription-polymerase chain reaction (qRT-PCR), using independent samples.

RESULTS: Microarray analysis revealed that 856 genes were up-regulated and 501 genes were down-regulated in Epab−/− mice. Biological processes involving regulation of transcription and chromosome and chromatin organization were significantly altered in the absence of EPAB. Microarray findings were validated by confirming the differential expression of chromatid remodeling genes (Smarcal1, Chd1, Pif1b, Mta2, and Kdm5a) in Epab−/− oocytes by qRT-PCR. We found significant fold change (p<0.01 for each) in the mRNA expression of these genes in Epab+/- oocytes.

CONCLUSION: Our findings establish EPAB as a key regulator of oocyte development, required for expression of genes that regulate transcription and chromatin remodeling.

OBJECTIVE: Recent studies have demonstrated the existence of a reproductive age-associated increase in meiotic chromosome segregation errors in the mouse egg, with frequencies paralleling those observed in humans. However, it is not known whether chromosome-specific segregation errors are prone to meiotic aneuploidies. Here we developed a new low pass whole genome sequencing approach to analyze chromosome-specific aneuploidy in the mouse.

DESIGN: Next generation sequencing of individual mouse eggs and their corresponding first polar bodies.

MATERIALS AND METHODS: 6 mice of advanced reproductive age (16-19 months old) were used to obtain MII-arrested eggs. The zona pellucida from each egg was removed using acidic tyrode’s solution, and the egg and its corresponding polar body were separated by aspiration. Single cell whole genome amplification was performed using the GenomePlex WGA4 kit. Next generation sequencing was performed on the Ion Proton P1 chip. Reads were aligned to the mouse genome sequence and counts per kb and strand were normalized to normal samples to assess reciprocal copy number in the matched eggs and polar bodies. Single trisomy 16 female and normal male mouse embryonic fibroblasts were used as controls.

RESULTS: 20 matched egg and polar body samples were evaluated, giving a total of 15.8 gigabases of sequence on 2 proton chips. Reciprocal errors were observed, including both premature separation of sister chromatids and classical nondisjunction of homologous chromosomes. Interestingly, several polar body samples displayed chaotic patterns of chromosome copy number, consistent with the well-documented rapid degradation of the mouse first polar body compared to the human. We also observed variation in the amount of mitochondrial (mt) DNA that segregated to the polar body, ranging from 0.2 to 8.1% of the matched oocyte quantity.

CONCLUSION: This study demonstrates the feasibility of a low-pass whole genome sequencing method that is applicable to the mouse and theoretically to any species with a published genome. The method can distinguish between chromatin and homologous chromosome imbalances, allowing the precise analysis of segregation errors with chromosome specificity, and provides the ability to characterize mtDNA segregation. Studies are ongoing to determine the frequency that each chromosome contributes to egg aneuploidy during meiosis in the aging mouse model.

SUPPLEMENTING OOCYTES WITH AUTOLOGOUS MITOCHON- DRIA ENHANCES FERTILIZATION OUTCOMES. J. C. St. John, Centre for Genetic Diseases, MIMR-PHI Institute of Medical Research, Clayton, Victoria, Australia.

OBJECTIVE: To determine if the introduction of autologous mitochondria into developmentally incompetent oocytes enhances developmental outcome following fertilization.

DESIGN: Using a pig fertilization and preimplantation development model, developmentally incompetent oocytes were isolated and supplemented with autologous populations of oocyte mitochondria. The development rates, mitochondrial DNA (mtDNA) copy number, gene expression and DNA methylation patterns up to the blastocyst stage were assessed.

MATERIALS AND METHODS: Developmental competence was determined by labeling cumulus-oocyte complexes with brilliant cresyl blue (BCB). Developmentally competent (BCB+) and developmentally incompetent (BCB-) oocytes underwent each of the following treatments: i) in vitro fertilization (IVF); ii) intracytoplasmic sperm injection (ICSI); and iii) oocyte mitochondrial supplementation and ICSI (mICSI). MtDNA copy number and gene expression were determined by real time PCR and RT-PCR, respectively. DNA methylation patterns were determined by bisul- fite sequencing. Statistical analysis was performed using ANOVA.

RESULTS: Blastocyst rates for mICSI treated BCB+ oocytes were 32.9% ± 16.5 whereas those from IVF equivalents were 7.6% ± 5.8 (P<0.05). Blastocyst rates from mICSI-derived BCB+ oocytes and BCB- oocytes derived through IVF were not significantly different. However, blastocyst rates derived from mICSI treated BCB+ oocytes had increased cell numbers (51) compared to BCB-ICSI derived blastocysts (32). BCB-mICSI-derived embryos increased mtDNA copy number by 444% at the 2-cell stage when compared to non-supplemented oocytes (P<0.01). This represents a 480-fold increase from the metaphase II to the blastocyst stages and that BCB-mICSI-derived blastocysts have similar mtDNA copy numbers to BCB+-derived blastocysts. The pluripotent genes, OCT4, SOX 2, NANOG and REX1, were all present in BCB-mICSI-derived blastocysts at levels comparable or greater than for IVF BCB+-derived blastocysts. Similar out- comes were observed for the trophectodermal marker CDX2 and the early developmental marker H19. DNA methylation of the mitochondrial specific replication factor, POLG, was similar for all blastocysts.

CONCLUSION: The results demonstrate that developmentally incompetent pig oocytes (BCB-) can be rescued by supplementation with autologous oocyte mitochondria during ICSI.

P-585 Wednesday, October 22, 2014


OBJECTIVE: With increasing periods of time following ovulation, the mammalian metaphase-II (MII) oocyte succumbs to a degradation process referred to as post-ovulatory ageing. Post-ovulatory ageing is associated with numerous pathologies including decreased receptivity for fertilization, and an elevated risk for the production of embryos and offspring with abnormal or retarded development. The current study aimed to identify the molecular mechanisms underpinning post-ovulatory ageing and apoptosis with a partic- ular focus on the role of the electrophilic aldehyde, 4-hydroxynonenal (4HNE).

DESIGN: Animal based research study.

MATERIALS AND METHODS: Levels of 4HNE in ‘fresh’ and in vitro ‘aged’ MII mouse oocytes were assessed using immunocytochemistry and Western blotting procedures. Upon determining stage-associated accumulation of 4HNE, the effects of 4HNE on the biochemistry and functionality of the oocyte was assessed in a time (1 - 24 h) and dose (0 – 200 μM) dependent study.

RESULTS: Identification of protein targets for 4HNE modification was conducted using immunoprecipitation techniques. Statistical significance was assessed using one way ANOVA analyses on a minimum of 5 experimental replicates.

RESULTS: We have demonstrated that the electrophilic aldehyde 4HNE is found to increase with extended periods of time post-ovulation (P<0.05) and the mitochondrial protein, succinate dehydrogenase (SDHA), was identified as a primary target. Time- and dose- dependent studies revealed that exposure to elevated levels of 4HNE causes increased mitochondrial ROS production (P<0.01), lipid peroxidation (P<0.001), loss of mitochondrial membrane potential (P<0.001) and expression of apoptotic markers (P<0.001) within the oocyte; presumably as a consequence of electron transport chain collapse due to SDHA impairment. Additionally, short term exposure to low doses of 4HNE was shown to dramatically impair the oocyte’s ability to participate in fertilization (P<0.01) and support embryo development (P<0.001).

CONCLUSION: This study has revealed that 4HNE accumulation is implicitly linked to post-ovulatory oocyte ageing; causing reduced fertility, oxidative stress, and apoptosis. These data highlight the importance of timely fertilization of the oocyte post-ovulation, and suggest a need for supplemen- tation of oocyte culture media with antioxidant compounds when extended periods of culture prior to fertilization are unavoidable.

Supported by: Funding was provided by the NHMRC.

P-586 Wednesday, October 22, 2014

DYNAMIC CHANGES OF THE RATIO OF GDF9 AND BMP15 mRNA EXPRESSION IN NORMAL AND PCOS OOCYTES DURING MATURATION. L.-N. Wei, R. Huang, L.-L. Li, C. Fang, X.-Y. Liang. Reproductive Medicine Research Center, The Sixth Affiliated Hospital of Sun Yat-Sen University, Guangzhou, Guangdong, China.

OBJECTIVE: Oocyte secreted factors, namely growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15), play crucial roles in the regulation of follicular development and oocyte maturation, and the ratio of GDF9 and BMP15 expression differs across a large range of species. The aim of this study was to investigate the ratio of GDF9 and BMP15 mRNA expression in human oocytes during maturation, and to compare the differences between normal and PCOS oocytes.

DESIGN: Case-control study.

Supported by: This research was Supported by OvaScience, Inc.
MATERIALS AND METHODS: One hundred and fifty-three oocytes (51 of GV, MI and MII, respectively) from 51 cases of normal ovulatory women and 52 of MI and MII, respectively) from 24 women with polycystic ovary syndrome (PCOS) were collected in the reproductive center from March to December in 2013. Nested quantitative real-time PCR was used to detect the transcript abundance of GDP9 and BMP15 in a single oocyte.

RESULTS: The data were expressed as medians with 25th-75th in parentheses. The ratio of GDP9 and BMP15 mRNA expression was 4.65 (1.83 - 9.05), 8.80 (2.83 - 14.69) and 11.63 (7.69 - 15.26) in normal oocytes, GV and MI, respectively. It was increased during oocyte maturation in normal oocytes (P<0.05). The results were 99.17 (19.21 - 242.82), 11.07 (1.20 - 166.01) and 2.50 (0.05 - 9.49) in PCOS oocytes of GV, MI and MII, respectively. It was reduced during oocyte maturation in PCOS oocytes (P<0.05).

CONCLUSION: The ratio of GDP9 and BMP15 mRNA expression demonstrates dynamic changes in human oocytes during maturation, and the trends are different between normal and PCOS oocytes. These results suggest that the expression pattern of GDP9 and BMP15 was abnormal in PCOS oocytes during maturation.

Supported by: The present study was Supported by the grants from National Natural Science Foundation of China (Grant No.81020476), Natural Science Foundation of Guangdong Province (Grant No. S2012400007770), National Doctoral Foundation of China (Grant No. 2012017120122), Medical Science and Technology Research Foundation of Guangdong Province (Grant No. B2012150).

P-587 Wednesday, October 22, 2014

OOCYTES FROM WOMEN WITH DIMINISHED OVARIAN RESERVE AND OBESITY HAVE SHORTENED TELOMERES. D. M. F. Antunes, a, b Y. Kramer, c D. M. F. Antunes, a E. Lazzaroni-Tealdi, c Departar, Cellular and Developmental Biology, Yale University, New Haven, CT; b Department of Obstetrics and Gynecology, New York University, New York, NY; cDepartment of Pathology, Fluminense Federal University, Niteroi, Rio de Janeiro, Brazil.

OBJECTIVE: Telomere shortening in mouse oocytes promotes genomic instability, apoptosis, spindle abnormalities and infertility. In humans, the highly correlated (R²=98%) polar body telomere length is associated with embryo aneuploidy, fragmentation and decreased pregnancy rate. To further test the hypothesis that telomere length reflects oocyte quality we examined the relationship between oocyte telomere length and factors associated with oocyte quality, including ovarian reserve and body mass index (BMI).

DESIGN: Prospective observational study.

MATERIALS AND METHODS: 143 arrested oocytes (MI and M2 stages) were collected three days after retrieval from consenting women undergoing IVF at NYU Fertility Center. BMI, age, anti-Müllerian hormone (AMH) and follicle-stimulating hormone (FSH) levels were obtained from medical records. A novel single-cell telomere length assay (SCT-pqPCR) measured telomere length relative to a reference gene (T/R) in individual oocytes (Wang et al., 2013). Mann-Whitney and Kruskal-Wallis Test were performed to compare means.

RESULTS: Telomere length in oocytes from women with < 0.8 mg/ml (n=20; 1.57±0.47) was significantly less than that from oocytes of women with AMH > 0.8 mg/ml (n=30; 2.84±0.65) (p=0.044). Oocytes from women with FSH > 10 IU (n=21) had significantly shorter telomeres (1.27±0.31) than oocytes from women with FSH < 10 IU (n=108; 2.23±0.27), p=0.026. Telomeres in oocytes from older women (n=99; 2.09±0.28) were shorter than from those younger women (<35 years old, n=44) (2.73±0.59), but this difference did not reach significance at the sample size studied (p=0.112). Oocytes from overweight women (n=51; 1.34±0.23) had shorter telomeres than those from women with normal BMI (n=67) (2.56±0.48) p=0.029. Oocytes from underweight women (n=11) had telomeres similar to those from women with normal BMI (2.38±0.62).

CONCLUSION: Oocytes from women with decreased low ovarian reserve and obesity, factors known to contribute to poor oocyte quality, have shortened telomeres. Polar body telomere length, which reliably estimates oocyte telomere length, may provide a valuable assay of oocyte quality.

Supported by: CAPES Brazil, Dept Obst/Gyn NYU Langone and NIH U01RR029893.

P-588 Wednesday, October 22, 2014

RNA HIGH THROUGHPUT SEQUENCING OF CUMULUS CELLS REVEALS NEW PATHWAYS INVOLVED IN HUMAN OOCYTE AGING. E. Molinariv, a P. Patrizio, a A. M. Pyle. a b Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT; b Yale Fertility Center, Yale University, New Haven, CT.

OBJECTIVE: To investigate the impact of aging on metaphase II oocytes by means of mRNA high throughput sequencing of human cumulus cells (CCs) and to identify the first comprehensive whole transcriptome signature analysis of CCs by assessing simultaneously both coding and non-coding transcripts.

DESIGN: Basic research comparative study.

MATERIALS AND METHODS: A total of 21 CCs were isolated from mature MI oocytes collected from patients aged <30 years (younger, n=10) and from patients aged >40 years old (older, n=11). Only cases of male factor infertility were considered. CCs were isolated at the time of CC retrieval, library preparation and sequenced on the Illumina HiSeq 2000 platform. Two different enrichment protocols were used to obtain information regarding mRNA and non-coding RNAs. The FastQC program was used for assessing overall read quality, whereas gene mapping, annotation, counting and differential expression was performed according to the programs in the Tuxedo pipeline [1]. Gene co-expressions and gene ontology analysis were used to define upregulated gene networks and interactions in the two cohorts [2, 3]. Significance of differentially expressed genes was assigned when both p value and false discovery rate were <0.05. Validation was performed with qPCR.

RESULTS: We found that 319 genes were differentially expressed between the younger and older cohorts. In CCs collected from the younger group, genes involved in oxidoreductase activity, extracellular matrix organization, and membrane composition were strongly upregulated (p<0.001 and FDR <0.05) compared to older women. By contrast, CCs from older patients show an overrepresentation of genes involved in the oxidative stress cascade and apoptosis (p<0.001, FDR <0.05). The whole transcriptomic analysis of CCs revealed an interesting complex of expressed transcripts, including a new set of potential long noncoding RNAs. CONCLUSION: This novel application of RNA high throughput sequencing on human cumulus cells reveals: a) The complete transcriptomic signature of CCs; b) The first RNAseq analysis to contrast younger and older patients and highlights pathways involved in diminishing female fertility. The unprecedented amount of information revealed by RNAseq will help identify markers related to oocyte aging, such as aneuploidy and reduced embryonic developmental competence.

P-589 Wednesday, October 22, 2014

MITOCHONDRIAL DNA ABUNDANCE DECLINES IN HUMAN CUMULUS GRANULOSA CELLS WITH AGE. V. A. Kushnir,a Y.-G. Wu, a b I.-C. Chen, c H.-J. Lee, a D. H. Barad,a,b E. Lazzaroni-Tealdi,a N. Gleicher,c b a Center for Reproductive Medicine, New York, NY; cFoundation for Reproductive Medicine, New York, NY.

OBJECTIVE: Cumulus granulosa cells (cGCs) are fundamental to oocyte maturation, supporting metabolism, disposal of waste and molecular signaling. Since mitochondrial (mt) contribution to reproductive aging is poorly understood, we examined whether mtDNA abundance changes in cGCs with advancing female age.

DESIGN: Prospective case control study.

MATERIALS AND METHODS: We examined effects of aging on mtDNA/nuclear DNA (nDNA) ratios using real-time PCR in human cGCs, collected at time of oocyte retrieval from 4 young oocyte donors (ages 21-30 years), 4 younger (30-37 years) and 4 older (44-46 years) infertility patients. Nuclear PCR probes targeted GAPDH, while mitochondrial PCR probes targeted ND5.

RESULTS: Relative mtDNA/nDNA ratios significantly decreased with advancing female age from 9.0 in young oocyte donors to 5.2 in younger infertility patients and 2.3 in oldest infertility patients (P=0.03).

CONCLUSION: These data demonstrate evidence for significant age-related decline in human cGCs mtDNA abundance, which can be assumed to play a role in reproductive aging. Functional studies are ongoing at our institute to further elucidate mitochondrial contribution to reproductive aging.

Supported by: Foundation for Reproductive Medicine, Center for Human Reproduction.

P-590 Wednesday, October 22, 2014

EFFECTS OF ADVANCING FEMALE AGE ON PROLIFERATION AND GENE EXPRESSION IN CULTURED HUMAN GRANULOSA CELLS FROM POST-HCG RETRIEVED FOLLICLES. Y.-G. Wu,a b I.-C. Chen, c H.-J. Lee, a D. H. Barad,a,b V. A. Kushnir,a E. Lazzaroni-Tealdi,a N. Gleicher,c b a Center for Human Reproduction, New York, NY; cFoundation for Reproductive Medicine, New York, NY.

OBJECTIVE: Granulosa cells (GCs) form the follicular microenvironment, which facilitates oocyte development, supplies energy, disposes of metabolic waste and provides the regulatory microenvironment for embryonic development. 

Supported by: CAPES Brazil, Dept Obst/Gyn NYU Langone and NIH U01 RR029893.
waste and participates in molecular signaling. Defects result in abnormalities in the oocyte’s nuclear and cytoplasmic maturation. Though poor oocyte quality is routinely observed in older women, the impact of reproductive aging on GC function has so far not been fully elucidated and, therefore, was subject of this investigation.

DESIGN: Prospective case control study.

MATERIALS AND METHODS: We examined effects of aging on cell proliferation and gene expression in vitro cultured human GCs in 4 young oocyte donors (ages 21-30 years), 4 younger (22-37 years) and 4 older (42-47 years) infertility patients. GC proliferation was examined by counting cell number with a hemacytometer, apoptosis by DAPI staining and gene expression by real-time PCR, following in vitro culture in presence or absence of 10IU/ml follicle stimulating hormone (FSH).

RESULTS: We observed statistically significantly lower proliferation and higher apoptosis with advancing female age during in vitro culture of GCs. FSH supplementation in culture stimulated GC growth and prevented luteinization, evaluated by FSH receptor and aromatase mRNA expression; but this effect completely dissipated with advancing female age.

CONCLUSION: These data demonstrate age-related functional declines in human GCs, characterized by changes in GC luteinization, proliferation, apoptosis and ability to respond to FSH during in vitro culture. Poor oocyte quality in older infertile women may, therefore, at least in part, be related to the loss of normal ovarian GC function.

Supported by: Foundation for Reproductive Medicine, Center for Human Reproduction.

P-591 Wednesday, October 22, 2014

THE CYCLE OUTCOMES OF GNRH AGONIST TRIGGERING WITH DIFFERENT LEUPROLIDE ACETATE DOSES IN HIGHER RISK PATIENTS.

OBJECTIVE: Purpose of the study is to compare the impact of different leuprolide acetate doses on cycle outcomes and OHSS rates in high-risk patients undergoing ovarian stimulation.

DESIGN: Retrospective cohort study of high-responder patients.

MATERIALS AND METHODS: After reviewing electronic database of IVF cycles during 3 years period in 2 different IVF centres. Group 1 was consisted of 38 patients who received 1 mg of agonist and group 2 was consisted of 39 patients who received 2 mg of agonist. In both centers, the criteria of agonist application are: being at high risk of OHSS by a high number of follicles (>12) measuring ≥12 mm and/or high serum estradiol levels (≥400 pg/mL) during the late follicular phase of the ovarian stimulation, even abnormal embryonic development compared to in vivo mature (IVO) oocytes. In this study, a mouse model was used to investigate the efficiency of the rescued IVM MI-MII oocytes which have been vitrified-warmed in order to adjust the maturity of ooplasm with precise IVM schedule and fertilization timing.

DESIGN: Experimental study.

MATERIALS AND METHODS: Metaphase I (MI; 7 h post hCG) and metaphase II (MII; 15 h post hCG) oocytes were collected form B6D2F1 mice. The surrounding cumulus cells of metaphase I oocytes were removed before another 8 h rescue IVM. The IVO MII and rescue IVM MI-MII oocytes were vitrified in a medium with 15% ethylene glycol (EG), 15% dimethyl sulfoxide (DMSO) and 0.5 M sucrose. In group 1, fresh IVO MII oocytes were injected with a sperm as a control. After oocyte warming, the vitrified IVO MII oocytes (group 2) and vitrified IVM MI-MII oocytes (group 3) were injected with a sperm to initiate subsequent embryo development.

RESULTS: The maturation rate was 84.0% (664/790) from IVM MI-MII oocytes. The survival rates were 95.3% (589/617) vs. 96.8% (644/664) (group 2 vs. 3, NS) following vitrification/warming. The cleavage rates were 73.3% (376/517) vs. 56.4% (341/617) vs. 50.7% (323/664) (group 1 vs. 2 and 1 vs. 3, NS) following vitrification/warming. The blastocyst formation rates were 56.1% (290/517) vs. 45.6% (274/617) vs. 32.4% (216/664) (group 1 vs. 2, 1 vs. 3 and 2 vs. 3, P<0.05).

OBJECTIVE: The in vitro maturation (IVM) of MI oocytes without surrounding cumulus cells, called rescue IVM oocytes, has triggered a significant interest on clinical applications recently. However, due to asynchronous cell cycle, it has been reported that these rescued human IVM MI-MII oocytes yield lower fertilization rates, multi-nucleation, and even abnormal embryonic development compared to in vivo matured (IVO) oocytes. In this study, a mouse model was used to investigate the efficiency of the rescued IVM MI-MII oocytes which have been vitrified-warmed in order to adjust the maturity of ooplasm with precise IVM schedule and fertilization timing.

CONCLUSION: 1 or 2 mg leuprolide acetate yields similar outcomes on cycle outcomes and OHSS rates in high-risk patients undergoing ovarian stimulation.

CONCLUSION: In this study, we examined the developmental competency of rescued IVM MI-MII oocytes following KCSI in a schedule controlled manner with the assistance of vitrification in a mouse model, and proved that live-offspring can be generated from the rescue IVM MI-MII oocytes with a satisfactory efficiency. The observations herein provide key insights into improving current rescue IVM system in human.

Table-1: Demographic characteristics of groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Leuprolide 1 mg</th>
<th>Leuprolide 2 mg</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) Mean ±SD</td>
<td>29.4±5.0 [28.7-31.5]</td>
<td>30.1±4.5 [28.7-31.6]</td>
<td>0.811</td>
</tr>
<tr>
<td>Duration of infertility (months) Median (min-max)</td>
<td>51.0 (8.0-240.0)</td>
<td>48.0 (12.0-240.0)</td>
<td>0.701</td>
</tr>
<tr>
<td>Day3 FSH (IU/L) Median (min-max)</td>
<td>5.5 (3.0-9.0)</td>
<td>5.3 (2.0-8.2)</td>
<td>0.920</td>
</tr>
<tr>
<td>Duration of stimulation (days) Median (min-max)</td>
<td>10.0 (6.0-20.0)</td>
<td>9.0 (8.0-12.0)</td>
<td>0.013</td>
</tr>
<tr>
<td>Total dose of gonadotropins (IU) Median (min-max)</td>
<td>1475.0 (635.0-2550.0)</td>
<td>1525.0 (1025.0-3500.0)</td>
<td>0.695</td>
</tr>
<tr>
<td>Number of retrieved oocytes Median (min-max)</td>
<td>17.5 (4.0-42.0)</td>
<td>15.0 (9.0-25.0)</td>
<td>0.510</td>
</tr>
<tr>
<td>Number of MI oocytes Median (min-max)</td>
<td>13.5 (3.0-40.0)</td>
<td>12.0 (5.0-20.0)</td>
<td>0.503</td>
</tr>
<tr>
<td>Number of fertilized oocytes (2PN) Median (min-max)</td>
<td>10.0 (3.0-32.0)</td>
<td>10.0 (1.0-16.0)</td>
<td>0.128</td>
</tr>
<tr>
<td>Fertilization rate (%) Median (min-max)</td>
<td>83.3 (42.8-100.0)</td>
<td>75.0 (20.0-100.0)</td>
<td>0.006</td>
</tr>
<tr>
<td>No. embryos transferred</td>
<td>1.2±0.43</td>
<td>1.17±0.38</td>
<td>0.541</td>
</tr>
<tr>
<td>No. good quality embryos transferred</td>
<td>0.76±0.67</td>
<td>0.61±0.54</td>
<td>0.227</td>
</tr>
<tr>
<td>Implantation rate (%) Median (min-max)</td>
<td>34.1% (27.9 - 57.8)</td>
<td>14.6% (9.4 - 54.1)</td>
<td>0.033</td>
</tr>
<tr>
<td>No. of recipient used</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>No. of embryos transferred</td>
<td>8</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>No. of pregnancy (%)</td>
<td>6 (75.0)</td>
<td>5 (71.4)</td>
<td>7 (77.8)</td>
</tr>
<tr>
<td>No. of offspring born (%)</td>
<td>2 (25.0)</td>
<td>2 (28.6)</td>
<td>3 (33.3)</td>
</tr>
</tbody>
</table>

In vivo development

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of recipient used</th>
<th>No. of pregnancy (%)</th>
<th>No. of embryos transferred</th>
<th>No. of offspring born (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>7 (87.5)</td>
<td>80</td>
<td>33 (34.3)ab</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>5 (71.4)</td>
<td>90</td>
<td>21 (20.2)ab</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>7 (77.8)</td>
<td>132</td>
<td>22 (13.8)ab</td>
</tr>
</tbody>
</table>
OTHER: ART - CLINICAL

P-593 Wednesday, October 22, 2014


OBJECTIVE: Blastocyst culture and vitrification technique have been almost established in the field of ART (Assisted Reproductive Technique), but the safety was not well-secured yet. Recently, there are some reports for the babies born with these techniques. For example, it was demonstrated that weight of placenta and baby’s body weight increase in frozen-thawed embryo compared with fresh embryo, and also increase body weight blastocyst compared with early embryo. In addition, it was well known that the better blastocyst grade, the higher pregnancy rate and birth rate. However, it is still unknown that relationship between blastocyst grade and pregnancy or abortion rate.

DESIGN: Retrospective study.

MATERIALS AND METHODS: We classified 187 cases (174 patients) which underwent single blastocyst transfer from April 2007 through March 2013 in our clinic retrospectively. We compared the placenta weight and baby’s body weight in fresh embryo and frozen-thawed embryo with Gardner’s classification which were counted Inner Cell Mass (ICM) and Trophoderm (TE) cell numbers.

RESULTS: There was no difference in placenta weight and baby’s body weight with ICM grade classification. In TE grade, the weight significantly increase as embryo grade is better. (P < 0.05). As for the case divided embryos between fresh and frozen-thawed, there was no difference in ICM grade; however, body weight increased in better TE grade.

CONCLUSION: The better TE grade, the larger weight of placenta and baby’s body weight; whereas ICM grade does not affect those weight. In comparison with ICM grade, TE grade is profoundly related not only to pregnancy rate and live birth rate, but also to embryo development and delivery process.

P-594 Wednesday, October 22, 2014

THE EFFECT OF ACUPUNCTURE ON PREGNANCY OUTCOMES IN IN-VITRO FERTILIZATION (IVF): A RANDOMIZED CONTROLLED TRIAL. L. C. Udolf, H. D. McClamrock, K. Chen, G. Zhang. Genetics and IVF Institute, Fairfax, VA; Reproductive Endocrinology, University of Maryland School of Medicine, Baltimore, MD; Center for Integrative Medicine and Department of Psychology, University of Maryland School of Medicine, Baltimore, MD; Grant Zhang Acupuncture, Ellicott City, MD.

OBJECTIVE: To evaluate whether acupuncture improves pregnancy and delivery rates in patients undergoing IVF.

DESIGN: Randomized, blinded, multi-center, sham treatment controlled trial.

MATERIALS AND METHODS: Patients planning to undergo IVF who met inclusion/exclusion criteria (age<40 years old, FSH<10mIU/mL, <3 prior failed IVF attempts, acupuncture naive) were randomly assigned to an acupuncture or sham treatment group. Treatment sessions occurred before gonadotropin start, before oocyte retrieval, the day before embryo transfer, and the day after embryo transfer. Acupuncture was performed using manual manipulation at 6 to 10 points depending on the timing of the treatment. Sham treated patients had needles placed in non-meridian points at a shallow depth. Patients were also given a questionnaire to guess their group assignment. Data was analyzed using chi-squared for categorical data shows no significant difference between acupuncture and sham control trial to study the impact of acupuncture on IVF success rates. Preliminary data shows no significant difference between acupuncture and sham treatment groups. Acupuncture was associated with a trend toward a higher delivery rate per transfer especially in those patients with an FSH level higher than the mean.

Supported by: Acknowledgment: This study was Supported by a grant from National Institute of Health (grant number: AT002651) to GZ.

P-595 Wednesday, October 22, 2014

ABSTRACT WITHDRAWN

P-596 Wednesday, October 22, 2014

A NEWLY DESIGNED OOCYTE ASPIRATION NEEDLE WITH A THIN TIP REDUCES PAIN AND BLEEDING COMPARED TO A STANDARD NEEDLE IN PATIENTS WITH A SINGLE FOLLICLE. K. Nakagawa, Y. Nishi, R. Sugiyama, H. Motoyama, R. Sugiyama. Division of Reproductive Medicine, Sugiyama Clinic, Setagaya, Tokyo, Japan; Center for Reproductive Medicine and Endoscopy, Sugiyama Clinic Marunouchi, Chiyoda, Tokyo, Japan.

OBJECTIVE: Since oocyte pick-up (OPU) can be painful even though under local anesthesia, several methods to reduce pain have been evaluated.1 When reducing the needle diameter significantly less pain was experienced. Reducing the needle diameter too much may however damage the oocytes. Another problem with a very thin needle is that it may miss the target, thereby making the retrieval technically more difficult and less efficient. Is the level of pain and bleeding in patients with a single follicle who do not receive any analgesic treatment, affected by the type of needle used during OPU?

DESIGN: From May through November 2013, we randomly assigned 100 patients to two groups on OPU day. Patients underwent OPU using either a reduced needle with tuare connection with a 18 gauge body but a 20 gauge tip of the needle (RN group) or a standard needle (SN group).

MATERIALS AND METHODS: Patients with a single follicle that had OPU without any analgesic procedure in a natural cycle or clomiphene citrate cycle t were recruited. We compared the rating of pain using Visual Analogue Scale (VAS), rates of mature oocytes, and frequency of bleeding between RN (n=50) and SN (n=50) groups.

RESULTS: The total number of punctured follicles and retrieved oocytes in the RN and SN groups were 1.1 and 1.0, and 1.1 and 1.1. There were no significant differences between two groups. The percentage of mature oocytes in the RN and SN groups were 86.0% and 86.5%, respectively. The mean VAS just after OPU in the RN group was 3.2±2.0 (mean±SD) and significantly lower than that in the SN group (4.9±2.2, p<0.01). The frequency of bleeding from vaginal wall after OPU in the RN group was 24.0%, and this was significantly lower than that in the SN group (52.0%, P<0.05). The fertilization rates were similar. The pregnancy rate in the RN group was 31.4% and comparable to the rate in the SN group (25.6%).

CONCLUSION: A newly designed oocyte aspiration needle with a thin tip reduced significantly perceived pain as well as bleeding immediately after operation in patients with a single follicle undergoing OPU without any analgesic procedure compared to a standard level. It did not affect the quality of the retrieved oocytes.

Supported by: The authors have received no funding for this study, and they have no financial interest in any companies. Therefore, there are no competing interests.
P-597 Wednesday, October 22, 2014

IMPROVING IUI OUTCOMES BY ADDING MYO-INOSITOL TO THE SEMEN PREPARATION PROCEDURE. R. Poverini, G. Carlonagamo, R. Lisi, F. Lisi, M. Montanino Oliva, "Centro Ricerca Medicina della Riproduzionen (CERMER), Rome, RM, Italy; R&D, Lo.Li. Pharma, Rome, RM, Italy.

OBJECTIVE: The aim of the present trial was to study whether myo-inositol (MI) treatment during semen preparation procedures is able to improve pregnancy rate after intrauterine insemination (IUI).

DESIGN: This preliminary trial was designed as a prospective study and was performed enrolling couples counseled for IUI from September 2013 to February 2014. A retrospective group (January to July 2013) matched for clinical fertility history and women age was used as control group. Semen samples from both groups were prepared with the swim-up procedure.

MATERIALS AND METHODS: The semen preparation media (Origio) routinely used in our practice were enriched with MI at the final concentration of 2mg/ml (Andrositol™, Lo.Li. Pharma).

RESULTS: In summary using MI in the semen preparation procedures increases the percentage of the clinical pregnancy during IUI. Furthermore, based on these results, we have performed a power analysis allowing us to identify the sample size for a future randomized controlled trial. In particular, enrolling 160 couples per group, we would be able to detect with a power of 80% a difference of 13% between the two groups.

CONCLUSION: It has previously shown that MI treatment increases spermatozoid mitochondrial membrane potential (MMP). Furthermore, following the swim-up procedure, MI treatment allows retrieving an increased number of spermatozoids in both normospermic (+71%/vs untreated) and OAT (+91% vs untreated)[1, 2] samples.

It is known that sperm progressive motility and the sperm fertilization capacity directly depend on the MMP of the sperm cells that fertilize the oocyte[3]. Having this in mind the drop of the MMP caused by the semen preparation procedures has to be considered one of the main issue that need to be addressed in order to improve ART efficiency[4].

According to ours findings, MI treatment by increasing the sperm MMP increase the percentage of clinical pregnancy after IUI.

Supported by: Lo.Li.pharma provided the product used in the study free of charge.

P-598 Wednesday, October 22, 2014

NUMBER OF OOCYTES ASPIRATED MAY BE A POWERFUL DETERMINANT OF IN VITRO FERTILIZATION (IVF) ONGOING PREGNANCIES IN ALL SOCIETY FOR ASSISTED REPRODUCTIVE TECHNOLOGY (SART) AGE GROUPS. J. L. Hall, E. Mor, A. Bayrak, M. Ghahremani, I. Biltz, Y. Zhang, P. Saadat. In Vitrotech Labs, West Hollywood, CA.

OBJECTIVE: To determine if a higher number of oocytes available at aspiration (oocyte yield) has a beneficial or detrimental effect on pregnancy outcome across all patient ages and if there exists an optimal oocyte number range for IVF success.

DESIGN: Retrospective analysis of consecutive IVF patient data.

MATERIALS AND METHODS: A total of 94 consecutive non-donor, non-pre-implantation genetic screening IVF patients underwent aspiration of all follicles greater than 8 mm followed by intra-cytoplasmic sperm injection of all mature oocytes, embryo culture and embryo transfer from January through August, 2013. Data for oocyte yield and live births or ongoing pregnancies were evaluated retrospectively according to each patient’s age using SART categories for IVF success rates in the Clinic Summary Report.

RESULTS: Live birth/ongoing pregnancy rate for all patients under 43 years of age was 59%. Four groups representing number of oocytes collected in each case (0-5, 6-12, 13-19, 20 and above) yielded an ongoing pregnancy rate of 30, 80, 53 and 36%, respectively. Combining SART age categories (less than 35, 35-37, 38-40, 41-42) and number of oocytes collected demonstrated that 6-12 oocytes resulted in the highest pregnancy rate for every age category (77,79,80 and 100%, respectively) and that pregnancies declined as the number of oocytes recovered increased.

CONCLUSION: These initial data are highly suggestive that ovarian stimulation and resulting oocyte numbers directly impact IVF success rate and that a clinical goal of obtaining higher oocyte yields may be counter-productive.

P-599 Wednesday, October 22, 2014


OBJECTIVE: Compare the pregnancy rates of egg receptors from donor patients diagnosed with polycystic ovarian syndrome (PCOS) to receptors from donors without ovarian pathology.

DESIGN: Retrospective case-control observational study.

MATERIALS AND METHODS: Total of 234 patients undergone into egg reception program. Those patients were separated into two groups: Group I - Receivers from PCOS donors (N=36); Group II - Receivers from donors without ovary pathologies (N=198). The PCOS patients were classified following the Rotterdam criteria. The medical records were reviewed and the following data were collected: age of donors and receptors; number of retrieved oocytes, number of mature oocytes (metaphase II) that were used by the receptors, the number of fertilized embryos on day 1, Fertilization, implantation and cumulative pregnancy rates were also calculated. Student T test were used to analyze the mean of the collected data between the groups and the Chi-square test was used to analyze the cumulative pregnancy, fertilization and implantation rates.

RESULTS: The mean age for both receptors groups were similar, 42 years old. The PCOS donors had an average age of 30 years against 29 years of donors from Group II. There was no statistical difference. PCOS patients had an average of 3.23 more oocytes collected, but there were no difference of the number of mature oocytes that were used for donation between the groups. We also observed that the number of transferred embryos were also not significantly different, as well as the fertilization and implantation rates. The cumulative clinical pregnancy rates were not significantly different: 28% and 26% in Group I and Group II, respectively.

Receptor age
Donor age
Oocytes Retrieved
Mature oocytes used
Day 1 embryos (2 pn)
Embryos Transferred
Implantation Rate (%)
Fertilization Rate (%)
cumulative pregnancy rate (%)
CONCLUSION: The use of PCOS donors for egg donation programs did not affect the results of the receptor, therefore, women with PCOS should not be excluded from egg donation programs.

Supported by: Vida Centro de Fertilidade.

P-600 Wednesday, October 22, 2014


OBJECTIVE: The Rotterdam definition for polycystic ovary syndrome (PCOS) states two out of three criteria must be met. However the clinical significance and management of isolated polycystic ovary (PCO) is still unclear. The aim of our study is to investigate the clinical significance of isolated PCO in assisted reproduction by comparing clinical and laboratory aspects of patients with morphologically normal ovaries (Nov), isolated PCO, and polycystic ovary syndrome (PCOS).

RESULTS: This is a retrospective medical record review study. Medical records of 444 patients that visited our infertility clinic between 2011 and 2013 were retrospectively reviewed. Patients in the three groups, isolated PCO, PCOS, and Nov, were subdivided into subgroups according to age, previous history of fertility or infertility. Cumulative embryo score (CES) and the number of good quality embryos were used to assess embryo quality.

RESULTS: The number of oocytes retrieved was significantly higher in PCO group compared to Nov group (12.9±4.11 vs. 8.03±7.4, respectively, p<0.05). The number of good quality embryo was lowest in patients with PCOS (mean±SD, 0.56±0.73), while the other two groups were similar (1.18±1.01 in the PCO group, 0.91±0.98 in the Nov group, respectively, statistically not significant). The mean CES score was lower in the PCOS group (49.2±33.9) than either PCO group (68.4±29.7) or Nov group (64.2±34.5), but failed to show a statistical significance (p=0.591). Overall pregnancy rates in the PCO group was highest, when compared to the overall pregnancy rates in the normal group and the PCOS group (p=0.082).

CONCLUSION: Patients with isolated PCO pattern may not only have high potentials for developing large number of follicles, but also produce equally high quality embryos as in the non-PCO patients, resulting in higher pregnancy rates with appropriate ovarian stimulation.

P-601 Wednesday, October 22, 2014


OBJECTIVE: To evaluate assisted reproductive technology(ART) outcomes including clinical pregnancies(CP) according to embryo age at the time of transfer, i.e. embryos of Day 5-6 vs Day 3-4 developed from in-vitro matured(IVM) oocyte cycles in Polycystic ovarian syndrome(PCOS) patients.

RESULTS: Design: This is a retrospective medical record review study. Medical records of 203 IVM cycles in 157 PCOS patients were included in this study. The patients underwent IVM cycles in Polycystic ovarian syndrome(PCOS) states two out of three criteria must be met. However the clinical significance and management of isolated polycystic ovary (PCO) is still unclear. The aim of our study is to investigate the clinical significance of isolated PCO in assisted reproduction by comparing clinical and laboratory aspects of patients with morphologically normal ovaries (Nov), isolated PCO, and polycystic ovary syndrome (PCOS).

RESULTS: The number of oocytes retrieved was significantly higher in PCO group compared to Nov group (12.9±4.11 vs. 8.03±7.4, respectively, p<0.05). The number of good quality embryo was lowest in patients with PCOS (mean±SD, 0.56±0.73), while the other two groups were similar (1.18±1.01 in the PCO group, 0.91±0.98 in the Nov group, respectively, statistically not significant). The mean CES score was lower in the PCOS group (49.2±33.9) than either PCO group (68.4±29.7) or Nov group (64.2±34.5), but failed to show a statistical significance (p=0.591). Overall pregnancy rates in the PCO group was highest, when compared to the overall pregnancy rates in the normal group and the PCOS group (p=0.082).

CONCLUSION: Patients with isolated PCO pattern may not only have high potentials for developing large number of follicles, but also produce equally high quality embryos as in the non-PCO patients, resulting in higher pregnancy rates with appropriate ovarian stimulation.

P-602 Wednesday, October 22, 2014

PROBABILITY OF CLINICAL PREGNANCY AND LIVE-BIRTH IN ART FOR WOMEN PREVIOUSLY TREATED FOR ART-RELATEDECTOPIC PREGNANCY. C. E. Boots, M. J. Hill, E. C. Feinberg, R. B. Lathi, E. J. Jungheim, Ob/Gyn, Division of REI, Washington University, St. Louis, MO; Reproductive and Adult Endocrinology, NIH/NIH Bethesda, MD; ‘Fertility Centers of Illinois, Highland Park, IL; Ob/Gyn, Division of REI, Stanford University, Stanford, CA.

OBJECTIVE: Ectopic pregnancy is an undesired yet somewhat common outcome after ART therapy. Women may consider additional ART treatment after this outcome, yet little published evidence exists to guide them in this decision. Thus, we sought to evaluate predictors of pregnancy and live-birth (LB) for women undergoing ART after previous treatment for an ART-related ectopic.

DESIGN: Retrospective cohort study of ART population included 204 women. Inclusion criteria were previous ART-related ectopic and treatment with methotrexate (MTX). Women were excluded if they did not plan on a fresh transfer. Women were evaluated for factors associated with pregnancy including age, BMI, race, antil follicle count (AFC), prior ovarian response to gonadotropins (defined as number of oocytes retrieved in previous ART cycle), number of prior MTX doses, time since MTX therapy, and prior salpingectomy for ectopic. Standard bivariate statistics were applied (Student’s t-test, chi-square analysis) for the entire cohort and for women stratified by age (<35 years, 35 and older) to identify relevant predictors. ROC curves were used to determine the strength of identified predictors (reported as area under the curve (AUC)).

RESULTS: Overall, 40% of women achieved a clinical pregnancy (CP) in the post-MTX ART cycle. LB data was available for 122 women. Of these women, 39% achieved a LB. Six (2.9%) women had another ectopic pregnancy. Probability of CP and LB was higher in women younger than 35 years of age (43.7%, 43.6% respectively) although no factors were identified that were predictive of CP or LB for these women. Probability of CP and LB was lower in women 38 years of age and older (33.8%, 30.7%). Number of oocytes retrieved in the previous ART cycle and current AFC were predictive of CP (AUC: 0.66, 0.7 respectively) and LB (AUC: 0.69, 0.7) for these older women.

CONCLUSION: Women with a history of ART-related ectopic pregnancy have a good chance of LB in a subsequent ART cycle (40%). Repeated doses of MTX do not impact this chance nor does salpingectomy. For women 38 years of age and older, prior response to gonadotropins and current AFC may be helpful tools to predict chance of CP and LB in future ART cycles. Supported by: NIH K12 HD063086 (ESJ).
ADVERSE PREGNANCY AND BIRTH OUTCOMES BY INFERTILITY DIAGNOSES WITH AND WITHOUT ART TREATMENT. J. E. Stern,1 B. Luke,2 M. Tobias,2 M. D. Hornstein,3 H. Diop.4 *Geisel School of Medicine at Dartmouth, Lebanon, NH; 2Michigan State University, East Lansing, MI; 3Boston University, Boston, MA; 4Brigham & Women’s Hosp, Boston, MA; 5Mass Dept of Public Health, Boston, MA.

OBJECTIVE: To compare the risks for adverse pregnancy and birth outcomes by infertility diagnoses with and without ART treatment to ART pregnancies with the diagnosis of male factor only.

DESIGN: Historical cohort.

MATERIALS AND METHODS: Pregnancies resulting in live births in Massachusetts from 2004-08 and linked to hospital discharge data were compared between women who had received ART treatment (ART, N=2,989) and women with no ART treatment in the current pregnancy (non-ART, N=4,097). Women had diagnoses of endometriosis, ovulation disorders and reproductive inflammatory disorders (non-ART only). The reference group was ART pregnancies with the diagnosis of male factor only, suggesting the absence of fertility issues for the female partner. Risks of gestational diabetes (GDM), prenatal hospitalizations (Admissions), prematurity (PTB, <37 weeks), low birth weight (LBW: <2,500 gms), and small-for-gestation (SGA) were modeled using multivariate logistic regression adjusted for maternal age, race, ethnicity, education, chronic hypertension, diabetes mellitus, and plurality (adjusted odds ratios, AORs, and 95% confidence intervals, CI).

RESULTS: Risks were increased for Admissions in both ART and non-ART pregnancies, GDM in the ART and non-ART ovulation disorders groups, PTB and LBW in the ovulation disorders ART group, and LBW and SGA in the inflammation non-ART group.

<table>
<thead>
<tr>
<th>Diagnosis-Treatment</th>
<th>GDM*</th>
<th>Admission*</th>
<th>PTB*</th>
<th>LBW*</th>
<th>SGA*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male factor-yes</td>
<td>1,906</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Endometriosis-yes</td>
<td>406</td>
<td>0.78[0.50,1.22]</td>
<td>1.68[1.15,2.47]</td>
<td>0.98[0.74,1.31]</td>
<td>0.94[0.68,1.31]</td>
</tr>
<tr>
<td>Endometriosis-no</td>
<td>590</td>
<td>1.08[0.72,1.62]</td>
<td>2.97[2.10,4.22]</td>
<td>1.00[0.75,1.34]</td>
<td>1.17[0.82,1.68]</td>
</tr>
<tr>
<td>Ovulatory Disorder-yes</td>
<td>678</td>
<td>1.76[1.32,2.36]</td>
<td>1.89[1.39,2.56]</td>
<td>1.44[1.15,1.80]</td>
<td>1.43[1.11,1.85]</td>
</tr>
<tr>
<td>Ovulatory Disorder-no</td>
<td>832</td>
<td>1.86[1.33,2.61]</td>
<td>2.38[1.71,3.32]</td>
<td>0.80[0.61,1.05]</td>
<td>1.18[0.86,1.62]</td>
</tr>
<tr>
<td>Infammation-no</td>
<td>2,675</td>
<td>0.93[0.67,1.31]</td>
<td>2.69[1.98,3.66]</td>
<td>0.81[0.64,1.04]</td>
<td>1.35[1.02,1.78]</td>
</tr>
</tbody>
</table>

* AOR[95%CI]

CONCLUSION: These findings indicate substantial excess perinatal morbidity associated with underlying infertility diagnoses, even in the absence of ART treatment.

Supported by: NIH R01HD064595 and R01 HD067270.

P-605 Wednesday, October 22, 2014

DIMINISHED OVARIAN RESERVE PREDICTS PREGNANCY LOSS IN OLDER BUT NOT YOUNGER PATIENTS UNDERGOING ART. L. A. Bishop,1 K. Devine,2 K. S. Richter,1 K. Moon.1 *Obstetrics & Gynecology, Georgetown University, Washington Hospital Center, Washington, DC; 2Program in Reproductive & Adult Endocrinology, NICHD, NIH, Bethesda, MD; 3Shady Grove Fertility, Rockville, MD.

OBJECTIVE: Previous studies have reported that women with diminished ovarian reserve (DOR), quantified by elevated follicle-stimulating hormone (FSH), have higher rates of pregnancy loss. However, these studies used relatively small cohort sizes or did not differentiate by age. This study aimed to assess the predictive value of ovarian reserve based on both FSH and antral follicle counts (AFC) for pregnancy loss in a large cohort of women under- going ART.

DESIGN: Retrospective Cohort Study.

MATERIALS AND METHODS: This study reviewed treatment cycles by all patients with recorded baseline FSH and AFC and having a positive serum LCG following autologous IVF treatment at a single fertility practice during 2012. Only the first treatment cycle per patient was included in the analysis. FSH < 10 IU/L was considered normal; FSH ≥ 10 IU/L was considered elevated. The associations between baseline measures
(age, FSH and AFC) and live birth were evaluated by chi-square and logistic regression analysis.

RESULTS: A total of 1997 patients (mean age 34.3 years, range 19-44) with recorded baseline FSH (1812 <10 and 185 ≥10) and AFC (mean 16.4) had positive serum hCG following IVF treatment during the study period. In univariate analyses, age (p<0.0001), FSH below vs above 10 (p=0.037), and AFC (p=0.008) were each significantly associated with live birth. Adjusting for age, the association between AFC and live birth was no longer significant (p=0.84). Among patients 40 years or older, elevated FSH was associated with a 19% decrease in live birth (p=0.025) though the elevated FSH sub-cohort was not older (41.11 vs 41.14, p=0.87). However, FSH was not significantly associated with live birth among patients under 40 years (p=0.66).

CONCLUSION: Patients 40 years and older with basal FSH ≥10 were 56% more likely to experience miscarriage following an ART cycle resulting in chemical pregnancy. Importantly, this effect was not seen among younger patients with DOR. Elevated FSH may be a marker not only for diminished ovarian reserve but also for diminished oocyte quality among older patients undergoing ART.

### P-606 Wednesday, October 22, 2014


**OBJECTIVE:** The objective was to describe clinical pregnancy, live birth, and multiple gestation rates resulting from blastocyst transfers in fresh anonymous oocyte recipient cycles. Even though in 2013, the American Society of Reproductive Medicine’s committee opinion on the number of embryos to transfer encouraged elective single embryo transfer (eSET) in donor oocyte recipients, many donor oocyte recipients are hesitant to transfer a single embryo.

**DESIGN:** Retrospective analysis.

**MATERIALS AND METHODS:** A review of anonymous donor oocyte recipient cycles from a single laboratory from January 2009 to June 2013 (N=237). Only fresh cycles with a single or double blastocyst transfers were included for analysis. Gestational carrier cycles were excluded. T-test and Chi squared were utilized where appropriate.

**RESULTS:** Forty-six (19.4%) eSET cycles and 191 (80.6%) double embryo transfer (DET) cycles were identified. Average age in the eSET group was 40.9 years compared to 41.3 years in the DET group (p=0.54). The clinical pregnancy rate (CPR) was 20/46 (43.5%) for eSET and 108/191 (56.5%) for DET, which was not significantly different with p=0.110. The live birth rate was 20/46 (43.5%) for eSET and 104/191 (54.4%) for DET which was not significantly different with p=0.181. Of the 104 live births in the DET group, there were 62 (59.6%) singletons and 42 (40.4%) twins. No triplets were described, but one DET case had 4 gestational sacs. In this case, there were only two live born babies, and it is unclear whether it was self reduced or selectively reduced. There was also one heterotopic pregnancy which resulted in a spontaneous loss of the intrauterine pregnancy.

**CONCLUSION:** While pregnancy rates and live birth rates are less in the eSET group compared to the DET, they are not significantly decreased. A 40.4% multiple gestation live birth rate in the DET group is clinically important. The average age in these oocyte recipients is greater than 40, and previous literature has reported that obstetrical complications are increased with singleton pregnancies and even higher risk with multiple gestations with increasing age. Interestingly, approximately 80% of cases still underwent DET. This can be hypothesized to be related to financial or emotional factors facing patients utilizing anonymous donor oocytes. The dramatically high multiples rate should be considered when determining eSET versus DET in this at risk population.

**OBJECTIVE:** AMH is a sensitive marker of ovarian reserve and a good predictor of poor or excessive response following controlled ovarian stimulation. We performed a systematic review of the medical literature and meta-analysis to assess whether AMH is a predictor of implantation and/or clinical pregnancy (CP) in women undergoing ART.

**DESIGN:** Meta-analysis.

**MATERIALS AND METHODS:** A systematic review and meta-analysis of the literature was performed. Studies were included if 2x2 tables for the outcomes implantation and/or CP in IVF in relation to AMH could be constructed. To minimize heterogeneity, studies including only women with diminished ovarian reserve (DOR) or polycystic ovary syndrome (PCOS) were analyzed separately from those with unknown ovarian reserve. Summary estimates of the diagnostic odds ratio (OR) and summary receiver operating characteristic (SROC) curves were developed using the random effects model for binary data for AMH as a predictor of implantation and CP rates.

**RESULTS:** Out of 525 studies, 24 met eligibility criteria and 17 studies (10 prospective and 7 retrospective), comprising 4889 women, had extractable data and thus were included in the meta-analysis. Nine studies reporting CP rates and 3 reporting implantation rates in women with unknown ovarian reserve were included. Four studies in DOR women and 4 studies in PCOS women reporting CP rates were included. The OR for AMH as a predictor of implantation in women with unknown ovarian reserve (n=1990) was 1.93 (95% confidence interval (CI): 1.55–2.4) while the area under the SROC curve (AUC) was 0.598 (95% CI: 0.568–0.628). The OR for AMH as a predictor of CP in these women (n=3664 women) was 1.94 (95% CI: 1.66–2.27) while the AUC was 0.629 (95% CI: 0.611–0.647). AMH predictive ability for PR was greatest in women with DOR (n=783), with OR and AUC of 3.95 (95% CI: 2.56–6.09) and 0.696 (95% CI: 0.641–0.751), respectively. In contrast, AMH had no significant predictive ability for PR in PCOS women (n=442), with OR and AUC of 1.04 (95% CI: 0.66–1.34) and 0.6 (95% CI: 0.547–0.653), respectively.

**CONCLUSION:** A comprehensive literature review demonstrates that AMH is associated with implantation and CP rates in ART. Its predictive ability for CP is weak but appears to be better in women with DOR. These data suggest that AMH may have some clinical utility in counseling women undergoing ART regarding CP rates, particularly those with DOR.
OBJECTIVE: To evaluate ART pregnancy outcomes by infertility diagnosis.
DESIGN: Historical cohort.

MATERIALS AND METHODS: ART data on women who were treated and gave birth in Massachusetts were linked to vital records and hospital utilization data. ART pregnancies were limited to those with only a single infertility diagnosis. Live births were categorized by eight mutually-exclusive ART diagnoses. Risks of prematurity, low birthweight (LBW), small-for-gestation (SGA), large-for-gestation (LGA), pregnancy hypertension, gestational diabetes, preterm hospitalizations, and primary cesarean delivery were modeled using logistic regression, adjusted for parental characteristics, treatment parameters, and plurality (adjusted odds ratios, AORs, and 95% confidence intervals); the reference group were pregnancies with the diagnosis of male factor.

RESULTS: Among the 7,354 singleton and twin pregnancies, there were nonsignificant differences in the risks for LBW, SGA, and LGA. Women with ovulation disorders were more likely to develop gestational diabetes (AOR 1.80, 95% CI 1.35-2.41) and deliver preterm (AOR 1.36, 95% CI 1.08-1.71); women with the diagnosis of other factors were also more likely to deliver preterm (AOR 1.33, 1.05, 1.67). Emergency room visits were greater for women with diminished ovarian reserve (AOR 1.45, 95% CI 1.01-2.08); preterm hospital admissions were greater for women with the diagnoses of endometriosis, tubal and other factors, ovulation disorders, and uterine factors (AORs 1.66-2.68). Women with the diagnosis of uterine factor were twice as likely to deliver by primary cesarean (AOR 1.96, 95% CI 1.15, 3.37).

CONCLUSION: Although the infant outcomes of LBW, SGA, and LGA were generally similar across diagnosis groups, specific diagnoses had greater risks for prematurity, gestational diabetes, preterm hospital utilization, and primary cesarean delivery.

Supported by: NIH R01 HD064595 and R01 HD067270.

P-610 Wednesday, October 22, 2014

EFFECT OF ETHNICITY ON IMPLANTATION AND CLINICAL PREGNANCY RATES AFTER IN VITRO FERTILIZATION OR INTRACYTOPLASMIC SPERM INJECTION TREATMENT.


Reproductive Medicine, St Mary’s Hospital, Central Manchester University Hospitals NHS Trust, Manchester, United Kingdom.

OBJECTIVE: To assess the relationship and determine whether ethnicity of women has any role to play in the clinical success rate of in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) treatment.

DESIGN: Retrospective Observational Cohort Study in one of the largest National Health Service (NHS) fertility unit.

MATERIALS AND METHODS: All of the women who underwent their first fresh cycle of assisted reproduction in year 2013. A total of 656 women, of which 502 were white European and 154 were of ethnic minority group mainly south Asians. Data analysed with SPSS 16 for windows (Statistical Package for Social Sciences; IBM, Chicago, IL, USA).

RESULTS: The pre-treatment variables (age, basal follicle stimulating, anti-mullerian hormone and antral follicle count) were slightly favourable in the ethnic group, but the implantation rate were lower (30.51%) in the ethnic group compared with white European group (42.62%). Clinical pregnancy rates were also lower in ethnic group (22.07%) compared to white European group (33.66%). These results remained similar even if woman had single or double embryo transfer or day of embryo transfer during assisted reproduction treatment.

CONCLUSION: Implantation rate and clinical pregnancy rate following first fresh IVF or ICSI treatment were lower in ethnic minority group compared with white European women. Ethnicity is also an important determinant of success following assisted reproduction treatment. Further research is needed to look into this very important issue and to estimate the degree of variation in success rates of IVF treatment from a much larger database. This would help us in better understanding of relationship between ethnicity and IVF outcome, leading to modifications in the clinical strategies to achieve equivalent success rates among all ethnic groups.

P-611 Wednesday, October 22, 2014

ABSTRACT WITHDRAWN

P-612 Wednesday, October 22, 2014

OVIULATION TRIGGER WITH LESS THAN 3 FOLLICLES IN STIMULATED IN VITRO FERTILIZATION (IVF) CYCLES: A RETROSPECTIVE STUDY.

C. Roumain,a,b N. Dean,a F. Dziemka,c I.-J. Kadoch,a,b S. Phillips,b L. Lapensee,a,b Centre de Procréation Assistée, Centre Hospitalier Université de Montréal, Montreal, QC, Canada; bClinique Ovo, Montreal, QC, Canada.

OBJECTIVE: In stimulated IVF cycles, ovulation trigger is usually performed in the presence of 3 or more mature follicles. This minimal number is based on clinical experience rather than on strong literature, and obtained by weighing the probability of implantation and pregnancy versus the risk of not reaching embryo transfer when so few mature follicles are present. This
study aims to evaluate if triggering ovulation in a stimulated IVF cycle with only 2 dominant follicles or less (measuring 14 mm or more) results in pregnancy rates comparable to those obtained with 3 follicles (the currently used triggering threshold).

DESIGN: We performed a retrospective cohort study over 2 years (2011 to 2013) based on chart review on a population of patients followed at Ovo and the CHUM fertility clinics, affiliated to the University of Montreal.

MATERIALS AND METHODS: 70 cycles triggered with 2 follicles or less were analyzed, versus 69 cycles with 3 follicles. The main outcome was clinical pregnancy rates. Secondary outcomes included implantation rates and spontaneous abortions.

RESULTS: The groups were identical in terms of age (39, range 30 to 45 versus 39, range 29 to 43), parity (0, range 0 to 3 versus 0, range 0 to 3), cause of infertility (p=0.05), AMH (0.4 ng/mL +/- 0.41 versus 0.6 +/- 0.58), stimulation protocol used (p=0.05), total dose of gonadotropins (5263 units, range 1200-12000 versus 5400 units, range 1800-10200), years of infertility (4 +/- 2.8 years versus 4.3 +/- 3.4 years) and smoking (p=0.24). Out of the 70 cycles triggered with 2 follicles or less, we only obtained one clinical pregnancy. The pregnancy rate in the 2 follicles or less group was less than in the 3 follicles group (1.4 versus 8.7%) although not statistically significant (p=0.06). However, the implantation rate was significantly lower in the 2 follicles or less group (6% versus 22%, p=0.03). The spontaneous abortion rate was higher in the group with 2 follicles or less, but not statistically significant (75 versus 50%, p=0.58).

CONCLUSION: Although not statistically significant, the extremely low clinical pregnancy rate obtained following a trigger with 2 follicles or less does not justify pursuing this practice. The higher implantation rate found in the 3 follicles group supports the use of this threshold in our practice. A larger sample would be necessary to increase power and reach statistical significance in the spontaneous abortions and clinical pregnancy rates.

P-613 Wednesday, October 22, 2014

PROGESTERONE LEVEL AT HCG TRIGGER AFFECTS CHEMICAL PREGNANCY RATE AFTER IVF BUT NOT PROGRESSION TO CLINICAL OR ONGOING PREGNANCY. K. A. Green," M. P. Coffey," L. J. Wolf," B. T. Miller." Obstetrics and Gynecology, Oakland University William Beaumont School of Medicine, Royal Oak, MI; Biostatistics, Beaumont Health System, Royal Oak, MI; Reproductive Medicine Associates of Michigan, Troy, MI.

OBJECTIVE: Evaluate IVF outcomes based on the progesterone (P) level at the time of hCG trigger.

DESIGN: Retrospective analysis.

MATERIALS AND METHODS: 681 fresh, autologous IVF cycles of 500 women from 2007 to 2014 were evaluated. Women were <35 years of age with FSH<10 and at least two follicles ≥17 mm on the day of hCG trigger. The primary objective was to evaluate whether P level at hCG trigger differentially affected chemical, clinical (fetal cardiac motion), or ongoing pregnancy rates. The effect of P was examined using a four level variable: Group 1: P<1.5 ng/mL, Group 2: 1 to <1.5, Group 3: 1.5 to <2, and Group 4: ≥2. Analyses used generalized linear models fit with GEE methodology to account for multiple IVF attempts.

RESULTS: There were 442 chemical pregnancies in 681 cycles (64.9%). Chemical pregnancy rates for Groups 1, 2, 3, and 4 were 70.71, 68.47, 51.82, and 50.72%, respectively. Clinical pregnancy rates for Groups 1, 2, 3, and 4 were 60.35, 53.15, 40.00, and 40.58%, respectively. Ongoing pregnancy rates for Groups 1, 2, 3, and 4 were 53.21, 49.55, 36.36, and 36.23, respectively. P level on the day of hCG trigger significantly affected chemical pregnancy (p=0.001). The odds of chemical pregnancy with P<1.5 ng/mL were 2.14 times the odds with P≥1.5 ng/mL (95%CI 1.49, 3.09). When chemical pregnancies were considered, P level did not affect progression to clinical or ongoing pregnancy (p=0.26 and 0.76, respectively). There was no difference in insensible method between P groups. P levels did not appear to differentially affect pregnancy in agonist versus antagonist cycles (p=0.60). The effect of P on chemical pregnancy persisted when peak estradiol was added to the model (p=0.0005).

CONCLUSION: P level on the day of hCG trigger significantly affects chemical pregnancy rate but does not appear to influence progression to clinical or ongoing pregnancy. The effect of P is significant regardless of peak estradiol levels. These findings support the theory that the negative effect of elevated P may be related to endometrial receptivity.

P-614 Wednesday, October 22, 2014

INHERITED THROMBOPHILIA AND OOCYTE QUALITY: AN AGE-RELATED CORRELATION. A. Nazzaro,a,b A. Salerno,a,b "Phy- siopathology of Human Reproduction, AORN “G. Rummo” Hospital, Benevento, Italy; Reproductive Medicine, Futuraivf - Center for Reproductive Medicine, Cava de’ Tirreni, Salerno, Italy.

OBJECTIVE: To investigate the association, if any, between thrombo- philic risk factors, oocyte quality and IVF outcome in an assisted reproduc- tive technologies (ART) setting.

DESIGN: This is a preliminary retrospective cohort study. 12 thrombo- philic genes mutations were detected in 539 women at their first IVF attempt from January 2010 to December 2013. Patients <35 were the group A. patients >35 were the group B. All women underwent ovarian reserve testing prior to treatment.

MATERIALS AND METHODS: 259 women (group A) and 280 (group B) were enrolled in a university hospital reproductive medicine unit. The presence of oocyte cytoplasmic and extracytoplasmic abnormalities, in the all women, were matched against thrombophilic mutations (MTHFRCT/AC,FV,FLFL,FXIII,FBG,PAH1,PAH1,ACE,FSR2,APOB,APOE). A STATA 12.1was used for statistical analysys and a p value <0.05 was considered positive.

RESULTS: Oocytes abnormalities were matched against the presence from 0 to 2, 2 to 4, 5 to 7 contemporary thrombophilic mutations in both groups. In group A despite that cytoplasmic abnormalities increase with the increasing in the number of mutations, the results are not statistically relevant, whereas, as well as the extracytoplasmic mutations is concerning, when 5 to 7 mutations are contemporary present, we observed a statistical difference (p<0.0067). In group B both, cyto- plasmic and extracytoplasmic, oocyte abnormalities were statistically related to the number of mutations, with a p<0.02017 when 5 to 7 gene mutations were present.These preliminary results may suggest that, in older women, the regulatory mechanisms of oocyte functioning may be worsened by a lower oxygen support due to a preexisting poor oocyte quality.

CONCLUSION: At the best of our knowledge, this is the first time age related oocyte abnormalities are matched against thrombophilic muta- tions. Our results, in the absence of clinical guidelines, may suggest an evidence base regarding thrombophilic screening and antithrombotic therapy in cases of reproductive failure in older women with poor oocyte quality, in order to try to improve IVF outcome. Other oocyte regulatory mechanisms, than thrombophilic factors and related oxygen support, may be present in older women affecting oocyte quality. Larger studies and multivariate analyses of other factors are needed to clarify the role, if any, of thrombophilic mutations on oocyte quality in women over 35 years.
P-616 Wednesday, October 22, 2014

PROGNOSTIC FACTORS FOR CLINICAL PREGNANCY OUTCOMES IN ASSISTED FERTILIZATION USING PITUITARY DOWN-REGULATION WITH DEPOT DOSE AND DAILY LOW-DOSE LUTEAL PHASE GNADOTROPIN RELEASING HORMONE AGONISTS. C. Liao, a,b,c,** R. Huang, a R. W. Scherer, a X.-Y. Liang. a Reproductive Medicine Research Center, 6th Affiliated Hospital, Sun Yat-sen University, Guangzhou, Guangdong, China; bDept. Epidemiology, Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD; cDept. Gynecology and Obstetrics, Johns Hopkins Hospital, Baltimore, MD.

OBJECTIVE: To summarize a single center’s experience in applying depot-dose and daily low-dose GnRH down-regulation protocols for IVF/ICSI and explore factors associated with clinical pregnancy outcomes in each of the two protocols.

DESIGN: This is a retrospective cohort study conducted at an infertility treatment center hospital.

MATERIALS AND METHODS: 3,405 controlled ovarian stimulation (COS) cycles using 1.3/1.0 mg depot or 0.1/0.05 mg daily low-dose GnRHa protocols for luteal phase down-regulation between June 2010 and October 2013 were analyzed, which comprised of 2,106 depot cycles and 1,299 daily low-dose cycles. We summarized patient characteristics and treatment outcomes of the two treatment groups respectively and conducted logistic regression analyses to identify prognostic factors for clinical pregnancy in each group. Regression models were first selected and applied to the data set with model-wise deletion, which automatically dropped observations with missing data in any of the outcome and predictor variables. Multiple imputations were then used to impute all the missing data for variables included in the logistic regression models selected under model-wise deletion and the models were subsequently applied to the complete data set. Results of regression analyses with these two approaches were compared.

RESULTS: In both of the two protocols, increased age is associated with lower odds of clinical pregnancy, while improved embryo quality and endometrium thickness on hCG day are associated with higher odds of clinical pregnancy in the depot group, but neither of these two factors was associated with pregnancy outcome in the daily-low-dose group. Some of the statistically significant associations observed under model-wise deletion regression analyses disappeared when the models were applied to the complete data set derived from multiple imputations.

CONCLUSION: Age, embryo quality and endometrium thickness on hCG day are the most important prognostic factors for COS utilizing depot or daily low-dose GnRHa protocols. Model-wise deletion or complete case analysis when some data are missing can lead to biased results and should be avoided.

P-617 Wednesday, October 22, 2014

ELECTIVE SINGLE VERSUS DOUBLE BLASTOCYST TRANSFERS IN GESTATIONAL CARRIERS USING FRESH ANONYMOUS DONOR OOCYTES. A. Rodgers, a A. Beltso, a S. Jasulaitis, b B. Kaplan, a C. Wagner- Coughlin, b J. Liebermann. b Fertility Centers of Illinois, Chicago, IL; b Aparent IVF, Highland Park, IL.

OBJECTIVE: The objective was to describe clinical pregnancy, live birth, and multiple gestation rates resulting from blastocyst transfers in gestational carriers using anonymous donor oocytes. In 2013, the American Society of Reproductive Medicine’s committee opinion on the number of embryos to transfer encouraged elective single embryo transfer (eSET) in donor oocyte recipients. Many intended parents using gestational carriers were hesitant to transfer a single embryo.

DESIGN: Retrospective analysis.

MATERIALS AND METHODS: A review of gestational carrier cycles in which anonymous donor oocytes were used from January 2009 to June 2013 (N=108). Only fresh cycles with single or double blastocyst transfers were included for analysis. Fisher’s Exact and Chi squared were utilized where appropriate.

RESULTS: Twenty-four (22.2%) eSET cycles and 84 (77.8%) DET cycles were identified. The clinical pregnancy rate (CPR) was 14/24 (58.3%) for eSET and 60/84 (71.4%) for DET, which was not significantly different with p=0.223. The live birth rate was 10/24 (41.7%) for DET and 56/84 (66.7%) for DET which was significantly different with p=0.027. Of the 56 live births in the DET group, there were 29 (48.3%) singletons, 26 (46.4%) twins, and 1 (1.7%) triplets. In the eSET group there was 1 set of twins.

CONCLUSION: Gestational carriers utilizing anonymous donor oocytes have both excellent prognosis embryos as well as an optimal uterine environment. Both CPR and live birth rates are less in the eSET group compared to the DET. However, a 48.1% multiple gestation rate in the eSET group is clinically important. Interestingly, approximately 78% of cases still underwent DET. The dramatically high multiples rate should be considered when determining eSET versus DET in this population with an excellent prognosis.

P-618 Wednesday, October 22, 2014


OBJECTIVE: Oocyte donors and women electing fertility preservation are at increased risk of developing ovarian hyperstimulation syndrome (OHSS). Different strategies are used to lower the risk, such as decreasing the hCG dose or using gonadotropin releasing hormone agonist (GnRHa) as an alternative to hCG. However, trigger failures can occur when using GnRHa alone. Alternatively, adding low dose hCG to GnRHa may allow a decreased incidence of OHSS while avoiding trigger failures.

DESIGN: Retrospective chart review of oocyte donation and fertility preservation cycles using GnRHa and long luteal protocols between 01/01/10 and 02/02/14.

MATERIALS AND METHODS: 364 total IVF/ICSI cycles were analyzed. Oocyte maturity was induced by either GnRHa, hCG, or GnRHa plus 1500U hCG. Correlation of post trigger LH and hCG
levels to maturation parameters were assessed in 199 cycles. Spearman’s correlation assessed correlation between post-GnRHa trigger LH with proportion of total and mature oocytes retrieved as well as fertilization. 1-way ANOVA was used to compare oocyte maturation between each different trigger.

RESULTS: All 3 failed triggers were with GnRHa only. No OHSS developed after GnRHa plus 150IU hCG. No difference was noted in proportion of metaphase II (MII) oocytes to mature size follicles measured on day trigger, MII oocytes to total oocytes retrieved, or the number of 2 pronuclei (2PN) to total oocytes retrieved between those that were triggered with either hCG, GnRHa, or GnRHa plus 150IU hCG. No difference was appreciated in the same proportions when comparing hCG trigger dose of 10000, 5000, and 3300 IU. There was a significant negative correlation of the LH level post-GnRHa plus hCG trigger to the proportion of 2PN and oocytes retrieved (p = 0.046) but not with GnRHa alone. No other significant correlations were found between parameters of oocyte maturity and post trigger LH.

OCCURRENT PREDICTORS - LAB: ART

P-619 Wednesday, October 22, 2014

RELATIONSHIP BETWEEN THE MEIOTIC SPINDLE SIZE IN HUMAN OOCYTES AND EMBRYO DEVELOPMENTAL POTENTIAL AFTER INTRACYTOPLASMIC SPERM INJECTION. H. Tomari, K. Honjou, K. Kunitake, N. Hidaka, K. Nishimura, Y. Nagata. Center of Reproductive Medicine, University of Tokyo, Tokyo, Japan.

OBJECTIVE: Recent studies suggest that meiotic spindle characteristics may be an indicator of oocyte quality. The meiotic spindle in human oocytes can be visualized in a non-invasive manner using polarized light microscopy (PolScope). We focused on the meiotic spindle size. The present study aimed to investigate the relationship between meiotic spindle size in human oocytes and embryo developmental potential after intracytoplasmic sperm injection (ICSI).

CONCLUSION: Adding low dose hCG to a GnRHa trigger may allow a decrease in OHSS incidence, and appears to not compromise oocyte maturity while avoiding failed oocyte maturation. Using this alternative form of trigger appears to be most optimal for donor and fertility preservation cycles that are at very high risk of developing OHSS.

P-620 Wednesday, October 22, 2014

ALTERED AMPHIREGULIN EXPRESSION INDUCED BY DIVERSE LH REACTIVITY IN PERI-OVULATORY GRANULOSA CELLS IS ASSOCIATED WITH IVF OUTCOMES. Y. Huang, Y. Zhao, Y. Yu, S. Lin, R. Li, J. Qiao. Center of Reproductive Medicine, China Medical University Hospital, Taichung, Taiwan.

OBJECTIVE: To evaluate the expression of specific genes involved in peri-ovulatory signaling pathway induced by LH surge in granulosa cells (GCs) and to analyze their relationships with the pregnancy outcomes in vitro fertilization/ intracytoplasmic sperm injection (IVF/ICSI) treatment.

DESIGN: A retrospective clinical study.

MATERIALS AND METHODS: 130 women undergoing IVF/ICSI at Division of Reproductive Center, Peking University Third Hospital form December 2012 to July 2013 were recruited. Mural and cumulus GCs were collected at the time of oocyte retrieval. mRNA levels of specific genes (LHR, Areg, Ereg, Egfr, Nppc, Npr2, connexin 43) induced by LH surge were measured by quantitative RT-PCR and their correlations to IVF outcomes were analysed. Mural GCs from 30 patients were cultured in vitro and stimulated with hCG for 2-24 hours to evaluate the biological activity of LH.

RESULTS: Amphiregulin (Areg) mRNA levels were much higher in pregnant patients than that in non-pregnant patients in both mural and cumulus GCs (p<0.05). Also, they were positively related with the parameters that represented oocyte quality (number of MII oocytes, 2PN zygotes and good quality embryos). Both Areg and Ereg mRNA expressions peaked at 4 hours after hCG stimulation, with much higher increases in pregnant group that in non-pregnant group (p<0.05). Meanwhile, the fold change of Areg expression was positively related with the number of good quality embryos.

CONCLUSION: Altered Areg expression induced by diverse LHR reactivity in GCs can be used as a predictive marker for oocyte quality and pregnancy outcomes.

Supported by: This research was Supported by National Natural Science Foundation of China (No.81170538).

P-621 Wednesday, October 22, 2014

IMPLEMENTING TIME-LAPSE TECHNOLOGY FOR ALL IVF PATIENTS. N. Zaninovic, Q. Zhan, Z. Ye, R. Clarke, R. Bodine, Z. Rosenwaks. Reproductive Medicine, WCMC, New York, NY.

OBJECTIVE: Test, evaluate and implement time-lapse embryo culture system for all IVF patients.

DESIGN: Embryological and clinical outcomes, blastocyst (BL) freezing rate were compared between standard incubator (S) and time-lapse instrument (EmbryoScope®, E).

CONCLUSION: Oocytes with a spindle area of 80–120 μm² showed higher blastocyst and pregnancy rates. These results suggest that quantitative measurement of the meiotic spindle area provides an indication of oocyte quality.
MATERIALS AND METHODS: Study I: Comparison of embryo development between S (20% O2) vs. E (20% or 5% O2) on sibling oocytes. Study II: Retrospective analysis of clinical pregnancy (CP), implantation (IMP) and miscarriages (MC) rate in E vs. S (20% O2, n=1121 pt. in 2013) vs. S (20% O2, n=1138 pt. in 2012) broken down by maternal age and day of ET. Blastocyst freezing rate per cycle and per embryo were compared. Patients with PGD/PGS and coculture were excluded.

RESULTS: Study I: In studies on sibling oocytes (E 20% O2 vs. S 20% O2, n=77 pts), embryos cultured in E showed better embryo development and ET selection rate compared to embryos in S group (ET: 58% vs. 42%). Embryos in E with 5% O2 (n=38 pt.) exhibited superior embryo development and morphology compared to E 20% O2, and more optimal embryos for transfer (55% vs. 44%) and freezing (60% vs. 40%). Study II: Retrospective analysis showed an overall increase in CP (43% vs. 38%) and IMP (25% vs. 19%) in E (5% O2) group compared to S, regardless of maternal age and day of ET (avg. number of embryos ET were the same). This increase was more pronounced in ICSI patients where the initial 24hr low O2 environment benefited embryo development. In insemination patients, IMP rates in E were higher than S (30% vs. 27%), while CP did not vary. Embryo exposure to reduced O2 resulted in overall lower MC rate (E 4% vs. S 7.7%). Regardless of maternal age, BL rate per cycle significantly increased in E vs. S group (35% vs. 18%). On average, one more BL was frozen from E group (avg. 3.4) compared to S group (avg. 2.4). In addition to providing a superior environment, time-lapse technology allows for the identification of embryos with abnormal cleavages (DUCS: Direct Unequal Cleavages, 1-3 cells). Approximately 20% of embryos showed this abnormal cleavage pattern and were excluded from the selection at the time for ET.

CONCLUSION: The time-lapse system (EmbryoScope®) with reduced oxygen showed superior incubator capabilities over standard incubators (ambient oxygen). Additionally, abnormal cleavages identified using time-lapse technology allowed for de-selection and more optimal embryo selection. We successfully implemented time-lapse system for all IVF patients with improvement in embryo quality and selection, as well as improved overall clinical outcome.

Supported by: Institutional.

P-622 Wednesday, October 22, 2014

TWO GOOD EMBRYOS ARE TOO GOOD: CAN A SIMPLIFIED EMBRYO SCORING SYSTEM YIELD FEWER TWINS? C. Chatzichristou,
W. Lieb, J. A. Jenkins, J. R. Stelling, M. A. Bray, Obstetrics and Gynecology, The Brooklyn Hospital Center, Brooklyn, NY; Reproductive Specialists of New York, Mineola, NY; Obstetrics and Gynecology, Stony Brook University School of Medicine, Stony Brook, NY.

OBJECTIVE: Many embryo grading systems are in use to predict clinical pregnancy. The one employed by Goto et al.(1) was studied using single, thawed embryo transfer cycles, grouping embryos into 6 categories. We evaluated the Goto et al. system on fresh single embryo transfers and on also on double embryo transfers in which the outcome of each embryo was known. We then developed a simplified modification (two categories) to make it more clinically applicable for use in prospectively choosing between elective single embryo transfer (eSET) versus elective double embryo transfer (eDET).

DESIGN: A retrospective study of 840 fresh embryo transfer cycles of which 326 were single and 514 were double. We compared age, ethnicity, BMI, FSH, parity, number of previous fresh cycles, trigger type, oocytes retrieved, fertilization rate, number of total and quality blastocysts, embryo stage, ICM grade, TE grade, clinical pregnancy rate and twin rate by embryo score and number of embryos transferred.

MATERIALS AND METHODS: Women undergoing IVF or ICSI from 2010 to 2013 were included in the study. Autologous, fresh, day 5 blastocyst transfers were included in the study. Women were categorized in age groups <35 and 35-37. The blastocyst quality was coded as category 1 – Optimal (including all hatched, hatching and selected good quality expanded blastocysts) and category 2 – Sub-optimal (including all others). Variables were analyzed using Chi-Square, Student’s t-test and ANOVA. P<0.05 was considered statistically significant.

RESULTS: Significant differences were identified in CPR and twin rates in all categories except 35-37 SET due to small (N).

| Clinical Pregnancy Rates by Age Group and Blastocyst Score |
|-----------------|-----------------|-----------------|
| Age Group | SET % (N) | DET % (N) |
| | Non Pregnant Singleton | Non Pregnant Singleton | Twin |
| <35 | Optimal 46 (80) 54 (95) | 61 (77) 13 (16) | 27 (34) |
| Suboptimal 71 (48) 29 (20) | 60 (115) 24 (46) | 15 (29) |
| 35-37| Optimal 61 (33) 39 (21) | 51 (40) 22 (17) | 28 (22) |
| Suboptimal 76 (22) 24 (7) | 72 (85) 20 (23) | 8 (10) |

† Statistically significant

CONCLUSION: Our modified Goto et al. embryo scoring system, significantly predicts clinical pregnancy in young SET and in all ages of DET. It further identifies cases at high risk of twinning. A prospective study is needed to determine if this scoring system could prevent inappropriately high twinning rate.

P-623 Wednesday, October 22, 2014


OBJECTIVE: To examine the birefringence of meiotic spindle of IVM-MI oocytes by polarized light microscopy (Polscope™), and verify the relationship between spindle angle visualization and blastocyst formation rate in IVM-MI oocyte from controlled ovarian hyperstimulation (COH) cycles.

DESIGN: Retrospective study.

MATERIALS AND METHODS: This study analyzed 44 patients who had MI-stage oocytes which matured in vitro between January-December 2013. Oocytes were characterized in terms of visible or non-visible spindle and its position in relation to the first polar body (PB): (Group 1) non-visible; (Group 2) 0°-29°; (Group 3) 30°-89°; (Group 4) ≥90°. Oocytes were retrieved 35 hours after human chorionic gonadotropin (hCG) injection. Oocytes were stripped and MI-stage oocytes were cultured overnight. Meiotic spindles of IVM-MI oocytes (n=161) were screened by Polscope™ before sperm injection and grouped depending on the spindle angle. Blastocyst formation rate was compared. Pearson’s chi-squared test was used for statistical analysis and P-values<0.05 were considered statistically significant.

RESULTS: Meiotic spindle was visualized in 98.8% of the IVM-MI oocytes and the distribution of the spindles location relative to the first PB was: 1.2% (group 1), 59% (group 2), 32.3% (group 3), 7.5% (group 4). Patient’s age was comparable in all groups. The overall fertilization was 68.9% (111/161 oocytes) and did not vary between the different groups (P=0.85). Additionally, the total blastocyst formation rate was 33.3% (37 out of 111 zygotes) without any statistically significant differences among...
the categories \(P=0.363\). Out of the total number of blastocysts reached, 40.5% of them were good quality blastocysts which was comparable between groups \(P=0.825\).

CONCLUSION: Meiotic spindle position is not associated with blastocyst developmental competence of in vitro matured metaphase I oocytes from controlled ovarian hyperstimulation cycles.

**P-624 Wednesday, October 22, 2014**


OBJECTIVE: The reduced potential of IVM oocytes has been attributed to a lack of synchrony between nuclear maturation and a loss or excess of cytoplasmic maturation. However, there is no direct evidence to describe the duration of MII arrest in order for the meiotic spindle to function normally without negatively effecting of the oocyte’s potential. The objective is to know what is more important between the timing of the first polar body (1PB) extrusion (nuclear maturation; NM) and duration of MII arrest to rescue the developmental potential of in vitro matured (IVM) human oocytes of stimulated cycles?

DESIGN: This is a retrospective study of patients who had metaphase I (MI) oocytes at retrieval at the MUHC Reproductive Center between January and December 2013 (n = 30 cycles). MATERIALS AND METHODS: MI oocytes were divided into two IVM MI groups according to the different timing of NM (1PB extrusion) and ICSI was performed after different durations of MII arrest. The embryo developments were compared between the two groups. The spindle of the oocytes was examined using the Polscope TM.

RESULTS: Group I in which 45 out of 109 MI oocytes became MII after 1~4 h of in vitro culture. Culture was extended for ICSI until next morning because spindle in early telophase I was observed by the Polscope. Group II where 53 IVM MI oocytes became MII during overnight culture and were inseminated by ICS next morning. The rates of fertilization and blastocyst formation in Group I were 84.4 and 36.8% respectively which are not significantly different with those in Group II which were 75.5 and 22.5% respectively. However, the freezeable good quality blastocyst rate in Group I was significantly higher than that in Group II (18.4 vs. 2.5 %).

CONCLUSION: Freezeable good quality blastocyst is significantly higher in early NM groups with longer MII arrest than that of late NM with shorter MII arrest. This suggests that the success of IVM depends on the NM timing rather than the duration MII. Therefore if it is necessary to increase the number of available embryos for transfer using IVM MI, the optimal timing of ICSI for them may be the next morning.

**P-625 Wednesday, October 22, 2014**


OBJECTIVE: To evaluate day 5, day 6 and total blastocyst formation rates in IVF and ICSI cycles occurring over a 16 year period.

DESIGN: A retrospective analysis of blastocyst formation from 6115 cycles beginning January 1, 1998 through December 31, 2013. MATERIALS AND METHODS: Embryos were cultured in Vitrolife (1998 to 2008), Sage (2008 to 2011) sequential media and Irvine continuous culture media (2012 to 2013). Extended culture was implemented in all patients undergoing IVF.

RESULTS: Rates of Blastocyst Formation.
CONCLUSION: This data suggest, although rates of blastocyst formation are reduced with advanced reproductive age, acceptable rates of blastocyst formation can be observed for all patients. A decrease in blastulation can be observed for all ICSI cycles versus traditional insemination. Rates of blastocyst formation were observed to increase over time.

P-626 Wednesday, October 22, 2014

THE MOST FREQUENT ANEUPLOIDIES IN HUMAN EMBRYO ARE SIMILAR TO THOSE OBSERVED IN THE EARLY PREGNANCY LOSS. E. Littman, V. Phan, D. Harris, M. Severino, A. La. Red Rock Fertility Center, Las Vegas, NV.

OBJECTIVE: To investigate whether the meiotic errors that are seen in cleavage stage embryo are the ones observed in the first trimester of pregnancy.

DESIGN: Retrospective Study in an In Vitro Fertilization Laboratory. MATERIALS AND METHODS: Embryos from 156 patients (average age 34.7 years ±5.5) were investigated. A-CGH was used to investigate 23 pairs of chromosomes of a single blastomere biopsied on day 3 of embryo development.

RESULTS: A total of 877 day 3 embryos were analyzed, of which 474 were euploid and 403 were aneuploid. Aneuploidy can happen in any chromosomes. In the following order, the most frequently involved in aneuploidy were chromosomes 22, 19, 16, 15, 21, XY, 9, 13, 18, 1, 20, 14, 11, 4, 12, 2, 6, 3, 7, 17, 8, 5, 10.

CONCLUSION: Aneuploidy is strongly affected by maternal age. Most embryos cannot survive with a missing or extra chromosome and are spontaneously aborted. This study partially corroborated with previous publications reporting chromosomal aberrations in first trimester abortion. Our results show that the most frequent aneuploidy involves the chromosome 22, 19, 16, 15, 21, XY, 9, 13, 18. As observed in abortus’ karyotypes; trisomy 22, 16 and 15 are common found in the first trimester miscarriages and account for 3.8%; 4.5%; 5.2% of SABs respectively. Viable trisomies have been observed for chromosomes 13,18 and 21. Monosomy X (Turner syndrome) is frequently observed and accounts for 4.3% of SABs. Our results demonstrate that a-CGH-for detection of aneuploidies in IVF embryos is an accurate diagnosis strategy. In addition, the transfer of normal embryos prevent miscarriage and the occurrence of pregnancies resulting in the birth of children with multiple healthy problems due to aneuploidy.

Supported by: Red Rock Fertility Center.

P-627 Wednesday, October 22, 2014


OBJECTIVE: The purposes of this study are: 1) to evaluate the relationship between the presence of spindle in metaphase II (MII) oocytes and their potential to develop blastocyst, and 2) to analyze the clinical pregnancy rate depending on the origin of the embryo transferred derived from the different spindle angle.

DESIGN: Retrospective study. MATERIALS AND METHODS: This study analyzed 58 patients who underwent IVF from January-December 2013. A total of 830 eggs were collected of which 648 were MII on retrieval day. Oocytes were screened using PolyscopeTM to visualize the meiotic spindle to avoid damaging the spindle during ICSI and characterized in terms of visible or non-visible spindle and its position in relation to the first polar body (PB). From the 648 MII oocytes, 581 (89%) had visible spindles and were separated into 3 groups: (group 1) 0°-29°; (group 2) 30°-89°; (group 3) ≥90° and those with no visible spindle into group 4. Blastocyst developmental potential and clinical pregnancy rate were analyzed depending on the origin of the single embryo transferred (SET). Data was analyzed using SPSS. Chi-square test and logistic regression were performed. P-values <0.05 were considered statistically significant.

RESULTS: There was no significant difference in fertilization and cleavage rates among the groups. Blastocyst formation rate in group 1 (0°-29°) was significantly higher than the other groups (P<0.001). Better clinical pregnancy rate was observed in the cycles where embryos transferred had been derived from group 1 oocytes (26/58, 44.9%) than in those where embryos were derived from other groups (group 2: 5/58, 8.6%; group 3: 3/58, 5.1%; group 4: 6/58, 10.3%), however this difference did not reach statistical significance.

IVF results based on spindle angle.

<table>
<thead>
<tr>
<th>Spindle Angle</th>
<th>Matured (%)</th>
<th>Fertilized (%)</th>
<th>Cleavage (%)</th>
<th>Blastocyst (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-29</td>
<td>292 (45.1)</td>
<td>202 (43.1)</td>
<td>204 (43.3)</td>
<td>111 (47.4)</td>
</tr>
<tr>
<td>30-89</td>
<td>219 (33.8)</td>
<td>167 (35.6)</td>
<td>167 (35.5)</td>
<td>87 (37.2)</td>
</tr>
<tr>
<td>≥90</td>
<td>64 (9.9)</td>
<td>51 (10.9)</td>
<td>51 (10.8)</td>
<td>25 (10.7)</td>
</tr>
<tr>
<td>No visible</td>
<td>73 (11.2)</td>
<td>49 (10.4)</td>
<td>49 (10.4)</td>
<td>11 (4.7)</td>
</tr>
<tr>
<td>Total</td>
<td>648 (78.1)</td>
<td>469 (73.5)</td>
<td>471 (56.7)</td>
<td>234 (28.2)</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.48</td>
<td>0.09</td>
<td>0.128</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

a vs. b P<0.01

CONCLUSION: Meiotic spindle of oocytes located between 0°-29° is a predictor for blastocyst developmental competence.
MALE FACTOR

**P-628 Wednesday, October 22, 2014**


**OBJECTIVE:** To determine whether clinical and laboratory outcomes with the use of cryopreserved and fresh collected testicular sperm for intracytoplasmic sperm injection (ICSI) are similar in patients with azoospermia due to spermatogenic dysfunction.

**DESIGN:** Retrospective Cohort Study, University Hospital.

**MATERIALS AND METHODS:** Totally 398 couples with azoospermia due to spermatogenic dysfunction and without any female indications between 2004-2014 at University Hospital were enrolled to this study. All data concerning ICSI cycle outcomes were evaluated and compared between fresh testicular sperm (F-TES) and cryopreserved-thawed testicular sperm (Cryo-TES) used ICSI cycles. Clinical pregnancy and fertilization rates are determined as main outcomes.

**RESULTS:** Of the 398 patients, F-TES (334 patients) and Cryo-TES (64 patients) were used in ICSI cycles. Due to un-retrieval or non-found of testicular sperm, the cycle was cancelled in 19.46% (65/334) of the cases where F-TES was used and ovarian stimulation implemented to female partners. The clinical pregnancy and fertilization rates 28% vs 29% and 66% vs 63%, in Cryo-TES and F-TES groups respectively. Cycle cancelation rates due to embryo development arrest and other parameters were all similar among groups.

**Table 1. The COH and Cycle Outcomes in the Groups**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>F-TES (n=334)</th>
<th>Cryo-TES (n=64)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol levels on HCG day</td>
<td>2223±1277</td>
<td>2197±1152</td>
<td>NS</td>
</tr>
<tr>
<td>&gt; 14 mm follicles on HCG day</td>
<td>7.9±5.3</td>
<td>9.2±4.7</td>
<td>NS</td>
</tr>
<tr>
<td>Total collected oocyte(s), n</td>
<td>9.5±5.3</td>
<td>10.7±5.8</td>
<td>NS</td>
</tr>
<tr>
<td>Collected MII oocyte(s), n</td>
<td>7.3±7</td>
<td>8.5±5</td>
<td>NS</td>
</tr>
<tr>
<td>Grade A embryo(s), n</td>
<td>2.4±1.9</td>
<td>1.8±1.1</td>
<td>NS</td>
</tr>
<tr>
<td>Transfered embryo(s), n</td>
<td>1.5±0.6</td>
<td>1.3±0.4</td>
<td>NS</td>
</tr>
<tr>
<td>Fertilization rates, %</td>
<td>66</td>
<td>63</td>
<td>NS</td>
</tr>
<tr>
<td>Cycle cancelation, due to un-retrieved testicular sperm (%)</td>
<td>19.46 (65/334)</td>
<td>-</td>
<td>Not Applicable</td>
</tr>
<tr>
<td>Cycle cancelation due to embryo arrest (%)</td>
<td>17.84 (48/269)</td>
<td>18.75 (12/64)</td>
<td>NS</td>
</tr>
<tr>
<td>Clinical Pregnancy rates, (%)</td>
<td>29 (78/269)</td>
<td>28.12 (18/64)</td>
<td>NS</td>
</tr>
<tr>
<td>Miscarriage rates, (%)</td>
<td>11.5 (9/78)</td>
<td>16.6 (3/18)</td>
<td>NS</td>
</tr>
</tbody>
</table>

**CONCLUSION:** There is no statistical difference between the use of fresh versus cryopreserved-thawed testicular sperm regarding fertilization and clinical or ongoing pregnancy rates in couples with azoospermia due to spermatogenic dysfunction undergoing ICSI. However almost 20% of men were drop out due to un-retrieved testicular sperm in fresh cycles wherein the female partner of these men received controlled ovarian stimulation protocols that let highly increased costs.

**P-629 Wednesday, October 22, 2014**

**POLYMORPHISM OF GAPDHS GENE IN INFERTILE MEN WITH DYSPLASIA OF SPERM TAIL FIBROUS SHEATH.** S. Khayat, E. Bragina, L. Kurilo.

**OBJECTIVE:** The fibrous sheath (FS) is a unique sperm tail periaxonemal structure, surrounding the axoneme in the principle part of the flagellum. It is known that the motility of sperm is provided mostly by glycolysis (1). Sperm-specific glyceraldehyde-3-phosphate dehydrogenase (GAPDHS), one of the enzymes of glycolysis, is tightly associated with FS (2). Oxidation of GAPDHS decreases sperm motility (3). The goal of the present work was to investigate of the gene GAPDHS in sperm samples of the patients with rare form of azoospermia - dysplasia of the FS (DFS), presumably of genetic origin.

**DESIGN:** As GAPDHS tightly associated with the sperm fibrous sheath is an essential enzyme for glycolysis, possible gene GAPDHS alterations may lead to low sperm motility.

**MATERIALS AND METHODS:** The sperm samples from 5 patients with total astenoospermia were investigated. Their family medical histories were unremarkable, physical examination, hormone assays, semen analysis and lymphocyte karyotype were performed for every patient. They were carried out by transmission electron microscopy (TEM). In order to investigate possible GAPDHS alterations semen samples from these 5 asthenozoospermic men with DFS and 5 sperm donors were collected. DNA was extracted from each semen sample. Analysis of the possible GAPDHS alterations was performed by PCR amplification and sequencing.

**RESULTS:** The FS of normal spermatozoa from donors is composed by the longitudinal columns, and the transverse ribs connecting them. Investigations by the TEM revealed ultrastructural abnormalities specific for DFS – a chaotic distribution of the longitudinal columns and ribs often in combination with axonemal defects (absence of the central pair of microtubules). Polymorphism rs29381 (660-22 G>A) was revealed in all DFS sperm samples in the heterozygous state with replacement of adenine to guanine, whereas DNA samples extracted from donated sperm correspond to reference GAPDHS sequence.

**CONCLUSION:** Single nucleotide polymorphism rs29381 of GAPDHS gene may be one of the reasons leading to decreasing of this glycolytic enzyme activity and therefore to low sperm motility. Further studies of other possible gene alterations are necessary to reveal dysplasia of the fibrous sheath genetic basis.

**P-630 Wednesday, October 22, 2014**

**MALE INFERTILITY AND ACCESS TO ASSISTED REPRODUCIVE TECHNOLOGY (ART) IN THE USA: A MAPPING APPROACH.** H. Pham, A. Odioso, A. K. Nangia, J. Sandlow, C. Herndon, J. F. Smith, University of California, San Francisco, San Francisco, CA; University of Kansas Medical Center, Kansas City, KS; Medical College of Wisconsin, Milwaukee, WI.

**OBJECTIVE:** To visually represent the prevalence and identify clustering of male infertility, map infertility hotspots, and determine their geospatial relationships.

**DESIGN:** Cross-sectional geospatial mapping study with spatial autocorrelation analysis and geographically-weighted spatial autoregression.

**MATERIALS AND METHODS:** Demographic data were compiled from the 2010 US Census and infertility data were gathered from the CDC ART Surveillance program for every clinic with data. Physician data were compiled from the National Provider and Enumeration System. Data were aggregated using population weights into Hospital Service Areas (HSAs), as defined by the Dartmouth Atlas. Data were prepared and analyzed using the R statistical environment. Mapping was performed with ArcGIS 10.2.

**RESULTS:** Spatial clustering analysis revealed a weak geographic relationship between male infertility (I=0.10, p<0.001) and the number of fresh, non-donor IVF cycles performed (I=0.05, p=0.001), and physician density metrics (GU (I=0.69, p<0.001), OB (I=0.10, p<0.001), and MD (I=0.09, p<0.001)). We found moderate geographic dependence between median household income (I=0.63, p<0.001), unemployment rates (I=0.48, p<0.001), rates of health insurance coverage (I=0.54, p<0.001), the proportion of people living under the poverty level (I=0.46, p<0.001), levels of education (I=0.50, p<0.001), and the proportion of the non-white that was Caucasian (I=0.49, p<0.001). Regression showed that urologist density (β=159.2, p<0.001) and education levels (β=23.6, p<0.001) were significant positive predictors of IVF cycles.

**Spatial Autoregression Model Predicting IVF Cycles in HSA**

For 1 IVF cycle increase: Rate of Change per 100,000 people p-value

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rate of Change</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Income</td>
<td>$0.0004 decrease</td>
<td>0.128</td>
</tr>
<tr>
<td>% Uninsured</td>
<td>8.32% decrease</td>
<td>0.074</td>
</tr>
<tr>
<td>GU</td>
<td>159.2 physician increase</td>
<td>0.0001</td>
</tr>
<tr>
<td>Education</td>
<td>23.6% increase in 4-year college degrees</td>
<td>0.0001</td>
</tr>
<tr>
<td>% White</td>
<td>2.2% decrease</td>
<td>0.127</td>
</tr>
</tbody>
</table>

\[ \lambda = 0.0865 \text{ Likelihood Ratio Test: 14.68 \ p < 0.0001} \]
CONCLUSION: Male infertility, IVF cycles, and all independent variables were significantly dependent on geography, suggesting geographic disparities in infertility diagnoses, ART utilization, and socioeconomic measures. Access to care may be determined by physician availability in a region and is highly predictive for ART utilization. Education level may explain the disparities in ART utilization, possibly through increased financial resources and health literacy for those with higher education.

P-631 Wednesday, October 22, 2014

ADVANCED PATERNAL AGE DOES NOT AFFECT EMBRYO DEVELOPMENT IN PREIMPLANTATION GENETIC SCREENING CYCLES. S. Morin,a B. Hodes-Wertzb, J. Grifo,b ¼ K. P. Davies,a K. Green,a R. T. Scott, Jr,a,b C. Junoa,b K. Green,a D. Taylor,a,b K. H. Hong,a,b M. D. Werner,a,b R. T. Scott, Jr,a,b ¼ RWJ Medical School, Rutgers University, New Brunswick, NJ; ¼ RMA of New Jersey, Basking Ridge, NJ; ¼ Beaumont School of Medicine, Royal Oak, MI.

OBJECTIVE: To determine whether advanced paternal age affects the ability of embryos to develop to blastocysts suitable for biopsy in preimplantation genetic screening (PGS) cycles.

DESIGN: Retrospective cohort.

MATERIALS AND METHODS: 646 PGS cycles between 2010 and 2014 were evaluated. Maternal age, paternal age, and the number of two pronuclear embryos (2PNs) produced in each cycle were recorded. In all cases, embryos were cultured to the blastocyst stage and trophectoderm (TE) biopsy was performed on all embryos deemed suitable for biopsy on either day 5 or 6. The percentage (% of 2PNs available for biopsy was then recorded. Statistical analysis was performed by separating male partners into two age groups: ≥ 50 years (n = 61, average age 56.8) vs. < 50 years (n = 585, average age 39.3). A student’s t-test was used to compare the % of 2PNs available for biopsy in each category. To eliminate the confounding effect of maternal age, the paternal age groups were then compared within the SART categories for maternal age. A linear regression was also run to evaluate the correlation between paternal age and % of 2PNs available for biopsy at the blastocyst stage.

RESULTS: There was no difference in the % of 2PNs ultimately available for TE biopsy between male partners ≥ 50 years old vs. < 50 years old (59% vs. 56%, p = 0.22). Within each SART category, there was also no difference in % of 2PNs available for biopsy between the two paternal age groups.

% 2PNs available for TE biopsy by SART criteria

<table>
<thead>
<tr>
<th>SART Group</th>
<th>Average paternal age (&lt;50)</th>
<th>Average paternal age (≥50)</th>
<th>% 2PNs available for biopsy (%50 vs. ≥50)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;35</td>
<td>38.6 (22–49)</td>
<td>55.1 (50–71)</td>
<td>60% vs. 70%</td>
<td>0.11</td>
</tr>
<tr>
<td>35-37</td>
<td>38.2 (29–49)</td>
<td>55.6 (50–59)</td>
<td>57% vs. 54%</td>
<td>0.74</td>
</tr>
<tr>
<td>38-40</td>
<td>40.8 (31–49)</td>
<td>58.8 (50–82)</td>
<td>56% vs. 53%</td>
<td>0.66</td>
</tr>
<tr>
<td>41-42</td>
<td>41.2 (33–48)</td>
<td>56.3 (50–71)</td>
<td>53% vs. 59%</td>
<td>0.27</td>
</tr>
<tr>
<td>≥42</td>
<td>43.1 (34–49)</td>
<td>52.2 (50–69)</td>
<td>52% vs. 56%</td>
<td>0.55</td>
</tr>
</tbody>
</table>

The linear regression demonstrated no correlation between paternal age and the ability for 2PNs to be available for biopsy at the blastocyst stage (r = 0.038, p = 0.32).

CONCLUSION: Paternal age ≥ 50 years was not associated with poor embryo development to the blastocyst stage in patients planning PGS. This was true at all maternal age groups. This finding is in contrast to previous reports which suggested that male age > 50 significantly decreased embryo development potential prior to the cleavage stage. These data demonstrate that in patients considering PGS, advanced male age should not discourage parents from culturing embryos to the blastocyst stage so that TE biopsy can be performed.

P-633 Wednesday, October 22, 2014

TOTAL MOTILE SPERM ON SEMEN ANALYSIS IS NOT PREDICTIVE OF SEX CHROMOSOME ANEUPLOIDY. J. M. Franiak,a,b C. Junoa,b K. Green,a D. Taylor,a,b K. H. Hong,a,b M. D. Werner,a,b R. T. Scott, Jr,a,b ¼ RWJ Medical School, Rutgers University, New Brunswick, NJ; ¼ RMA of New Jersey, Basking Ridge, NJ; ¼ Beaumont School of Medicine, Royal Oak, MI.

OBJECTIVE: It is often suggested that poor parameters on semen analysis (SA) are associated with increased incidence of sex chromosome abnormalities. However, large scale embryonic data are lacking. This data seeks to determine if progressive oligospermia impacts the prevalence of sex chromosome aneuploidy risk.

DESIGN: Retrospective cohort.

MATERIALS AND METHODS: All patient undergoing IVF/ICSI at a single institution from 2008-2014 were included. The SA from the day of ICSI, which is performed routinely when CCS is planned, was collected. The total motile sperm were utilized to separate the patients into groups for comparison. Groups were compared using Chi-square tests.

RESULTS: A total of 4848 individual patients who produced 23363 embryos met inclusion criteria. CCS was performed on all which resulted in 9016 aneuploid embryos (38.6%). The total number of embryos with sex chromosome abnormalities of any kind was 579 (2.5%). When comparing rates amongst patients with varying levels of total motile sperm, no difference was found in autosomal aneuploidy (p = 0.27) or sex chromosome aneuploidy rates (p = 0.49).

No difference in autosomal or sex chromosome aneuploidy rates is seen with worsening male factor as defined by progressive oligospermia.

<table>
<thead>
<tr>
<th>Total Motile Sperm on SA</th>
<th>Total Embryos, n (%</th>
<th>Total Embryos, n (%)</th>
<th>Total Embryos, n (%)</th>
<th>Embryos containing sex chromosome abnormality, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;500,000</td>
<td>1309 (5.6)</td>
<td>807 (5.6)</td>
<td>502 (5.6)</td>
<td>28 (4.8)</td>
</tr>
<tr>
<td>500,000-999,999</td>
<td>160 (0.7)</td>
<td>103 (0.7)</td>
<td>57 (0.7)</td>
<td>2 (0.3)</td>
</tr>
<tr>
<td>≥1,499 million</td>
<td>1341 (5.7)</td>
<td>854 (5.6)</td>
<td>487 (5.4)</td>
<td>31 (5.4)</td>
</tr>
<tr>
<td>Total</td>
<td>23363</td>
<td>14347 (61.4)</td>
<td>9016 (38.6)</td>
<td>579 (2.5)</td>
</tr>
</tbody>
</table>

CONCLUSION: When male factor is stratified by total motile sperm on SA, no difference in aneuploidy is detected in autosomes or sex chromosomes amongst groups. This large scale embryonic aneuploidy data set does not support the notion that poor SA parameters are associated with aneuploidy and specifically demonstrates that total motile sperm as an indicator of severity of male factor infertility is not predictive of aneuploidy.
RESULTS: Opiorphin treatment resulted in a 1.2-fold change in motility in men with asthenozoospermia (p=0.01) bringing 40% of cases into the normal motility range. Conversely, opiorphin did not affect sperm motility in the control group.

CONCLUSION: Overall, these exciting preliminary data suggest that the endogenous NEP inhibitor, opiorphin, may offer potential benefit for the treatment of idiopathic male infertility characterized by asthenozoospermia.

Supported by: This work was partly Supported by NIH/NIDDK (Grant# DK087872) awarded to KPD.

P-634 Wednesday, October 22, 2014

1H NMR BASED METABOLOMICS PROFILING IN SEMINAL PLASMA OF ASTHENOOZOSPERMIC MEN: A PILOT STUDY. S. Singh, a E. Subramani, b R. Chattopadhyay, a S. Yasmin,a K. Chowdhury, a B. Chakravarty, a Reproductive Medicine, Institute of Reproductive Medicine, Kolkata, West Bengal, India; bSchool of Medical Science and Technology, Indian Institute of Technology, Kharagpur, West Bengal, India.

OBJECTIVE: Sperm motility is usually evaluated in research and infertility clinics for assessing the male fertility. However, there is a need to identify metabolite markers for the improved diagnosis and treatment of male infertility.[1] The present pilot study, therefore, aims to identify differentially expressed metabolites in seminal plasma of asthenozoospermic men using proton NMR based metabolomics.

DESIGN: Asthenozoospermic semen samples (n=19) were randomly collected from subjects (28–40 years) reporting at the Institute of Reproductive Medicine, Salt Lake City, Kolkata for male factor infertility treatment. Proven fertile men (n=19) whose partners had delivered healthy babies during the last six months without assisted reproductive technology were considered as controls.

MATERIALS AND METHODS: Proton NMR spectra of seminal plasma were recorded using 700 MHz Bruker Avance AV III spectrometer. Following phased and baseline correction of all NMR(2) spectra using Mest ReNova, spectral binning of the data was performed. The acquired data were analyzed using multivariate principal component analysis, partial least-squares discriminant analysis, and orthogonal projection to latent structure with discriminant analysis and metabolites were identified.

RESULTS: A significant alteration in metabolites including lactate, alanine, choline, glycerophosphocholine, tyrosine, histidine, phenylalanine and isoleucine was observed in seminal plasma of asthenozoospermic men as compared with controls.

CONCLUSION: The metabolic changes in seminal plasma of asthenozoospermic men produced a distinct pattern which helps in differentiating asthenozoospermia from controls. The altered endogenous metabolites in the seminal plasma of patients showed glycolysis intermediates, amino acids, and molecules related to lipid catabolism. These findings suggest that the metabolomic profiling may contribute for the diagnosis and treatment of infertility. However, this study warrants further investigation with large sample size.

P-635 Wednesday, October 22, 2014

SEMEN VARIATION IN A POPULATION OF FERTILE CANDIDATES FOR SPERM BANK. C. Borghi, A. Nabel, F. Aguirre, S. Papier, M. A. Barros, C. Alvarez Sedo. CEGYR - Genetics and Reproductive Medicine, Capital Federal, Buenos Aires, Argentina.

OBJECTIVE: The seminal parameters related to male fertility have been studied for many years by the World Health Organization (WHO), in their last manual (2010) many of the values suffered important changes in relation to the 1999 version. The aim of this study was to compare the seminal parameters of fertile candidates for sperm banking with the current WHO values.

DESIGN: Prospective blind study.

MATERIALS AND METHODS: Since one year, our institution is recruiting men for creating a sperm bank. After passing the medical and psychological evaluation, a total of 80 candidates were considered for the evaluation of a semen sample. The evaluation was conducted using the guidelines of the WHO (2010) and blinded to the personal history of each candidate. Mainly, we proceeded to measure: volume, concentration / mL, progressive motility, vitality and sperm morphology. DNA fragmentation (TUNEL), is not considered as an essential parameter by the WHO, but it was also evaluated. Subsequently, we proceeded to check out the medical history each candidate, stating that 28 were fertile, but only 20 were fertile in a period not exceeding two years, so it was decided to take this group of men for the analysis.

RESULTS: Considering the average value of each seminal parameter, all of them were over the threshold established by WHO (table 1). However, some candidates showed values under the cut-off for specific parameters. In that sense, 25% of fertile men had abnormal volume values, 35% for concentration, 20% for progressive motility and 10% for vitality. Sperm morphology was the only parameter that didn’t have an abnormal value. There were not candidates who had two or more altered parameters.

The average of DNA fragmentation levels was 8.4±3.9.

CONCLUSION: In our experience, the seminal parameters of fertile men had normal average values according to WHO, but some individual cases may be below the cutoff values. The sperm concentration, is the seminal parameter that suffered more variation between the candidates, and morphology seems to be the most stable parameter.

Supported by: CEGYR Foundation.

P-636 Wednesday, October 22, 2014

OUTCOME OF INTRACYTOPLASMIC SPERM INJECTION USING FRESH AND CRYOPRESERVED-THAWED TESTICULAR SPERMATOZOA IN 83 AZOOSPERMIC MEN WITH KLINEFELTER SYNDROME. K. Vично, a C. Akarsu, a E. Sützen, a B. Buluç, a A. Vично, a K. Biberoglu. a Private Ankara IVF Center, Ankara, Turkey; bDepartment of Genetics, Ankara University Medical School, Ankara, Turkey; cDepartment of Obstetric and Gynecology, Gazi University Medical School, Ankara, Turkey.

OBJECTIVE: To report the outcome of intracytoplasmic sperm injection (ICSI) using fresh or cryopreserved-thawed testicular spermatozoa in patients with Klinefelter syndrome.

DESIGN: Retrospective clinical study.

MATERIALS AND METHODS: Medical records of 83 azoospermic men with Klinefelter syndrome who underwent 88 TESE procedures between 2003-2013 were reviewed. The clinical parameters for predicting sperm recovery, the sperm retrieval, pregnancy and live birth rates of ICSI cycles were evaluated.

RESULTS: Seventy seven out of 83 azoospermic men had classic and the remaining 6 had mosaic Klinefelter syndrome. A total of 88 TESE procedures were performed of which conventional method consisting of multiple tissue biopsies and microsurgery were applied in 48 and 40 of the cases, respectively. Spermatozoa were found in 35 men with a retrieval rate of 39.7%. The age, volume of testes, serum FSH and testosterone levels, presence of mosaicism, the method of sperm retrieval and smoking were not found to be predictive in obtaining testicular spermatozoa. A total of 41 embryo transfer cycles were carried out using fresh testicular spermatozoa in 30, cryopreserved-thawed spermatozoa in 10 and frozen thawed embryo replacement in one. Twenty two clinical pregnancies were established, including 14 singleton, 5 twin, 2 triplet and 1 quadruplet gestation (53.6%). Fifteen women delivered singleton and 7 women twin fetuses. In total, while 21 of the newborns (15 female and 6 male) were healthy, 5 newborns died following delivery due to various reasons. The outcome of three fetuses was unknown because the mothers were lost to follow-up. Karyotype analysis were available in 12 of the healthy newborns and all were normal.

CONCLUSION: Testicular sperm extraction (TESE) and ICSI provide high sperm recovery and pregnancy rates in infertile couples with azoospermia due to Klinefelter syndrome. There is no clinical, epidemiological or laboratory finding that predicts sperm retrieval in TESE procedures.

FERTILITY & STERILITY®
ASSESSING WHO 2010 SEMEN ANALYSIS PARAMETERS FOR PREDICTING PREGNANCY IN AN INFERTILE POPULATION. M. K. Mays, E. P. New, D. Chelmow, R. S. Lucidi. Obstetrics, Gynecology and Women’s Health, University of Louisville, Louisville, KY. Obstetrics and Gynecology, Virginia Commonwealth University, Richmond, VA.

OBJECTIVE: The World Health Organization’s (WHO) 2010 laboratory manual for the evaluation of human semen provides threshold values for evaluating semen based on studies of fertile men, but it does not provide probabilities of conception in couples with infertility. The goal of this study was to determine which semen analysis thresholds best predict conception in infertile couples with timed intercourse (TI) and intrauterine insemination (IUI).

DESIGN: Retrospective case-control study.

MATERIALS AND METHODS: The records of 204 couples undergoing TI or IUI for infertility at our outpatient clinic from January 2009 through December 2010 were retrospectively reviewed. Semen analyses were available for 119 couples including 435 total treatment cycles and were performed in accordance with the WHO 2010 laboratory manual (1). Logistic regression was performed including the semen parameters of count, motility and morphology. Female age, infertility diagnosis, and method of ovulation induction were also included in the analysis to control for known confounders.

RESULTS: Of the 435 treatment cycles, 15.2% resulted in pregnancy. In the logistic regression models, only morphology was significant as a predictor of pregnancy. Further analysis using morphology threshold values of ≤2%, 3-4%, and ≥5% normal forms showed that pregnancy rates were similar for values above 2% (18.9%) but significantly lower with 2% or fewer normal forms (10.0%) (P = 0.037).

Odds Ratio (95% CI) for pregnancy

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>TI</th>
<th>IUI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female Age</td>
<td>0.96</td>
<td>0.99</td>
<td>0.94</td>
</tr>
<tr>
<td>Semen Volume</td>
<td>0.84</td>
<td>0.81</td>
<td>0.89</td>
</tr>
<tr>
<td>Sperm Concentration</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Sperm Motility</td>
<td>0.55</td>
<td>0.20</td>
<td>0.18</td>
</tr>
<tr>
<td>Sperm Morphology</td>
<td>1.12</td>
<td>1.25</td>
<td>1.13</td>
</tr>
</tbody>
</table>

A sub-analysis was performed to measure separately the predictive value of sperm morphology on TI and IUI cycles. In the timed intercourse group, sperm morphology correlated with statistically significant increased odds of pregnancy OR 1.251 (1.09-1.44). In the IUI group, it was not statistically significant OR 1.129 95% CI (0.95-1.34).

CONCLUSION: Morphology was the only semen analysis parameter that predicted pregnancy. When patients are undergoing IUI, a lower threshold for normal sperm morphology (<2%) has a pregnancy rate comparable to normal morphology values (>4%) when intercourse is employed as the method of conception.

P-638 Wednesday, October 22, 2014

PREDICTORS OF SUCCESS AFTER MICROSCOPIC SUBINGUINAL VARICOCELECTOMY. B. A. Harnisch, D. Johnson, A. Zganjar, J. J. Sandlow. Urology, Medical College of Wisconsin, Milwaukee, WI.

OBJECTIVE: Varicocele is found in 35-40% of men with infertility. Although several meta-analyses have demonstrated that varicocelectomy improves semen parameters and pregnancy rates, it is still unclear as to which patients will benefit from this procedure. The aim of this study was to determine pre-operative clinical and laboratory predictors of success of microscopically selected subinguinal varicocelectomy as defined by a 50% improvement in total motile sperm count.

DESIGN: An Institutional Review Board retrospective study.

MATERIALS AND METHODS: An Institutional Review Board retrospective study was conducted on all patients undergoing microscopic subinguinal varicocelectomy as defined by a 50% improvement in total motile sperm count. Pre-operative clinical and laboratory predictors of success of microscopic subinguinal varicocelectomy were examined. The single cell gel electrophoresis assessment requires a higher level of nuclear decondensation while still focusing on the individual sperm cell and provides a valuable option towards the identification of spermatozoa sensitive to a poor pregnancy outcome similarly to SCSA.

CONCLUSION: The popularity of a sperm chromatin integrity assay as an add-on to a standard semen analysis, aims at identifying deleterious paternal genomic contribution to the new conceptus. Because of the poor and insensitive predictability of sperm DFI, particularly when individual spermatozoa are arbitrarily selected for insemination, we are on the quest for a more sensitive assay. The single cell gel electrophoresis assessment requires a higher level of nuclear decondensation while still focusing on the individual sperm cell and provides a valuable option towards the identification of spermatozoa with DNA compaction defects or target of oxygen-free radicals.

Supported by: Reproductive Medicine, Weill Cornell Medical College.

P-640 Wednesday, October 22, 2014

IMMATURE CHROMATIN SPERM (HDS) DETERMINED WITH Sperm CHROMATIN STRUCTURE ASSAY (SCSA) ARE RELATED TO OVARIATE STRESS. M. Brassesco, R. Lafuente, G. Lopez-Granillos, C. Brassesco, J. Benet, R. Ribas-Maynou, A. Garcia-Periñó. "Human Reproduction and Infertility Center (CIRH), Corachan Center, Barcelona, Spain; "Biologia Cellular, Fisiologia i Immunologia, Universitat Autonoma de Barcelona, Bellaterra, Barcelona, Spain; "Centro de Infertilidad Masculina y Analisis de Barcelona (CIMAB), Bellaterra, Barcelona, Spain.

OBJECTIVE: To test a sensitive assay capable of detecting single (ss) and double strand (ds) DNA breaks due to sperm nuclear DNA compaction errors or oxygen-free radical damage.

DESIGN: We tested a single cell gel electrophoresis method to assess DNA damage in patients considered for IVF due to DNA damage as measured using standard DFI, a non-specific predictability of sperm DFI, particularly when individual spermatozoa are arbitrarily selected for insemination, we are on the quest for a more sensitive assay. The single cell gel electrophoresis assessment requires a higher level of nuclear decondensation while still focusing on the individual sperm cell and provides a valuable option towards the identification of spermatozoa sensitive to a poor pregnancy outcome similarly to SCSA.

CONCLUSION: The popularity of a sperm chromatin integrity assay as an add-on to a standard semen analysis, aims at identifying deleterious paternal genomic contribution to the new conceptus. Because of the poor and insensitive predictability of sperm DFI, particularly when individual spermatozoa are arbitrarily selected for insemination, we are on the quest for a more sensitive assay. The single cell gel electrophoresis assessment requires a higher level of nuclear decondensation while still focusing on the individual sperm cell and provides a valuable option towards the identification of spermatozoa with DNA compaction defects or target of oxygen-free radicals.

Supported by: Reproductive Medicine, Weill Cornell Medical College.
**P-641 Wednesday, October 22, 2014**


OBJECTIVE: The aim of this study was to evaluate the efficiency of the hyaluronic acid (HA) sperm selection and intracytoplasmic morphologically selected sperm injection (IMSI) technique compared with conventional ICSI in patients with teratozoospermia.

DESIGN: Comparative prospective study.

MATERIALS AND METHODS: This study was conducted in a private fertility hospital, between May 2013 and April 2014. A total of 126 patients with infertility related to teratozoospermia (strict morphologically normal sperm < 4%) and no or one more previous conventional ICSI failure. All patients were divided into three groups of randomized trial by spermatozoa selection methods. Conventional ICSI group: spermatozoa were injected performed into oocytes under 200× magnification of TE 2000U (Nikon, Japan); PICSI group: spermatozoa were selected with a HA-bound of PICS1™ dish (Biocout, Inc., Horsham, PA, USA); IMSI group: spermatozoa were selected under high magnification observation (> 6,000×) with the use of IMSI-StrictTM (Hamilton Thorne, Inc., Beverly, MA, USA). We compared the fertilization rates, embryonic development and clinical outcomes among groups. Statistical analysis utilized Chi-squared test and ANOVA.

RESULTS: See the table below.

Comparisons of clinical outcomes.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Conventional ICSI</th>
<th>PICSI</th>
<th>IMSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICSI cycles (n)</td>
<td>50</td>
<td>31</td>
<td>46</td>
</tr>
<tr>
<td>Maternal age (mean±SD)</td>
<td>35.2±4.6</td>
<td>37.0±4.0</td>
<td>37.1±4.4</td>
</tr>
<tr>
<td>No. of oocytes (mean±SD)</td>
<td>505 (10.1±6.3)</td>
<td>336 (10.8±7.0)</td>
<td>495 (10.8±8.5)</td>
</tr>
<tr>
<td>No. of MII oocytes (mean±SD)</td>
<td>405 (8.1±5.1)</td>
<td>258 (8.3±5.3)</td>
<td>384 (8.3±5.8)</td>
</tr>
<tr>
<td>No. of oocytes fertilized (mean±SD)</td>
<td>315 (77.6, 6.3±4.3×10^6)</td>
<td>213 (8.2, 6.9±5.0)</td>
<td>326 (84.9, 7.1±5.4×10^6)</td>
</tr>
<tr>
<td>No. of good-quality embryos (%)</td>
<td>20 (6.3)</td>
<td>65 (2.5±10.5)</td>
<td>102 (2.2±10.7)</td>
</tr>
<tr>
<td>No. of embryo transferred (mean±SD)</td>
<td>125 (2.5±0.6)</td>
<td>57 (4.8±3.8)</td>
<td>90 (4.2)</td>
</tr>
<tr>
<td>No. of blastocysts developed (%)</td>
<td>71 (19.0)</td>
<td>15 (23.1)</td>
<td>25 (24.5)</td>
</tr>
<tr>
<td>No. of embryos implanted (%)</td>
<td>22 (17.6)</td>
<td>15 (23.1)</td>
<td>25 (24.5)</td>
</tr>
<tr>
<td>No. of clinical pregnancies (%)</td>
<td>16 (32.0)</td>
<td>13 (41.9)</td>
<td>20 (43.5)</td>
</tr>
</tbody>
</table>

* ab p = 0.036; cd p = 0.0001; ce p = 0.002; de p = 0.046

**FERTILITY & STERILITY**

**CONCLUSION**: These results demonstrated that PICSI and IMSI improved the fertilization rates, good-quality embryo rates and clinical outcomes compared with conventional ICSI. Therefore, this study suggests that PICSI or IMSI could be an effective option for patients with teratozoospermia.

---

**P-642 Wednesday, October 22, 2014**

NOVEL GENE BIOMARKERS OF SPERMATOGENESIS - POTENTIAL FOR SPERMATOGENESIS ASSESSMENT AND TREATMENT MONITORING. M. K. Kurpisz,1 A. Waclawska,2 M. K. Kurpisz,3 P. Jedrzejczuk,2 W. Zietkowicz,2 1Department of Reproductive Biology and Stem Cells, Institute of Human Genetics, Polish Academy of Sciences, Poznan, Wl, Poland; 2Department of Nuclear Medicine and Endocrine Oncology, Cancer Center and Institute of Oncology Gliwice, Gliwice, GS, Poland; 3Department of Infertility and Reproductive Endocrinology, Institute of Gynecology and Obstetrics, Poznan, Wl, Poland; 4Department of Gynecology and Obstetrics, Regional Hospital, Kalisz, Wl, Poland.

OBJECTIVE: To search for novel markers of human spermatogenesis of diagnostic and prognostic value for non obstructive azoosperma.

DESIGN: Retrospective study of novel genes by using male gonad microarrays and prospective study with treatment intervention and designed gene expression previously defined in retrospective gene survey.

MATERIALS AND METHODS: Twenty seven testicular biopsies were collected from 27 males with non obstructive azoosperma with different degree of spermatogenic arrest based on histopathology (from SCSEO to hypospermatogenesis). Control group was obtained from commercial RNA sources. Total RNA was extracted and postpurified. Samples were analyzed with the GeneChip Human Gene 1.0 ST array (Affymetrix which targets 33,297 independent transcripts). All the statistical analyses were performed in the R/Bioconductor programming environment. We used the unpaired Student t-test for groups comparison, false discovery rates (FDR) were computed to dismiss multiple testing effect. Validation of selected genes was performed by RT-PCR. Western blotting and immunohistochemistry.

For prospective study, pre-selected post-meiotic genes as well as Class II HLA genes were used to monitor non obstructive azoospermia treatment. Global gonadal transcriptome was carried out as previously. Gene validation was performed with different probes (including HLA-DQ locus) and allelic determination of 6 treated patients performed by DNA sequencing.

RESULTS: Out of selected 4,9436 genes differentially expressed 14 were found to be significantly down (AKAP4, UBQLN3, CAPN11, SPACA4, SPAT13, FAM171F2) or up-regulated (WBSCR2B, ADCY10, TMEM225, SPATS1, FSCN3, GTF5F1, GSG1) signifying different degree severity of azoosperma and differentiating between fertile versus normal individuals. Class II HLA gene (DQB1) came out as critical for treatment prognosis taking into account sperm appearance.

CONCLUSION: Fourteen not previously observed genes in human spermatogenesis are candidates for novel biomarkers while allelic variations of HLA-DQB1 gene may become promising tools for therapy prognosis of azoosperma.

Supported by: The Ministry of Science and Higher Education (grant No NR13 0066 06) and by the National Science Center (Poland) , grant No 2012/05/N/ZNS 00893.
P-643 Wednesday, October 22, 2014

1Molecular Endocrinology, National Research Institute for Child Health and Development, Setagaya-ku, Tokyo, Japan; 2The Reproduction Center, Kiba Park Clinic, Koutou-ku, Tokyo, Japan; 3Urology, Dokkyo Medical University Koshigaya Hospital, Koshigaya-shi, Saitama, Japan; 4Pediatrics, Ichikawa General Hospital, Tokyo Dental College, Ichikawa-shi, Chiba, Japan; 5Pediatrics, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan; 6Comprehensive Reproductive Medicine, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, Japan; 7National Center for Child Health and Development, Setagaya-ku, Tokyo, Japan.

OBJECTIVE: Microdeletions in the azoospermia factor (AZF) region including those named the gr/gr and b2/b4 deletions have been postulated as risk factors for spermatogenic failure, while the association between AZF microduplication and the disease phenotype remains controversial. The aim of this study was to clarify the role of AZF variation in the development of non-obstructive azoospermia (NOA).

DESIGN: Molecular analysis of 58 NOA patients and 38 fertile males. Patients with apparent sex chromosomal abnormalities were excluded from the study.

MATERIALS AND METHODS: Genomic DNA samples were obtained from peripheral leukocytes. Copy-number alterations in the AZF region were examined by multiplex ligation-dependent probe amplification (MLPA).

RESULTS: MLPA indicated genomic alterations in ~60% of the patients and ~35% of the controls. While the gr/gr deletion was observed in patients and controls at similar frequencies, the b2/b4 and AZFb deletions were exclusively detected in the patient group. Eight patients carried duplication or triplication, three of which were accompanied by deletion. Of the 38 control males, only one had duplication.

CONCLUSION: The results provide further evidence for the genetic diversity and clinical significance of AZF variation. Importantly, our data imply that duplication/triplication in the AZF region constitutes one of the major risk factors for NOA.

---

P-645 Wednesday, October 22, 2014

SEMEN QUALITY AND THE SECONDARY SEX RATIO. J. Bae,a,b S. Kim,a Z. Chen,a M. L. Eisenberg,a G. M. Buck Louis,c 1Division of Intramural Population Health Research, Eunice Kennedy Shriver National Institute of Child Health and Human Development, Rockville, MD; 2Department of Preventive Medicine, Catholic University of Daegu School of Medicine, Daegu, Republic of Korea; 3Department of Urology, Obstetrics and Gynecology, Stanford University School of Medicine, Stanford, CA.

OBJECTIVE: To evaluate the association between semen quality and the secondary sex ratio (SSR), defined as the ratio of male to female live births.


MATERIALS AND METHODS: Of the 235 (47%) couples who had a singleton birth following enrollment, 227 (97%) male partners provided one and 200 (85%) provided two semen samples. Semen analysis was performed to assess 35 parameters including 5 general characteristics, 8 computer-assisted sperm analysis (CASA) motility measures, 6 sperm head measures, 14 morphology measures, and 2 sperm chromatin stability assay (SCSA) measures. Logistic random effects regression models were used to estimate the odds ratio (OR) and 95% confidence interval (CI) for a male birth for each semen parameter, after adjusting for sample age, research site, paternal body mass index, paternal and maternal annual income, maternal age, delta (paternal age - maternal age), and maternal parity.

RESULTS: Percentage of hypo-osmotic swelling (OR, 1.03; 95% CI, 1.00-1.06) and linearity as a marker of motility (OR, 1.02; 95% CI, 1.00-1.04) were associated with an excess of male births. On the contrary, % bicepspheric sperm (OR, 0.81; 95% CI, 0.66-0.99) was associated with an excess of female births.

CONCLUSION: Only 3 of the 35 semen parameters were significantly associated with an increase or decrease in the SSR. Although the findings appear to be consistent with previous research suggesting that human sex selection may be influenced by male fecundity1, they need to be corroborated through further investigation given the exploratory approach of this study.

Supported by: This study was supported by the Intramural Research Program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development (contracts #001-HD-3-3355, N01-HD-3-3356, N01-HD-3-3358). Jisuk Bae was supported by the Korea-US Visiting Scientist Training Award (VSTA) of the Eunice Kennedy Shriver National Institute of Child Health and Human Development (award #VFTB057303).
TELOMERE LENGTH DETERMINATION IN HUMAN SPERMATOGENESIS. M. Brassesco, R. Lafuente, G. Lopez-Granollers, C. Brassesco, J. Benet, J. Ribas-Maynou, A. Garcia-Peiro. Human Reproduction and Infertility Center (CIRH), Corachan Center, Barcelona, Spain; Biologia Cel·lular, Fisiologia i Immunologia, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain; Centro de Infertilidad Masculina y Análisis de Barcelona (CIMAB), Bellaterra, Barcelona, Spain.

OBJECTIVE: The objective of the present study was to assess the relative telomere length in human spermogenesis and testicular spermatozoa.

DESIGN: From 15 human testicular biopsy samples, telomere length was analyzed in all human spermogenesis stages and testicular spermatozoa through Fluorescent In Situ Hybridization using Peptide Nucleic Acid probes (PNA-FISH). The fluorescence intensity was used as a relative measure of telomere length.

MATERIALS AND METHODS: Human testicular biopsies had received a previous hypotonic treatment before Carnoy fixation. Afterwards, PNA-FISH technique was performed on slides. This technique consists of a DNA denaturation step followed by a 1-hour hybridization with PNA probes at room temperature. A minimum of 30 cells per stage were captured and the fluorescence intensity of each telomere was analyzed using TFL-Telo software.

RESULTS: Mean telomere intensity was expressed as arbitrary units ± standard deviation: 1189 ± 1118 in interphase; 1257 ± 865 in leptotene; 1431 ± 1170 in zygote; 1006 ± 834 in pachytene; 994 ± 744 in metaphase I; 1022 ± 624 in metaphase II, and 2109 ± 2050 in testicular sperm.

CONCLUSION: Results suggest an uniform telomere length during meiosis, interestingly, telomere length showed about 2-fold increase in testicular sperm cell. These results suggest an important telomere function during spermiogenesis.

Supported by: Centro de Infertilidad y Reproducción Humana and Instituto de Salud Carlos III (FIS, Project: PI11/00630).

P-647 Wednesday, October 22, 2014

EFFECTS OF INTRA UTERINE GROWTH RESTRICTION ON TESTES. S. Demirbag,* U. Harkness,* J. Parvadia,* D. Alae,* T. Cramblethorne,* Pediatric Surgery, Gulhane Military Medical Academy, Ankara, Turkey; Pediatric Surgery, Cincinnati Children’s Hospital Medical Center, Cincinnati, OH.

OBJECTIVE: There is a recognized association between the intrauterine growth restriction (IUGR) and chronic diseases such as, type 2 diabetes, hypertension, and heart disease. We have previously shown that placental gene transfer of Insulin-like Growth Factor-1 (IGF-1) corrects growth in models of IUGR. We hypothesized that there is a relationship between IUGR and subfertility in males. To test this hypothesis we examined the effects of surgically induced IUGR on postnatal testes histology.

DESIGN: An experimental model.

MATERIALS AND METHODS: Time mated Sprague-Dawley rats were treated on day 18 of a 23-day gestation; Control (n = 4), Uterine Artery Ligation (UAL, n = 3). Adult male rats were sacrificed at 36 weeks and testicles were harvested. Morphometric analysis was performed was using Seminiferous Tubule Area (STA), Germinal Layer Area (GLA), as well as quantification of Sertoli cell, Spermatogonia, and Spermatocyte counts per tubule. Statistical analysis was performed using ANOVA.

RESULTS: UAL resulted in a significant reduction in STA compared to control (0.035±0.01 vs. 0.04±0.08 mm², p<0.001). UAL resulted in a significant lower GLA compared to control rats (0.01±0.001 vs. 0.03±0.001 mm², p=0.001). Sertoli cell counts were significantly lower in UAL compared to control (11.1±3 vs. 33.7±1.8, p<0.007) Spermatocyte counts were significantly lower in UAL compared to control (103.7±7.9 vs. 229.3±21.71, p<0.01). Spermatogonia counts were significantly lower in UAL compared to Control (39.66±11.54 vs. 66.66±2.99, p=0.03).

CONCLUSION: UAL mediated IUGR results in sub-fertility in adult males. These results provide proof of concept that placental gene therapy may be an effective strategy for correction of adult disease related to IUGR such as male sub-fertility. These results may become implemented for other adult diseases associated with IUGR.

Supported by: Supported by American Association of Obstetricians and Gynecologists Foundation.

P-648 Wednesday, October 22, 2014

INEQUITY EXISTS BETWEEN MALE AND FEMALE INFERILITY COVERAGE IN STATE INSURANCE LAWS. J. M. Dupree, R. Dickey, G. M. Langille, R. Ramasamy, J. Kovac, L. I. Lipshultz, Scott Department of Urology, Baylor College of Medicine, Houston, TX.

OBJECTIVE: With the Affordable Care Act silent about federal mandates for insurance coverage for infertility work-up and treatment, the issue will be left to individual states. A minority of states currently have laws that address insurance coverage for infertility evaluation or treatment. While several studies have analyzed female infertility coverage in these states, none have evaluated coverage for male infertility. Male-factor infertility alone accounts for 20% of all infertility and is associated with an increased risk of cardiovascular disease, cancer, and early mortality. We hypothesize that male infertility evaluation and treatment is disproportionately excluded in state laws that address insurance coverage for infertility.

DESIGN: We identified and compared states with laws or codes related to insurance coverage for infertility.

MATERIALS AND METHODS: We used the National Conference of States Legislatures and the National infertility Association to identify state laws and performed a primary review and analysis of the laws or codes to specify identify coverage for male infertility services.

RESULTS: There are 16 states with laws addressing insurance coverage for infertility (AR, CA, CT, HI, IL, LA, MD, MA, MN, MT, NJ, NY, OH, RI, TX, WV). Of the 16 states, 10 (62.5%) clearly mandate evaluation or treatment for female infertility. Only 6 states (37.5%) clearly mandate male factor evaluation or treatment. In two states (WV, MT), infertility coverage is listed only as part of covered basic health care services but is not further defined. In CA and TX, infertility coverage must be offered to employers, but employers may elect to include or exclude infertility coverage for their employees. Three states (MA, NY, NJ) specifically exempt coverage for elective sterilizations, including vasectomy reversal.

CONCLUSION: Despite the Centers for Disease Control and Prevention’s and the American Society for Reproductive Medicine’s recommendations to include both male and female partners in infertility evaluation and treatment, only 37.5% of states with laws concerning infertility clearly include coverage for the male partner. Excluding men from infertility coverage risks missing opportunities to diagnosis serious health conditions in men, correct reversible causes of infertility, and provide cost-effective treatments that can down-grade the intensity of intervention required for the couple to achieve a pregnancy.

FERTILITY & STERILITY®

P-649 Wednesday, October 22, 2014

EFFECTIVENESS OF SPERM WASHING BY DISCONTINUOUS DENSITY GRADIENT CENTRIFUGATION TO REMOVE ANTI-BODIES BOUND TO SPERM MEMBRANE. D. T. Schneider, C. M. Feijó, S. Verza Junior, S. C. Esteves, Androfert, Campinas, São Paulo, Brazil.

OBJECTIVE: We evaluated the effectiveness of sperm washing using the discontinuous two-layer density gradient centrifugation method to remove antisperm antibodies attached to the sperm surface.

DESIGN: Prospective study.

MATERIALS AND METHODS: We prospectively enrolled sixty-six men with unexplained infertility seeking evaluation. Each patient delivered one semen specimen for the study. We determined antisperm antibodies (ASA) levels using the direct immunofluorescent test (IIFT). Specimens were classified into two groups according to the pre-washing levels of antibody-bound spermatozoa: group 1 (low ASA levels, IBT<20%; n=54) and group 2 (high ASA levels, IBT>20%; n=12). Sperm washing was carried out using the discontinuous two-layer colloidal density gradient centrifugation method. Pre- and post-wash levels of antisperm antibodies were compared in the groups.

RESULTS: Pre- and post-wash percentage of spermatozoa with anti-sperm antibodies attached to their surface was 11% and 6.5% in group 1 (mean difference = 40%; p=0.01), and 30% and 19.5% in group 2 (mean difference=36.8%; p=0.02), respectively. The effectiveness of density gradient centrifugation in removing antisperm antibodies was not different between the groups, but individual variation from 52.3% to 3.9% was observed.
Table 1. Effect of density gradient centrifugation on ASA levels before and after sperm washing in patients with low (IBT <20%) and high (IBT >20%) ASA.

<table>
<thead>
<tr>
<th>% Antisperm antibodies</th>
<th>Group 1 (IBT &lt;20%)</th>
<th>Group 2 (IBT &gt;20%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-wash</td>
<td>11.0 (7.0; 15.0)</td>
<td>30.0 (24.0; 32.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Post-wash</td>
<td>6.5 (4.0; 11.0)</td>
<td>19.5 (13.0; 27.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Percent change</td>
<td>-40.0 (-57.1; -16.6)</td>
<td>-36.8 (-52.3; -3.9)</td>
<td>0.75</td>
</tr>
<tr>
<td>p value</td>
<td>&lt;0.01</td>
<td>0.02</td>
<td></td>
</tr>
</tbody>
</table>

Data reported as median and 95% confidence interval. Percent change calculated as median of individual changes. Woloxon rank sum test used to compare pre- and post-wash ASA levels, as well as ASA levels between the groups.

CONCLUSION: Sperm washing by density gradient centrifugation is an overall effective method to remove antibodies bound to sperm membranes, regardless of the levels of ASA in the neat semen. Due to an inter-individual variation in the effectiveness of the method, we recommend that each patient be tested before applying sperm washing by density gradient centrifugation in intrauterine insemination.

P-650 Wednesday, October 22, 2014


OBJECTIVE: The correlation of maternal age and aneuploidy has been well studied but the effect of paternal age on aneuploidy is still not clear. With the current trend of couples starting a family at a later age this becomes an important emerging issue. Here we examined the effect of paternal age on aneuploidy when controlling for maternal age.

DESIGN: Retrospective, single clinic study.

MATERIALS AND METHODS: Patients undergoing IVF with comprehensive chromosome screening at the blastocyst stage were included in the study. Patients were divided into four groups based upon paternal and maternal age: maternal age <35 years and paternal age <40 years (group 1), maternal age <35 years and paternal age ≥40 years (group 2), maternal age ≥35 years and paternal age <40 years (group 3), and maternal age ≥35 years and paternal age ≥40 years (group 4).

RESULTS: A total of 43, 16, 55, and 55 patients were included in groups 1, 2, 3, and 4, respectively. Average paternal age between group 1 and group 2 was significant, 34.0±2.7 years and 39.8±5.0 years, respectively (P=0.0001). Average paternal age between group 1 and group 2 was not significant, 31.6±2.1 years and 31.3±1.6 years, respectively (P=0.5782). Blastocyst aneuploidy rates were not significantly higher in group 1 compared to group 2, 107/172 (62.2%) and 50/77 (64.9%), respectively (P=0.7873). Average paternal age between group 3 and 4 was significant, 36.8±2.5 years and 45.3±5.9 years, respectively (P=0.0001). Average maternal age between group 3 and 4 was not significant, 38.2±2.2 years and 38.9±2.1 years, respectively (P=0.0536). Blastocyst aneuploidy rates were significantly higher in group 3 compared to group 4, 93/196 (47.5%) and 70/202 (34.6%), respectively (P=0.0127).

CONCLUSION: Our results indicate that paternal age has little to no influence in a younger maternal population (<35 years). However, there is a significant increase in blastocyst aneuploidy rates in an older maternal population (>35 years) when the male is >40 years compared to younger men (<40 years old). This may indicate that oocytes from older women cannot compensate for the paternal contribution to aneuploidy at the same rate as oocytes from young women.

Supported by: Reproductive Endocrinology Associates of Charlotte.

P-652 Wednesday, October 22, 2014

IVF OUTCOME IN AZOOSPERMIC CANCER SURVIVORS. S. Dar, J. Levron, J. Haas, R. Machtinger, A. kedem, I. Gat, I. Madgar, R. Orsieto, G. Raviv. "IVF Unit, Chaim Sheba Medical Center, Ramat Gan, Israel; trichology, Chaim Sheba Medical Center, Ramat Gan, Israel.

OBJECTIVE: The number of cancer survivors is increasing constantly and an average of 15-30% of them remain sterile in the long term. As such, concerns about fertility and family planning are more relevant than ever. Successful pregnancies have been reported with IVF/ICSI using testicular sperm. We therefore aim to evaluate the IVF outcome in azoospermic cancer survivors, who underwent testicular sperm extraction (TESE) and IVF/ICSI in our tertiary university-affiliated IVF unit.

DESIGN: A retrospective cohort study.

MATERIALS AND METHODS: All consecutive azoospermic cancer survivors who underwent TESE consisting of multiple random biopsies and IVF/ICSI, between 1996 to 2011 were evaluated. ICSI procedure was performed using sperm retrieved. Fertilization was confirmed by the presence of two pronuclei (PN) on the day after IVF. Embryo transfer (ET) was done on day 2 or 3. Clinical pregnancy was defined as the presence of an intrauterine gestational sac with embryonic pole diagnosed by ultrasonography. Demographic information, pretreatment hormones levels and TESE and ICSI outcomes were analyzed.

RESULTS: During the study period, 31 cancer survivors underwent 39 TESE combined with IVF/ICSI cycles. The mean patients’ age and serum FSH level were 34.0±7.0 years and 18.8±10.4 IU/L, respectively. The average left and right testicular volumes were 17.2±4.2 ml and 13.4±8.8 ml, respectively. The mean time from chemotherapy to TESE was 8.2±2.6 years. Sperm was retrieved on the day of oocyte retrieval (fresh cycle). Sperm was successfully retrieved in 11 out of 31 patients (35.5%) on initial TESE, with an overall sperm retrieval rate of 38.4% (15 of 39). The average number of retrieved oocytes was 14.0±4.0 per cycle, with clinical pregnancy and live birth rates per successful TESE were 60% (9 of 15) and 53.3% (8 of 15), respectively. In IVF, serum FSH, testicular volume and time from chemotherapy to TESE were not significantly different between patients with successful TESE to those without successful TESE. No Hodgkin lymphoma 1 of 3, leukemia 0 of 3, solid tumors 4 of 11, Hodgkin lymphoma 4 of 11 and Seminoma
4 of 11. Patients who had seminoma had significantly higher sperm retrieval rate comparing to patients who had Hodgkin lymphoma or solid tumors (P<0.009).

CONCLUSION: Post chemotherapy azospermia, can be successfully treated with TESE and ICSI, whenever cryopreservation of sperm prior to chemotherapy was not done.

SPERM PREPARATION

P-653 Wednesday, October 22, 2014

SHORTENED INTERVAL FROM SEMEN PROCESSING TO INTRAUTERINE INSEMINATION DOES NOT AFFECT PREGNANCY RATES. L. B. Craig,a K. R. Hansen,a J. S. Graves,a L.-C. Pan,a J. Peck,b M. T. Zavy,a J. J. S. Graves,a L. B. Craig,a T. Miura,a L. B. Craig,a J. S. Graves,a L. B. Craig,a J. S. Graves,a L. B. Craig,a J. S. Graves,a L. B. Craig,a J. S. Graves,a J. S. Graves,a A. Quas,a M. T. Zavy,a J. Peck,b Department of Obstetrics & Gynecology, University of Oklahoma Health Sciences Center, Oklahoma City, OK; Department of Biostatistics and Epidemiology, University of Oklahoma Health Sciences Center, Oklahoma City, OK.

OBJECTIVE: The objective of this study was to determine whether a shortened time interval from semen processing to IUI affects pregnancy and delivery rates, given that the time for the sperm to capacitate is decreased. Previous studies have focused on the time of semen collection to processing but few studies have evaluated the optimal time from semen processing to IUI procedure.

DESIGN: Retrospective study.

MATERIALS AND METHODS: Pregnancy and delivery rates from IUIs performed during the week (approximately 2.5 hours after semen processing) were compared to IUIs performed on weekends and holidays (within 30 minutes of semen processing). IUI cycles performed July 2007-June 2012 at a university-based clinic were reviewed. The first cycle for each couple was included in the analysis. Donor sperm cycles were excluded. The primary outcome was quantitative hCG per cycle. Delivery per cycle was evaluated as a secondary outcome. The risk ratios (RR) and 95% confidence intervals (95% CI) were calculated using a generalized estimating equation method to estimate modified Poisson regression models.

RESULTS: A total of 1947 IUI cycles were performed on 650 women (mean female age 31.8 ± 4.9). Limiting analysis to the first IUI cycle, resulted in 449 cycles during the week and 201 cycles on the weekend/holiday. There was no significant difference in pregnancy rates when comparing IUI cycles performed on a weekend or weekday (15.6% versus 13.9% respectively; RR 0.89, 95%CI 0.60-1.34). Likewise, there was no association between delivery and the timing of IUI (RR 0.80, 95%CI 0.48-1.37). These results were consistent when examined by year and by age group. The results were unchanged in multivariable analysis controlling for years of infertility, fertility medications, BMI, total motile sperm count for IUI, infertility diagnosis and female age (adjusted RR for pregnancy 0.94, 95% CI 0.62-1.42).

CONCLUSION: Based on these findings, patients and physicians can be reassured that shortening the time interval from semen processing to IUI procedure on weekends and holidays has no adverse effects on pregnancy or delivery rates.

Supported by: Menicon Co., Ltd.

P-654 Wednesday, October 22, 2014

SORTING OF SPERM WITH REVERSE PROGRESSIVE CHARACTERISTIC MAY PROVIDE ANOTHER OPTION FOR ACQUIRING SPERMATOZOA WITH SIGNIFICANT IMPROVEMENT IN FERTILITY RELATED QUALITY FOR PATIENTS WITH OLGOSPERMIA. L.-C. Pan,a F.-C. Hsu,a K. Nishimura,a W.-S. Yu,a F.-G. Tseng,b C.-W. Wang,a C.-R. Tseng,a b Center for General Education, Taipei Medical University, Taipei City, Taiwan; Department of Gynecology and Obstetrics, Taipei Medical University Hospital, Taipei City, Taiwan; Department of Engineering and System Science, National Tsing Hua University, Hsinchu City, Taiwan.

OBJECTIVE: Previous study had reported a sub-group from progressive motile sperm sub-population with characteristics often referred to as upstream swimming or reversed progressive [1]. However, until recently this sub-group can be separated with minimum damage. Therefore, through the use of a novel microfluidic biochip design, it is the purpose of current study to examine whether or not spermatozoa thus acquired could show improvements not only in sperm motility but also in DNA integrity for normal as well as oligospermia patients.

DESIGN: Sperms of with reverse progressive characteristic was performed by the construction of a flow velocity gradient between 20-180 μm/sec within the flow channel of a diffuser type microfluidic system [3]. Thereafter, targeted sperms would include sperms that were able to withstand or swim against the forward flow stream, but not slower ones, ones with forward progressive characteristic, or debris.

MATERIALS AND METHODS: In this study, 17 males subjects were recruited from couples undergoing ART cycles in Taipei Medical University Hospital. Approval was obtained from Joint IRB of Taipei Medical University (TMU-JIRB: 201209014). Samples were grouped according WHO and ASRM guidelines [4]: normal (sperm concentration ≥ 15 x 106 cells/ml, motility ≥ 40%), sub-fertile (sperm concentration < 15 x 106 cells/ml) and severe oligospermia (sperm concentration < 5 x 106 cells/ml). Two semen processes would be used for comparative purpose, centrifugation and chip-sorting. The adopted key quality measures included sperm motility (via Computer assisted sperm analysis, C.A.S.A.), sperm apoptosis (via TUNEL assay) and DNA fragmentation (via Halo sperm assay).

RESULTS: In normal and sub-fertile, although centrifugation and flow chip process would both promote sperm motility (in progressiveness, and linearity). However, no statistical differences were observed in all experimental measures. Meanwhile, in oligospermia group, chip sorted sample showed significantly (P<0.01) improvement in sperm apoptosis rate (from 86% down to 69% in TUNEL assay), as well as in DNA fragmentation rate (from 92% down to 74% in Halo sperm assay).

FERTILITY & STERILITY®
CONCLUSION: Sorting of sperms with reverse progressive characteristic may have significant clinical efficacy in ART treatment, especially for patients with severe oligospermia.

Supported by: This study was Supported by a research grant from Ministry of Health and Welfare (MOHW-103-TDU-PB-211-123009) of Taiwan.

TESTIS

P-656 Wednesday, October 22, 2014

QUO VADIS? NO EFFECTIVE FOLLOW-UP ALGORITHM IN PEDIATRIC TESTICULAR MICROCALCIFICATION. I. Surer,1 S. Demirbag,1 M. Kocaoglu,2 Pediatric Surgery, Gulhane Military Medical Academy, Ankara, Turkey; 1Radiology, Gulhane Military Medical Academy, Ankara, Turkey.

OBJECTIVE: Testicular microthiatis (TM) is a benign condition which hydroxyapatite microcalcifications are located within the spermatic tubules. The importance of this clinical condition is primarily due to its association with testicular malignancy and the concern of increased risk for future malignancy in TM patients. Although there is no consensus about on the follow-up algorithm in the literature for TM. The aim of the study was to review our yearly basis long term follow-up protocol.

DESIGN: This study was designed as retrospective chart review and prospective yearly basis ultrasonographic examination of the TM patients.

MATERIALS AND METHODS: A total of 25 testicles in 15 children were diagnosed with typical microthiatis formations by ultrasonography with high frequency (12-17 MHz) linear transducers and followed up in our clinic between 2000-2014. All charts were evaluated retrospectively and undescended testicle (8 pts), varicocele (2 pts), acute scrotal (2 pts), trauma (1 pt), developmental delay (1 pt) and scrotal pain (1 pt) were the complaints in these patients. After the initial diagnosis, the patients were followed up with yearly basis with ultrasonographic examination.

RESULTS: Median age was 9.3 (3-19) years. Follow up period after initial US scan was changing between 1-13 years (Mean 8 yrs) under ultrasonographic surveillance in every 6 mo. before 2005) or 12 mo. (2005-2014) basis. In most boys (13 boys, 87%) TM were stable at follow-up studies but in 2 boys TM increased gradually to snow storm appearance within 7 years. In these cases, primary pathological condition was the undescended testicles. No malignancy was detected in follow-up period.

CONCLUSION: Testicular microthiatis is more common in undescended testicle. But due to its rarity for tumor development in children and adolescence period, intensive US screening program is not cost-effective and would add little benefit to improve outcomes in testicular malignancies before second decades. Also frequent hospital visits may affect the psychological status of the children. Instead of frequent examination, the patients and parents should be educated for self-examination. The frequency of office examinations can be decreased by this way and advised in pediatric age group. Our current protocol is no regular control until second decade if there is no complaints or abnormality with self exam. The parents should be informed about the long term follow up necessity due to possible malignancy and infertility problems in the future.

EFFECTS OF RESVERATROL TREATMENT ON THE RAT TESTIS INJURY IN AN EXPERIMENTAL MODEL OF METABOLIC SYNDROME. E. Guzel,1 A. Gamusel1, S. Ozkan,2 G. Tanriverdi,2 M. B. C. Balci,3 S. Develi Is1, A. I. Hazar,2 S. Bekpinar,2 M. Uysal1.1 Department of Histology & Embryology, Istanbul University Cerrahpasa Faculty of Medicine, Istanbul, Turkey; 2Urology Clinic, GOP Taksim EAH, Istanbul, Turkey; 3Department of Biochemistry, Istanbul University Medical Faculty, Istanbul, Turkey.

OBJECTIVE: Metabolic syndrome (MS) triggers inflammation and oxidative stress which result in dysfunction of crucial organs including testis (1). However, there are limited data about the histological and ultrastructural changes of testis after induction of MS. Therefore, this study was conducted to evaluate the rat testis morphology in a MS model and possible protective effects of resveratrol, an anti-inflammatory and anti-oxidant agent.

DESIGN: An in vivo animal study assessing testis morphology in induced MS model.

MATERIALS AND METHODS: 32 male Wistar rats were divided into four (n=8): control (C), fructose (F), fructose+resveratrol (F+R) and resveratrol (R) groups those received tap water, 20g/kg/day fructose, 20g/kg/day fructose+4.5 mg/kg/day resveratrol, and 4.5 mg/kg/day resveratrol respectively for 2 months. Light- and electron-microscopic evaluations were performed. Testicular histomorphometric analysis including seminiferous tubular diameter and seminiferous epithelial (SE) height, and percentage of seminiferous tubules (ST) with germ cell loss were measured. Apoptotic cell index was assessed through the TUNEL method.

RESULTS: F group had significantly increased glucose and triglyceride levels and HOMA index (p<0.05), confirming the MS model. C group revealed normal testicular histology. In F group, some of the ST had irregular shape with reduced diameter and epithelial height, and exhibited detachment of epithelial cells. These parameters were improved in F+R group. In R group showed slight alteration in testicular histology such as shrinkage of few ST. Ultrastructurally, F group showed increased amount of lipid droplets forming aggregates within the cytoplasm of Sertoli cells and late spermatids. Mitochondrial damage was observed in Sertoli cells. Some of the spermatids fail to form the acrosomal cap. In the F+R group, the amount of lipid droplets decreased but still were present, there was a recovery for mitochondrial damage as well. Most of the ST cell components were normal. In R group, loss of cristae in the mitochondria of Sertoli cells was noted. TUNEL assay revealed that the amount of apoptotic cells in the ST increases with MS.

CONCLUSION: This study has demonstrated that resveratrol partially improves morphological changes of testis induced by MS.

A GENOME-WIDE DNA METHYLATION STUDY IN AZOOSPERMIC TESTES. K. Louie, N. Ng, V. Chow, S. Ma. Department of Obstetrics and Gynaecology, University of British Columbia, Vancouver, BC, Canada.

OBJECTIVE: To identify genomic locations that are aberrantly methylated in the testis of men with azoospermia compared to men with normal spermatogenesis and proven fertility.

DESIGN: Testicular tissue from 12 men, 6 undergoing vasectomy reversals (VR), 5 with obstructive azoospermia (OA), and 1 with non-obstructive azoospermia (NOA) were extracted for DNA. Epigenome-wide DNA methylation was assessed with the Illumina HumanMethylation450 BeadChip assay (485,512 probes).

MATERIALS AND METHODS: Men were recruited at a fertility clinic presenting with azoospermia. Testicular tissue was retrieved for this study during testicular biopsies for sperm retrieval. Phenotypes were confirmed by pathology. The tissue was washed in human tubal fluid (Vitrolife) and incubated with hypo-extraction buffer. DNA was extracted with the Centra Puregene Tissue Kit (Qiagen) according to manufacturer’s instructions. DNA quality control, bisulfite conversion, and application to the Infinium array were completed at McGill University and Génome Québec Innovation Centre, Montréal, Canada. Data was directly imported into R 3.1.0 (http://www.r-project.org). Data processing and statistical analyses were executed with R open source software, Bioconductor 2.14, and associated minfi and shinyMethyl packages. Probe exclusion was addressed with external references.

RESULTS: Through quality control assessments, a total of 1 OA sample and 58,519 probes were excluded. No CpG sites were found significant between 4 OA and 6 VR samples (false discovery rate (FDR) >0.05). Five CpG sites (FDR = 0.008, 0.028, 0.033, 0.045, 0.045) were found to be significantly differentially methylated between 1 NOA and 6 VR samples with M-value differences of 1.7, 0.4, 3.4, 2.9, and 4.3, respectively. The genes associated with the significant sites include vitamin B6 synthesis enzymes, dynein proteins, metalloproteinsases, and GTase-activating proteins.

CONCLUSION: With the most advanced methylation array platform available, we have identified 5 potential regions of the human genome that may be involved with non-obstructive azoospermia. In contrast, the methylome profiles of obstructive azoospermic men show remarkable similarities to normal men. However, caution must be taken in interpreting these results due to the small sample size. Nevertheless, the preliminary data presented here highlight a novel potential mechanism regarding sperm development and testis environment. The future addition of more samples will evaluate the preliminary results and elucidate the value of this technical approach.

Supported by: The study is Supported by the Canadian Institute of Health Research (CIHR). Grant awarded to Sai Ma.
OVEREXPRESSION OF X-LINKED E1F2S3X CAN SUBSTITUTE FOR THE LOSS OF Y-LINKED E1F2S3Y AND ALLOWS FOR SPERMATOGONIAL PROLIFERATION AND DIFFERENTIATION IN THE MOUSE. V. A. Ruthig, Y. Yamauchi, J. M. Riel, M. J. Mitchell, M. A. Ward. Institute for Biogenesis Research, Department of Anatomy, Biochemistry and Physiology, University of Hawaii; John A. Burns School of Medicine, Honolulu, HI; Medical Genetics and Functional Genomics, INSERM, Marseille, Codex 05, France.

OBJECTIVE: Mice with a single X chromosome transgenic for Sry (XOSry) develop testes populated with spermatogonia. Without other Y chromosome genes these spermatogonia undergo proliferation arrest, and meiotic and post-meiotic stages of spermatogenesis are absent. Spermatogonial proliferation block can be overcome by transgenic addition of Y chromosome gene E1f2s3y. In XOSry,E1f2s3y males spermatogenesis progresses but with meiotic and postmeiotic arrest allowing only for occasional appearance of round spermatids, and never sperm. E1f2s3x is an X chromosome gene with high homology to E1f2s3y but whether it has similar functional potential remains unknown. We aimed to determine if overexpression of E1f3s3x can substitute for lack of E1f2s3y and allow for spermatogonial proliferation and differentiation in XOSry mice.

DESIGN: Research study.

MATERIALS AND METHODS: Mice transgenic for E1f2s3x were generated and the transgene placed in the context XOSry by breeding. Quantitative analysis of spermatogenesis progression was performed on staged histological sections from XOSry,E1f2s3x males (n=11), XOSry,E1f2s3y (n=3), XOSry (n=3) and XY (n=3). For each male 10 tubules were examined per stage category, the number of spermatogonia (sg), round spermatids (rs), and Sertoli cells (sc) counted, and the data expressed as germ cell/Sertoli cell ratio. Counts and categorization of cellular abnormalities were also performed.

RESULTS: Spermatogonial proliferation was similar in XOSry, E1f2s3x and XOSry,E1f2s3y males (sg/sc: 0.54±0.03 vs 0.41±0.09, P<0.001); both male types had fewer spermatogonia than XY controls (0.79±0.08, P<0.05). Meiotic and postmeiotic arrests were present in both XOSry,E1f2s3x and XOSry,E1f2s3y, with rare development to round spermatids. The efficiency of spermatid production in XOSry,E1f2s3x was about 10-fold lower than in XOSry,E1f2s3y (rs/sc: 0.09±0.04 vs 0.92±0.15, P=0.0001) and >80-fold lower than in XY (7.88±0.50, P=0.001). Abnormalities of seminiferous epithelium were observed in both XOSry,E1f2s3x and XOSry,E1f2s3y males, with the latter specifically exhibiting a large population of apoptotic cells at meiotic metaphase.

CONCLUSION: E1f2s3x, when present in sufficient levels, can replace the function of E1f2s3y and act as the initiator of spermatogonial proliferation but with decreased progression to postmeiotic stages.

Supported by: HCF13ADVC-60314, NIH HD072380 & RR024206 grants to MAW.

P-660 Wednesday, October 22, 2014

EXPOSURE TO ELEVATED TEMPERATURES RESTORES FERTILITY IN “JUVENILE SPERMATOGONIAL DEPLETION” MUTANTS. M. A. Ward, P. B. Comish, L. Y. Liang, Y. Yamauchi, C. C. Weng, G. Shetty, R. A. Naft, M. Meistrich. Institute for Biogenesis Research, University of Hawaii, Honolulu, HI; University of Texas, M.D. Anderson Cancer Center, Houston, TX.

OBJECTIVE: “Juvenile spermatogonial depletion” (jsd) mice are homozygous for the mutated Utp14b gene and have spermatogenic arrest. Several waves of spermatogenesis proceed in the immature jsd mice, but at 6 wks of age spermatogonial differentiation arrests and spermatocyte numbers decline, resulting in the only germ cells being type A spermatogonia by 10 wks. We previously observed that suppression of testosterone or elevation of testicular temperature by cryptorchidization restored spermatogonial and spermatocyte differentiation. In this study we tested if maintaining jsd mice at higher temperature enables spermatogenesis to proceed.

DESIGN: Research study.

MATERIALS AND METHODS: Jsd mice were placed in humidified incubators at elevated ambient temperatures. This treatment resulted in spermatogonial differentiation to the spermatocyte stage, but it inhibited spermiogenesis and sperm production. The effects of elevated ambient temperatures on spermiogenesis were therefore tested in wild-type mice and revealed that sperm production and controlled study. Based on these results, a 24-day induction period in 35°C incubators was used to induce spermatocyte formation in jsd mice, and then the mice were transferred to 32°C incubators for 24 days to allow spermiogenesis and sperm production to proceed.

RESULTS: Incubation of jsd mice at 35°C increased the percentage of testis with spermatocytes from 0% in untreated mice to over 80%. Subsequent maintenance at 32°C resulted in progression of spermatid differentiation so that up to 42% of tubules had late spermatids. About half of the mutant mice treated with the induction/progression regimen had sperm in testicular cell suspensions. These sperm were used for intracytoplasmic sperm injection (ICSI) and viable, healthy, fertile offspring were obtained.

CONCLUSION: Maintenance of jsd mice at elevated temperatures with a regime compatible with both spermatogonial differentiation and spermatogenesis progression overcomes their infertility and allows them to reproduce with the help of assisted fertilization. In humans, one of the UTP14 genes, UTP14C, is expressed exclusively in germ cells and its mutations result in azoospermia or severe oligospernia. If a standard TESE-ICSI treatment of azoospermic patients with the UTP14C mutation have failed, those patients might benefit from a second round of TESE-ICSI following a mild testicular warming regime.

Supported by: NIH HD40397 to MM and NIH HD072380 to MAW.

P-661 Wednesday, October 22, 2014

HUMAN GERM CELL SECRETING FACTOR NODAL REGULATES SERTOLI CELL FUNCTIONS. R. Tian, S. Yang, Z. Zhu, J. Wang, Z. He, Z. Li. Urology, Shanghai Human Sperm Bank, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China; Urology, Shanghai Human Sperm Bank, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China; Urology, Shanghai Human Sperm Bank, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China; Urology, Shanghai Human Sperm Bank, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China; Urology, Shanghai Human Sperm Bank, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China; Urology, Shanghai Human Sperm Bank, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China.

OBJECTIVE: To explore the regulatory effects of germ cells and germ cells secreting factor Nodal on the function of Sertoli cells derived from obstructive azoospermia and non-obstructive azoospermia patients.

DESIGN: Comparative and controlled study.

MATERIALS AND METHODS: Human Sertoli cells and germ cells were isolated using two-steps enzymatic digestions from the testes of azoospermia patients. Expressions of Nodal signaling components in Sertoli cells and germ cells were identified by PCR and immunochemistry. Human germ cells and Sertoli cells were cocultured in vitro to evaluate their effects on Sertoli cells. Human recombinant nodal and its receptor inhibitor SB431542 were added in the Sertoli cells culture medium to study their effects on cells functions. QPCR and western blot were applied to assess the expression of functional Sertoli cell genes.

RESULTS: Human germ cells down-regulated blood-testis-barrier associated genes (CLDN11, OCLN) expressions of Sertoli cells in coculture system. Nodal was expressed in germ cells but not in Sertoli cells, whereas its receptors ALK4, ALK7 and ActR-IIB were detected on Sertoli cells, which indicated Nodal signaling pathway play roles in the regulation of germ cells to Sertoli cells. Nodal could promote the proliferation of human Sertoli cells, while the proliferative activity was inhibited by SB431542. Nodal could enhance the expressions of functional Sertoli cell genes (NOS1, PDX1, BMP4, and ABP), while SB431542 decreased their expressions. In contrast, Nodal decreased the expression of blood-testis-barrier associated genes (CLDN11, OCLN), while SB431542 increase their expressions.

CONCLUSION: Human Sertoli cell functions could be regulated by germ cells via paracrine pathway. Human germ cells secrete Nodal which could regulate Sertoli cell functions.

Supported by: This work was supported by National Science Foundation of China (31201109) and China National Key Project (2010CB945200).
DISCRIMINATION OF SERTOLI-CELL-ONLY SEMINIFEROUS TUBULES WITH TUBULES CONTAINING SPERMATOGENESIS IN A MOUSE MODEL USING RAMAN SPECTROSCOPY. Y. Liu Z. Li. Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China.

OBJECTIVE: To determine if Raman spectroscopy can non-invasively distinguish SCO tubules and tubules with spermatogenesis in Mouse model.

DESIGN: Paired experiments to compare Raman spectra of seminiferous tubules from mice with normal spermatogenesis and Sertoli-cell-only syndrome.

MATERIALS AND METHODS: 14 Adult male ICR mice were divided into two groups: test group (SCO tubules) and control group (tubules with complete spermatogenesis). Mice in SCO group were treated with single intraperitoneal injection of busulfan (40 mg/kg), while mice in control group were treated with same dose of 0.9% NaCl solution. After 4 weeks, mice were sacrificed and the testis weight were measured. One testis was scanned by Raman spectroscopy, and the other testis was processed with histopathological analysis. For Raman scanning, a single seminiferous tubule was placed at the center of a glass bottomed dish and hydrated with PBS solution. Linear equidistant points (a total of 5 points with the interval of 5μm) at the midline were scanned for each tubule with a 10 seconds period per point. Each mouse had 5 randomly selected tubules scanned, with an averaged Raman spectrum representing its Raman signal. For data analysis, the real-time Raman spectra were acquired automatically by OPUS software, baseline corrected and normalized. Then the spectral data were loaded in the Matlab platform for discriminant analysis and standard ‘leave-one spectrum out’ cross-validation, which aimed to classify the spectra and predict the histological diagnosis for each spectrum.

RESULTS: Mice testis weight in SCO group was (40.43±9.05) mg; in control group was (136.50±13.44) mg. The mean Johnsen Score in SCO group was 3.86±0.74; in control group was 8.28±0.57. SCO tubules had obviously intensified Raman peak intensities at 1,001 cm⁻¹, 1,096 cm⁻¹, 1,124 cm⁻¹ than tubules with spermatogenesis. Using Discriminant analysis combined with standard ‘leave-one spectrum out’ cross-validation, Raman spectroscopy was able to distinguish SCO tubules with tubules of spermatogenesis at a sensitivity of 88.24% and specificity of 90.91%.

CONCLUSION: Raman spectroscopy was able to distinguish SCO tubules with tubules containing spermatogenesis, future studies on human testicular tissue shall be necessary to determine if Raman spectroscopy could have some clinical application potentials.

Supported by: Science and Technology Commission of Shanghai Municipality (No: 10JC1409900), National Basic Research Program of China (No: 2011CB944504) and National Natural Science Foundation of China (Key Program:31230048).
<table>
<thead>
<tr>
<th>Author</th>
<th>Vol Page(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Behre, C.</td>
<td>O-85</td>
</tr>
<tr>
<td>Bekpinar, S.</td>
<td>P-657</td>
</tr>
<tr>
<td>Beloff, C.</td>
<td>O-608</td>
</tr>
<tr>
<td>Bello, A.</td>
<td>P-469</td>
</tr>
<tr>
<td>Bello, S.</td>
<td>O-333</td>
</tr>
<tr>
<td>Belliver, J.</td>
<td>O-70, O-209</td>
</tr>
<tr>
<td>Belohlavek, A.</td>
<td>O-43</td>
</tr>
<tr>
<td>Belongie, S.</td>
<td>O-292</td>
</tr>
<tr>
<td>Belotte, J.</td>
<td>O-213</td>
</tr>
<tr>
<td>Beltran, D.</td>
<td>P-559</td>
</tr>
<tr>
<td>Beltos, A.</td>
<td>O-245, P-99, P-575, P-606, P-617</td>
</tr>
<tr>
<td>Ben Slama, C.</td>
<td>P-381</td>
</tr>
<tr>
<td>Benadvi, C.</td>
<td>O-157, O-336, P-298, P-357, P-564, P-578</td>
</tr>
<tr>
<td>Ben-Ami, M.</td>
<td>O-65, P-207</td>
</tr>
<tr>
<td>Bendarsky, O.</td>
<td>P-124</td>
</tr>
<tr>
<td>Bendikson, K.</td>
<td>O-304, O-404</td>
</tr>
<tr>
<td>Bener, A.</td>
<td>P-519</td>
</tr>
<tr>
<td>Benet, J.</td>
<td>P-640, P-646</td>
</tr>
<tr>
<td>Benner, A. T.</td>
<td>P-131, P-136, P-199</td>
</tr>
<tr>
<td>Benoit, A.</td>
<td>P-79</td>
</tr>
<tr>
<td>Benoit, J.</td>
<td>O-62</td>
</tr>
<tr>
<td>Benrick, A.</td>
<td>O-85</td>
</tr>
<tr>
<td>Ben-Yosef, D.</td>
<td>O-54, P-289</td>
</tr>
<tr>
<td>Berberoglugil, M.</td>
<td>O-88</td>
</tr>
<tr>
<td>Berbir, M.</td>
<td>O-400</td>
</tr>
<tr>
<td>Berenbaum, K.</td>
<td>O-4</td>
</tr>
<tr>
<td>Berger, B.</td>
<td>O-77, O-241</td>
</tr>
<tr>
<td>Berger, B. M.</td>
<td>O-358</td>
</tr>
<tr>
<td>Berger, D. S.</td>
<td>P-363</td>
</tr>
<tr>
<td>Berger, J. J.</td>
<td>O-271</td>
</tr>
<tr>
<td>Bergh, C.</td>
<td>O-313</td>
</tr>
<tr>
<td>Bergh, P. A.</td>
<td>O-219</td>
</tr>
<tr>
<td>Bergin, J. K.</td>
<td>O-338, P-81</td>
</tr>
<tr>
<td>Bergman, K.</td>
<td>O-41</td>
</tr>
<tr>
<td>Berkeley, A.</td>
<td>P-54, P-122, P-252</td>
</tr>
<tr>
<td>Berker, B.</td>
<td>O-215, P-181, P-251, P-544, P-628</td>
</tr>
<tr>
<td>Bernardi, L.</td>
<td>O-257, P-35</td>
</tr>
<tr>
<td>Bernie, A. M.</td>
<td>O-18</td>
</tr>
<tr>
<td>Berrodo, C.</td>
<td>O-264</td>
</tr>
<tr>
<td>Bersinger, N.</td>
<td>O-144</td>
</tr>
<tr>
<td>Bertolla, R.</td>
<td>O-335</td>
</tr>
<tr>
<td>Bertolla, A. E.</td>
<td>P-62, P-385, P-407</td>
</tr>
<tr>
<td>Bates, Jr.</td>
<td>O-271</td>
</tr>
<tr>
<td>Batukan, M.</td>
<td>O-88</td>
</tr>
<tr>
<td>Barad, D. H.</td>
<td>O-330, P-298, P-357, P-564, P-578</td>
</tr>
<tr>
<td>Barret, E. S.</td>
<td>P-321, P-411</td>
</tr>
<tr>
<td>Bartsch, J.</td>
<td>O-241</td>
</tr>
<tr>
<td>Batsiti, M.</td>
<td>O-226</td>
</tr>
<tr>
<td>Baxhkaj, H.</td>
<td>O-17</td>
</tr>
<tr>
<td>Beraldi, B.</td>
<td>O-400</td>
</tr>
<tr>
<td>Bhatte, N.</td>
<td>P-305</td>
</tr>
<tr>
<td>Bhatte, N. S.</td>
<td>P-408</td>
</tr>
<tr>
<td>Bhatte, N.</td>
<td>P-305</td>
</tr>
<tr>
<td>Bhatte, N. S.</td>
<td>P-408</td>
</tr>
<tr>
<td>Bee, D.</td>
<td>O-72</td>
</tr>
<tr>
<td>Blevins, A. N.</td>
<td>P-467</td>
</tr>
<tr>
<td>Blitz, I.</td>
<td>P-598</td>
</tr>
<tr>
<td>Blum, J.</td>
<td>P-522</td>
</tr>
<tr>
<td>Blumenthal, P.</td>
<td>P-522</td>
</tr>
<tr>
<td>Blyth, E.</td>
<td>O-167</td>
</tr>
<tr>
<td>Boada, M.</td>
<td>P-568</td>
</tr>
<tr>
<td>Bocca, S.</td>
<td>P-31, P-293, P-477</td>
</tr>
<tr>
<td>Bodine, R.</td>
<td>P-621</td>
</tr>
<tr>
<td>Bodhi, D.</td>
<td>O-170, P-253</td>
</tr>
<tr>
<td>Boekelheide, K.</td>
<td>O-1</td>
</tr>
<tr>
<td>Bohler, H.</td>
<td>O-118, P-376</td>
</tr>
<tr>
<td>Bohler, H. C. L.</td>
<td>O-367, P-467</td>
</tr>
<tr>
<td>Bohrer, C.</td>
<td>P-129, P-518, P-583</td>
</tr>
<tr>
<td>Boivin, J.</td>
<td>P-325</td>
</tr>
<tr>
<td>Bolkas, M.</td>
<td>O-369, P-116</td>
</tr>
<tr>
<td>Bolli, S.</td>
<td>P-501</td>
</tr>
<tr>
<td>Bolnick, A.</td>
<td>O-160</td>
</tr>
<tr>
<td>Bolnick, A. D.</td>
<td>O-247, P-471</td>
</tr>
<tr>
<td>Bolnick, J.</td>
<td>O-160, P-359</td>
</tr>
<tr>
<td>Bolnick, J. M.</td>
<td>O-247, P-471</td>
</tr>
<tr>
<td>Bolyakov, A. O.</td>
<td>O-316</td>
</tr>
<tr>
<td>Bondy, C. A.</td>
<td>O-274, P-346</td>
</tr>
<tr>
<td>Bonetti, T. C.</td>
<td>O-172</td>
</tr>
<tr>
<td>Bono, S.</td>
<td>O-276</td>
</tr>
<tr>
<td>Boostanfar, R.</td>
<td>O-328, O-377</td>
</tr>
<tr>
<td>Boots, C. E.</td>
<td>O-337, P-602</td>
</tr>
<tr>
<td>Bord, I.</td>
<td>P-280</td>
</tr>
<tr>
<td>Borgatta, L.</td>
<td>O-32, P-7</td>
</tr>
<tr>
<td>Borges Jr., E.</td>
<td>O-346, P-201, P-474</td>
</tr>
<tr>
<td>Borghi, C.</td>
<td>O-635</td>
</tr>
<tr>
<td>Bornmann, C. L.</td>
<td>O-298</td>
</tr>
<tr>
<td>Borroto, C.</td>
<td>O-509</td>
</tr>
<tr>
<td>Borschet, J.</td>
<td>O-326</td>
</tr>
<tr>
<td>Bortoletto, P.</td>
<td>P-487</td>
</tr>
<tr>
<td>Bosdou, J. K.</td>
<td>P-375</td>
</tr>
<tr>
<td>Borsler, J. S.</td>
<td>P-633</td>
</tr>
<tr>
<td>Bossert, N. L.</td>
<td>P-24, P-343</td>
</tr>
<tr>
<td>Bosti, A. M.</td>
<td>O-184</td>
</tr>
<tr>
<td>Botsiura, G.</td>
<td>P-276</td>
</tr>
<tr>
<td>Boudhar, S. O.</td>
<td>O-142</td>
</tr>
<tr>
<td>Bouknight, J. M.</td>
<td>O-37, P-8, P-43</td>
</tr>
<tr>
<td>Boulet, S. L.</td>
<td>O-46, O-112, O-195</td>
</tr>
<tr>
<td>Bounemer, L.</td>
<td>O-235</td>
</tr>
<tr>
<td>Bowler, J.</td>
<td>O-241</td>
</tr>
<tr>
<td>Boyd, B.</td>
<td>P-107</td>
</tr>
<tr>
<td>Brackett, N.</td>
<td>O-21</td>
</tr>
<tr>
<td>Bradford, A. O.</td>
<td>O-4</td>
</tr>
<tr>
<td>Braga, D. P. A.</td>
<td>O-346, P-201, P-474</td>
</tr>
<tr>
<td>Bрагай, O. S.</td>
<td>P-265</td>
</tr>
<tr>
<td>Bрагина, Е. P.</td>
<td>P-629</td>
</tr>
<tr>
<td>Brahms, P.</td>
<td>P-217</td>
</tr>
<tr>
<td>Branham, J.</td>
<td>O-283</td>
</tr>
<tr>
<td>Brant, W. O.</td>
<td>P-149</td>
</tr>
<tr>
<td>Brasile, D. R.</td>
<td>P-257</td>
</tr>
<tr>
<td>Brassesco, C.</td>
<td>O-640, P-646</td>
</tr>
<tr>
<td>Brassesco, M.</td>
<td>O-640, P-646</td>
</tr>
<tr>
<td>Braverman, A. M.</td>
<td>O-42</td>
</tr>
<tr>
<td>Braverman, R. M.</td>
<td>O-212</td>
</tr>
<tr>
<td>Bray, M. A.</td>
<td>O-163, P-622</td>
</tr>
<tr>
<td>Brazert, M.</td>
<td>P-378</td>
</tr>
<tr>
<td>Bray, M. A.</td>
<td>O-163, P-622</td>
</tr>
<tr>
<td>Brame, A. M.</td>
<td>O-640, P-646</td>
</tr>
<tr>
<td>Bremain, J. P.</td>
<td>P-513</td>
</tr>
<tr>
<td>Brenner, A. T.</td>
<td>P-107</td>
</tr>
<tr>
<td>Brew, Z.</td>
<td>O-41</td>
</tr>
<tr>
<td>Brezina, P.</td>
<td>O-89, P-60</td>
</tr>
<tr>
<td>Brezina, P. R.</td>
<td>O-107, P-131, P-136, P-199, P-273</td>
</tr>
<tr>
<td>Bringer-Deutsch, S.</td>
<td>P-481</td>
</tr>
<tr>
<td>Bristow, S. L.</td>
<td>O-402</td>
</tr>
<tr>
<td>Author</td>
<td>Page(s)</td>
</tr>
<tr>
<td>-----------------</td>
<td>---------</td>
</tr>
<tr>
<td>Coeffey, M. P.</td>
<td>P-613</td>
</tr>
<tr>
<td>Cohen, A.</td>
<td>O-108</td>
</tr>
<tr>
<td>Cohen, B.</td>
<td>O-48, P-608</td>
</tr>
<tr>
<td>Cohen, M.</td>
<td>P-374</td>
</tr>
<tr>
<td>Cohen, M. A.</td>
<td>O-369, P-123</td>
</tr>
<tr>
<td>Cohen, T.</td>
<td>O-54, P-289</td>
</tr>
<tr>
<td>Cohn, A. S.</td>
<td>P-509</td>
</tr>
<tr>
<td>Cokelez, K.</td>
<td>O-329</td>
</tr>
<tr>
<td>Collazo, I.</td>
<td>P-274</td>
</tr>
<tr>
<td>Collins, G. G.</td>
<td>O-218</td>
</tr>
<tr>
<td>Collins, S. C.</td>
<td>O-81</td>
</tr>
<tr>
<td>Colls, P.</td>
<td>O-60, O-77, P-288</td>
</tr>
<tr>
<td>Colvin, R.</td>
<td>P-32</td>
</tr>
<tr>
<td>Comhaire, F.</td>
<td>P-70</td>
</tr>
<tr>
<td>Comish, P. B.</td>
<td>P-660</td>
</tr>
<tr>
<td>Comstock, I.</td>
<td>P-522</td>
</tr>
<tr>
<td>Conaghan, J.</td>
<td>O-168, O-377, P-391</td>
</tr>
<tr>
<td>Contino, E.</td>
<td>O-39</td>
</tr>
<tr>
<td>Connell, M. T.</td>
<td>P-294</td>
</tr>
<tr>
<td>Conover, L. F.</td>
<td>P-46</td>
</tr>
<tr>
<td>Considine, R. V.</td>
<td>P-334</td>
</tr>
<tr>
<td>Constance, E. S.</td>
<td>P-6, P-69</td>
</tr>
<tr>
<td>Convissar, S. M.</td>
<td>O-383</td>
</tr>
<tr>
<td>Conway, D.</td>
<td>P-534</td>
</tr>
<tr>
<td>Cook, C.</td>
<td>P-559</td>
</tr>
<tr>
<td>Cook-Andersen, H.</td>
<td>O-115, P-379</td>
</tr>
<tr>
<td>Cookingham, L. M.</td>
<td>O-150, P-55</td>
</tr>
<tr>
<td>Cool, D.</td>
<td>P-385</td>
</tr>
<tr>
<td>Coomarasamy, A.</td>
<td>O-236, P-486</td>
</tr>
<tr>
<td>Cooney, A. J.</td>
<td>O-118</td>
</tr>
<tr>
<td>Cooper, A.</td>
<td>P-92, P-313</td>
</tr>
<tr>
<td>Cooper, A. R.</td>
<td>O-103, P-18, P-32</td>
</tr>
<tr>
<td>Copperman, A. B.</td>
<td>O-104, O-225, O-243, O-270, O-338, O-382, P-27, P-81, P-95, P-118, P-249, P-261</td>
</tr>
<tr>
<td>Coral, L.</td>
<td>P-651</td>
</tr>
<tr>
<td>Corpuz, E.</td>
<td>P-537</td>
</tr>
<tr>
<td>Correa, F. A.</td>
<td>P-349</td>
</tr>
<tr>
<td>Correa, L.</td>
<td>O-119</td>
</tr>
<tr>
<td>Corselli, J. U.</td>
<td>P-408, P-515</td>
</tr>
<tr>
<td>Costa, E. M. F.</td>
<td>P-347</td>
</tr>
<tr>
<td>Cotarello, R. P.</td>
<td>O-185</td>
</tr>
<tr>
<td>Cotroneo, E.</td>
<td>O-267</td>
</tr>
<tr>
<td>Cotteone, G.</td>
<td>O-276</td>
</tr>
<tr>
<td>Coughman, G.</td>
<td>O-184</td>
</tr>
<tr>
<td>Coughlan, C.</td>
<td>P-244</td>
</tr>
<tr>
<td>Coughlin, C.</td>
<td>P-99</td>
</tr>
<tr>
<td>Coutifaris, O. C.</td>
<td>O-124, O-385, P-326</td>
</tr>
<tr>
<td>Cox, J.</td>
<td>O-358, P-340, P-403, P-446, P-448</td>
</tr>
<tr>
<td>Cox, J. M.</td>
<td>O-122, O-352, P-419, P-478</td>
</tr>
<tr>
<td>Coyne, K. D.</td>
<td>P-361</td>
</tr>
<tr>
<td>Cozzubbo, T.</td>
<td>O-176, O-289, P-89, P-146, P-639</td>
</tr>
<tr>
<td>Craig, J.</td>
<td>O-183</td>
</tr>
<tr>
<td>Craig, L.</td>
<td>P-499</td>
</tr>
<tr>
<td>Craig, L. B.</td>
<td>P-30, P-653</td>
</tr>
<tr>
<td>Crain, J.</td>
<td>P-650</td>
</tr>
<tr>
<td>Crain, J. L.</td>
<td>P-134</td>
</tr>
<tr>
<td>Crawford, N. M.</td>
<td>P-10, P-414</td>
</tr>
<tr>
<td>Crawford, S.</td>
<td>P-548</td>
</tr>
<tr>
<td>Crawford, T. N.</td>
<td>P-343</td>
</tr>
<tr>
<td>Crec, L. M.</td>
<td>O-57</td>
</tr>
<tr>
<td>Crichton, M.</td>
<td>P-577</td>
</tr>
<tr>
<td>Criscuoli, L.</td>
<td>O-356</td>
</tr>
<tr>
<td>Criscuolo, T.</td>
<td>O-172</td>
</tr>
<tr>
<td>Crofoot, J.</td>
<td>O-360, P-552</td>
</tr>
<tr>
<td>Crombleholme, T.</td>
<td>P-647</td>
</tr>
<tr>
<td>Croxatto, D.</td>
<td>P-454</td>
</tr>
<tr>
<td>Sokmay, J. M.</td>
<td>O-397</td>
</tr>
<tr>
<td>Cueva, L.</td>
<td>O-343</td>
</tr>
<tr>
<td>Cui, L.</td>
<td>P-173</td>
</tr>
<tr>
<td>Cui, Y.</td>
<td>P-338, P-387</td>
</tr>
<tr>
<td>Cui, Z.</td>
<td>P-144, P-178</td>
</tr>
<tr>
<td>Cullen, M. R.</td>
<td>P-149</td>
</tr>
<tr>
<td>Czeresnia, C. E.</td>
<td>P-103, P-104</td>
</tr>
<tr>
<td>D’Alloja, P.</td>
<td>P-501</td>
</tr>
<tr>
<td>D’Elia, P. Q.</td>
<td>P-140</td>
</tr>
<tr>
<td>D’Hooghete, T.</td>
<td>O-139, O-141, O-299, P-482</td>
</tr>
<tr>
<td>Da Broi, M. G.</td>
<td>O-228, P-425</td>
</tr>
<tr>
<td>Dabaja, A.</td>
<td>P-147, P-153</td>
</tr>
<tr>
<td>Dabizzi, S.</td>
<td>O-356</td>
</tr>
<tr>
<td>Dada, R.</td>
<td>O-249</td>
</tr>
<tr>
<td>Daftary, G.</td>
<td>O-119</td>
</tr>
<tr>
<td>Dahan, M. H.</td>
<td>O-331</td>
</tr>
<tr>
<td>Dai, J.</td>
<td>P-423, P-424, P-471</td>
</tr>
<tr>
<td>Dalleac, A.</td>
<td>O-333</td>
</tr>
<tr>
<td>Dalilou, M.</td>
<td>O-38</td>
</tr>
<tr>
<td>Damario, M. A.</td>
<td>P-24</td>
</tr>
<tr>
<td>Dambhaeva, S.</td>
<td>O-398</td>
</tr>
<tr>
<td>Daneshmand, S. T.</td>
<td>O-181, O-351</td>
</tr>
<tr>
<td>Danich, M.</td>
<td>P-343</td>
</tr>
<tr>
<td>Danielle, G.</td>
<td>P-559</td>
</tr>
<tr>
<td>Danzer, H.</td>
<td>O-91, O-171, O-360, P-552, P-553, P-609</td>
</tr>
<tr>
<td>Dar, S.</td>
<td>O-64, P-652</td>
</tr>
<tr>
<td>Darder, M.</td>
<td>P-99</td>
</tr>
<tr>
<td>Darzykiewicz, Z.</td>
<td>O-320</td>
</tr>
<tr>
<td>Das, D.</td>
<td>P-134, P-650</td>
</tr>
<tr>
<td>Davie, J.</td>
<td>O-221</td>
</tr>
<tr>
<td>Davies, E. B.</td>
<td>P-257</td>
</tr>
<tr>
<td>Davies, K. P.</td>
<td>P-633</td>
</tr>
<tr>
<td>Davis, K. P.</td>
<td>P-572</td>
</tr>
<tr>
<td>Davis, J. W.</td>
<td>P-235</td>
</tr>
<tr>
<td>Davis, O.</td>
<td>O-344</td>
</tr>
<tr>
<td>Davis, O. K.</td>
<td>P-187</td>
</tr>
<tr>
<td>Davis, P.</td>
<td>P-534</td>
</tr>
<tr>
<td>Davis, S.</td>
<td>O-336</td>
</tr>
<tr>
<td>de Andrade, A. Z.</td>
<td>O-228</td>
</tr>
<tr>
<td>de Chavez, P.</td>
<td>O-257, P-35</td>
</tr>
<tr>
<td>de Jong, P. G.</td>
<td>O-239</td>
</tr>
<tr>
<td>de la Garza, J. L.</td>
<td>O-150</td>
</tr>
<tr>
<td>de los Santos, J. M.</td>
<td>O-252</td>
</tr>
<tr>
<td>De Luca, R.</td>
<td>O-501</td>
</tr>
<tr>
<td>De Neubourg, D.</td>
<td>O-299</td>
</tr>
<tr>
<td>de Paz, C. P.</td>
<td>P-425</td>
</tr>
<tr>
<td>De Sutter, P.</td>
<td>O-113</td>
</tr>
<tr>
<td>Dean, L.</td>
<td>P-451</td>
</tr>
<tr>
<td>Dean, N.</td>
<td>P-612</td>
</tr>
<tr>
<td>DeAngelis, A. M.</td>
<td>P-350</td>
</tr>
<tr>
<td>Debrock, S.</td>
<td>O-299</td>
</tr>
<tr>
<td>Dechaud, H.</td>
<td>O-144, P-476</td>
</tr>
<tr>
<td>Decheneray, A.</td>
<td>O-122, P-428, P-478</td>
</tr>
<tr>
<td>DeCherney, A.</td>
<td>O-8, O-10, O-14, O-248, O-303, P-87, P-275, P-294, P-367</td>
</tr>
<tr>
<td>Declercq, E.</td>
<td>O-7, O-48, P-608</td>
</tr>
<tr>
<td>Degelos, S.</td>
<td>O-263</td>
</tr>
<tr>
<td>Degerblad, M.</td>
<td>P-65</td>
</tr>
<tr>
<td>Deignan, K.</td>
<td>P-244</td>
</tr>
<tr>
<td>Dekker-Ensink, N. G.</td>
<td>P-53</td>
</tr>
<tr>
<td>Delaney, A. A.</td>
<td>O-367, P-376</td>
</tr>
<tr>
<td>Delpaere, A.</td>
<td>O-324, P-245</td>
</tr>
<tr>
<td>De Mayo, F.</td>
<td>O-374</td>
</tr>
<tr>
<td>De Mayo, F. J.</td>
<td>O-102</td>
</tr>
<tr>
<td>Dernestere, I.</td>
<td>O-324</td>
</tr>
<tr>
<td>Demirbag, S.</td>
<td>O-105, P-647, P-656</td>
</tr>
<tr>
<td>Demirici, U.</td>
<td>P-320</td>
</tr>
<tr>
<td>Demirtas, S.</td>
<td>P-420, P-421</td>
</tr>
</tbody>
</table>
Le, B., O-133
Le, M., P-165
Leach, R., O-152, O-153
Leader, B., O-237
Leo, R. B. F., P-263
Leath, C. A., P-43
Lebovitz, O., O-98
Lechtenberg, L., O-125, P-16
Lee, B. S., P-453, P-475
Lee, C. S., P-265
Lee, D., P-397, P-430
Lee, D. H., O-398
Lee, D. O., O-1
Lee, E., P-371
Lee, E. H., P-205
Lee, H. S., O-191, P-268, P-545
Lee, H.-J., P-20, P-185, P-404, P-589, P-590
Lee, H.-L., O-372, P-97, P-105, P-110, P-138
Lee, I. H., P-56
Lee, J., O-382, P-50, P-50
Lee, J. A., O-104, O-225, O-338, P-27, P-81, P-95, P-118, P-249, P-261
Lee, J. E., P-601
Lee, J. H., P-188, P-191, P-565
Lee, J. R., P-50, P-371, P-551
Lee, J.-W., P-218
Lee, K., O-52, O-384, P-190
Lee, K. H., P-56, P-188, P-191
Lee, K. S., P-391
Lee, L. S., P-197
Lee, M.-S., P-341
Lee, R. K.-K., P-525
Lee, S. C., P-265
Lee, S. H., P-82
Lee, S. M., O-221, P-416
Lee, S.-K., P-218
Lee, T.-H., P-541
Lee, T.-L., P-138
Lee, W., P-49
Lee, W. D., P-203, P-204, P-205
Lee, W. S., P-601
Lee, Y., P-601
Lee, Y. H., O-140
Lee, Y. J., P-453, P-475
Lee, Y.-J., P-536
Lefebvre, J., O-62
Lefko, S. C., O-178, P-128
Legro, R. S., O-2, P-326
Lei, Z., O-118
Leis, L., P-324
Leonard, P. H., P-343
Leone Roberti Maggiore, U., O-214, O-227
Leor, R., O-137
Leung, M., P-415
Levanduski, M., P-240
Levens, E., O-248, O-352
Levy, H. R., O-264
Levi Setti, P., O-379
Levin, L., O-108
Levy, B., O-281, P-129
Levy, M., O-122, P-478
Levy, M. J., O-248, O-325
Leyland, N. A., O-27
Leyleko, O. A., O-22
Li, F., O-394, P-115
Li, J., P-139, P-479
Li, J. Y., O-234
Li, L.-J., P-139
Li, L.-L., P-586
Li, M., P-106, P-114
Li, P., P-19, P-316, P-372
Li, Q., O-308, P-139
Li, R., O-407, P-517, P-620
Li, R. H. W., O-327
Li, S., P-296
Li, T., P-159, P-570
Li, W., P-570
Li, X., O-118, O-374
Li, X. J., P-492
Li, Y., P-39
Li, Z., O-198, O-309, P-161, P-161, P-316, P-661, P-662
Lian, Y., P-93
Liang, L. Y., P-660
Liang, M., P-161
Liang, X., P-39, P-159, P-570
Liang, X.-Y., P-586, P-615
Liao, C., P-615
Libby, V., P-255, P-374, P-394
Liberman, R. F., O-7
Librach, C., P-526
Librach, C. L., O-17, O-200
Licciardi, F., O-71, P-54, P-122, P-292, P-535
Lico, D., P-14
Lieb, W., P-622
Liebermann, J., P-574, P-575, P-606, P-617
Lieman, H., P-392
Likes III, C. E., P-516
Likes, C., O-194
Lim, B., O-171
Lim, E.-J., P-49
Lim, J., O-122, O-358
Lim, J. H., O-290, P-203, P-204, P-205
Lim, J.-H., P-641
Lim, K. T., P-56
Lim, R. M., P-509
Lin, C.-C., P-412, P-413
Lin, H.-H., P-143, P-377
Lin, J., O-3
Lin, M.-H., P-525
Lin, S., P-33, P-620
Lin, S.-Y., P-525
Lin, Y.-L., P-655
Lin, Y.-M., P-162
Lilian, A., P-216
Lincoln, S. R., O-361
Linde, V., O-401, P-462
Lindheim, S. A., O-433
Lindheim, S. R., P-361, P-385
Lindsey, J., P-320
Linehan, W. M., P-444
Link, M., P-170, P-179
Linn, J., P-78, P-86, P-217, P-563, P-572
Lipari, C., P-456
<table>
<thead>
<tr>
<th>Author</th>
<th>Page(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moore, R.</td>
<td>P-598</td>
</tr>
<tr>
<td>Mor, E.</td>
<td>P-128, P-129, P-200, P-577</td>
</tr>
<tr>
<td>Moran, A.</td>
<td>P-651</td>
</tr>
<tr>
<td>Moravek, M. B.</td>
<td>O-110, P-299</td>
</tr>
<tr>
<td>Morbeck, D. E.</td>
<td>O-246, P-221</td>
</tr>
<tr>
<td>Moreau, C.</td>
<td>P-554</td>
</tr>
<tr>
<td>Moreno, H.</td>
<td>P-651</td>
</tr>
<tr>
<td>Mori, Y.</td>
<td>O-294, P-156</td>
</tr>
<tr>
<td>Morimoto, Y.</td>
<td>O-61, O-207, O-357</td>
</tr>
<tr>
<td>Morin, S.</td>
<td>O-76, P-631</td>
</tr>
<tr>
<td>Morin-Papunen, L.</td>
<td>O-78</td>
</tr>
<tr>
<td>Morita, M.</td>
<td>O-387</td>
</tr>
<tr>
<td>Moriyama, H.</td>
<td>P-502</td>
</tr>
<tr>
<td>Morriss, A.</td>
<td>P-509</td>
</tr>
<tr>
<td>Morse, C. B.</td>
<td>O-142</td>
</tr>
<tr>
<td>Moschini, R. M. A.</td>
<td>O-225</td>
</tr>
<tr>
<td>Moskovtsev, S. I.</td>
<td>O-17, O-200</td>
</tr>
<tr>
<td>Mostafa, M. I.</td>
<td>O-370</td>
</tr>
<tr>
<td>Mostafa, T.</td>
<td>O-285, P-70</td>
</tr>
<tr>
<td>Motan, T.</td>
<td>O-386</td>
</tr>
<tr>
<td>Motato, Y.</td>
<td>P-203</td>
</tr>
<tr>
<td>Motoyama, H.</td>
<td>P-596</td>
</tr>
<tr>
<td>Motta, E. L. A.</td>
<td>O-172</td>
</tr>
<tr>
<td>Mouhyar, Y.</td>
<td>O-235</td>
</tr>
<tr>
<td>Moy, F.</td>
<td>P-34</td>
</tr>
<tr>
<td>Moy, L.</td>
<td>P-430</td>
</tr>
<tr>
<td>Moy, V.</td>
<td>O-403, P-392</td>
</tr>
<tr>
<td>Moyle, E.</td>
<td>P-504</td>
</tr>
<tr>
<td>Mu, Y.</td>
<td>P-338</td>
</tr>
<tr>
<td>Muasher, S. J.</td>
<td>O-47, O-49, P-546, P-555</td>
</tr>
<tr>
<td>Mueller, E.</td>
<td>O-218</td>
</tr>
<tr>
<td>Mueller, M.</td>
<td>O-12</td>
</tr>
<tr>
<td>Mueller, M. D.</td>
<td>O-144</td>
</tr>
<tr>
<td>Muenke, M.</td>
<td>O-274, P-346</td>
</tr>
<tr>
<td>Muhammad, A.</td>
<td>O-36</td>
</tr>
<tr>
<td>Mukaida, T.</td>
<td>O-300</td>
</tr>
<tr>
<td>Mukherjee, T.</td>
<td>P-95, P-118, P-261</td>
</tr>
<tr>
<td>Mullet, T.</td>
<td>O-179</td>
</tr>
<tr>
<td>Mullin, C.</td>
<td>P-255, P-374, P-394</td>
</tr>
<tr>
<td>Mumford, S.</td>
<td>O-14, O-131</td>
</tr>
<tr>
<td>Münch, J.</td>
<td>P-163</td>
</tr>
<tr>
<td>Muneyyirci-Delale, O.</td>
<td>O-38</td>
</tr>
<tr>
<td>Murakami, M.</td>
<td>O-296</td>
</tr>
<tr>
<td>Murakawa, H.</td>
<td>P-593</td>
</tr>
<tr>
<td>Murata, N.</td>
<td>P-344</td>
</tr>
<tr>
<td>Murphy, E.</td>
<td>P-451</td>
</tr>
<tr>
<td>Murphy, E. M.</td>
<td>P-187, P-287, P-494, P-557</td>
</tr>
<tr>
<td>Murphy, K.</td>
<td>P-168, P-177</td>
</tr>
<tr>
<td>Murugappan, G.</td>
<td>O-155, O-277</td>
</tr>
<tr>
<td>Musali, N.</td>
<td>P-181</td>
</tr>
<tr>
<td>Myers, D.</td>
<td>O-75</td>
</tr>
<tr>
<td>Myers, J. B.</td>
<td>P-149</td>
</tr>
<tr>
<td>Nabel, A.</td>
<td>P-635</td>
</tr>
<tr>
<td>Nadal, A.</td>
<td>O-95</td>
</tr>
<tr>
<td>Naft, K. A.</td>
<td>O-660</td>
</tr>
<tr>
<td>Nagai, S.</td>
<td>P-37</td>
</tr>
<tr>
<td>Nagai, Y.</td>
<td>O-345</td>
</tr>
<tr>
<td>Nagao, K.</td>
<td>P-156</td>
</tr>
<tr>
<td>Nagase, Y.</td>
<td>O-388, P-219, P-270</td>
</tr>
<tr>
<td>Nagata, Y.</td>
<td>P-531, P-619</td>
</tr>
<tr>
<td>Nagayoshi, M.</td>
<td>P-183, P-254</td>
</tr>
<tr>
<td>Nagorny, Y.</td>
<td>P-227</td>
</tr>
<tr>
<td>Nagy, P. Z.</td>
<td>P-86</td>
</tr>
<tr>
<td>Nagy, Z. P.</td>
<td>P-78, P-217, P-563, P-572, P-592</td>
</tr>
<tr>
<td>Nail, E. J.</td>
<td>P-55</td>
</tr>
<tr>
<td>Nair, S.</td>
<td>O-260</td>
</tr>
<tr>
<td>Najari, B. B.</td>
<td>O-31</td>
</tr>
<tr>
<td>Nakada, Y.</td>
<td>O-343</td>
</tr>
<tr>
<td>Nakagawa, K.</td>
<td>P-596</td>
</tr>
<tr>
<td>Nakagawa, M.</td>
<td>P-497</td>
</tr>
<tr>
<td>Nakajima, S. T.</td>
<td>O-367, P-376</td>
</tr>
<tr>
<td>Nakajo, Y.</td>
<td>O-294, P-156, P-569</td>
</tr>
<tr>
<td>Nakamura, F.</td>
<td>P-325</td>
</tr>
<tr>
<td>Nakamura, R.</td>
<td>O-82, P-539</td>
</tr>
<tr>
<td>Nakamura, Y.</td>
<td>P-569</td>
</tr>
<tr>
<td>Nakano, T.</td>
<td>O-207</td>
</tr>
<tr>
<td>Nakaoka, Y.</td>
<td>O-61, O-207</td>
</tr>
<tr>
<td>Nakayama, K.</td>
<td>P-182, P-440</td>
</tr>
<tr>
<td>Nakayama, M.</td>
<td>O-312</td>
</tr>
<tr>
<td>Nangia, A. K.</td>
<td>P-165, P-630</td>
</tr>
<tr>
<td>Napoli, A.</td>
<td>P-367</td>
</tr>
<tr>
<td>Nascimento, P. F.</td>
<td>P-263</td>
</tr>
<tr>
<td>Nath, N. M.</td>
<td>O-175</td>
</tr>
<tr>
<td>Nathan, E.</td>
<td>O-186</td>
</tr>
<tr>
<td>Navarrrete, G.</td>
<td>O-52, O-384, P-190</td>
</tr>
<tr>
<td>Navarro, P. A.</td>
<td>O-228</td>
</tr>
<tr>
<td>Navarro, P. A. A. S.</td>
<td>P-425</td>
</tr>
<tr>
<td>Nayak, S. R.</td>
<td>P-305</td>
</tr>
<tr>
<td>Nayar, K. D. E. V.</td>
<td>O-189</td>
</tr>
<tr>
<td>Nazem, T. G.</td>
<td>P-252</td>
</tr>
<tr>
<td>Nazzaro, A.</td>
<td>P-614</td>
</tr>
<tr>
<td>Neal, S. A.</td>
<td>P-112, P-124, P-247</td>
</tr>
<tr>
<td>Neal-Perry, G.</td>
<td>O-128, O-403</td>
</tr>
<tr>
<td>Neal-Perry, G. S.</td>
<td>P-633</td>
</tr>
<tr>
<td>Neff, L. M.</td>
<td>O-257</td>
</tr>
<tr>
<td>Neitzel, D.</td>
<td>P-513</td>
</tr>
<tr>
<td>Nejat, E.</td>
<td>P-253</td>
</tr>
<tr>
<td>Nelson, S.</td>
<td>P-21</td>
</tr>
<tr>
<td>Neri, O.</td>
<td>O-176, O-284, O-289, O-292, O-395, P-89, P-146, P-174, P-521, P-639</td>
</tr>
<tr>
<td>Nesle, E. C.</td>
<td>O-290</td>
</tr>
<tr>
<td>Neubauer, B. R.</td>
<td>P-426</td>
</tr>
<tr>
<td>New, E. P.</td>
<td>P-637</td>
</tr>
<tr>
<td>Nezhat, A.</td>
<td>O-95</td>
</tr>
<tr>
<td>Nezhat, C.</td>
<td>O-95</td>
</tr>
<tr>
<td>Nezhat, F.</td>
<td>O-146</td>
</tr>
<tr>
<td>Ng, E. H. Y.</td>
<td>O-327</td>
</tr>
<tr>
<td>Ng, R.</td>
<td>P-160, P-658</td>
</tr>
<tr>
<td>Ng, S.</td>
<td>P-511</td>
</tr>
<tr>
<td>Nguyen, H.</td>
<td>P-194</td>
</tr>
<tr>
<td>Nguyen, K.-H.</td>
<td>P-401</td>
</tr>
<tr>
<td>Ni, W.</td>
<td>O-196</td>
</tr>
<tr>
<td>Nichols, J.</td>
<td>O-451</td>
</tr>
<tr>
<td>Nicholson, T. M.</td>
<td>O-321</td>
</tr>
<tr>
<td>Nickerson, M.</td>
<td>P-320</td>
</tr>
<tr>
<td>Nicoliciel, M.</td>
<td>O-172</td>
</tr>
<tr>
<td>Nicotra, P.</td>
<td>O-293</td>
</tr>
<tr>
<td>Niederberger, C.</td>
<td>P-148</td>
</tr>
<tr>
<td>Nielsen, H. S.</td>
<td>P-301</td>
</tr>
<tr>
<td>Nieman, L. K.</td>
<td>P-447, P-448</td>
</tr>
<tr>
<td>Niemaskis, E. E.</td>
<td>P-71</td>
</tr>
<tr>
<td>Nisenbaum, M. G.</td>
<td>P-103, P-104</td>
</tr>
<tr>
<td>Nishi, Y.</td>
<td>P-596</td>
</tr>
<tr>
<td>Nishijima, C.</td>
<td>P-66</td>
</tr>
<tr>
<td>Nishimura, K.</td>
<td>P-531, P-619, P-654</td>
</tr>
<tr>
<td>Niu, J.</td>
<td>P-19, P-372</td>
</tr>
<tr>
<td>Nodar, F.</td>
<td>O-293</td>
</tr>
<tr>
<td>Noel, M.</td>
<td>O-80, O-409, P-286</td>
</tr>
<tr>
<td>Negales, M.</td>
<td>P-216</td>
</tr>
<tr>
<td>Noncett, E.</td>
<td>O-100</td>
</tr>
<tr>
<td>Nononora, K.</td>
<td>P-502</td>
</tr>
<tr>
<td>Norberg, C. M.</td>
<td>P-225</td>
</tr>
<tr>
<td>Norian, J. M.</td>
<td>P-275</td>
</tr>
<tr>
<td>Noriega, L.</td>
<td>P-210</td>
</tr>
<tr>
<td>Notrica, D. E.</td>
<td>P-171</td>
</tr>
<tr>
<td>Name</td>
<td>Page Numbers</td>
</tr>
<tr>
<td>---------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Rajagopal, V.</td>
<td>P-242</td>
</tr>
<tr>
<td>Radworth, G.</td>
<td>P-44</td>
</tr>
<tr>
<td>Radin, O.</td>
<td>P-207</td>
</tr>
<tr>
<td>Radwirth, G.</td>
<td>P-44</td>
</tr>
<tr>
<td>Rai, E.</td>
<td>P-654</td>
</tr>
<tr>
<td>Rajagopal, V.</td>
<td>P-242</td>
</tr>
<tr>
<td>Ramasamy, R.</td>
<td>O-16, O-18, O-40, O-136, P-142, P-189, P-648</td>
</tr>
<tr>
<td>Ramirez, P. T.</td>
<td>P-44</td>
</tr>
<tr>
<td>Randolph, J.</td>
<td>P-29, P-337</td>
</tr>
<tr>
<td>Rao, P. K.</td>
<td>O-133</td>
</tr>
<tr>
<td>Rapisarda, J.</td>
<td>P-574, P-575</td>
</tr>
<tr>
<td>Rappolee, D. A.</td>
<td>O-308</td>
</tr>
<tr>
<td>Rataraj, P.</td>
<td>P-313</td>
</tr>
<tr>
<td>Ratts, V. S.</td>
<td>P-32</td>
</tr>
<tr>
<td>Raviv, G.</td>
<td>P-652</td>
</tr>
<tr>
<td>Raviv, S.</td>
<td>O-54</td>
</tr>
<tr>
<td>Ray, L. J.</td>
<td>P-206</td>
</tr>
<tr>
<td>Ray, M.</td>
<td>P-224</td>
</tr>
<tr>
<td>Recht, H.</td>
<td>O-255, P-329</td>
</tr>
<tr>
<td>Reda, C. V.</td>
<td>O-378</td>
</tr>
<tr>
<td>Redding, S. D.</td>
<td>O-118</td>
</tr>
<tr>
<td>Reddy, S.</td>
<td>O-133, P-313</td>
</tr>
<tr>
<td>Reed, S.</td>
<td>O-30</td>
</tr>
<tr>
<td>Reeka, N.</td>
<td>P-512</td>
</tr>
<tr>
<td>Reeves, V. L.</td>
<td>P-362</td>
</tr>
<tr>
<td>Reichman, D.</td>
<td>O-344, O-414, P-74</td>
</tr>
<tr>
<td>Reichman, D. E.</td>
<td>P-187</td>
</tr>
<tr>
<td>Reljo Pera, R.</td>
<td>O-59, O-250</td>
</tr>
<tr>
<td>Reilly, S. D.</td>
<td>O-206</td>
</tr>
<tr>
<td>Reis, A. B.</td>
<td>P-169</td>
</tr>
<tr>
<td>Remis, A. B.</td>
<td>P-509</td>
</tr>
<tr>
<td>Remohi, J. O-70, O-252, O-301</td>
<td></td>
</tr>
<tr>
<td>Remorguida, V. O-227, P-454</td>
<td></td>
</tr>
<tr>
<td>Ren, J.</td>
<td>P-90</td>
</tr>
<tr>
<td>Ren, R.</td>
<td>O-136</td>
</tr>
<tr>
<td>Renzi, A. O-197, O-335, O-350, P-281</td>
<td></td>
</tr>
<tr>
<td>Restekovas, N. P-111</td>
<td></td>
</tr>
<tr>
<td>Revellic, A.</td>
<td>O-356</td>
</tr>
<tr>
<td>Reyes, M.</td>
<td>P-232</td>
</tr>
<tr>
<td>Rhee, J.</td>
<td>O-117</td>
</tr>
<tr>
<td>Rhee, J. H.</td>
<td>P-370</td>
</tr>
<tr>
<td>Rhee, J. S. O-9, O-103, P-18</td>
<td></td>
</tr>
<tr>
<td>Riaz, A.</td>
<td>O-400</td>
</tr>
<tr>
<td>Ribas-Maynou, J.</td>
<td>P-640, P-646</td>
</tr>
<tr>
<td>Ribbeck, K.</td>
<td>P-510</td>
</tr>
<tr>
<td>Ribeiro, D. M. R.</td>
<td>O-145</td>
</tr>
<tr>
<td>Ribustello, L.</td>
<td>O-77, O-275</td>
</tr>
<tr>
<td>Ricard, J. P-274</td>
<td></td>
</tr>
<tr>
<td>Richardson, M.</td>
<td>O-148</td>
</tr>
<tr>
<td>Richter, K. S. O-8, O-122, O-325, P-478, P-524, P-605</td>
<td></td>
</tr>
<tr>
<td>Richter, W. O-321</td>
<td></td>
</tr>
<tr>
<td>Ridgeway, A. D. O-16</td>
<td></td>
</tr>
<tr>
<td>Riel, J. P-176</td>
<td></td>
</tr>
<tr>
<td>Riel, J. M. O-6, P-659</td>
<td></td>
</tr>
<tr>
<td>Rinaudo, P. P-195, P-503</td>
<td></td>
</tr>
<tr>
<td>Rincón-Bertolín, A.</td>
<td>P-466</td>
</tr>
<tr>
<td>Riqueros, M.</td>
<td>O-90</td>
</tr>
<tr>
<td>Roan, N. N. P-163</td>
<td></td>
</tr>
<tr>
<td>Roberge, S.</td>
<td>O-21</td>
</tr>
<tr>
<td>Roberts, C.</td>
<td>O-13</td>
</tr>
<tr>
<td>Roberts, J. O-287</td>
<td></td>
</tr>
<tr>
<td>Roberts, R. M. P-319</td>
<td></td>
</tr>
<tr>
<td>Robinson, S.</td>
<td>P-433</td>
</tr>
<tr>
<td>Robins, J. C. P-35, P-299</td>
<td></td>
</tr>
<tr>
<td>Robinson, B.</td>
<td>O-18</td>
</tr>
<tr>
<td>Robinson, Jr. L. G.</td>
<td>O-307</td>
</tr>
<tr>
<td>Robinson, R. D. P-241</td>
<td></td>
</tr>
<tr>
<td>Rocafort, E. P-441</td>
<td></td>
</tr>
<tr>
<td>Rocha, K. A. P-579</td>
<td></td>
</tr>
<tr>
<td>Rodgers, A.</td>
<td>P-617</td>
</tr>
<tr>
<td>Rodgers, A. K. P-606</td>
<td></td>
</tr>
<tr>
<td>Rodrigo, L. O-70</td>
<td></td>
</tr>
</tbody>
</table>
Wang, L., O-307, P-132
Wang, M., P-161
Wang, N., O-126
Wang, T., P-52
Wang, W., O-151
Wang, X. Q., P-310
Wang, Y., P-296, P-383, P-496
Wang, Z.-L., P-143
Wantman, E., O-148
Ward, K., O-22, O-23, O-26, O-222
Ward, M., P-176
Ward, M. A., O-6, P-175, P-659, P-660
Ward, W. S., P-194, P-196
Warner, L., O-195, O-318
Watanabe, A., O-187
Watanabe, H., P-84
Watanabe, N., P-152
Watanabe, S., P-220
Watanabe, Y., P-654
Watanabe, Z., P-215
Watcharaseranee, N., P-226
Weckstein, L. N., O-178, P-128
Weghofer, A., P-427
Wei, L.-N., P-586
Weinerman, R. S., O-124
Weitzel, P., O-306, P-428
Weitzel, R. P., P-288
Weitzman, G., O-134
Wellons, M., O-258
Wells, D., O-60, O-73, O-280, P-193
Welt, C., P-388
Wemmer, N., P-126
Wen, Z., P-161
Weng, C. C., P-660
Wennerholm, U.-B, O-297
Werner, E. F., P-111
Werner, M. D., O-74, O-92, O-121, O-219, O-378,
O-396, P-98, P-112, P-213, P-247, P-422,
P-438, P-506, P-507, P-518, P-632
Wertenberger, R. A., O-5
Wessels, J. M., O-27
Weston, G., P-242
Westphal, L., P-522
Wetendorf, M., O-374
Whigham, L. D., P-361
Whitcomb, B., O-37, O-352
Whitcomb, B. W., P-8
White, A., P-32
White, M. V., O-156
White, S., P-470
Whitehouse, M., O-382, P-27, P-249
Whitehouse, M. C., P-95, P-118
Whitney, J. B., O-205, O-279
Wicklund, C., P-141
Widra, E., O-248
Wiemer, K., O-275
Wieneke, C., P-6
Wilcox, J., O-177
Wild, R. A., P-499
Wiley, A. S., P-644
Wilken, N., P-142
Wilkerson, J., P-59
Willard, B., P-144, P-178
Williams III, J., O-413, P-439
Williams, C., P-443
Williams, D. H., O-321
Williams, J., P-366
Williams, L., O-318

e374

Author Index

Williams, M., O-152, O-153
Williams, P. L., O-20, P-530
Williams, S. E., P-516
Willis, R., P-362
Willman, S. P., O-178, P-128
Wing, R., P-650
Winikoff, B., P-522
Winkel, C., P-442, P-460
Winkelman, W., O-75
Winkle, T., P-237, P-512
Winston, N. J., O-269, O-383
Winter, A., P-397
Winter, A. G., O-316
Wirleitner, B., P-540
Wirth, T., P-163
Wise, L. A., O-259, P-644
Witkin, G., O-338, P-81
Witkin, S., O-174, O-375
Witkin, S. S., P-300
Witmer, J., O-50
Wolf, L. J., P-613
Wolff, A. B., P-367
Wolff, E., P-428
Wolff, E. F., O-303, O-306, P-288, P-367
Wonchockier, R., P-324
Wong, C., O-400
Wong, T. H., P-579
Woodard, S., O-95
Woodard, T., O-99
Woodruff, T. K., P-55, P-57, P-141, P-583
Word, R. A., O-231, P-297
Worrilow, K. C., O-263
Wosnitzer, M., P-397
Wosnitzer, M. S., P-147
Woyton, R., P-437
Wright, D. L., O-20, O-117, P-530
Wu, C. Q., O-226
Wu, E. Q., P-442, P-460
Wu, G., O-118
Wu, H. L., P-234, P-238
Wu, J., P-67, P-490
Wu, M., O-14
Wu, Y.-G., P-20, P-185, P-404, P-589, P-590
Wu, Y.-Y. W., P-356
Wun, W.-S., O-359
Wun, W.-S. A., P-550
Xia, P., P-579
Xia, X., O-97, P-52
Xiao, K., P-151
Xiao, Z., P-296
Xie, Y., P-106, P-114
Xing, L.-F., O-45
Xiong, L., O-341
Xu, G., P-479
Xu, G.-F., O-67
Xu, J., O-268, P-592
Xu, K., P-119, P-137
Xu, N., O-413
Xu, P., P-161
Xu, W., P-139, P-338
Xu, X., P-107, P-131, P-199
Xu, Y., P-39
Xuan Hoi, N., P-538
Xue, L., P-579
Xue, S., P-267
Yabuuchi, A., P-344
Yaegashi, N., P-215
Yaklic, J. L., P-361

Yalcinkaya, E., O-329
Yalcinkaya, T. M., P-353
Yallampalli, C., O-374
Yamada, K., P-593
Yamada, Y., O-199, O-282
Yamagata, K., O-61
Yamaguchi, A., O-44
Yamaguchi, T., P-37
Yamamoto, Y., O-388, P-219
Yamanaka, N., P-220
Yamauchi, Y., O-6, P-175, P-176, P-659, P-660
Yan, G., P-405
Yan, H., P-153
Yan, J., O-97, P-52
Yan, L., O-97, P-52
Yan, X., P-387
Yan, Z., P-267
Yang, H., O-369, P-116, P-442, P-460
Yang, J., P-151
Yang, K. M., P-56, P-283
Yang, M., O-66, P-253, P-413
Yang, R., P-383
Yang, S., O-309, P-316, P-661
Yang, S.-H., P-641
Yang, X., P-159
Yang, Y., O-89, O-308
Yang, Y.-S., O-410, O-411, P-356
Yang, Z., P-113
Yango, P. L., O-319
Yankee, T., P-429
Yano, J. C., P-308, P-309
Yao, S., O-35, O-347, P-1, P-4
Yao, Y., P-132
Yarci, A., P-591
Yasmin, S., P-634
Yata, M., O-162
Yates, M. M., P-67
Yauger, B. J., O-397, P-275
Yay, A., P-17
Yazdi Lahav, V., P-207
Ye, H., P-234, P-238
Ye, Y., P-173
Ye, Z., P-225, P-621
Yeargin-Allsopp, M., O-46
Yearian, C., P-1
Yeboah, E., P-200
Yee, B., O-193
Yeh, J. S., O-47, O-49, P-546, P-555
Yelke, H. K., P-206
Yen, M.-F. A., P-356
Yesildaglar, N., O-105
Yi, H. J., P-283
Yildirim, G., O-25
Yilmaz, B., P-591
Yilmaz, N., O-130, P-373
Yin, P., O-110
Yin, T., O-97, P-52
Ying, Y., P-543
Yinon, Y., O-238
Yokota, H., P-497
Yokota, M., P-497
Yokota, Y., P-497
Yonaha, H., P-220
Yondorf, C., O-128
Yonezawa, J., O-388
Yoo, J.-H., P-327, P-567
Yoo, J.-Y., O-28, P-484
Yoo, Y. J., P-246

Vol. 102, No. 3, Supplement, September 2014


AUTHOR AND SPOUSE/PARTNER DISCLOSURES INDEX

All speakers at the 2014 ASRM Annual Meeting and Postgraduate Courses were required to complete a disclosure form. These disclosures were reviewed and potential conflicts of interest resolved by the Subcommittee on Standards of Commercial Support of the Continuing Medical Education Committee. Each abstract or video author is listed below along with any relationships their partners/spouses disclosed.

Abuzeid, M. I.  Actavis Pharmaceuticals, Speakers bureau

Adamson, G. D.  Advanced Reproductive Care, Inc., Company officer; Auxogyn, Research/Principal Investigator; Bayer Pharmaceuticals, Paid consultant; Ziva Medical, Paid consultant; LabCorp, Research/Principal Investigator; Glycotope, Paid consultant

Albertsen, H. M.  Juneau Biosciences, LLC, Full-time company employee; Juneau Biosciences, LLC, Company officer

Alikani, M.  Fertilitech, Honoraria; Reprogenetics, LLC, Direct stockholder; IVF Online, Paid consultant

Alper, M.  EMD Serono, Honoraria; Good Start Genetics, Paid consultant; Reprosource, Direct stockholder; Ferring, Honoraria

Antoni, D.  Channel Medical, Paid consultant

Apter, D.  Bayer HealthCare, Received financial support to undertake research studies and to speak at educational meetings and conferences; Merck/MSD, Received financial support to undertake research studies and to speak at educational meetings and conferences; GSK, Received financial support to undertake research studies and to speak at educational meetings and conferences

Arav, A.  FertilSafe, Company officer

Arce, J.-C.  Ferring Pharmaceuticals, Full-time company employee

Asgari, S.  Recombine, Full-time company employee

Baker, V. L.  Good Start Genetics, Advisory Board; Roche, Grant recipient; NIH, Grant recipient; TEVA, Advisory Board; Ovuline, Advisory Board

Bannister, W. M.  UnitedHealth Group, Full-time company employee

Barad, D. H.  Fertility Nutraceuticals, LLC, receive patent royalties

Barmat, L.  Intuitive Surgical, Inc., Surgical proctor; Abbvie Pharmaceuticals, Lecturer

Barnhart, K. T.  Swiss Percision Diagnostics, Paid consultant; Bayer, Paid consultant; NMS labs, Paid consultant

Barrett, C. B.  Reprosource, Paid consultant

Behr, B.  Cooper Surgical, Paid consultant; Auxogyn, Direct stockholder; Iviron, Direct stockholder

Bennett, S.  Johnson & Johnson, Full-time company employee; Merck, Full-time company employee

Belongs, S.  Merck, Speakers bureau; Ferring, Speakers bureau; EMD Serono, Speakers bureau; Ferring, EMD Serono, Merck, Teva, Watson, Grant recipient

Bergh, C. M.  Omnia Education, Honoraria; Med Software, LLC, Company officer

Bergman, K.  Growing Generations, LLC, Company officer; HIV Assisted Reproductive Technologies, Company officer

Bianchi, P. H. M.  Recombine, Company officer; Recombine, Direct stockholder; Recombine, Full-time company employee

Bisignano, A.  Merck S.A., Honoraria

Blackburn, E.  Telome Health, Inc, founder of company to measure telomere length

Blesa, D.  IVIOMICS, Full-time company employee

Bocca, S. M.  Merck-Organon, Speakers bureau

Boelkelheide, K.  Global Alliance for TB Drug Development, Paid consultant; Zafgen, Paid consultant; Akros, Paid consultant

Boostanfar, R.  Actavis, Grant recipient

Borgatta, L.  Bayer HealthCare, Grant recipient
Borroto, C. GenePeeks, Inc., Full-time company employee

Borseth, J. A. Pfizer Inc., Direct stockholder; Covidien PLC, Direct stockholder

Brannian, J. Sanford Health, Full-time company employee; SCSA Diagnostics, Paid consultant

Brant, W. O. American Medical Systems, Paid consultant; Coloplast, Paid consultant; Auxilium, Speakers bureau; American Medical Systems, grant recipient

Brennan, J. Good Start Genetics, Inc., Full-time company employee

Bristow, S. L. Recombine, Full-time company employee

Bromer, J. G. OvaScience, Member of Product Advisory Board

Broome, J. INVOBioscience, Direct stockholder

Buhling, K. Bayer HealthCare, Speakers bureau; MSD, Speakers bureau; Jenapharm, Speakers bureau; Dr Kade, Speakers bureau

Burger, C. W. Roche Switzerland, Paid consultant

Buster, J. E. Previo Genetics, LLC, Company officer

Camargo, F. Ingenes, Company officer

Cameron, K. A. MERCi (Medical Error Reduction and Certification, Inc.), Co-investigator on contracted research

Carlomagno, G. LoLLI pharma, Full-time company employee

Carr, B. R. Pfizer, Speakers bureau; Noven, Speakers bureau

Carrascosa, P. General Electric Company, Paid consultant

Carson, S. Previo Genetics, LLC, Direct stockholder

Carter, D. Nora Therapeutics, Inc., Full-time company employee

Castello, D. KITAZATO-DIBIMED, Product Manager

Castrillon, D. H. Molecular MD, Paid consultant

Caswell, W. A. Irvine Scientific, Paid consultant

Cataldo, N. A. Good Start Genetics, Paid consultant

Catherino, W. H. EMD Serono, Full-time company employee

Cedars, M. I. Nora Therapeutics, Grant recipient; Ferring Pharmaceuticals, Grant recipient

Cesario, M. Previo Genetics, LLC, Company officer

Chakraborty, A. Ferring Pharmaceuticals, Full-time company employee

Chang, C.-C. My Egg Bank, Direct stockholder

Chang, R. Ansh Labs, Research collaboration

Chen, S. H. Hologic, Speakers bureau; Merck, Speakers bureau

Chettier, R. Juneau Biosciences, LLC, Full-time company employee; Juneau Biosciences, LLC, Direct stockholder

Chokkeri-Singh, A. Ethicon Endo Surgery, Advisory Board Member, Speakers bureau; Bayer, Advisory Board Member, Speakers bureau
Driggers, P. H. Eisai Inc., Speakers bureau; Axio Research, Data Safety Monitoring Committee, Orexigen CVOT
Du, E. X. AbbVie, I am an employee of Analysis Group, which has received support from AbbVie for this project
Duleba, A. Channel Medical, Paid consultant
Dumesic, D. A. Ferring Pharmaceuticals Inc, Paid consultant
Duncan, F. E. Ferring Pharmaceuticals, Contracted Research (9/1/2013-8/31/2014; $14,883.48 salary)
Dupree, J. M. P&G, Direct stockholder
Durrett, R. Recombine, Full-time company employee
Earl, T. Donor Egg Bank USA, Full-time company employee
Eisenberg, M. I. Sandstone Diagnostics, Advisor
Eisenberg, M. L. Sandstone Diagnostics, Direct stockholder
Elashoff, M. Celmatix, Inc., Full-time company employee
Epel, E. Telome Health, Inc, founder of company to measure telomere length
Elashoff, M. Celmatix, Inc., Full-time company employee
Erguder, B. I. Ankara University Faculty of Medicine, Full-time company employee
Everson, D. P. SCSA Diagnostics, Company officer
Farrington, P. Juneau Biosciences, LLC, Company officer
Farrington, P. Juneau Biosciences, LLC, Direct stockholder
Farrington, P. Juneau Biosciences, LLC, Company officer
Farrington, P. Juneau Biosciences, LLC, Direct stockholder
Farrington, P. Juneau Biosciences, LLC, Company officer
Farrington, P. Juneau Biosciences, LLC, Direct stockholder
Faulkner, M. Abbvie, Paid consultant; Ferring Pharmaceuticals, Paid consultant
Feinberg, E. C. Abbvie, Paid consultant; Ferring Pharmaceuticals, Paid consultant
Forstein, D. Dusheney, USA, Speakers bureau
Frederick, J. Actavis, Speakers bureau
Frumovitz, M. Novadaq, Paid consultant
Fuldeore, M. Abbvie, Full-time company employee
Gardner, D. K. Vitrolife AB, Grant recipient
Gargiulo, A. Omniguide Inc, Paid consultant
Geltinger, M. E. Genetics & IVF Institute, Full-time company employee
Gemzell-Danielsson, K. Bayer HealthCare, Honoraria; Merck/MSD, Honoraria; Gedeon Richter, Honoraria; Exelgy, Honoraria; HRA-Pharma, Honoraria
Giménez, C. Reprogenetics Spain, Company officer
Ginsburg, E. S. Up To Date, Honoraria; Springer Inc, Honoraria; Nora Therapeutics Inc, Industry study- money to my department for participation
Giritharan, G. Nevada Center for Reproductive Medicine, Full-time company employee
Giudice, L. C. Pfizer, Direct stockholder; Merck, Direct stockholder; Pfizer, Direct stockholder; Merck, Direct stockholder
Givens, C. Merck, Paid consultant
Gleicher, N. Fertility Nutraceuticals, LLC, own shares; receive patent royalties
Goering, M. C. MedTech for Laboratory Solutions, Paid consultant
Goldstein, E. Allergan Inc., Full-time company employee
Goldstein, M. Therologix, Medical Advisory Board
Gole, J. Good Start Genetics, Direct stockholder; Good Start Genetics, Full-time company employee
Gómez, E. IVIOMICS, Full-time company employee
Gore, K. Merck & Co., Inc., Full-time company employee
Gore, K. Good Start Genetics, Direct stockholder; Good Start Genetics, Full-time company employee
Gorelick, A. CELMATIX, Inc., Full-time company employee
Gorelick, A. Ferring, Grant recipient; Roche, Grant recipient
Gore, T. Good Start Genetics, Inc., Full-time company employee
Gömez, E. Lilly, Actavis, Abbott, Paladin, Honoraria; Lilly, Abbott, Paladin, Speakers bureau
Grossman, M. Bayer Pharma AG, Full-time company employee
Hallam, J. Western IVF, Direct stockholder; Medical Director Western IVF, Full-time company employee
Hansen, K. R. Good Start Genetics, Inc., Full-time company employee
Hanssen, K. R. Ferring, Investigator for a Ferring sponsored study; Roche, Investigator for a Roche sponsored study
Hammond, K. R. Menicon Co., Ltd., Grant recipient
Hara, T. Bayer, ZEG is conducting PASS studies requested by EMA and/or FDA which are supported by unconditional grants from Bayer; Merck, ZEG is conducting PASS studies requested by EMA and/or FDA which are supported by unconditional grants from Merck.
Hart, R. Bayer, ZEG is conducting PASS studies requested by EMA and/or FDA which are supported by unconditional grants from Bayer; Merck, ZEG is conducting PASS studies requested by EMA and/or FDA which are supported by unconditional grants from Merck.
holder of early stage start up company
SpermDx, Company officer; Andro360, Company officer
Le, B.
Huang, A. Ferring, Speakers bureau
Legro, R. S. Fertility, Honoraria
Huddleston, H. Merck Serono, sponsorship to national meetings
McDonald, J. A. Astra Zeneca, Paid consultant; Euroscreen, Paid consultant; Ferring, Paid consultant
Hunter, T. J. Merck Serono, Honoraria; MSD, Honoraria; Ferring, Paid consultant; Mochida Pharmaceuticals, Honoraria
FertileSafe, medical advisor
Ishihara, O. Merck Serono, Honoraria; MSD, Honoraria; Ferring, Paid consultant; Mochida Pharmaceuticals, Honoraria
Affymetrix Inc., Paid consultant; Natera Inc., Honoraria
Isley, L. California Cryobank, Full-time company employee
Shady Grove Fertility Center, Company officer; Donor Egg Bank, USA, Direct stockholder
Iwaszko, M. A. Genetics & IVF Institute, Full-time company employee
Merck, Speakers bureau
Jain, J. K. Santa Monica Fertility, I own Santa Monica Fertility, my Fertility Practice and lab that provides oocyte cryopreservation services
Origio/Sage, Paid consultant; Irvine Scientific, Speakers bureau
Jasulaitis, S. Merck, Speakers bureau
GenePeeks, Inc., Full-time company employee
Kadoch, I.-J. YAD-TECH, Direct stockholder; FERRING Pharmaceuticals, Paid consultant
Teleome Health, Inc, Founder of company to measure telomere
Karvir, H. V. Celmatix, Inc., Full-time company employee
Bayer, Full-time company employee
Kaser, D. J. Healthcare consultant for McKinsey & Company, Full-time company employee
Illumina/BlueGnome, Full-time company employee
Keller, L. M. Essen Bioscience, Full-time company employee
Natera, Full-time company employee
Kellogg, G. Recombine, Full-time company employee
Beckman Coulter, Full-time company employee
Kiehl, M. Natera, Inc, Full-time company employee
Glowing, Company officer
Kimble, T. D. Merck Pharmaceuticals, Speakers bureau; Bayer Healthcare, Grant recipient
OvaScience, Paid consultant
Klein, B. M. Ferring Pharmaceuticals, Full-time company employee
Up To Date, author (royalties); Merck U.S.A., Speakers bureau
Kokocinski, F. Illumina Inc, Full-time company employee
Virotile, Paid consultant
Kolibianakis, E. M. MSD, Merck Serono, Ferring, Glycotope, Honoraria
Natera, Inc., Full-time company employee
Krisher, R. L. Serono GFI grant recipient, Grant recipient
FertileSafe, medical advisor
Kumar, A. Anshlabs, Full-time company employee
Bayer Pharma, ZEG is conducting PASS studies requested by EMA and/or FDA which are supported by unconditional grants from Bayer.; Merck, ZEG is conducting PASS studies requested by EMA and/or FDA which are supported by unconditional grants from Merck.
Kumar, N. Recombine, Full-time company employee
Kokocinski, F. Illumina Inc, Full-time company employee
Kolibianakis, E. M. MSD, Merck Serono, Ferring, Glycotope, Honoraria
Kolibianakis, E. M. MSD, Honoraria; Merck Serono, Honoraria; Ferring, Honoraria; American Medical Systems, Grant recipient
LaMarca, B. Abbott Laboratories, ABT-627 used in this study is a gift from Abbott Laboratories
Bayer Pharma, ZEG is conducting PASS studies requested by EMA and/or FDA which are supported by unconditional grants from Bayer.; Merck, ZEG is conducting PASS studies requested by EMA and/or FDA which are supported by unconditional grants from Merck.
LaMarca, B. Beckman Coulter Inc, Grant recipient
Lambert-Messerlian, G. MSD, Honoraria; Merck Serono, Honoraria; Ferring, Honoraria; MSD, Honoraria; Merck Serono, Honoraria; Ferring, Honoraria
Lan, V. T. N. MSD, Honoraria; Merck Serono, Honoraria; Ferring, Honoraria; American Medical Systems, Grant recipient
Moehner, S. Bayer Pharma, ZEG is conducting PASS studies requested by EMA and/or FDA which are supported by unconditional grants from Bayer.; Merck, ZEG is conducting PASS studies requested by EMA and/or FDA which are supported by unconditional grants from Merck.
Moley, K. H. | OvaScience, Scientific Advisory Board company employee | Speakers bureau; Antares, Paid consultant
Montegriffo, E. | Bayer HealthCare, Full-time employee | Merck, Paid consultant
Morbeck, D. E. | Fertilitech, Scientific advisory board; Fertilitech, Research instrument loan | Celmatix, Inc., Full-time company employee
Morriss, A. | GenePeeks, Inc., Company officer | FertilSafe, medical advisor
Motan, T. | Ferring, Grant recipient; Merck, Speakers bureau; Serono, Journal Club Sponsorship | Ferring Pharmaceuticals, Speakers bureau; Cooper Surgical, Paid consultant
Mueller, E. R. | Astellas, Paid consultant; Astellas/Allergan, Principal Investigator | Biomedical Supply, S.L. (DIBIMED), IVI stockholder; UNISENSE FERTILITECH A/S, IVI stockholder; IVIOMICS S.L., IVI stockholder
Munne, S. | Reprogenetics, Company officer; Recombine LCC, Company officer | OvaScience, Clinical Advisory Board; Nora Therapeutics, Clinical Advisory Board; ReproSource, Board of Directors; Ferring Pharmaceuticals, UIT Scientific Committee Member
Munne, S. | Reprogenetics, Company officer; Recombine, Company officer; Reprogenetics, Direct stockholder; Reprogenetics, Full-time company employee | Natera, Inc., Full-time company employee; Natera, Inc., Direct stockholder
Nadal, A. | Previvo Genetics, LLC, Full-time company employee | Synergyne Imaging Technology, Inc, Company officer; Synergyne ART Analytics, Inc, Company officer; Ferring Pharmaceuticals AS, Paid consultant; GlaxoSmithKline, Paid consultant; ADE Therapeutics, Inc, Scientific Advisor
Nagy, Z. P. | My Egg Bank, Direct stockholder; Origio, Paid consultant; Fertilitech, Paid consultant; MERCK MSD, Speakers bureau | ASRM, Grant recipient; NICHD, Grant recipient
Neff, L. | GI Dynamics, Grant recipient | Good Start Genetics, Company officer; Good Start Genetics, Direct stockholder; Good Start Genetics, Full-time company employee
Neitzel, D. | Good Start Genetics, Inc., Full-time company employee | ABBVIE, Grant recipient; Bayer, Grant recipient; AIUM, Board of Directors
Nelson, S. | Beckman Coulter, Honoraria; Ferring Pharmaceuticals, Honoraria; MSD, Honoraria; Merck Serono, Honoraria; Roche Diagnostics, Honoraria; SSIF, Honoraria | LifeGlobal Group, Paid consultant; Auxogyn, Paid consultant
Niederberger, C. | NexHand, Company officer; American Society for Reproductive Medicine, Journal Co-Editor in Chief; American Urological Association, Journal Section Editor; UCLA, Speakers bureau | AstroZenea, Clinical trial of investigational drug; Merck, Paid consultant; Medtronic, Grant recipient; Stryker, Royalties; Paradigm Spine, Direct stockholder; Pioneer/Bonovo, Direct stockholder; Nocimed/ouroBoros, Direct stockholder
Nieman, L. K. | HRA Pharma, grant to NIH for research; PregLem, Royalties | California Cryobank, Full-time company employee
Oehninger, S. C. | Ferring, Paid consultant | Bayer Pharma, ZEG is conducting PASS studies requested by EMA and/or FDA which are supported by unconditional grants from Bayer.; Merck, ZEG is conducting PASS studies requested by EMA and/or FDA which are supported by unconditional grants from Merck.
Ohl, D. A. | Coloplast Corporation, Paid consultant; American Medical Systems, Paid consultant; Pfizer, Paid consultant | Auxiliary Inc., Co-Founder
Oktay, K. | OvaScience, Medical Advisory Board Member | GenePeeks, Inc., Full-time company employee
Omturtag, K. R. | RegularRateRhythmSoftware, Paid consultant | IVOIOMICS, Full-time company employee
Orkunoglu-Suer, F. | Quest Diagnostics, Full-time company employee |
<table>
<thead>
<tr>
<th>Name</th>
<th>Affiliation</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rizk, B.</td>
<td>Hologic, Inc, Grant recipient; Hologic, Inc, Speakers bureau</td>
<td>Shah, M.</td>
</tr>
<tr>
<td>Robins, J. C.</td>
<td>Ovascience, Paid consultant; Vivere Health, Paid consultant</td>
<td>Gilead, Direct stockholder; Johnson and Johnson, Direct stockholder; Ironwood, Direct stockholder</td>
</tr>
<tr>
<td>Robinson, R. D.</td>
<td>Merck, Honoraria; AbbVie, Grant recipient</td>
<td>Keryx, Direct stockholder; Merck, Grant recipient; Watson, Grant recipient; Glycostep, Paid consultant; Merck, Paid consultant</td>
</tr>
<tr>
<td>Rodgers, A. K.</td>
<td>Merck, Speakers bureau; Warner Chilcott, Speakers bureau; Vitamed, Speakers bureau; Ferring, Speakers bureau; AbbVie, Full-time company employee</td>
<td>Ferring, Grant recipient; Ferring, Speakers bureau</td>
</tr>
<tr>
<td>Rodrigues, A.</td>
<td>Lipid Genomics, Inventorship rights to molecular diagnostics of SCARB1 variants</td>
<td>Endo Pharmaceuticals, Speakers bureau; Auxilium, Speakers bureau</td>
</tr>
<tr>
<td>Rodriguez-Oquendo, A.</td>
<td>Lipid Genomics, Inventorship rights to molecular diagnostics of SCARB1 variants</td>
<td>Natera, Full-time company employee; GenePeeks, Inc., Full-time company employee</td>
</tr>
<tr>
<td>Rombauts, L.</td>
<td>MONASH IVF, Direct stockholder; Merck Serono, MSD, Grant recipient</td>
<td>IVIOMICS, Direct stockholder; IVIOMICS, Co-author of the ERA patent; IVIOMICS, Board membership; Equipo IVI investigacion, Direct stockholder</td>
</tr>
<tr>
<td>Rosen, K.</td>
<td>Bayer HealthCare, Full-time company employee</td>
<td>Simón, C.</td>
</tr>
<tr>
<td>Rosenberg, L.</td>
<td>McNeil Pharmaceuticals, Paid consultant; Boehringer Ingelheim International, Paid consultant</td>
<td>Bayer Healthcare AG, Grant recipient; Episona, Direct stockholder; AbbVie Inc., Full-time company employee</td>
</tr>
<tr>
<td>Roth, K.</td>
<td>Bayer Pharma AG, Full-time company employee</td>
<td>Multitector A/S, Frederiksberg, Denmark, Direct stockholder; Astellas, Paid consultant; Berlin Chemie, Paid consultant; Eli Lilly, Paid consultant</td>
</tr>
<tr>
<td>Rothman, K. J.</td>
<td>Quintiles, Full-time company employee</td>
<td>Merck, Paid consultant; Agile Therapeutics, Paid consultant; Merck &amp; Co., Company officer; Glaxo Smith Klein, Full-time company employee</td>
</tr>
<tr>
<td>Ruiz Alonso, M.</td>
<td>IVIOMICS, Full-time company employee</td>
<td>Allergan, Clinical Trials Agreement; Irvine Scientific, Honoraria; 3D Biomatrix, Have stock options; PHASIQ, Direct stockholder</td>
</tr>
<tr>
<td>Rybowski, S.</td>
<td>Bayer HealthCare, Full-time company employee</td>
<td>Beckman-Coulter, Medical consultant; WINFertility, Medical advisory board</td>
</tr>
<tr>
<td>Sakkas, D.</td>
<td>Ferring, Paid consultant; Ferring, Grant recipient; Merck, Speakers bureau; Unisense, Scientific Advisory Board; Origo, Scientific Advisory Board</td>
<td>Beckman-Coulter, Medical consultant; WINFertility, Medical advisory board</td>
</tr>
<tr>
<td>Sakkas, D.</td>
<td>Ferring, Paid consultant; Ferring, Grant recipient; Merck, Speakers bureau; Unisense, Scientific Advisory Board; Origo, Scientific Advisory Board</td>
<td>Beckman-Coulter, Medical consultant; WINFertility, Medical advisory board</td>
</tr>
<tr>
<td>Sandalinas, M.</td>
<td>Reprogenetics Spain, Company officer</td>
<td>Beckman-Coulter, Medical consultant; WINFertility, Medical advisory board</td>
</tr>
<tr>
<td>Santoro, N.</td>
<td>Bayer, Inc, Grant recipient; Menogenix, Stock options</td>
<td>Beckman-Coulter, Medical consultant; WINFertility, Medical advisory board</td>
</tr>
<tr>
<td>Sarver, D. B.</td>
<td>BAROnova, Paid consultant; Enteromedics, Paid consultant</td>
<td>Beckman-Coulter, Medical consultant; WINFertility, Medical advisory board</td>
</tr>
<tr>
<td>Sasaki, K. J.</td>
<td>Ethicon Endo-Surgery, Presenter at Resident Course</td>
<td>Beckman-Coulter, Medical consultant; WINFertility, Medical advisory board</td>
</tr>
<tr>
<td>Sato, K.</td>
<td>Menicon Co., Ltd., Grant recipient</td>
<td>Beckman-Coulter, Medical consultant; WINFertility, Medical advisory board</td>
</tr>
<tr>
<td>Saucier, J.</td>
<td>Natera, Inc, Full-time company employee</td>
<td>Beckman-Coulter, Medical consultant; WINFertility, Medical advisory board</td>
</tr>
<tr>
<td>Schattman, G. L.</td>
<td>Abbvie, Speakers bureau; Theralogix, medical advisory board; Femasys, medical advisory board; Ferring, Speakers bureau</td>
<td>Beckman-Coulter, Medical consultant; WINFertility, Medical advisory board</td>
</tr>
<tr>
<td>Schlegel, P. N.</td>
<td>Ferring Pharmaceuticals, Paid consultant; GNYUTES, Inc, Company officer; Theralogix, Inc, Direct stockholder</td>
<td>Beckman-Coulter, Medical consultant; WINFertility, Medical advisory board</td>
</tr>
<tr>
<td>Schust, D. J.</td>
<td>UNIFY, non-paid consultant</td>
<td>Beckman-Coulter, Medical consultant; WINFertility, Medical advisory board</td>
</tr>
<tr>
<td>Sciandra, S.</td>
<td>Walgreens, Honoraria</td>
<td>Beckman-Coulter, Medical consultant; WINFertility, Medical advisory board</td>
</tr>
<tr>
<td>Scott, Jr., R. T.</td>
<td>Ferring Pharmaceutical, Grant recipient</td>
<td>Beckman-Coulter, Medical consultant; WINFertility, Medical advisory board</td>
</tr>
<tr>
<td>Seifer, D. B.</td>
<td>Rutgers Medical School/ MGH with Beckman Coulter, Receive royalties from licensing agreement between Rutgers Medical School/ MGH and Beckman Coulter for using AMH to determine ovarian reserve; Ferring, Scientific consultant; Univfy, Medical advisory board;</td>
<td>Pfizer - grant to Yale Univ., Grant recipient; OvaScience- grant to Yale Univ., Grant recipient; Abbvie, Pfizer, Ovascience, Merk, Paid consultant</td>
</tr>
<tr>
<td>Name</td>
<td>Disclosure Details</td>
<td>Company/Position</td>
</tr>
<tr>
<td>---------------------</td>
<td>------------------------------------------------------------------------------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>Taylor, J. J.</td>
<td>City Fertility Centre, Full-time company employee</td>
<td>Biosciences, LLC, Full-time company employee;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Affiliated Genetics, Inc., Direct stockholder;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Juneau Biosciences, LLC, Direct stockholder;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Juneau Biosciences, LLC, Company officer</td>
</tr>
<tr>
<td>Taylor, R. N.</td>
<td>Abbvie, Paid consultant; Takeda, Paid consultant</td>
<td>Reprogenetics, Direct stockholder;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Merck Serono, Grant recipient; Life Technologies,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Honoraria;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BlueGnome, Paid consultant</td>
</tr>
<tr>
<td>Taylor, T. H.</td>
<td>Biodiseno, Part time contract employee</td>
<td>Up to Date, Writer; Astra Zeneca, Paid consultant;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Endocrine Society, Committee Member, Editor</td>
</tr>
<tr>
<td>Thomas, M. A.</td>
<td>Smith and Nephew, Speakers bureau; Merck, Grant recipient</td>
<td>Natera, Full-time company employee; Natera, Direct</td>
</tr>
<tr>
<td></td>
<td></td>
<td>stockholder</td>
</tr>
<tr>
<td>Thouas, G. A.</td>
<td>Vitrolife AB, Employed on commercial grant</td>
<td>Counsyl, Paid consultant</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Atherotec, Paid consultant; ORWH NIH, Advisor; FDA,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Honoraria; Abbvie, Paid consultant; Merck, Direct</td>
</tr>
<tr>
<td></td>
<td></td>
<td>stockholder; Previvo Genetics, Paid consultant;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Previvo Genetics, Direct stockholder</td>
</tr>
<tr>
<td>Toledo, A. A.</td>
<td>My Egg Bank, Direct stockholder</td>
<td>Watson Pharmaceuticals, Speakers bureau</td>
</tr>
<tr>
<td>Tong, J. K.</td>
<td>Nora Therapeutics, Company officer</td>
<td>Watson Pharmaceuticals, Speakers bureau</td>
</tr>
<tr>
<td>Tulandi, T.</td>
<td>Actavic Inc., Advisor</td>
<td>Watson Pharmaceuticals, Speakers bureau</td>
</tr>
<tr>
<td>Tuong, H. M.</td>
<td>Ferring, Honoraria; MerckSerono, Honoraria; Ferring, Honoraria; MerckSerono, Honoraria; Ferring, Honoraria; MSD, Honoraria; MSD, Honoraria</td>
<td>Watson Pharmaceuticals, Speakers bureau</td>
</tr>
<tr>
<td>Tuppurainen, M.</td>
<td>MSD, Finland and Angen, Finland, Member of Scientific Board; Roche, Paid consultant; AptivSolutions, Paid consultant; Bayer, Paid consultant</td>
<td>MSD, Finland and Angen, Finland, Member of Scientific Board; Roche, Paid consultant; AptivSolutions, Paid consultant; Bayer, Paid consultant</td>
</tr>
<tr>
<td>Udoff, L. C.</td>
<td>Watson Pharmaceuticals, Speakers bureau</td>
<td>Woodard, S.</td>
</tr>
<tr>
<td>Uhler, M.</td>
<td>Merck, Speakers bureau</td>
<td>Merck, Speakers bureau</td>
</tr>
<tr>
<td>Ullhoa-Aguire, A.</td>
<td>Astrazeneca, Mexico, Speakers bureau</td>
<td>LifeAire Systems, LLC, Company officer</td>
</tr>
<tr>
<td>Umbarger, M. A.</td>
<td>Good Start Genetics, Direct stockholder; Good Start Genetics, Full-time company employee</td>
<td>AbbVie, I am an employee of Analysis Group, which has received support from AbbVie for this project</td>
</tr>
<tr>
<td>Unutmaz, D.</td>
<td>Affymetrix, Honoraria; Karyopharm, Paid consultant; AOBIOME, Direct stockholder</td>
<td>Wultrilow, K. C.</td>
</tr>
<tr>
<td>Venetis, C. A.</td>
<td>Merck, Sharp &amp; Dome, Honoraria; IPSEN Hellas E.P.E., Paid consultant; Merck, Sharp &amp; Dome, Travel grant</td>
<td>AbbVie, I am an employee of Analysis Group, which has received support from AbbVie for this project</td>
</tr>
<tr>
<td>Venkatesan, A.</td>
<td>Philips Healthcare, Collaborative Research and Development Agreement (CRADA) with Philips Healthcare</td>
<td>Biomedical Devices of Kansas, Grant recipient; Biomedical Devices of Kansas, Paid consultant</td>
</tr>
<tr>
<td>Verweij, P.</td>
<td>MSD Oss B.V., Full-time company employee</td>
<td>Roche Pharmaceuticals, Grant recipient</td>
</tr>
<tr>
<td>Vollenhoven, B. J.</td>
<td>Monash IVF, Direct stockholder</td>
<td>Celmatix, Inc., Full-time company employee</td>
</tr>
<tr>
<td>Ward, K.</td>
<td>Juneau Biosciences, LLC, Company officer; Juneau Biosciences, LLC, Direct stockholder; Juneau</td>
<td>Roche Pharmaceuticals, Grant recipient</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cemal, Direct stockholder</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cadence Pharmaceuticals, Grant</td>
</tr>
<tr>
<td></td>
<td></td>
<td>recipient</td>
</tr>
</tbody>
</table>