DISTRIBUTION OF THE FMR1 GENE IN FEMALES BY RACE-ETHNICITY: WOMEN WITH DIMINISHED OVARIAN RESERVE VERSUS WOMEN WITH NORMAL FERTILITY (SWAN STUDY). L. Pastore, S. L. Young, A. Manichaikul, V. Baker, Stony Brook Medicine, Stony Brook, NY; UNC School of Medicine, Chapel Hill, NC; University of Virginia, Charlottesville, VA; Stanford University, Stanford, CA.

OBJECTIVE: Premutation level trinucleotide repeat lengths in the FMR1 gene (CGG 55-199) are associated with primary ovarian insufficiency before age 40. However, an association between the repeat length with other ovarian aging phenotypes is not established. We examined whether reported associations between the FMR1 CGG repeat lengths in the intermediate, high normal, or low normal range differentiate women diagnosed with diminished ovarian reserve (DOR) from those with normal reproductive histories, and whether associations vary by race-ethnic group.

DESIGN: DOR cases (n=129) enrolled from 5 US fertility clinics vs. normal fertility female controls (n=803) from the US-based Study of Women's Health Across the Nation (SWAN).

MATERIALS AND METHODS: Cases’ (95 Caucasians, 22 Asian, 12 other) and controls’ (386 Caucasians, 219 African-Americans, 102 Japanese, 96 Chinese) banked DNA was analyzed for FMR1 CGG repeat lengths. Cases were clinically diagnosed with DOR, with regular menses and no fragile X syndrome family history. Controls had >1 menstrual period in the 3 months pre-enrollment, >1 pregnancy, no history of infertility or hormonal therapy, and menopause ≥40 years. In a previous analysis the SWAN Chinese and Japanese groups had similar FMR1 CGG repeat lengths, so those two groups were combined. We used Fisher’s exact tests to analyze data.

RESULTS: We found fewer CGG trinucleotide repeats in the lower of the two FMR1 alleles (i.e., allele with fewer repeats) in DOR cases relative to controls among Caucasians (p<0.0001), but not Asians (p=0.24). Caucasian DOR cases were more likely to have fewer CGG repeats in the lower allele compared with Asian DOR cases (p=0.027). No significant differences were found in the CGG repeat length distribution of the higher FMR1 allele among DOR cases compared with controls (p=0.20), or by race/ethnic group (p=0.41).

CONCLUSIONS: This study did not find an association between DOR and normal high/normal/intermediate repeats, but there was an association between DOR and low normal repeats (<20 CGG) in Caucasians. Our study design is distinguished by its analysis of FMR1 CGG repeat lengths by race-ethnic group and its large comparison group of women with normal fertility and normal menopausal histories. Variation in the expected distribution of CGG repeat lengths by race/ethnicity should be considered when determining whether distributions in patient populations are truly abnormal. A clear association between the trinucleotide repeat length and ovarian phenotypes, or lack thereof, will need to be demonstrated to allow patients, clinicians, and genetic counselors to correctly interpret FMR1 test results and make informed reproductive decisions.

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Materials and methods: Peripheral blood was collected from women with FMR1-POI and non-FMR1-POI. Mean telomere length was measured by quantitative polymerase chain reaction with the Rotor-Gene SYBR Green Kit (Quagen). Telomere length for each sample is based on the telomere to single copy gene ratio (T/S ratio) [C(telomere)/C(single gene)] normalized to the reference sample. Telomere lengths were compared to age matched controls using a standard curve generated from healthy female subjects.

RESULTS: Women with FMR1-POI (n=22, age 21 to 46 years) were significantly younger than women with non-FMR1-POI (n=15, age 29 to 79 years) in this cohort. Women with non-FMR1-POI demonstrated a trend towards increased telomere length compared to age matched controls (40.39 STDEV, p=0.09). Women with FMR1-POI exhibited a reduction in telomere length which did not reach statistical significance (-0.18 standard deviation (STDEV) compared to age matched healthy controls, p=0.41). Age differences between the FMR1-POI and non-FMR1-POI cohorts were controlled for by evaluating the STDEV of the cohorts as compared to age matched controls. Women with FMR1-POI demonstrated a significant reduction in telomere lengths compared to non-FMR1-POI women (+0.18 STDEV, p=0.04).

CONCLUSIONS: This is the first direct comparison of women with and without FMR1 POI, where FMR1-POI was associated with a significant reduction in telomere length compared to non-FMR1-POI. These results suggest different mechanisms for reproductive aging in women with FMR1 and non-FMR1 POI.

References: NIH

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OBJECTIVE: Fragile X mental retardation 1 (FMR1) codes for a nucleocytoplasmic shuttling protein (FMRP). In males, premutation of FMR1 is associated with reduced telomere length. However, FMRP may be important in telomerase complex trafficking to the nucleus where telomere lengthening occurs. In contrast, women with premature ovarian insufficiency (POI) have been reported to have longer telomer, but the effect of FMR1 premutation in women with POI is not known. The objective of this study was to compare telomere length in women with FMR1-POI and non-FMR1-POI in order to further evaluate reproductive aging in POI.

DESIGN: Cohort study.
membrane permeability and compromises oocyte quality. These findings contribute to understanding of the premature ovarian insufficiency and infertility seen commonly in women with classic galactosemia.

O-144 Tuesday, October 20, 2015 12:30 PM

SELECTING EMBRYOS WITH OPTIMAL CLEAVAGE KINETICS IMPROVES ONGOING PREGNANCY RATE FOLLOWING BLASTOCYST TRANSFER IN A MOUSE MODEL. R. S. Weinerman, T. S. Ord, R. F. Feng, C. Coutifaris, M. A. Mainigi. Division of Reproductive Endocrinology and Infertility, University of Pennsylvania, Philadelphia, PA; Department of Biostatistics and Epidemiology, University of Pennsylvania, Philadelphia, PA.

OBJECTIVE: To develop a model predicting blastocyst (blast) formation based on early cleavage parameters in the mouse and to determine if the model predicts pregnancy outcome following blast transfer. Microarray analysis was performed to identify genes associated with early embryo development.

DESIGN: Laboratory research.

MATERIALS AND METHODS: 313 2-pronuclear (PN) embryos were collected from superovulated (SO) female mice and were cultured to blast in 5% (n=180) and 20% (n=133) oxygen (O2). Time-lapse videos were collected in the Eeva™ system (Progyny). Classification and regression tree analysis (CART) was used to predict blast formation based on 4 cleavage parameters: 1st cytokinesis duration, and time from 2-3 cell, 3-4 cell, and 4-5 cell stages. The model was built in the 20% O2 cohort and validated in the 5% O2 cohort. For the transfer experiments, 2PN embryos were collected from a single injection of SO females to transgenic male mice heterozygous for green-fluorescent protein (GFP) and cultured in the Eeva™ system. blast cells were rated as having "optimal" (Opt) or "suboptimal" (Subopt) timing based on the CART model. 10 blasts were transferred into each of 10 pseudo-pregnant recipients, using GFP status to tag the embryos as Opt or Subopt. Pregnancy outcomes were assessed at mid-gestation (E10.5). Differences in outcomes were assessed using Fisher’s exact test. Single-embryo microarray analysis was performed using the Affymetrix GeneChip Mouse Gene ST 1.0 Array on Opt and Subopt blasts.

RESULTS: The final model utilized 2 parameters, cleavage times from 2-3 cell and 3-4 cell. The model predicted blast formation with a 97.5% sensitivity and 62.5% specificity in 5% O2. Following transfers, 8 mice became pregnant and were included for analysis. The overall implantation rate per embryo transfer did not differ significantly between groups (86% Opt vs. 77% Subopt, p=0.03). The resorption rate (implantation sites with placental tissue only) among implanted embryos was significantly higher in the Subopt embryos (60% (35/58) vs. 32% (7/22), p=0.04). Gene expression differences were seen in 74 genes between the Opt and Subopt groups (FDR<0.05). Pathway analysis revealed enrichment for genes encoding membrane-bound proteins including amino acid and ion transporters.

CONCLUSIONS: We have validated a mouse model of time-lapse microscope with morphokinetic parameters that predicts blast formation and improved ongoing pregnancy rates following blast transfer. We have also shown that cleavage kinetics are correlated with gene expression changes. The data from this model suggest that time-lapse may allow selection of blast cells with the highest probability of live birth.


EMBRYO TRANSFER

O-145 Tuesday, October 20, 2015 11:15 AM

GESTATIONAL SURROGACY / EGG DONOR IVF: BEHAVIOR OF INTENDED PARENTS WITH RESPECT TO NUMBERS OF EMBRYOS TRANSFERRED. G. Sylvestre-Margolis, R. S. Weinerman, E. Rauch, Obstetrics and Gynecology, Flushing Hospital Medical Center, Flushing, NY; Obstetrics and Gynecology, RMA New Jersey, Freehold, NJ.

OBJECTIVE: The objective of this study was to characterize the attitudes of gay men intended parents (IP) with respect to the number of embryos transferred into their gestational carrier during a gestational surrogacy/egg donation (GS/ED) IVF cycle.

DESIGN: Questionnaire / survey.

MATERIALS AND METHODS: Intended parents were surveyed via a Facebook site called “Men Having Babies”, a popular website destined to gay men wishing to become parents via gestational surrogacy in the United States. They were asked 10 questions pertaining to the number of embryos they transferred, their motivations towards transferring one or multiple embryos and the outcome of their surrogacy journey.

RESULTS: 104 men responded to the survey. The majority of men were aged between 36-45 years old (62%) and 90% were in a relationship. During their first embryo transfer, 68% of couples or individuals transferred 2 embryos, 19% 1 embryo and 13% transferred 3 or more embryos. The rate of twins was 50%. The most common quoted reasons toward multiple embryo transfers were a perceived higher chance of successful IVF outcome (42%), a desire to have twins (26%) and a desire to have embryos from each partner (11%). Poor embryo quality (4%) and economical reasons (9%) were less frequent motivations to have multiple embryos transferred. There were a few adverse outcomes, mostly in the patients who have multiple embryos transferred (three pre-viable twin losses, one anomalous twin, and two sets of triplets).

CONCLUSIONS: Despite a very high IVF success rate and high risk nature of multifetal pregnancies, the majority of gay men IP attempting parenthood via GS/ED IVF transfer more than one embryos specifically to have twins. Interestingly, their behavior about the numbers of embryos transferred is similar to infertile women in the same age group, who have less favorable IVF outcomes. This contrasts with younger infertile women, who are more likely to desire a singleton pregnancy (Latar, 2014). Gay men IP and their gestational carriers need to be counseled about a much higher rate of successful pregnancy and the risks of multifetal pregnancies when more than one embryo is transferred.

O-146 Tuesday, October 20, 2015 11:30 AM


OBJECTIVE: With quantitative real-time polymerase chain reaction (qPCR)-based comprehensive chromosome screening (CCS), it’s possible to biopsy blastocyst stage embryos, receive a genetic interpretation overnight and transfer within a 12-hour period. Although CCS gives insight into the genetic composition of an embryo, laser hatching, embryo biopsy, and extend culture can result in an embryo that is fully hatched and potentially fragile, particularly at the time of transfer. We sought to investigate if the replacement of a euploid fully hatched affects implantation rates compared to less expanded matched controls.

DESIGN: Retrospective.

MATERIALS AND METHODS: Patients undergoing a fresh IVF cycle with qPCR-based CCS who had a fresh euploid ET on day 6 from September 2013 to January 2015 were included. Trophectoderm biopsies were performed on Day 5, contingent upon expansion eligibility criteria (embryos ≥3BB). Embryos were graded before ET according to the Gardner scale. Embryos were classified by either partially expanded (expansion grade 4 and 5) or fully hatched (expansion grade 6) at ET. Embryos with expansion 3 or less were excluded. Biochemical and clinical pregnancies were analyzed. Fisher exact test on contingency tables was computed on frequencies with significance at p<0.05.

RESULTS: A total of 234 patients were included in the study, (Gardner 4-5: n=112; Gardner 6: n=122). Patient’s demographics were similar between groups (Table 1). There was no significant difference in biochemical (65.2% vs. 64.8%) or clinical (54.5% vs. 53.3%) PRs from single euploid ETs whether or not the embryo was fully hatched prior to ET (p=0.05) (1.02 OR [0.58-1.82 95% CI]).

CONCLUSIONS: This study corroborates former reports that the transfer of a single euploid blastocyst results in high implantation rates. Additionally, it demonstrates that a fully hatched embryo is neither more friable nor less likely to implant after an embryo transfer. In our study, implantation, clinical pregnancy, and miscarriage rates where similar when blastocysts were transferred fully hatched as compared to non-fully hatched embryos.

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