Ovaries were dissected and granulosa cells were isolated from large antral follicles by the needle puncture method. Cells were either frozen at -80° immediately, or plated in McCoy's medium with 10% FBS, 1% Pen/strep at 37C in 5% CO2 humid incubator. After culture for 24 hours, cells were rinsed and changed to serum-free medium consisting of McCoy's, 0.01% BSA, 10ul/ml ITS+ and Pen/Strep for 24 hours. Then, cells were treated with FSH (1 IU/ml) and/or activin (10 ng/ml). After 24 hours incubation, cells were lysed and total RNA isolated utilizing TRIzol Reagent. Total RNA (0.5-2.0ug) was used to synthesize cDNA for real-time PCR. Real-time PCR analysis was performed on cDNA from three independent experiments using a custom kit from ABI. In each experiment, TATA was measured in each sample as an internal control and data were analyzed by the comparative CT method.

RESULTS: Expression of the mRNA for aromatase in whole ovaries from Smad3 -/- mice was less than 30 % that of wild type ovaries. Fresh granulosa cells from Smad3 -/- mice also had aromatase mRNA expression less than 50% of that expressed in wild type granulosa cells. When wild type granulosa cells were cultured with FSH or activin or both together, FSH or activin induced a more than two-fold increase in aromatase mRNA expression, however FSH + activin treatment increased aromatase mRNA by almost 6-fold. In contrast, in the Smad3 deficient granulosa cells, there was no stimulation of aromatase expression by either FSH or activin.

CONCLUSION: Neither FSH nor activin was able to increase aromatase expression in the absence of Smad3. While this is not surprising with activin which signals directly via Smad3, it is more puzzling with FSH. The interactions of FSH downstream signaling with other pathways are just coming to light. Further studies will be necessary to delineate the exact role of Smad3 in FSH receptor signaling. Yet it seems clear that at least part of the cause of infertility in the Smad3 knockout mouse is the reduced ability to produce estrogen with appropriate FSH stimulus. These studies may also have implications for infertility and poor ovarian response to gonadotropins in humans.

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P-714

MATERNAL BIOLOGICAL AGE IMPACTS FIRST GENERATION FEMALE OFFSPRING REPRODUCTIVE SUCCESS DURING INFERTILITY TREATMENT. Z. P. Nagy, T. Elliott, C. Elsner, S. Slayden, D. Shapiro, H. Kort. Reproductive Biology Associates, Atlanta, GA.

OBJECTIVE: During the early human life, somatic cells differentiate and specialize to certain functions through intense epigenetic modifications. The gametes' genome also undergoes these epigenetic modifications, however, they are then later erased (except gonad specific imprinting) contributing to the preservation of their longevity and the potential to produce a new individual. Gametes in the female gonad present an age dependent decrease of quality and related decline of female fertility is also well described. This fertility decline also relates to the biological/functional age of the ovary characterized by the age at the onset of menopause. Thus the scientific question arises: Will this affect the fertility potential of a woman if she was conceived from "young" egg (age relative to menopause) or if she was conceived from an "aged" egg? For this reason, our objective was to compare pregnancy outcomes taking into account the ovarian aging of the mother of the infertile patient.

DESIGN: Longitudinal, observational, currently ongoing study.

MATERIALS AND METHODS: The study was initiated in October 2005. IVF patients were included if reproductive age was less than 35 years. Female patients were asked 3 questions: 1, Age of the mother at the time of delivery; 2, Age of the mother at the time of menopause and 3, Age of the father at the time of delivery. Ovarian stimulation was performed using GnRH agonist in combination with rFSH and HCG. All laboratory procedures were standardized and uniform. Clinical and laboratory parameters were recorded and analyzed using the One-Way ANOVA and chi-square tests. $P < 0.05$ was considered to be statistically significant.

RESULTS: There were 48 patients who qualified to participate in the analyses (22 had positive FCA and 26 had negative FCA). Mean female age was 30.8 (+/-2.3 S.D.) and 31.2 (+/-1.9) (NS), in the pregnant and in the not pregnant groups, respectively. Egg number, fertilization rate, embryo development, and all examined clinical parameters were the same in the two groups. The mothers mean ages at time of birth were 26.0 (+/-4.7) and 28.1 (+/-4.6) ($P=0.12$); fathers mean ages were 28.6 (+/-5.2) and 31.6 (+/-6.8) ($P=0.11$); and onset of menopause of mothers was 50.5 (+/-4.7) and 48.4 (+/-4.3) ($P=0.12$) in the pregnant and in the not pregnant groups, respectively. Average time span between age of delivery (mother) and menopause was 24.6 (+/-6.2) and 19.9 (+/-6.4) ($P<0.02$) in the pregnant and in the not pregnant group respectively.

CONCLUSION: The present study shows a trend that parental age may affect infertility treatment outcome of female offspring. However, this difference has not reached statistical significance, possibly due to the currently low number of patients evaluated. On the other hand, the results of the present study demonstrate that maternal age at childbirth - relative to the onset of menopause - significantly relates to assisted reproduction success in female patients. It seems conceivable that oocytes from ovaries of advanced reproductive age may increasingly develop impairment of epigenetic regulators that will remain latent until the formation of the gametes in the fetus, resulting in considerable infertility conditions in the female offspring at adult age. The data of this study suggests that ovarian/gamete aging in women is critical and it not only effects the individual's own infertility potential, but it has consequences for the next generation's fertility of female offspring.

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P-715

OVARIAN FOLLICULAR VOLUME AND FOLLICULAR SURFACE AREA ARE MORE ACCURATE INDICATORS OF FOLLICULAR GROWTH AND MATURATION, RESPECTIVELY, THAN FOLLICULAR DIAMETER ALONE. Y. Yavas, M. R. Selub. Florida Institute for Reproductive Sciences and Technologies (F.I.R.S.T.), Weston, FL.

OBJECTIVE: Serial ultrasonography monitors diameters of individual follicles for growth, whereas serial blood sampling monitors serum estradiol-17β (E_2) for overall functional maturation. However, the two-dimensional follicular diameter cannot accurately reflect the three-dimensional growth, and serial measurement of serum concentrations of E_2 monitors the overall functional maturation of all, but not of individual, follicles. The objective was to develop accurate and non-invasive methods to monitor the three-dimensional growth and maturation (E_2 production) of individual follicles. An accurate method to monitor the three-dimensional follicular growth is to monitor the three-dimensional follicular volume. An accurate, non-invasive method to monitor maturation (E_2 production) of an individual follicle is to monitor the follicular surface area where E_2-precursor (testosterone)-producing theca interna cells are located.

DESIGN: Hypothetical, mathematical model of the relationships among the diameter, surface area and volume of individual follicles.

MATERIALS AND METHODS: Because of its curvilinear (parabolic) relationship both to follicular surface area and follicular volume, the follicular diameter can be used to calculate both the follicular surface area and the follicular volume. Assuming follicles are spherical, the surface area (S) and the volume (V) of a follicle can be calculated using the following formulae: $S = 4\ (D/2)^2\ \pi$, and $V = 4/3\ (D/2)^3\ \pi$, where D is the diameter of the follicle. Using these formulae, the relationships among follicular diameter, follicular surface area and follicular volume were evaluated.

RESULTS: When follicular diameter (D) increases from D_1 to D_2, the surface area (S) increases from $S_1 = 4\ (D_1 / 2)^2\ \pi$ to $S_2 = 4\ (D_2 / 2)^2\ \pi$, and the volume (V) increases from $V_1 = 4/3\ (D_1 / 2)^3\ \pi$ to $V_2 = 4/3\ (D_2 / 2)^3\ \pi$. If we express the increases in diameter, surface area and volume as folds of D_1, S_1, and V_1, respectively, i.e., as D_2 / D_1, S_2 / S_1, and V_2 / V_1, respectively, then $S_2 / S_1 = (D_2 / D_1)^2$ and $V_2 / V_1 = (D_2 / D_1)^3$. If $D_2 / D_1 = a$, then $S_2 / S_1 = a^2$, and $V_2 / V_1 = a^3$. Therefore, $D_2 = a.D_1$, $S_2 = a^2.S_1$, and $V_2 = a^3.V_1$. Thus, when diameter increases from D_1 to D_2 (or to $a.D_1$), i.e., from D_1 to 'a' folds of D_1, surface area increases from S_1 to S_2 (or to $a^2.S_1$), i.e., from S_1 to 'a^2' folds of S_1, and volume increases from V_1 to V_2 (or to $a^3.V_1$), i.e., from V_1 to 'a^3' folds of V_1. Thus, the fold increase in surface area is the square of the fold increase in diameter, and the fold increase in volume is the cube of the fold increase in diameter. For example, a 2-, 5- or 10-fold increase in diameter corresponds to a 4-, 25- or 100-fold increase in surface area, respectively, and to an 8-, 125- or 1,000-fold increase in volume, respectively.

CONCLUSION: The three-dimensional functional maturation and growth of follicles occur at much greater extents than they are assessed by a mere increase in the two-dimensional diameter. Follicular surface area and

volume are more accurate indicators of functional maturation (E_2 production) and growth, respectively, than diameter alone.

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P-716

PROTEOMICS OF HUMAN FOLLICULAR FLUID. R. Wang, G. Dolios, M. Luna, J. Barritt, M. Tham, A. B. Copperman. Mount Sinai School of Medicine, New York, NY; Reproductive Medicine Associates of New York, New York, NY.

OBJECTIVE: Human follicular fluid (HFF) proteins have been poorly characterized, and variation between groups of patients not well described. Proteins identified by proteomic analysis of follicular fluid may emerge as candidates for specific functions during folliculogenesis and could prove useful biomedical markers for follicular competence and/or oocyte maturation. This study was designed to comprehensively characterize proteins in HFF and to identify substantially changed proteins in HFF associated with Diminished Ovarian Reserve (DOR) and Polycystic Ovarian Syndrome (PCOS) in order to gain molecular insights of DOR and PCOS.

DESIGN: This is a pilot study in which 3 HFF specimens from each group were analyzed. Relative protein expression in each specimen was analyzed by quantitative proteomic analysis using stable-isotope labeling technology together with mass spectrometry. Protein classification was analyzed for HFF proteome and statistical analysis conducted to estimate the bio-variability and identify any significant changes in protein expression associated to DOR and PCOS.

MATERIALS AND METHODS: The follicular fluid was aspirated during oocyte retrieval under an IVF-ET IRB protocol. HFF samples were fractionated into two fractions, €~high abundant HFF proteins€™ and €~low abundant HFF proteins€™ using either Seppro anti-HAS IgY Microbeads (GenWay Biotech) or ProteoPrep 20 Plasma Immunodepletion Kit (Sigma-Aldrich). The low abundant HFF proteins were subjected to reduction/alkylation, trypsin digestion and iTRAQ (Applied Biosystems) labeling. The labeled protein samples were mixed and subjected to two-dimensional HPLC-MS/MS analysis using a QSTAR XL quadrupole time-of-flight (QqTOF) tandem mass spectrometer (Applied Biosystems) coupled with an UltiMate Capillary/Nano LC System (Dionex). Protein identification and quantitation were carried out with software of ProID, ProQuant and ProGroup based on the NCBI non-redundant sequence database.

RESULTS: HFF protein analysis after fractionations with Seppro anti-HAS IgY Microbeads ProteoPrep 20 Plasma Immunodepletion Kit revealed that high abundant proteins were similar to those found in human plasma. Of note was a significantly decreased quantity of immunoglobulin across all specimens of HFF. With the combined technologies, more than 250 proteins have been identified. The use of iTRAQ, a multiplexed set of four isobaric reagents, allowed us to detect relative protein level changes. Comparative proteomic analysis showed no significant differences found among groups when analyzing Antichymotrypsin, a variety of glycoproteins, apolipoproteins, complement components, extracellular matric proteins, plasma protease (C1) inhibitors,vitronectin, and Zinc finger proteins.

CONCLUSION: As has been previously reported, the protein content of HFF is similar to human plasma protein. A number of predominantly intracellular proteins were isolated along with traditional expected components of follicular fluid. It is not clear whether these proteins were isolated from exfoliated granulosa cells or were transmitted via blood. A comprehensive HFF proteome data set was established through this pilot study. Quantitative proteomic analysis of HFF may allow detection of protein changes associated with DOR and PCOS and may help us gain insight into the physiological and pathological alterations in the ovarian developmental maturation.

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OVARIAN RESERVE

P-717

OVARIAN RESERVE TESTS HAVE NO PREDICTIVE VALUE FOR SPONTANEOUS PREGNANCY IN SUBFERTILE COUPLES WITH A FAVOURABLE PROGNOSIS. M. L. Haadsma, H. Groen, E. M. Roeloffzen, E. R. Groenewoud, M. J. Heineman, A. Hoek. Univ Medical Centre Groningen, Groningen, The Netherlands.

OBJECTIVE: Reproductive ageing is the result of a decrease in quantity and quality of the ovarian follicle pool. The quantity of the ovarian reserve can be estimated by ovarian reserve tests (ORT). ORT are reliable predictors of the ovarian response to hyperstimulation in artificial reproductive techniques (ART). However, the chance to conceive after ART is poorly predicted by these tests. Little is known about the predictive value of ORT for spontaneous pregnancy. Especially in couples with only a mild semen factor or unexplained subfertility, ovarian reserve may play an important role. The aim of this study is to assess the value of ORT for the prediction of spontaneous pregnancy in subfertile couples with a favourable prognosis for spontaneous conception.

DESIGN: Prospective cohort study.

MATERIALS AND METHODS: From 1999 to 2003, 475 subfertile couples were recruited after basal fertility evaluation at the University Medical Centre Groningen and the Martini Hospital, Groningen, the Netherlands. The main inclusion criteria were 1) subfertility for at least twelve months, 2) regular ovulatory cycle, 3) semen analysis with a total motile count of at least one million, 4) at least one open Fallopian tube. After inclusion an antral follicle count (AFC) was performed and inhibin B (InhB) and baseline follicle stimulating hormone (bFSH) were measured on cycle day 2, 3 or 4. The result of the AFC was defined as the total number of follicles $\leq$ 10 mm. Subsequently the participants took clomiphene citrate 100 mg on cycle days 5 to 9. Seven days after initial measurement FSH was measured again. The result of the Clomiphene Citrate Challenge Test (CCCT) was defined as the sum of bFSH and FSH after seven days. For each couple the chances of spontaneous conception were estimated using the prognostic model of Eimers et al., which includes female age, duration of subfertility, fertility history, postcoital test and semen motility. Expectant management was proposed to couples with an estimated chance to conceive spontaneously of at least 30% in the following year up to a maximal duration of subfertility of three years (two years in case of female age $\geq$ 37 years). These couples with a favourable prognosis for spontaneous conception were selected for analysis (n=204). Cox regression analysis was performed to analyse the influence of the various ORT on the occurrence of spontaneous clinical pregnancy.

RESULTS: Of the 204 couples selected, 151 were diagnosed with unexplained subfertility (74,0%) and 53 with mild semen factor (26,0%). Mean age was 32,1 years (range 19,9-44,7). 69 couples (33,8%) achieved a spontaneous clinical pregnancy. Couples were censored at start of treatment (n=57; 27,9%), duration of subfertility of resp. three or two years (n=74; 36,2%) or last date of follow-up (n=4; 2,0%). Cox regression analysis showed a cumulative spontaneous pregnancy rate after one year of 38,5%. No significant influence of AFC, bFSH, InhB and CCCT on spontaneous pregnancy rate was found. Analysis of the subgroup diagnosed with unexplained subfertility (n=151) did not show any significant influence of the various ORT on spontaneous pregnancy rate either.

CONCLUSION: In this analysis various ovarian reserve tests, i.e. AFC, bFSH, InhB and CCCT, have no predictive value for spontaneous clinical pregnancy in selected couples with a favourable prognosis for spontaneous conception.

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EFFECTS OF LOW OVARIAN RESERVE VERSUS MATERNAL AGE ON OVARIAN RESPONSIVENESS, PREEMBRYO QUALITY AND CLINICAL OUTCOME IN ASSISTED REPRODUCTION. R. Berrios, R. N. Clarke, R. Gosden, H. Bang, N. Zaninovic, L. Veeck Gosden. Cornell Medical Center, New York, NY.

OBJECTIVE: Decreased ovarian reserve is associated with a decline in oocyte number and quality, higher Day 3 (D3) serum Follicle Stimulating Hormone (FSH) levels, an increase in chromosome abnormalities and potentially lower pregnancy rates. However, it is not clear whether the association between decreased ovarian reserve and preembryo quality is causal. In addition, the relative importance of chronological versus biological age in declining fertility rates is controversial. The objectives of the present study were to determine 1) the effect of basal D3 FSH (a marker of ovarian reserve) on preembryo quality in ART patients, and 2) the relative importance of maternal age and D3 FSH on declining pregnancy rates.

DESIGN: A retrospective analysis of 1381 ART patients attending the Weill-Cornell Center for Reproductive Medicine and Infertility from 2002