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,[a] S. Desai,[a] M. Hill,[b] E. C. Feinberg,[c] S. A. Fowler,[a] E. S. Jungheim.[a] [a]OB/Gyn, Division of Reproductive Endocrinology & Infertility, Washington University, St. Louis, MO; [b]Program in Reproductive and Adult Endocrinology, Eunice Kennedy Shriver NICHD & NIH, Bethesda, MD; [c]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 complied. With IRB approval, primary data from our center was also included. Meta-analysis of the data was performed using a DerSimonion-Laird random effects model. Results are reported as weighted mean difference and 95% confidence interval.

RESULTS: Seven studies and our center's primary data were 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.

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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] [a]Reproductive Medicine Associates of New York, New York, NY; [b]Department of OBGYN 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).

Ooctye Retrieval: IVF vs. EOC

	IVF (n=12,065)	EOC (n=722)	p-value
<35	16.7±8.8 (n=4098)	16.3±10.4 (n=108)	0.7
35-37	13.8±7.8 (n=2637)	14.5±8.2 (n=239)	0.18
38-40	12.2±7.6 (n=2827)	13.1±9.3 (n=291)	0.06
>40	10.3±6.9 (n=2503)	9.2±6.5 (n=84)	0.14

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.

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ABSTRACT WITHDRAWN

OOCYTE BIOLOGY

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MICRORNAS REGULATE EXPRESSION OF AGED HUMAN CUMULUS CELLS GENES THAT ARE ESSENTIAL FOR OOCYTE QUALITY. T. Al-Edani,[a] S. Assou,[a] A. Ferrières,[b] C. Brunet,[b] O. Aït-Ahmed,[a] S. Hamamah.[c] [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 FGF2 (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 SPSB) 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. IGFBP3 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 aneuploidy oocyte.

Supported by: Ferring and Genevrier companies.

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SPECIAL RESEARCH PRESENTATION: BMPR SIGNALING IN THE MAMMALIAN OOCYTE IMPROVES FERTILIZATION AND INFLUENCES EARLY EMBRYO DEVELOPMENT. A. M. Zamah,[a] A. M. Laeno,[b] H. Cakmak,[b] L. Xiong.[b] [a]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 polysome 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% 55%, 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.

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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] [a]National Foundation for Fertility Research, Lone Tree, CO; [b]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 involved 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.

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REGULATION OF FOXO3 SUBCELLULAR LOCALIZATION BY KIT LIGAND IN THE NEONATAL MOUSE OVARY. M. Ezzati,[a] M. Baker,[b,c] G. Aloisio,[b] C. Pena,[b] Y. Nakada,[b] I. Cuevas,[b] B. R. Carr,[a] D. H. Castrillon.[b] [a]Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, University of Texas Southwestern Medical Center, Dallas, TX; [b]Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX; [c]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 Image J 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.