RESULTS: RNA sequencing revealed 1,079 and 623 differentially expressed genes in KO CCs compared to WT in 3- and 6-month-old mice, respectively. Among them, 163 genes were common differentially expressed at both age groups. Pathway analysis revealed apoptosis and phagosome-lysosome pathways to be uniquely affected at 3- and 6-month-old KO CCs, respectively. IF microscopy for apoptotic and cell proliferation markers in ovarian sections and COCs confirmed RNAseq findings with increased immunoreactivity for TUNEL and decreased expression of Ki67 and PCNA ($p<0.05$). Electron microscopy revealed significant impairment of mitochondrial dynamics in *Clpp*-deficient cumulus cells with lower aspect ratio (length/width; 1.92 ± 0.04 vs. 1.64 ± 0.04, $p<0.0001$). qRT-PCR showed a significant decrease in expression of genes involved in mitochondrial dynamics, *Mfn1*, *Mfn2* and *Opa1* ($p<0.05$).

CONCLUSIONS: Impaired mitochondrial stress response in cumulus cells with targeted deletion of *Clpp* is associated with significant changes in CC transcriptome and mitochondrial dynamics that culminate in increased apoptotic cell death and accelerated follicular depletion. The relevance of these parameters in women undergoing IVF and whether they can be exploited to improve treatment outcomes remain to be investigated.

O-238 Wednesday, October 10, 2018 11:30 AM

OOCYTE SECRETED FACTORS REGULATE INSULIN GROWTH FACTOR 2 (IGF2) EXPRESSION IN HUMAN PRIMARY CUMULUS GRANULOSA CELLS.

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OBJECTIVE: We have reported that human primary cumulus granulosa cells (hGCs) express and respond to IGF2; however, the mechanisms involved inthe regulation of IGF2 and the role of oocyte-secreted factors (OSFs) on IGF2 expression remain unknown. The purpose of our study was to determine the role of the OSFs: growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15) on IGF2 production in primary hGCs.

DESIGN: Prospective in vitro studies using human primary cumulus granulosa cell cultures.

MATERIALS AND METHODS: Follicular aspirates from 24 women undergoing in vitro fertilization at a University clinic were used. Cumulus cells were mechanically separated from the oocyte, seeded on culture dishes precoated with extracellular matrix at a density of $6x10^4$ cells/mL and cultured for 24-72 hours before treatment. Cells were treated with a combination of GDF9, BMP15, recombinant follicle stimulating hormone (FSH), cyclic adenosine monophosphate (cAMP) and SMAD inhibitors for 48 hours. Cells were harvested for analysis. IGF2 mRNA levels were quantified using real-time PCR. Data was analyzed by two-way ANOVA and $P<0.05$ was considered significant.

RESULTS: Treatment with GDF9 or BMP15 alone, or in combination (G+B) had no effect on IGF2 mRNA levels. Interestingly, FSH-stimulation of IGF2 mRNA expression was significantly potentiated in the presence of the G+B combination (IGF2 mRNA fold increase relative to control: FSH: 2.3 ± 0.2 vs FSH+G+B: 35.6 ± 8.8, $P<0.0001$). Using a fixed concentration of FSH (50ng/ml), the addition of G+B induced a dose-dependent increase in IGF2 expression in cells treated with 0.6, 2.5, 5 and 10ng/ml of G+B with maximal effect achieved at 5ng/ml ($P<0.0001$).The additionof SMAD2/3 and SMAD3 inhibitors prevented this effect ($P<0.01$) but did not affect FSH stimulation. The combination of G+B also potentiated the increase in IGF2 expression observed in the presence of dbcAMP, an analog of cAMP ($P<0.03$). To determine whether G+B controls IGF2 mRNA transcription, the promoter 3 of the IGF2 gene was cloned into a luciferase reporter. Using this reporter, we observed that although FSH stimulated IGF2p3-Luc activity, treatment with FSH+G+B had no additive effect on promoter activity. Moreover, inhibition of insulin-like growth factor 1 receptor partially blocked G+B potentiation of FSH actions, suggesting other mechanisms of action in addition to IGF2 positive feedback ($P<0.009$).

CONCLUSIONS: Our data are the first to show that the oocyte actively participates in the regulation of IGF2 expression in primary hGCs. We demonstrated that the specific combination of G+B potently synergized FSH actions on IGF2 via a SMAD2/3 and SMAD3 receptor pathway and that G+B actions target mechanisms downstream of cAMP in the FSH pathway.

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O-239 Wednesday, October 10, 2018 11:45 AM

SERUM H19 EXPRESSION IS DECREASED IN WOMEN WITH DIMINISHED OVARIAN RESERVE.

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OBJECTIVE: The Anti-müllerian hormone (AMH) is an informative marker for the assessment of ovarian reserve, but its regulation remains poorly understood. We previously demonstrated that AMH is a novel target of the microRNA let-7, which itself is regulated by the long noncoding RNA H19, and that H19 knockout mice have decreased AMH, accelerated follicular recruitment, and diminished fertility. In this study we sought to determine whether alterations in serum H19 might be associated with diminished ovarian reserve in a sample of patients undergoing fertility treatment.

DESIGN: Experimental study

MATERIALS AND METHODS: Serum was collected from women (n=69) presenting for baseline assessment prior to initiation of a controlled ovarian hyperstimulation/in vitro fertilization cycle. 26 women were categorized as having diminished ovarian reserve (defined as antral follicle count <5-7 and AMH < 0.5-1.1). 43 patients with were identified as a comparison group and further categorized into: 1) unexplained infertility (24 patients) and 2) controls (19 patients with tubal or male factor). H19 total RNA was isolated from discarded serum collected on cycle day 2, and cDNA was synthesized by reverse transcription. RNA levels were analyzed using quantitative real-time PCR. H19 levels were normalized to β-actin and presented as relative expression levels using the comparative C_t method. Statistical analysis was performed using one-way ANOVA.

RESULTS: Women with DOR had significantly lower serum H19 expression levels as compared to control women and women with unexplained infertility and normal ovarian reserve ($p<0.001$). Of note, women with DOR were significantly older than their counterparts (39.6 years vs 35.0 and 35.2 years, $p<0.01$), with lower AMH levels (as expected; 0.4 ng/mL vs 3.0 ng/mL and 2.5 ng/mL, $p<0.01$) and higher baseline estradiol levels (69.9 pg/mL vs 50.9 and 30.5 pg/mL, $p<0.01$). BMI. serum progesterone, and FSH and LH did not differ among groups. Serum H19 was positively correlated with serum AMH ($p<0.01$, $r=0.307$).

CONCLUSIONS: This study for the first time suggests that circulating H19 levels may be diminished in women with diminished ovarian reserve. In light of our prior findings suggesting that H19 may have a role in the regulation of AMH, further study with an expanded sample size is necessary to determine whether serum H19 could be of use as a novel biomarker for diminished ovarian reserve, or whether H19 has a role in the pathogenesis of diminished ovarian reserve.

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O-240 Wednesday, October 10, 2018 12:00 PM

HUMAN BONE MARROW MESENCHYMAL STEM CELL SECRETOME STIMULATES PROLIFERATION AND STEROIDOGENESIS IN HUMAN UNLUTEINIZED GRANULOSA CELLS.

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OBJECTIVE: There is increasing clinical evidence on the potential benefit of human bone marrow mesenchymal stem cell (hMSC) therapy in the treatment of premature ovarian insufficiency. The mechanism of action of hMSC

in the human ovary remains unknown. The purpose of our study was to determine the role of hMSC secretome on immortalized unluteinized gonadotropin dependent human granulosa cell line (GC).

DESIGN: Prospective in vitro studies using immortalized unluteinized gonadotropin dependent human granulosa cell line.

MATERIALS AND METHODS: GCs were seeded on culture dishes precoated with extracellular matrix at a density of 6x104 cells/ml and cultured for 24 hours before treatment. Media was then collected, and either replaced by control media (C) or conditioned media (Cm). Cm was previously prepared by collecting the media of hMSC when those reached 90% confluence in their culture plate. A subset of those two groups was treated with recombinant follicle stimulating hormone (FSH) at a concentration of 50 ng/ml. Forty eight hours after media change and FSH treatment, cells and supernatant were collected for analysis. Proliferation assay based on Ki67 analysis with flow cytometry was performed. Genes mRNA and protein expression levels were quantified by real-time PCR and Western blot, respectively. Estrogen levels were measured by ELISA.

RESULTS: Human GCs cultured in hMSC Cm showed significantly higher proliferation rates when treated with Cm compared to the control group based on the percentage of gated Ki67^{+}cells (4.02 % ±0.78 vs 30.9 ± 4.62, P<0.001). The addition of FSH to both groups showed a trend of increased proliferation in the Cm group, however this did not reach statistical significance (52.7 ± 2.45 vs 53.1 ± 2.6, P>0.05). Human aromatase mRNA expression was 20 fold increased with Cm compared to the C group (P < 0.05), and 30 fold increase with CmF compared to the CF group (P < 0.05). This was also shown at the level of human StAR mRNA without FSH (14 fold increase, P<0.05), and with FSH (30 fold increase, P < 0.05). Protein studies and other gene expression profiles, in addition to results with molecular pathway inhibitors are being analyzed and will be presented during the meeting in October 2018.

CONCLUSIONS: These findings show, for the first time, that hMSC secretome promotes human granulosa cell proliferation and regulates gene expression involved in folliculogenesis, such as aromatase. Further investigation, including co-culture and analysis of Cm is warranted to fully understand the effect of hMSC on human granulosa cells.

Supported by: UIC start-up fund

OVARIAN STIMULATION 2

O-241 Wednesday, October 10, 2018 10:45 AM

MOLECULAR STIMULATION FOR PROMOTING GROWTH OF EARLY STAGE FOLLICLES BY IGF-1.

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OBJECTIVE: Most of the follicles in human ovaries remain dormant, and only a fraction are mobilized into the growing pool. The growth of these follicles is mediated in part by upregulation of Akt signaling. Insulin-like growth factor 1 (IGF-1) is a potent physiological activator of the AKT pathway. Using our unique xenograft model, we co-transplanted endothelial cells (ECs) that overexpress IGF-1 and achieved both restoration of vascular perfusion and the direct paracrine delivery of IGF-1 in the vicinity of human ovarian tissue. We investigated the potential for ECs expressing IGF-1 to modulate the growth of follicles within the grafts.

DESIGN: Xenograft of human ovarian tissue into NSG mice with co-transplantation of control ECs and ECs expressing human IGF-1.

MATERIALS AND METHODS: To test the modulation of follicles in grafts, we have generated lentiviral vectors expressing human IGF-1. ECs modified by the adenoviral gene fragment E4-ORF1 were transduced with these particles. Xenograft of human ovarian tissue into NSG-oophorectomized mice with co-transplantation of IGF-1 ECs served as the study group. Patient-matched ovarian tissue co-transplanted with non-IGF-1-producing ECs served as controls. We co-transplanted multiple grafts and harvested at 3 (Ctrl, n=10; study, n=9), 8 (Ctrl, n=2; study, n=2), and 14 (Ctrl, n=7; study, n=9) weeks. The ratio of follicles in each treatment was assessed in histologic sections.

RESULTS: The study group demonstrated at 3 and 8 weeks a higher proportion of secondary follicles (35.38±9.78 vs. Ctrl 21.36±8.16; p=0.035) and a lower proportion of primary follicles (42.86±5.6 vs. Ctrl 55.49±6.63; p=0.017); no difference was found between the ratios of primordial follicles. Interestingly, at 14 weeks, the proportion of primordial follicles was reduced in the study group (4.73±4.726% vs. Ctrl 22.63±7.04%; p=0.03), while the primary follicle rate was increased (64.27±9.16 vs. Ctrl 50.55±5.29; p=0.05). While there was no difference in the short term, the total number of follicles per mm^2 in the study group was lower in the long-term grafts (4±3.6 vs. 7.5±2.05; p=0.05).

CONCLUSIONS: In this distinctive xenograft model, we have measured the effect of IGF-1 on the growth and development of early-stage follicles that are not responsive to gonadotropins and, therefore, cannot be influenced using conventional in vitro fertilization stimulation protocols. Importantly, long-term exposure resulted in a "burn out" phenotype. Nevertheless, a short-term "molecular stimulation" approach could provide a means of promoting the growth of early stage follicles and fostering their survival to more advanced stages. This approach would benefit patients with unmet need (e.g., poor responders) by optimizing the growth and survival of residual follicles that typically undergo atresia before maturing to the hormone-responsive stages.

References: NA

Supported by: Internal CRMI

O-242 Wednesday, October 10, 2018 11:00 AM

ADJUVANT RECOMBINANT LH(RLH) OR GROWTH HORMONE (GH) TO THE ANTAGONIST PROTOCOL IN POOR RESPONDERS UNDERGOING IVF.

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OBJECTIVE: To evaluate the effectiveness of the addition of recombinant LH(rLH) or Growth hormone (GH) to the antagonist protocol cycles in poor responders undergoing IVF(In Vitro Fertilisation)

DESIGN: Single centre prospective randomised control trial at a tertiary care infertility centre from 1st April 2017 to 30th March 2018.

MATERIALS AND METHODS: One hundred and twenty poor responder patients selected as per Poseidon Group 3 and 4 for IVF were enrolled the study and were randomly divided into two groups. Group A (n = 64) received rLH (75 IU)+rFSH (225 IU) from Day 2 (D 2) of cycle with addition of GnRh antagonist from D 6 and group B (n = 56) received GH (4 IU)+rFSH (225 IU) from D 2 with addition of GnRh antagonist from D 6 . The primary outcome measured was pregnancy rate. The secondary outcomes measured were number of oocytes retrieved, number of embryos formed and miscarriage rates.

RESULTS: The number of retrieved oocytes was significantly higher in rLH/rFSH/GnRH antagonist group (Gp A) than GH/rFSH/GnRH antagonist group (Gp B), 9.35 ± 3.09 vs. 6.58 ± 2.91(p = 0.002) and the number of obtained embryos was also significantly higher in rLH/rFSH/GnRH antagonist group than GH/rFSH/GnRH antagonist group, 6.96 ± 2.82 as compared to 4.08 ±1.72 (p < .001). There were no significant differences between these group A & B regarding implantation, clinical pregnancy and miscarriage rate

Comparison of means for number of oocytes retrieved and number of embryos formed

Variables	Therapy	Number of patients	Mean	Std. Deviation	Std. Error	Unpaired t-test p-value
Number of oocytes retrieved	rLH	64	9.35	3.098	0.607	0.002
	GH	56	6.58	2.914	0.572	
Number of embryos formed	rLH	64	6.96	2.821	0.553	<0.001
	GH	56	4.08	1.719	0.337	

CONCLUSIONS: Addition of recombinant LH in antagonist protocol in poor responder patients significantly increased number of oocytes retrived and embryos formed when compared to addition of Growth hormone but there was no difference in pregnancy rates between the two groups.

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