P-289 Tuesday, October 15, 2013

SMALL RNAS: NEW CANDIDATES FOR THE REGULATION OF THE HUMAN CUMULUS-OOCYTE COMPLEX CROSSTALK. S. Assou, T. Al-Edani, A. Gala, D. Haouzi, A. Ferrieres, S. Hamamah. Université Montpellier 1, CHU Montpellier, Institute for Research in Biotherapy, Inserm U1040, Montpellier, Herault, France.

OBJECTIVE: To identify and quantify small RNAs, including microRNAs, in human cumulus cells (CCs) and mature MII oocytes and to characterize the biological relationships between miRNAs and the messenger RNA expression profiles of MII oocytes and CCs.

DESIGN: Mature MII oocytes and CCs were collected from women who underwent IVF/ICSI under COS.

MATERIALS AND METHODS: Using Illumina/deep-sequencing technology, we dissected the small RNAome of pooled mature MII oocytes and CCs (n=24 and n=20 respectively). The correlation between these microRNAs and their corresponding validated mRNA targets was investigated using in silico prediction algorithms. Using oligonucleotide microarrays, genome-wide gene expression was studied in mature MII oocytes or CCs. TaqMan miRNA assays were used to confirm the sequencing results. The functional roles of microRNAs, were validated in an in vitro system of primary cultures of human CCs.

RESULTS: Deep sequencing of small RNAs yielded more than one million raw reads. We identified known microRNAs that were abundant in MII oocytes (MIR100 and MIR10A) or CCs (MIR29a, MIR30d, and the LET7 family). Predicted target genes of the oocyte miRNAs were associated with regulation of transcription and cell cycle, whereas, genes targeted by CC miRNAs were involved in extracellular matrix and apoptosis. Comparison of the predicted miRNA target genes and mRNA microarray data resulted in a list of 224 target genes that were differentially expressed in MII oocytes and CCs, including CTGF (fold: 38, $p<0.0001$) and BMPR1B (fold: 15.4, $p<0.0001$) that are important for cumulus-oocyte communication.

CONCLUSION: This study provides the first characterization of the microRNA profile in human CCs and mature MII oocytes.. These results might help improving our understanding of the roles of miRNAs in oocyte maturation. Moreover, many of the identified miRNAs might be used as a tools to monitor the oocyte health, viability and competence and consequently to improve IVF/ICSI outcome.

Supported by: Ferring and Genevrier companies.

P-290 Tuesday, October 15, 2013

IMMATURE OOCYTE SYNDROME: OVULATION TRIGGERING WITH COMBINED hCG /GnRH AGONIST OR INCREASED DOSES OF HCG? P. Massart,[a,b] L.-M. Durand,[c] C. Sifer,[a] P. Piver,[c] J.-N. Hugues.[a] [a]Médecine de la Reproduction, Hopital Jean Verdier, Bondy, France; [b]Gynécologie, AMP, Centre Hospitalier Départemental, La Roche sur Yon, France; [c]Gynécologie Obstétrique, AMP, CHU de Limoges, Limoges, France.

OBJECTIVE: Immature Oocyte Syndrome (IOS) is usually defined by $<$ 70 % of retrieved metaphase II (MII) oocytes in IVF cycle. It can be explained by intrinsic abnormalities or inadequate stimulation. Ovulation triggering is critical for resumption of meiosis. As both LH and FSH are needed for oocyte final maturation in natural cycle, it has been suggested to trigger ovulation with a combination of hCG and GnRH agonist in case of oocyte immaturity. In this study we evaluated the interest of 2 strategies of ovulation triggering (dual trigger with combined hCG/GnRH agonist versus increased doses of hCG alone) in women with previous IOS.

DESIGN: Retrospective cohort pilot study.

MATERIALS AND METHODS: We included 66 patients with a history of IOS in a prior IVF cycle. 51 were excluded due to inadequate stimulation or oocyte pick-up difficulties (total oocytes/follicle 14 mm $<$80%) or premature triggering (MII/follicle 14 mm $>$80%). The other 15 patients were stimulated with an antagonist protocol, and randomized to receive either dual triggering (250μg r-hCG/Triptorelin 0,2 mg, group 1, n = 7) or high dose of hCG (500μg r-hCG, group 2, n = 8). The main outcome was the number of mature oocytes (MII) retrieved. Paired comparison was performed for statistical analysis.

RESULTS: Ovarian stimulation parameters did not differ significantly between both groups. In group 1, the mean number of MII oocytes and embryos significantly increased with the new trigerring strategy (3.4$\pm$0.7 vs 8.9$\pm$2.1 and 2$\pm$0.3 to 6.3$\pm$2.2 respectively). In group 2, the mean number of MII oocytes significantly increased (3.1$\pm$0.2 to 5$\pm$1.1) but the mean number of embryos/patient was not statistically different.

CONCLUSION: This pilot study assessing the comparative efficacy of dual trigger and increased doses of hCG for meiosis resumption shows that both strategies are effective. However, in terms of safety, dual trigger should be preferred to reduce the risk of hyperstimulation Syndrome. Further studies are required to compare the safety issue.

P-291 Tuesday, October 15, 2013

SELECTION OF IMMATURE HUMAN OOCYTES BY TIME-LAPSE TECHNOLOGY. M-J. Escribá, L. Escrich, N. Grau, Y. Galiana, A. Pellicer, M. Meseguer. IVF Laboratory, Instituto Universitario IVI Valencia, Valencia, Spain.

OBJECTIVE: To assess the presence of the meiotic spindle (MS) in *in vitro* matured (IVM) oocytes according to their maturational origin (GV or MI), nuclear maturation (NM) timing and post-MII aging (PA) and to correlate these variables with the oocyte's ability to activate parthenogenetically.

DESIGN: Experimental.

MATERIALS AND METHODS: Sixty-one VG and 37 MI oocytes were recovered from stimulated ovaries and cultured in a time-lapse incubator (Embryoscope) for 19.9$\pm$1.7hr. Following the protocol described by Escrich et al. (F&S, 2012), ova that reached the MII stage at $\leq$20.3hr of culture were selected and assessed for presence of the MS using PolScope technology (Oosight, EMB). Ova were activated at different PA by calcium ionophore and puromyicin and, their activation was assessed after 16-20hrs.

RESULTS: Significantly more MI matured than GV (73.0% vs. 49.2%), and the former required a shorter culture period in order to mature (6.2$\pm$7.0hr vs. 16.1$\pm$6.0hr; $p<0.01$). The percentage of MS detected was comparable regardless of maturational origin (average: 66.7%; $p>0.05$). However, this percentage was affected by the timing of NM; the MS was observed more frequently in GV that matured $\leq$16.8hr than in those that matured later (100% vs. 50%; p=0.006). Similarly, more MS were observed in MI that matured at $\leq$17.8hr than in those that matured later (70.8% vs. 0%; p=0.043). Concerning the effect of PA, the MS was detected more frequently in GV-derived ova aged for $\geq$2hrs than in younger samples (83.3% vs. 41.7%; p=0.018). In the MI group, no effect of PA was observed, probably because these oocytes were aged for longer than 2hr (95CI: 11.6-17.4hr). Activation rates were not affected by any of the variables studied (average: 84.2%).

CONCLUSION: Time-lapse technology allows the selection of ova that: 1) complete NM within a certain time frame; 2) have the MS; and 3) are capable of responding to an artificial stimulus.

Supported by: IVI Valencia.

P-292 Tuesday, October 15, 2013

DOES OOCYTE MORPHOLOGY ABNORMALITIES AFFECT THE PREGNANCY RATES OF SINGE EMBRYO TRANSFER ON DAY 5 OR DAY 3? L. Karakoç Sökmensüer, S. Gunalp, G. Bozdag, I. Selçuk, I. Esinler. Obstetrics and Gynecology IVF Unit, Hacettepe University, Ankara, Turkey.

OBJECTIVE: Aim of this study was to evaluate the impact of oocyte morphology on pregnancy rates in intracytoplasmic sperm injection (ICSI) cycles with the single embryo transfer (SET).