GENOMICS OF CUMULUS CELLS: NOVEL CONCEPT FOR PROVIDING BIOMARKERS CANDIDATES TO ASSESS IVF EFFICIENCY

S. Hamamah

ART/PGD Department, INSERM U° 1040, 'Early embryo development and human embryo stem cells'
Aranud de Villeneuve hospital; Montpellier-34295, France

Whatever the controlled ovarian hyperstimulation protocoles (COH) for classical IVF or ICSI, the cumulus-oocyte complex (COC) controls the early as well as the final stage of folliculogenesis. We have profiled protein expression in cumulus cells (CCs) of MII oocytes from a single patient undergoing cycles of classical IVF under two different protocols of ovarian hyper stimulation: recombinant-FSH versus hMG. We show that Cumulus cells express approximately 800 proteins of which the majority are expressed during the first 20 h after oocyte collection for classical IVF (Hamamah et al, 2006). When comparing protein expression profiles in cumulus cells from fertilized and unfertilized oocytes from the same hyperstimulation protocol, we have identified relatively small differences in protein expression, increased or decreased expression and de novo protein synthesis. In contrast, comparison of proteins expression patterns in cumuli from oocytes obtained from rFSH and hMG protocols reveals much more significant differences.

By using the DNA chip technique, our group observed, that 2,600 genes are over-expressed in human CCs when compared with MII oocyte samples (Assou et al., 2006). Among these genes, we confirmed the presence of previously-described genes such as *PTX3*, CYP19A1, PRDX2, versican and *HAS2*. We also reported other over-expressed genes in CCs in comparison with MII oocyte samples such as *TNFSF13/APRIL*, a ligand of the TNF superfamily, which was described to bind to proteoglycan inducing a survival signal conveyed by this cytokine to target cells. Because CCs over-express several proteoglycans such as *versican*, *APRIL* could mediate a comparable trophic signal from the oocyte to the surrounding CCs. In addition, tumour necrosis factor alpha-induced protein 6 (*TNFAIP6*), a hyaluronan binding protein involved in CCs expansion. Beyond of its role as biomarker, *TNFAIP6* gene has also been reported to be associated with the expression of common receptor of luteinizing hormone/human chorionic gonadotrophin (*LH/hCGR*) in CC (Haouzi et al., 2009).

Thousands of genes screened in CCs, were intensively used to identify the biomarkers related to oocyte competence, which is defined as the intrinsic ability of oocytes to undergo meiotic maturation, fertilization and embryonic development Using the same approach, genes expressed in CCs used as biomarkers associated with embryo quality and pregnancy outcome (Assou et al., 2008; 2010) have also been identified. All these studies target the gene expression profile of human CCs, a source of cells reflecting the biology and competence of both oocytes and embryos and leading consequently towards a non-invasive method of predicting IVF outcome. However, to date, although microarray technologies have allowed gene expression profiling of such tiny specimens as oocytes and CCs, limited data are known regarding the close dialogue between the human oocyte and their associated CCs. The unraveling of this information is fundamental to understanding the complexity of gamete physiology, including the dialogue between the oocyte and its environment which is necessary for both oocyte and CC function.

By comparing gene expression profiles of CCs according to embryonic quality and pregnancy outcome, our group reported for the first time a specific transcriptomic signature, including 630 genes associated with pregnancy outcome. The majority of the differentially expressed genes were up-regulated, suggesting that transcriptional activation in CCs is essential to the acquiring embryonic competence. Among up-regulated CC genes correlated with a pregnancy, we validated BCL like protein 11 (BCL2L11) and phosphoenolpyruvate carboxykinase 1 (PCK1) expressions, which are involved in apoptosis and gluconeogenesis, respectively (Assou et al., 2008). We also reported the decreased expression of the nuclear factor 1B (NFIB) gene in CCs, which is a nuclear transcription factor that has previously been shown to play a role in regulating tissue-specific gene expression during mammalian embryogenesis.

The objectives of my lecture are to update studies on transcriptomic profiles of the oocyte–cumulus complex and to suggest CCs as biomarkers for oocyte and embryo selection. In addition, we propose testing these biomarkers to predict embryo and pregnancy outcomes by conducting a study in which the embryo selection occurs according gene expression profile of CCs.

References:

Hamamah S, Matha V , Anahory T, Loup V, Dechaud H, Hedon B, Fernandez A, Lamb N. Coparative protein expression profiling in human cumulus cells in relation to oocyte fertilization and ovarian stimulation protocol. RBM Online. 2006, 13: 807- 814. ▪ Assou S, Anahory T, Pantesco V, Pellestor F, Klein B , Dechaud H, de Vos J, Hamamah S. The human cumulus-oocyte complex gene expression profile. Human Reproduction, 2006, 21 : 1705-1719 ▪ Assou S, Houzi D, Mahmoud K, Aouacheria A, Guillemin Y, Pantesco V, Reme T, Dechaud H, de Vos J, Hamamah S. A non-invasive test for assessing embryo potential by gene expression profiles of human cumulus cells: a proof of concept study. Mol Hum Reprod. 2008; 14: 711-719 ▪ Haouzi D, Assou S, Mahmoud K, Hedon B, de Vos J, Dewailly D, Hamamah S. LH/hCGR gene expression in human cumulus cells is linked to the expression of the extracellular matrix modifying gene TNFAIP6 and to serum estradiol levels on day of hCG administration.

Human Reproduction. 2009, 24:2868-2878 ▪ Assou s, Haouzi D, de Vos J, Hamamah S Human cumulus cells as biomarkers for embryo and pregnancy outcomes Mol Hum Reprod. 2010;16:531-538