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Transcriptomic analysis of follicular cells provides information on the chromosomal status and competence of unfertilized oocytes

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"A noninvasive test based on cumulus cells, allowing the identification of the embryo with the highest potential to survive and implant, would revolutionize assisted reproduction techniques..."

A 'competent' oocyte is one that is able to undergo the necessary cytoplasmic and meiotic maturation, go through fertilization, support early embryonic development and lead to a successful pregnancy and a healthy live birth. Among the many events critical to oocyte viability, those occurring during the preovulatory stage within the egg and its surrounding somatic cells are considered to be of particular importance. The somatic cells that enclose the oocyte are called cumulus cells (CCs) and, together with the egg, form what is known as the cumulus–oocyte complex. CCs are differentiated granulosa cells arranged in layers surrounding the oocyte in antral follicles. A bidirectional communication between the oocyte and its CCs is established with the formation of gap junctions, enabling a continuous exchange of proteins and metabolites between the two types of cells. These intimate connections allow the CCs to fulfil a vital role in the support and resourcing of the maturing oocyte.

During assisted reproduction techniques (ART), oocytes are collected from the ovaries and the CCs are typically removed. However, these discarded cells may harbor valuable information concerning the oocyte with which they were so closely associated. We have hypothesized that the chromosome content of the oocyte and information about its general competence may be reflected in a residual impression left in the transcriptome of the surrounding CCs. This article will focus on insights into oocytes gained from gene-expression analysis of CCs, highlighting their potential as markers of oocyte quality.

The influence of the follicular environment on oocyte development & competence

Meiosis in the human begins in the fetal ovary at around 11–12 weeks of gestation when newly formed oocytes enter prophase I [1]. Recombination and exchange of genetic material between homologous chromosomes follows, and once completed, the oocytes enter a protracted arrest stage known as dictyate. At this stage, oocytes are surrounded by flattened pregranulosa cells and together form primordial follicles. These follicles remain quiescent until puberty, when under the influence of two gonadotrophins, follicle-stimulating hormone and luteinizing hormone, they are recruited to further mature and develop. Usually only one follicle will complete growth each month, and its enclosed oocyte will resume and complete meiosis I with the extrusion of the first polar body, and arrest again at metaphase

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of meiosis II [2]. The second meiotic division will only resume after fertilization. The surrounding follicular cells also undergo cytodifferentiation and proliferation, under hormonal influence [3]. This leads to the formation of two distinct cellular populations, the mural granulosa cells, which line the wall of the follicle and the CCs, which surround the oocyte [4,5].

> "...cumulus cells surrounding aneuploid oocytes were less transcriptionally active, compared with the cumulus cells of normal oocytes."

One of the main functions of CCs is to provide nutritional and metabolic support to the oocyte, as the latter is a poor metabolizer of glucose and is unable to generate the amino acids necessary for energy production [6,7]. Such substrates are thought to be provided by the CCs via an oocyte–CC regulatory loop. It was recently demonstrated in mice, that two secreted factors of oocyte origin, namely growth differentiation factor 9 and bone morphogenetic factor 15 (BMP15), regulate both glycolysis and cholesterol biosynthesis in its surrounding CCs prior to the luteinizing hormone surge [6]. Transcriptomic analysis of CCs obtained from human oocytes that led to successful pregnancies compared with those associated with failure of embryo implantation yielded similar results [7]. It was evident that in CCs from oocytes producing pregnancies there was a general upregulation of phosphoenolpyruvate carboxykinase 1 (*PCK1*), which during gluconeogenesis catalyses the formation of phosphoenolpyruvate from oxaloacetate. Other genes associated with *PCK1* were also upregulated. These two studies lead to the conclusion that an active CC metabolism is essential for both oocyte maturation and competence.

Another factor that could have an adverse effect on the oocyte is oxidative stress caused by reactive oxygen species. CCs are capable of protecting the oocyte by producing antioxidant enzymes, such as superoxide dismutases (SOD). Interestingly, a decline in SOD activity has been observed in CCs in relation to advancing female age. Increasing age is the single most powerful factor in oocyte competence, with the risks of aneuploidy, poor preimplantation embryo development and failure to implant in the uterus all rising dramatically with advancing years, particularly from the mid-30s onwards. Increased SOD activity has been associated with successful ART outcomes [8]. Hence, CCs appear to constantly monitor the follicular environment and, by regulating expression of antioxidants and other molecules, ensure optimal oocyte development.

As well as maintaining an optimal microenvironment, CCs have a crucial function mediating the transmission of signals to and from the developing oocyte. Signaling pathways regulating CC proliferation and apoptosis are likely to be of prognostic significance. Inferior oocyte quality and poor embryo development have both been correlated with a high incidence of CC apoptosis [9,10]. A recent study has shown that, similar to CC metabolism, the oocyte secreted factors BMP15 and BMP6 act to protect the surrounding CCs from apoptosis, by establishing a morphogenic paracrine gradient of BMPs [11].

The identification of novel oocyte viability biomarkers

The fact that CCs share the same follicular environment as the oocyte and are in close communication with it via gap junctions and local paracrine factors, suggests their analysis may serve as a surrogate for direct analysis of the oocyte itself. In addition, the fact that CCs can easily be collected without compromising the oocyte, makes them attractive targets for the development of noninvasive assays of oocyte competence [12].

A combination of microarray and real-time PCR technology was employed in an attempt to elucidate specific patterns of CC gene expression associated with embryo morphology (a key indicator of viability) and pregnancy outcome [7]. This study showed that upregulation of two CC genes, *BCL2L11* and *PCK1*, involved in apoptosis and glugoneogenesis, respectively, and downregulation of the transcription factor *NFIB* was related to the ability of oocytes to generate embryos capable of implanting and producing live births. This research group has gone on to apply these findings clinically, using these three genes as biomarkers to select competent oocytes [7].

> "...initial data are promising, suggesting that the wide-scale clinical application of cumulus cell-based tests may not be far off."

In a different study, the expression of 13 genes regulating CC metabolism, signaling and extracellular matrix formation was examined, so as to identify patterns associated with the oocyte's ability to lead to a live birth, after elective single embryo transfer [13]. The findings obtained suggested that CCs from developmentally competent oocytes had a general upregulation of the investigated genes. In addition, among the 13 genes, *VCAN*, *PTGS2*, *GREM1* and *PFKP* were highlighted as having expression patterns indicative of good quality oocytes [13].

The aforementioned studies focused on the examination of CC gene expression in relation to oocyte/embryo quality and viability. However, another factor, not considered in those investigations, is aneuploidy. Chromosome abnormality is perhaps the single most important factor in oocyte potential. It is extremely common (affecting more than half of all oocytes from women over 40 years of age) [14] and almost always lethal to the developing embryo or fetus. The nature of female meiosis makes it prone to chromosome malsegregation errors, a problem that increases dramatically with advancing age. Methods for testing oocytes for aneuploidy are already well established, based upon biopsy of the polar bodies followed by testing using methods such as microarray comparative genomic hybridisation. However, such methods are labor intensive, expensive and somewhat invasive, involving the breach of the zona pellucida, the protective envelope covering the oocyte [14]. Noninvasive methods for testing oocyte aneuploidy are highly desirable.

It is possible that the presence of a chromosome abnormality in the oocyte could disrupt signaling and intercellular communication, leading to alteration of gene expression and protein production in the surrounding CCs. It might also be the case that an inappropriate follicular microenvironment (e.g., hypoxic

or otherwise poorly resourced) could predispose the oocyte to meiotic errors while simultaneously affecting the gene expression of somatic cells in the vicinity. To further investigate this idea, we set out to compare the gene expression patterns seen in CCs of normal and aneuploid oocytes, hoping to gain an insight into the follicular environment of oocytes that become chromosomally abnormal and to identify potentially useful noninvasive markers of oocyte physiology, aneuploidy and competence [15,16]. An interesting finding was that the CCs surrounding aneuploid oocytes were less transcriptionally active, compared with the CCs of normal oocytes. A reduction in overall mRNA content seems to be one of the few consistent findings between different studies assessing CC gene expression and oocyte potential. Of the 29,098 genes initially examined via microarray, 729 were differentially expressed between the two groups of samples, the vast majority of these (457 genes) being downregulated in the CCs of aneuploid oocytes [15]. Ninety six of the candidate genes were selected for further validation using real-time PCR. This analysis highlighted 14 genes with highly statistically significant expression differences ($p < 0.01$), all of which were underexpressed in CCs associated with aneuploid oocytes. The genes were involved in the regulation of various biological processes, including metabolism, signaling, apoptosis, and transport. Among these genes was *SPSB2*, the expression of which seemed to correlate both with the chromosome status of the oocyte and also with its ability to lead to a live birth. SPSB2 is involved in proteasomal degradation, and its downregulation in the CCs of aneuploid oocytes may result in the accumulation of excessive levels of abnormal/redundant proteins [16].

The development of noninvasive tests for oocyte assessment

CC gene expression is affected by the conditions within the follicle, an environment also experienced by the oocyte during the final stages of its nuclear and cytoplasmic maturation. This has led to the suggestion that it might be possible to define a transcriptomic signature predictive of a 'healthy' oocyte. A noninvasive test based on CCs, allowing the identification of the embryo with the highest potential to survive and implant, would revolutionize ART, potentially leading to dramatic improvements in clinical pregnancy rates. Significant reductions in the incidences of miscarriage and aneuploid syndromes (such as Down's syndrome) are also likely. Furthermore, a detailed understanding of the interactions between the oocyte and its CCs could lead to improvements in oocyte *in vitro* maturation protocols and the optimization of gonadotrophin dosing during ART cycles, maximizing the availability of high quality euploid oocytes. A significant amount of work remains to be done, but initial data are promising, suggesting that the wide-scale clinical application of CC-based tests may not be far off. However, it remains the case that most transcriptomic studies undertaken to find novel CC biomarkers have identified different candidate genes. The lack of uniformity or overlap between studies suggests that there may be confounding variables affecting gene expression, perhaps of a patient, clinic, treatment or etiology-specific nature. The characterization of a universally applicable CC-based test for oocyte competence may prove to be a challenge, but the potential benefits make it a goal well worth pursuing.

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