Impact of maternal aging on the molecular signature of human cumulus cells

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Objective: To investigate the impact of maternal aging on the molecular signature of cumulus cells.
Design: Experimental study.
Setting: Research laboratory.
Patient(s): Patients, young fertile oocyte donors (n = 40) and infertile women of advanced maternal age (40–45 years; n = 48), donated, with Institutional Review Board consent, cumulus cells during routine infertility treatment.
Intervention(s): None.
Main Outcome Measure(s): Proteomic and gene expression profiles of cumulus cells.
Result(s): Proteomic analysis identified a total of 1,423 cumulus cell proteins. Statistical analysis revealed 110 (7.7%) proteins to be differentially expressed in relation to female aging (>1.5-fold change). Pathway annotation revealed significant involvement in metabolism (ACAT2, HSD17B4, ALDH9A1, MVK, CYP11A1, and FDFT1), oxidative phosphorylation (OP; NDUFA1, UQCRC1, MT-ATP6, ATP5I, and MT-ATP8), and post-transcriptional mechanisms (KHSRP, SFPQ, DDX46, SNRPF, ADAR, NHPL1, and U2AF2) relative to advanced maternal age. Gene expression analysis also revealed altered profiles in cumulus cells from women in their early to mid-40s.
Conclusion(s): This novel study reveals that the cumulus cell molecular signature, at both the gene and protein level, is impacted by advanced maternal aging. A compromised follicular environment is evident with altered energy metabolism and post-transcriptional processes. (Fertil Steril® 2012;98:1574–80. ©2012 by American Society for Reproductive Medicine.)
Key Words: Cumulus cells, aging, proteomics, metabolism and splicing

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With increased life expectancy and changing sociological factors, women in developed countries are choosing to have children later in life. However, there is an abrupt decline in fertility as women enter into their fifth decade, resulting in decreased implantation and pregnancy rates as well as increased spontaneous miscarriage. In part, this is due to the progressive loss of oocytes, which accelerates as women reach their early 40s. But perhaps the major factor in the etiology of age-related female infertility is the decline in oocyte quality associated with factors including but not limited to chromosomal aneuploidy and mitochondrial dysfunction (1, 2).

At birth, women have their complete oocyte pool in the form of primordial follicles. These follicles contain oocytes arrested in meiotic prophase I that will remain quiescent until recruited to complete oogenesis (3). This recruitment is a steady process activated by a complex array of bidirectional signaling between the oocyte and the surrounding somatic cells of the follicle. Oogenesis is coordinated with folliculogenesis, which includes complex molecular interactions between the oocyte and neighboring somatic granulosa cells (GCs) within the follicle. Oocytes secrete important mitogenic factors, such as growth differentiation factor-9 (GDF-9) and bone morphogenetic factor 15 (BMP-15), that influence follicular growth and development (4, 5). These factors are in turn regulated by the GCs themselves.

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This bidirectional communication between the oocyte and GCs is essential for oocyte competence.

Cumulus cells (CCs) are a specialized type of GCs that are in immediate vicinity of the oocyte with direct channels called transzonal projections (TZP) for molecular communication (4, 6). CC expansion that takes place in the periovulatory period is necessary for normal oocyte development in vivo and denotes the optimal stage for fertilization (7). CCs play a critical role in the development of the follicle and therefore may have the potential to provide meaningful information regarding oocyte viability.

To date several groups have used quantitative real-time polymerase chain reaction (Q RT-PCR) and microarray technologies to analyze CC gene expression profiles in association with oocyte quality, embryo development, and pregnancy outcome, with promising results (8). In addition, a comparative study of CC protein expression profiles in relation to oocyte fertilization and ovarian stimulation protocols indicated that the type of hormone treatment was significant and that there were dissimilarities in protein patterns between patients even when using the same stimulation protocol (9). Further, Lee et al. investigated CC gene expression with female reproductive aging (10). In this study, two genes, CKB and PRDX2, involved in energy maintenance and redox regulation, respectively, were observed to be increased in CCs from infertile women >38 years old.

Little is known about the effect of maternal aging on CC biological function. The aim of this novel study was to analyze the molecular signature of CCs at both the protein and gene level to identify age-related changes that could potentially reflect the decline in oocyte developmental potential.

MATERIALS AND METHODS

Patients/Subjects

Patients donated CCs with Institutional Review Board consent during routine infertility treatment at the Colorado Center for Reproductive Medicine between 2010 and 2011. CCs were obtained from infertile women of advanced maternal age (AMA; 40–45 years old; n = 48) and young fertile oocyte donors (20–33 years old; n = 40). In more detail, patient samples used for liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis in part I included AMA (n = 8) and donor (n = 8). Protein expression validation (part IIA) was performed on the same patient samples as in part I in addition to patient samples from AMA (n = 8). Part IIB gene expression analysis included patient samples from AMA (n = 32) and donor (n = 32; see Supplemental Fig. 1). Subjects were considered good ovarian responders on agonist protocols based on infertility history, prior response, and the results of ovarian reserve testing. As anticipated, young fertile oocyte donors presented with no known fertility problems and a better profile of ovarian reserve markers (Table 1). Routine oocyte retrieval, intracytoplasmic sperm injection, embryo culture, and transfer were performed as described elsewhere (11).

CC Collection

CCs were isolated by mechanical stripping after oocyte collection.

Protein isolation. Pooled CCs from only sibling metaphase II oocytes were washed twice with phosphate-buffered saline (PBS; Fisher Scientific) and centrifuged at 1500 *g* for 5 minutes at room temperature. CC pellets were frozen in MPER buffer containing Halt protease inhibitor cocktail (Thermo Scientific) and stored at −80°C until analysis (Supplemental Fig. 1).

RNA isolation. Pooled CCs from only sibling metaphase II oocytes were washed in PBS/10% bovine serum albumin and centrifuged at 3,000 *g* for 3 minutes at room temperature. CCs pellets were resuspended in 10 μL Arcturus picopure RNA extraction buffer (Applied Biosystems) before being snap frozen in liquid nitrogen and stored at −80°C until analysis (Supplemental Fig. 1).

Protein Sample Preparation

CC samples were thawed on ice and centrifuged at 10,000 *g* for 10 minutes at 4°C to remove cellular debris. For mass spectrometry (MS) analysis, CC samples were pooled from eight AMA infertile women and from eight young oocyte donor controls. Total protein concentration was determined using a Bradford assay (Invitrogen). A portion of the samples (15 μg) was separated by one-dimensional SDS-polyacrylamide gel electrophoresis (Invitrogen), and 24 individual bands were cut out from each sample lane. A standard in-gel digestion protocol was used before MS analysis as described elsewhere (12). Further details can be found in Supplemental Methods.

LC-MS/MS and Analysis

Digested CC protein samples were analyzed on a LTQ Orbitrap Velos mass spectrometer (Thermo Fisher Scientific) coupled to an Eksigent nano-LC-2D system through a nanoelectrospray LC-MS interface (Supplemental Methods). All samples were run in duplicates. MS/MS spectra were extracted from raw data files and converted Mascot generic files using an in-house script. Mascot (ver. 2.2; Matrix Science Inc.) was used to perform database searches against the human subset SwissProt database of the extracted MS/MS data. A detailed description of the LC-MS/MS run and analysis can be found in Supplemental Methods.

Scaffold (ver. 2, Proteome Software) was used to validate MS/MS-based peptide and protein identifications. Total peptides per protein was calculated in each group and normalized to total peptides per sample. These values were used for semiquantitative comparison between the two groups. Student's *t*-test was used for assessing the statistical significance where proteins were considered to have significant differential expression when $P<.05$ and there was at least 1.5-fold difference in the mean total peptides per protein. Differentially expressed proteins were subjected to pathway analysis using DAVID (EASE ver. 2.0).