TRANSCRIPTOME ANALYSIS OF HUMAN CUMULUS CELLS REVEALS HYPOXIA AS THE MAIN DETERMINANT OF FOLLICULAR SENESCENCE

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Objective: To further characterize pathways leading to human oocyte aging using cumulus cells (CCs) RNA sequencing, immuno-fluorescent staining and weighted gene co-expression network analysis
Design: Basic research comparative study.
Materials and methods: Cumulus cells clumps (CCs) were isolated from MII oocytes collected from patients 35 years ("younger", n=10) and 40 years old ("older", n=11) undergoing ICSI for male factor infertility. CCs were individually processed for RNA extraction, library preparation and sequenced on Illumina HiSeq 2000 platform. Gene enrichment and gene ontology analysis were used to define upregulated gene networks and interactions in the two cohorts. Significance of differentially expressed genes was assigned when both p value and false discovery rate were 0.05. Validation was performed with qPCR. Immuno-fluorescent (IF) staining was used to assess iNOS protein expression in CCs from both Older and Younger patients. Co-expression network analysis (WGCNA) was applied to identify clusters of co-expressed genes whose expression is altered in senescent CCs.
Results: A core of 45 genes was differentially expressed between the younger and older cohorts. In CCs collected from the younger group, genes involved in the extracellular matrix (ADAMTS14) organization and membrane composition were significantly upregulated (p0.001 and FDR 0.05) compared to the older. WGCNA analysis corroborated findings of significantly increased expression of genes involved in cellular response and those participating in mitochondrial translation, Wnt signaling and mTOR pathways in the younger (p0.001 and FDR 0.05). CCs of oocytes from older patients showed an overrepresentation of genes involved in the oxidative stress cascade (iNOS), cell death (NR4A3) and genes involved in the Hypoxia-related pathway (p0.001, FDR 0.05). Network analysis revealed a significant enrichment of apoptosis and advanced glycation end-products pathways. IF staining of CCs revealed higher amount of iNOS protein confirming hypoxic follicular environment in older patients (p0.05).
Conclusions: Hypoxic stress, apoptosis and advanced glycation end-products are markers of oocyte aging. Chronic stress from the hypoxic follicular environment impairs the CCs trophic support and is the most likely event responsible for oocyte aging. CCs can be new markers to assess oocyte senescence and competence