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IMPAIRED MITOCHONDRIAL STRESS RESPONSE IN CUMULUS CELLS IS ASSOCIATED WITH INCREASED APOPTOSIS AND ACCELERATED FOLLICULAR DEPLETION.

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OBJECTIVE: Caseinolytic peptidase P (CLPP) mediates degradation of mitochondrial misfolded proteins to maintain homeostasis under metabolic stress. CLPP is a key factor for mitochondrial function and targeted global germline deletion of Clpp results in female infertility and accelerated follicular depletion. In this study, we aimed to determine the mechanism of reproductive dysfunction in this model, by investigating molecular and ultrastructural changes that take place in cumulus cells lacking CLPP.

DESIGN: Experimental study.

MATERIALS AND METHODS: RNAseq analysis was performed in pooled CCs (approx 50), derived from cumulus oophorus complexes (COCs) collected from 3- and 6- months of Clpp-knockout (KO) and wild type (WT) female mice (n¼3 mice per group), 48 hours after 5 IU PMSG injection. cDNA was amplified with Smart-seq V4 Ultra Low Input RNA kit. RNAseq libraries were constructed with Nextera XT kit (Illumina) and sequenced on Illumina HiSeq 2500 platform. Reads were aligned to mouse (mm10) genome by Tophat (v.2.0.10), and gene expression values were calculated as FPKM using Cufflinks 2.1.1. Genes were deemed differentially expressed if they showed an adjusted FDR p-value < 0.05. Pathway analysis was performed using IPA. Cumulus cell mitochondrial morphology and dynamics were assessed using Tecnai 12 Biotwin Electron Microscope. Quantitative reverse transcription polymerase chain reaction (qRT-PCR) was used to determine mRNA expression. Immunofluorescence (IF) was used to detect markers of apoptosis (TUNEL) and cell proliferation (PCNA, Ki67) in ovarian sections and COCs. FERTILITY & STERILITY_ e99

RESULTS: RNA sequencing revealed 1,079 and 623 differentially expressed genes in KO CCs compared to WT in 3- and 6-month-old mice, respectively. Among them, 163 genes were common differentially expressed at both age groups. Pathway analysis revealed apoptosis and phagosomelysosome pathways to be uniquely affected at 3- and 6-month-old KO CCs, respectively. IF microscopy for apoptotic and cell proliferation markers in ovarian sections and COCs confirmed RNAseq findings with increased immunoreactivity for TUNEL and decreased expression of Ki67 and PCNA ($p<0.05$). Electron microscopy revealed significant impairment of mitochondrial dynamics in Clpp-deficient cumulus cells with lower aspect ratio (length/width; 1.92 _ 0.04 vs. 1.64 _ 0.04, $p<0.0001$). qRT-PCR showed a significant decrease in expression of genes involved in mitochondrial dynamics, Mfn1, Mfn2 and Opa1 ($p<0.05$).

CONCLUSIONS: Impaired mitochondrial stress response in cumulus cells with targeted deletion of Clpp is associated with significant changes in CC transcriptome and mitochondrial dynamics that culminate in increased apoptotic cell death and accelerated follicular depletion. The relevance of these parameters in women undergoing IVF and whether they can be exploited to improve treatment outcomes remain to be investigated.