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Mitochondrial stress responses in bovine cumulus cells and oocytes matured under lipotoxic conditions: A proteomic insight

W.F.A. Marei*[1], G. Van Raemdonck[2], G. Baggerman[2,3], P.E.J. Bols[1], J.L.M.R. Leroy[1];

[1]Gamete Research Centre, Department of Veterinary Sciences, University of Antwerp, Wilrijk, Belgium, [2]Centre for Proteomics (CFP), University of Antwerp, Wilrijk, Belgium, [3]VITO, MOL, Belgium

Obesity and upregulated lipolysis are commonly associated with increased free fatty acid (FFA) concentrations, predominantly palmitic acid (PA), in blood and ovarian follicular fluid, which have been strongly linked with reduced oocyte quality. Mitochondria are known to play a central role in regulating cellular stress responses to liptoxicity, which is well described in somatic cells. Although mitochondrial functions in oocytes are crutial for developmental competence, their stress response capacity has not been clearly described. Networking with endoplasmic reticulum unfolded protein responses (UPRs) and protein translation may be different in oocytes compared to somatic cells. Understanding these mechanisms is important to develop treatments. The aim of this study was to compare PA-induced stress responses in oocytes to those in cumulus cells (CCs). We exposed bovine cumulus oocyte complexes to pathophysiological PA concentration (150µM) or solvent during in vitro maturation (24h) as a model. Then, the CCs were separated from the oocytes (from pools of 120 COCs per treatment per replicate, 3 replicates) and their proteomic profiles were examined using shotgun proteomic analysis with tandem mass tags. Functional analysis of the differentially regulated proteins (DRPs; $P<0.05$, fold change >10%, FDR <5%) was done using Ingenuity® Pathway Analysis (IPA®; Qiagen). A total of 1843 and 1275 proteins were identified in CCs and oocytes, respectively, of which 86 and 54 were differentially regulated by PA. In CCs, 20/86 proteins were mitochondrial, 16 of which were downregulated. Canonical pathway analysis in CCs showed that pro-apoptotic UPRs, mitochondrial dysfunction and other related redox regulatory, metabolic and apoptotic pathways were the most affected. In the enclosed oocytes, 12/54 proteins were mitochondrial, 8 of which were upregulated. Functional analysis of the DRPs in oocytes suggests that pro-survival mechanisms were predominant. Mitochondrion-specific H_2O_2-scavenging enzyme (peroxiredoxin-3), mitochondrial trifunctional protein (HADHB), heat shock protein A8 (HSPA8), as well as the NRF2-mediated Oxidative Stress Response, among others, were key regulatory mechanisms induced by PA in the oocytes. However, an increase in the relative abundance of Cytochrome C was evident, which may trigger apoptosis. This was accompanied by SLC24A5 upregulation that negatively regulates mitochondrial outer membrane permeabilization and may prevent such apoptotic trigger. These data show that the mitochondria in oocytes, despite being structurally immature, regulate adaptive signalling pathways in response to metabolic stress. Although the proteomic changes in oocytes were predominantly anti-apoptotic, certain defective pro-apoptotic changes were identified. These data provide a unique insight into the mitochondrial adaptive signalling pathways in metabolically-compromised oocytes, and indicate specific mitochondrial target pathways through which the developmental capacity of metabolically-compromised oocytes can be improved or protected.

KEYWORDS
Infertility
Lipotoxicity