Animal Reproduction

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Folliculogenesis, oogenesis and superovulation

DISORDERS OF PYRUVATE METABOLISM ALTER THE METABOLIC AND THE TRANSCRIPTIONAL PROFILE OF BOVINE OOCYTES

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Resumo

Previous research from our group has shown the importance of pyruvate metabolism in the epigenetic reprogramming of bovine oocytes during maturation. More specifically, oocytes matured in the presence of sodium dichloroacetate [DCA, a stimulator of pyruvate oxidation in acetyl-CoA] or sodium iodoacetate [IA, a glycolysis inhibitor] showed increased mitochondrial membrane potential (MMP) and changes in the dynamic of lysine 9 histone 3 (H3K9) deacetylation. In the present work, we characterize the metabolic pathways involved in this mechanism and the molecular consequences of the modulation of pyruvate metabolism during in vitro maturation (IVM). Oocytes were IVM for 24h in three experimental groups: Control [IVM medium], DCA [IVM medium supplemented with 1.5 mM DCA], or IA [IVM medium supplemented with 5 µM IA]. The metabolic profile of single oocytes (at least 5 oocytes/3 replicates/ group) was analyzed at the end of IVM (metabolome, Raman spectroscopy; lipid droplets, Nile Red, Sigma; and reactive oxygen species (ROS), CellRox Green®, ThermoFisher Scientific). The images were acquired using a fluorescence microscope and analyzed by Fiji software. Raman spectra were processed and analyzed using the Spectrograpy 1.2.15 software. Peak attributions were done according to previous references. Results were compared by Student's t-test (treatment vs. control) considering $P<0.05$. Changes in transcript content were assessed by RNASeq analysis (5 oocytes/3 replicates/per group). Differentially expressed genes (DEGs) were assessed using the DESeq2 R package considering a $P<0.05$ and absolute log2 fold change >1 and the enrichment analysis was done by submitting the lists of DEGs to ClusterProfiller. The oocytes from DCA and IA groups had a decrease in lipid droplets (DCA: $P=0.003$; IA: $P=0.004$) and an increase in the intensity of Raman bands attributed to fatty acids (DCA: $P=0.0001$ and $P=0.0005$; IA: $P=0.0285$ and $P<0.0001$), suggesting that beta-oxidation may be responsible for the higher MMP previously identified. This was followed by higher levels of ROS content in treated groups (DCA: $P=0.0036$; IA: $P<0.0001$). A total of 148 and 356 DEGs were identified in DCA and IA groups, respectively. The enrichment analysis revealed that the control group presented more transcripts related to the ROS pathway than the DCA group while mRNA surveillance and oxidative phosphorylation pathways were enriched in the control oocytes compared to the IA group. In conclusion, disorders in pyruvate metabolism during maturation alter the lipid and the mitochondrial metabolism, with consequences for the mRNA content of bovine oocytes.

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