Animal Reproduction

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Supporting biotechnologies Cryopreservation and cryobiology, diagnostic imaging, molecular biology and "omics"

Metabolic screening of inner cell mass of bovine blastocysts correlates with its epigenetic and molecular status

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Resumo

The metabolic dynamics of the preimplantation embryo is a consistent matter when focusing on its viability and adequate developmental status. Nevertheless, recently, it has been intelligible the relationship between the metabolism and the transcriptional profile of the embryo as well as the mechanisms that build up this scenery, such as chromatin availability and the distribution of epigenetic marks. We demonstrate this connection by matching the metabolic screening of inner cell mass (ICM) of bovine blastocysts by Raman spectroscopy (Bruker), with their epigenetic and molecular status. Bovine embryos were cultured into 3 experimental groups according to culture medium [synthetic oviductal fluid with amino acids (SOFaa) + 4% bovine serum albumin]: Control (no additional supplementation), sodium dichloroacetate (DCA; 2 mM; acetyl-CoA conversion stimulator) and sodium iodoacetate (IA; 2 µM; glycolysis inhibitor). Blastocysts were collected on Day 7 and their ICM analyzed for mitochondrial membrane potential (4 blastocysts/rep./group; 4 rep. - fluorescence), H3K9 and H3K27 acetylation and H3K27 trimethylation [Nucleus=experimental unit (min. 100, max. 360/ antibody/group) – immunostaining] and transcriptional profiling by RNA sequencing [3 ICM/group/rep. (3 rep. – Illumina RNA-Seq)]. Data were submitted to normality test and treatment groups were compared to control using t-test or Mann–Whitney test for non-parametric data (mean ± s.e.) considering $P < 0.05$. RNA-Seq data were analyzed by DESEq 2 and transcripts with Padj <0.05 were submitted to gene ontology by DAVID. ICM metabolomics showed DCA group with lower levels of fructose-6-P, phosphoenolpyruvate and an unexpected decrease in acetyl-CoA levels, suggesting a higher influx to the pentose phosphate pathway, which may represent an adaptive response to DCA. The lower levels of acetyl-CoA in blastocysts were followed by lower mitochondrial membrane potential. Still in DCA group, higher levels for H3K27ac were found together with lower levels of H3K9ac, suggesting that the presence of acetyl-CoA may be decisive for H3K9ac. IA-derived blastocysts also presented lower acetyl-CoA levels when compared to control, as expected by the metabolic impairment proposed on the glycolytic pathway. Raman spectroscopy indicated increase in fatty acids, suggesting an attempt of the embryo to maintain the energy production by mobilizing fatty acids through beta-oxidation. Furthermore, blastocysts from the IA group, showed lower levels of H3K27ac together with higher levels of H3K27me3, indicating the competitive profile regarding these two modifications. Transcriptome analysis indicates that those metabolic and epigenetic alterations resulted in molecular differences mainly associated to metabolic processes, establishment of epigenetic marks, control of gene expression and cell cycle, outlining the complex and close relationship composing the metaboloepigenetics boundaries in the preimplantation bovine blastocyst.