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Differential Transcript Usage in Bovine Blastocysts Exposed to Varying Concentrations of Energy Substrates

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ABSTRACT

According to the Developmental Origins of Health and Disease (DOHaD), energetic imbalance during critical developmental periods may impact long-term the offspring health, predisposing to the development of cardiovascular and metabolic diseases in adulthood. While epigenetic processes have been proposed as bona fide mediators of DOHaD, the mechanisms establishing its early onset remain poorly understood. Recently, the expression of different transcript isoforms of given genes, defined as differential transcript usage (DTU), has been observed during development and implicated in the onset of various diseases, making DTU a possible player in the landscape of developmental plasticity. Notably, transcript isoforms can arise by different mechanisms, among which change of the transcription start/end sites, alternative splicing, or activation/repression of different promoters. Consequently, functionally different transcript isoforms are produced, carrying changes in the coding sequence or the regulatory, untranslated regions. Therefore, even in the absence of noticeable changes in gene expression, the production of two or more transcript isoforms of a gene can exert biological effects. DTU, however, goes mainly undetected when routinely analyzing RNAseq datasets if specific pipelines are not applied. With this in mind, we aimed to analyze the expression of transcript isoforms in bovine blastocysts developing in varying concentrations of energy substrates. The preimplantation embryo development has been chosen as the window of exposure for the intense genome reprogramming and epigenetic remodeling to capture the earliest events of DOHaD. Bovine ovaries were obtained from a slaughterhouse and cumulus-oocyte complexes were collected from follicles of 2-8 mm in diameter. After in vitro maturation and in vitro fertilization with X-sorted semen, zygotes were cultured in a serum-free system with varying concentrations of energy substrates, i.e. increasing or decreasing concentrations of glucose, pyruvate, lactate, citrate, and amino acids. Expanded blastocysts (pools of 5 blastocysts/group, 3 biological replicates) were harvested for RNA extraction giving a minimum yield of 289 pg/µL and RIN>8.6 and sequenced on an Illumina platform. Transcript levels were quantified and genes having multiple transcript isoforms were filtered with DRIMSeq. Subsequently, DEXSeq was employed to distinguish which of these isoforms were differentially expressed (adjusted $p<0.1$). Almost 6000 genes with multiple isoforms were identified, of which approximately 90 had differential isoform expression in response to varying concentrations of energetic levels, for a total of 250-280 isoforms in the decreased and increased energy substrates, respectively. Enrichment analysis conducted to understand which molecular functions were mainly affected by energy supplementation revealed a general implication of 'palmitoyl-CoA' and 'acyl-CoA hydrolase activity' that seem concordant with the experimental model. Notably, other molecular functions involved were 'histone deacetylase binding' in the condition of increased supplementation and 'transcription

factor binding' when energy levels were decreased, pointing at possible interferences with epigenetic and nuclear reprogramming mechanisms. To validate these observations, the acetylation of histone residues was monitored by immunofluorescence and confocal microscopy on expanded blastocysts (7-8 blastocysts/group, 2 biological replicates), revealing a 25-50% increase in the relative intensity of the acetylated signal when embryos were cultured with higher energetic substrates ($p<0.01$, unpaired T-test, two-tailed). Overall, these observations contribute to a deeper understanding of how energetic excess impacts early embryonic development at the molecular level, showing for the first time the implication of DTU in the early onset of epigenetic modifications that might lead to DOHaD.

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