Developmental Patterns and Gene Expression of Bovine Blastocysts Exposed to Imbalanced Energetic Levels

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The Developmental Origins of Health and Disease (DOHaD) states that an imbalanced maternal diet has long-term effects on the health of the offspring. In humans and animal models, an inadequate nutrient supply during pregnancy may impact post-natal life, predisposing to cardiovascular and kidney diseases, metabolic disorders, and poor female fertility in the absence of overt developmental defects. Given the extensive genome and epigenome reprogramming during the preimplantation embryo development, in this study we asked if exposure to nutritional imbalance during this window triggers changes in developmental patterns and gene expression in the blastocyst. We modelled this stage using in vitro bovine embryo production to strictly control the environmental conditions and reduce the number of animals used for experimentation. Furthermore, the amount of in vitro produced bovine embryos has increased exponentially in the last decades, surpassing the number of embryos produced in vitro since 2016, posing the need to carefully evaluate the long-term safety of embryo culture.

Bovine blastocysts were produced in vitro from abattoir-derived oocytes using X-sorted semen. The zygotes were cultured for 8 days in a modified, serum-free culture medium. The composition of the medium was changed to contain either 0.5 (energetic restriction) or 1.5-fold (energetic excess) of the standard content of energetic substrates. RNA was extracted from a pool of 5 expanded blastocysts per treatment and amount and quality were assessed by BioAnalyzer. Three biological replicates per experimental group with a minimum RNA amount of 289 pg/µl and RNA integrity number 8.6-10 were used in downstream procedures. Libraries were prepared and sequenced on NovaSeq 6000 generating 150 bp paired-end reads. Sequenced reads were mapped and quantified using Salmon or HISAT2/HTSEQ. Differential gene expression analysis was performed in R using DESeq2 package. Genes with adjusted $p<0.1$ were considered differentially expressed. Markers of cell lineage specification, SOX2, CDX2, and NANOG, were traced by immunofluorescence. The obtained blastocysts were also characterized morphologically by recording the overall blastocyst rate and morphology, and nuclei counting.

Principal component analysis evidenced that increased levels of energetic substrates induced a clear clustering compared to the control, in contrast to energetic restriction. Furthermore, only a few genes were differentially expressed, without substantial differences between the two bioinformatic pipelines, when conducting paired comparisons. When considering gene expression patterns across the 3 treatments, 2 patterns were generated: increasing and decreasing expression along with increased energy substrates. Consistent with these findings, the blastocyst rate, comprising the expansion and hatching rates, the number of cells, and the expression of the first cell lineage markers were comparable.

A lack of major disruption of the expression profile and normal development patterns seem in line with previous observations made in vivo that a mild nutrient restriction in the mother does not prevent the development to term and the birth of healthy-looking offspring, which will only be affected later in life. We are therefore tempted to speculate that at this stage of development embryo plasticity allows to adjust in response to energetic imbalance. However, such adjustments might come at the cost of heritable alterations of the epigenetic profiles that will impact cells and organ functions later in life. For instance, overexpression of *MUC13*, as consistently observed in the energetic excess treatment, has been linked with changes in the methylation levels of its promoter and with higher glucose uptake.