Investigating the Presence of Acetylated Histone Post-Translational Modifications in Bovine Preimplantation Embryos

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Following fertilization, the preimplantation embryo must undergo a series of cellular and molecular changes that are essential for successful development. A key event in preimplantation development is the activation of embryonic transcripts, or zygotic genome activation (ZGA). This process of switching from maternally provided transcripts to activating the embryonic genome is essential for survival of the embryo and the development of embryonic tissue and placental membranes. Throughout preimplantation embryo development, gene expression is regulated by several different epigenetic mechanisms, including DNA methylation, chromatin conformation, small non-coding RNAs, and histone post-translational modifications (PTMs). Changes to the epigenetic landscape at different stages of preimplantation embryo development is a critical component in the precise transcriptional regulation of genes that are necessary for embryo survival and lineage differentiation. One essential mechanism of epigenetic regulation is the attachment of PTMs to histone tails, thus altering chromatin accessibility and inducing or silencing gene transcription. The addition of acetyl groups to histone lysine residues activates gene transcription by opening the chromatin, thus allowing binding of DNA polymerase and other transcription factors. While the specific timing and location of histone PTMs during preimplantation embryo development has been well characterized in the mouse and human, little is known about the presence and timing of histone PTMs during bovine preimplantation embryo development. The aim of this study was to investigate the presence of activating histone PTMs and associated chromatin modifying enzymes in bovine preimplantation embryos. We first analyzed previously published RNAseq data from bovine blastocysts and found robust expression of several histone acetyltransferases, including: *Kat5*, *Kat7*, *Kat8*, *Hat1*, *CBP*, and *p300*. We next utilized immunofluorescence to determine the abundance and location of two activating epigenetic marks. Acetylation of lysine 27 on histone 3 (H3K27ac) is associated with active enhancers and promoters. Acetylation of lysine 9 on histone 3 is associated with active promoters (H3K9ac). Both have demonstrated regulatory roles in gene expression and may indicate important roles during and after ZGA. Immunofluorescence was also performed for the histone acetyltransferase CBP, which acetylates H3K9ac and H3K27ac. Frozen, IVF generated embryos collected at day 7.1 of development were used for this initial study. Immunofluorescence was utilized to determine the presence of H3K27ac, H3K9ac, and CBP at the blastocyst stage of development. Both H3K27ac and H3K9ac are localized to the nuclei of the inner cell mass and trophectoderm of the blastocysts. Interestingly, CBP appears to be expressed in the cytoplasm of blastocysts, thus suggesting other histone acetyltransferases are involved with H3K9/27ac in the bovine embryo. The study sought to answer 1) Is the quantity and location of H3K27ac and H3K9ac consistent with previous findings in other mammals, 2) What are the specific epigenetic mechanisms regulating the early bovine embryo, and 3) Can the localization of activating histone modifications indicate quality of the blastocyst. The findings indicate the location of these modifications throughout bovine preimplantation development

could be potential biomarkers for assessing quality and viability and that these epigenetic marks are conserved across species.