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Characterization of histone lysine β-hydroxybutyrylation in bovine tissues, cells, and cumulus-oocyte complexes.

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Resumo

In addition to their canonical roles as energy sources, short-chain fatty acids act as metabolic regulators of gene expression through histone posttranslational modifications. Ketone body β-hydroxybutyrate (BHB) causes a novel type of epigenetic modification, histone lysine β-hydroxybutyrylation (Kbhb), which is associated with genes upregulated in starvation-responsive metabolic pathways. Dairy cows increase BHB in early lactation, and the effects of this increase on cellular epigenomes are unknown. On this basis, there seems to be an intriguing and complex connection between bovine metabolism and epigenetics that can be further understood by studying Kbhb biology. To achieve this goal, we carried out a series of experiments aimed at (1) determining whether histone lysine β-hydroxybutyrylation (Kbhb) is physiologically present in several tissues/organs in dairy cows; (2) examining whether supplementation with BHB in vitro increases Kbhb in a dose-dependent manner in bovine and human fibroblast cultures; (3) exposing cumulus-oocyte complexes (COCs) during in vitro maturation (IVM) to investigate whether BHB affects Kbhb levels in cumulus cells and oocytes; (4) determining whether the exposure of COCs to different concentrations of BHB during IVM alters the oocyte's ability to complete meiotic maturation and develop to the blastocyst stage following parthenogenetic activation or in vitro fertilization (IVF); and (5) characterizing the alterations in cumulus cell gene expression patterns (RNA-seq) in COCs exposed to ketone body β-hydroxybutyrate during IVM. As a result, we identified that Kbhb is present in bovine tissues in vivo and confirmed that this epigenetic mark is responsive to BHB in bovine and human fibroblasts cultured in vitro in a dose-dependent manner. Maturation of cumulus-oocyte complexes with high concentrations of BHB did not affect the competence to complete meiotic maturation or to develop until the blastocyst stage. IVF blastocysts derived from oocytes treated with BHB during IVM increased the expression of NANOG compared with the control group. BHB treatment strongly induced H3K9bhb in cumulus cells, but faintly in oocytes. RNA-seq analysis in cumulus cells indicated that BHB treatment altered the expression of 345 genes. The downregulated genes were mainly involved in glycolysis and ribosome assembly pathways, while the upregulated genes were involved in mitochondrial metabolism and oocyte development. Our data showed that BHB is a strong epigenetic modifier in bovines. The full consequences of the transcriptional alterations on the pregnancy establishment and fetal development need to be investigated. The genes and pathways altered by BHB will provide entry points to carry out functional experiments aiming to mitigate metabolic disorders and improve fertility in cattle. Grant Numbers: Research was supported by São Paulo Research Foundation (FAPESP) grant #2016/13416-9 and #2018/09552-0 (JRS); #2013/08135-2 (FVM).