The oxidative metabolism dictates the histone lactylation levels during *in vitro* maturation of bovine oocytes

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The lysine histone lactylation (Kla) has emerged as a newly identified epigenetic marker, playing a crucial role in maintaining the transcriptional machinery of cells, with its reliance on lactate metabolism. Our latest findings reveal the presence of Kla and its regulation dependent on oxygen metabolism during the bovine morula and blastocyst stages. However, the existence of Kla and its relationship with oxygen regulation during oocyte maturation remains unknown. The objectives of this study were: 1) to confirm the existence of Kla in bovine oocytes and 2) to assess the global nuclear levels of Kla during the maturation of bovine oocytes cultured under two different oxygen tensions (low [5% O_2] or high oxygen tension [20% O_2]). For this purpose, during *in vitro* maturation (IVM), the oocytes were randomly assigned to two groups [5% O_2 or 20% O_2] and subsequently collected at 8 or 24 hours of IVM. The oocyte Mitochondrial Membrane Potential (MMP) was assessed using Mitotracker™ Red CMXRos dye (ThermoFisher), Reactive Oxygen Species (ROS) were measured using CellRox Green® (ThermoFisher), and the nuclear levels of Kla were determined through immunostaining with the polyclonal antibody for pan histone lactylation (PTM-1401). Image acquisition was performed with a fluorescence microscope, and subsequent analysis was conducted using ImageJ software. Data analysis was carried out using Student's t-test, with significance set at $p<0.05$. We observed an elevated level of MMP in oocytes cultured in 5%O_2 compared to 20%O_2 at 8h of IVM ($p=0.0400$), but no significant differences were found at 24h of IVM ($p=0.4642$). ROS analysis did not reveal any alterations in either period of IVM (8h - 5%O_2 vs 20%O_2 - $p=0.4628$ / 24h - 5%O_2 vs 20%O_2 - $p=0.1777$). However, at 8h of IVM, both oxygen treatments led to increased MMP and ROS levels compared to 24h of IVM (MMP: 5%O_2 - 8h vs 24h - $p<0.0001$; 20%O_2 - 8h vs 24h - $p=0.0097$ / ROS: 5%O_2 - 8h vs 24h - $p<0.0001$; 20%O_2 - 8h vs 24h - $p<0.0001$). Furthermore, we identified a positive correlation between MMP and ROS levels at 8h (5%O_2 - $p=0.0012$ - $r=0.4419$; 20%O_2 - $p=0.0139$ - $r=0.362$), indicating heightened oxidative metabolism activity at the beginning of IVM. The differences in oxygen tension did not influence oocyte Kla levels at 8h (5%O_2 vs 20%O_2 - $p=0.3882$) or at 24h (5%O_2 vs 20%O_2 - $p=0.5121$) of IVM. Upon closer examination, we observed an increase in KLA levels at 8h when compared to 24h of IVM in oocytes exposed to 20%O_2 (5%O_2 - 8h vs 24h - $p=0.0970$; 20%O_2 - 8h vs 24h - $p<0.0001$), suggesting that the oxidative metabolism coordinates the global Kla levels during IVM. In summary, to the best of our knowledge, this study presents the first description of Kla in a bovine oocyte model. It proposes that variations in O_2 availability during IVM elicit alterations in oxidative metabolism, thereby influencing the lactylation levels, reinforcing the association between the metabolic status and the epigenetic mechanisms responsible for regulating embryonic transcriptional machinery during IVM.

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