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Effects of palmitic acid-induced lipotoxicity on epigenetic programming in zygotes and morulas during bovine in vitro embryo production

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Maternal metabolic disorders are associated with subfertility. Upregulated lipolysis causes a rise in non-esterified fatty acids in the blood, which is reflected in the follicular and oviductal micro-environment. This has a lipotoxic effect on oocyte and embryo development, mainly due to elevated palmitic acid (PA) concentrations. Surviving embryos may exhibit persistent defects in later life due to epigenetic alterations as oocyte maturation and early embryo development involve dynamic changes in epigenetic reprogramming and may therefore be vulnerable to changes in their micro-environment. We hypothesized that short-term exposure to PA during oocyte maturation and early embryo development can alter epigenetic patterns in the resulting embryos. To test this hypothesis, a validated bovine IVP model was used, where oocytes were exposed to standard (CONT) or solvent (SCONT) media, or a pathophysiological concentration of PA (150µM, BSA 7.5 mg/ml) during IVM (24h). Oocytes were then *in vitro* fertilized (for 20h) and presumptive zygotes were cultured in the corresponding CONT, SCONT or PA (230µM, BSA 20 mg/ml) media, respectively (3 groups in total). 12 replicates were performed (858-1630 COC's/treatment). Cleavage rates were recorded at 48h post insemination (p.i.) (12 replicates) and blastocyst rates at 8d p.i. (4 replicates). Zygotes (60/treatment, 6 replicates) were collected at 20h p.i. and morulas (70/treatment, 8 replicates (not used to record blastocyst rates)) were collected at 4.7d p.i. and were fixed for 5mC and H3K9ac/H3K9me2 immunostaining and confocal microscopy to assess global DNA methylation and histone acetylation/methylation, respectively. Developmental competence data were analysed using logistic regression, and numerical data with one-way ANOVA (5mC/H3K9ac) or Kruskal-Wallis test (H3K9me2) with post-hoc Bonferroni correction depending on normality of distribution. For developmental competence, 5mC, and H3K9ac, no effects of the solvent could be detected compared to CONT. H3K9me2 mean gray intensity was significantly lower in SCONT compared to CONT in zygotes (22.2% reduction, P=0.011) and morulas (13.5% reduction, P=0.001). Exposure to PA during IVM and IVC resulted in significant reduction of cleavage (63.9 ± 4.7% vs. 79.5 ± 2.6%, P<0.001) and blastocyst rates (25.2 ± 4.9% vs. 39.2 ± 2.6%, P=0.005) compared to SCONT. 5mC mean grey intensity was not altered in PA-exposed zygotes (P=0.432) but was increased in morulas (27.4% increase, P<0.001). H3K9ac was significantly increased in zygotes exposed to PA (32.5% increase, P<0.001), but not in morulas (P=0.913). H3K9me2 was significantly increased in PA-exposed zygotes (46.3% increase, P<0.001) and morulas (15.5% increase, P=0.039). We conclude that a lipotoxic micro-environment during bovine IVM results in increased histone acetylation and methylation already at the zygote stage. Continued exposure during IVC was associated with increased histone and DNA methylation in the morulas. These upregulated epigenetic marks may cause altered gene expression and imprinting, resulting in aberrant embryonic cell differentiation. We are currently investigating potential mechanisms through which these changes occur.

Keywords: bovine in vitro embryo, epigenetics, lipotoxicity