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Effect of embryo culture conditions on developmental potential of bovine oocytes matured under lipotoxic conditions

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Metabolic disorders such as negative energy balance in cows are being linked to subfertility. They are associated with lipolysis and elevated saturated (stearic; SA, palmitic; PA) and mono-unsaturated (oleic; OA) fatty acids (FAs) in serum. These FA concentrations are reflected in the follicular fluid and have a direct detrimental impact on oocyte quality. However, embryo culture conditions after fertilization may improve its developmental potential. Insulin-transferrin-selenium (ITS), which is used in serum-free culture systems, may enhance development of metabolically compromised oocytes due to its mitogenic and anti-oxidative properties, however this has not been tested yet. In this study, metabolically compromised bovine oocytes were generated by exposure to elevated pathophysiological concentrations of PA (150 µM) or a combination of high PA (150 µM), SA (75 µM) and OA (200 µM) (HIGH COMBI) during *in vitro* maturation. They were compared to oocytes exposed to a combination of physiologically relevant concentrations of PA (23 µM), SA (28 µM) and OA (21 µM) (BASAL). After fertilization, the presumptive zygotes were cultured in the presence or absence of ITS. We evaluated cleavage and fragmentation rates 48 hours post insemination (p.i.) and blastocyst rates at day 7 and 8 p.i. (n=905 oocytes, 3 repeats). At day 8, total cell number and apoptotic cell index in blastocysts (n=227) were also assessed by staining with Hoechst and anti-cleaved caspase 3 antibody. Categorical data were analysed using binary logistic regression. Numerical data were analysed using ANOVA and Bonferroni-corrected for multiple testing. In the absence of ITS during culture, PA supplementation during maturation significantly reduced cleavage rate (64.2% *vs.* 78.3%), proportion of ≥4-cell embryos (38.4% *vs.* 57.5%) at 48h p.i., and blastocyst rate at day 7 (12.4% *vs.* 24.3%) compared to the BASAL control ($P<0.05$). Blastocyst rate of PA-treated oocytes at day 8 tended to be lower (22.0% *vs.* 32.5%, $P=0.098$). In the presence of ITS, supplementation with either PA or HIGH COMBI had no significant effect on cleavage and blastocyst rates. Within the PA-treated group, ITS supplementation increased embryo cleavage rate (by 15.1%, $P<0.05$), proportion of ≥4-cell embryos (by 13.9%, $P<0.05$) and tended to increase blastocyst rate at day 7 (by 8.2%, $P=0.076$) compared to the PA-treated group cultured in the absence of ITS. Supplementation with ITS had no significant influence on embryo development in BASAL and HIGH COMBI groups. Total cell numbers were similar among all treatments, however, a significant increase in apoptosis was observed in embryos from PA and HIGH COMBI-treated oocytes. This was not alleviated by ITS supplementation during culture. We conclude that ITS supplementation during embryo culture enhances development of embryos derived from metabolically compromised oocytes (PA-treated), however produced embryos were still inferior in quality as evident in higher apoptosis.