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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 obesity and type II diabetes in women or negative energy balance in dairy cows are 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 in humans and farm animals. Several embryo culture media formulations are commercially available, some of which are supplemented with antioxidants (e.g. selenium) to enhance embryo development. Insulin supplementation has also been tested in clinical trials. Insulin-transferrin-selenium (ITS) is routinely used in serum-free bovine *in vitro* embryo production systems due to its mitogenic and anti-oxidative properties. We hypothesize that supportive culture media (e.g. with ITS supplementation) can rescue embryo development and quality of metabolically-compromised oocytes matured under elevated lipotoxic NEFA concentrations. In this study, bovine oocytes were exposed during *in vitro* maturation 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). 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 control). After fertilization, 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 indices in blastocysts (n=227) were assessed by staining with Hoechst and anti-cleaved caspase 3 antibody. 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, PA and HIGH COMBI exposure had no significant effect on cleavage and blastocyst rates compared with controls. 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 blastocysts derived 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, the produced embryos were still inferior in quality as evident in higher apoptosis. Our data indicate that supportive culture conditions may compensate for unfavourable oocyte maturation conditions in terms of improved embryo developmental capacity but not in terms of embryo quality.