Enhancement of the developmental capacity of metabolically compromised bovine oocytes and embryos by water soluble vitamin E (TROLOX) depends on the timing of the treatment

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Maternal metabolic disorders are associated with elevated concentrations of free fatty acids (FFA) in serum, follicular- and oviductal fluid. Previous studies have shown that pathophysiological FFA concentrations, and in particular the saturated palmitic acid (PA), jeopardize bovine oocyte and embryo developmental competence in vitro. Moreover, gene expression and proteomic analysis of FFA exposed bovine oocytes point towards oxidative stress related pathways. As such, antioxidants may be a key factor in improving oocyte and embryo developmental competence. We investigated if the use of TROLOX, a water soluble vitamin E analogue and antioxidant, during IVM or IVC could enhance developmental competence of PA-exposed oocytes and embryos in vitro. Hereto, 1279 bovine oocytes were routinely matured, fertilized and cultured until day 8 in 2 different experiments (6 repeats each).

In Exp.1, oocytes were exposed to pathophysiological follicular PA concentrations (150µM), after which the zygotes were cultured under solvent control (ethanol, PA-SC) or TROLOX (100µM, PA-TROLOX) conditions. In Exp.2, oocytes were matured under SC or TROLOX (100µM) conditions, then exposed to pathophysiological oviductal PA concentrations (230µM) during culture (SC-PA, TROLOX-PA). In each experiment a solvent control was included (SC-SC). Cleavage (48h post insemination, pi), blastocyst rates (D8 pi), the rates of D8 blastocysts/cleaved zygotes and the rates of D8 expanded and hatched blastocysts/total blastocysts were calculated. Developmental competence data were compared using a binary logistic regression model and Bonferroni post-hoc test (IBM SPSS Statistics 24). In Exp.1, cleavage of PA-SC (71%) was not significantly different from SC-SC (79%, P=0.133). D8 blastocyst rates of PA-SC (22%) tended to be lower compared with SC-SC (32%; P=0.064). Compared to PA-SC, we showed that TROLOX during IVC was not able to neutralize the PA insult during IVM (PA-TROLOX, 23%; P>0.100). The rates of total D8 blastocysts/cleaved zygotes and D8 expanded and hatched blastocysts/total blastocysts were not significantly different. In Exp.2, cleavage, D8 blastocyst rates and D8 blastocysts on total cleaved zygotes of SC-PA (59%, 9%, 14%, respectively) were significantly reduced compared with SC-SC (79%, 32%, 39%, respectively; P<0.0001). Cleavage and D8 blastocysts/cleaved zygotes of TROLOX-PA (68% and 24%, respectively) tended to be improved compared with SC-PA (P<0.1). Moreover, the addition of TROLOX during IVM could significantly increase D8 blastocyst rates (17%) of PA-exposed embryos (P=0.022), but not to control levels (32%). TROLOX during IVM significantly improved blastocyst development into expanded and hatched blastocysts when embryos were exposed to PA (SC-PA, 49% vs. TROLOX-PA, 68%; P=0.025) to levels similar to controls (SC-SC, 63%). In conclusion, the antioxidant TROLOX can protect oocytes from metabolic stress insults after fertilization, but metabolically compromised oocytes cannot be rescued by the addition of TROLOX during embryo culture.