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.