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Trolox during *in vitro* maturation of bovine oocytes can protect embryos from palmitic acid induced lipotoxicity during development: effects on mRNA transcript abundance

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Maternal metabolic disorders are associated with elevated concentrations of palmitic acid (PA), which is known to jeopardize bovine oocyte and embryo development and quality. Molecular analyses of PA exposed bovine oocytes and embryos point towards oxidative stress (OS) pathways. Previous research has shown that the detrimental effects of PA-exposure during oocyte IVM cannot be alleviated by antioxidant (AO) supplementation, e.g. Trolox (TR, water soluble VitE), during IVM or IVC. Exposing oocytes with TR during IVM protected subsequent embryo development under PA conditions (De Bie *et al.* 2018, AETE). In the present study, we examined the effects of TR on the quality of the produced blastocysts at the transcriptome level.

Bovine COCs were matured, fertilized and cultured in 2 different experiments (min 3 repeats each). In **EXP1,** COCs (n=1565) were exposed to pathophysiological follicular PA concentrations (150 µM, Sigma-Aldrich, BE), subsequent embryos were cultured under solvent control (ethanol) conditions (PA-SC). TR was added during IVM or IVC (100 µM, Thermo Fisher, BE; PATR-SC, PA-TR). In **EXP2,** COCs (n=1477) were matured under solvent control conditions, subsequent embryos were exposed to pathophysiological oviductal PA concentrations (230 µM; SC-PA). TR was added during IVM or IVC (100 µM; TR-PA, SC-PATR). In each experiment a solvent control was included (SC-SC). Pools of min 10 day 8 blastocysts per treatment were examined for relative transcript abundance of genes (normalized to *H2AFZ* and *YWHAZ*) involved in OS (C*AT, GPX, SOD1, SOD2, PRDX1, PRDX3, NRF2*), mitochondrial function (*TFAM, HSPD1*), lipid metabolism (*PPARg*) and apoptosis (*BAX*) and analyzed by one-way ANOVA.

A significant increase in *NRF2* and *TFAM* was found in blastocysts from PA exposed COCs (PA-SC) and embryos (SC-PA) compared with controls (SC-SC). Increased *NRF2* in blastocysts from PA exposed COCs (PA-SC) returned to control levels when TR was added during IVM or IVC (PATR-SC, PA-TR). In contrast, when embryos were exposed to elevated PA (SC-PA), adding TR during IVM or IVC (TR-PA, SC-PATR) was not able to alleviate elevated *NRF2* expression to control levels, suggesting activation of OS defence mechanisms. The addition of TR in each EXP significantly reduced *TFAM* gene expression to levels similar to controls (SC-SC), suggesting normalisation of mitochondrial biogenesis. In EXP1, a significant increase in *CAT* was found in PA exposed oocytes (PA-SC) compared with their control counterparts. Adding TR during IVM or IVC (PATR-SC, PA-TR) significantly reduced blastocyst *CAT* expression to levels lower than controls. No significant PA-induced changes were found in the expression of other genes.

In conclusion, the enhancement of the developmental capacity of PA-exposed bovine oocytes and embryos by TR is most promising when oocytes are protected by TR prior to the PA insult. Moreover, subsequent blastocysts appear to have control levels of expression of genes related to OS and mitochondrial function and increased expression of genes involved in OS relief.