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Trolox during *in vitro* maturation of bovine oocytes protects developing embryos from palmitic acid-induced lipotoxicity

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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. Analyses of PA exposed bovine oocytes and embryos point towards oxidative stress (OS) related 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. In contrast, supplementing TR during IVM could protect developing zygotes from PA-induced lipotoxicity by increasing their development into 4-cell embryos and D8 blastocysts. In the present study, we examined the effects of TR during IVM or IVC on PA-exposed oocytes and embryos by evaluating OS and mitochondrial membrane potential (MMP) of the produced ≥2-cell Day 2 embryos.

Bovine COCs were matured, fertilized and cultured in 2 experiments. In **EXP1,** COCs were exposed to pathophysiological follicular PA concentrations (150µM), and subsequent embryos were cultured under solvent control (ethanol) conditions (PA-SC). TR treatment was applied during IVM or IVC (100µM; PATR-SC, PA-TR). In **EXP2,** COCs were matured under SC conditions, and subsequent embryos were exposed to pathophysiological oviductal PA concentrations (230µM; SC-PA). TR treatment was applied during IVM or IVC (100µM; TR-PA, SC-PATR). In each EXP a SC was included (SC-SC). A total of 126 and 137 Day 2 embryos were stained with JC-1 and CellRox Deep Red in EXP1 and EXP2 (3 repeats), respectively. MMP was evaluated as active/total mitochondria and OS as OS/total mitochondria and analysed by one-way ANOVA.

Exposure of oocytes and embryos to PA significantly increased OS and MMP in Day 2 embryos compared to controls. Regardless of the moment of PA exposure, TR treatment during IVM increased MMP even more. The increased MMP levels in PA-exposed oocytes and embryos were not influenced by TR treatment during IVC. Regardless the AO effect of TR, increased embryonic OS levels observed in PA exposed oocytes and embryos could not be reversed to control levels by TR treatment during IVM or IVC. However, when OS levels were expressed on active mitochondria, we found that TR treatment prior to the PA insult (TR-PA) generated D2 embryos that displayed significant lower OS/active mitochondria rates compared with SC-PA treated embryos. Taken together, we may conclude that the combination of relatively low OS levels with highly active mitochondria may be a mechanism implicated in the protective effect of TR prior to the PA insult on embryo developmental competence.

		Controls	PA-exposure	TR during IVM	TR during IVC
EXP1		SC-SC	PA-SC	PATR-SC	PA-TR
	MMP	0.39±0.02^{a}	0.53±0.02^{b}	0.65±0.02^{c}	0.50±0.02^{b}
	OS	0.55±0.04^{a}	0.68±0.04^{b}	0.78±0.03^{b}	0.59±0.03^{ab}
EXP2		SC-SC	SC-PA	TR-PA	SC-PATR
	MMP	0.39±0.02^{a}	0.50±0.02^{b}	0.65±0.02^{c}	0.58±0.02^{b}
	OS	0.55±0.04^{a}	0.69±0.04^{b}	0.70±0.03^{b}	0.71±0.02^{b}

^{abc} Different letters in the same row indicate significant differences (P<0.05)