A321 Support biotechnologies: Cryopreservation and cryobiology, diagnosis through imaging, molecular biology, and "omics"

L-carnitine supplementation during vitrification did not improve survival and quality rates, but altered CrAT and PRDX1 expression in *in vivo*-produced ovine embryos

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Embryo cryodamage is observed mainly at metabolic and molecular aspects and it impairs post warming quality and survival rates. This study aimed to evaluate the effect of L-carnitine (LC) supplementation during either vitrification or post warming solutions on the 6-7th day of in vivo-produced ovine embryos. LC (3.72 mM) was added to vitrification (Experiment 1; C1: control; LC1: supplemented embryos) or warming solutions (Experiment 2; C2; LC2). In vitro culture (IVC) of warmed embryos was performed for 72 h at 38,5 °C, 5% CO2 and 5% O2 to evaluate survival rates in both Experiments. In Experiment 1, reactive oxygen species (ROS) levels were measured by CellROX Green staining, total cell number (TCN) by Hoechst 33342, number of apoptotic cells by caspase-3 immunofluorescence staining protocol, apoptotic index evaluation in both groups. Gene expression analysis of carnitine palmitoyltransferase 1 and 2 (CPT1 and CPT2), carnitine O-acyltransferase (CrAT) and peroxiredoxin 1 (PRDX1), were performed by RT-qPCR (ACTB as endogenous control) in Experiments 1 and 2 and results were compared to fresh embryos (FE). Averages of survival rates were compared by the Chi-Square test. Means of TCN, apoptotic cells, apoptotic index and fluorescence intensity were compared by Student's t-test, at 5% significance level. Survival rates were similar between groups ($p > 0.05$) in Experiments 1 (68.7%, C1 vs 81.8%, LC1) and 2 (48.5%, C2 vs 64.7%, LC2). In Experiment 1, ROS levels at 24 h of IVC (85.83 ± 68.37 x 1010, C1 vs 89.04 ± 84.48 x 1010, LC1), total cell number at 24 h (89 ± 22, C1 vs 82.2 ± 28, LC1) and 72 h (86 ± 19.9, C1 vs 68.5 ± 25.26, LC1), apoptotic cells (3.75 ± 1.48, C1 vs 4.50 ± 4.72, LC1) and apoptotic index (4.37 ± 1.45, C1 vs 5.23 ± 4.72, LC1) at 72 h of IVC did not differ ($p > 0.05$) between C1 and LC1. Gene expression analysis showed no differences in CPT1 and CPT2 mRNA relative abundance in embryos of both experiments compared to FE, however, CrAT was downregulated ($p < 0.05$) in C1 and PRDX1 was downregulated ($p < 0.05$) in both C1 and LC1, compared to FE. Moreover, CrAT and PRDX1 were upregulated ($p < 0.05$) in C2 and CrAT was downregulated ($p < 0.05$) in LC2, in relation to FE. In conclusion, although the short-term LC supplementation at 3.72 mM during cryopreservation did not improve post-warming survival and morphological parameters of the evaluated embryos, it was able to modulate expression of genes related to energy homeostasis (CrAT) and oxidative stress (PRDX1), proving to be beneficial, in both forms of supplementation, to in vivo-produced ovine embryos.

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