Animal Reproduction

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Post-warming culture with beta-mercaptoethanol does not alter embryo survival but improves the quality of in IVP bovine embryos post vitrification

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The aim of the present study was to investigate the relationship between vitrification and excessive generation of reactive oxygen species (ROS) and the effects of oxidative stress attenuation on the survival and quality of bovine in vitro produced (IVP) embryos. To test the hypothesis that oxidative stress was related to lower post-vitrification embryo survival, the antioxidant beta-mercaptoethanol was used in the post-warming culture and then the survival and quality of embryos were assessed. In experiment 1, we evaluated the levels of ROS in grade I blastocysts (at day 7 after IVF) from fresh (F) and post-warming vitrified (V) embryos. In experiment 2, F and V embryos were submitted to 48 hours of culture (SOF) in the presence (F+, V+) or absence (F-, V-) of beta-mercaptoethanol (100 µM) (Gibco BRL, Grand Island, NY) for posterior assessment of re-expansion, hatching, number of cells and oxidative index. All IVP stages (as well as post-warming culture) were carried out at 38.5ºC in 5% CO2 in high humidified air. Re-expansion was evaluated at 0, 2 and 4 hours after warming. In both, experiments 1 (2h after warming) and 2 (48 h after warming), the embryos from each group were stained with CellRox Green (Invitrogen Molecular Probes, USA) and Hoechst 33342 (Sigma-Aldrich, USA). Then, images of each embryo were captured and evaluated using ImageJ software to measure the levels of ROS (average pixels) and number of cells. According to the present results, increased concentration of ROS was detected in vitrified embryos considering the fluorescence levels (F= 57.25±4.17, V= 103.71±8.68, P<0.05; T Test, n= 117, 53-64 per group). No differences were observed regarding the re-expansion rate of vitrified embryos cultured in the presence or absence of beta-mercaptoethanol (0 hrs: V+ = 63.86%, V- = 62.64%; 2hs: V+ = 90.36%, V- = 95.60%; 4 hs: V+ = 92.77%, V- = 98.90%, P>0.05, Fisher's Exact Test, n= 174, 83-91, per group). The hatching rates of vitrified embryos (V+ and V-) were similar to F- embryos but lower than F+ embryos (V+ = 49%, V- = 48%, F+ = 71%, F- = 66%, P<0.05, Exact Test Fisher, n=229, 79-81-34-35, per group, respectively). Still, the total number of cells in V+ embryos was similar to F- and F+ blastocysts (F- = 158.04±14.53, F+ = 163.19±9.21, V- = 117.40±5,50, V+ = 148.04±7.26, P>0.05, Post Tukey Test, n= 214, 23-32-81-78, per group, respectively). The oxidative index (at the end of 48 h of culture) was not different among the experimental groups (F- = 309.51±29.25, F+ = 342.67±28.26, V- = 384.19±26.65, V+ = 375.49±26.99, P>0.05; Post Dunn's Test, n=196, 22-32-67-75, per group, respectively). We conclude that, although it did not affect embryo survival, the attenuation of oxidative stress improved the quality of vitrified embryos.

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