Animal Reproduction

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Reactive oxygen species attenuation in vitrified-warmed bovine embryos

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Vitrification is the most used methodology to cryopreserve *in vitro* produced (IVP) embryos. Still, despite providing an efficient protocol that allows the survival of 95% of IVF embryos and good pregnancy rates (40%) after vitrification and direct transfer in cattle (Oliveira *et al*., Cryobiology 97:222-225, 2020) it is still necessary to increase the quality of vitrified warmed. Hence, the aim of this study was to investigate the effects of modulating reactive oxygen species in vitrified bovine IVP embryos. Grade 1 blastocysts were selected and vitrified using a two-step protocol. Vitrification was performed in groups of five embryos and holding media was supplemented with 10% fetal calf serum. Selected embryos were transferred 200µl 7.5%DMSO +7.5% ethylenoglycol for 3 min. Next, washed in 200µl 16%DMSO +16% ethylenoglycol +0.5M sucrose media and placed in an open vitrification device in a 0.5 µl droplet. After 30s, the device was immersed in liquid nitrogen. We first compared ROS production in fresh and vitrified-warmed blastocysts and then we evaluated the effects of antioxidant supplementation (100 µM of 2-mercaptoethanol; BME) on ROS levels in vitrified-warmed blastocysts. At the end, we compared the development of fresh and vitrified-warmed blastocysts in the presence (100 µM BME) or absence (Control) of antioxidants. The oxidative index and total number of cells were compared by T-test and hatching rates with Fisher's Exact Test. Oxidative index was analyzed with Kruskall-Wallis and Dunn's Post Test, and the total number of cells using ANOVA and Tukey with 5% of significance (Minitab, 21.4.1.0). It was observed (n = 117 blastocysts obtained in two replicates, 52-64 per group) higher ROS production (Fresh: 50.75 ± 2.79 *vs* Vitrified: 81.21 ± 7.59; P<0.05) and lower cell number in vitrified compared to fresh embryos (Fresh: 132.79 ± 6.10 *vs* Vitrified: 105.02 ± 4.61; P<0.05). We also detected that antioxidant supplementation reduced the ROS levels (Vitrified: 38.24 ± 1.27 *vs.* Vitrified/BME: 33.54 ± 1.08; P<0.05) and increased the cell number in treated embryos (Vitrified: 100.65 ± 3.98 *vs*. Vitrified/BME: 112.95 ± 3.72; P<0.05). The BME neutralizes ROS levels by inducing the synthesis of intracellular glutathione, which occurs by reducing cystine to cysteine. Thus, the results demonstrated that 100 µM BME supplementation in the culture medium reduced ROS levels and increased the total number of cells in vitrified embryos. Although the embryo hatching rate did not differ (P>0.05) among embryos from the fresh, vitrified and vitrified/BME groups, the total cell numbers were higher (P<0.05) in vitrified embryos supplemented with BME (148.04 ± 7.26) than in vitrified embryos without BME (117.40 ± 5.50) but similar (P>0.05) to fresh embryos cultured with (163.19 ± 9.21) and without BME (158.04 ± 14.53). It was concluded that the vitrification and warming process increased ROS levels and its attenuation with BME antioxidant improved embryonic quality. Acknowledgment: CAPES and Embrapa.