COMPARISON OF TWO VITRIFICATION PROTOCOLS FOR IN VITRO PRODUCED BOVINE EMBRYOS

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In vitro production of bovine embryos associated to simplest and fastest cryopreservation techniques has a great potential to accelerate the multiplication and dissemination of high genetic merit animals. The aim of this work was to compare two vitrification protocols, evaluating the hatching rate and ultrastructural modifications of *in vitro* produced bovine embryos. Cumulus-oocyte complexes were aspirated from ovaries of slaughter cows and matured in medium 199 for 24 hours and fertilized for 22 hours. The zygotes were cultured in CR2aa medium, for seven days at 38.5°C and 5% CO_2 in air. Blastocysts of grade I and II were distributed randomly in the following treatments: T1) not vitrified (n=35); T2) embryos vitrified by OPS method in vitrification solution with DMSO 20% + ethylene glycol 20% (n=37); T3) embryos vitrified by OPS method in glycerol 25% + ethylene glycol 25% (n=43). Embryos of T2 were warmed at 39ºC and then transferred to solutions with different concentrations of sucrose (0.25M; 0.15M), at 39ºC. Embryos of T3 were warmed at room temperature and transferred to solutions of sucrose (1M; 0.5M; 0.25M). After that, embryos were cultured in medium 199, for 72 hours, when hatching rate was evaluated. Other embryos were fixed two hours after beginning of in vitro culture and processed for the transmission electronic microscopy. The data of the hatching rate was evaluated by analysis of variance (ANOVA) and the Scott-Knott test for comparison of the averages. The hatching rate did not differ ($P < 0.05$) between embryos in T1 and T2 (83.6% vs 67.4%, respectively). However, significant difference ($P < 0.05$) was found between these treatments and T3 (29.72%). The preliminary ultrastructural analysis of T2 (n=2) and T3 (n=2) showed many alterations in embryos of both treatments. The most external region of the zona pellucida was more porous and presented an increased perivitelline space with many cellular debris. The trophectoderm cells showed sparse and shortness microvilli with many mitochondria presenting electron lucent matrix and few transverse cristae. Those cell also showed great number of intracytoplasmatics vacuoles. Debris cellular were found within the blastocoele, which also had a reduced volume. Increased intercellular spaces were found among cells of the internal cellular mass (ICM). These cells also presented a great number of intracellular vacuoles. In both treatments the cells of the ICM showed better ultrastructural preservation than trophectoderm cells. The plasma membrane and cellular junctions of the trophectoderm cells of the T2 embryos were more conserved than of the T3 embryos, which presented some ruptures of membrane. However embryos of T2 presented larger intracytoplasmatics vacuoles than T3 Nuclear membrane integrity of trophectoderm and ICM cells of embryos in both treatments was conserved and characteristic forms of apoptosis were not observed. Moreover the embryos had few lipid drops. These data suggest that vitrification solution with DMSO + ethylene glycol (T2) may be more adequate for cryopreservation of *in vitro* produced bovine embryos.