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Supporting biotechnologies Cryopreservation and cryobiology, diagnostic imaging, molecular biology and "omics"

In vitro effect of C-type natriuretic peptide supplementation on the cryotolerance of bovine blastocysts

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Resumo

Modulations of cAMP and cGMP concentrations have already been associated with an increase or decrease in lipid content in bovine oocytes and embryos. Thus, the use of C-type natriuretic peptide (CNP) - a modulator of intracellular concentrations of cAMP and cGMP - can be used to modulate the lipid profile of embryonic cells in the in vitro culture (IVC) of bovine embryos. This study aimed to evaluate the effect of the addition of CNP during the IVC of bovine embryos (Bos taurus indicus), on the blastocyst rate and the re-expansion of blastocyst after cryopreservation with OPS (Open Pulled Straw). Two concentrations of CNP were used (100 nM - CNP-100 group and 400 nM - CNP-400 group) besides fresh Control and cryopreservation Control groups, in the IVC with high oxygen tension (20%). Eight replicates were performed with approximately 1,000 presumptive zygotes/group. On days 7 and 8, only expanded and grade I blastocysts (according to the IETS criterium) were submitted to vitrification by the OPS technique, with a total of 142 vitrified. Data were tested for normal distribution and homogeneity of variance using the Shapiro-Wilk test. Normally distributed data were submitted to analysis of variance by one-way. (significance was considered when P≤0.05). On day 7, the blastocyst rate for fresh Control, cryopreservation Control, CNP-100, and CNP-400 groups were, respectively, 32.0 ± 2.1; 26.1±7.8; 24.8±7.6 and, 31.5 ±10.7 (mean± standard deviation of percentage). The re-expansion rate at 12 hours after warming was 51.2% ± 1.6; 50.0% ± 1.8 and 45.4% ± 2.1, respectively for cryopreservation Control, CNP-100 and CNP-400. Respectively, the hatching rate was evaluated at the following times: 12 hours [9.5 ± 0.4; 4.3 ± 0.3 and, 4 ± 0.3], 24 hours [39.1 ± 0.7; 39.1 ± 0.9 and, 32.0 ± 0.9] and, 48 hours after warming [28.5 ± 0.7; 17.3 ± 1.0 and, 40.0 ± 1.1]. Finally, there was no difference in the total hatching [76.1% ± 0.9; 60.8% ± 1.9 and, 76.0% ± 1.6, respectively (P≤0.05)]. The results indicate that the use of CNP in IVC was not able to change the embryonic response to the cryopreservation technique when re-expansion and hatching were the endpoints evaluated. Although, other studies from our research group suggest that the use of CNP changes the lipid content, however, more studies are needed to better investigate molecular changes.

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