A305 Support biotechnologies: Cryopreservation and cryobiology, diagnosis through imaging, molecular biology, and "omics"

Evaluation of L-carnitine supplementation on the production and vitrification of bovine embryos produced *in vitro*

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In vitro produced embryos (IVP) present low survival to conventional methods of cryopreservation. In order to improve production and survival rates after vitrification, L-carnitine (CA) was used in different doses and different cultivation moments of in vitro embryonic development. For this purpose, the oocytes IVM was performed in TCM199 medium supplemented with 25 mM of sodium bicarbonate, 1.0 µg/mL of FSH, 50 Ul/mL of hCG, 1.0 µg/mL of estradiol, 0.2 mM of sodium pyruvate, 83.4 µg/mL of amikacin and 10% of fetal bovine serum. After 24 hours of IVM, oocytes were co-incubated with semen in a Talp-IVF medium supplemented with 6 mg/mL of BSA for approximately 20 hours. The development culture (CIV) was performed in a SOFaa medium with 6 mg/mL of BSA and 2.5% of SFB. According to the design, CA was used in the concentrations of 0.0mM (control); 1.0 mM; 2.5 mM and 5.0 mM, from 96 or 144 hours after fertilization (hpf). All IVP cultures were performed in an incubator at 38.5 ºC and a CO2 atmosphere of 5% in air. At the seventh day, IVCs were evaluated for blastocysts rates, with the embryos being submitted to vitrification. The embryonic viability post-vitrification was evaluated by the re-expansion rate and embryos hatching after re-heating and cultivation for 48 hours within the same conditions of IVC. Embryos production had a completely randomized design with a factorial scheme 4 x 2 (four concentrations and two days) and four replicates, with data being transformed in arc sine and submitted to a variance analysis, with means compared by the Tukey's test at 1% probability, with the aid of the software SAS. Regarding categorical data analysis (expansion and hatching), the Chi-square test was used, considering the effects of concentration and day, as for P values equal or inferior to 0.01 ($p<0.01$), the differences between these effects were considered as significant. The moment of CA use did not interfere on blastocyst production ($P=0.3$), however the use of 2.5 mM resulted in a greater production of embryos (62.0±0.07%, $P=0.02$), followed by the groups 0.0 mM, 1.0 mM and 5.0 mM, respectively (53.6±0.02, 57.7±0.07 e 49.4±0.11, $P<0.05$). Both the embryonic re-expansion and hatching were influenced by the moment of application and the CA concentration. The use from 96 hpf (38.4 and 27.9) was better that 144 hpf (27.9 and 13.5), while the concentration of 2.5 mM resulted in a greater percentage of re-expanded and hatched embryos (37.7 and 27.0), when comparing to groups 0.0 mM, 1.0 mM and 5.0 mM, respectively (19.6 and 14.3; 22.8 and 15.7; 23.6 and 16.7 $P<0.05$). Considering the moment and concentration, the group 96 hpf/2.5 mM presented the greatest expansion rates of 60.7% ($P=0.008$) and hatching of 41.4% ($P=0.04$). Based on the obtained results, it was concluded that when using L-carnitine from 96 hpf with the concentration of 2.5 mM, the embryonic survival to vitrification is improved.