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The use of linoleic acid in *in vitro* culture of bovine embryos and its effects on production and survival to vitrification

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In the attempt to produce in vitro bovine embryos more resistant to vitrification and to maintain the embryo production levels, the trans-10; cis-12 conjugated linoleic acid (CLA t10, c12) was use in different dosages and different moments of culture. For this purpose, the oocytes IVM (in vitro maturation) was performed in TCM 199 medium supplemented with 25 mM of sodium bicarbonate, 1.0 µg/mL of FSH, 50 UI/mL of hCG, 1.0 µg/mL of estradiol, 0.2 mM of sodium pyruvate, 83.4 µg/mL of amikacin and 10% of bovine fetal serum. After 24 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 in vitro culture (IVC) was performed in a SOFaa medium with 6 mg/mL of BSA and 2.5% of SFB. According to the experimental design, CLA t10, c12 concentrations were 0.0 µM (control); 50.0 µM; 100.0 µM and 150.0 µM from 96 or 144 hours after fertilization (hpf). All IVP (in vitro production) cultures were made in incubators at 38.5 ºC and CO2 atmosphere of 5% in air. At the seventh day of IVC the produced embryos indexes were evaluated, being the blastocysts of good quality submitted to vitrification. The embryo viability post-vitrification was evaluated by the re-expansion and hatch rate of embryos after re-heating and cultivation for 48 hours at the same IVC conditions. The embryo production had a completely randomized design with a factorial scheme 4 x 2 (four concentrations and two days) with six replicates and data were 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. The Chi-square test was used for the analysis of categorical variables (expansion and hatching), 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. Were treated from 96 hpf 1564 zygotes, 0.0 µM (278); 50.0 µM (401); 100.0 µM (448), 150.0 µM (437); and from 144 hpf 1566 zygotes, 0.0 µM (278); 50.0 µM (469); 100.0 µM (441), 150.0 µM (378). Using CLA t10, c12 did not alter the in vitro production of embryos, regardless of dosages 0.0 µM (56.1±0.08), 50.0 µM (50.1±0.06); 100.0 µM (50.8±0.05) and 150.0 µM (50.1±0.08) or the moment of application 96 hpf (51.2±0.07) or 144 hpf (50.1±0.08) (P=0.4). The embryos' re-expansion and hatching after vitrification was positively influenced with the use of CLA t10, c12 with any dosage 50.0 µM (63.6 e 43.8%), 100.0 µM (64.6 e 39.6%) and 150.0 µM (70.3 e 53.3%) when comparing to the control group (34.7%; 16%) and to the start day of supplementation, with 96 hpf being more efficient than 144 hpf (70.4% and 50% vs. 48.2% and 28.4%, respectively), regardless of the used concentration (P=0.01). Considering both the moment and concentration, the group 96 hpf/150 µM presented the greatest expansion (85.5%) and hatching (70.9%) rates post-vitrification ($P<0.05$). Based on the results, it was concluded that when CLA t10, c12 is used from 96 hpf at the concentration of 150 µM, the embryo survival to vitrification is improved, without impairing production.