A327 Support Biotechnologies: Cryopreservation and cryobiology, diagnosis through imaging, molecular biology and "omics"

Cryotolerance and pregnancy rates after exposure of bovine *in vitro*-produced embryos to forskolin and linoleic acid before vitrification

M. Meneghel[1], P. Chediek Dall Acqua[1], M. Ambrogi[1], L.M. Gouvêa[2], M.A. Achilles[3], B.C.S. Leão[1], E. Nogueira[4], N.A.S. Rocha-Frigoni[1], G.Z. Mingoti[1]

[1]Universidade Estadual Paulista Júlio de Mesquita, Jaboticabal; [2]Concepção Reprodução Bovina, Araçatuba; [3]Achilles Genetics - Biotecnologia Em Reprodução Animal, Garça; [4]EMBRAPA Pantanal, Corumbá.

Keywords: Linoleic acid, forskolin, cryopreservation.

The objective of the present study was to evaluate the effects of supplementation of *in vitro* culture (IVC) medium with drugs that stimulates the lipolysis (Forskolin: Forsk) and inhibit the lipogenesis (Linoleic Acid LA) on the intracytoplasmic lipid content and cryotolerance of bovine embryos (Experiment 1), as well as to evaluate the effect of treatment of embryos with Forsk on the pregnancy rates after transfer to synchronized recipients (Experiment 2). In the experimente 1, the oocytes (n = 1242) were matured in vitro for 22h at 38.5°C and 5% CO2 in air in medium TCM199 with 10% FCS and hormones. After fertilization, presumptive zygotes were cultured in SOF medium (Control group) supplemented with: 100 μM LA throughout the culture period (LA group); or 5 μM Forsk from the 6th day to the end of the culture (Forsk group); or with the association of LA and Forsk, as described above (LA+Forsk group). The IVC was conducted at 38.5°C and 5% CO2 in air, for 7 days. Embryonic development was assessed on day 7 of culture (D7), when blastocysts were stained with 1% Sudan Black B to determine the intracytoplasmic lipid content. The embryos were evaluated by light microscopy and the images were analyzed by Q-Capture Pro Image software. The Control group was chosen as a calibrator and the measured value of each treatment was divided by the mean of the calibrator to generate the relative expression levels of pixels, expressed in arbitrary units. The expanded blastocysts were vitrified (Vitri Ingá®), warmed and cultured for 24h in SOF to evaluate the re-expansion rates. The data were analyzed by ANOVA followed by Tukey's test and rates of re-expansion by qui-square ($P < 0.05$). No differences were observed between treatments ($P > 0.05$) in blastocysts production rates (47.2 ± 3.9% to 51.6 ± 5.5%). Intracytoplasmic lipid content was decrease ($P < 0.05$) in embryos from Forsk group (0.86 ± 0.04) compared to Control (0.99 ± 0.02) and LA (1.02 ± 0.02); however, all these treatments were similar ($P > 0.05$) to LA+Forsk group (0.95 ± 0.03). There were no differences ($P > 0.05$) between treatments in the rates of re-expansion immediately after warming (0h: 30.8% to 41.9%); after 24h of culture post-warming the re-expansion rates were higher ($P < 0.05$) in Forsk group (71.4%) compared with Control (46.2%) and LA+Forsk (45.2%) groups, but there were no diferences between LA group (65,1%) and the other groups ($P > 0.05$). Based on these results, treatment with 5 μM Forsk was chosen for the Experiment 2: oocytes (n = 1947) were aspirated from 22 Nelore donnors and were matured and fertilized in vitro as described above. Presumptive zygotes were cultured according to the treatments Control and Forsk as described above, and in D7 the expanded blastocysts were vitrified and warmed, before being transfered to synchronized recipients. The data of pregnancy rates were analyzed using the command PROC GLIMMIX (SAS Inst. Inc.). The pregnancy rates after transfer of vitrified-warmed embryos were similar ($P > 0.05$) between Control (15.1 ± 3.6%) and Forsk (19.7 ± 4.0%) groups. According to the results, treatment with Forsk was effective to promote the reduction of intracytoplasmic lipids content in *in vitro*-produced bovine embryos and improves their cryotolerance. Nevertheless, it did not increase the pregnance rates.

Acknowledgments: CAPES.