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Blastocoel collapse associated with melatonin in the culture media improves bovine embryo viability after vitrification

T.C. Marques[1], T.O. Diesel[1], C.G. da Silva[2], E.C.S. Santos[3], C.F. Martins[2], M.A.N. Dode[4], M.L. Gambarini[1]

[1]Universidade Federal de Goiás, Goiania; [2]EMBRAPA Cerrados/CTZL, Brasília; [3]National Institute Agrobiological Sciences, Tsukuba; [4]Embrapa/Cenargen, Brasília.

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Melatonin treatment (MEL) and blastocoel collapse (BC) have been suggested to be potential options to enhance embryo development and viability after cryopreservation of bovine embryos (Wang M et al, J Pineal Res, 56, 333-42, 2014; Wang F et al, PLoS One, 9, e93641, 2014; Min SH eta al, Cryobiology, 66, 195-9, 2013). We investigated the effect of BC performed before the vitrification of bovine embryos produced in vitro in a culture medium supplemented with MEL. Viable oocytes were in vitro matured (IVM) for 24 hours at 38.5°C at atmosphere of 5% CO2 and fertilized with conventional semen (Nellore, 1x106/mL). After 18 hours presumed zygotes were cultured without (Control) or with MEL 10-9 M (IVC+M10-9) for seven days under the same IVM conditions. On Day 3, feeding was done according to the treatment. On Day 7, half of the expanded blastocysts (Bx) Grade I and II of the IVC+M10-9 treatment was submitted to BC (IVC+M10-9 BC). In the sequence the embryos Grade I and II were vitrified (n = 618), thawed and cultured in the IVC medium for 72 hours. Re-expansion rate (RR) after 2 and 24 hours and hatching rates (HR) after 24, 48 and 72 hours were evaluated. Total number of cells (TC) and number of apoptotic cells (NAC) count was performed by TUNEL. TC and NAC presented normal distribution (Shapiro-Wilk test) and were compared by Tukey's test (ANOVA). RR e HR was performed by Chi square. MEL supplementation improved RR and HR at 24, 48 and 72 hours ($P < 0.05$), regardless the BC procedure. After 2 hs Control RR (n = 198), IVC+M10-9 (n = 215) and IVC+M10-9 BC (n = 205) were 29.3%, 86.7% and 89.3%, and after 24 hours were 78.8%, 89.7% and 87.7%, respectively. Control embryos required more time during re-culture (24 hs) for all expansion compared with MEL groups ($P < 0.05$). HR at 24 hs and 48 hs were 6.1% and 6.7%; 18.7% and 37.9%; 27.3% and 41.5%, respectively ($P > 0.05$). At 72 hs HR were 20.3%, 56.1% and 66.8% respectively for control, IVC+M10-9 and IVC+M10-9 BC ($P > 0.05$). HR was higher in IVC+M10-9 BC in all the evaluation moments ($P < 0.05$). Although Control and IVC+M10-9 showed the same HR at 24 hs ($P > 0.05$), MEL added in IVC enhances HR at 48hs and 72 hs ($P < 0.05$). The TC and NCA for fresh embryos were 135.6 ± 5.5 and 7.8 ± 0.6 (Control, n = 15) and 135.4 ± 5.6 and 3.4 ± 0.6 (IVC+M10-9, n = 15) and in the vitrified embryos were 139.8 ± 6.1 and 12.5 ± 0.6 (Control, n = 15), 136.1 ± 5.6 and 4.9 ± 0.6 (IVC+M10-9, n = 15), 140.1 ± 5.8 and 3.7 ± 0.6 (IVC+M10-9 BC, n = 15), respectively. There was no difference in the TC ($P > 0.05$). Embryos produced with MEL had similar NAC regardless of vitrification and BC ($P > 0.05$), but smaller compared with embryos cultured without MEL ($P < 0.05$). In conclusion, the addition of melatonin (10-9 M) in IVC improved embryo quality and viability, independently of BC.

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