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Effect of melatonin in culture medium and blastocoel collapse on viability of vitrified bovine embryos

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Melatonin treatment and blastocoel collapse (BC) had been suggested as potential alternatives to enhance embryo development and viability after cryopreservation of bovine embryos. We investigated the effects of BC before vitrification of embryos produced *in vitro* in the presence of melatonin. The re-expansion rates after 2 and 24 hours, and hatching rates after 24, 48 and 72 hours from heating were evaluated in three treatments: 1- Control (embryos produced without melatonin and vitrified), 2- CIV+M (embryos cultured with 10^{-9} M melatonin and vitrified), 3- CIV + M 10^{-9} (removal of the blastocoel fluid from embryos cultured with 10^{-9} M melatonin). Total number of cells, number of apoptotic cells and expression of genes related to cellular metabolism, oxidative stress, cell repair, placentation and implantation were evaluated in expanded blastocysts from all treatments. Independent of BC, melatonin supplementation during embryo culture at 10^{-9} M improved re-expansion rate ($P < 0.05$). Hatching rate from BC in association with melatonin treatment at 24, 48 and 72 hours was higher than the others groups ($P < 0.05$). Embryos from control group needed more than 24 h of culture for total re-expansion in relation to the groups supplemented with melatonin ($P < 0.05$). The number of apoptotic cells was similar in the groups supplemented with melatonin, independent of blastocoel fluid removal ($P > 0.05$), but lower than those without melatonin. The BC procedure did not affect messenger RNA expression of genes related to cellular metabolism (SLC2A1, SLC2A3), oxidative stress (HSPB1, HSPA5, HSP1A1, SOD2), cell repair (MSH6), placentation (KRT8) and implantation (FOSL1) ($P > 0.05$). This research demonstrated a beneficial effect of melatonin supplementation at 10^{-9} M to the culture medium on embryo quality, but its association with BC was important to increase the hatching rate of vitrified and thawed bovine embryos. Financial support: Embrapa 01130600104.03.04.