THE OPTIMUM CONCENTRATION AND TIMING OF MELATONIN SUPPLEMENTATION DURING IVP TO IMPROVE BOVINE EMBRYO DEVELOPMENT

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The modern techniques for Animal Reproduction allowed Brazil to become a leader in the bovine embryo production (IVP). However, the efficiency is still low when compared with *in vivo* results. One of the problems of IVP is the sensitivity of embryos to reactive oxygen species (ROS). For this reason, antioxidants are commonly used to inhibit the action of ROS and improve the embryo production. Recent studies demonstrated that melatonin (MEL) could enhance embryo quality and cryotolerance due the antioxidant action. Nevertheless, the optimum concentration and embryo stage has not been clarified yet. The objective of this study was to determine the most suitable concentration and timing of MEL supplementation to maximize blastocyst development. Two experiments (E1 and E2) were performed using cumulus oocyte-complexes (COCs) (n=2767) obtained from ovaries collected in slaughterhouses and matured *in vitro* (IVM) in TCM 199 for 24h at 38.5 °C in 5% of CO_2. *In vitro* fertilization (IVF) was performed in FERT-TALP for 18h followed by *in vitro* culture (IVC) for 7 days in SOFaa, both under the same conditions as IVM. Cleavage (CLI) was verified on D3 and Blastocyst Development (BL) on D7 (Day 0= IVF). In E1, three concentrations of MEL were added in IVM media (IVM 10^{-7} M, n= 260; IVM 10^{-9} M, n=253; IVM 10^{-11} M, n= 261; control 0 M, n=254), four replicates, and in IVC media (IVC 10^{-7} M, n=223; IVC 10^{-9} M, n=228; IVC 10^{-11} M, n= 228; control 0 M, n= 221), three replicates. In the E2 the best results for BL in both media were associated. BL was evaluated as in E1 (six replicates) and the rate of apoptotic cells (AC) was determined in D7 embryos by the TUNEL assay (Control n= 15; IVM10^{-9} n= 15; IVC10^{-9} n= 18; IVM/IVC10^{-9} n= 15) 2 replicates. Chi-square analysis was performed for CLI and BL rates and Kruskal-Wallis was performed for AC. In E1 no differences in BL rates using MEL during IVM (IVM10^{-7} 36.2%; IVM10^{-9} 42.3%; IVM10^{-11} 44.4%; control 39.8%) were verified ($P<0.05$). However, using MEL in IVC, BL rate in the IVC10^{-9} was better (47.4%) than IVC10^{-7} (43.5%); IVC10^{-11} (31.1%) and control (37.1%). In E2, when this concentration was used in combination with the media (either IVM or IVC or both), IVC10^{-9} rate for BL was higher (52.8%) than IVM10^{-9} (47.3%); IVM/IVC^{-9} (42.2%) and control (41.7%) ($P<0.05$). AC for IVC10^{-9} was 3.0 ± 0.5 which was significantly lower than that in control (6.5 ± 0.6), IVM10^{-9} (5.3 ± 0.6) and IVM/IVC^{-9} (5.4 ± 1.0). In conclusion, supplementation of IVC media with MEL 10^{-9} M could improve bovine embryo development, because it provides high embryo quality and reduces the number of apoptotic cells.