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Addition of melatonin to the maturation medium of bovine oocytes subjected to heat shock: effects on nuclear maturation, apoptosis, reactive oxygen species, mitochondrial activity, and gene expression

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The effects of melatonin addition to IVM medium of bovine oocytes under heat shock (HS) on nuclear maturation, apoptosis, mitochondrial activity, reactive oxygen species (ROS) and *GDF9* gene expression were evaluated. Cumulus-oocytes complexes (COCs) were recovered from 3-8 mm follicles of crossbreed *Bos indicus ovaries* collected at a slaughterhouse. COCs were matured under HS (12h at 41.5°C and 7% CO_2 followed by 12h at 38.5°C and 5% CO_2) in medium with 0, 10^{-12}, 10^{-9}, 10^{-6} and 10^{-3} M melatonin (Sigma-Aldrich, St. Louis, USA). In the non-stress (NS) group oocytes were matured for 24h at 38.5°C and 5% CO_2 without melatonin. Oocytes were processed for TUNEL assay (Promega, Madison, USA) and stained with DAPI (Vector Lab., Burlingame, USA) to evaluate apoptosis and maturation rates (six replicates, 140±36 CCOs/replicate). For mitochondrial activity (three replicates, 133±18 CCOs/replicate) and ROS (four replicates, 130±20 CCOs/replicate) oocytes were stained in MitoTrackerRed CMX-Ros (Thermo Fisher Scientific, Waltham, USA) and DCFDA (Sigma-Aldrich) and analyzed under a fluorescence microscope. Images were analyzed by the software Image J 1.49. The *GDF9* gene expression was evaluated by RT qPCR (Applied Biosystems 7300 Real-Time PCR System, Thermo Fisher Scientific, Waltham, USA; three replicates, 10 CCOs/replicate). It was considered a randomized block design. Data were analyzed by the GLIMMIX procedure (SAS® 9.3), using binomial (maturation and apoptosis rates) or gamma (mitochondrial activity and ROS) distribution. The *GDF9* gene expression was analyzed by the software REST® and the results expressed regarding the calibrator NS. Melatonin did not improve ($P>0.05$) the maturation rate under HS (67.8±0.6; 75.2±0.2; 59.5±0.3; 67.6±0.2 and 55.8±0.5% in the 0, 10^{-12}, 10^{-9}, 10^{-6} and 10^{-3} M, respectively). The maturation rate did not differ ($P>0.05$) between 0, 10^{-12}, 10^{-6} M and NS (76.6±0.14%). Apoptosis rate in the NS group (0.6±0.6%) was lower ($P<0.05$) than in the groups 0, 10^{-12}, 10^{-9} and 10^{-6} M (4.4±1.0; 3.9±0.9; 4.0±1.2; 3.2±0.9%, respectively) and did not differ from 10^{-3} M (2.1±0.4%). Mitochondrial activity was lower ($P<0.05$) in the 10^{-3} M (42.9±0.1 arbitrary units - AU) than in the other groups (0 M: 63.9±0.1; 10^{-12} M: 62.4±0.1; 10^{-9} M: 59.8±0.1; 10^{-6} M: 58.0±0.1 AU) and it was greater in 0 M than in NS (57.1±0.1 AU). ROS production was lower ($P<0.05$) in the 10^{-3}, 10^{-6} and 10^{-9} M (13.5±0.2; 16.2±0.2 and 16.1±0.2 AU, respectively) than in 10^{-12} M (32.5±0.2 AU) and 0 M (31.2±0.2 AU). ROS was greater in 10^{-12} M and 0 M than in NS (25.0±0.2 AU). *GDF9* gene expression was greater in the 10^{-3}, 10^{-6} and 10^{-9} M (5.8±1.6; 2.5±0.8; 1.7±0.4 folds) compared to NS. Melatonin at 10^{-6} M in the IVM protects oocytes from the damage caused by HS, as demonstrated by maturation rate similar to that observed on oocytes from NS, lower ROS production, and greater *GDF9* gene expression. Financial support: CNPq (427476/2016-0), FAPEMIG and CAPES (Financial code 001).