Proceedings of the 29th Annual Meeting of the Brazilian Embryo Technology Society (SBTE); Gramado, RS, Brazil, August 20th to 23rd, 2015, and 31st Meeting of the European Embryo Transfer Association (AETE); Ghent, Belgium, September 11th and 12th, 2015. Abstracts.

A230 Embryology, Developmental Biology and Physiology of Reproduction

Effect of melatonin on DNA fragmentation and in vitro maturation of bovine oocytes subjected to heat shock

I.J. Ascari[1], N.G. Alves[1], L.S.A. Camargo[2], J. Jasmin[3], C.C.R. Quintão[2], J.A.S. Oliveira[4], T.D. Araújo[5]

[1]Universidade Federal de Lavras; [2]EMBRAPA; [3]Universidade Federal do Rio de Janeiro; [4]CES; [5]Universidade Federal de Juiz de Fora.

Keywords: DNA fragmentation, heat shock, *in vitro* maturation.

The aim of this study was to evaluate the effect of different concentrations of melatonin added to the medium IVM in DNA fragmentation and maturation of oocytes subjected to heat shock. Immature oocytes aspirated from ovaries obtained from slaughterhouse were selected and randomly allocated in factorial experiment design 3x2. Three concentrations of melatonin (0 M, 10-6 M and 10-4 M; M5250 - Sigma, St. Louis, MO , USA) added to the medium and two MIV incubation conditions (conventional: 24 hours at 38.5°C and 5% CO_2; or heat shock: 12 hours at 41°C followed by 12 hours at 38.5°C and 5% CO_2) were tested, resulting in treatments: M1 (0 M; 38.5°C; n = 156), M2 (10-6 M; 38.5°C; n = 154), M3 (10-4 M; 38.5°C; n = 161), M4 (0 M; 41°C; n = 154), M5 (10-6 M; 41°C; n = 143) and M6 (10-4 M; 41°C; n = 159). The IVM was performed in Nunc plate containing 400 µL of TCM-199 (Tissue Culture Medium 199 - Invitrogen, California, USA) supplemented with 20 µg/mL of FSH (Pluset®, Calier Laboratories, Spain) and 10% of estrus cow serum. After the maturation period, the cumulus-oocytes complex were denuded in a solution of PBS plus 0.1% hyaluronidase (Sigma, St. Louis, USA) by vortexing for 5 minutes and washed twice in PBS containing 0.1% PVP. The oocytes were fixed in 4% paraformaldehyde in PBS for one hour and evaluated by the TUNEL assay (deadend ™ Fluorometric TUNEL System - Promega, Madison, WI, USA) about the percentage of TUNEL positive oocytes (DNA fragmentation) and percentage of nuclear maturation (percentage of oocytes in metaphase II). Four replicates were performed. Data were analyzed by Proc Genmod of SAS software (version 9.1; SAS Institute Inc., Cary, NC, USA) considering effects of repetition, melatonin concentration, incubation conditions and interaction between the factors. Values shown are the mean ± s.e.m. Addition of melatonin did not affect ($P > 0.05$) the percentage of TUNEL positive oocytes (M1 = 2.1% ± 0.7; M2 = 1.9% ± 1.9; M3 = 1.9% ± 1.3; M4 = 5.1% ± 2.7; M5 = 2.3% ± 1.5; and M6 = 1.9% ± 0.7) and there was no interaction between concentration and incubation conditions. Melatonin did not affect the percentage of nuclear maturation in the temperature of 38°C ($P > 0.05$), however, in the heat shock, the percentage of maturation was higher in M6 treatment when compared to M4 ($P < 0.05$) (M1 = 85.8% ± 2.9^{a}; M2 = 84.0% ± 2.8^{a}; M3 = 79.5% ± 2.9^{ab}; M4 = 61.6% ± 6.9^{c}; M5 = 62.8% ± 8.5^{cd}; M6 = 72.6% ± 5.3^{bd}). The DNA fragmentation was not influenced by melatonin supplementation to the medium MIV. However, there was an increase in the percentage of maturation of oocytes subjected to heat shock in maturation medium with a concentration of 10-4 M in comparison with 0M concentration.

Acknowledgments: Fapemig, CNPq and Embrapa.