IVF/IVP

157 EFFECT OF MELATONIN ON EMBRYO QUALITY OF BOVINE OOCYTES SUBJECTED TO HEAT SHOCK

N. G. Alves[A], *I. J. Ascari*[A], *L. S. A. Camargo*[B], *J. Jasmin*[C], *C. C. R. Quintão*[B], *J. A. S. Oliveira*[B], *E. D. Souza*[D], *C. M. A. Assunção*[E], *V. R. A. Mendes*[F], *and R. R. Lima*[A]

[A]Federal University of Lavras, Lavras, MG, Brazil;
[B]Embrapa Dairy Cattle, Juiz de Fora, MG, Brazil;
[C]Federal University of Rio de Janeiro, Rio de Janeiro, MG, Brazil;
[D]Federal University of Espírito Santo, Vitoria, ES, Brazil;
[E]Federal University of Juiz de Fora, Juiz de Fora, MG, Brazil;
[F]Federal University of Viçosa, Viçosa, MG, Brazil

The aim of this study was to evaluate the effect of different concentrations of melatonin added to *in vitro* maturation (IVM) medium of oocytes subjected to heat shock on embryo quality. Immature oocytes aspirated from ovaries obtained from a slaughterhouse were selected and randomly allocated in factorial experiment design (3 × 2). Three concentrations of melatonin (0, 10^{-6}, and 10^{-4} M; M5250, Sigma, St. Louis, MO, USA) were added to the IVM medium and 2 incubation conditions (conventional: 24 h at 38.5°C and 5% CO_2; heat shock: 12 h at 41°C followed by 12 h at 38.5°C and 5% CO_2) were tested, resulting in treatments: M1 (0 M; 38.5°C; $n = 15$), M2 (10^{-6} M; 38.5°C; $n = 15$), M3 (10^{-4} M; 38.5°C; $n = 15$), M4 (0 M; 41°C; $n = 15$), M5 (10^{-6} M; 41°C; $n = 15$), and M6 (10^{-4} M; 41°C; $n = 15$). The IVM was performed in a Nunc plate (144444 – Thermo, Fisher Scientific Inc., Pittsburgh, PA, USA) containing 400 µL of TCM-199 (Invitrogen, Carlsbad, CA, USA) supplemented with 20 µg mL^{-1} of FSH (Pluset®, Calier Laboratories, Barcelona, Spain) and 10% oestrus cow serum. Oocytes were IVF in FERT-TALP medium for 20–22 h and incubated at 38.5°C and 5% CO_2. After IVF, the presumptive zygotes were denuded and cultured in CR2aa medium supplemented with 2.5% FCS (Nutricell, Campinas, Brazil) in an incubator at 38.5°C under 5% CO_2, 5% O_2, and 90% N_2, and saturated humidity for 8 days. Blastocysts with 8 days post-fertilization from different treatments were fixed in 4% paraformaldehyde in PBS for 1 h and analysed by TUNEL assay (Deadend™ Fluorometric TUNEL System, Promega, Madison, WI, USA) to evaluate embryonic quality. Data were analysed by generalised linear models considering the Poisson distribution and using the Proc Genmod of SAS software (version 9.1; SAS Institute Inc., Cary, NC, USA) considering effects of melatonin concentration, incubation conditions, and interaction between the factors. Values shown are the mean ± s.e.m. The interaction between melatonin concentration and incubation conditions was no significant ($P > 0.05$). The total number of cells was not affected ($P > 0.05$) by melatonin, but it was decreased ($P < 0.05$) by heat shock (M1 = 117 ± 6.7^a; M2 = 118 ± 4.2^a; M3 = 120 ± 6.3^a; M4 = 102 ± 6.2^b; M5 = 106 ± 5.7^b; M6 = 108 ± 8.9^b). Melatonin and heat shock did not affect ($P > 0.05$) the index of embryo apoptotic cells (M1 = 15.3% ± 2.0; M2 = 15.5% ± 1.3; M3 = 13.6% ± 2.0; M4 = 14.9% ± 1.5; M5 = 13.3% ± 1.3; M6 = 13.5% ± 1.2) and the index of trophoblast cells (M1 = 74.6% ± 2.3; M2 = 75.0% ± 1.7; M3 = 75.2% ± 1.9; M4 = 78.4% ± 2.3; M5 = 76.4% ± 3.0; M6 = 75.2% ± 2.6). The melatonin and heat shock affected the index of the inner cell mass (ICM; $P < 0.05$), and the heat shock reduced the index of the ICM of oocytes not treated with melatonin (M1 = 25.4% ± 2.3^a; M2 = 25.0% ± 1.7^a; M3 = 24.8% ± 1.8^a; M4 = 21.6% ± 2.3^b; M5 = 23.6% ± 3.0^a; M6 = 24.8% ± 2.6^a). In conclusion, melatonin supplementation to the medium IVM of oocytes subjected to heat shock had no effect on blastocyst total cell number, general apoptotic index, or index of the trophoblast cells, but increased index of the ICM.

Research was supported by Fapemig, CNPq, Embrapa, and CAPES.

Reproduction, Fertility and Development

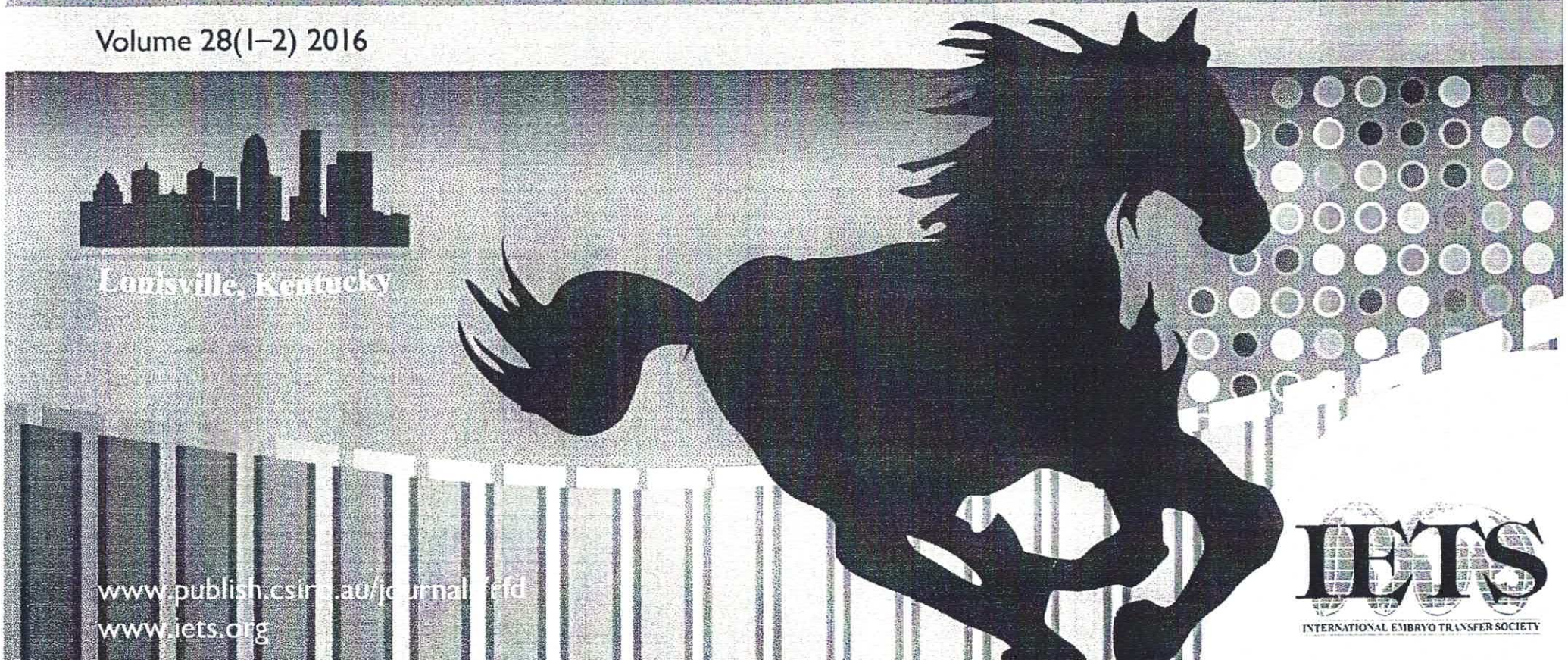
Contents

Search

Exit

Proceedings of the Annual Conference of the International Embryo Transfer Society, Louisville, Kentucky, 23–26 January 2016

Volume 28(1–2) 2016



www.publish.csiro.au/journals/rfd
www.iets.org

IETS
INTERNATIONAL EMBRYO TRANSFER SOCIETY