



A191 Embryology, Developmental Biology and Physiology of Reproduction

**Induction of survival mechanisms to thermal stress in bovine embryos****C.S. Oliveira<sup>1</sup>, P.H.E. Guedes<sup>1,3</sup>, S.C.S. Marques<sup>1</sup>, J.V.G. Silva<sup>1</sup>, A.J.R. Camargo<sup>2</sup>, C. Freitas<sup>1</sup>,  
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Thermal stress during embryonic development is associated with increased gestational losses in cattle. An alternative to avoid the negative effects of thermal stress is to induce thermotolerance, a phenomenon that involves the expression of HSP proteins and makes the cells resistant to subsequent stress. The aim of this study was to develop a protocol for the thermal treatment of Girolando (3/4) IVF embryos, which would induce thermotolerance without causing negative effects over development. For this, embryos produced *in vitro* from Girolando cows (1/2) were used (CEUA-EGL 3956180316). After some tests, we standardized a temperature increase curve ranging from 38.5 to 40.5°C for 6 hours, applied in morulae (144hpi) – TT group, which did not alter the production of blastocysts/ morulae at d7 (C 70.20%, TT 71.67%,  $p = 0.82$ , Fisher exact test,  $n = 371$  morulae, 173-198 per group). Immunofluorescence analysis showed that the treatment induced increased nuclear expression of HSP70 protein after 12h (C  $43.27 \pm 9.22$ , TT  $62.80 \pm 18.10$ ,  $p = 0.01$ , T Test,  $n = 19$  blastocysts, 9- 10 per group), measured by fluorescence intensity using Photoshop software (pixels 0-255). There was no effect on the blastocyst total cell number (C  $70.53 \pm 20.06$ , TT  $78.00 \pm 14.87$ , T-test,  $n = 27$  blastocysts, 12 to 15 per group). In a second experiment, the TT embryos were exposed to thermal shock at 40.5 ° C for 2 hours at d7, and survived 15% more than control embryos (C 71.42%, TT 86.66%,  $p = 0.004$ , Fisher's exact test,  $n = 263$  blastocysts, 124-139 per group). The effects of treatment after implantation are described in another abstract. The future prospects of the study include embryo transfer of TT embryos in lactating cows, during the summer, to validate the thermotolerance of the embryos, and to follow gestations till birth to prove the safety of this treatment. Therefore it is concluded that the induction of survival mechanisms can be an important strategy to mitigate the effects of thermal stress.

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