A135 Embryology, developmental biology and physiology of reproduction

In vitro production of bovine thermotolerant embryos

Sheila Costa de Souza Marques[1,3], João Victor Gonçalves da Silva[1,4], Agostinho Jorge dos Reis Camargo[2], Luiz Sérgio de Almeida Camargo[1], Clara Slade Oliveira[1]

[1]EMBRAPA - Empresa Brasileira de Pesquisa Agropecuária, Juiz de Fora, MG, Brasil; [2]Pesagro Rio - Pesagro Rio, Niterói, RJ.Brasil; [3]UBM - Centro Universitário Barra Mansa, Barra Mansa, RJ, Brasil; [4]UGB - Centro Universitário Geraldo di Biase, Barra do Piraí, RJ, Brasil.

Considering the climatic changes and the elevation of temperature, the reproductive indices in dairy herds of countries with tropical climate are critical. High metabolic rate and the high uterine temperature are related to embryonic death. This experiment was designed to develop a heat treatment protocol for bovine embryos in order to induce thermotolerance in Girolando embryos. Subsequently, those embryos will be characterized (HSP protein expression) and tested as an alternative to conventional embryos during the summer season. For this experiment, oocytes were collected from F1 (½ Gir and ½ Holstein) donors by ultrasound guided follicular aspiration (OPU, ovum pick-up) (CEUA EGL 3956180316). Oocytes were in vitro fertilized with Holstein bull semen for production of ¾ Holstein embryos. A mild heat treatment protocol was designed and tested at 96, 120 or 144h. p.i.. The embryos were submitted to the heat treatment at a temperature of 38.5 to 40.5 °C for 6 hours. A homemade incubator was settled with a water filled plastic box placed at a heat stage. Temperature increase or decrease was controlled manually by opening or closing the plastic box. Treated embryos were moved to cryotubes with 200ul medium and 200ul of mineral oil and remained for 40 min in the incubator for gas equilibration with a loose cover. After that, cryotube was tightly closed and sealed with parafilm and transferred to heat treatment chamber. Heat treatment was carried out as six 1h cycles. At each cycle, every 7.5 min a 0.5° C increase was induced up to 40.5°C, and then every 7.5 min a 0.5°C decrease was induced until 38.5°C. Control remained in the incubator at 38.5 °C. Two replicates were performed, and blastocyst rates at d7 were evaluated as well as the number of cells and apoptosis rate of the blastocysts. No differences were observed in the blastocyst rates (C = 28.57, TT96hpi = 35.14, TT120hpi = 23.81, TT144hpi = 19.77, p> 0.05, Fisher's exact test, n = 298 cleaved embryos) or on the apoptosis index (C = 6.47 ± 3.93, TT96hpi = 7.41 ± 4.05, TT120hpi = 7.07 ± 5.21, TT144hpi = 4.54 ± 2.71, p> 0.05, ANOVA and Dunnett, n = 50 blastocysts). The mean number of cells did not differ in any treatment compared to the control group (C = 70.53 ± 20.03, TT96hpi = 67.46 ± 13.65, TT120hpi = 57.80 ± 8.74, TT144hpi = 78, 00 ± 14.87, p> 0.05, ANOVA and Dunnett, n = 50 blastocysts). The results allow us to conclude that heat treatment developed can be used at any of the tested moments without being harmful to embryos. The 144h.p.i. can be preferred due to the proximity to embryo transfer (168 h.p.i.), so that the post-transfer effects are prolonged. Acknowledgements: Fapemig and CNPq.