IVF/IVP

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## 228 EVALUATION OF EMBRYO VIABILITY AFTER PROLONGED TRANSPORT IN DIFFERENT MEDIA AND AT DIFFERENT TEMPERATURES

R. Z. Puelker<sup>A</sup>, A. G. R. Pupulim<sup>B</sup>, C. H. O. Constant<sup>B</sup>, M. A. Andreazzi<sup>B</sup>, and I. P. Emanuelli<sup>B,C</sup>

<sup>A</sup>PROGEST, Botucatu, São Paulo, Brazil; <sup>B</sup>CESUMAR, Maringá, Paraná, Brazil; <sup>C</sup>UEM, Maringá, Paraná, Brazil

In vitro-produced embryos (IVP) are known to have poor quality and be susceptible to heat and oxidative stress. In addition, they constantly undergo long-distance transport, which causes low conception rates. The aim of this study was to search for alternatives to long-distances transport of embryos produced in vitro by evaluating the use of different media and different temperatures. Bos indicus cumulus-oocyte complexes (COC; 823, quality I and II) were matured in TCM-199 bicarbonate-10% FBS (38.5°C, 5% CO<sub>2</sub>, in air) for 24 h. The fertilization was performed in TALP-IVF medium for 18 h of incubation. Presumptive zygotes were transferred to SOF medium and in vitro culture in a controlled atmosphere (5% O<sub>2</sub>, 5% CO<sub>2</sub>, 90% N<sub>2</sub>) for 7 days at 38.5°C. At Day 7, 366 embryos (quality I and II, IETS), were randomly allocated to the designated experimental groups. For Experiment 1, blastocysts were filled into straws and kept for 12 h at 36°C, using the medium as an independent variable according to the following groups: GSup, embryo support medium without FCS and amino acids (n = 115); and GHSOF, embryo culture medium SOF (HSOF) with FCS and amino acids (n = 105). Both media were buffered with HEPES. For Experiment 2, blastocysts were filled into straws with HSOF medium and sustained in transportation for 12 h, using temperature as the independent variable according to the following groups: G36,  $36^{\circ}C$  (n = 65); and G38,  $38^{\circ}C$  (n = 81). After 12 h of transport in both experiments, embryos were evaluated and classified as viable or nonviable blastocysts and were recultured in the same conditions mentioned. On Day 10, hatching rates and degeneration were evaluated. Logistic regression was used to compare the groups in each experiment. In Experiment 1, blastocyst viability after 12 h of transport was higher in the culture medium (GHSOF: 91.4%) than the support medium (GSup: 75.6%; P < 0.001). The hatching capacity in the culture medium (GHSOF: 72.4%) was higher than that in the support medium (GSup: 34.5%; P < 0.001). In Experiment 2, blastocyst viability post-transport was the same in both temperatures (G36: 92.3%, G38: 82.7%; P > 0.09). Blastocyst hatching capacity at 36°C (G36: 75.4%) was superior to that at 38°C (G38: 49%; P < 0.001). The hatching capacity immediately after 12 h of transport was minimal, so it was not possible to apply the logistic regression method in any experiment. In conclusion, the culture medium was more efficient for long-distance transport than the support medium; moreover, a temperature of 36°C for the culture medium increased embryo development compared with a temperature of 38°C during transport.

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## 229 EVALUATION OF OOCYTE QUALITY RECOVERED FROM GIR BREED COWS WITH SYNCHRONIZED FOLLICULAR WAVE ON *IN VITRO* EMBRYO PRODUCTION

L. T. Iguma<sup>A</sup>, J. N. S. Sales<sup>B</sup>, R. I. T. P. Batista<sup>A,C</sup>, M. M. Pereira<sup>A,C</sup>, C. C. R. Quintão<sup>A</sup>, R. S. Verneque<sup>A</sup>, L. S. A. Camargo<sup>A</sup>, J. H. M. Viana<sup>A</sup>, and P. S. Baruselli<sup>B</sup>

## <sup>A</sup>Embrapa Dairy Cattle, Juiz de Fora, MG, Brazil;

<sup>B</sup>University of São Paulo, São Paulo, SP, Brazil; <sup>C</sup>Federal University of Juiz de Fora, Juiz de Fora, MG, Brazil

Recent data reveal that approximately 80% of bovine embryos produced *in vitro* worldwide are from (Brazil J. H. M. Viana, personal communication). Adoption of this biotechnology by Brazilian producers might be attributed to particularities of the *Bos indicus* subspecies. Zebu breeds provide 2 to 3 times more viable oocytes than *Bos taurus* breeds per ovum pickup (OPU) session. This work aimed to evaluate the quality of cumulus–oocyte complexes (COC) retrieved from Gir breed cows (*B. indicus*) by OPU with a synchronized follicular wave and subjected to an *in vitro* embryo production (IVP) technique. All COC were obtained by OPU of 14 Gir cows performed every 14 days. There were 4 OPU sessions, preceded by synchronization of the follicular waves. Wave synchronization was achieved by an injection of 2 mg of oestradiol benzoate followed by the insertion of a norgestomet implant that stayed in place for 5 days. On Day 5, follicles of 2 to 8 mm were aspirated. The oocytes recovered were distributed according to classification: Group 1, with  $\geq$ 3 cumulus cell layers and homogeneous or slightly heterogeneous cytoplasm (n = 314); and Group 2,  $\leq$ 2 cumulus cells layers and slightly or coarsely granulated cytoplasm (n = 236). Oocytes from both groups were followed to *in vitro* maturation–IVF–fertilization–*in vitro* culture. Two (Day 2) and 8 (Day 8) days after the beginning of fertilization, the cleavage and blastocyst rates were measured, respectively. The statistical analyses were performed using a chi-square test (P < 0.05). The mean cleavage rates observed were 72.93 and 69.07% (not significant), whereas the mean blastocyst rates were 37.90 and 17.80%, and were significantly different for Groups 1 and 2, respectively. The results suggest that the morphology of oocytes (number of cumulus cell layers and cytoplasm homogeneity) is a strong indicator of the *in vitro* developmental capacity of oocytes recovered from Gir cows with synchronized follicular waves, although we did not observe compromised cleavage

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## 230 OVUM PICKUP–IN VITRO PRODUCTION EFFICIENCY FOR CONSERVATION OF THE FLAMENGA CATTLE BREED

F. C. Zago<sup>A,B</sup>, S. Gaudêncio Neto<sup>B</sup>, L. H. Aguiar<sup>B</sup>, L. F. Schutz<sup>B</sup>, F. Forell<sup>B</sup>, L. R. S. Marinho<sup>B</sup>, J. Machado, Jr<sup>B</sup>, A. Mezzalira<sup>B</sup>, and M. Bertolini<sup>B,C</sup>

<sup>A</sup>Agropecuary Research and Rural Extension Company of Santa Catarina State (EPAGRI), Lages, SC, Brazil; <sup>B</sup>Center of Agronomy and Veterinary Sciences, Santa Catarina State University (UDESC), Lages, SC, Brazil; <sup>C</sup>School of Medicine, University of Fortaleza (UNIFOR), Fortaleza, CE, Brazil

The introduction of more specialised and productive dairy breeds into commercial herds in recent decades has caused a gradual loss of interest in dualpurpose cattle breeds, such as the Flamenga. Because of its importance to biodiversity, the high risk of loss of this small Brazilian herd, located in one



