168 CORRELATION OF DEVELOPMENT KINETICS AND SEX OF *IN VITRO*-PRODUCED BOVINE EMBRYOS

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Approaches to improve the culture medium for *in vitro* production (IVP) of bovine embryos have been continuous because of the high commercial demand and a portion of this attempts the production of female cattle (dairy cows and stud cattle). However, in some embryonic *in vitro* culture systems, the development kinetics is faster in male than in female embryos (Avery 1992 *Mol. Reprod. Dev.* **32**, 265–70; Xu 1992 *Mol. Reprod. Dev.* **31**, 249–50). The aim of this work was to relate the kinetics of blastocyst expansion with the production rates of male and female embryos. Cumulus–oocyte complexes ($n = 917$; classes I and II) of cows from a slaughterhouse were matured with TCM-199 bicarbonate and 10% FCS (38.5°C, 5% CO_2) for 24 h and fertilized with frozen-thawed semen in TALP-IVF medium for 18 h. Presumptive zygotes were culture in SOF medium supplemented with 10% FSB (5% O_2, 38.5°C). Seven days after IVF, embryos were divided in 2 groups according to their kinetic stage of development: nonexpanded blastocysts ($n = 175$), or hatched and expanded blastocysts ($n = 146$). Hence, embryos were individually frozen in LN and stored in cryotubes. After thawing, Proteinase K (16 mg mL^{-1}) was added to each tube and the tubes were incubated for 60 min at 37°C. Proteinase was denatured at 98°C for 10 min and the contents of each tube were divided into 2 samples (A and B) and subjected to the PCR technique. Two pairs of primers for the specific sequence of the Y chromosome were used to amplify the sequence of 210 and 250 bp for the male bovine and 1 pair of primers was used for the autosomal bovine sequence with a 280-bp fragment. Female embryos with a 280-bp product were observed in sample A and none were observed in sample B. The presence of 2 amplicons (280 and 210 bp) in sample A and 1 amplicon of 250 bp in sample B indicated that the embryo was male. A chi-square test was used to evaluate homogeneity. An analysis of the percentage of males and females between the experimental groups was performed by logistic regression and significance was considered when $P < 0.05$. There was no difference in the proportions of males and females in the nonexpanded blastocyst group (49.71 and 50.29%; $P > 0.05$). In the hatched and expanded blastocyst group, the proportion of males (65.75%) was statistically different from the proportion of females (34.25%); that is, the chance of the embryo being male was twice as high ($P < 0.0038$). These results suggest that there is a difference in the kinetics of embryo development between male and female embryos and that blastocyst expansion can point that out. *In vitro* culture media with FCS support the development of expanded male blastocysts. Further research in culture medium modifications (FCS, the energy source, amino acids and others) are needed to respond to the trend in the production of sex-defined embryos.

169 THE RELATIONSHIP BETWEEN OOCYTE RECOVERY AND EMBRYO PRODUCTION IN *BOS INDICUS*

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The ovarian follicular population has been used as a parameter to evaluate fertility and also the potential of donors undergoing assisted reproductive procedures in both human medicine and animal practice. There is a high correlation between follicular population and oocyte recovery by ovum pickup (OPU), but the relationship between oocyte recovery, embryo production and pregnancy rates may not be fully understood. The aim of the present study was to evaluate whether the conversion rate of oocytes to embryos and further pregnancies could be positively related to the number of cumulus–oocyte complexes (COC) recovered after OPU in cattle. For this purpose, records of 626 OPU sections from 251 nonlactating Gyr cows (dairy Zebu breed) were analysed. The animals had a good body condition score, were kept in a good feeding pasture (*Brachiaria* spp.) and were supplemented with corn silage and a mixture of corn, soybeans and vitamin and minerals, according to their nutritional requirements. For each ovarian aspiration, the ovarian follicular wave was previously synchronized with an auricular implant (Norgestomet-Crestar®), IM injections of 2 mg of oestradiol benzoate (Gonadiol®) and 0.25 mg of D-cloprostenol (Sincrocio®). The OPU procedures were performed using an ultrasound device (Aquila Vet, Esaote, São Paulo, Brazil) equipped with a vaginal sector 7.5-MHz probe, disposable 20 G needles and a vacuum pressure of 80 mmHg. The cows were ranked in quartiles regarding the total number of COC recovered. To reduce bias related to the eventual fluctuation of OPU results, for the present analysis the authors used only the recorded OPU session of each cow with the highest number of COC recovered. Viable COC were fertilized with sex-sorted (X) semen of Gyr bulls previously tested for *in vitro* embryo production. Conversion rates (%) of the total and viable oocytes to embryos, viable oocytes to pregnancy and embryo to pregnancy were evaluated for each quartile. Differences between the first and fourth quartiles were accessed by Fisher's exact test. In the 251 OPU, 4246 total and 3173 viable COC were recovered, resulting in the production of 1001 embryos (31.5%) and 453 pregnancies (45.3%). The cows ranked in the first, second, third and fourth quartiles produced >30 (41.6 ± 10.6), 21 to 30 (25.2 ± 3.0), 12 to 20 (15.9 ± 2.6) and <12 (6.7 ± 3.1) total oocytes. The average viable oocyte (29.1 ± 11.0, 18.1 ± 5.3, 11.1 ± 3.7 and 4.5 ± 2.7, respectively) and embryo production (8.6 ± 5.7, 5.2 ± 3.6, 3.8 ± 2.8 and 1.8 ± 1.8, respectively) were different ($P < 0.0001$) among all quartiles. Pregnancy rates, however, did not differ (46.0, 44.9, 43.9 and 45.6%, respectively; $P > 0.05$). Interestingly, the conversion rates (viable oocytes to embryos and viable oocytes to pregnancies) were higher ($P < 0.0001$ and 0.002) in cows from the last quartile (51.1 and 31.9%) compared with those from the first quartile (23.7 and 14.7%). In conclusion, the number of COC recovered by OPU (and consequently the ovarian follicular count) can further predict the total number of embryos and pregnancies produced, but it is not directly related to the oocyte development potential.

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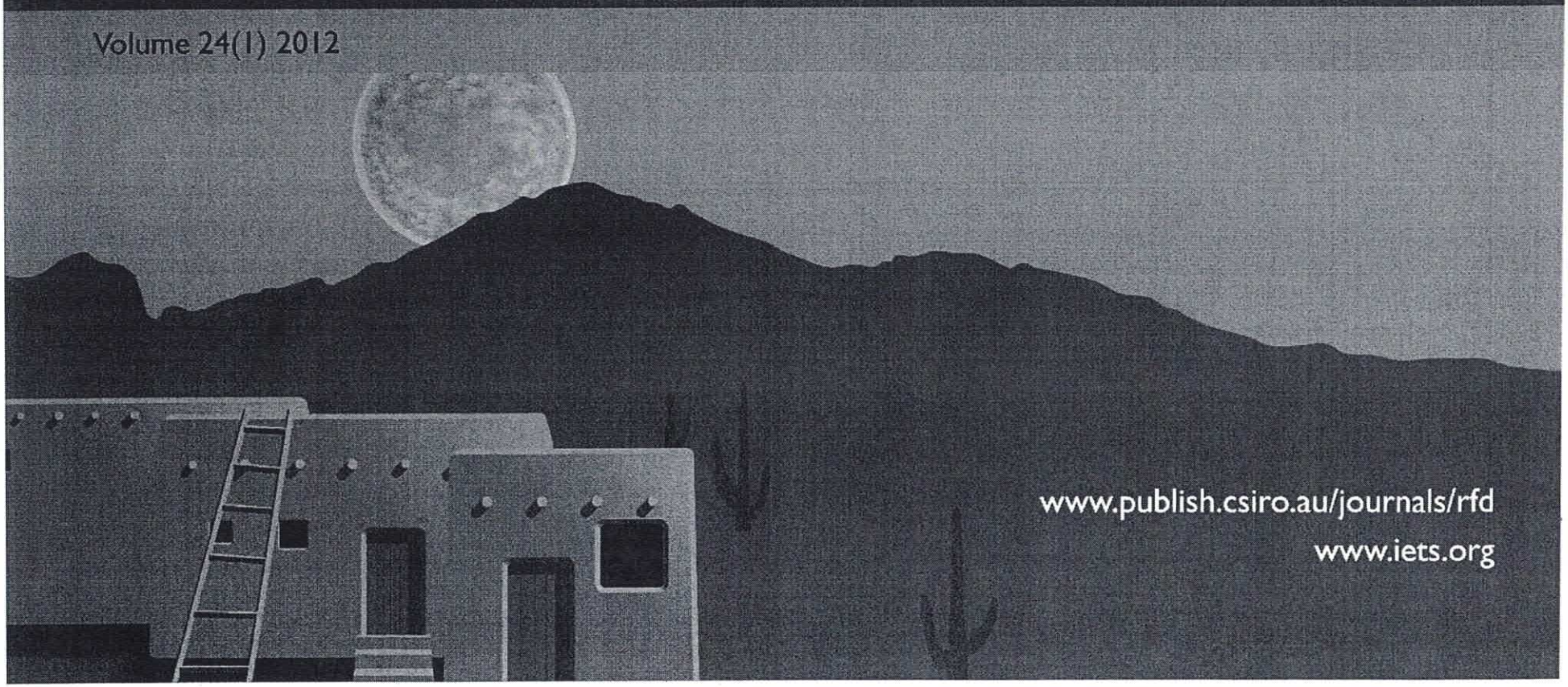

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