PREGNANCY RATE, LENGTH AND GENDER OF IN VITRO PRODUCED EMBRYOS IN BOVINE

ARASHIRO¹, E.K.N.; CAMARGO², L.S.A.; VIANA², J.H.M.; PALHÃO³, M.P.; FONSECA¹, J.F.; SÁ², W.F.; FERREIRA², A.M.

Introduction
The use of assisted reproductive techniques (ART) in Brazilian cattle industry has increased significantly in the last few years. The in vitro embryo production is one of them and has been used to increase offspring of genetically superior animals and accelerate genetic improvement; moreover, it allows preservation of wild or rare species. However, efficiency of these techniques is still limited. Embryo production rates are usually low, and the proportion of pregnancy abnormalities and stillbirth is high. Under in vitro culture conditions, embryos are exposed to an environment different from which it would have in vivo, and this is one of the supposed causes of the developmental problems observed (YOUNG et al., 1998). Low pregnancy rates, high embryonic losses, oversized calves and consequent dystocia are common. The aim of this study was to evaluate pregnancy characteristics in recipients inovulated with embryos produced by oocyte pick-up and in vitro fertilization.

Material and Methods
The present study was performed in the Embrapa’s Dairy Cattle Research Center, in Juiz de Fora, MG. Holstein (n=5) and Gir (n=8) cows were used as cumulus-oocyte complexes (COCs) donors. Oocyte pick-up was performed using a portable ultrasound device equipped with a 7.5 sector intravaginal transducer. The COCs recovered and morphologically classified as viable were transported in Talp-Hepes medium to the in vitro fertilization laboratory, and underwent maturation in TCM 199 medium for 22h, under controlled culture conditions. In vitro fertilization was performed with previously capacitated sperm of bulls from the same breed of the donors. Presumptive zygotes were transferred to microdrops of CR-2 and co-cultured with granulosa cells for seven days. The blastocysts produced were non-surgically transferred to recipients, previously synchronized and evaluated. Pregnancy diagnosis was performed by ultrasound 20 to 23 days after transfer, and confirmed at the time of embryo sexing, at 55 to 60 days of pregnancy. Pregnant recipients were maintained in separated lots, and monitored until parturition.

Results and Discussion
The overall pregnancy rate was 48.89% (22/45), similar to results obtained by other groups with in vitro produced embryos (KRUIP et al., 1997) but below pregnancy rates observed with in vivo fresh embryos (HASLER, 2001). However, there was a 9% of pregnancy losses between 30 and 60 days. Embryo development or placental abnormalities may be one of the reasons for pregnancies loss of in vitro produced embryos, which can reach up to 47% (SCHMIDT et al., 1996). There was a bias in the expected proportion of gender distribution (1:1), with 70% (14/20) of males and 30% (6/20) of females, and this phenomenon was observed in both breeds (75% of males for Gir and 66.7% for Holstein). Higher proportion of males has been reported for in vitro produced embryos and may be associated with the sperm preparation technique used (swim-up) or with differences in developmental rate under cultured conditions of the different gender embryos. Gestation length of Holstein (282.17±6.49 days) and Gir (292.21±5.81 days) in vitro produced calves was similar to those observed in natural bred or artificial insemination.

Conclusion
The transfer of in vitro produced embryos resulted in pregnancies of normal length in Holstein and Gir breeds, but pregnancy rates are still below that obtained with in vivo fresh embryo, and there is a trend for the birth of male calves in both breeds.

References
KEY WORDS: bovine, embryo, parameters

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¹ Médico Veterinário autônomo
² Méd. Veterinário, DSc., Pesquisador da Embrapa Gado de Leite, Juiz de Fora, MG
³ Méd. Veterinário, Mestrando, DZQ-UFV, Viçosa, MG
⁴ Méd. Veterinário, DSc., Pesquisador Embrapa Caprinos, Sobral, CE

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