Effect of the postpartum days on the pregnancy rate of cows submitted to fixed-time embryo transfer

Efeito dos dias pós-parto sobre a taxa de prenhez de receptoras submetidas à transferência de embriões em tempo fixo

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ABSTRACT

The present study aimed to analyze the influence of the postpartum period on the pregnancy rate of cows submitted to fixed-time embryo transfer (FTET). It was used one hundred thirty-one crossbreed embryo recipient cows, on the 79.35 ± 28.0 days postpartum and BCS between 2.75 to 3.0. The cows received a hormonal protocol initiated on a random day of estrus cycle (D0), beginning with an intravaginal device containing 1.9 g of progesterone and intramuscular administration (IM) of 2 mg of estradiol benzoate. In D8, the intravaginal device was removed, and it was administered a 0.15 mg D-cloprostenol, 0.5 mg estradiol cypionate and 300 IU eCG IM. The FTET of Senepol IVP embryos was performed on D17, without previous estrus observation. The recipient cows were divided into two experimental groups: until the seventh day postpartum (N = 70) and after the seventh day postpartum (N = 61). The pregnancy diagnosis was performed by transrectal ultrasonography (Mindray DP2200, 7.5 Mhz) on the 23th day after ET. The pregnancy rate of the recipient’s cows was 52% (68/131) and there was no difference (p>0.05) on the pregnancy rate of the recipient’s cows regarding the embryo development stage, neither the postpartum period. In conclusion, the use of calving cows as embryos recipients may be a viable alternative, resulted in adequate pregnancy rates, optimizing the use of this animal category in embryo transfer programs.

Keywords: Bovine, Reproductive efficiency, Embryo, Postpartum.
de prenhez satisfatórias, otimizando o uso desta categoria em programas de transferência de embriões.

**Palavras-chave:** Bovino, Eficiência reprodutiva, Embrião, Pós-parto.

### 1 INTRODUCTION

The reduction of reproductive efficiency is one of the major problems for improving the productivity of beef cattle herds. Beef cows usually present a prolonged anestrus period after calving, caused by nutritional disorders associated or not with nursing (Sá Filho et al., 2013), which is the main factor in increasing the calving interval. Biotechnologies such as estrus and ovulation synchronization, artificial insemination (AI) and embryo transfer (ET) in reproductive management have been applied in order to increase reproductive efficiency. The use of pharmacological control for fixed-time insemination has been effective in reducing the calving interval and allowing conception at the beginning of the breeding season in beef cows (Bó, Baruselli, & Mapletoft, 2013; Sá Filho et al., 2013).

The response of bovine embryo transfer programs is influenced by nutrition, management and estrus detection of the recipients (Baruselli et al., 2004; Bó, Baruselli, & Mapletoft, 2012; Bó et al., 2012). However, other factors must be considered in an embryo transfer program, such as the stage of embryo development, the synchrony between embryos, donors and recipients cows, as well as the quality of the embryo, even produced in vivo or in vitro, and the corpus luteum quality, which may have a direct effect on the pregnancy rate (Andrade et al., 2012; Grázia et al., 2016; Peixoto et al., 2007; Spell et al., 2001). Reliable results have been demonstrated using intravaginal devices of progesterone and estradiol for follicular growth control, which were associated with ovulation induction employing estradiol esters to allow fixed-time embryo transfer (FTET), putting an end to the estrus observation (Baruselli et al., 2004; Bó et al., 2012; Dantas et al., 2018).

From the advance of IVP in Brazil, there was a need to use calving cows to reach the demand of these embryos (IETS, 2016). However, there are few studies on the reproductive efficiency of beef cows as embryo recipients. Therefore, the present study aimed to analyze the influence of the postpartum period on the pregnancy rate of beef cows submitted to fixed-time embryo transfer.

### 2 MATERIAL AND METHODS

#### 2.1 ETHICAL ASPECTS

This study was approved by the Institutional Ethics Committee under number 04/2016. All procedures followed federal law Nº. 11,794 of October 8, 2008.
2.2 LOCATION, ANIMALS AND MANAGEMENT

The study was carried out on a property located in the city of Itapagipe, state of Minas Gerais. It were used as embryo recipients, 131 crossbred (Bos taurus x Bos indicus) cows with BCS (body condition score) between 2.75 to 3.0 (scale from 1 to 5, Lowman, Scott, & Somerville, 1976), on the 79.35th ± 28.0 postpartum day. The animals were maintained in Urochloa brizantha pasture with mineral supplementation ad libitum and free access to water. The females underwent previous gynecological exam, through external inspection and transrectal palpation, to evaluate the uterine tone and possible changes. Only cows that did not present abnormalities in the genital system were considered apt for the hormonal protocol administration and for the embryo reception.

2.3 HORMONAL PROTOCOL FOR FTET AND PREGNANCY DIAGNOSIS

The recipients were synchronized with a hormonal protocol initiated on a random day of the estrous cycle. On day 0 (D0), an intravaginal device containing 1.9 g of progesterone (CIDR®, Zoetis, Brazil) was inserted associated by an intramuscular administration (IM) of 2 mg of estradiol benzoate (Gonadiol, Zoetis®, Brazil). On day 8 (D8) the intravaginal device was removed and 0.15 mg D-cloprostenol (Sincrocio®, Ouro Fino, Brazil), 0.5 mg estradiol cypionate (ECP®, Zoetis, Brazil) and 300 IU eCG- equine chorionic gonadotropin (Novormon, Zoetis®, Brazil), were administered intramuscularly. On day 17 (D17), FTET was performed without previous estrus observation (Figure 1). The embryos transferred were from commercial in vitro production and from Senepol oocytes donor cows submitted to follicular aspiration in D9 of the recipients FTET protocol. After IVP, it were assigned to FTET only the embryos classified as grade 1 according to Wright (1998) and between the initial blastocyst, blastocyst or expanded blastocyst stages. The transfer procedure was performed using the transcervical technique, with the embryo deposition in the cranial portion of the uterine horn, ipsilateral to the corpus luteum. The pregnancy diagnosis was conducted on the 23th day after the insemination, approximately 30 days of pregnancy, by transrectal ultrasound. (Mindray DP2200, 7.5 Mhz).

Figure 1- Hormonal protocol of Bos taurus x Bos indicus cows submitted to FTET and OPU from Senepol donors cows, Itapagipe - MG, 2016-2017 breeding season.
2.4 STATISTICAL ANALYSIS

The variable postpartum period did not present a normal distribution by the Kolmogorov-Smirnov test and the division of the experimental groups was performed according to the median.

The recipients were divided into two experimental groups: less than 70days postpartum (N = 70) and more than 70days from the delivery (N = 61).

The data were analyzed by logistic regression (p < 0.05) using the software SigmaStat (1999). In the statistical model, dependent variables were included: body condition score, postpartum period and embryonic development stage. Pregnancy was considered as an independent variable to verify the existence of interactions.

3 RESULTS

The present study analyzed the pregnancy rate of 131 crossbred postpartum cows and submitted to TETF of Senepol PIV embryos. The results obtained showed that, for pregnancy rates, there was no interaction regarding the embryonic development stage, BCS and postpartum days (Table 1).

| Effect                  | Pregnancy rates (%) | p-value |
|-------------------------|---------------------|---------|
| Embryo development stage| Initial blastocyst and blastocyst | 0.395   |
|                         | 47.1 (25/53)        |         |
|                         | Expanded blastocyst |         |
|                         | 55.1 (43/78)        |         |
| BCS                     | 2.75                |         |
|                         | 3.0                 |         |
|                         | 53.0 (26/49)        |         |
|                         | 51.2 (42/82)        | 0.824   |
| Postpartum days         | 26 to 70            |         |
|                         | 71 to 157           |         |
|                         | 50.0 (35/70)        | 0.603   |
|                         | 54.0 (33/61)        |         |

p<0.05

4 DISCUSSION

The initial hypothesis that the postpartum period interferes with the pregnancy rates of cows submitted to TETF has not been confirmed under the present study conditions. The pregnancy rates obtained were similar to those reported by Nasser et al. (2004) and Scanavez, Campos, & Santos (2013). A previous study, in which a retrospective analysis was performed on pregnancy rates in Nellore cows, were found superior results (p < 0.001) in postpartum cows [58.1% (1232/2590)] compared to nursing cows [47.5% (617/1062)] submitted to large-scale FTET (Blaschi et al., 2015). Likewise, the use of FTET programs in lactating dairy cows provided an increase in reproductive efficiency by rising the service rate, previously reduced by failure to detect estrus (Pontes et al., 2010).
Although most *Bos indicus* females have anestrus in the postpartum period (Barreiros, Hidalgo, et al., 2014; Sá Filho et al., 2013), the use of pharmacological control for FTAI in cows from the 30th to the 45th postpartum day has resulted in a pregnancy rate above 50% (Sá Filho et al., 2013; Torres-Júnior et al., 2014). These results are probably due to the progesterone effect as a cyclicity inducer and the administration of eCG in the removal of progesterone devices, which contribute to the dominant follicle growth and enables a better ovulation rate induced by estradiol esters (Barreiros, Blaschi, et al., 2014). The eCG may be considered a fundamental substance in FTAI programs for postpartum beef cows, promoting an increase in the pregnancy rate in postpartum females (Ferreira et al., 2018; Pessoa et al., 2016).

Such as the postpartum period, the stage of embryonic development did not influence pregnancy rates. This result was also observed in other studies that demonstrated similar pregnancy rates between the stages of IVP blastocysts (Dantas et al., 2018; Hasler et al., 2002; Ortlibas et al., 2015; Veloso Neto et al., 2014). In addition, embryos transferred in more advanced stages (blastocysts) usually show an increase in the number of pregnancies when compared to the transfer of morulae (Dantas et al., 2018; Leese, 2012) possibly because they present biochemical differences and better use of uterine substrates (Leese, 2012).

In the present study, FTET was used as a synchronization method, and, therefore, variables such as the presence of corpus luteum (CL) for the recipients classification and the synchrony between the embryo and the recipient were not analyzed. However in field study, considering the presence of CL or mature follicles among embryo recipients, there was no difference in pregnancy rates, indicating that the final results were similar (Dantas et al., 2018). Corroborating with a study by Leal et al. (2009) who stated that the larger CL produces a higher concentration of progesterone than those of medium or small size, however, without interfering in the pregnancy rate, suggesting that the presence of CL is more relevant than the classification regarding the size.

Asynchrony between the embryonic development stage development and the estrus manifestation day in recipients can influence the pregnancy rate. According to studies (Andrade et al., 2012; Veloso Neto et al., 2014) a variation in the pregnancy rate was observed in bovine recipients submitted to the transfer of IVP embryos of the Nelore and Senepol breeds that presented estrous 24 hours before, on the same day or 24 hours after the donor OPU. Nevertheless, some studies in which estrus observation was used, there were no variations in pregnancy rates in relation to asynchrony (Hasler et al., 1995; Spell et al., 2001).

In ovulation synchronization protocols for FTET, ovulations occur, on average, 72 hours after removal of intravaginal progesterone devices and application of estradiol cypionate (Torres-Júnior
et al., 2014). Therefore, in the present study, possibly the variation in the occurrence of ovulations and the reduction of asynchrony may not have interfered on the pregnancy rates reduction.

5 CONCLUSION

It can be concluded that the postpartum period had no influence on pregnancy rates of the recipients. The body condition score associated with an efficient synchronization protocol, resulted in a high proportion of pregnant recipients with satisfactory pregnancy rates, enabling the use of this category in fixed-time embryo transfer programs.

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