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Research Article

Temporary Calf Removal and Equine Chorionic Gonadotropin (Ecg) Administration after Progesterone-Based Protocol Improves the Reproductive Performance of Beef Cattle

Jose Carlos dos Santos Breda[1], Alisson Morais Giacomeli[2] , Luiz Ernandes Kozicki[3], Marcio Saporski Segui[3], Romildo Romualdo Weiss[4], Ivo Walter dos Santos[4] and Melina Andrea Formighieri Bertol[4]

[1]Veterinary and Master's Graduate Program in Animal Science
[2]Veterinarian autonoumus
[3]Veterinarian and Professor at the Pontifical University Catholic of Paraná
[4]Veterinarian and Professor at the Federal University of Paraná

Correspondence should be addressed to: Luiz Ernandes Kozicki; kozicki.l@pucpr.br

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Abstract

This study aimed to evaluate the effects of equine chorionic gonadotropin (eCG) administration and the temporary 48-h calf removal (CR) in a long-term progesterone (P4)-based protocol in suckled Nelore cows. In all, 150 cows were randomly submitted to P4 protocol as follows. At day 0 (d 0), an intravaginal device containing 1.9 g P4 was inserted, and 1 mg estradiol benzoate (EB) was injected intramuscularly (im). On d 8, the P4 device was removed, and cows were im injected 500 µg of prostaglandin F group 2 alpha ($PGF_{2\alpha}$). On the same day, 75 cows (eCGCR group) received 400 IU im of eCG and were separated from their calves for 48 h. The other 75 cows (NoeCGCR group) stayed with the calves and did not receive eCG. On d 10, the calves were returned to the eCGCR group. After 8 days, all the cows were observed for estrus. The ovulatory follicle size was measured using ultrasonography at the day estrus was detected, and artificial insemination (AI) was performed 12 h later. Pregnancy was diagnosed 35 days after AI. The respective parameters for the eCGCR and NoeCGCR groups were as follows: pregnancy rate, 74.6% and 50.6% ($p < 0.01$); estrus induction rates, 80.0% and 64.0% ($p < 0.05$); ovulatory follicle diameter, 11.3 and 10.5 mm; estrus observation after d 8, 2.1 and 3.5 days ($p > 0.05$). Thus, eCGCR after a long-term P4 protocol markedly improved pregnancy rates and estrus induction.

Keywords: Progesterone, eCG, calf removal, estrus observation, AI, Nelore.

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Introduction

Brazil has the largest cattle herd in the world; however, the productivity levels of these cattle are relatively very low. This is because large number of cows show delayed pregnancy after parturition. This delay ranges to about 100 days or more, resulting in considerable productivity loss. The resumption of ovarian activity is delayed after the postpartum period, and significant percentage of cows become anestrous. Early postpartum anestrus in cattle is a physiological event that causes uterine involution, and the ovaries become more active. Anestrus becomes a pathological event when the anestrus interval exceeds the average range (McDougall, 1994). The duration of anestrus interval is influenced by various factors such as age, race, and environmental and genetic factors (Hopkins, 1986).

Hormone therapy after calving allows greater number of cows to resume estrus and become pregnant in a short period (Baruselli et al., 2004). Reproductive biotechnologies like estrus synchronization (ES) followed by timed AI are auxiliary methods to improve herd productivity. These programs ensure high pregnancy rates (number of pregnant cows/number of synchronized cows) across different places and situations.

Studies evaluating different P4 based protocols show that long-term treatment for spontaneous regression of the corpus luteum (CL) synchronizes estrus (Rajamahendran et al., 2001). Treatments that induce regression of follicles lead to emergence of a new wave of follicular growth, thereby improving pregnancy rates (Bo et al., 2004). Treatment with estrogen (E2) and P4 have been increasingly employed in ES programs in the Brazilian cattle. This treatment involves placing a device containing P4 and administration of E2 on day 0 (for the emergence of a new wave of follicular growth and prevent persistent follicles), administration of $PGF_{2\alpha}$ (days 7, 8, or 9) after P4 removal, induction of luteolysis, and subsequent application of E2 (0.5 to 1.0 mg) after 24 h or GnRH after 48–54 h for the synchronization of ovulation. Estrogen might act as an agent that synchronizes ovulation by inducing an LH surge by positive feedback of GnRH (Moreira, 2002). Other hormones such as equine chorionic gonadotropin (eCG) (Ereno et al., 2007; Pinheiro et al., 2009; Sá Filho et al., 2010a,b; Marquesini et al., 2013) have been used to stimulate the development of ovarian follicles as well as to synchronize ovulation by using the human chorionic gonadotropin (hCG; Fantini et al., 2004).

Considering the pregnancy rates of national livestock, beef cattle are known to have an interval of 21 months between births (Zimmer and Euclides Filho, 1997). The calving interval depends of the quantity and frequency of suckling after parturition (Belows and Short, 1994). This prevents the resumption of the pulsatile secretion of LH in the puerperium, which is required to support the final development and maturation of the preovulatory follicles (Williams and Griffith, 1995). In Nelore cows, postpartum anestrus can be more pronounced because of the greater frequency of suckling by calves (average 8 times per day; Cubas et al., 1985). The absence of LH pulsatility in the first 4 weeks postpartum is known to deplete gonadotropin concentrations in the anterior pituitary. After the gonadotropin concentrations are restored, full or temporary weaning (48 or 96 h) increases the LH pulse frequency (; Shively and Williams, 1989; Marquesini et al., 2013) leading to ovulation by the LH surge. Studies on temporary weaning (Pencai et al., 2011) and eCG use postpartum showed improved follicular development in anestrus cows (Yavas and Walton, 2000; Marquesini et al., 2013). Our hypothesis was that by associating the temporary removal of the calf and eCG administration could improve the pregnancy rate in commercial herd of beef cattle.

This study aimed to evaluate the combined effects of eCG and of 48-h temporary calf removal in order to synchronize estrus and pregnancy rates in lactating Nelore cows.

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Material and Methods

A total of 150 Nelore cows' pluriparous and suckled were used for present study. The cows were provided *Brachiaria decumbens*, *Cynodon* spp., and mineral salt *ad libitum*. The body condition score was 3.0 (1 = thin to 5 = obese). In the seventh week postpartum, ovaries were examined by ultrasound. The following criteria were used for anestrus: cows without CL and ovarian follicles smaller than 7.0 mm (Borges et al., 2004). All cows were submitted to progesterone protocol: day 0 (d 0), an intravaginal device containing 1.9 g of P4 (CIDR; Intervet Schering-Plough, SP, Brazil) was inserted, and 1 mg estradiol benzoate (EB; Estrogin-Farmavet, SP, Brazil) was injected im. On d 8, the P4 device was removed, and cows received 500 µg im of $PGF_{2\alpha}$ (Ciosin, MSD, Brazil). On the same day, 75 cows (eCGCR group) received 400 IU im of eCG (Folligon, MSD, Brazil) and were separated from their calves for 48 h. The other 75 cows (NoeCGRC group) remained with the calves and did not receive eCG. On d 10, the calves were returned to the eCGCR group. After d 8 of the protocol all the cows were observed visually for estrus for 1 h each in the mornings, noon, and afternoons. Cows in estrus were kept separately and inseminated after 12 h. Just before AI, the diameter of the biggest follicle was measured by ultrasonography (Ultrasound, Aloka SSD 500; probe 5.0 Mhz, Fujihira Industry Co. Ltd., Tokyo, Japan,). Pregnancy was diagnosed 35 days after AI. The animals that did not show estrus within 5 days after P4 removal and $PGF_{2\alpha}$ administration were considered as unresponsive to the protocol.

Protocols of the eCGCR and NoeCGCR groups respectively,

eCGCR (=75 animals)

P_4 + EB -P_4 + PG + eCG +CR -CR US OF+ AI

I_____I_____ _I_____I_____

D0 D8 CR(48 h) D10 +Estrus observation

NoeCGCR (=75 animals)

P_4 +EB -P4+PG US OF+ AI

I_____I_____I_____

D0 D8 + Estrus observation

P_4= 1,9 g of progesterone (CIDR, Intervet Schering-Plough, SP, Brazil); EB=estradiol benzoate 1 mg, Estrogin Farmavet; PG=cloprostenol, 500 µg, Ciosin, MSD, Brazil; eCG=equine chorionic gonadotropin, Folligon 400 UI, MSD, SP, Brazil; CR=temporary 48-h calf removal; OF= ovulatory follicle; US=ultrasound.

Statistical analysis

The experiment was carried out as a completely randomized design. The analysis of variance (ANOVA) was used for comparisons of means (x±s). Differences in the groups were conducted using the SAS System for Windows (SAS Institute Inc.,

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Cary, NC, USA, 2003). Differences were considered significant at P<0.05.

Results and Discussion

The results are shown in Table 1.

Table 1: Data from Animals with Visible Estrus, Artificial Insemination, Estrus Observation after P_4 Removal, Pregnancy Rate, and Diameter of the Ovulatory Follicle in Anestrus Postpartum of Nelore Cows (n = 150).

Group	Animals (n)	Cows in visible estrus (n, %)	Animals inseminated (n,%)	Estrus after P_4 removal (days)(x±s)	Pregnancy rate (n, %)	Ø OF in day of AI (mm)(x±s)
eCGCR	75	60/75 (80.0)[a]	60/75 (80.0)[a]	2.1±1.1[a]	56/75 (74.6)[c]	11.3±1.1[a]
NoeCGCR	75	48/75 (64.0)[b]	48/75 (64.0)[b]	3.5±2.1[a]	38/75 (50.6)[d]	10.5±3.3[a]

Different letters in columns indicate significance being (a:b= P < 0.05 and (c:d P < 0.01)
Data calculated under ANOVA, Tukey Test – SAS 2003.

Administration of eCG in acyclic dairy cows previously treated with P4 has been recommended (Pinheiro et al., 2009, Bryan et al., 2013; Marquezini et al., 2013) since FSH and LH can induce ovarian follicle development and ovulation (; Yavas and Walton, 2000). The present study evaluated the effects of eCG treatment along with 48-h calf removal after an 8-day P4 protocol in suckling Nelore cows. Nelore cows in anestrous postpartum were submitted to AI after estrus observation, resulting in a pregnancy rate of 74.6% and 50.6% in the eCGCR and NoeCGCR groups, respectively (P < 0.01). Our results were better than those obtained by Belloso et al., (2002) who performed a similar experiment in *Bos indicus* primiparous cows under anestrous conditions. They submitted the cows to P4 protocols with fixed-time artificial insemination (TAI) and TW (temporary weaning) for 96 h (G1) and with eCG (G2) achieving conception rates of 61.5% and 67.7%, respectively. Similarly, in another study, Marquesini et al., (2013) used eCG after 7 days of intravaginal P4 and observed increased dominant follicle (DF) diameter at the TAI, but there was no influence on the pregnancy rate of *Bos taurus* beef cattle. Sá Filho et al., (2010 b) observed improvement in pregnancy rates when eCG was used along with PGF administration using a controlled internal drug-releasing device and CR. Our results (PR) can be assigned the cows were inseminated after the visible estrus. Protocols combining progestin, eCG, and temporary removal of calves are important tools that can be used to synchronize estrus (; Meneghetti et al., 2001; 2009; Sá Filho et al., 2010b). Others studies using eCG, with or without TW, did not yield consistent results. Temporary calf removal, with or without eCG administration, did not significantly alter the pregnancy rates in TAI by suckling Nelore cows (Pinheiro et al., 2009) or in *Bos indicus* heifers (Butler et al., 2011), emphasizing that these authors employed fixed-time artificial insemination (FTAI). Vasconcelos et al., (2009) showed that the temporary removal of calves increased the estrus rate and improved reproductive performance in Angus × Nelore crossbred anestrus cows; estrus induction was observed even in cows with a DF having a size of less than 7 mm. This corroborates the results of the present study, indicating

the reason for the difference between the eCGCR group and NoeCGCR ($p < 0.05$) on the visible manifestation of estrus. Our hypothesis was that the association of eCG and CR could exert beneficial effects, because eCG would induce the development of ovarian follicles and ovulation in lactating dairy cows previously treated with progesterone (Yavas and Walton, 2000) and the CR would increase the frequency of LH pulses up to the final follicle maturation and ovulation (; Kawashima et al., 2008).

Regarding the size of ovulatory follicles (OFs) on the AI day, Meneghetti et al., (2001) suggested that the temporary removal (48 h) of calves resulted in increased size of the DF and ovulation rate in anestrus Nelore cows compared to the cows that remained with calves. In the present study, the diameter of OFs was larger, but not statistically significant, in the treated group than in the control. Our data are in agreement to those obtained in Nelore cows by Borges et al., (2003) who reported that the diameter of OFs was 11.0 mm, which is very close to the diameter found in our study (11.3mm; table 1) and different from that reported by Ferraz et al., (2009) (9.1 mm). This difference could be assigned to the difference in body condition score.

In conclusion, the use of eCG + CR after a long-term P4 protocol yielded better results and improved pregnancy rates and estrus induction.

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Jose Carlos dos Santos Breda, Alisson Morais Giacomeli, Luiz Ernandes Kozicki, Marcio Saporski Segui, Romildo Romualdo Weiss, Ivo Walter dos Santos and Melina Andrea Formighieri Bertol (2013), International Journal of Veterinary Medicine: Research & Reports, DOI: 10.5171/2013. 781569.