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Pregnancy rate in crossbred cows subjected to FTAI protocol and application of a GNRH analog 12 days after AI¹

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Abstract

This study aimed to evaluate the effect of GnRH administered 12 days after FTAI on pregnancy rate and progesterone levels in crossbred beef cows. Fiftynine non-pregnant crossbred cows (*Bos taurus indicus x Bos taurus taurus*) previously selected by gynecologic examination with a body condition score (BCS) of 3.0 (scale of 1 - 5). Cows were randomly assigned to two treatments: TEB (n = 30), on day 0, an intravaginal progesterone releasing device (Primer[®]) was inserted and cows received i.m. 2.0 mg of estardiol benzoate (EB, Estrogin[®]); on day 8, the PRIMER was removed and cows received i.m. 300 IU of eCG (Novormon[®]) and 0.15 mg of PGF_{2a} (Prolise[®]); on day 9, cows

received i.m. 1 mg of EB. FTAI was carried out 48-56 hours after PRIMER removal; and TEBGnRH12 (n = 29): similar protocol to TEB, but with administration of 25 μ g GnRH (Lecirelin, Gestran Plus[®]) on the 12th day after AI. On the 35th day after AI, diagnosis of pregnancy was performed by transrectal ultrasound. The pregnancy rate was analyzed by logistic regression. The results showed that 53.33% of the cows (16/30) in TEB and 37.93% (11/29) in TEBGnRH12 became pregnant after the first service. No significant difference was found among the pregnancy rate of animals in the treatments (P> 0.05). There was no difference between the P4 concentrations (TEB = 3.88 ng/mL and TEBGnRH12 = 3.12 ng/mL) among the heifers in the treatments (P> 0.05). The administration of GnRH analogs 12 days after TAI did not influence pregnancy rate or the progesterone concentration.

Keywords: beef cattle, estradiol benzoate, lecirelin, hormone, progesterone, synchronization of ovulation

Taxa de prenhez em vacas mestiças submetidas ao protocolo de IATF e aplicação de análogo de GnRH 12 dias após a IA

Resumo

Objetivou-se avaliar o efeito do GnRH, administrado 12 dias após IATF, sobre a taxa de prenhez e os níveis de progesterona sérica em vacas de corte mestiças. Foram utilizadas 59 fêmeas bovinas mestiças (*Bos taurus indicus x Bos taurus taurus*), não-gestantes, previamente selecionadas por exame ginecológico, com escore de condição corporal (ECC) 3,0 (escala de 1 – 5). Os animais foram alocados ao acaso, em dois tratamentos: TBE (n = 30) - no dia 0 inseriu-se o dispositivo intravaginal de progesterona (Primer[®]) mais 2,0 mg de BE (Estrogin[®]), im; no dia 8, retirou-se o PRIMER e aplicaram-se 300 UI de eCG (Novormon[®]) e 0,15 mg de PGF_{2a} (Prolise[®]), im; no dia 9, aplicou-se 1 mg de BE, im, e realizou-se a IATF 48-56 horas após a retirada do PRIMER; e TBEGnRH12 (n = 29): o protocolo foi similar ao do TBE, porém com administração de 25 µg de GnRH (Gestran Plus[®], Lecirelina) no dia 12 após a

IA. No dia 35, após IA, foi feito o diagnóstico de gestação por exames ultrassonográficos pela via transretal. A taxa de prenhez foi analisada por regressão logística. Observou-se que 53,33% das vacas (16/30) no TBE e 37,93% (11/29) no TBEGnRH12 ficaram gestantes após o primeiro serviço. Não foi observada diferença entre a taxa de prenhez dos animais dos tratamentos (P > 0,05). Não se observou diferença entre as concentrações de P₄ (TBE= 3,88 ng/mL e TBEGnRH12 = 3,12 ng/mL), entre as fêmeas dos tratamentos (P > 0,05). A administração do análogo de GnRH, no dia 12 após a IATF, não influenciou a taxa de prenhez e nem a concentração de progesterona.

Palavras-chave: benzoato de estradiol, gado de corte, hormônio, lecirelina, progesterona, sincronização de ovulação

INTRODUCTION

Synchronization and induction of estrus are important tools in beef cow production systems, as they allow most of the herd to return to cyclicity and/or to begin a new gestation in a shorter period of time (Lucy et al. 2001 Borges, 2008). Baruselli et al. (2004), Kasimanickam et al. (2006) and Smith et al. (2008) showed that hormone protocols that eliminate the need for estrus detection, thus favoring fixed-time artificial insemination (FTAI), are more attractive, however they present pregnancy rates ranging between 25 and 67%. This variation occurs because of the percentage of cows that initiate estrous cycles in the postpartum period, the environment influence and nutritional condition of the animals and also the hormone combination that was used.

Nascimento (2005) compared the pregnancy rate after synchronization of ovulation and estrus among three hormonal protocols and obtained satisfactory results, similar to those achieved by AI with estrus detection, with fixed-time artificial insemination using a combination of the hormones estradiol benzoate, progesterone, prostaglandin and eCG. It has been suggested that the administration of GnRH analogs or after AI (11 to 14 after estrus), period

coinciding with the largest diameter of the dominant follicle in the first follicular wave, the initiation of pregnancy recognition and the onset of the luteolytic mechanism, can act in the ovary, enhancing the formation of an accessory corpus luteum and thereby increasing the concentration of P4 serum, which contributes to embryo survival (Peters et al., 2000, Borges et al., 2001).

The objective of this study was to evaluate the effect of the GnRH analog Lecirelin administered 12 days after FTAI on the pregnancy rate and progesterone serum levels in crossbred beef cows.

MATERIAL AND METHODS

The experiment was carried out at Monte Verde Farm, in the municipality of Dores do Rio Preto, Espírito Santo, located at the coordinates 20°41'26" South and 41°50'48" West, from March 25 to May 10 2008.

Fifty-nine non-pregnant, non-lactating, crossbred heifers (*Bos taurus indicus x Bos taurus taurus*), previously selected by gynecologic examination with a body condition score (BCS) of 3.0 (scale of 1 - 5) (Vieira et al., 2005) were used in the experiments. The animals were allotted, in a complete randomized design, into two treatments: TEB (n = 30) on day 0, an intravaginal progesterone releasing device (Primer[®]) was inserted and cows received i.m. 2.0 mg of EB (Estrogin[®]); on day 8, the PRIMER was removed and cows received i.m. 300 IU of eCG (Novormon[®]) and 0.15 mg of PGF_{2a} (Prolise[®]); on day 9, cows received i.m. 1 mg of EB. FTAI was carried out 48-56 hours after PRIMER removal; and TEBGnRH12 (n = 29): similar protocol to TEB, but with administration of 25 µg GnRH (Lecirelin, Gestran Plus[®]) on the 12th day after AI (adapted from Nair, 2005).

Inseminations were performed by a single inseminator, using semen from a single Nellore bull obtained from the Semen Center, which is associated with the Brazilian Association of Artificial Insemination (ASBI).

The heifers were kept under extensive management on predominantly Brachiaria pasture (*Brachiaria brizantha* cv. Marundu), with commercial mineral supplement and water *ad libitum*.

The animals were separated, weighed and underwent gynecologic examination by transrectal palpation. ECC was analyzed up to seven days before starting the experimental period.

The pregnancy rate of cows of each experimental group was determined by dividing the number of pregnant heifers by the total number of heifers used in each treatment. The diagnosis of pregnancy was performed by transrectal ultrasound, using an Aloka SSD-500 Portable Ultrasound Unit equipped with a 5.0 MHz linear transducer. Ultrasound examinations were performed on the 35th day after AI.

Blood samples for P4 serum measurement were collected from the coccygeal artery/vein in 30% of animals of each treatment on the day AI was performed and on days 0, 5, 12 and 20 after TFAI.

Blood samples were left to coagulate at room temperature in the shade for 2 hours and then stored at 4 °C for 24 hours. Serum was collected after centrifugation at 2500 rpm (1.439 g) for 10 minutes and maintained at minus 20 °C until hormonal analysis by chemiluminescence using commercial kits (AECCess[®] Progesterone, Beckman Coulter).

All statistical analyses were done using SAS 8.0 software (1999), at 5% probability level. Data were analyzed in a complete randomized design. Live weight (LW) was analyzed by analysis of variance and body condition score by the Mann-Whitney test. The presence of corpus luteum and pregnancy rate were analyzed by logistic analysis.

The effects of treatment and day on the P4 concentration were analyzed in a split plot design, with the effect of days in the main plot, in a mixed model, considering error and repetition as random effects and mean comparisons were done using the Tukey-Kramer test. Spearman's correlation was performed between the state of pregnancy and P4 concentration on the different days.

RESULTS AND DISCUSSION

Body weight (BW) and body condition score (BCS) were found uniform (P > 0.05) among the animals of the different treatments (Table 1), allowing

the statement that the treated animals had suitable weight and body condition score for reproduction (adapted from Oliveira et al. 2006).

Considering that the presence of corpus luteum confirms the cyclicity of the animals, there was evenly distribution among the cyclic and/or anoestrus cows in the treatments (Table 1).

Table 1 –Weight (BW), body condition score (BCS) and presence of CL of cows at the day of progesterone device insertion according to the treatments

Variable	Treatments ¹			
	TEB	TEBGnRH12	P Value	VC (%) ²
BW (kg) ³	357.67 ±	354.31 ±	0 7020	12 70
	1,68	1,65	0.7939	13.79
BCS (1-5) ⁴	$2.93 \pm 0,01$	$2.95 \pm 0,01$	0.7336	-
CL (%)	33.33(10/30)	13.79(4/29)	0.0738	-

 ${}^{1}\text{TEB}$ = synchronization of ovulation using EB; TEBGnRH12 = estrus synchronization using EB with application of 25µg of leciralin on day 12 after FTAI; ${}^{2}\text{Variation Coefficient}$; ${}^{3}\text{Differences}$ between treatments were not significant by F test (P>0.05). ${}^{4}\text{Differences}$ between treatments were not significant by CP>0.05).

No significant difference (P> 0.05) was found for pregnancy rate among cows treated for synchronization of ovulation. TEB and TEBGnRH12 showed pregnancy rates at the first service of 53.33% (16/30) and 37.93% (11/29), respectively (Table 2). The pregnancy rates of the two protocols corroborates the results obtained in other studies (Cutaia et al. 2003; Mialot et al. 2003; Nascimento, 2005; Cutaia et al., 2006, Martins et al., 2006). However, the administration of a Lecirelin dose on day 12 after FTAI in cows of TEBGnRH12 did not result in any difference (P> 0.05) when compared with the TEB protocol. Comparing with results obtained with the use of GnRH analogs, these findings corroborate those of Mann et al. (1999), Tefera et al. (2001) and Szenci et al. (2006) that the administration of 10 to 250 μ g of GnRH analogs in the diestrus phase of the estrous cycle of dairy cows did not influence

pregnancy rate at the first service. However, other studies revealed an increase in pregnancy rates in cows treated with 10 - 250 μ g of GnRH analog in the diestrus period (at the time of AI or 12 days after AI) (Macmillan et al. 1986; Lajil et al., 1991, Lopez-Gatiuz et al., 2006). According to Thatcher et al. (1989), Chenault et al. (1990) and Yániz et al. (2004), the positive effect of GnRH at the time of AI, especially as a stimulator of LH release, probably favors the increase in ovulation rate and increase in P4 serum concentrations.

Variable	Treatments ¹			
	TEB	TEBGnRH12	P Value	
Pregnancy (%) ²	53.33 (16/30)	37.93 (11/29)	0.2341	

Table 2 – Crossbred cows pregnancy rate subjected to two protocols FTAI

¹TEB = synchronization of ovulation using EB; TEBGnRH12 = estrus synchronization using EB with application of 25µg of leciralin on day 12 after FTAI; ²Differences between treatments were not significant by logistic function (P>0.05).

The results of this study may be related to the time of application of GnRH analogs, which has been delayed, causing no luteinization of the dominant follicle of the first follicular wave after estrus, or, it has been earlier, not causing the same effect on the dominant follicle of the second follicular wave. Or, it may be that the application of 25 μ g of Lecerelin 12 days after FTAI is not a sufficient dose to influence the formation of accessory corpora lutea, thereby increasing the P4 production.

Table 3 shows the mean concentrations of serum progesterone in heifers from the treatments TEB and TEBGnRH12 on 0, 5, 12 and 20 days. There was no effect of the protocols on the days of collection and neither of the interaction treatment x day (P> 0.05), however there was effect of day (P <0.05), as reported by Mann et al. (1995) and Szenci et al. (2006). On the other hand, Macmillan et al. (1986), Lajil, et al. (1991) and Lopez-Gatius et al. (2006) showed that a single dose of GnRH at the AI can increase the serum concentration of P4 and the number of accessory corpora lutea in pregnant

cows treated after insemination. The authors showed that stimulation of luteal function was increased with application of GnRH analogs in the mid-luteal phase. This effect was not observed in Licerelin-treated cows of the treatment TEBGnRH12, indicating that this GnRH analog did not influence the formation of accessory corpora lutea, probably for the same reasons already mentioned.

Dav	Treat	Average		
Day	TEB(ng/mL) TEBGnRH12		- //veruge	
0	1.79 ± 0.45	1.74 ± 0.35	1.76 ^b	
5	2.49 ± 0.73	1.63 ± 0.27	2.06 ^b	
12	4.69 ± 1.32	4.75 ± 1.32	4.72 ^a	
20	6.56 ± 1.87	4.37 ± 1.37	5.46ª	
Average	$3.88^{a} \pm 0.61$	$3.12^{a} \pm 0.49$	-	

Table 3 - P₄ concentration (ng/mL) on the days o, 5, 12 and 20 crossbred cows subjected to two FTAI protocols

¹TEB = synchronization of ovulation using EB; TEBGnRH12 = estrus synchronization using EB with application of 25 g of lecirelin on day 12 after FTAI; There was no effect treatment and treatment/day, but there was a day effect by Tukey-Kramer Test (P < 0.05).

A possible luteotropic action of GnRH analogs in increased secretion of progesterone should not be overlooked when considering the potential mechanism by which this treatment improves the pregnancy rate (Mann et al., 1995). But in the present study, no difference in progesterone levels was observed between the pregnant and non-pregnant cows from the treatments and control (Figure 1).

P4 serum concentrations were similar in all cows on days 0, 5 and 12. However, on day 20, the plasma progesterone concentration was higher in pregnant than in non-pregnant cows in all treatments (P> 0.05) (Table 4). These findings are consistent with the normal parameters of cattle reproductive physiology reported by several authors (Thibault et al., 1993, Mann et al., 1995, Hafez and Hafez, 2004).



Picture 1- Average serum P₄ concentration of pregnant and non-pregnant cows in treatments TEB and TEBGnRH12

Table 4 – Correlation between pregnancy and serum P₄ concentration at different days after FTAI

	D0	D5	D12	D20
Pregnancy	-0.2463 ^{1/}	0.3428	0.3127	0.5966
	(0.3245) ^{2/}	(0.1637)	(0.2216)	(0.0147)*

¹Spearman correlation coefficient ; ²P- Value

CONCLUSIONS

The administration of GnRH analog (Lecirelin) at 12 days after FTAI did not affect the pregnancy rate in crossbred cows. Lecirelin also did not affect the progesterone concentration among the treatments on days 0, 5, 12 and 20 after FTAI, probably not acting in the formation of the accessory corpus luteum or in the increase of the luteal function.

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