Original

Effect of follicular growth promoters on timed artificial insemination in dairy cows

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ABSTRACT

Objective. To evaluate the efficiency of equine chorionic gonadotropin (eCG) and follicle stimulating hormone (FSH) on the pregnancy rate of crossbred cows from different body condition score (BCS) in the postpartum period in timed artificial insemination (TAI). **Material and methods.** The cows were subjected to the same hormone protocol: Day zero (D0), progesterone-release intravaginal device (PRID) and 2 mg estradiol benzoate (EB); D8, withdrawal of PRID, application 0.150 mg of prostaglandin. On D8, the animals were distributed into three treatments: 1 – (TeCG; n = 98) injection of 300 IU eCG; 2 – (TFSH; n = 84) injection of 20 mg of FSH and; 3 – (Control; n = 115) without further treatment. On D9, all animals received 1 mg of EB; D10, TAI of all the cows. After 30 days from TAI, ultrasound diagnosis of pregnancy was performed. **Results.** Pregnancy rate did not differ among eCG (52.04%), FSH (40.47%), and Control (40.00%) treated cows (p>0.05). In cows with low body condition, the pregnancy rate was higher (p<0.05) for cows treated with eCG (41.46%) when compared to FSH (18.18%) and Control (17.07%) cows. The postpartum interval among treatments was similar (p>0.05) (35–65 or 66–95days). **Conclusions.** The use of eCG or FSH did not result in an increase in the pregnancy rate when compared to the control treatment; however, eCG improved the pregnancy rate of cows with low body condition scores.

Keywords: d(*Source: USDA*).

RESUMEN

Objetivo. Evaluar la eficacia de la gonadotropina coriónica equina (eCG) y la hormona estimulante del folículo (FSH) en la tasa de preñez de vacas cruzadas con diferentes puntajes de condición corporal (PCC) en el período posparto en inseminación artificial de tiempo fijo (IATF). **Materiales y métodos.** Las vacas se sometieron al mismo protocolo hormonal: día cero (D0), inserción de un dispositivo de progesterona intravaginal (DPI) y aplicación intramuscular de 2 mg de benzoato de estradiol (BE); D8, abstinencia DPI, aplicación de 0.150 mg de prostaglandina. En D8, los animales se distribuyeron

en tres tratamientos: 1-(TeCG;n=98) aplicación de 300 UI de eCG; 2-(TFSH; n=84) aplicación de 20mg de FSH y; 3-(Control; n=115) sin tratamiento adicional; en D9, todos los animales recibieron 1mg de BE; D10, IATF de todas las vacas. Después de 30 días de IATF, se realizó un diagnóstico de embarazo por ultrasonido. **Resultados.** No hubo diferencias (p<0.05) en relación con la tasa de preñez para vacas en los tratamientos TeCG (52.04%), TFSH (40.47%) y Control (40.00%). La tasa de preñez fue mayor (p<0.05) para TeCG (41.46%) en vacas con baja condición corporal en comparación con TFSH (18.18%) y Control (17.07%). No hubo diferencias (p<0.05) en relación con los días posparto (35-65 o 66-95) en relación con la tasa de embarazo. **Conclusiones.** El uso de eCG o FSH no resultó en aumento en la tasa de embarazo en comparación con el tratamiento de control, sin embargo, eCG mejoró la tasa de preñez de vacas con puntajes bajos de condición corporal.

Palabras clave: eCG; FSH; eficiencia reproductiva; reproducción animal; tasa de embarazo (*Fuente: USDA*).

INTRODUCTION

The Brazilian dairy herd is composed predominantly (74%) of crossbred animals, Holstein x Zebu, which, added to non-specialized animals (20%), constitute 94% of the herd. This panorama demonstrates the social and economic importance of crossbred cows for the country, and their exploitation appears as a viable alternative for several production systems that seek to reduce the cost of animals kept under grazing. Crossbred cows have desirable characteristics such as rusticity, productive capacity, and adaptation to the limitations existing on most farms, as well as greater disease resistance and good productivity (1).

Reducing the calving interval increases the number of births and milk production during the productive life of crossbred cows. The birth interval of 12 to 13 months brings economic benefits, but it is difficult to obtain in practice due to the management problems associated with the reproductive cycle of the cow (2). Strategies to anticipate the return to cyclicity and increase estrus synchronization can be used 30 days after conception in animals with adequate body condition. In this context, there is the use of hormonal protocols aiming at timed artificial insemination (TAI), which aims at the synchronization and induction of estrus and ovulation, reducing the occurrence of failures in the identification of estrus and the interval of births (3).

Several TAI protocols have been used in bovine females, with an increasing use of protocols based on progesterone and estrogen (4). These protocols are associated with the use of follicular growth promoters in order to increase the size of the preovulatory follicle, the ovulation rates, the progesterone production by the corpus luteum, and consequently, the pregnancy rates (5,6). Among the promoters of follicular growth, equine chorionic gonadotropin (eCG) and follicle stimulating hormone (FSH), which are the most recommended (7,8) stand out. In this context, some adjustments to TAI protocols, including the use of ovulation inducers such as eCG and FSH, can provide improvement in the results in terms of pregnancy rate, since the success rates with TAI are stationary around 35% for dairy cattle (9).

The objective was to evaluate the efficiency of equine chorionic gonadotropin and follicle stimulating hormone on the pregnancy rate of crossbred dairy cows of different body condition scores in the postpartum period.

MATERIALS AND METHODS

Animal care. This work was in accordance with the ethical principles for animal experimentation, adopted by the Ethics Committee on the Use of Animals, under number 017/2014.

Study site. The experiment was carried out on a property located in the Municipality of Dores do Rio Preto, State of Espírito Santo, Brazil, located at geographic coordinates 20° 41' South, 41° 50' West and 774 m altitude, between October 2014 and February 2015. The predominant climate in the region is high altitude tropical, with hot and rainy summers and cold, dry winters. The average annual temperature in the region is 19.2°C and annual rainfall is 1.000 mm.

Animals and feeding. A total of 297 milking crossbred cows (*Bos taurus indicus* x *Bos taurus taurus*) within the first and fourth lactation were selected based on their fertility background and lack of clinical signs of infectious or metabolic

diseases, retention of placenta, or abnormal genitals during gynecologic examination. Cows were kept on pastures of *Brachiaria brizantha* cv. Marandu and water and mineral salt were administered *ad libitum*, and supplemented with concentrate containing 22% of crude protein at 1 kg/3L of milk.

Experimental treatments. During the application of the progesterone-release intravaginal device (PRID), the postpartum period was recorded in relation to the beginning of the TAI protocol (day 0). Milk production and cows' body weight (BW) were also evaluated. The BCS was measured, ranging from one for very thin cows to five for fat cows (10). Cows were uniformly divided into groups based on their parturition order, milk production, and BCS.

The cows were submitted to the same synchronization protocol: day zero (D0), insertion of PRID plus intramuscular (IM) injection of 2 mg of estradiol benzoate (EB); day eight (D8), PRID was removed and 0.150 mg of prostaglandin (PGF2α) was injected intramuscularly. On D8, the animals were distributed into three treatments: 1) TeCG – application of 300 IU eCG (n=98); 2) TFSH – application of 20 mg of FSH (n=84) and; 3) Control – no hormone treatment (n=115). On day 9 (D9), intramuscular injection of 1mg of estradiol benzoate was administered; on day 10 (D10), cows were artificially inseminated 52 hours after removal of the PRID.

Variables analyzed. Cows were artificially inseminated by the same technician with semen collected from Girolando bulls, which was obtained from the Brazilian Association of Artificial Insemination. Pregnancy was determined 30 days after the artificial insemination by ultrasound evaluations and the pregnancy rate was recorded in each treatment (number of pregnant cows divided by the total number of cows within the treatment).

The effect of postpartum days (PD) and BCS on the reproductive efficiency of treatments was evaluated. Females were divided into two subgroups: females who started the TAI protocol between 35–65 PD and females who started the TAI protocol between 66–95 PD. The pregnancy rate was determined according to the body condition score, which ranged from 2.0 to 3.5 for the animals of each treatment.

Statistical analysis. All statistical analyses were performed using the SAS Studio program (11) at a 5% probability of error, in a completely randomized design, considering the effect of the treatments. Categorical variables were assessed using the chi-square test on contingency tables to verify their association or adherence. Continuous variables were assessed using the Shapiro–Wilk normality test to verify the null hypothesis that the data were sampled from a normal distribution (12). As none were normally distributed, the evaluation was performed by the Wilcoxon test or Kruskal–Wallis analysis of variance.

RESULTS

The cows had an average weight of 411.3±26.4 kg, 416.0±30.4 kg, and 418.4±25.6 kg for TeCG, TFH, and Control, respectively. The BCS was 2.31±0.2, 2.48±0.3, and 2.34±0.2 for TeCG, TFH, and Control, respectively. Average delivery was 2.4±0.3, 2.2±0.2, and 2.1±0.2 for TeCG, TFH, and Control, respectively. Average milk production was 11.6±0.8, 11.2±0.6, and 11.1 ± 0.5 liters for TeCG, TFH, and Control, respectively and mean postpartum days were 59.1±21.2, 57.5±20.8, and 60.22±21.2 for TeCG, TFSH, and Control, respectively.

There was no statistical difference between treatments and pregnancy rate (p>0.05; Table 1).

Table 1. Pregnancy rate (%) of cows subjected to treatments with equine chorionic gonadotropin (TeCG), follicle stimulating hormone (TFSH), and without additional treatment (Control).

Treatments	n	P	V	Pregnancy (%)
TeCG	98	51	47	52.04
TFSH	84	34	50	40.47
Control	115	46	69	40.00
Total	297	131	166	44.10

Chi-square adherence test (χ^2 = 3.26; p=0.19); n = number of cows evaluated; P = number of pregnant cows; V = number of non-pregnant cows.

There was a difference between treatments (p<0.05) in cows with low body condition scores (Figure 1). In this context, treatment with equine chorionic gonadotropin (TeCG) showed a higher pregnancy rate than the other treatments when the animals presented a BCS = 2.
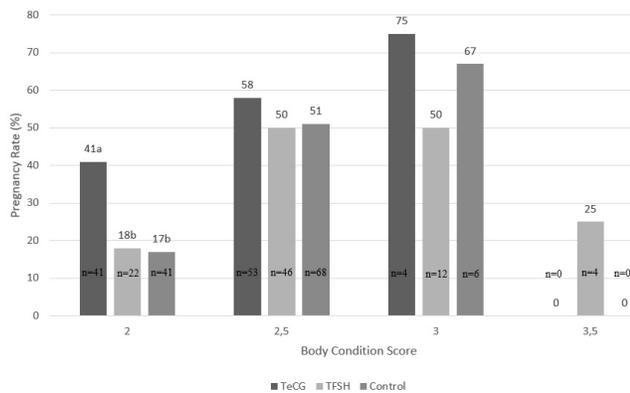
Figure 1. Pregnancy rate (%) as a function of body condition scores of cows subjected to treatments with equine chorionic gonadotropin (TeCG), follicle stimulating hormone (TFSH), and without additional treatment (Control). Chi-square adherence test ($\chi^2 = 9.93$; p=0.007).

In the present study, the pregnancy rate did not differ (p > 0.05) in relation to the days elapsed postpartum at the beginning of the protocol (35–65 days or 66–95 days) according to the treatments TeCG, TFSH, and Control (Table 2).

Table 2. Pregnancy rate (%) of cows subjected to treatments with equine chorionic gonadotropin (TeCG), follicle stimulating hormone (TFSH), and without additional treatment (Control) depending on the postpartum days (PD), 35–65 and 66–95 days.

Treatments	PD	n	P	Pregnancy (%)
TeCG	35–65	63	33	52.38
	66–95	35	18	54.54
TFSH	35–65	57	22	38.59
	66–95	27	12	44.44
Control	35–65	71	27	38.02
	66–95	44	19	43.18

Kruskal–Wallis test = 0.014; p = 0.99; respective medians and interquartile ranges (TeCG = 50.0; 40.0–76.0; TFSH = 46.5; 42.0–80.0; Control = 48.5; 40.0–78.0). n = number of cows evaluated; P = number of pregnant cows.

DISCUSSION

The timed artificial insemination protocols studied were efficient in promoting pregnancy in crossbred dairy cows in the early postpartum period. The pregnancy rate of cows submitted to TeCG showed pregnancy rates considered satisfactory, above 50% (13).

With the withdrawal of the intravaginal progesterone device in TAI protocols, it is possible to induce an increase in the frequencies of the luteinizing hormone (LH) pulses sufficient for the final growth of dominant follicles, to stimulate estrous behavior and ovulation (14), a factor that may explain why the results for pregnancy rates do not differ between treatments in the present study.

The results found in the present study corroborate the data found by other authors (15), who, working with Nellore cows, found no difference in pregnancy rates when using eCG as a follicular growth promoter (46.4%) or FSH (46.3%) compared to the control (41.2%). On the other hand, some authors, comparing the use of eCG and FSH in Zebu cows, found pregnancy rates of 55.9 and 51.4% respectively, which differed significantly from control cows, with a 38.9% pregnancy rate (8). Other authors did not report differences in pregnancy rates when they tested the use of eCG (60.2%) or FSH (59.7%); however, they claimed a significant difference when comparing treatments (eCG and FSH) with 29.9% in cows in the control group (7). There is a great diversity in the types of hormonal protocols for the reproduction of cows. In this context, the success of the implementation of this reproductive biotechnology, characterized by satisfactory pregnancy rates is also varied, with pregnancy rates ranging from 16 to 68% in dairy cows (13,16,17,18).

Working with Nellore cows in postpartum anestrus, superior results (p<0.05) were found for cows treated with eCG (41.5%) when compared to cows treated with FSH (22.7%) or control (26.3%); the eCG treatment being superior to the others (19). The authors report that treatment with FSH at the time of removal of the progesterone device is not indicated in cattle herds, unlike eCG, recommended for herds that have a high percentage of cows in anestrus (20). One of the assumptions of the better results for eCG in other studies is attributed to its concentration of LH. Anestrus cows are known to have low blood concentrations of LH (21). Thus, the differences of FSH in relation to eCG in the pregnancy rate may be related to the differences between the concentrations of LH in the blood of cows.

It has been reported that eCG is superior to FSH to stimulate pregnancy rates in cows; however, other authors have reported that there was no difference in the use of FSH and eCG in cows

subjected to TAI (7,22). Such authors also reported that eCG has an effect on FSH as well as LH, and has a longer half-life, remaining active in the blood for a longer time, from five to seven days after its administration (15). The FSH used in the present study, on the other hand, contained 87% FSH, and its metabolism seems to be fast, since, after intramuscular administration, the FSH half-life and disappearance were estimated at 5 and 12 minutes, respectively (23,24). These half-life differences between eCG and FSH were reported in studies of superovulatory protocols, in which a single dose of intramuscular eCG was sufficient to stimulate the growth of multiple follicles (25,26), while to superovulate cows with FSH it is necessary to use several intramuscular administrations (27).

Studies show that the use of eCG improves the pregnancy rate in the postpartum period of cows, especially with a more pronounced effect on those with low body condition scores. In this work, the pregnancy rate with the use of eCG (41.46%), proved to be beneficial in cows with a BCS = 2 (scale from 1 to 5), when compared to the use of FSH (18.18%) and the control (17.07%). In corroboration, other authors found a difference in the pregnancy rate with the use of eCG (66.7%) when compared to FSH (25.5%) and control (34.7%) in cows with a BCS <2.75, but did not find differences in cows with a BCS ≥2.75 (19).

Corroborating the results of the present study, a positive effect of the use of eCG was found on the pregnancy rate in *Bos indicus* cows TAI with a BCS ≤ 3 (21). The increase in eCG is not necessary in animals with a good BCS (≥ 2.5) or that are cyclical, as in these conditions eCG does not promote an improvement in pregnancy rates, since they have already reached the minimum condition score (≥ 2.5) recommended for carrying out TAI protocols in cattle (28), a fact that helps to explain the results of the present study.

When eCG is administered at the time of removal of the progesterone device, the dominant follicle tends to become larger after discontinuation of progestogen treatment. Thus, it creates more favorable conditions for the greater development of the dominant follicle and, consequently, ovulation, leading to an improvement in pregnancy rates for cows with a lower BCS (29), a fact that can explain the better results for eCG treatment in the present study.

Regarding the rate of pregnancies and the days elapsed postpartum (Table 2), similar results were found by other authors (30), comparing the pregnancy rate of cows under 45 days (40%) or more than 45 days (48%) postpartum, with no difference between treatments. Corroborating the results of the present study, other authors found no difference when analyzing pregnancy rates in cows with an early postpartum period (30–60 days) or a late postpartum period (61–90 days), with pregnancy rates of 45.77 and 48.80%, respectively (13).

The use of growth promoters such as eCG and FSH increase the appearance of the LH wave, and when these hormones are administered together with progesterone and estrogen in TAI protocols, they increase the cyclicity and the rate of ovulation and pregnancy of animals in anestrus or in a postpartum period of less than 60 days, especially in beef cows in the postpartum period and breastfeeding (22); facts that can explain the results of this work.

Usually, a postpartum period of 30 days is recommended for uterine involution to occur, but this period can extend up to 100 days postpartum. The ideal is to use females over 45 days postpartum in TAI protocols (31). However, 30 days after the puerperium, there is already a release of gonadotropin-releasing hormone (GnRH) and pulses of luteinizing hormone, thus characterizing signs of the restoration of the hypothalamic-pituitary-ovarian-uterine axis (32).

Techniques aimed at inducing or synchronizing estrus in the postpartum period are essential. These protocols using progesterone and estrogen associated with follicular growth promoters, facilitate a joint action in order to generate hormonal changes mimicking the physiological events in the bovine female and allowing cows in anestrus to resume cyclicity and may establish a new pregnancy earlier.

In conclusion, in the present study, the use of equine chorionic gonadotropin or follicle stimulating hormone did not result in an increase in pregnancy rate when compared to the control treatment, nor did it influence the pregnancy rate due to the days elapsed postpartum. However, equine chorionic gonadotropin improved the pregnancy rate of cows with low body condition scores.

Conflict of interests

Acknowledgments

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