GONADOTROPIN RELEASING HORMONE (GnRH) AND EQUINE CHORIONIC GONADOTROPIN (ECG) IMPROVE THE PREGNANCY RATE ON PROTOCOLS FOR TIMED-ARTIFICIAL INSEMINATION IN BEEF CATTLE

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ABSTRACT

The study aimed to verify the influence of GnRH and eCG in TAI protocols to improve the pregnancy rate (PR) in beef cattle, treated with previous intravaginal progesterone long-acting, in the breeding season 90 days. A total of 660 females were divided into five groups: (TAI1) injection of 1.0mg estradiol benzoate (EB) and P4 on d0, P4 removal and 0.075mg prostaglandin F2α on d9, 1.0mg EB on d10, and TAI 32h (n = 120); (TAI2) same as TAI1, but inclusion of 500μg GnRH at TAI (n = 120); (TAI3) same as TAI1, but inclusion of 400IU eCG on d10 (n = 120); (TAI4) same as TAI3, but inclusion of 500μg GnRH at TAI (n = 120); (5) no estrus synchronization, but served by natural service (NS; n = 180). Forty-five days after the TAI, the TAI1, TAI2, TAI3, and TAI4 group were run with "clean-up" bulls for 45 d. Pregnancy was determined by ultrasonography on d45 for TAI PR and on d120 after TAI for all treatments for overall PR. There were no differences in PR among the four TAI protocols on d45 (cows: TAI1=48.0%, TAI2=53.8%, TAI3=57.6%, TAI4=58.3%; heifers: TAI1=53.3%, TAI2=57.1%, TAI3=57.1%, TAI4=56.2%). The PR after the 90-day breeding season was greater in all TAI groups of cows ($P<0.0001$) compared with NS, except for heifers ($P>0.05$). Cows and heifers were analyzed together, and no differences among groups were found. It was concluded that protocols for TAI using GnRH or eCG alone or together contribute significantly to improve the pregnancy rate in beef cattle females, when subjected to previous treatment with progesterone in the reproductive season.

Keywords: fixed-time artificial insemination, beef cattle, estrus synchronization, GnRH, eCG.

O FATOR LIBERADOR DE GONADOTROFINAS (GnRH) E A GONADOTROFINA CORIÔNICA EQUINA (eCG) MELHORA A TAXA DE PRENHEZ EM PROTOCOLOS PARA A INSEMINAÇÃO ARTIFICIAL EM TEMPO FIXO EM BOVINOS DE CORTE

RESUMO

O estudo teve como objetivo verificar a influência do GnRH e eCG em protocolos de IATF para melhorar a taxa de prenhez (TP) em bovinos de corte, tratados anteriormente com

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progesterona intravaginal de ação prolongada, em estação de monta de 90 dias. Um total de 660 fêmeas foi dividido em cinco grupos: (TAI1) injeção de 1,0mg de benzoato de estradiol (BE) e P4 em d0, remoção de P4 e 0,075mg de prostaglandina F2a em d9, 1,0mg de BE no d10, e TAI 32h (n = 120); (TAI2) mesmo que TAI1, mas a inclusão de 500ug de GnRH em IA (n = 120); (TAI3) mesmo que TAI1, mas a inclusão de 400 UI de eCG no d10 (n = 120); (TAI4) mesmo que TAI3, mas a inclusão de 500ug de GnRH em IA (n = 120); (5) não sincronização do estro, mas servido por monta natural (MN, n = 180). Quarenta e cinco dias após a TAI, a TAI1, TAI2, TAI3 e grupo TAI4 foram deixados com os touros por 45 d. A prenhez foi determinada por ultrassonografia em d45 depois da TAI e em d120 após todos os tratamentos. Não houve diferenças na TP entre os quatro protocolos de TAI em d45 (vacas: TAI1 = 48,0%, TAI2 = 53,8%, TAI3 = 57,6%, TAI4 = 58,3%; novilhas: TAI1 = 53,3%, TAI2 = 57,1%, TAI3 = 57,1%, TAI4 = 56,2%). A TP, após a estação de monta de 90 dias foi maior em todos os grupos de vacas TAI (P<0,0001) em comparação com MN, com exceção de novilhas (P>0,05). As vacas e novilhas foram analisadas em conjunto, e não foram encontradas diferenças entre os grupos. Concluiu-se que os protocolos de TAI, utilizando GnRH ou eCG sozinho ou em conjunto, contribuem de forma significativa para melhorar a taxa de prenhez em fêmeas bovinas, quando submetidas a um tratamento prévio com progesterona na fase reprodutiva.

Palavras-chave: IATF, GnRH, eCG, sincronização de cio, gado de corte.

EL FACTOR LIBERADOR DE GONADOTROPINA (GNRH) Y LA GONADOTROPINA CORIÓNICA EQUINA (ECG) MEJORA DE LA TASA DE EMBARAZO EN PROTOCOLOS PARA LA INSEMINACIÓN ARTIFICIAL EM TIEMPO FIJO EN GANADO DE CARNE

RESUMEN

El estudio tuvo como objetivo verificar la influencia de la GnRH y eCG en protocolos de IATF para mejorar la tasa de preñez (TP) en ganado para carne, previamente tratados con progesterona intravaginal una acción prolongada en la temporada de cría de 90 días. El total de 660 hembras fueron divididas en cinco grupos: (TAI1) inyección de 1,0 mg de benzoato de estradiol (BE) y P4 en dia 0(d0), remoción de la P4 + 0,075 mg prostaglandina F2a en d9, 1,0 mg de EB en d10 y TAI 32h (n = 120); (TAI2) lo mismo TAI1, pero la inclusión de 500µg de GnRH en TAI (n = 120); (TAI3) lo mismo TAI1, pero la administración de 400 UI eCG en d10 (n = 120); (TAI4) lo mismo TAI3, pero la administration de 500µg de GnRH en TAI (n = 120); (5) sin la sincronización de celo, pero servida por monta natural (MN, n = 180). Cuarenta y cinco días después de la TAI, el TAI1, TAI2, TAI3, y el grupo TAI4 quedarón con los toros por 45 d. La gestatión fue determinada por ultrasonografía en 45d despues de TAI y en 120d después para todos los tratamientos. No hubo diferencias en TP entre los cuatro protocolos de TAI en d45 (vacas: TAI1 = 48,0%, TAI2 = 53,8%, TAI3 = 57,6% = TAI4 58,3%; vaquillas: TAI1 = 53,3%, TAI2 = 57,1%, TAI3 = 57,1% , TAI4 = 56,2%). El TP después de la temporada de cría de 90 días fue mayor en todos los grupos de vacas TAI (P<0,0001) en comparación con MN, a excepción de las novillas (p>0,05). Vacas y novillas fueran analizadas en conjunto, y no se encontraron diferencias entre los grupos. Se concluyó que el TAI usando protocolos de la GnRH o ECG solos o juntos, colaboran significativamente a mejorar la tasa de preñez en el ganado hembra cuando se somete a un tratamiento previo con la progesterona en la fase reproductiva.

Palabras clave: IATF, GnRH, eCG, sincronización de estros, bovino.

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INTRODUCTION

Brazil's national cattle herd has recently been quantified at 209.5 million head (1). Among these, about 50% are females capable of reproduction. However, only 10% of these females are subjected to artificial insemination (AI) (2). Artificial insemination has contributed to improved genetics, hygiene, and reproduction control, but inaccurate observation of estrus (3) and lack of skilled labor (4) are limitations in this technique. Consequently, there has been significant economic interest in development of protocols involving hormonal synchronization of estrus and ovulation in beef cattle for fixed-time artificial insemination (TAI) without estrus observation (3).

To avoid the need for estrus detection in TAI, various hormonal protocols have been developed using combinations of progesterone (P4), estrogen, and prostaglandin F2alpha (PGF2α) (5-8). Pregnancy rate did not differ with or without estrus detection (9).

To increase pregnancy rates, drugs (e.g., eCG and GnRH) and management techniques (e.g., interrupted temporary weaning) have been added to the conventional TAI protocols (10-13). Several studies investigating the usefulness of hormonal protocols in commercial beef cattle reported pregnancy rates of more than 50% in animals submitted to TAI (10,14), and TAI hormonal protocols followed by natural service in the breeding season resulted in an overall pregnancy rate of more than 80% (15,13,16).

Studies on bovines submitted to TAI protocols reported increased ovulation rates, indicating that P4 exposure improves ovarian activity (17). This becomes more relevant when there is a need for an annual female replacement rate of 20% (18).

Baruselli et al. (15) compared protocols based on P4, estrogen, and prostaglandin with protocols based only on GnRH or PGF2α and found significantly superior pregnancy rates of 42.7% and 15.0%, respectively, in *Bos indicus* cows. The Ovsynch protocol based on GnRH and PGF2α has not shown good reliability in Nelore cows, resulting in low pregnancy rate. We hypothesized that hormonal protocols for TAI using eCG at P4 removal or GnRH on the day of TAI would produce better pregnancy rates than protocols without the use of eCG or GnRH or the group with natural service. The present study aimed to verify the effects of GnRH and eCG in TAI protocols for improving the pregnancy rate (PR) in lactating beef cows and heifers, treated with previous intravaginal progesterone long-acting in 90-days breeding season.

MATERIALS AND METHODS

The present study used 660 animals (*B. indicus*, Nelore; *B. indicus* × *B. taurus*, Simmental × Nelore) and was conducted during the breeding season of 2011/2012. The study population included 387 lactating multiparous cows with suckling calves and 273 pubertal (presence of corpus luteum or follicles >8.0 mm diameter) heifers from a commercial herd. The cows were all within the 90-day (45–93 days) postpartum period and average values were as follows: age, 4.5 (3–5) years; parity, 3 (2–4) calvings; weight, 480 (450–550) kg; and body condition score (BCS), 6.0 (4.5–7.0) (1 = emaciated/9 = very fat). Average values for the heifers were as follows: age, 2.4 years (1.9–3.2); weight, 335 (290–350) kg; and BCS, 6.0 (4.0–7.0). The animals were kept on *Cynodum* sp. pasture plus mineral supplementation and water *ad libitum*. The animals were divided according to a 2 × 2 factorial arrangement into 4 groups: TAI1, TAI2, TAI3, and TAI4 groups received different hormonal protocols (Figure 1) and TAI with frozen semen (19), and a natural service (NS) group remained with fertile bulls for 90 d. Animals in the TAI1, TAI2, TAI3, and TAI4 groups underwent pregnancy diagnosis on d45 before staying with the bulls for natural service for 45 days. Pregnancy diagnosis was

performed in all animals on d120 by using an ultrasound machine (D-Chison 600VET, 6-MHz linear transducer; China).

Protocols used in TAI 1, TAI2, TAI3, and TAI4 groups of cows and heifers.

TAI1 (n = 75 cows and 45 heifers)

P4+EB	-P4+PG	EB	+32 h TAI	US +NS	-NS
I_____	I_____	I_____	I_____	I_____	I_____
D0	D9	D10	D11	D45	D90

TAI2 (n = 78 cows and 42 heifers)

P4+EB	-P4+PG	EB	+32 h GnRH +TAI	US +NS	-NS
I_____	I_____	I_____	I_____	I_____	I_____
D0	D9	D10	D11	D45	D90

TAI3 (n = 78 cows and 42 heifers)

P4+EB	-P4+PG	EB +eCG	+32 h TAI	US+NS	-NS
I_____	I_____	I_____	I_____	I_____	I_____
D0	D9	D10	D11	D45	D90

TAI4 (n = 72 cows and 48 heifers)

P4+EB	-P4+PG	EB +eCG	+32 h GnRH +TAI	US+NS	-NS
I_____	I_____	I_____	I_____	I_____	I_____
D0	D9	D10	D11	D45	D90

NS (n=84 cows and 96 heifers)

NS	- NS
I_____	I__
D0	D 90

Legend: P4: Intra vaginal device (0,558 g Progesteron = Cronipress, Biogenesis Bago); EB: (1 mg Estradiol Benzoate/mL. IM, Cronibest, Biogenesis Bago); PGF2α (0.075 mg / ml Cloprostenol = Croniben, Biogenesis Bago); eCG (400 IU Equine Chorionic Gonadotropin = Novohormon, Coopers); GnRH (500 µg/mL Gonadorelin = Profertil, Tortuga, SP); NS = natural service.

STATISTICAL ANALYSIS

The data were analyzed using a statistical software program (20). The experimental data were analyzed as a 2 × 2 factorial arrangement, and conception rate, eCG, and GnRH were considered as main effects. The differences in the postpartum period (DPP), weight, age, parity, and eCC among the treated groups of cows were analyzed by ANOVA (PROCTTEST procedure). The differences in the weight, age, and ECC among the heifer groups were analyzed by ANOVA (PROCTTEST procedure). The pregnancy rate data of the TAI for cows and heifers were analyzed by PROC FREQ of SAS. Cows and heifers were analyzed together to find differences using PROC FREQ of SAS. The model included main effects for CR, eCG, and GnRH and their respective interactions. Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Results of pregnancy rate in multiparous and nulliparous animals are shown in Tables 1, 2, and 3. Table 3 shows the combined data of the cows and heifers analyzed within their respective groups. The DPP, weight, age, parity, and BCS before the beginning of the treatment did not differ among the groups. No interaction between TAI groups of cows (P = 0.55) and heifers (P = 0.15) was found.

Table 1. Pregnancy rate in multiparous cows (*Bos indicus* and *Bos indicus* x *Bos taurus*) subjected to different hormonal protocols to fixed-time artificial insemination (TAI) and natural service (NS) in the breeding season of 2011/2012.

	Pregnancy rate		
Group	TAI (%)	TAI+45 days NS(%)	90 days of NS(%)
TAI1(n=75)	48.00 (36/75)[a]	84.00 (63/75)[b]	
TAI2(n=78)	53.84 (42/78)[a]	84.61(66/78)[b]	
TAI3(n=78)	57.69 (45/78)[a]	88.46 (69/78)[b]	
TAI4(n=72)	58.33(42/72)[a]	91.66 (66/72)[b]	
NS5(n=84)			57.14(48/84)

Different letters in the same rows or column are significantly different, $P < 0.0001$

Table 2. Pregnancy rate in nulliparous (*Bos indicus* and *Bos indicus* x *Bos taurus*) subjected to different hormonal protocols to fixed-time artificial insemination (TAI) and natural service (NS) in the breeding season of 2011/2012.

	Pregnancy rate		
	TAI (%)	TAI + 45 days (%)	90 days of NS(%)
TAI1(n=45)	53.33 (24/45)[a]	86.66 (39/45)[b]	
TAI2(n=42)	57.14 (24/42)[a]	85.71 (36/42)[b]	
TAI3(n=42)	57.14 (24/42)[a]	92.85 (39/42)[b]	
TAI4(n=48)	56.25 (27/48)[a]	93.75 (45/48)[b]	
NS5(n=96)			84.37 (81/96)

Different letters in the same rows or column are significantly different, $P < 0.0001$

Table 3. Pregnancy rate in nulliparous and multiparous cows (*Bos indicus* and *Bos indicus* x *Bos taurus*) subjected to different hormonal protocols to fixed-time artificial insemination (TAI) and natural service (NS) in the breeding season of 2011/2012.

	Pregnancy rate		
Group	TAI (%)	TAI+45 days NS(%)	90 days of NS(%)
TAI1(n=120)	50.0 (60/120)[a]	85.0 (102/120)[b]	
TAI2(n=120)	55.0 (66/120)[a]	85.0 (102/120)[b]	
TAI3(n=120)	57.5 (69/120)[a]	90.0 (108/120)[b]	
TAI4(n=120)	57.5(69/120)[a]	91.6(111/120)[b]	
NS5(n=180)			71.6 (129/180)

Different letters in the same rows or column are significantly different, $P < 0.0001$

The use of P4 intravaginal devices together with eCG or GnRH has been tested in *B. Indicus* (14, 21-27) and in *B. taurus* (28-33), with no consistent results. Overall, Brazilian data of TAI in beef cattle has shown a pregnancy rate of 49.6% (14).

Animals in groups TAI1, TAI2, TAI3, and TAI4 had pregnancy rates of 48.0%, 53.8%, 57.6%, and 58.3%, respectively (Table 1) ($P = 0.55$). Although there were no significant differences among the four groups ($P > 0.05$), TAI4 (eCG + GnRH) yielded a greater pregnancy rate (10.0% more) than TAI1. A 10% increase in pregnancy rate with the use of eCG and GnRH, in the same protocol, compared with the use of neither eCG nor GnRH is economically attractive.

Pregnancy rates for TAI vary and there is no consistent pattern among researchers. According to Ereno et al. (10) and Sá Filho et al. (26), the use of eCG or GnRH in TAI programs did not improve pregnancy rate in beef cows. Other studies, however, showed an increase (22,23,32,34). In our study, TAI using eCG or GnRH yielded pregnancy rates of 53.8%–57.6%, very close to those of Ereno et al. (10) (54.7%) and de Sá Filho et al. (7) (55.7%). Other authors state that eCG or GnRH administration with a progesterone-based protocol has advantages regarding ovulation and pregnancy rates (22,34).

Certain aspects have not been sufficiently clarified, such as timing of eCG or GnRH administration. Peres et al. (22) employed eCG 48 h, and Sa Filho et al. (24) 54 h and 32 h, after removal of P4, with no significant differences in results. Other variables, such as the administration of eCG earlier after P4 removal (22), anestrus cows (7), BCS (30,32), breed, diameter of the largest follicle (25), pre-synchronization (31), temporary weaning (10), and parity (30), should be considered for better results in TAI. In the present study, the TAI3 and TAI4 groups were treated with eCG on the day of P4 removal, and GnRH was injected in the TAI2 and TAI4 groups at 32 h after P4 removal following AI, and there were no differences in the pregnancy rate among the three groups.

Considering pregnancy rates after TAI followed by use of "clean-up" bulls (natural service) (Table 1), the difference between TAI1 and TAI4 (84.0% versus 91.6%) was less than when TAI was applied alone. However, when comparing pregnancy rates of any treated cows (TAI + 45 days NS) with those of cows that remained under the regime of natural service for 90 days, differences in favor of the TAI groups were strongly accentuated ($P < 0.0001$). This corroborates the results of Baruselli et al. (15), who reported the beneficial effects of TAI protocols with exposure of animals to P4 on induction of ovarian activity and synchronization of estrus in beef cows with TAI followed by natural service (35). In addition, no significant interaction was observed between GnRH and eCG for multiparous cows ($P = 0.55$), as well as for nulliparous animals ($P = 0.15$).

Regarding heifers submitted to TAI1, TAI2, TAI3, and TAI4, there was no significant difference among groups (Table 2). Further, there were no significant differences in pregnancy rate between groups treated with TAI + natural service and the group treated with natural service alone (NS5). This could lead to the misguided view that there is no benefit of TAI protocols in heifers. However, from a practical standpoint, the heifers submitted to TAI probably will calved earlier in the next breeding season. Azeredo et al. (35) reported that 82.0% of heifers submitted to TAI calved in the first 40 days of the next breeding season. As a result, heifers have increased postpartum rest in the next breeding season, parturitions can be timed according to nutritional availability, and herd health and management as well as marketing are improved. Time artificial insemination can also facilitate the use of breeds that would not be viable for natural service because of poor adaptability. Heifers submitted to TAI reached at least 53.3% pregnancy, and the products of these animals may have higher value because they are derived from genetically superior animals. The adoption of TAI in heifers is justified by a significantly superior pregnancy rate in the next breeding season (35). Analyzing the cow and heifer groups together yielded no difference between the protocols for TAI (Table 3), but there was a difference among the treated groups and the NS alone indicating that exposure of animals to previous P4 can result in improved pregnancy rate.

CONCLUSIONS

In conclusion, protocols for TAI using GnRH or eCG alone or together improve significantly the pregnancy rate in beef cattle females, when subjected to previous progesterone treatment in the reproductive season.

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