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Impact of Equine Chorionic Gonadotropin Associated with Temporary Weaning, Estradiol Benzoate, or Estradiol Cypionate on Timed Artificial Insemination in Primiparous Bos Indicus Cows

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ABSTRACT

The study aimed to determine the impact of equine chorionic gonadotropin (eCG) associated with different timed artificial insemination (TAI) protocols on the pregnancy rate (PR) in Bos indicus cows previously treated with progesterone. Five hundred and fifty-seven primiparous cows were subjected to the following treatments: on day 0 (d0), GeCGTW (group equine Chorionic Gonadotropin+Temporary Weaning;n=178) received 0,558 g intravaginal progesterone (P4)+1.0 mg of estradiol benzoate (EB) (IM); on d8 (P4 removal+0,075 mg D-cloprostenol + 400 IU eCG + TW for 48 h); on d10, TAI + calves return to dam; GeCGEB (group equine Chorionic Gonadotropin+Estradiol benzoate; n=176) the same as GeCGTW without TW + application of 1.0 mg of EB on d9; GeCGEC (group equine Chorionic Gonadotropin+Estradiol Cypionate; n=203), the same as GeCGTW without TW+1.5 mg EC (IM). On d35, post TAI, pregnancy diagnosis (PD) was performed. Non-pregnant animals remained under clean-up bulls for 90 days. After this period, the animals were subjected to PD using ultrasound. The PR of TAI was 51.1%, 47.1%, and 47.8% for GeCGTW, GeCGEB24, and GeCGEC (P>0.05) respectively. The PR under clean-up bulls was 88.3%, 47.3%, and 31.1% (P<0.05). The final PR (TAI+clean-up bulls) of the groups was 94.4%, 72.1%, and 64.0%, respectively (P<0.05). It was concluded that no differences in PR among the protocols related to TAI were detected; PR in the GeCGTW protocol under clean-up bulls was higher compared to others (P<0.05); the overall PR of cows subjected to TAI+clean-up bulls was significantly higher in GeCGTW than in the other groups.

Key words: Beef catlle, Equine Chorionic Gonadotropin, Fixed time artificial insemination.

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INTRODUCTION

Although conventional artificial insemination (AI) has become one of the most efficient reproductive biotechnologies, resulting in significant benefits to cattle herds, it is not been widely used in tropical countries (Barreiros et al. 2014). Some limitations of its applicability on a large scale include the necessity of daily observations for estrus detection, low cyclicality rate, low service rate in cows after calving, and workforce shortage. Researchers have been conducting studies to improve and overcome the challenges of AI by adopting timed artificial insemination (TAI). This biotechnology not only provides the advantages of conventional AI, but also includes other benefits such as the ability to perform AI in a large number of animals without estrus observation, and the relatively short duration (Sales et al. 2015).

Many hormonal protocols related to TAI have been developed for beef cattle in the last decade by using long-acting progesterone (P_4), EB, or gonadotropin-releasing hormone (GnRH) on the first day of treatment (Pursley et al.1995; Wheaton and Lamb 2007), followed by eCG, EC, or even temporary weaning (TW), when the intravaginal P_4 is removed (day 8 after the start of the protocol) (Campos et al. 2013; Barreiros et al. 2014).

To improve ovarian follicle quality, ovulation, and pregnancy rate (PR), eCG was administered on the day of P_4 removal in TAI protocols (Peres et al. 2009; Sá Filho et al. 2010; Campos et al. 2013). Peres et al. (2009) reported the enhancing effect of eCG on serum P_4, ovulation, and PR in non-lactating *Bos indicus* cows. Small et al. (2009) determined the effects of estrus presynchronization using intravaginal P_4 and eCG at P_4 removal stating that eCG had no significant effect on the diameter of the pre-ovulatory follicle, but signaled improved PR in primiparous cows. Sá Filho et al. (2010) established positive eCG effects (increased growth rate of the largest follicle, larger follicle diameter, increased ovulation rate, and increased PR) after P_4 removal in TAI in Nellore cows.

To improve reproductive efficiency several protocols for TAI have been proposed, including eCG and TW. Soto Belloso et al. (2002) administered P_4 plus eCG or TW to induce fertile estrus, reduce the interval between estrus, and lower birth-conception interval in anestrus *B. indicus* cows. Meneghetti et al. (2009) conducted studies in a commercial herd aiming to develop a protocol for TAI, to reach a PR of 40% to 55% in *B. indicus* cows. They concluded that the TAI protocols should contain 400 IU eCG associated with TW.

The combination of eCG and TW in *Bos indicus* lactating cows was also used for estrus resynchronization. Campos et al. (2013) verified that the association between eCG and more TW was the best protocol for TAI in *B. indicus* lactating cows.

For induced ovulation studies, EC was administered at the time of P_4 removal. This resulted in ovulation between 67 h and 70 h after treatment (Sales et al. 2008). Penteado et al. (2006) administered EC to *B. indicus* lactating cows on the day of P_4 removal, resulting in 49.4% PR, indicating that it can be used on the day of P_4 removal. Sales et al. (2012) synchronized ovulation in *B. indicus* cows using EB or EC. They concluded that both esters effectively induced a preovulatory LH peak resulting in synchronized ovulation either by applying EC on P_4 removal or EB 24 h after P_4 removal. Sá Filho et al. (2011) administered EC at P_4 removal, resulting in improved ovarian response due to greater follicles in TAI, higher ovulation rate, biggercorpus luteum, higher P_4 concentration and more efficient PR. The hypothesis of this study is that primiparous cows treated with a protocol of eCG and TW of a 48-h duration (previous treatment P4), could result in higher PR at the end of the breeding season, than the protocol EB 48 h after the application of eCG or EC on the same day.

This study aimed to determine the impact of eCG followed by TW for 48 h; eCG followed by EB 24 h after or eCG more estradiol cypionate in the same day, in primiparous *B. indicus* cows previously subjected to an intravaginal device with long-acting P_4 on the PR in the postpartum period.

MATERIAL AND METHODS

Selection, management, and feeding of animals

We used 557 primiparous *B. indicus* cows (Nellore breed) from a commercial farm, with an average age of 36 months, body condition score (BCS) = 2.8 ± 0.4 (2.0 to 4.0, considering 1 = thin, 5 = obese), 64 ± 20.1 days open after delivery (30–112 days), 320 kg average weight. The animals grazed on *Brachiaria brizantha*, with provision of mineral salt and water *ad libitum*. The cows, were divided in groups, subjected to the same management, and inseminated in the same day. Pregnancy was performed by use of ultrasound machine (Tringa Ultrasound model, 5-MHz transducer; Pie Medical, Netherlands) on day 35 after TAI and after 90 days of the breeding season period.

On day 35, non-pregnant animals remained in the same groups along the breeding season (90 days) for clean-up bulls in the proportion of one bull for 20 cows. Before the clean up of the bulls, semen analysis according to the rules of the CBRA (1998) was performed.

Experimental design

The animals were assigned into three groups (G) and subjected to the following protocols (Fig.1): GeCGTW48 (Group eCG+ Temporary Weaning=calf separated out of dam for 48 hours; n=178); GeCGEB24 (eCG + estradiol benzoate 24 h after P_4 removal; n=176) and GeCGEC (eCG + estradiol cypionate; n=203).

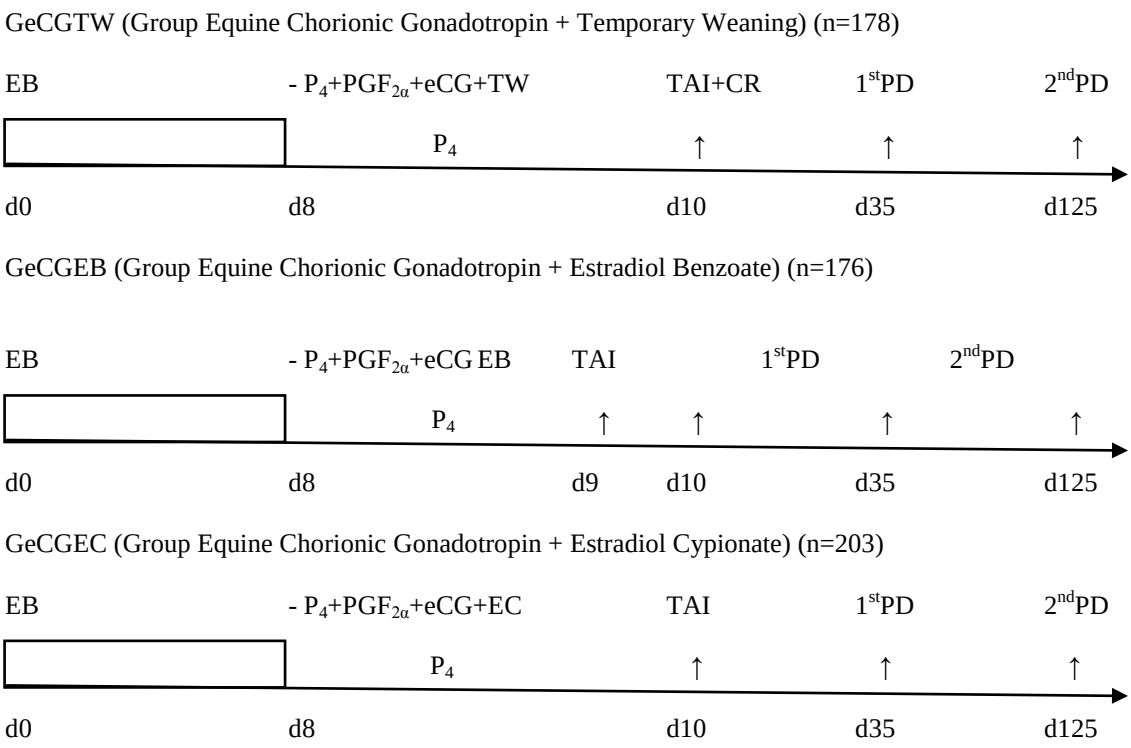

Figure 1 –Graphical representation of applied protocols to groups of primiparous *Bos indicus* cows for timed-artificial insemination (n=557); (P_4 - Cronipress monodosis – 0.558 g progesteron= Cronipress, Biogenesis Bago); EB(Cronibest - 1 mg estradiol benzoate, Biogenesis Bago); equine Chorionic Gonadotropin= 400 UI (Folligon, MSD – Animal health, São Paulo, Brazil); $PGF_{2\alpha}$(0.075 mg D-Cloprostenol - Croniben, Biogenesis Bago); EC (1.5 mg – Zoetis, São Paulo, Brazil); CR= calf return; PD=pregnancy diagnosis.

Statistical analysis
Data related to the PR for TAI and to the clean-up bulls were compared using the chi-square test with Yates' correction (X^2) at $P<0.05$ level of significance (Soft GraphPad Prism, version 5.0, 2014).

RESULTS AND DISCUSSION

In recent years, hormonal protocols related to TAI in cattle provided significant advances in the replacement of conventional AI. These studies have been addressed to an increased understanding of ovarian follicular dynamics, and hormonal reproductive physiology.
Primiparous suckling cows are a special class of animals, because they are more sensitive and more susceptible to nutritional imbalance along the postpartum period than pluriparous cows (Vasconcelos 2011). Primiparous cows are those that have recently passed from heifer to cow condition, an event that heavily affects the BCS (tendency to slimming), especially in beef cattle. One of the causes of this imbalance can be attributed to nursing, depleting essential nutrients in the primiparous cow (Short et al.1990; Wiltbank et al. 2002). Regarding this matter we hypothesized that the hormonal protocol eCG + TW would resulted in better PR than the other groups at the end of the breeding season. This was confirmed because GeCGTW demonstrated PR higher than the other groups (P=0.0001), related to the clean-up bulls and also to TAI + clean-up bulls in the final rate of the breeding season (Table 1).

Table 1 – Pregnancy rate (PR) in primiparous Nellore cows, related to timed artificial insemination (TAI) and clean-up bulls (n=557).

Groups	PR of TAI (n/%)	PR of clean-up bulls(n/%)	PR at end of breeding season (TAI+clean-up bulls) (n-%)
GeCGTW (n=178)	92/178 (51.7)	76/86 (88.3)[a]	168/178 (94.4)[a]
GeCGEB (n=176)	83/176 (47.1)	44/93 (47.3)[b]	127/176 (72.1)[b]
GeCGEC (n=203)	97/203 (47.8)	33/106 (31.1)[c]	130/203 (64.0)[b]
Total (n=557)	272/557(48.8)	153/285 (53.7)	425/557 (76.3)

Different letters in the same column are significantly different (P<0.05).

Over the last decade, PR in beef cows has varied greatly among studies related to TAI. PR may extend from 27.0% (Peres et al. 2009), 42.4 (Hill et al. 2014), 49.6% (Sá Filho et al. 2009), 50.7% (Baruselli et al. 2012) to 62.4% (Dobbins et al. 2009). The results of the present study showed no differences between treatments applied in three groups of cows to TAI (table 1).
However our results (PR) of TAI in primiparous shown to be superior to Sá Filho et al. (2009) and Hill et al. (2014), which worked with *Bos taurus indicus* and *Bos taurus taurus*. On the other hand data from this study confirm very good results in PR when there wasclean up of the bulls for cows previously submitted to P_4 and EB in the end of the breeding season, corroborating studies of Baruselli et al. (2012), which found strong increase in PR in cows pretreated with progestin and EB (Table 1). The PR at the end of the breeding season (results from TAI + clean-up bulls), GeCGWT showed differences (P<0.05) compared to other groups (22.3% more than GeCGEB, and 30.4% more than GeCGEC). Studies confirm an increase of 15% to 20% in PR, by using TW associated with eCG towards protocols without TW in the other

two groups (Barreiros et al. 2014). This result could be attributed to the TW itself. Increaseof the GnRH and LH pulses frequency, resulting in a higher ovulation rate, essential for good results in AI (Edward 1985).

Temporary weaning leads to a good combination of results increasing the fertility rate in *B. indicus* cows, stimulating LH secretion (Edward 1985). This mechanism can be optimized after eCG administration, increasing PR. Potentially eCG offers improvement in follicular development rate, and additionally provides more detailed E_2 profiles during the proestrus and diestrus (P_4) phase, optimizing fertility (Sá Filho et al. 2009). The hypothesis of this study was that primiparous cows submitted to hormonal protocols with eCG + TW (48 h) after previous exposure to long-action P4 would result in better PR at TAI. Partly this happened because the group GeCGTW showed higher PR by bulls clean up alone and TAI + clean up at the end of the breeding season. In fact, this was confirmed because GeCGEB and GeCGEC displayed no differences between themself at the end of the breeding season (Table 1).Working on five groups of beef cows, Campos et al. (2013) administered 300 IU eCG in one group and subjected the other group to eCG + TW, resulting in higher PR, than in other groups using estrus resynchronization protocols. Yelich et al. (1995) used TW after the previous use of oral progestin. They found that P_4 + TW treatment was more effective than that in other groups, as well as TW + eCG that resulted in better PR rates (Sá Filho et al. 2010). *B. indicus* cows treated with norgestomet + 500 IU eCG after removal of the implant, or treated only with TW (96 h) showed greater efficiency by reducing the estrus interval/first service, and increasing the conception rate (Soto Belloso et al. 2002). Cows milked twice daily without calves, presented a shorter duration between parturition/first estrus and additionally showed better reproductive efficiency than cows with suckling calves. The calf suckling action activates mechanisms that inhibit the hypothalamic-pituitary-ovarian axis of the cow after delivery for longer than 90 days (Soto Belloso et al. 2002). Although the mechanism of this process is not fully understood, the response of estrus depends partly on the number of follicles and their level of maturity when the calf is removed (Dunn et al.1985). Treatment with eCG at P4 removal increased conception rate in anestrus *B. indicus* cows (Baruselli et al. 2004; Sá Filho et al. 2004). Additionally, the use of eCG led to improve follicular development, increasing the P_4 concentration in the luteal phase after ovulation (Sá Filho and Vasconcelos 2010). By application of eCG, *B. indicus* cows with small or medium follicle sizes, presented better PR (Cutaia et al. 2003; Baruselli et al. 2004), and ovarian cyclicity was induced, even when affected by nutritional factors (Bó et al. 2003), or under BCS adverse conditions (hard ovaries and small follicles) after delivery. In the present study the cows carried average BCS of 2.8. The eCG + TW may have contributed to a higher follicular development due to absence of endogenous opioids and the higher flushing of pituitary FSH, than other groups of cows that received eCG + EB or eCG + EC only. This provided a more effective and ovarian activity (cyclicity) and ovulation confirming the findings of Yelich et al. (1995).

CONCLUSION

The eCG + TW protocol was more efficient than the eCG + EB or eCG + EC, related to clean-up bulls, or TAI + clean-up bulls, at the end of the breeding season in primiparous cows.

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