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# Effects of Equine Chorionic Gonadotropin on Follicular, Luteal and Conceptus Development of Non-Lactating Bos Indicus Beef Cows Subjected to a Progesterone Plus Estradiol-Based Timed Artificial Insemination Protocol

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PAPER

# Effects of equine chorionic gonadotropin on follicular, luteal and conceptus development of non-lactating *Bos indicus* beef cows subjected to a progesterone plus estradiol-based timed artificial insemination protocol

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## Abstract

The aim of this study was to evaluate the effects of equine chorionic gonadotropin (eCG) on ovarian follicular responses, *corpus luteum* (CL) development and conceptus length on day 16 after timed artificial insemination (TAI). A total of 124 cows at day 0 (D0) received 2 mg of estradiol benzoate (EB) and the insertion of a progesterone (P4) intravaginal device. Eight days later, the device was removed, and cows received 0.15 mg of prostaglandin and 0.5 mg of estradiol cypionate (EC), and were randomly assigned to 1 of 2 treatments: eCG (n=60), in which cows received 300 U of eCG; and control (n=64). Cows were TAI 48 h after P4 device removal. The diameter of the largest follicle (LF) present on D8 and D10 and of CL on D15 and D26 were measured. Conceptus recovered rate, conceptus length, CL diameter and weight were determined at slaughter on D26. Plasma P4 concentration was determined on D15 and D26. Follicular growth from D8 to D10 (P=0.03), the diameter of CL at D15 (P=0.03) and D26 (P=0.003) and the CL weight at day 26 (P=0.04) were greater in the eCG group than the control. However, there was no effect of eCG treatment on oestrus occurrence, conceptus recovery rate and length, or P4 concentrations on either D15 or D26. In conclusion, although eCG increases follicular responses and the diameter of the CL, this gonadotropin treatment does not influence the length of the conceptus or the P4 concentration on the subsequent oestrus cycle.

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Key words: Nelore, Reproduction, Timed artificial insemination, Progesterone, Embryo survival.

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## Introduction

Timed artificial insemination (TAI) protocols are commonly used to control follicular growth and synchronise ovulation, allowing for artificial insemination and abolishing the necessity of oestrus detection. In particular, TAI programmes have been applied in postpartum anovular beef cows, anticipating the first postpartum insemination and improving reproductive efficiency under tropical conditions (Baruselli *et al.*, 2004; Sales *et al.*, 2012). The use of equine chorionic gonadotropin (eCG) has improved the efficacy of TAI protocols and increased ovulatory responses and pregnancy rates (Cutaia *et al.*, 2003; Sá Filho *et al.*, 2009, 2010c; Sales *et al.*, 2011). Equine chorionic gonadotropin is a long half-life molecule produced by mare's endometrial cup cells (Murphy and Martinuk, 1991), and it has a stimulatory effect on FSH and LH hormones, leading to increased follicular growth and ovulation (Sá Filho *et al.*, 2010a; Sales *et al.*, 2011). The injection of eCG at the moment of progesterone (P4) intravaginal device removal has been demonstrated to be efficient in increasing the largest follicular (LF) growth rate, the diameter of LF at TAI, the probability of ovulation (Sá Filho *et al.*, 2010a; Sales *et al.*, 2011), the diameter of resulting *corpus luteum* (CL) and the P4 concentration five days after insemination (Fátima *et al.*, 2012; Sá Filho *et al.*, 2010c).

Embryo mortality causes serious economic losses in livestock production (Diskin *et al.*, 2012). In high-producing dairy cows, the major part of these gestation losses occurs before D16 of the conception (43% of the total mortality) (Diskin *et al.*, 2012; Dune *et al.*, 2000). It is well recognised that P4 has a pivotal function in establishing gestation and adequate embryonic development (Mann and Lamming, 2001; Spencer, 2004; Bazer *et al.*, 2009). Several studies have described the positive effect of exogenous P4 supplementation on embryo growth, survival and pregnancy establishment (Larson *et al.*, 2007; Lonergan *et al.*, 2007; Carter *et al.*, 2008; Beltman *et al.*, 2009; Lonergan, 2011). Therefore, the present hypothesis was that eCG treatment would increase ovarian follicular responses, CL development, P4 plasma concentration and conceptus length.

## Materials and methods

### Animals and management

A total of 124 non-lactating Nelore cows (*Bos indicus*) with an average body condition score (BCS) of 3.33±0.50 from 3 commercial farms located in Mato Grosso, Brazil, were used in this experiment. The study was conducted from October 2010 to March 2012. All of the cows were kept in pastures of *Brachiaria brizantha* and *Brachiaria humidicola* with *ad*

*libitum* access to water and mineral supplement. On the first day of the synchronisation protocol (D0), the body condition score (BCS) was determined according to Ayres *et al.* (2009) on a scale of 1 to 5 (1=emaciated, 5=obese) stepped of 0.5 points.

## Reproductive management

Cows were synchronised using the protocol depicted in Figure 1. Regardless of the stage of the oestrus cycle (D0), all the cows received 2 mg of estradiol benzoate (EB) intramuscular (i.m.) (Gonadiol®; MSD Animal Health, Summit, NJ, USA) and a P4 intravaginal device (1.9 g of P4, CIDR®; Pfizer Animal Health, Florham Park, NJ, USA). On day 8, the device was removed, and the cows received 0.15 mg of D-cloprostenol i.m. (Prostaglandina Tortuga®; Tortuga Companhia Zootécnica Agrária, São Paulo, Brazil) and 0.5 mg of estradiol cypionate (EC) i.m. (ECP®; Pfizer Animal Health). Cows were randomly assigned into two groups: eCG (n=60), in which the animals received 300 U of eCG i.m. (Novormon®; MSD Animal Health) according to Sá Filho *et al.* (2009); and control (n=64), in which females did not receive any additional treatment. Cows were timed inseminated 48 h after P4 device removal. Frozen semen from single ejaculates of 3 sires was homogenously distributed among experimental groups. All of the animals were slaughtered 16 days (D26) after TAI in a certified slaughterhouse.

## Ultrasonic examinations and detection of oestrus

All the cows were submitted to ultrasonography exams (Mindray 2200 VET-China; Mindray, Shenzhen, China) to determine the diameter of the largest follicle (LF) on D8 and D10 and of the CL on D15 and D20. Oestrus was determined using a self-adhesive heat detection patch (Estrotect®; IVP, Spring Valley, WI, USA) placed on the cows' tail head concurrent with removal of the intravaginal device. The ovulation rate was calculated using the CL presence on D15.

## Serum samples and progesterone radioimmunoassay

On D15, blood samples were collected by puncture of the median coccygeal vein or artery from a subset of cows (n=40). The blood samples on D26 were collected from the carotid artery at the moment of bleeding. Blood was refrigerated at 4°C for 24 h and centrifuged at 900 g for 12 min. Serum was removed and frozen at –20°C until assays were performed. Serum P4 concentrations were determined by a validated solid-phase radioimmunoassay without extraction using a commercial kit (Siemens, Muenchen, Germany) that had been previously validated in our laboratory (Santos and Vasconcelos, 2006). The intra-assay coefficient of variation was 2.9%, and the assay sensitivity was 0.006 ng/mL.

## Conceptus and *corpora lutea* recovery

Immediately after slaughter, the reproductive tracts were collected, placed in a sealed and numbered plastic bag and then in a polystyrene box with ice for transport to the laboratory. Conceptuses were recovered by flushing the uterus with 60 mL of phosphate-buffered saline (PBS). The recovered conceptuses were photographed alongside a scale bar (Figure 2). The size was determined by measuring the

**Table 1. Follicular dynamics, CL development, P4 plasma levels and conceptus length in *Bos indicus* cows submitted to a timed artificial insemination protocol in equine chorionic gonadotropin and control groups.**

| | Control | eCG | P |
|---|---|---|---|
| Number of animals | 64 | 60 | - |
| Largest follicle diameter at day 8, mm | 9.4±0.43 | 9.6±0.39 | 0.70 |
| Largest follicle diameter at day 10, mm | 11.0±0.41 | 12.2±0.44 | 0.06 |
| Follicular growth rate (day 8 to 10), mm | 2.6±0.24 | 3.5±0.32 | 0.03 |
| Occurrence of oestrus, % | 68.7 (44/64) | 66.7 (40/60) | 0.99 |
| Ovulation rate % | 78.13 (50/64) | 86.7 (52/60) | 0.23 |
| CL diameter at day 15, mm | 14.7±0.49 | 16.3±0.53 | 0.03 |
| CL diameter at day 26, mm | 17.9±0.42 | 19.6±0.32 | 0.003 |
| Weight of the CL at day 26, g | 2.4±0.11 | 2.8±0.12 | 0.04 |
| Concentration of P4 plasma at day 15, ng/mL | 1.5±0.34 | 1.4±0.20 | 0.82 |
| Concentration of P4 plasma at day 26, ng/mL | 5.5±0.83 | 5.5±0.76 | 0.94 |
| Conceptus recovered, % | 29.7 (19/64) | 30.0 (18/60) | 0.97 |
| Conceptus length, mm | 98.1±17.24 | 118.5±21.78 | 0.23 |

eCG, equine chorionic gonadotropin; CL, *corpus luteum*.

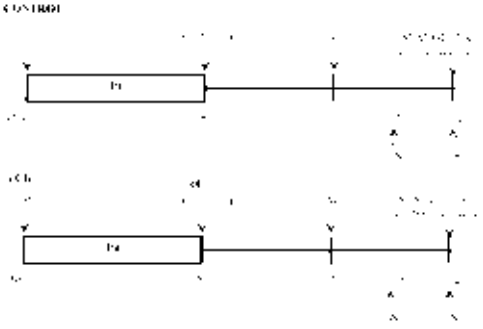

**Figure 1. Schematic diagram of the experimental design. Control and equine chorionic gonadotropin groups were equal, except for the administration of 300 U of equine chorionic gonadotropin on day 8 in the treatment group.**

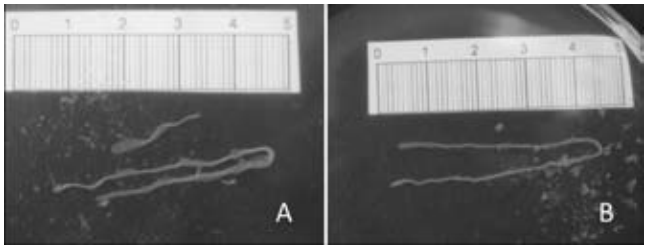


**Figure 2. *Bos indicus* conceptuses recovered on day 16 after timed artificial insemination from equine chorionic gonadotropin (A) and control (B) groups.**

total length of the conceptus using AutoCAD 2007® software. The CLs were dissected from the ovary and weighed, and the diameter was measured using a caliper.

### Statistical analysis

Statistical analysis was performed using the statistical analysis system software for Windows® (SAS, 2000). Continuous variables were evaluated for normality of residuals by the univariate procedure and submitted to the Bartlett's test to analyse the homogeneity of variances. Following these analyses, the general linear model (GLM) procedure and Tukey's test were used for analysis of variance and to determine differences between treatments. Binomial variables (occurrence of oestrus, ovulation rate and conceptus recovered) were analysed by the GLIMMIX procedure of SAS®. In both analyses, the factors included in the models were treatment (control and eCG) and BCS levels (2.5, 3, 3.5 and 4) at the first day of the synchronisation protocol, and their interaction. $P<0.05$ was considered significant. The parametric dependent variables are expressed as the mean±the standard error of the mean (mean±SEM), and the binomial variables are expressed as percentages.

## Results and discussion

All the results can be found in Table 1. There was no interaction between eCG treatment and BCS on analysed variables ($P>0.05$). However, cows with BCS 4 present at the moment of P4 insertion (D0) showed lower diameter of the largest follicle at day 8 (10.8±0.4 for BCS 2.5; 10.6±0.5 for BCS 3; 9.7±0.8 for BCS 3.5; 7.1±0.3 for BCS 4; $P=0.001$) and the diameter of the largest follicle at day 10 (12.8±0.4 for BCS 2.5; 13.1±0.7 for BCS 3; 12.4±0.7 for BCS 3.5; 9.4±0.3 for BCS 4; $P=0.001$). At the moment of P4 device removal, the experimental groups had similar follicular diameters of LF. The follicular growth rate from day 8 to 10 was higher in the eCG group than the control. The administration of eCG did not increase the occurrence of oestrus ($P=0.99$). In addition, eCG administration increased the diameter of CL at D15 ($P=0.03$) and D26 ($P=0.003$). Furthermore, CL weight at the day of slaughter (day 26) was greater in the eCG group than the control ($P=0.04$). However, there was no difference between treatments in ovulation rate ($P=0.23$), conceptus recovery rate ($P=0.97$), conceptus length ($P=0.23$), or P4 concentration on D15 ($P=0.82$) or D26 ($P=0.94$).

The current study found that eCG treatment at the end of the oestrus and ovulation synchronisation protocol increases the ovarian follicular growth and the development of CL in the subsequent oestrus cycle. Despite being the first report regarding the effects of exogenous administration eCG associated to the conceptus length, no differences were found in blood P4 concentrations or the length of the conceptus.

Cows treated with eCG had greater final follicular development, which is in agreement with studies by other authors who used eCG in postpartum Nelore cows (Sá Filho *et al.*, 2009, 2010a; Sales *et al.*, 2011). Additionally, the results of the present trial showed a greater diameter of the LF at TAI after eCG treatment. The optimisation of follicle size and health is an important objective in current TAI programmes (Wiltbank *et al.*, 2011). Larger ovulatory follicles exhibited a greater growth and ovulation rate and resulted in a greater number of pregnancies per artificial insemination in beef cattle (Perry *et al.*, 2007; Sá Filho *et al.*, 2010b). In addition to the increased ovulation rate, the ovulation of LFs could be responsible for other events, such as the improvement of endogenous E2 production, oocyte competence, CL diameter and concentration of P4 in the subsequent oestrus cycle, which may increase the fertility of beef cows following TAI. The effect of eCG increasing the diameter of the large follicle could be related to the capacity of its gonadotropin to bind to FSH and LH on follicular cell receptors (Murphy and Martinuk, 1991).

Cows treated with eCG presented an enlarged CL diameter that resulted in a heavier CL. The greater weight of CL can be due to the increase in the LF at TAI (Sá Filho *et al.*, 2010b), an increase in the number of large luteal cell or an increase in the proportion between large and small luteal cells (Rigoglio *et al.*, 2012). However, although eCG-treated cows presented larger and heavier CLs, these cows did not present a higher concentration of P4 in the dioestrus. This conflicting result has also been found in the literature (Lucy *et al.*, 1995); however, other authors found a positive relationship between increased CL diameter and weight and higher circulating progesterone concentration (Sartori *et al.*, 2002; Baruselli *et al.*, 2010; Mann, 2009). Also, the size of ovulatory follicle has been correlated with the weight of formed CL, each millimeter of ovulatory follicle size corresponding to 1.5 g of CL weight (Fields *et al.*, 2012).

The effects of eCG are likely to be dependent on the severity of anoestrus at the onset of the synchronisation protocol. The difference in LH blood concentration could differ among breeds or change under different environmental conditions (even within the same breed). In this way, the inclusion of eCG treatment in TAI synchronisation protocols was shown to be advantageous only in cows with a low body condition score at the beginning of the protocol (Souza *et al.*, 2009; Sales *et al.*, 2011) or in anoestrus cows (Garcia-Ispierto *et al.*, 2012). Sales *et al.* (2012) found a significant interaction between eCG treatment and bovine BCSs at the onset of the synchronisation protocol. These authors found that cows presenting a lower BCS had greater pregnancy responses after eCG treatment, and no effect of eCG administration was found when cows presented a greater BCS at onset of the synchronisation protocol (Sales *et al.*, 2011).

The role of P4 as the key hormone in the maintenance of pregnancy is well established. In the present study, eCG administration did not change P4 concentration or conceptus length. This result could be explained by the use of non-lactating cows presenting a high BCS. Previous studies did not found effect of the eCG treatment in cows with high BCS (Bó *et al.*, 2006), although the inclusion of eCG treatment in TAI synchronisation protocols was shown to be advantageous only in cows with a low BCS at the beginning of the protocol (Souza *et al.*, 2009).

The initial hypothesis was that the increase in the CL diameter would lead to a higher P4 concentration and conceptus length. A relationship between conceptus size and progesterone concentration before day 7 has been previously described (Beltman *et al.*, 2009). Several studies showed that exogenous supplementation of P4 could regulate embryonic length and development (Garrett *et al.*, 1988; Lonergan *et al.*, 2007). However, the present study did not find an effect of eCG on the conceptus length, most likely due to the similarity in P4 plasma levels between groups. The potential effect of eCG on embryo development would be most likely observed in anoestrus or lower BCS cows.

## Conclusions

In conclusion, despite the lack of a clear eCG effect on CL functionality, as measured by P4 production and conceptus lenght, positive effects of eCG were found on dominant follicle final growth and on the diameter and weight of CL.

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