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Manipulation of the proestrous by exogenous gonadotropin and estradiol during a timed artificial insemination protocol in suckled Bos indicus beef cows

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Abstract

The objective of this study was to evaluate the effects of an exogenous administration of equine chorionic gonadotrophin (eCG) and eCG plus estradiol cypionate (eCG+ECP) during the proestrous period on the occurrence of estrus (based on the activation of a self-adhesive heat detection patch), on ovarian responses, and on pregnancy per AI (P/AI; 30 d after artificial insemination) in suckled beef cows subjected to timed artificial insemination (TAI) protocols. A total of 386 suckled cows received an intravaginal device containing 1.9 g progesterone (P4) and 2.0 mg estradiol benzoate IM on the first day of the synchronization protocol. Eight days later, the P4 device was removed, and $PGF_{2\alpha}$ (0.15 mg of D-cloprostenol) was administrated, followed by TAI, plus 100 µg of GnRH IM 48 h later. At the time of the removal of the P4 device, the cows received one of three treatments: 300 IU of eCG (eCG; *n*=134); 300 IU of eCG plus 1 mg of ECP IM (eCG+ECP; *n*=123) or no additional treatment (Control; *n*=129). A subset of the cows (*n*=96) were evaluated according to the occurrence of estrus between the P4 device removal and the TAI. Their ovarian follicles were evaluated using ultrasound at the time of P4 device removal and at TAI, and the corpus lutea (CL) formed from follicles 5 days after TAI. A greater P/IA (*P*=0.002) was noted in the cows receiving the eCG+ECP treatment (50.4%; 62/123) than in the control group (29.5%; 38/129). Whereas the eCG group (41.8%; 56/134) was intermediate. More cows in the eCG+ECP treatment (56.3%; 18/32) displayed estrus (*P*=0.002) compared to those cows in the eCG treatment (24.2%; 8/33) or the cows from the control group (16.1%; 5/31). In addition, the cows receiving the eCG+ECP treatment had a greater ovulation rate (90.6%; 29/32) than the control group (64.5%; 20/31), with the eCG group (84.9%; 28/33) remaining intermadiate. The eCG+ECP group also tended (*P*=0.08) to have a larger CL diameter 5 days after the TAI (16.3±0.43 mm) than the cows that received only GnRH (14.7±0.57 mm) and eCG (15.4±0.44 mm). In conclusion, exogenous estradiol supplementation at the time of P4 device removal increased the proportion of cows that displayed estrus and an ovulatory response after the synchronization protocol for TAI. The manipulation of the proestrous period by exogenous gonadotropin plus estradiol improved the occurrence of estrus, the ovarian response and the pregnancy outcomes of suckled beef cows subjected to an estradiol/P4-based synchronization protocol for TAI.

Keywords: eCG; Estradiol cypionate; Nelore; Beef cow; Fixed time artificial insemination; Reproduction

1. Introduction

Estradiol (E2)- and progesterone (P4)-based estrus synchronization protocols have been successfully used to control follicular and luteal dynamics in cattle and to synchronize ovulation when inseminating without estrus detection (Baruselli et al., 2004, Bó et al., 2002, Sá Filho et al., 2010b and Sales et al., 2012). Common among these protocols is the insertion of an intravaginal P4 device or a progestin ear implant, plus the administration of an E2 ester on Day 0 [estradiol benzoate (EB) or estradiol valerate to induce the emergence of a new follicular wave], prostaglandin (PG) $F_{2\alpha}$ on the day of device withdrawal (to ensure luteolysis) and an ovulation inducer used at implant removal, 24 h later or concurrent with the TAI to induce synchronized ovulation (Baruselli et al., 2004, Sá Filho et al., 2011a and Sales et al., 2012).The treatment of cattle with equine chorionic gonadotropin (eCG) has been suggested as an effective tool to increase follicular development and pregnancy per AI (P/AI) in suckled beef cows that exhibit a high prevalence of anestrus or a low body condition score (Baruselli et al., 2004, Sá Filho et al., 2010a, Sá Filho et al., 2010c and Sales et al., 2011). The efficiency of this hormone is related to its FSH- and LH-like activities (Murphy and Martinuk, 1991), which stimulate the continuation of follicular growth in cows with compromised gonadotropin secretion. Females treated with eCG at the time of the removal of the P4 source had increased final ovarian follicle growth, greater diameter of the dominant follicles at TAI, and greater ovulation rates (Sá Filho et al., 2010a, Sá Filho et al., 2010c and Sales et al., 2011). Proestrous E2 concentrations from either endogenous or exogenous sources may play an important role in sperm transport and viability and in the modulation of the uterus for the subsequent luteal phase (Hawk, 1983 and Pohler et al., 2012). This priming may be important for the induction of the endometrial progesterone receptors and reduces the synthesis of oxytocin receptors to avoid premature luteolysis and short cycles (Mann and Lamming, 2000 and Robinson et al., 2001). Previous studies demonstrated that ECP supplementation during the proestrous phase increased the proportion of cows that displayed estrus after the induction of luteolysis (Hillegass et al., 2008 and Sá Filho et al., 2011b) and increased endometrial thickness (Souza et al., 2007) compared to cows that received only GnRH as an ovulatory stimulus. Furthermore, some authors reported greater pregnancy outcomes in suckled beef cows (Sá Filho et al., 2011b) or lactating dairy cows (Cerri et al., 2004) receiving ECP as an ovulatory stimulus compared to cows receiving GnRH. However, other authors failed to find these positive effects of estradiol supplementation on fertility (Hillegass et al., 2008 and Sá Filho et al., 2011a). Thus, the objectives of the current study were to evaluate: the effects of eCG administration or its association with ECP (eCG+ECP) at the time of P4 device removal on the occurrence of estrus, on the ovarian response and on P/AI following an E2-plus-P4-based TAI synchronization protocol in suckled beef cows.

2. Materials and methods

2.1. Animals and general management

2.1.1. Cows and management

This experiment was conducted during the 2009/2010 and 2010/2011 spring-summer breeding seasons. A total of 386 (108 primiparous and 278 multiparous) suckled Nelore (*Bos indicus*) beef cows at 30–60 days postpartum from two commercial farms in Mato Grosso state, Brazil, were enrolled in this study. All of the cows were maintained on *Brachiaria brizantha* and *Brachiaria humidicola* pastures with free access to water and given mineral supplementation. At the initiation of the TAI protocol, information about parity (multiparous or primiparous) and a body condition score (BCS; range, 1=emaciated to 5=obese; with 0.5 scale) (Ayres et al., 2009) were collected from each cow. The lowest BCS value observed was 2.0 and the highest value was 4.0.

2.1.2. Reproductive management

After calving, the cows were allocated into breeding groups according to calving date. At 30–60 d post-partum, females were synchronized using an E2-plus-P4-based TAI protocol. Briefly, the cows received an intravaginal device containing 1.9 g of P4 (CIDR®, Pfizer Animal Health, São Paulo, Brazil) plus 2.0 mg of estradiol benzoate IM (Gonadiol®, MDS Animal Health, São Paulo, Brazil). Eight days later, the devices were removed, and the cows received 0.15 mg of D-cloprostenol IM (Prostaglandina Tortuga®—Tortuga Companhia Zootécnica Agrária, São Paulo, Brazil). The cows were artificially inseminated 48 h after removal of the P4 device and received 100 μg of gonadorelin (Profertil®—Tortuga Companhia Zootécnica Agrária, São Paulo, Brazil) immediately after TAI. Inseminations were performed by a single technician using frozen–thawed semen from two bulls of proven fertility, homogenously distributed among the experimental groups (Bull A,Control; *n*=64, eCG+ECP; *n*=62, eCG; *n*=67, Bull B, Control; *n*=65, eCG+ECP; *n*=61, eCG; *n*=67). The bulls used had been previously used in TAI programs and had satisfactory (∼50%) pregnancy results.

2.1.3. Treatments

*At the time of removal of the P4 device, the cows were randomly assigned to receive one of three treatments: the eCG group (*n*=134) cows received 300 IU of equine chorionic gonadotropin IM (eCG, Folligon®, Intervet-Shering Plow, Boxmeer, Netherlands); the eCG+ECP group (*n*=123) cows received 300 IU of eCG plus 1 mg of estradiol cypionate IM (ECP®, Pfizer Animal Health, Sao Paulo—Brazil); and the control group (*n*=129) cows received no additional treatment (Fig. 1).*

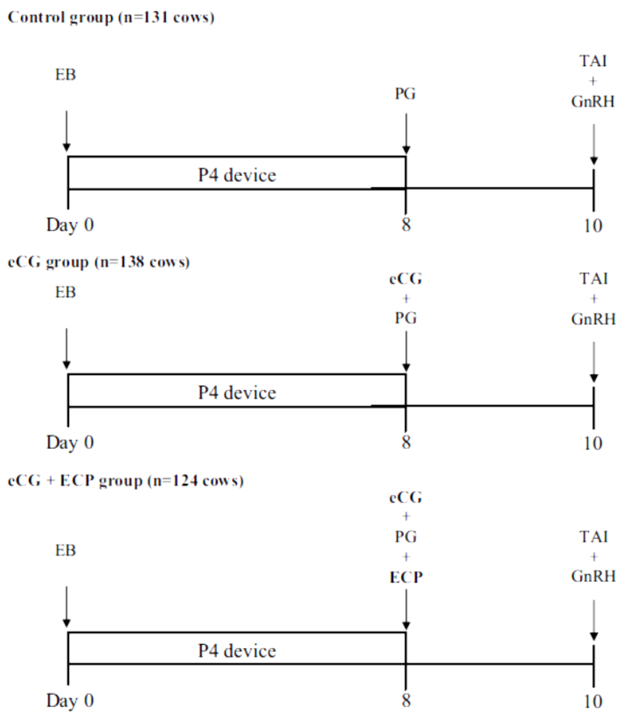

Fig. 1. Schematic diagram of the synchronization of ovulation protocol in suckled *Bos indicus* cows. EB = 2 mg of estradiol benzoate; eCG = 300 IU of equine chorionic gonadotropin; GnRH = 100 µg of gonadorelin; ECP = 1 mg of estradiol cypionate; PG = 0.25 mg of cloprostenol sodium; TAI = timed artificial insemination performed 48–52 h after removal of the progesterone (P4) device.

2.2. Detection of estrus

Estrus was determined using a self-adhesive heat detection patch (Estrotect®, IVP, Spring Valley, Wisconsin, USA). At the time of removal of the P4 device, a subgroup of multiparous cows (*n*=96) received the self-adhesive heat detection patch, placed between the hips and the tail head. Estrus was determined at TAI by the activation of each device.

2.3. Ultrasonographic examination

In the subset of 96 multiparous cows, both ovaries were scanned at the time of the removal of the P4 device and immediately before TAI to measure the diameter of the largest follicle (LF) and 5 d later to determine the presence and the diameter of the resulting corpus luteum (CL). A real-time ultrasonic scanner equipped with a 7.5 MHz linear transducer (Mindray 2200 VET-China) was used. Ovulation was defined as the appearance of a CL on the same ovary where the LF was detected at the time of the removal of the P4 device and the TAI.Pregnancy was diagnosed by transrectal ultrasonography examination 30 d after TAI. Detection of an embryonic vesicle with a viable embryo (presence of heartbeat) was used as an indicator of pregnancy. The pregnancy per AI (P/AI) was calculated as the proportion of cows pregnant 30 d after TAI divided by the total number of cows inseminated.

2.4. Statistical analysis

A binomial distribution was assumed for the categorical response variable. The occurrence of estrus and the P/AI were analyzed using the GLIMMIX procedure of SAS, with cows as a random effect. Variables initially included in the models were breeding group (two breeding groups); treatments (eCG, eCG+ECP or Control), parity (primiparous or multiparous), BCS at the first day of the synchronization protocol and interactions. For the final logistic regression model, variables were removed through backward elimination based on the Wald statistics criterion when $P>0.20$. The variables included in the final model for analysis of the P/AI were treatment, parity and BCS.In the subset of cows, the treatment group was the only explanatory variable included in the statistical model. The dependent variables [i.e., diameter of the LF at device removal, diameter of the LF at TAI, CL diameter 5 d after TAI] were analyzed using the GLIMMIX procedure from SAS. The response variables were tested according to their homogeneity and the normality of variances using Guide Data Analysis from SAS. The data are presented as mean±SE.

3. Results

Similar BCS at Day 0 ($P=0.97$) was observed among the treatment groups (Control Group=2.85±0.05; ECP+eCG Group=2.93±0.05 and eCG group=2.90±0.05). No difference was found among the treatments for the diameter of the LF at the time of the P4 device removal

Table 1
Overall occurrence and effects of different treatments on follicular and luteal development in an estradiol/progesterone-based synchronization protocol on suckled *Bos indicus* beef cows (subset of cows; $n=96$).

	Treatments [1]			
	Control	eCG	eCG + ECP	*P*-value
N	31	33	32	
BCS [2]	3.6 ± 0.07	3.6 ± 0.09	3.6 ± 0.07	0.72
Diameter of the largest follicle at progesterone device removal (mm)	8.0 ± 0.35	8.0 ± 0.39	7.8 ± 0.31	0.92
Diameter of the largest follicle at TAI (mm)[3]	10.7 ± 0.46	11.8 ± 0.46	11.2 ± 0.52	0.14
Ovulation rate (%)	64.5 (20/31)^{b}	84.9 (28/33)^{a,b}	90.6 (29/32)^{a}	0.03
Diameter of CL on day 15 (mm)[4]	14.7 ± 0.57	15.4 ± 0.44	16.3 ± 0.43	0.08

a, b means within a row with different superscripts are different ($P < 0.05$).
[1] Cows received an intravaginal device containing 1.9 g of progesterone (P4) and 2.0 mg of estradiol benzoate on the first day of the estrus/ovulation synchronization protocol (D0). The P4 device was removed eight days later, and cows received different hormonal stimulus during the proestrus (ECP = 1 mg of estradiol cypionate and eCG = 300 IU of equine chorionic gonadotropin). All cows received GnRH (100 µg of gonadorelin) at fixed time AI. Cows from eCG treatment receiving eCG at P4 removal. Cows from eCG + ECP received eCG plus ECP at P4 removal. Cows from Control treatment did not received any additional treatment.
[2] BCS = body condition score collected at D0.
[3] TAI = timed artificial insemination.
[4] CL = corpus luteum five days after the TAI.

($P=0.92$) or at TAI ($P=0.14$). The cows from the eCG+ECP group exhibited a greater ovulation rate ($P=0.03$). Moreover, the diameter of the formed CL five days after TAI tended to be larger ($P=0.08$) in the eCG+ECP group than in the control and eCG groups (Table 1).

The overall proportion of cows displaying estrus between the time of the P4 device removal and TAI moment was 32.3% (31/96). An increased BCS on the first day of the synchronization protocol was associated with increases in the occurrence of estrus [High BCS, 22.6% (35/155) and Low BCS, 7.4% (17/231); $P=0.006$]. Moreover, more cows that received the eCG+ECP supplementation displayed estrus than cows that did not receive this treatment (Fig. 2).

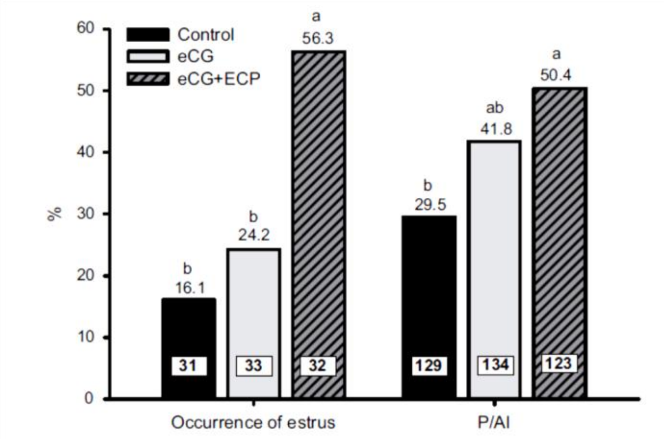

Fig. 2. Occurrence of estrus and pregnancy per AI (P/AI) in suckled *Bos indicus* beef cows according to the manipulation of the proestrous by exogenous gonadotropin (eCG) and estradiol (ECP). Bars without a common letter differ ($P < 0.05$). The numbers in boxes indicate the number of cows.

No interactions (P>0.05) were found among parity, BCS with the treatment on P/AI. The BCS also had no effect on P/AI. However, the primiparous cows [25.0% (27/108)] exhibited lower P/AI than the multiparous cows [46.4% (129/278); P=0.001]. Moreover, the cows treated with eCG+ECP exhibited greater P/AI (P=0.002) than the cows from the control group (Fig. 2), whereas pregnancy results from the eCG group were intermediate.

4. Discussion

To our knowledge, this is the first report regarding the effects of exogenous administration of eCG associated to ECP during the proestrous period on the occurrence of estrus, on ovarian responses and on pregnancy per AI in suckled *Bos indicus* beef cows subjected to TAI protocols. A greater rate of ovulation and P/AI was observed in the cows receiving eCG plus ECP supplementation at the onset of the proestrous period. Moreover, E2 supplementation during proestrous increased the number of cows in estrus at the time of insemination. However, no additional effect of E2 supplementation on P/AI was found when the cows received eCG at the time of the removal of the P4 device comparing treatments eCG and ECP. Regardless of the exogenous supplementation hormone used during proestrous, the multiparous cows exhibited greater P/AI than the primiparous cows

Timed AI has been routinely utilized during reproductive programs in *Bos indicus* cattle to improve the reproductive efficiency in beef herds (Baruselli et al., 2002, Sá Filho et al., 2012 and Sales et al., 2012). Several factors have been reported to influence reproductive success following TAI protocols in beef cows (Perry et al., 2005, Sá Filho et al., 2009 and Sá Filho et al., 2010b). Synchronized cows displaying estrus before TAI exhibited larger dominant follicles, greater luteal function on the subsequent estrus cycle and greater P/AI when compared to cows that did not display estrus (Perry et al., 2007, Sá Filho et al., 2010b, Sá Filho et al., 2011b and Fields et al., 2012). The present study demonstrated that *Bos indicus* cows that received exogenous ECP associated with eCG treatment in E2-plus-P4-based TAI protocols had a greater proportion of cows displaying estrus and ovulating after the synchronization protocol, allowing greater pregnancy outcomes following TAI than the cows receiving only GnRH as an ovulatory stimulus.

Estrus behavior is modulated by the direct action of E2 on the hypothalamus (Allrich, 1994), and E2 concentration is directly related to the diameter of the ovulatory follicle (Perry et al., 2007). The onset of estrus behavior and the surge release of LH are coincident with the estradiol peak (Glencross et al., 1981). The primary endogenous source of E2 responsible for inducing estrus behavior and the preovulatory surge of LH is the dominant follicle (Dobson, 1978). A positive relationship has been observed between the concentration of estradiol in circulation and the duration and/or intensity of estrus (Katz et al., 1980 and Lopez et al., 2004). Therefore, greater estrus occurrence in cows receiving eCG+ECP at the onset of proestrous, compared to the non-supplemented cows, could be related to the greater E2 concentration and greater number of cows reaching the threshold of estrus occurrence.

The gonadotropin eCG, has an affinity for the LH and FSH receptors on the granulosa cells (Murphy and Martinuk, 1991). Studies have shown that eCG increased the final follicular development and the follicular diameter at TAI and the ovulation rate in *Bos indicus* cows submitted to TAI (Sá Filho et al., 2010a and Sales et al., 2011). Moreover, cows with a larger dominant follicle at TAI had an increased probability of ovulation after the synchronization protocol (Sá Filho et al., 2010c). The eCG treatment has also increased the pregnancy outcomes when administered during the synchronization of ovulation in TAI programs in suckled *Bos indicus* beef cows. Treatment with eCG increased fertility in *Bos indicus* cows submitted to TAI, promoting improvement in the final follicular development, which raises the ovulation rate in *Bos indicus* cows (Sá Filho et al., 2010a and Sales et al., 2011). In addition, there is evidence that eCG increases estradiol production during proestrous (Soumano et al., 1998) and increases P4 in the subsequent estrous cycle (Sá Filho et al., 2010c).

The hormonal milieu of steroids during the proestrous, estrus and diestrous periods promotes a critical influence necessary to optimize uterine receptivity to conception. Previous exposure to P4 before estrus, following greater concentrations of E2 during the proestrous phase and, finally, a greater concentration of P4 during the estrous cycle subsequent to the AI, are important to the establishment of pregnancy (Moore, 1985 and Wilmut et al., 1986). Proestrous E2 concentrations from either endogenous or exogenous sources may play an important role in sperm transport and viability and in modulation of the uterus for the subsequent luteal phase (Hawk, 1983 and Pohler et al., 2012). This priming may be important for the induction of the endometrial progesterone receptors (Lamming and Mann, 1995 and Robinson et al., 2001) to avoid premature luteolysis and short cycles (Kieborz-Loos et al., 2003) and to increase the P/AI (Lopes et al., 2007). Therefore, the positive effect of the ECP treatment on the occurrence of estrus, on the ovulatory response and on pregnancy outcomes could be related to the greater E2 concentration during proestrous, determined by the exogenous ECP supplementation and by the greater steroidogenic capacity of the dominant follicle following the eCG treatment.

For establishment of pregnancy the conceptus needs to attach on endometrium. The attachment is outcome of a series of highly synchronized secretory activity, cellular and molecular modulations are necessary between maternal and fetal tissues (Bazer et al., 2009). Gene expression of bovine endometrium changes according to the phase of the estrous cycle and is closely controlled by circulating concentrations of E2 and P4 and the ratio between these steroid receptors in target tissues (Bazer et al., 2009 and Pohler et al., 2012). In suckled beef cows, high concentrations of E2 during the proestrus, induce changes in the uterus throughout the subsequent estrous cycle regarding mRNA expression for E2 and P4 receptors (ERS1 and RMP, respectively), oxytocin receptors, and various proteins such as cyclooxygenase-2, and beta subunit inhibin serpin-14 (Ulbrich et al., 2009). These changes are reduced when females have lower concentrations of E2 during the proestrous (Pohler et al., 2012 and Ulbrich et al., 2009). Furthermore, beef heifers exposed to a longer exposure of E2 during the proestrous, present alterations in the pattern of receptor expression and other proteins associated with the uterine receptivity pregnancy (Bridges et al., 2012). Therefore, the concentration of E2 during the proestrus either from ovarian follicle or exogenous administration could influence the uterine environment and fertility after AI (Allrich, 1994, Fields et al., 2012, Lyimo et al., 2000, Perry et al., 2007 and Sá Filho et al., 2011b).

In the present study, the multiparous cows had a greater P/AI than the primiparous cows. Primiparous beef cows maintained under pasture conditions are known to experience longer periods of postpartum anestrus (Wiltbank, 1970 and Randel, 1990), resulting in reduced P/AI in TAI programs compared with multiparous cows (Sá Filho et al., 2009). In addition, several studies showed a increase on reproductive performances on multiparous beef cows compared to the primiparous beef cows (Meikle, 2004 and Pinheiro et al., 2009). The energy requirements for growth in addition to the requirements for maintenance and lactation are believed to account for the greater negative effects on cyclicity and the reproductive performance of primiparous cows under pasture conditions (Randel, 1990 and Wiltbank, 1970).

In conclusion, exogenous supplementation with eCG plus ECP increased the occurrence of estrus, the ovarian response and the pregnancy outcomes of suckled beef cows submitted to TAI following an E2-plus-P4-based synchronization protocol.

Conflict of interest

None.

Acknowledgments

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