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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.

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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; 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; 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; 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; 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; 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; 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; 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; 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).

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.