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A comparison of two different esters of estradiol for the induction of ovulation in an estradiol plus progestin-based timed artificial insemination protocol for suckled *Bos indicus* beef cows

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ABSTRACT

The abilities of two different estradiol esters to induce ovulation in a timed AI (TAI) synchronization protocol in suckled *Bos indicus* cows were evaluated. In Experiment 1 (synchrony of ovulation), 31 cows were submitted to an estradiol/progestin-based synchronization protocol (Day 0) and randomly assigned to one of three treatments at the time of progestin removal on Day 8: 0.5 or 1.0 mg of estradiol cypionate (EC) at that time or 1.0 mg of estradiol benzoate (EB) 24 h later (Day 9). To determine the timing of ovulation, ultrasound examinations were performed every 12 h from ear implant removal to 96 h after the removal. Orthogonal comparisons were performed to determine the effects of estradiol ester and the effects of the dose of EC on reproductive parameters. Although neither the E2 ester ($P = 0.83$) nor the dose of EC ($P = 0.55$) affected the ovulation rate, the interval from progestin removal to ovulation was longer ($P = 0.04$) in EC-treated cows (1.0 mg EC = 71.1 ± 3.6 and 0.5 mg EC = 78.0 ± 3.5) than EB-treated cows (EB = 66.0 ± 2.3) was detected. Ovulation in 0.5-mg-EC-treated cows was less synchronous than that in 1.0-mg-EC-treated cows (distribution curves compared using kurtosis). In Experiment 2 (pregnancy per AI; P/AI), 660 cows at two different locations received the same synchronization protocol ($n = 361$ at Farm A and $n = 299$ at Farm B) and were treated with estradiol esters as in Experiment 1 [0.5 mg EC ($n = 220$) or 1.0 mg EC ($n = 219$) at the time of progestin removal or 1.0 mg EB ($n = 221$) 24 h later]. The cows were inseminated 54 to 56 h after progestin removal. As applied in the Experiment 1, orthogonal comparisons were performed to evaluate the effect of estradiol ester and the dose of EC on P/AI. Although the type of estradiol ester used did not affect the P/AI ($P = 0.57$; EB – 43.0% vs. EC – 44.6%), the P/AI was higher ($P = 0.03$) in cows treated with

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1.0 mg EC (55.7%) than in those treated with 0.5 mg EC (38.6%). In summary, the administration of 0.5 mg EC at the time of progestin removal altered the distribution of ovulation and resulted in a lower P/AI when compared with the use of 1.0 mg EC in suckled *B. indicus* cows. However, the P/AI following the administration of 1.0 mg EC at the time of progestin removal did not differ from that after the administration of 1.0 mg EB 24 h later.

1. Introduction

Synchronization of ovulation protocols for timed artificial insemination (TAI) has been efficiently applied for the reproductive management of suckled *Bos indicus* beef cattle (Baruselli et al., 2004; Sá Filho et al., 2013). Hormonal therapies enhance the use of artificial insemination, save time and hasten a herd's genetic gain and cow-calf operation profitability (Rodgers et al., 2012; Sá Filho et al., 2013).

The combination of progesterone/progestin and estradiol is the most common hormonal treatment used to synchronize ovulation for TAI in cattle in South America (Bó et al., 2002; Carvalho et al., 2008; Sá Filho et al., 2011). These protocols consist of the insertion of an exogenous progesterone/progestin source on the first day of the protocol plus an intramuscular treatment with an estradiol ester (estradiol benzoate or estradiol valerate) to induce the emergence of a new ovarian follicular wave (Sá Filho et al., 2011). Eight or nine days after treatment, the progesterone/progestin source is removed, and a dose of prostaglandin is administered to ensure luteolysis. In addition, treatment with equine chorionic gonadotropin (eCG) has been used to increase dominant follicular development and pregnancy rates in TAI programs, especially in postpartum anestrous cows, which frequently exhibit an insufficient pulsatile release of LH to support the final stages of ovarian follicular development and ovulation (Baruselli et al., 2004; Sá Filho et al., 2010a; Sá Filho et al., 2010b; Sales et al., 2011).

To achieve a satisfactory synchronization of ovulation, TAI synchronization protocols should have an ovulatory stimulus treatment. In GnRH-based protocols, GnRH has been applied to synchronize ovulation for TAI in beef cattle (Geary et al., 2001). Additionally, in the E2 plus P4-based TAI protocols, estradiol benzoate (EB) or estradiol cypionate (EC) have been successfully used for the induction of ovulation (Macmillan et al., 2003; Peres et al., 2009; Sá Filho et al., 2009a; Sá Filho et al., 2011).

EC is an estradiol ester with low solubility in water and slow release from the injection site, which prolongs the plasma concentrations of E2 for 98–170 h after administration of high doses (5–10 mg) (Burton et al., 1990; Vynckier et al., 1990). However, previous studies demonstrated that treatment with EC at the time of intravaginal P4 device removal is less effective for synchronizing the LH surge (Souza et al., 2009) and ovulation (Martins et al., 2005) than the use of GnRH or EB. Nonetheless, in spite of the increased variability in the timing of the LH peak and ovulation in suckled *B. indicus* beef cows, satisfactory pregnancy per AI (~50%) following the use of EC as an inducer of ovulation has been described (Sá Filho et al., 2009b; Sá Filho et al., 2011; Sales et al., 2012).

Thus, the objective of this experiment was to evaluate the effect of two different estradiol esters (EB vs. EC) and two different doses of EC (0.5 vs. 1.0 mg EC) as inducers of ovulation on the synchrony of ovulation and the P/AI of suckled *B. indicus* beef cows. The tested hypothesis stated that the estradiol esters and doses do not differentially affect the ovarian or fertility response.

2. Materials and methods

2.1. Experiment 1: estradiol ester on the synchrony of ovulation

2.1.1. Animals and handling

This experiment was conducted at a commercial beef farm located in Vera Cruz do Oeste, Parana state, Brazil. Cows were maintained on a *Brachiaria brizantha* pasture with free access to mineralized salt and water. Thirty-one anestrous suckled Nelore (*B. indicus*) cows that were 38 to 52 d postpartum and that scored 2.9 ± 0.7 on a 1 to 5 scale of body condition score (BCS; 1 = emaciated, 5 = obese) were used.

2.1.2. Experimental design

The cows were synchronized using a progestin plus estradiol-based synchronization protocol for TAI. At the beginning of the protocol (Day 0), cows received a norgestomet ear implant (Crestar®, MSD Animal Health, Sao Paulo, Brazil) that had been previously used for nine days, and 2 mg of estradiol benzoate (EB, Estrogin®, Farmavet, Sao Paulo, Brazil; i.m.) (Fig. 1). Eight days later, the ear implants were removed, and the cows received 0.150 mg of D-Cloprostenol ($PGF_{2\alpha}$, Preloban®, Intervet, Sao Paulo, Brazil) and 400 IU of eCG (Folligon®, MSD Animal Health, Sao Paulo, Brazil) intramuscularly. Moreover, the cows were randomly assigned to one of three treatment groups: 1.0 mg EB ($n = 10$) 24 h after ear implant removal or 0.5 mg ($n = 11$) or 1.0 mg of estradiol cypionate ($n = 10$) (EC; ECP®, Zoetis Animal Health, Sao Paulo, Brazil) intramuscularly at ear implant removal (Fig. 1).

2.2. Experiment 2: effect of estradiol esters on pregnancy per TAI

2.2.1. Animals and handling

Six hundred and sixty suckled Nelore (*B. indicus*) cows that were 30–60 d postpartum and scored 2.9 ± 0.7 on a 1 to 5 scale of body condition score (BCS; 1 = emaciated, 5 = obese) were enrolled in the study. The cows were from two different commercial beef farms that were located in Camapua, Mato Grosso state (Farm A; $n = 361$)

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