



Effects of melengestrol acetate supplementation after fixed-timed artificial insemination on pregnancy rates of *Bos indicus* beef cows



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ARTICLE INFO

Keywords:

Artificial insemination
Beef cows
Melengestrol acetate
Pregnancy rate

ABSTRACT

This experiment compared pregnancy rates in *Bos indicus* cows assigned to temporary calf weaning (TCW) or eCG administration during estrus synchronization, with or without melengestrol acetate (MGA) supplementation after artificial insemination (AI). A total of 3042 lactating, multiparous, non-pregnant Nelore cows were managed in 48 groups, and assigned to an estrus synchronization + fixed-time AI protocol (d –11 to 0, with AI on d 0). On d –11, groups were randomly assigned to receive 1 of 2 gonadotropic stimulus, which were either 48-h TCW from d –2 to AI (n = 9 groups, 604 cows) or 300 IU i.m. injection of eCG on d –2 (n = 39 groups, 2438 cows). On d 1, groups were assigned to receive, in a 2 × 3 factorial arrangement, 1 of 3 MGA treatments: 0.5 mg of MGA/cow from d 5–18 (M5to18; n = 16 groups, 1074 cows) or from d 13–18 (M13to18; n = 16 groups, 971 cows), or no MGA supplementation (CON; n = 16 groups, 997 cows). Estrus expression was evaluated by painting the tailhead of each cow on d –2, and evaluating the presence of tailhead paint at AI. Body condition score (BCS; 1–9 scale) was recorded at AI, and cows were classified as adequate (≥ 4.5) or inadequate (< 4.5) BCS. Pregnancy rates on d 30 and 80 were greater ($P \leq 0.05$) in M5to18 and M13to18 compared with CON cows (62.9%, 62.9%, and 55.3% on d 30, 58.1%, 59.2%, and 50.5% on d 80, respectively; SEM = 3.1), and similar ($P \geq 0.79$) between M5to18 and M13to18 cows. Pregnancy rates on d 30 and 80 were similar ($P \geq 0.17$) between cows assigned to eCG and TCW (58.1% and 62.6% on d 30, 54.3% and 57.6% on d 80, respectively; SEM = 2.7). The MGA supplementation × gonadotropic stimulus interaction was not significant ($P \geq 0.41$) whereas no interactions of main treatment effects with cow BCS and estrus expression were detected ($P \geq 0.21$) for pregnancy outcomes. Hence, supplementing *B. indicus* beef cows with MGA post-AI increased pregnancy rates compared with non-supplemented cows, and this outcome was independent of period and length of MGA supplementation, gonadotropic stimulus, cow BCS status, and estrus expression during the synchronization protocol.

1. Introduction

Early embryonic loss is a major reproductive challenge in cow-calf systems, and is defined as losses that occur from conception to d 27 of gestation (Humbolt, 2001). Hence, strategies to enhance early embryonic survival are warranted for optimal reproductive and overall efficiency of cow-calf operations. Progesterone (P4) is a key hormone involved in early pregnancy by regulating histotroph secretion and composition (Faulkner et al., 2013; Lonergan and Forde, 2014), modulating expression of endometrial genes associated with pregnancy establishment (Satterfield et al., 2006; Forde et al., 2009, 2011a), and influencing conceptus elongation (Lawson and Cahill, 1983; Mann et al., 2006; Clemente et al., 2009). Accordingly, circulating P4 concentrations after breeding have been positively associated with pregnancy rates in cattle (Robinson et al., 1989; Stronge et al., 2005).

Several strategies to manipulate post-breeding P4 concentrations were developed to enhance reproductive efficiency in beef females (Vasconcelos et al., 2014). As an example, our research group supplemented beef cows with melengestrol acetate (MGA; synthetic progestogen) from d 13–18 after fixed-time artificial insemination (AI), with the original intent of stimulating and synchronizing estrus resumption of cows that failed to conceive (Aono et al., 2008). Interestingly, cows supplemented with MGA had greater pregnancy rates to fixed-time AI compared with non-supplemented cows, suggesting that post-AI MGA supplementation may be an alternative to enhance pregnancy success in cattle. Nevertheless, Demetrio et al. (2007) reported that pregnancy rates in cattle are influenced by serum P4 concentrations on d 7 but not on d 14 after AI. Based on these outcomes, we hypothesized that beginning MGA supplementation prior to d 7 after fixed-time AI will further increase the MGA benefits on pregnancy success reported by

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Aono et al. (2008).

In South America and many parts of the world, estrus synchronization + fixed-time AI protocols often include additional gonadotropic stimulus such as eCG administration or temporary calf removal (TCW; Vasconcelos et al., 2014). Although the adoption of either or both gonadotropic stimuli have resulted in similar benefits on pregnancy rates to fixed-time AI (Sá Filho et al., 2009a), Carvalho et al. (2016) reported that circulating P4 concentrations 7 d after fixed-time AI were less in cows assigned to TCW compared with cows receiving eCG. Hence, we also hypothesized that MGA supplementation would be of further benefit if provided to beef cows assigned to an estrus synchronization + fixed-time AI protocol including TCW compared with eCG administration.

To test these hypotheses, this experiment was designed to compare pregnancy rates in *Bos indicus* cows assigned to TCW or eCG administration during an estrus synchronization protocol, and receiving or not MGA supplementation during different periods after fixed-time AI.

2. Materials and methods

This experiment was conducted from October 2013 to March 2014 in a commercial cow-calf ranch located in Porto Estrela, Mato Grosso, Brazil. All animals utilized were cared for in accordance with the practices outlined in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010). Cows were managed similarly independently of MGA supplementation or gonadotropic stimuli treatments, following the existing nutritional, reproductive, and sanitary procedures of the ranch.

2.1. Animals and treatments

A total of 3042 lactating, multiparous, non-pregnant Nelore cows [body condition score (BCS) = 4.51 ± 0.01 according to Wagner et al. (1988)], between 40 and 80 d postpartum, were assigned to the experiment. Hence, cows were enrolled in this experiment within the recommended voluntary waiting period for *B. indicus*-influenced cattle to optimize pregnancy rates to timed-AI (Cooke et al., 2009) and maintain a 365-d calving interval (Vasconcelos et al., 2014). Cows were managed in 48 groups, with an average of 65 cows in each group (range = 35–115 cows/group) according to the general management scheme of the operation, and this arrangement was maintained throughout the experimental period (d –41 to 80). Groups were maintained in individual *Brachiaria brizantha* pastures with ad libitum access to water and a commercial mineral supplement (Nutrideal Nutrição Animal, Cuiabá, MT, Brazil).

All groups were assigned to an estrus synchronization + fixed-time AI protocol (Meneghetti et al., 2009; d –11 to 0). More specifically, cows received a 2 mg injection (i.m.) of estradiol benzoate (Gonadiol; Zoetis, São Paulo, SP, Brazil) and an intravaginal P4 releasing device (CIDR, originally containing 1.9 g of P4; Zoetis) on d –11, a 12.5 mg injection (i.m.) of PGF_{2α} (Lutalyse; Zoetis) on d –4, CIDR removal in addition to 0.6 mg injection (i.m.) of estradiol cypionate (ECP; Zoetis) on d –2, and fixed-time AI on d 0. On d –11, however, groups were randomly assigned to receive: 1) 48-h TCW from d –2 (immediately after CIDR removal) to AI (n = 9 groups, 604 cows total), or 2) 300 IU injection (i.m.) of eCG (Novormon, Zoetis) administered concurrently with estradiol cypionate on d –2 (n = 39 groups, with 2438 cows total). The commercial operation favored the use of eCG instead of TCW due to labor availability, hence the difference in number of groups assigned to each gonadotropic stimuli.

After fixed-time AI, groups were randomly assigned to receive, in a 2 × 3 factorial arrangement: 1) 0.5 mg of MGA per cow from d 5–18 after fixed-time AI (M5to18; n = 16 groups, with 1074 cows total), 2) 0.5 mg of MGA per cow from d 13–18 after fixed-time AI (M13to18; n = 16 groups, with 971 cows total), or 3) no MGA supplementation after fixed-time AI (CON; n = 16 groups, with 997 cows total). Daily intake

of the commercial mineral supplement was evaluated within each group from d –41 to –11 of the experiment. The MGA was included in the mineral supplement to provide 2.28 mg of MGA Premix (Zoetis) per cow daily, based on the average intake (d –41 to –11) of the commercial mineral supplement of each group as previously described (Sá Filho et al., 2009b; Martins et al., 2015). The MGA premix + mineral mixture was offered to M5to18 and M13to18 groups daily at 0700 h. Cows were inseminated by 1 of 15 technicians with semen from 50 different sires. The proportion of cows inseminated by each technician, sire, and the combination of technician and sire was balanced within each treatment combination.

To facilitate cattle management and sampling procedures, groups (n = 6) started the supplement evaluation and estrus synchronization + fixed-time AI protocol 2–3 d apart, totaling 8 enrollment events. Treatments assignment to groups was balanced within and across enrollment events. Hence, all groups started and completed their experimental procedures within 30 d, but following the same experimental schedule (d –41 to 80).

2.2. Evaluations

On d –2 of the estrus synchronization protocol, immediately after CIDR removal, the tailhead of each cow was painted (Walmur Instrumentos Veterinários, Porto Alegre, RS, Brazil). At fixed-time AI on d 0, cows with missing or disrupted paint strip were considered to have expressed estrus activity between CIDR removal and AI (Bridges et al., 2008).

Cow BCS was evaluated at AI (Wagner et al., 1988), and cows were classified based on BCS as either adequate (BCS ≥ 4.5) or inadequate (BCS < 4.5) as described by Sá Filho et al. (2010). Pregnancy status was verified by detecting a viable conceptus or fetus, respectively, on d 30 and 80 via transrectal ultrasonography (Mindray-2200VET DP; Mindray Bio-Medical Electronics Co., Shenzhen, China). Pregnancy loss was considered in cows that were pregnant on d 30 but non-pregnant on d 80.

2.3. Statistical analysis

Group was considered the experimental unit, given that MGA treatments were administered to each group. Quantitative (BCS) and binary (estrus expression and pregnancy outcomes) data were analyzed, respectively, with the MIXED and GLIMMIX procedures of SAS (SAS Inst., Inc., Cary, NC, USA) and Satterthwaite approximation to determine the denominator degrees of freedom for the tests of fixed effects. The model statement used for analysis of BCS and estrus expression contained the fixed effects of MGA supplementation (CON, M5to18, or M13to18), gonadotropic stimulus (eCG or TCW), and the resultant interaction, with group(MGA supplementation × gonadotropic stimulus) and cow(group) as random variables. The model statement used for pregnancy data contained the fixed effects of MGA supplementation (CON, M5to18, or M13to18), gonadotropic stimulus (eCG or TCW), estrus expression from d –2 to 0, BCS (adequate or inadequate), and all resultant interactions. Pregnancy data were analyzed using group(MGA supplementation × gonadotropic stimulus), cow(group), sire, and AI technician as random variables. Results are reported as least square means and separated using least square differences. Significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and ≤ 0.10 .

3. Results

The MGA supplementation × gonadotropic stimulus interaction was not significant for any of the variables reported herein ($P \geq 0.41$); hence, results are reported according to main treatment effects.

Cow BCS at fixed-time AI was similar ($P \geq 0.56$) between eCG and TCW cows, as well as CON, M5to18, and M13to18 cows (Table 1).

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