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Impact of estradiol cypionate prior to TAI and progesterone supplementation at initial diestrus on ovarian and fertility responses in beef cows

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ABSTRACT

In cattle, early diestrus progesterone (P4) supplementation modulates endometrial function to exert pro- and anti-pregnancy establishment effects; specifically, P4 stimulates conceptus growth, but also induces early onset of luteolysis. This paradoxical effect is frequently related to the inconsistent fertility outcomes that result from P4 supplementation experiments. Aim was to investigate the impact of exogenous estradiol (E2) treatment at the end of timed fixed AI (TAI) on frequency of early luteolysis and pregnancy of beef cows supplemented with P4. Ovulations (D0 of study) of suckled multiparous (n = 643) and primiparous (n = 193) Nelore cows (*Bos indicus*) were synchronized with an E2/P4-based protocol for TAI and assigned to receive 1.0 mg of estradiol cypionate (CP) or nothing (NoCP) on D−2 and 150 mg of injectable long-acting P4 (iP4) or Placebo (NoiP4) on D4 on a 2 × 2 factorial arrangement. On D15, the iP4 supplementation increased ($P < 0.05$) the frequency of early luteolysis (NoCP + iP4: 26.0%; [13/50] vs. NoCP: 8.0% [4/50]), but CP prevented this effect (CP + iP4: 8.3% [4/48] and CP: 6.4% [3/47]). The CP improved pregnancy/AI (P/AI) of multiparous (CP: 51.6% [165/320] and NoCP: 35.0% [113/323]; $P < 0.001$) and primiparous cows (CP: 40.4% [40/99] and NoCP: 24.5% [23/94], $P < 0.05$), regardless of iP4 treatment. The iP4 supplementation affected P/AI of CP and NoCP treated cows according to follicle size at TAI. For the CP treated cows, the iP4 supplementation improved P/AI of sub-populations of cows with follicles <12.35 mm (42.0% [34/81] vs. 53.1% [34/64]), while for NoCP treated cows, the improvements occurred in subpopulations of cows with follicles ≥12.35 mm (46.1% [35/76] vs. 58.7% [37/63]). In conclusion, strategies associating E2 and P4 supplementation decrease the incidence of early onset of luteolysis and improve P/AI of suckled beef cows with smaller follicles.

1. Introduction

In addition to genetic gains, timed artificial insemination (TAI) programs in beef cattle improves reproductive efficiency because it overcomes challenges associated to long anestrous periods and estrus detection, that delay the time to first service post-partum. Despite of such benefits, cows induced to ovulate dominant follicles smaller than 11.0 mm at TAI present low pregnancy per AI (P/AI) [1–3]. Such poor results are mainly attributable to the insufficient uterine exposure to estradiol (E2) and progesterone (P4) at the pre-ovulatory (proestrus/estrus) and post-ovulatory (diestrus) periods [4,5], respectively. Indeed, aiming to achieve an adequate sequential uterine exposure to E2 and P4, different strategies to stimulate follicle growth in beef cattle submitted to TAI were proposed [6,7]. For example, consistent fertility gains in TAI protocol have been achieved by extending the proestrus period [8,9] or adding exogenous E2 [5,10,11]. An adequate uterine exposure to E2 affects positively the fertilization process [12], reduces the incidence of early luteolysis [13,14], and provides an uterine

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environment favourable to the establishment of pregnancy [15,16]. Regarding P4 concentrations during diestrus, the relationship between P4 concentrations and fertility are generally positive [3,6,17]. Progesterone is critical for successful maternal recognition and maintenance of pregnancy [18,19]. This steroid stimulates endometrial secretions [20] associated with conceptus growth [21–23] and interferon-τ production [24,25]. Therefore, manipulation of the endocrine environment by the addition of exogenous E2 and/or P4 can potentially improve fertility outcomes in TAI programs.

Forde et al. [23] demonstrated that insertion of an intravaginal P4-releasing device between days 3 and 7 after estrus advanced expression of endometrial genes related to energy provision and histotroph constituents. Such alterations seems to be associated with greater conceptuses growth during late diestrus, that was also observed by others using a similar approaches [22,23]. Commonly, the benefits of P4 supplementation on fertility are observed when administration occur at early diestrus (i.e., days 3–7 after ovulation) [26,27]. However, in many studies, P4 supplementation at this period causes a greater incidence (30–35%) of early luteolysis (i.e., by day 14), that may impair maternal recognition and maintenance of pregnancy [24,28]. This paradoxical effect is one of the main explanations for the inconsistent fertility results obtained in response to different strategies to supplement P4 at early diestrus. Indeed, studies reported no effect [29–31], a negative effect [29,32,33], or a positive effect [34–36] of P4 supplementation on P/AI of beef and dairy cattle.

One plausible explanation for the incidence of early luteolysis in P4-supplemented cows is that the early increase in circulating P4 is related to advances in the timing of nuclear P4 receptor (PGR) downregulation in the endometrial epithelia [37]. The downregulation of the PGR is closely followed by an increase in epithelial estrogen receptor alpha (ERα) and oxytocin receptors (OXTR), resulting in the pulsatile release of PGF2α, which induces luteolysis [38,39]. Furthermore, inconsistent fertility results can be a consequence of differences in the timing, duration, source and dose of P4 treatments [26,27].

Recently, a long-acting injectable P4 formulation (iP4) was evaluated by Pugliesi et al. [40] to supplement P4 at early diestrus in a manner that would be more practical than the use of the P4 intravaginal device or multiple P4 injections. By a single administration of 150 or 300 mg iP4 on days 2 or 3 post-ovulation, the authors observed that supplementation efficiently increased the circulating P4 concentrations for ≥3 days during early luteal phase in non-suckled Nelore cows, but also increased the frequency of early luteolysis (0% vs. 40.7%). Despite of this apparent negative aspect, in a subsequent fertility trial using an E2/P4-based TAI protocol, Pugliesi et al. [35] verified that 150 mg of iP4 administered 4 days post TAI (~3 days post-ovulation) increased the P/AI of anestrous suckled beef cows by 20%. Thus, our recent findings highlight the somewhat paradoxical effects of P4 supplementation when given in the early diestrus, as shown previously by others [24]; there are both beneficial effects regarding uterine receptivity and conceptus elongation and potentially negative effects associated with reduced CL lifespan. Thus, it is critical to find strategies to minimize the negative, while emphasizing the beneficial effects of P4 supplementation to maximize efficiency of this technology. Here, we propose to test the effects of supplementing E2 concurrent with the withdrawal of the P4-releasing device (i.e., at the beginning of proestrus) as a strategy to support the beneficial effects of exogenous P4 administration to improve fertility in beef cattle.

The exposure of the endometrium to the rising proestrus concentrations of E2 stimulates ERα and PGR [41,42], and this is indirectly responsible for the decrease of OXTR during metaestrus and early diestrus [13]. Thus, it is possible that E2 supplementation could cause a greater increase of ERα and PGR than the endogenous E2 pre-ovulatory surge. A greater initial rise in PGR could compensate the advanced disappearance of PGR caused by P4 supplementation. This represents a possible alternative to bypass the detrimental aspects of P4 supplementation. In fact, there is evidence for a positive role of proestrus E2 alone on uterine [15] and luteal [8,13,15] functions during diestrus, establishment of pregnancy [5,43,44] and fertility [5,10,11]. However, the associated response to supplemental E2 and P4 is unknown. Therefore, in this study, we aimed to evaluate the role of E2 supplementation at the P4 device withdrawal on the incidence of advanced luteolysis and fertility outcome after iP4 supplementation at early diestrus. Specifically, we tested the hypothesis that the E2 supplementation (1) decreased the incidence of short luteal lifespan and (2) improved the fertility response obtained by diestrus iP4 supplementation.

2. Materials and methods

2.1. Animals

This experiment was carried out during the summer and early fall on a commercial beef operation located in Mato Grosso do Sul, Brazil. Suckled multiparous (n = 643) and primiparous (n = 193) Nellore cows used in this study exhibited an average of days of postpartum of 52.1 and 50.8 (range, 31 to 85) and body condition scores of 3.48 and 3.00 (BCS, range, 2.25 to 5.00; 1 = emaciated to 5 = obese [45], using 0.25 increments), respectively. The cows were kept in grazing conditions (*Brachiaria brizantha*) with *ad libitum* access to water and minerals. The multiparous cows were split into 5 and 2 allotments from farms 1 (n = 524) and 2 (n = 119), respectively, while the primiparous cows were split into 4 allotments on farm 1.

All animal procedures were approved by the Ethics and Animal Handling Committee of the School of Veterinary Medicine and Animal Science of the University of Sao Paulo under the protocol number CEUA-6236220316.

2.2. Experimental design

Within each allotment, cows were subjected to an estrus synchronization protocol based on a single administration of 2 mg of estradiol benzoate (2.0 mL, i.m., Sincrodiol® Ourofino Saúde Animal) and insertion of an intravaginal P4-releasing device (1.0 g, Sincrogest® Ourofino Saúde Animal) followed by visual evaluation of BCS, on day −10. On this day, a transrectal ultrasonography exam was performed to exclude any cows with abnormalities of the reproductive tract and to establish the ovarian status. The ovarian status was determined based in three predefined categories, presence of CL, absence of CL and presence of follicles <8.0 mm and absence of CL and presence of follicles ≥8.0 mm.

On day −2, P4 devices were removed, and cows received 0.53 mg of sodium cloprostenol (2.0 mL, i.m., Sincrocio® Ourofino Saúde Animal) followed by administration of 300 IU of equine chorionic gonadotropin (1.5 mL, i.m., SincroeCG® Ourofino Saúde Animal). At the time of device removal, Estrotect™ patches (Western Point Inc., Apple Valley, MN) were applied halfway between the hip and tail head to determine the occurrence of mounting behavior associated with estrus. Concurrent with TAI (day 0), all cows received 10 μg of gonadotropin releasing hormone analogue (buserelin acetate, 2.5 mL, i.m., Sincroforte® Ourofino Saúde Animal).

Cows were blocked based on the BCS (low: L-BCS, 2.00 to 2.50, moderate: M-BCS, 2.75 to 3.50 or high: H-BCS, 3.75 to 5.00) to receive one of four treatments: injection of 1.0 mg of estradiol cypionate (CP, 1.0 mL, i.m, SincroCP® Ourofino Saúde Animal) or