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Role of progesterone concentrations during early follicular development in beef cattle: II. Ovulatory follicle growth and pregnancy rates

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A R T I C L E   I N F O

A B S T R A C T

Two experiments were conducted to investigate the role of relatively lesser and greater progesterone (P4) concentrations during early follicular development on ovulatory follicle growth and pregnancy rate in beef cattle. In Experiment 1, time of ovulation was synchronized with the 5 d CO-Synch + CIDR (Controlled Internal Drug Release) program in multiparous cows ($n = 241$). Six days after the 2nd GnRH injection of the pre-synchronization program (d 0), ablation of follicles ≥ 5 mm in the ovaries was performed and cows were assigned to receive either a previously used CIDR and 2x-25 mg PGF2α doses 8 h apart (LoP4), or a new CIDR (HiP4). On d 5, CIDR were removed from all cows, 2x-25 mg PGF2α were administered, and estrous detection tail paint was applied. Timed artificial insemination (TAI) was performed on d 8. On d 5, P4 concentrations were greater ($P < 0.01$) in the HiP4 (4.9 ± 0.13 ng/mL) than LoP4 (1.0 ± 0.06 ng/mL) treatment group. Conversely, d 5 estradiol (E2) concentrations and follicular diameter were greater ($P < 0.01$) in the LoP4 (5.0 ± 0.23 pg/mL and 8.9 ± 0.20 mm) than HiP4 (1.5 ± 0.12 pg/mL and 7.4 ± 0.15 mm) treatment group. Follicular diameter at TAI (12.0 ± 0.12 mm, Table 1) and TAI pregnancy rate did not differ ($P > 0.10$) between treatment groups. In Experiment 2, a new follicular wave was induced with estradiol benzoate on d -7, and cows ($n = 275$) were assigned on d 0 to receive 25 mg PGF2α and either have the CIDR replaced with a new CIDR (HiP4) or the used CIDR was left in place (LoP4).Furthermore, all cows received GnRH on d 0. The CIDRs were removed from all cows on d 5 and two doses of -25 mg PGF2α were administered. Estrous detection combined with AI 12 h later (Estrus-AI) was performed for 60 h after CIDR removal with TAI coupled with GnRH administration at 72 h if estrus was not detected. The concentrations of P4 on d 5 were greater ($P < 0.01$) in the HiP4 (2.8 ± 0.10 ng/ml) than LoP4 (1.7 ± 0.05 ng/mL) treatment group. For cows that were detected in estrus after PGF2α administration, estrous response (83.5%) and interval to estrus (55.0 ± 0.5 h) did not differ between treatment groups. Pregnancy rate (combined Estrus-AI and TAI) that resulted from breeding at the time of the synchronized time of estrus was similar between treatment groups

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(HiP4: 77.1%; LoP4: 82.3%). In conclusion, differences in P4 concentrations during early follicular development do not effect pregnancy rate in beef cows when the cows are inseminated at the time of a synchronized estrus if the cows have similar intervals of proestrus.

1. Introduction

Reproductive efficiency is a major limiting factor in cattle production, and estrous synchronization programs have been used as a beneficial technique to enhance estrous detection and fertility. Luteinizing hormone (LH) pulse frequency is regulated by progesterone (P4) concentrations throughout the estrous cycle (Rahe et al., 1980; Schallenberger and Prokopp, 1985), and increasing evidence suggests that greater LH stimulation during follicular development positively effects fertility in beef cattle. Bridges et al. (2010) reported that prolonging the follicular phase when there is a relatively lesser compared with greater P4 concentration, which would increase LH stimulation, resulted in greater preovulatory estradiol (E2) concentration and increased pregnancy rate to AI. Similarly, early luteolysis in progesterone-based estrous synchronization protocols increased ovulatory follicle diameter and fertility (Carvalho et al., 2008; Dias et al., 2009; Meneghetti et al., 2009).

In *Bos indicus* influenced cattle, relatively lesser P4 concentrations during follicular growth was induced with the use of a previously used Controlled Internal Drug Release (CIDR; Dias et al., 2009; Claro et al., 2010). Heifers that received the previously used CIDR during the early follicular growth have ovulations from a larger diameter follicle and had a greater pregnancy rate to AI (Dias et al., 2009; Claro et al., 2010). In contrast, cows with three waves of ovarian follicular development during an estrous cycle have lesser P4 concentrations during late follicular growth. Some evidence suggests that cows with three waves of follicular development may be more fertile; potentially, increased LH stimulation may be one of the mechanisms involved with fertility enhancement (Bleach et al., 2004; Townson et al., 2002). The objective of the present study, therefore, was to investigate the effect of relatively lesser P4 concentrations during early follicular development on pregnancy rates. It was hypothesized that relatively lesser circulating concentrations of P4 during early follicular development would enhance pregnancy rates to AI in beef cattle.
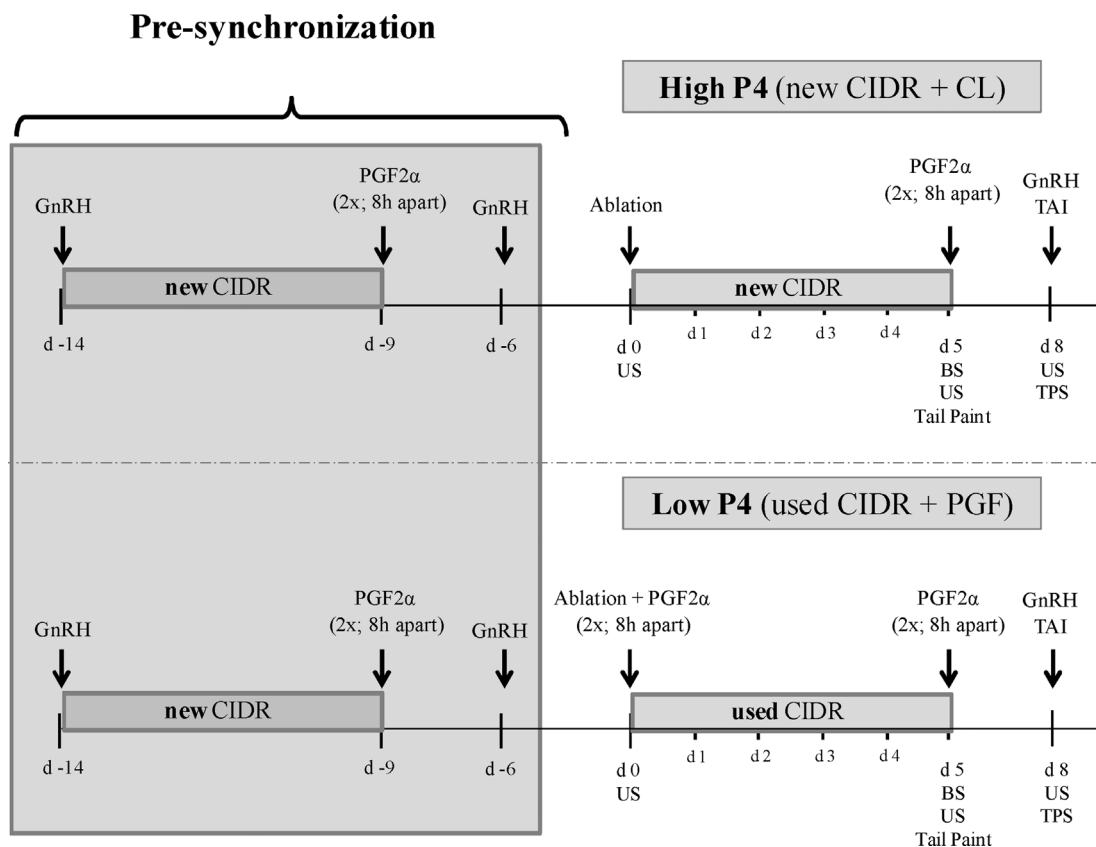

Fig. 1. Diagram of treatments performed in Experiment 1.
Treatments = High Progesterone (HiP4) and Low Progesterone (LoP4); BS = Blood Sample US = Ultrasonography. CIDR (controlled internal drug release; new = never used; used = previously used for 5 d); PGF2α = Prostaglandin F2α; TAI = Timed-AI; TPS = Tail Paint Score.

2. Materials and methods

2.1. Animals and treatments

All procedures involving animals used in this research were approved by The Ohio State University Agricultural Animal Care and Use Committee (Experiment 1) or the Fort Keogh Livestock and Range Research Laboratory Animal Care and Use Committee (Experiment 2).

2.1.1. Experiment 1

Multiparous postpartum (64.9 ± 14.0 d postpartum) Angus and Angus crossbred cows (n = 241) ranging in age from 3 to 15 years of age from three locations (Belle Valley, BV, n = 98; Columbus, COL, n = 68; and Jackson, JK, n = 75) were used. Ovulation was pre-estrous synchronized with the 5 d CO-Synch + CIDR program (Fig. 1; CIDR [Zoetis, New York, NY, USA] + GnRH [Cystorelin®, 100 µg, i.m., Merial, Inselin, NJ, USA] on d -14, followed 5 d later with CIDR removal and two injections of PGF2α [Lutalyse®, 25 mg each, i.m., Zoetis; d – 9] and GnRH [100 µg, i.m.] 3 d after PGF2α; d - 6). Cows with a corpus luteum (CL) present (d 0 of the experiment) were stratified by age and days post-partum within location and assigned to receive a high (HiP4, n = 118) or low (LoP4, n = 123) P4 treatment group. Transvaginal ultrasonic-guided aspiration (ablation) was also performed on d 0 to remove the dominant follicle of the first wave and other follicles with a diameter ≥ 5 mm. This was expected to synchronize the time of follicle wave emergence 1 to 2 d later (Mussard et al., 2007; Bridges et al., 2010). Cows in the HiP4 treatment group received a new CIDR (d 0) to supplement P4 being produced by the CL. Cows in the LoP4 treatment group received a CIDR (d 0) that had been used previously for 5 d and two injections of PGF2α (25 mg, i.m.) 8 h apart to induce luteolysis of the CL reducing endogenous P4 secretion. On d 5, the CIDR were removed and all cows were administered two doses of PGF2α (25 mg, i.m. each) to maintain a similar synchronization protocol between groups. In addition, tail paint (Tell Tail; Fil Agritech, Little York, NY) was applied to all cows for estrous detection. On d 8, administration of 100 µg of GnRH and timed-AI (TAI) were performed (72 h after CIDR removal; Fig. 1) and tail paint score (TPS; 1 = paint completely removed; 2 = paint partially removed; 3 = paint largely undisturbed and no evidence of mounting) was recorded.

Transrectal ultrasonography (US; Fig. 1) was performed using a 7.5 MHz linear array transducer (Aloka 500 V; Aloka, Wallingford, CT) to characterize ovarian structures in all cows on d 0, 5 and 8. Pregnancy diagnosis was conducted using a 5.0 MHz transrectal linear array transducer (Aloka 500 V) 30–40 d after TAI in all locations. Blood samples were collected (BS; Fig. 1) via jugular venipuncture into 10 ml EDTA vacutainer tubes (BD Vacutainer®, Franklin Lakes, NJ) on d 5 (immediately before/after CIDR removal) to assess circulating P4 and E2 concentrations.

2.1.2. Experiment 2

Multiparous postpartum (56.5 ± 13.3 d postpartum) Angus crossbred cows (n = 275) ranging from 3 to 14 years of age from a single location were used. All cows received estradiol benzoate (EB; 1 mg/500 kg BW, i.m. [beta-Estradiol 3-Benzoate (Sigma-Aldrich
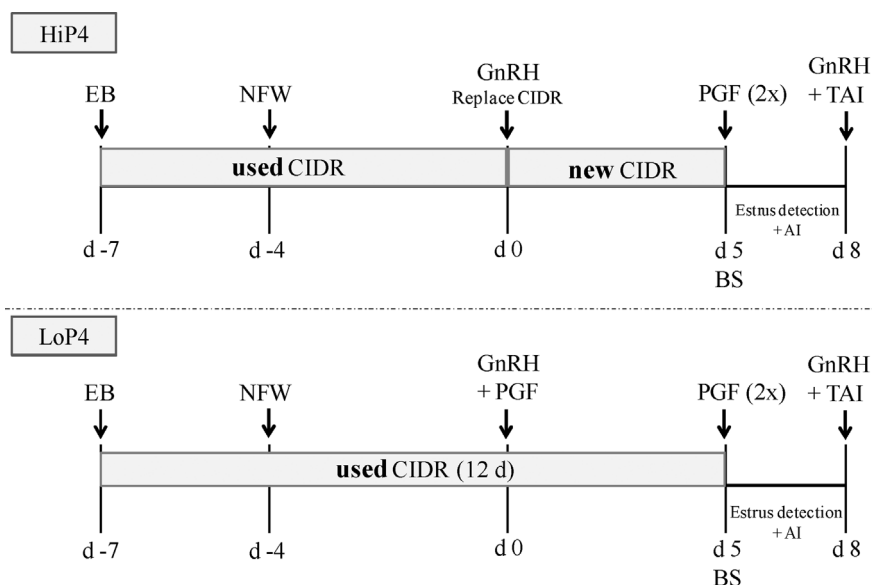

Fig. 2. Diagram of treatments performed in Experiment 2.
Treatments = High Progesterone (HiP4) and Low Progesterone (LoP4); BS = Blood Sample; EB = Estradiol Benzoate; NFW = New Follicular Wave Emergence; CIDR = intravaginal progesterone device (new = never used; used = previously used for 5 d); PGF2α = prostaglandin F2α; TAI = Timed-AI.

Co. LLC, Milwaukee, WI, USA) in 10% Benzyl Alcohol (Sigma-Aldrich Co. LLC) and 90% sesame oil]) and a previously used (for 7 d) CIDR on d -7, to induce emergence of a new wave of ovarian follicular development approximately 3 d later (d -4; Burke et al., 2003). On d 0, all cows received 100 µg of GnRH (i.m., Factrel®, Zoetis, New York, NY, USA) and were randomly assigned to a HiP4 or LoP4 treatment (Fig. 2). In the HiP4 treatment (*n* = 136), the CIDR was replaced with a new CIDR on d 0. In the LoP4 treatment (*n* = 139), the CIDR was not replaced and 25 mg PGF2α was administered on d 0. On d 5, all cows received 2 PGF2α injections (25 mg, i.m. each; administered at the same time), CIDR were removed, and an Estrotect estrous detection patch (Rockway Inc., Spring Valley, WI; positive estrus = $\geq$ 50% of bright color of patch is exposed) was applied on their tailhead. Estrous detection was performed at least twice daily for 60 h with AI approximately 12 h after estrous detection (Estrus-AI). Cows not detected in estrus within this period received 100 µg GnRH at 60 h and were inseminated by TAI at 72 h (Fig. 2). Blood samples were collected (Fig. 2) via tail veni-puncture into 10 ml vacutainer tubes (Fisher Scientific, Pittsburg, PA) on d 5 to assess circulating P4 concentrations. Pregnancy diagnosis was performed by ultrasonography approximately 30 to 40 d after inseminations.

2.2. Blood samples handling and radioimmunoassay

In Experiment 1, blood samples were collected and stored on ice during transport to the laboratory (less than 4 h from collection). Samples were then centrifuged at 1500 x *g* at 4 °C for 20 min, plasma was removed and stored in cryovials at – 20 °C until analyses. In Experiment 2, samples were placed in ice immediately after collection, incubated for 24 h at 4 °C followed by centrifugation at 1200 × *g* for 25 min. Serum was collected and stored at -20 °C until analyses.

Plasma concentrations of E2 were analyzed using a double antibody assay previously validated in the laboratory where the assay procedures for the present study was conducted (Abreu et al., 2018, submitted). Average intra-assay CV was 6.2%, inter-assay CVs (three assays) for low (2.3 pg/mL), medium, (7.6 pg/mL), and high (16.3 pg/mL) pools were 27.0%, 15.4%, and 13.0%, respectively. The sensitivity of the assay was 0.97 pg/mL.

Circulating concentrations of P4 were determined using a Coat-a-Count® RIA kit (Siemens, Los Angeles, CA) as previously de-scribed (Bellows et al., 1991; Burke et al., 2003). Average intra-assay CV was 6.1%, inter-assay CV (2 assays) for pooled plasma samples containing 0.29, 1.30, and 3.50 ng/ml of P4 were 6.7%, 9.2% and 9.2%, respectively, and the average sensitivity of the assay was 0.072 ng/mL.

2.3. Used CIDR sanitation

After removal from the cows, CIDR were immediately rinsed in water to remove debris followed by a final rinse in 2% chlor-hexidine solution (Novalsan®, Zoetis, New York, NY, USA). The devices were then allowed to air dry, and later packaged and stored at room temperature for future use. At time of insertion, CIDR were first submerged either in a 0.1% solution of povidone iodine (Betadine®, Purdue Frederick, Norwalk, CT, USA) in Experiment 1 or again in 2% chlorhexidine solution in Experiment 2, followed by the manufacturer recommended protocol for insertion.

2.4. Data and statistical analyses

2.4.1. Experiment 1

Data from cows identified without a CL at ablation (*n* = 11), with a CL on d 5 of the LoP4 treatment (n = 1), or without a CL on d 5 when the HiP4 treatment was imposed (*n* = 1) were excluded from the analyses. Final analyses were performed on a total of 228 cows (HiP4, *n* = 113; LoP4, *n* = 115). Data were analyzed using a model that included treatment, location, and the interaction of these two variables. Concentrations of P4 and E2, follicle diameters on d 5 and 8, and follicular growth rate (difference between follicle diameter at TAI (d8) and at PGF2α (d 5) divided by the number of d (3) between these two measurements) were compared using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC, USA, version 9.3). Pregnancy rates were compared with the GLIMMIX procedure of SAS. The distribution of TPS between treatments was compared using the FREQUENCY procedure of SAS. Data are expressed as the mean ± SEM.

2.4.2. Experiment 2

Data for all cows that had lost a CIDR by d 0 (*n* = 10) or d 5 (*n* = 4) were removed from further analyses, resulting in 261 cows included in all analyses (HiP4, *n* = 131; LoP4, *n* = 130). Data were analyzed using a model that included treatment only. Estrous response (proportion of cows detected in estrus during the 60 h period after CIDR removal) and pregnancy rates (estrus-AI, TAI, and overall) were analyzed using the GLIMMIX procedure of SAS. The MIXED procedure of SAS was used to analyze interval to estrus (from PGF2α administration; proestrus interval) and serum concentrations of P4 on d 5. Distribution of estrus was compared using the FREQUENCY procedure of SAS. Serum concentrations of P4 and either estrus response or length of proestrus were analyzed with the CORR procedure of SAS. Data are expressed as the mean ± SEM.

3. Results

3.1. Experiment 1

At all of the physical locations, P4 concentrations at CIDR withdrawal (d 5) were greater (*P* < 0.01) in the HiP4 than LoP4

treatment group (Table 1). A treatment by location interaction ($P < 0.01$) was detected and this was predominantly due to elevated P4 concentrations at one location (BV: 4.9 ± 0.2 and 0.7 ± 0.1 ng/mL; COL: 5.8 ± 0.2 and 1.4 ± 0.2 ng/mL; JK: 4.1 ± 0.2 and 0.9 ± 0.1 ng/mL). Concentrations of E2 on d 5 were greater ($P < 0.01$) in the LoP4 than HiP4 (Table 1) treatment group. Greater concentrations of E2 in the LoP4 treatment group (BV: 4.6 ± 0.3; COL: 6.1 ± 0.5; JK: 4.6 ± 0.3) at one location resulted in a treatment by location interaction ($P < 0.05$). Diameter of the ovulatory follicle was greater ($P < 0.05$) in the LoP4 than HiP4 treatment group on d 5, but did not differ between treatment groups on d 8 when TAI was performed (Table 1). Accordingly, follicle growth from d 5 to 8 was greater ($P < 0.05$) in the HiP4 than in the LoP4 (Table 1) treatment group. Distribution of TPS (Fig. 3) differed ($P < 0.05$) between treatments with a majority of cows in the HiP4 treatment group having TPS 3 (56%) and a majority in the LoP4 group with TPS 1 (54%). Cows with TPS 1 or 2 had a greater ($P < 0.05$) pregnancy rate (73.6 and 73.9%, respectively) than cows with a TPS 3 (57.1%). Pregnancy rate, however, did not differ between the HiP4 and LoP4 treatment groups (Table 1). Ovulation before the time of TAI (determined by disappearance of the largest follicle between d 5 and 8) occurred in 20% of the cows in the LoP4 and no cows in the HiP4 treatment group (Table 1). Pregnancy rate to TAI of cows that had ovulations was 56.5% (13/23 cows). Overall, pregnancy rate to TAI did not differ between the LoP4 and HiP4 treatment groups; regardless of whether the data for the cows that had ovulations early were included in this comparison (Table 1).

3.2. Experiment 2

Serum P4 concentrations at CIDR withdrawal (d 5) were greater ($P < 0.01$) in the HiP4 than LoP4 (Table 2) treatment group. Estrous response within the 60 h after PGF2α administration (Table 2), interval from PGF2α to estrus (Table 2), and estrous distribution (Fig. 4) did not differ between the HiP4 and LoP4 treatment groups. Pregnancy rate due to insemination at the synchronized time of estrus (combination of Estrus-AI and TAI) was similar between HiP4 and LoP4 treatment groups ($P > 0.10$; Table 2). Across treatments, pregnancy rates were greater ($P < 0.01$) with Estrus-AI (82.9%) than TAI (63.6%). Regardless of treatment, concentrations of P4 on d 5 were negatively correlated ($P < 0.01$; r = - 0.19) with estrous response and positively correlated ($P < 0.01$; r = 0.2) with interval to estrus.

4. Discussion

The effect of altering blood P4 concentrations during follicular development on pregnancy to AI in multiparous beef cows was evaluated in these experiments. In both experiments, P4 concentrations at CIDR withdrawal were, as designed, greater in the HiP4 than the LoP4 treatment group. The administration of PGF2α on d 0 to induce luteolysis in the LoP4 treatment group prior to emergence of the new wave of follicular development, in conjunction with either insertion (Experiment 1) or maintenance (Experiment 2) of a previously used CIDR, resulted in relatively lesser P4 concentrations at the time of emergence and early development of the ovulatory follicle. It was determined that the relatively lesser circulating P4 concentrations during follicular development in the present study resulted in greater E2 concentrations and increased follicle diameter at CIDR withdrawal on d 5 (Experiment 1), but did not influence pregnancy rate when inseminations occurred at the synchronized estrus in either experiment. A treatment by location interaction was observed for P4 and E2 concentrations in Experiment 1. Mean P4 concentration was elevated in the samples from the COL location compared to BV and JK locations. Moreover, mean concentration of E2 was greater for the LoP4 group at the COL location. The slight variation in P4 and E2 values did not appear to negatively affect the outcome and the exact cause for these differences is unclear.

The different concentrations of circulating P4 were experimentally-induced with the aim to induce different LH stimulation of the ovulatory follicle throughout its development. Frequency of episodic release of LH pulses varies throughout the estrous cycle and is mainly regulated by circulating concentrations of P4 (Rahe et al., 1980; Schallenberger and Prokopp, 1985), with the number of LH

Table 1
Effect of high progesterone (HiP4) or low progesterone (LoP4) treatment during follicular growth[a] on response variables (Mean ± SE) in Experiment 1.

	HiP4 (n = 113)	LoP4 (n = 115)	P value
Progesterone concentration on d 5, ng/ml	4.9 ± 0.13	1.0 ± 0.06	< 0.01
Estradiol concentration on d 5, pg/ml	1.5 ± 0.12	5.0 ± 0.23	< 0.01
Follicle diameter on d 5, mm	7.4 ± 0.15	8.9 ± 0.20	< 0.05
Follicle diameter at TAI[b] (d 8), mm	11.8 ± 0.15	12.1 ± 0.19	> 0.10
Follicle growth rate (d 5 to d 8), mm/d	1.5 ± 0.06	1.3 ± 0.06	< 0.05
Ovulation rate before TAI (d 8), % (n)	0 (0/113)	20 (23/115)	> 0.10
Pregnancy rate of cows with ovulations[c], % (n)	–	56.5 (13/23)	na
Pregnancy rate without cows without ovulations, % (n)	67.3 (76/113)	70.7 (65/92)	> 0.10
TAI pregnancy rate with all cows, % (n)	67.3 (76/113)	67.8 (78/115)	> 0.10

[a] Follicular growth rate was calculated by the difference between follicle diameter at TAI (d8) and at PGF2α (d 5) divided by the number of d (3) between these two measurements).

[b] TAI = timed-AI.

[c] Cows with ovulations refer to the pregnancy rate of the 23 cows in the LoP4 treatment that had ovulations before TAI.
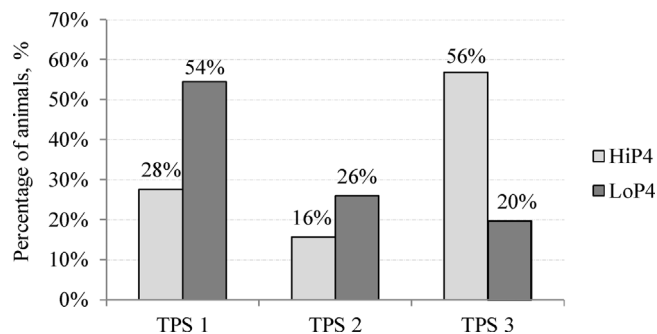
Fig. 3. Distribution of Tail Paint Score (TPS) on d 8 (Timed-AI) in Experiment 1.
TPS index = 1 = paint completely removed; 2 = paint partially removed; 3 = paint largely undisturbed and no evidence of mounting.
Distribution of TPS differed ($P < 0.05$) between treatments.

Table 2
Effect of high progesterone (HiP4) or low progesterone (LoP4) treatment during follicular growth on response variables (Mean ± SE) in Experiment 2.

	HiP4 (n = 131)	LoP4 (n = 130)	P value
Progesterone concentration on d 5, ng/ml	2.8 ± 0.10	1.7 ± 0.05	< 0.01
Estrous response within 60 h after PGF2α, %	81.7	85.4	> 0.10
Interval from PGF2α to estrus, h	56.1 ± 0.7	54.0 ± 0.7	> 0.10
Estrous synchronized pregnancy rate, % (n)[a]	77.1 (101/131)	82.3 (107/130)	> 0.10

n = number.
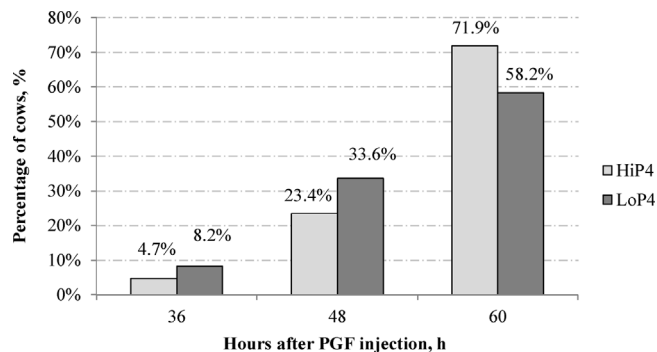[a] Combined Estrus-AI and TAI.

Fig. 4. Distribution of estrus within 60 h after PGF2α administration on d 5 in Experiment 2 between HiP4 and LoP4 groups; A cow was considered in estrus when ≥ 50% of the bright color of patch was exposed.
Treatments = High Progesterone (HiP4) and Low Progesterone (LoP4).

pulses being relatively less during the luteal phase (6–8 pulses/24 h; Rahe et al., 1980) and greater after luteal regression (20–30 pulses/24 h; Rahe et al., 1980; Abreu et al., 2018). In Experiment 1, follicle diameter and E2 concentrations at cessation of P4 treatment were greater in the LoP4 than HiP4 treatment group and this result was similar to those in previous reports (Pfeifer et al., 2009; Cerri et al., 2011). It is presumed that this acceleration in growth of the dominant follicle and E2 secretion was the result of increased LH pulse frequency. Indeed, 20% of the cows in the LoP4 group had already had ovulations at TAI, 72 h after CIDR removal. It is speculated that an increase in LH pulse frequency in the LoP4 treatment group occurred in response to the sub-luteal P4 concentrations during follicular development (Ireland and Roche, 1982; Roberson et al., 1989; Abreu et al., 2018) and enhanced growth and E2 production of the dominant follicle (Stegner et al., 2004). The study reported in a companion manuscript evaluated the LH secretion pattern using a similar animal model (Abreu et al., 2018). As a result, decreased P4 concentrations during early follicular development resulted in a greater LH pulse frequency and increased E2 concentrations in comparison to cows that were in the HiP4 treatment group (Abreu et al., 2018).

Estrous response and interval to estrus did not differ between treatment groups in Experiment 2. The relative difference in P4 concentrations between the LoP4 and HiP4 treatment groups was much greater in Experiment 1 than 2 and may explain the apparent inconsistency for time of estrus between the two experiments. With the animal model used in Experiment 2, it is possible that some cows in the HiP4 treatment group did not have a functional CL at the time of insertion of the new CIDR, however, with

ultrasonography not being performed it cannot be ascertained whether there was a functional CL present. Hence, the only source of P4 in these cows was the new CIDR. Conversely, all cows in the HiP4 treatment group in Experiment 1 had a CL visible on ultrasonic assessment at the time of insertion of the new CIDR. Previous studies have reported that small doses of progesterone (< 3 ng/mL) increased LH pulse frequency compared with mid-luteal concentrations of progesterone (Roberson et al., 1989). Thus, in Experiment 2, the small difference in P4 concentrations between cows in the HiP4 and LoP4 groups could have resulted in increased LH stimulation of the growing follicle; leading to follicles that grew more rapidly and with greater steroidogenic capacity than others in the same treatment with a CL. This occurrence could also contribute to the lack of difference in pregnancy rates observed in Experiment 2.

Increased follicle diameter at AI in response to relatively lesser P4 concentrations during follicular development has been reported in lactating dairy cows (Cerri et al., 2011), beef heifers (Carvalho et al., 2008; Dias et al., 2009; Pfeifer et al., 2009; Mantovani et al., 2010; Martins et al., 2014), and mature cows (Pfeifer et al., 2009). Although follicle diameter at CIDR withdrawal on d 5 was greater in the LoP4 than HiP4 treatment groups in Experiment 1, diameter of the ovulatory follicle did not differ between treatments at TAI 72 h later. It appears that the 72 h period when concentrations of P4 were low from d 5 to 8 allowed follicles developing in the existing endocrine milieu to attain an adequate developmental competence by the time of ovulation. Cessation of P4 treatment resulted in an increased (compensatory) follicular growth rate during proestrus in the HiP4 treatment group and this may have resulted in a compensation for any functional differences in follicular development created by differences in P4 during earlier development, as ascertained in a previous study (Dadarwal et al., 2013).

When there are relatively lesser P4 concentrations during early follicular development, there may be a benefit in fertility that is supported by previous data from our laboratory (Abreu et al., 2013) and research with Nelore (*Bos indicus*) cattle in Brazil (Martins et al., 2014). In our previous study, oocytes were recovered from follicles by ovum pick-up after they developed in a high or low P4 concentration milieu. There was a greater number of follicles that developed when there was relatively low circulating P4 concentrations and this resulted in a greater number of follicles being aspirated, increased oocyte recovery rate and enhanced oocyte quality compared with follicles developing when there was a relatively greater P4 concentration milieu (Abreu et al., 2013). Moreover, *in vitro* fertilization of oocytes collected from cows with decreased P4 concentrations yielded blastocysts with greater numbers of cells than oocytes from cows in the high P4 treatment group (Kruse et al., 2013). In studies conducted with *Bos indicus* beef cattle, there has been an inverse relationship of P4 concentrations during estrous synchronization and pregnancy rate to TAI (Dias et al., 2009; Peres et al., 2009; Claro et al., 2010). In the current experiment, however, pregnancy rates after AI with fixed-time or at estrus were not affected by the different circulating P4 concentrations. This finding is consistent with those studies with *Bos Taurus* cattle where it was observed that there was no differences (Pfeifer et al., 2009; Hill et al., 2014; Mercadante et al., 2015) or only marginal differences (Sparks et al., 2012; Mellieon et al., 2012) in pregnancy rates when there were relatively greater or lesser P4 concentrations. It is speculated that in *Bos taurus* cattle the sensitivity to relatively greater P4 concentrations is less than in Nelore females, and sufficient gonadotropin stimulation of follicular development occurs when there is a wider range of P4 circulating concentrations. Indeed, Cerri et al. (2011) reported no difference in the proportion of good quality embryos and oocytes, proportion of degenerated embryos and unfertilized oocytes recovered 6 d after AI in lactating dairy cows with different serum P4 concentrations during follicular development. The reasons for the different response between *Bos indicus* and *Bos taurus* beef cattle regarding P4 concentrations and fertility remain unclear and require further investigation.

In summary, when there were relatively lesser P4 concentrations during early follicular development in the present study there were greater E2 concentrations and greater follicle diameter at the end of the P4 treatment as compared with when there were relatively greater P4 concentrations. In groups with the greatest differences in P4, this resulted in altered timing of estrus and ovulation during the period after cessation of P4 treatment. The differences in dynamics of follicular development and time of estrus, however, did not effect pregnancy rate to either estrus-AI or TAI. It is suggested that if there is an appropriate interval of time when circulating P4 is basal after cessation of a period of differential P4 treatment, differences in early developmental rate of follicles do not influence fertility of *Bos taurus* cows.

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Conflict of interest

Mention of a proprietary product does not constitute a guarantee or warranty of the product by USDA or the authors and does not imply its approval to the exclusion of other products that may also be suitable. USDA-ARS, is an equal opportunity/ affirmative action employer. All agency services are available without discrimination.

Declaration of interest

None.

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