Evaluating the effects of a high-concentration dose of prostaglandin $F_{2\alpha}$ in a 5-d CO-Synch + controlled internal drug release protocol on fertility in beef cows[1]

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INTRODUCTION

Estrous synchronization is a useful management tool to facilitate the use of fixed-time artificial insemination (AI) and improve fertility in beef herds. Addition of progesterone (P_4) in the form of a controlled internal drug release (CIDR) device between the initial gonadotropin-releasing hormone (GnRH) and $PGF_{2\alpha}$ injections (e.g. 5- and 7-d CO-Synch + CIDR [5-d and 7-d protocols, respectively]) has been shown to improve synchrony of ovulation and overall fertility (Lamb et al., 2001; Gunn et al., 2016). It has been hypothesized that prolonged exposure to P_4 in the 7-d protocol may increase the incidence of persistent follicles and aged oocytes (Santos et al., 2010). By reducing duration of CIDR exposure from 7 to 5 d, in the 5-d protocol, incidence of prolonged follicular dominance is decreased, thus improving oocyte quality and pregnancy response (Bridges et al., 2008; Cerri et al., 2011; Santos et al., 2010). However, there is a limitation when the length of follicular dominance is shortened, as the success of synchronization relies on the ability of $PGF_{2\alpha}$ to regress a newly formed corpus luteum (CL) by the time of AI.

New and immature (<5-d old) bovine CL are refractory to $PGF_{2\alpha}$ (Tsai and Wiltbank, 1998). Therefore, previous research concluded that two 25 mg $PGF_{2\alpha}$ injections 7 to 24 h apart at CIDR removal (as opposed to a single 25 mg $PGF_{2\alpha}$ dose in the 7-d protocol) are required to reliably induce complete luteolysis by the time of AI in a 5-d protocol (Bridges et al., 2008; Whittier et al., 2010). Although two doses of conventional (5 mg/mL) $PGF_{2\alpha}$ (2PG) in a 5-d protocol have shown improved pregnancy per AI (P/AI) in some studies (Kasimanickam et al., 2009), the addition of a second $PGF_{2\alpha}$ treatment adds another handling of cattle; increases cost, labor, and time requirements; and potentially decreases protocol compliance. In the 5-d protocol, Spencer et al. (2018) used a high-concentration $PGF_{2\alpha}$ product (HighCon; 12.5 mg/mL) and showed that one 25 mg dose of HighCon administered at CIDR removal is as effective as 2PG (5 mg/mL) at inducing luteolysis and thus reducing P_4 to optimal concentrations (P_4 < 0.5 ng/mL) by the time of AI. According to Zoetis HighCon reaches a greater maximum plasma concentration, has a longer half-life, and undergoes less blood level fluctuations following administration than conventional $PGF_{2\alpha}$ (Zoetis Inc., 2015). However, there is a lack of information on the effect of HighCon in a 5-d protocol on P/AI in beef cows. Therefore, the objectives

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of this study were to examine the effects of one high-concentration dose of $PGF_{2\alpha}$ or two doses of conventional $PGF_{2\alpha}$ 7 to 11 h apart on P/AI and P_4 concentrations at the time of AI in beef cows synchronized with a 5-d protocol.

MATERIALS AND METHODS

All procedures and protocols were in compliance with the University of Idaho Animal Care and Use Committee. Two-hundred primiparous and multiparous Angus-Hereford crossbred cows were used in this study. Animals were maintained on irrigated pasture and supplemented with alfalfa hay before and at the time of breeding. All cows were an average of 63 d postpartum (DPP) and had an average body condition score (BCS) (1 to 9 scale, 1 = emaciated, 9 = obese) of 5.55 ± 0.05 at the initiation of the experiment.

Experimental Design and Treatments

To determine cyclicity, blood samples were collected from all cows on d -7 and d 0 (Fig. 1). On d 0, GnRH (100 µg, i.m.; Factrel; Zoetis, Kalamazoo, MI) was administered to all animals and a CIDR (1.38 g [P_4]; EAZI-breed CIDR cattle insert; Zoetis) was inserted (Fig. 1). On d 5, CIDR inserts were removed and cows were stratified by BCS, age, and DPP and randomly assigned to receive either one 25 mg dose of HighCon (i.m., *n* = 100; Lutalyse *HighCon*; Zoetis) or two 25 mg doses of conventional $PGF_{2\alpha}$ (i.m., *n* = 100; Lutalyse; Zoetis) 7 to 11 h apart. On d 8, all cows received a second GnRH and simultaneous TAI with semen from six sires by two trained technicians. A final blood sample was collected on d 8 for P_4 analysis (Fig. 1). All cows were exposed to bulls 5 to 14 d after AI.

Estrus Detection and Pregnancy Diagnosis

Estrotect patches were applied to the tail head of all cows as an estrus detection aid, and estrual behavior was visually observed three times daily until d 8 (Fig. 1). Animals were considered to have been in estrus if visually observed in standing estrus or if Estrotect patches were ≥50% activated at the time of insemination. Cows with missing patches were also considered to have been in estrus. Pregnancy status was determined by transrectal ultrasonography (ReproScan XTC, ReproScan, Winterset, IA or Ibex Evo, E.I. Medical Imaging, Loveland, CO) either 48 d (*n* = 113) or 57 d (*n* = 87) after insemination.

Blood Sampling and P_4 Quantification

Blood samples were collected via venipuncture of the coccygeal artery or vein using a 10-mL vacutainer tube (Coviden LLC, Mansfield, MA). All samples were placed on ice immediately after collection and then stored at 4°C for 24 h. Samples were centrifuged at 2,400 × *g* and 4°C for 20 min. Serum was harvested and stored at -20°C until assayed for P_4 concentration. P_4 concentration was analyzed using a single-antibody coated tube radioimmunoassay (MP Biomedicals; ImmuChem Coated Tube Progesterone ^{125}I RIA Kit; Costa Mesa, CA). The standard curve ranged from 0.15 to 20 ng/mL, and all samples and standards were run in duplicate, with an intraassay coefficient of variance of 3.91%.

Statistical Analyses

Differences in P_4 concentrations and the descriptive variables of BCS, BW, DPP, and age between treatments were analyzed using the GLM procedure in SAS 9.4 (SAS 2015 Inc., 2015, Cary, NC). The LOGISTIC procedure of SAS was used to examine the effects of treatment

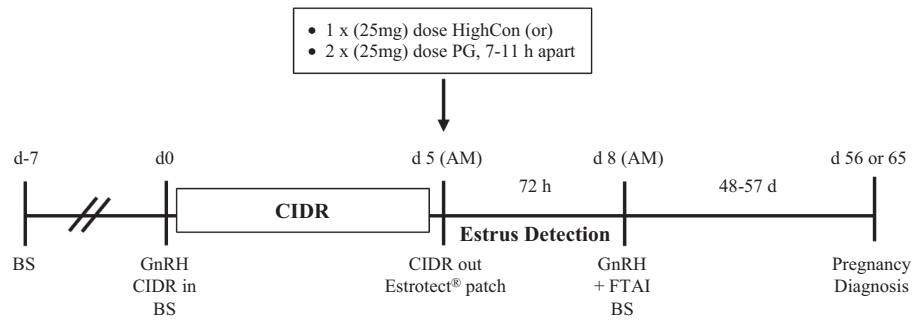

Figure 1. Schematic of experimental design to examine the effect of HighCon (*n* = 100, 25 mg, i.m. [12.5 mg/mL]) or 2PG (*n* = 100, 25 mg, i.m. [5 mg/mL]) on P_4 concentrations and AI pregnancy rates in Angus-Hereford crossbred beef cows subjected to a 5-d protocol. On d -7, 0, and 8, a blood sample was collected via venipuncture of the coccygeal vein. All blood samples were analyzed for P_4 concentration to examine cyclicity (P_4 > 1.0 ng/mL) prior to synchronization (d -7 and 0) or to assess incidence of luteolysis (P_4 < 0.5 ng/mL) in response to treatment. Pregnancy diagnosis using ultrasonography was conducted on d 56 (*n* = 113) or on d 65 (*n* = 87).

(2PG or HighCon) on AI pregnancy rate, estrus expression before AI, and incidence of luteolysis (defined as P_4 < 0.5 ng/mL). For P/AI analysis, the model included the effects of treatment, estrus expression, cyclicity before protocol initiation (P_4 > 1.0 ng/mL on d -7 and/or d 0), and appropriate interactions between treatment and the main effects. The models for estrus expression and incidence of luteolysis included treatment and cyclicity.

RESULTS AND DISCUSSION

There were no differences in average BCS (P = 0.18), DPP (P = 0.93), BW (P = 0.26), or age (P = 0.84) between treatments (Table 1). Before synchronization (d -7 or d 0), 82.1% of all cows were cyclic (P_4 > 1.0 ng/mL). Mean P_4 concentrations for both treatments on d -7 and d 0 were greater than 1.0 ng/mL (Table 2). Mean P_4 concentrations on d 0 (P = 0.29) and proportion of cyclic cows before synchronization (P = 0.59) did not differ between treatments (Tables 2 and 3). At the time of CIDR insertion, 62.3% of all cows had a luteal tissue present on the ovary based on serum P_4 concentrations.

No treatment effect was detected in P/AI (P = 0.18; Table 3). Similarly, there were no differences (P = 0.16) in estrus expression before AI between treatments; however, cows detected in estrus had greater (P = 0.02) P/AI (75%) than cows not detected in estrus (58%). This is consistent with previous research that showed a positive relationship between estrus expression and fertility in a fixed-time AI protocol (Pereira et al., 2016). There was no treatment by estrus interaction (P = 0.60) on P/AI. Additionally, cyclicity status before protocol initiation did not have an effect on P/AI (P = 0.56). Overall breeding season pregnancy rate was 95.5% and was similar (P = 0.99) between HighCon and 2PG (93% vs. 96.9%).

Mean concentration of P_4 for both treatment groups was below 1.0 ng/mL at the time of AI. However, average P_4 concentrations at AI were slightly greater in HighCon than in 2PG (P < 0.01; Table 2). Some cows in the HighCon group had abnormally high P_4, with concentrations ranging from 2.5 to 11.0 ng/mL, which may have skewed the treatment mean. Overall, irrespective of treatment, cows pregnant to AI had smaller (P < 0.01) P_4 concentrations on d 8 (0.14 ± 0.02 ng/mL) than cows not pregnant to AI (0.71 ± 0.21 ng/mL). In addition, a greater proportion (P < 0.01) of animals in 2PG underwent luteolysis (defined as P_4 < 0.5 ng/mL) than animals in HighCon (Table 3).

To improve fertility, the original 5-d protocol, which calls for two 25 mg doses of $PGF_{2\alpha}$ 8 to 12 h apart, was developed with the notion that animals may form a new CL in response to GnRH administered at CIDR insertion that is refractory to a single injection of $PGF_{2\alpha}$ (Bridges et al., 2008). In fact, previous research showed the use of 2PG injections in a 5-d protocol improves P/AI, compared with one $PGF_{2\alpha}$ injection (Kasimanickam et al., 2009). Authors

Table 1. Mean BCS, BW, days postpartum, and age (±SEM) in beef cows administered either HighCon (n = 100; 1 × 25 mg, i.m.) or 2PG (n = 100; 2 × 25 mg, 7-11 h apart, i.m.) following CIDR device removal in a 5-d protocol

	Treatment		
Item	HighCon	2PG	Overall
BCS	5.48 ± 0.07	5.61 ± 0.07	5.45 ± 0.05
BW, kg	570.96 ± 9.07	585.56 ± 9.07	578.26 ± 6.42
Days postpartum	63.24 ± 1.46	63.05 ± 1.46	63.14 ± 1.03
Age, yr	6.03 ± 0.36	6.13 ± 0.36	6.08 ± 0.25

Table 2. Mean serum P_4 concentrations (±SEM) on d -7, 0, and 8 in cows synchronized with a 5-d protocol that included either HighCon or 2PG 7-11 h apart at CIDR removal

	P_4, ng/mL		
Item	HighCon	2PG	P-value
d -7	3.11 ± 0.46[a]	4.48 ± 0.46[b]	0.04
d 0	3.96 ± 0.48	4.68 ± 0.48	0.29
d 8	0.54 ± 0.10[a]	0.13 ± 0.10[b]	<0.01

[a,b]Values within a row without a common superscript differ (P < 0.05).

Table 3. Pregnancy rate to AI, estrus expression, incidence of luteolysis, cyclicity status, and CL presence at the time of CIDR insertion in cows synchronized with a 5-d CO-Synch + controlled internal drug release (CIDR) protocol that included either one high-concentration dose of prostaglandin $F_{2\alpha}$ (HighCon) or two conventional doses of prostaglandin $F_{2\alpha}$ (2PG) 7-11 h apart at CIDR device removal.

	Treatment		
Item	HighCon	2PG	P-value
P/AI[1]	62 (62/100)	71 (71/100)	0.18
Estrus[2]	45 (45/100)	55 (55/100)	0.16
Luteolysis[3]	80 (80/100)[a]	98 (98/100)[b]	<0.01
Cyclic[4]	79.80 (79/99)	82.83 (82/99)	0.59
CL on d 0[5]	64 (64/100)	61 (61/100)	0.66

[a,b]Values within a row without a common superscript differ (P < 0.05).

[1]Number of cows pregnant to FTAI divided by the number of cows synchronized.

[2]Number of cows observed in estrus, with Estrotect patches ≥50% activated or missing a patch at AI.

[3]Luteolysis defined as P_4< 0.5 ng/mL on d 8.

[4]Cyclicity defined as P_4>1.0 ng/mL on either d -7 or d 0.

[5]Cows with a CL on d 0 defined as P_4>1.0 ng/mL.

hypothesized that improved P/AI may be attributable to the efficacy of 2PG in causing complete luteolysis. The findings of our study differ from this research in that no difference in P/AI was detected between one HighCon and 2PG injections. Notably, in the previous study, cows receiving one $PGF_{2\alpha}$ treatment were administered conventional $PGF_{2\alpha}$ dinoprost, whereas the present study used a high-concentration $PGF_{2\alpha}$ dinoprost with a longer half-life (HighCon).

Our previous research showed that one injection of HighCon was as effective as 2PG to cause luteolysis and decrease P_4 by AI (Spencer et al., 2018). In contrast, the current study observed a difference in proportion of animals with complete luteolysis between HighCon and 2PG; nevertheless, no difference in P/AI was detected between treatments. As previously determined by Colazo et al. (2017), we defined luteolysis as $P_4 < 0.5$ ng/mL at AI. However, other studies describe luteolysis in beef cattle as <1.0 ng/mL (Bridges et al., 2012; Stevenson and Lamb, 2016). If incidence of luteal regression is redefined as $P_4 < 1.0$ ng/mL in the present study, no differences ($P = 0.94$) in luteolysis between HighCon (89%) and 2PG (100%) are detected. Therefore, more research is needed to determine the optimal prebreeding P_4 concentration used to define luteolysis and improve fertility in beef cattle.

IMPLICATIONS

Investigation of the use of one, instead of two, $PGF_{2\alpha}$ injections was established to assess the potential for reducing labor, animal handling, time, and overall synchronization costs for beef producers without compromising fertility to artificial insemination. Although two $PGF_{2\alpha}$ injections were more effective in causing luteolysis by the time of insemination, there is not sufficient evidence to indicate that it improves pregnancy response compared with one injection of HighCon in the 5-d protocol, as no difference in pregnancy rate was detected between treatments. Additional research is needed to accurately determine if a single dose of high-concentration $PGF_{2\alpha}$ with a longer plasma half-life results in optimal pregnancy rates and whether it may replace the conventional double-dose scheme in the 5-d protocol.

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