



Research Article

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One Injection of High-Concentration Prostaglandin $F_{2\alpha}$ is as Effective as Two Injections of Conventional Prostaglandin $F_{2\alpha}$ in Causing Luteolysis for Dairy Cows Subjected to A 5-Day CIDR-Cosynch Protocol



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Abstract

The objective of this study was to examine the effects of one or two doses of prostaglandin $F_{2\alpha}$ (PG, dinoprost tromethamine), or one dose of high-concentration formula PG (HighCon) on progesterone (P4) concentration profiles, and luteolysis ($P4 < 0.5$ ng/mL) in lactating dairy cows subjected to a 5-day CIDR-Cosynch protocol. On day 0 (d0), 67 cows received GnRH, and a CIDR was inserted. On day 5 (d5), CIDR was removed, and cows were assigned randomly to receive one dose (5 mL) PG (1PG; 5 mg/mL; $n = 24$), two doses (5 mL/dose) PG 12 h apart (2PG; 5mg/mL; $n = 22$), or one dose (2 mL) of HighCon (12.5 mg/mL; $n = 21$). One h after CIDR removal and before treatment, a blood sample was collected to measure baseline P4 concentrations (d5). After treatment, blood samples were collected every 12 h from d 5 to 8 to measure P4. On day 8 (d8), all cows received a second GnRH and were inseminated. Ovaries were examined by ultrasonography on d0, d5, and d8. A repeated measures analysis assuming a lognormal distribution was used to assess treatment differences in P4 concentrations. All cows ($n = 67$) used for analysis had elevated P4 concentrations and corpora lutea before treatment (d5). Blood P4 concentrations decreased over time in all treatments ($p < 0.01$). There was an effect of treatment by time interaction on P4 ($p = 0.05$). By 24 h after treatments, P4 concentrations were smaller, and remained at a smaller ($p < 0.01$) throughout the experiment for 2PG and HighCon compared with 1PG. There was no difference in P4 concentrations between 2PG and HighCon at 72 h, and the P4 averages were 1.02, 0.05, and 0.22 ng/mL for 1PG, 2PG and HighCon, respectively. The proportion of cows that went through luteolysis ($P4 \leq 0.5$ ng/mL) were greater in 2PG and HighCon compared with 1PG. The results provide evidence that 1PG was not as effective as 2PG and HighCon in causing luteolysis, and that one injection of HighCon was as effective as 2PG in decreasing blood P4 and causing luteolysis by the time of Insemination (AI).

Keywords: Prostaglandin Dose; Luteolysis; Dairy Cow

Abbreviations: TMR: Total Mixed Ration; CIDR: Controlled Internal Drug Release; TAI: Fixed-time AI; CL: Corpus Luteum; DIM: Days in Milk; TBRD: Times Bred; GLM: Generalized Linear Model; AI: Artificial Insemination; PG: Prostaglandin $F_{2\alpha}$, P4: Progesterone

Introduction

Effective systematic breeding program is a critical segment of the integrated management program in an efficient dairy cow production system. Reproductive efficiency represents the future profitability and genetic improvement of the herd. Expanded use of AI and/or adoption of emerging reproductive technologies for dairy cattle led to development of several breeding protocols to better control estrous cycle. Fixed-time AI (TAI) breeding

protocols, such as Ovsynch or Ovsynch + controlled internal drug release (CIDR) insert, and their modified versions, are attractive reproductive management tools that enhance AI submission rate. However, AI pregnancy rates (P/AI) achieved through these programs vary significantly in dairy cows. For example, P/AI for first insemination have been reported to range between 30 to 37% for dairy cattle [1].

The addition of progesterone (P4) in the form of CIDR inserts between the initial GnRH and prostaglandin F2 α (PG) injections (7-day CIDR-Cosynch) aid in preventing premature estrus and ovulation before PG and have been shown to improve synchronization and P/AI [2-6]. In beef cows, the reduction in duration of CIDR treatment to the induction of luteolysis by 2 days (from 7-day to 5-day) in the CIDR-Cosynch, has been found to enhance P/AI rates [7-9]. However, these authors concluded that two injections of PG (2PG), administered 7 to 24 h apart on day 5, were necessary to consistently induce luteolysis by the time of AI. It was hypothesized that improved P/AI may be attributable to efficacy of 2PG in causing complete luteolysis and lowering P4 concentrations by the time of AI compared with one PG (1PG) injection.

The hypothesis postulated that shortening the timeframe between the initiation of a GnRH-induced new follicular wave and luteolysis induction by PG injection by 2 days could enhance P/AI rates in lactating dairy cows [10]. Considering the assumption that a sole injection of PG might not adequately regress a recently formed corpus luteum (CL) 5 days post-ovulation induction, the authors concluded that a second dose of PG treatment 24 h later is required. It should be noted that in that study cows did not receive CIDR, and although P4 concentrations at the time of AI were lesser in cows received 2PG than 1PG, P/AI between these two treatment groups were not assessed.

Incorporating a second PG treatment into a 5-day CIDR-Cosynch protocol, however, brings about additional animal handling, increases expenses, demands more labor and time, and could potentially reduce adherence to the protocol. A recent study conducted in our laboratory [11] evaluated the efficacy of a high-concentration PG product, HighCon (dinoprost tromethamine, 12.5 mg/mL, Zoetis Inc.), within a 5-day CIDR-Cosynch protocol for suckling beef cows. The objective was to determine whether a single dose of HighCon could achieve luteolysis as effectively as two injections of conventional PG. Zoetis reports that HighCon attains greater peak plasma levels, has an extended half-life, and experiences fewer fluctuations in blood levels after administration compared to conventional PG [12]. In our study, we demonstrated that a single 25 mg dose of HighCon, given at CIDR removal, achieves similar effectiveness as 2PG (25 mg/dose) in triggering luteolysis, thereby lowering P4 levels to optimal concentrations (P4 < 0.5 ng/mL) by the time of AI in beef cows [11].

Based on our previous finding in beef cows, it is plausible that one dose of HighCon is as effective in causing luteolysis as 2PG hence potentially reduce above mentioned difficulties associated with two PG injections in lactating dairy cows. Nevertheless, the direct effects of one concentrated PG(HighCon) on P4 profiles and luteolysis have not been examined in dairy cows subjected to a 5-day CIDR-Cosynch estrous synchronization protocol. Therefore, the objectives of this study were to examine the effects of 1PG, 2PG (12 h apart), and one high concentration, HighCon PG on luteolysis and P4 profiles in lactating Holstein cows subjected to a

5-day CIDR-Cosynch protocol.

Materials and Methods

All procedures and protocols followed the University of Idaho, Animal Care and Use Committee. Sixty-seven lactating Holstein cows from the University of Idaho (Moscow, Idaho) dairy research facility were used for this study. All cows were on average 127 days in milk (DIM) at the initiation of the study.

Initially, 87 lactating Holstein cows were enrolled into this study and synchronized using a 5-day CIDR-Cosynch protocol [7]. Twenty cows were eliminated from this study as they did not have a CL at and (or) elevated concentrations of P4 on d5 before treatment (7 cows), or they were acyclic and blood samples from d-7, d0, and d5 were all < 0.5 ng/mL (13 cows). All animals were housed in free stall barns and milked twice daily. Cows had free access to water and a total mixed ration (TMR) balanced for lactating dairy cows [13].

Experimental design and treatments

Seven days before estrus synchronization, coccygeal blood samples were collected from all cows (Figure 1). On d0 (initiation of synchronization), all cows received GnRH (100 μ g, i.m., Factrel, Fort Dodge Animal Health, Fort Dodge, IA), and a coccygeal blood sample was collected. Simultaneously, a CIDR (1.38 g P4, Eazi-Breed CIDR, Zoetis, Florham Park, NJ) was vaginally inserted (Figure 1). All cows were subjected to transrectal ultrasonography (Aloka SSD-500 V, Aloka, Tokyo, Japan), and categorized into two groups based on presence or absence of a CL at initiation of synchronization (d0). Five days later CIDR inserts were removed, and cows were stratified by presence or absence of a CL on d0, parity, DIM, and the presence of one or two CL on d5 (existing CL and ovulated to initial GnRH). Once stratified cows were assigned randomly to one of three treatments: one PG injection (1PG, n = 24, 25 mg, i.m., Lutalyse, Zoetis, Florham Park, NJ), two PG injections (2PG, n = 22, 50 mg total, i.m., Lutalyse, Zoetis, Florham Park, NJ) given 12 h apart, or one high concentration PG (HighCon, n = 21, 25 mg, i.m., Lutalyse HighCon, Zoetis, Florham Park, NJ) (Figure 1). Sixty min after CIDR removal and before treatments, coccygeal blood samples were taken, and ultrasonography was conducted to confirm the presence of luteal tissue. Following treatment, blood samples were collected every 12 h from d5 to d8 to measure P4 concentrations (Figure 1). Between d5 and d8, cows were monitored every 4 to 6 h for estrual behavior through the observation of tail paint removal. Regardless of expression of estrus, all cows received a second GnRH (100 μ g) on TAI on d8 (Figure 1).

Ovarian examination and luteolysis

On d0 and d5 transrectal ultrasonography (Aloka SSD-500 V, Aloka, Tokyo, Japan) was conducted to assess ovarian structures. Cows were categorized as developing a new CL or possessing an old CL at the time of treatment. The presence of a dominant follicle (≥ 10 mm diameter) on d0, and the presence of a CL on d5 in the

same location identified cows ($n = 31$) that ovulated to the initial GnRH and formed a new CL. Cows presenting a CL on d0 and d5 in the same location were classified as possessing an old CL at the

time of treatment. Luteolysis was defined as P4 concentrations ≥ 1 ng/mL on d5, and P4 ≤ 0.5 ng/mL on d8.

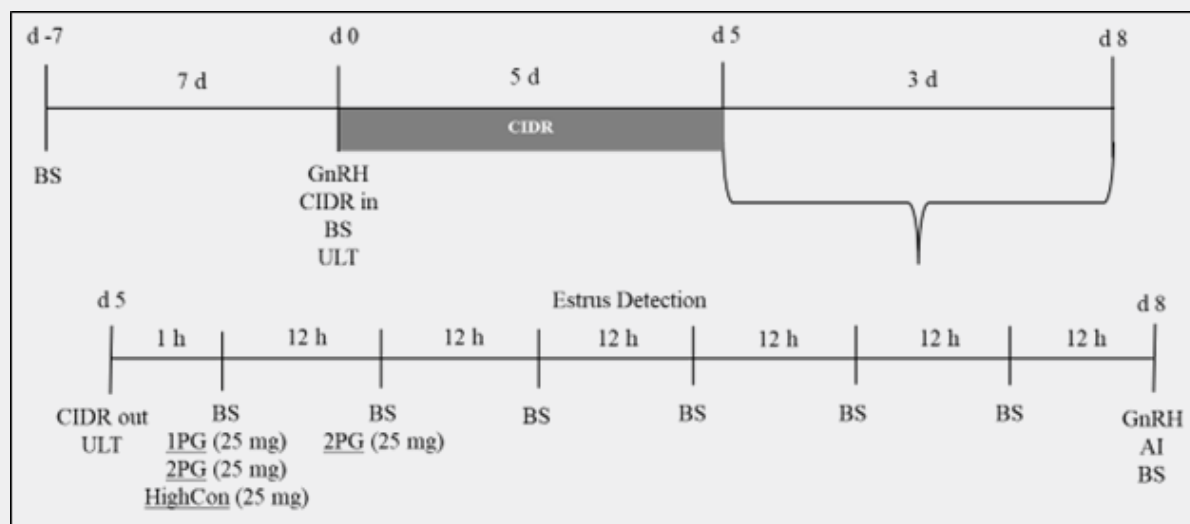


Figure 1: Schematic of the experimental design to examine the effect of one prostaglandin F $_{2\alpha}$ (PG) injection (1PG, 25 mg i.m., $n = 24$), two PG injections (2PG, 50 mg i.m., $n = 22$) administered 12 h apart, or one high concentration, long-lasting PG (HighCon, 25 mg i.m., $n = 21$) injection on progesterone (P4) concentrations in lactating dairy cows subjected to a 5-day controlled internal drug release (CIDR)-Cosynch protocol. On d0 and d5, ovarian structures were recorded for all cows via transrectal ultrasonography (ULT). On d5, cows were stratified by presence or absence of a CL on d0, parity, DIM, and the presence of one or two CL on d5 (existing CL and ovulated to initial GnRH). One h after CIDR removal, treatments were administered, and coccygeal blood samples (BS) were collected every 12hr until d8 (72 h). All BS were analyzed for P4 concentrations. Cows were observed every 4 to 6 h between d5 and d8 for estrus behavior via tail paint removal.

Blood samples and progesterone quantification

Blood samples were collected via puncture of the medial caudal coccygeal vein or artery using a 20-gauge single use blood collection needle. Blood samples were collected into a 10 mL vacuumed tube (Covidien LLC, Mansfield, MA). All samples were placed on ice and stored at 4°C for 18 to 24 h. Samples were then centrifuged for 20 min at $2,400 \times g$ and 4°C. Serum was harvested and stored at -20°C until assayed for P4 concentrations.

Progesterone concentrations were assessed via a double antibody radioimmunoassay (RIA) method (MP Biomedicals, Costa Mesa, CA), conducted under equilibrium conditions. The standard curve spanned from 0.05 to 25 ng/mL. Each sample and standard underwent duplicate runs, with both intra- and inter-assay coefficients of variation recorded at 3.6% and 6.7%, respectively.

Statistical analysis

Data for weekly milk production during the experiment, parity, DIM at initiation of synchronization, and number of times bred (TBRD) prior to treatment, were analyzed using the analysis of variance procedure to test for treatment effects, using

a generalized linear model (GLM) procedure in SAS (v. 9.4, SAS® Institute Inc.) [14]. The analysis of variance was also used to determine differences on d0, d5, and d8 P4 concentrations. The model included the main effect of treatment.

A univariate analysis was conducted indicating a non-normal distribution for P4 data after treatment. A repeated measure generalized linear mixed model [14] was used to determine differences in P4 concentrations across time assuming a lognormal distribution [15]. The model included the fixed effects of treatment, time, and the time by treatment interaction. Initial P4 concentration prior to treatment (d5) were also included in the analysis as a covariate. Cows within treatments were considered random effects, and the correlation structure for the repeated measures followed an ARMA (1,1) structure.

The differences in proportion of cows between treatments with the presence of a CL on d0 and proportion of cows with luteolysis on d8 were analyzed using a generalized linear model (GLIMMIX), assuming a binomial distribution. The differences in proportion of cows between treatments with the presence of a CL on d0, development of a new CL (ovulatory follicle ≥ 10 mm on d0 and presence of CL on d5 in same location), remnants of a CL

on d8, and P4 concentrations ≤ 0.5 ng/mL on d7 (60 h after CIDR removal) and d8 (72 h after CIDR removal) were analyzed using GLIMMIX, assuming a binomial distribution.

Non-orthogonal predetermined contrasts were made between groups for ovarian structures on d0 (presence or absence of CL), d5 (development of new CL), and incidence of luteolysis (60 and 72 h after treatments). Using Bonferroni adjusted for multiple comparison [16], differences between 1PG vs. 2PG, 1PG vs. HighCon, and 2PG vs. HighCon were examined. All statistical computations were carried out using SAS v. 9.4 (SAS Institute Inc. 2015). Significance was declared at $p \leq 0.05$ and a tendency at $p \leq 0.1$.

Results

Descriptive Data

There were no differences between 1PG, 2PG, and HighCon groups regarding in parity ($p = 0.28$; 2.0, 2.1, 1.9 ± 0.1 , respectively) or TBRD ($p = 0.61$; 0.46 ± 0.06 , 0.55 ± 0.06 , 0.48 ± 0.07 , respectively). There were small differences ($p < 0.05$) in daily milk production being less for 2PG (34.6 ± 0.6 kg/d) compared with 1PG (37.3 ± 0.6 kg/d) but not different from HighCon (36.1 ± 0.6 kg/d). Mean DIM for all cows was 127 ± 7.6 d. Based on the P4 concentrations measured on days -7 and 0, all cows enrolled in this study were determined to be cyclic, with at least one blood sample showing a

P4 concentration greater than 1 ng/mL.

Ovarian Structures

The percentage of cows with an existing CL, as detected by ultrasonography, on d0 of the experiment tended ($p = 0.09$) to differ between treatments (71%, 89%, and 60%, for 1PG, 2PG, and HighCon, respectively; Table 1). More cows in 2PG had a CL present on d0 (initiation of synchronization) than HighCon but that did not differ from 1PG. In addition, the presence of a CL on d0 did not differ between 1PG and HighCon treatments.

All cows, by study design, had at least one CL present on their ovary on d5, and no difference in the proportion of cows that ovulated to the initial GnRH and developed a new CL was detected ($p = 0.39$). Overall, ovulation incidence to the initial GnRH injection was 40% regardless of treatment.

Progesterone Concentrations and Profiles

Mean P4 concentrations on d0 (initiation of synchronization) did not differ between treatments ($p = 0.98$) and were 3.21 ± 0.78 for 1PG, 3.25 ± 0.82 for 2PG, and 3.40 ± 0.84 ng/mL for HighCon (Figure 2). Similarly, mean P4 concentrations did not differ ($p = 0.26$) between treatments on d 5. Mean concentrations on d5 were 4.16 ± 0.67 , 3.07 ± 0.70 , and 2.60 ± 0.72 ng/mL for 1PG, 2PG, and HighCon, respectively (Figure 2).

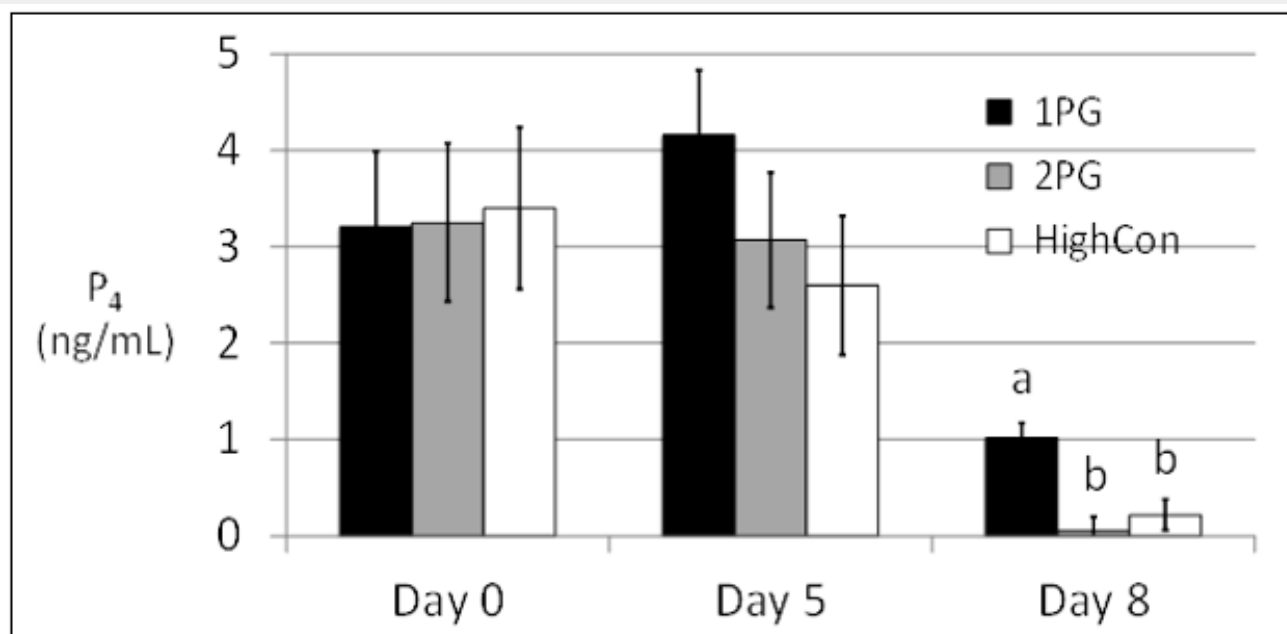


Figure 2: Mean $\pm$ SEM progesterone (P4) concentrations (ng/mL) on Day 0, Day 5, and Day 8 (72 h after treatment) of the experimental protocol for lactating Holstein dairy cows subjected to a 5-day controlled internal drug release (CIDR)-Cosynch protocol. One h after CIDR removal (Day 5) cows were treated with either one prostaglandin F2 α (PG) injection (1PG, n = 24), two PG (2PG, n = 22) injections 12 h apart, or one high concentration PG (HighCon, n = 21) injection.

a,b Bars with different letters within d differ $p < 0.01$.

The P4 data from 12 to 72 h after treatment were skewed indicating a non-normal distribution with an unstable variance. Univariate analyses confirmed a large variation, and hence, P4 data were assumed to follow a lognormal distribution. Progesterone concentrations on d5 were used as a covariate in the model to account for concentrations before treatment administration.

As expected, P4 concentrations after treatments decreased over time ($p < 0.01$) in all treatments. There was an effect of

treatment ($p < 0.01$) and treatment by time ($p = 0.05$) on P4 concentrations from h 12 to 72 after treatments (Figure 3) indicating that the decline in P4 concentrations over time were not similar among treatments. From 24 to 72 h after initial treatment, P4 concentrations were smaller in 2PG and HighCon compared with 1PG; however, P4 concentrations during this sampling period did not differ between 2PG and HighCon. The serum P4 concentrations (non-transformed data) from d5 to d8 are shown in Figure 4.

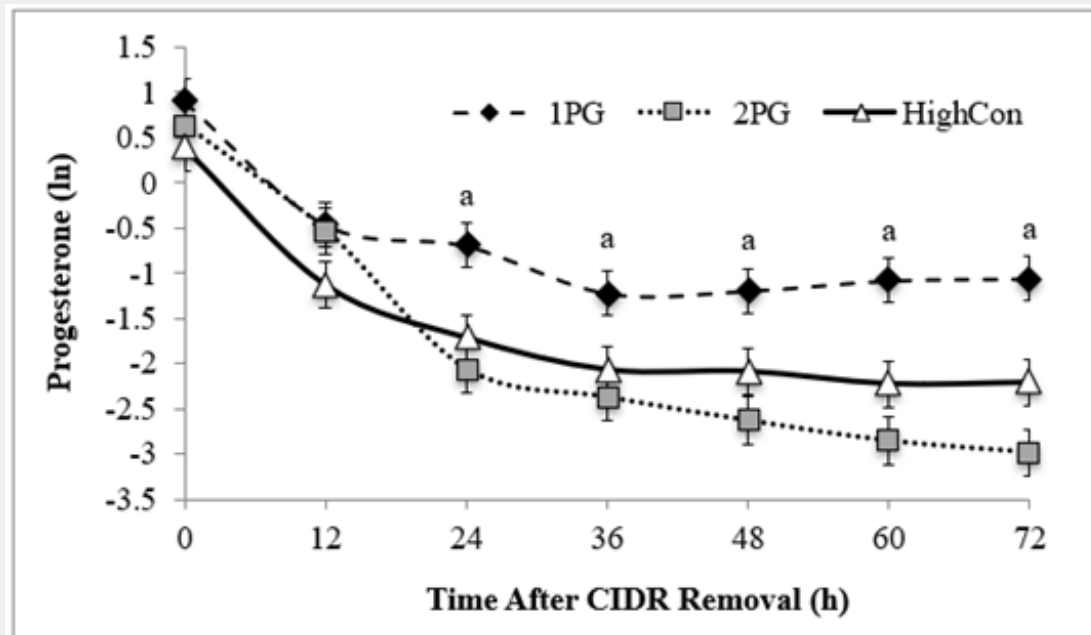


Figure 3: Mean blood progesterone concentrations after lognormal data transformation in lactating dairy cows receiving one prostaglandin F_{2α} (PG) injection (1PG, $n = 24$), two PG injections 12 h apart (2PG, $n = 22$), or one high concentration PG injection (HighCon, $n = 21$) in a 5-day controlled internal drug release (CIDR)-Cosynch protocol. Time 0 is one h after CIDR removal before treatments were administered. Blood samples were collected every 12 h for three days after treatment administration.

aMeans for 2PG or HighCon differ from 1PG ($p < 0.05$).

Mean P4 concentrations on d8 (72 h after treatment) differed between treatments ($p < 0.01$). The 1PG had the greatest P4 concentrations (1.02 ± 0.15 ng/mL), followed by HighCon (0.22 ± 0.16 ng/mL), and 2PG which had the lowest P4 concentrations on d8 (0.05 ± 0.15 ng/mL) (Figure 2). The P4 concentrations on d8 did not differ between HighCon and 2PG but both groups P4 concentrations were smaller than 1PG (Figure 2).

The differences in P4 concentrations 60 and 72 h (d8) after treatments were further examined to determine the proportion of cows with $P4 \leq 0.5$ ng/mL, indicating functional luteolysis, between treatments. There was a difference ($p < 0.01$) between treatments for the proportion of cows with $P4 \leq 0.5$ ng/mL at both 60 and 72 h after treatments (Table 1). At 60 and 72 h, 54% and 50% of 1PG cows exhibited $P4 \leq 0.5$ ng/mL. The HighCon treatment had a greater proportion of cows with $P4 \leq 0.5$ ng/mL

at 60 and 72 h with 90% and 81%, respectively. No difference in incidence of luteolysis was detected between 2PG and HighCon. However, it should be noted that the small number of observations in the current study limited the statistical power to detect these numerical differences. The HighCon treatment did not differ from 2PG, where 100% of cows had $P4 \leq 0.5$ ng/mL at 60 and 72 h (Table 1).

Discussion

It is evident that incomplete luteolysis is a major contributing factor that influences fertility in dairy cows [17-20]. Therefore, the purpose of this study was to investigate the effects of one or two doses of conventional PG and one high-concentrated PG on P4 profiles and luteolysis in lactating dairy cows subjected to a 5-day CIDR-Cosynch protocol. Most studies have examined P4 concentrations at the initiation of an estrous synchronization

protocol, at the time of PG administration, and at the time of AI [10, 17, 19, 21, 22], however, the P4 profile from PG injection until the time of AI have not been examined, and thus the effect of PG

dose on rate of P4 concentration decline has not been defined. In addition, the use of one HighCon PG injection versus 2PG injections has not been examined in lactating dairy cows.

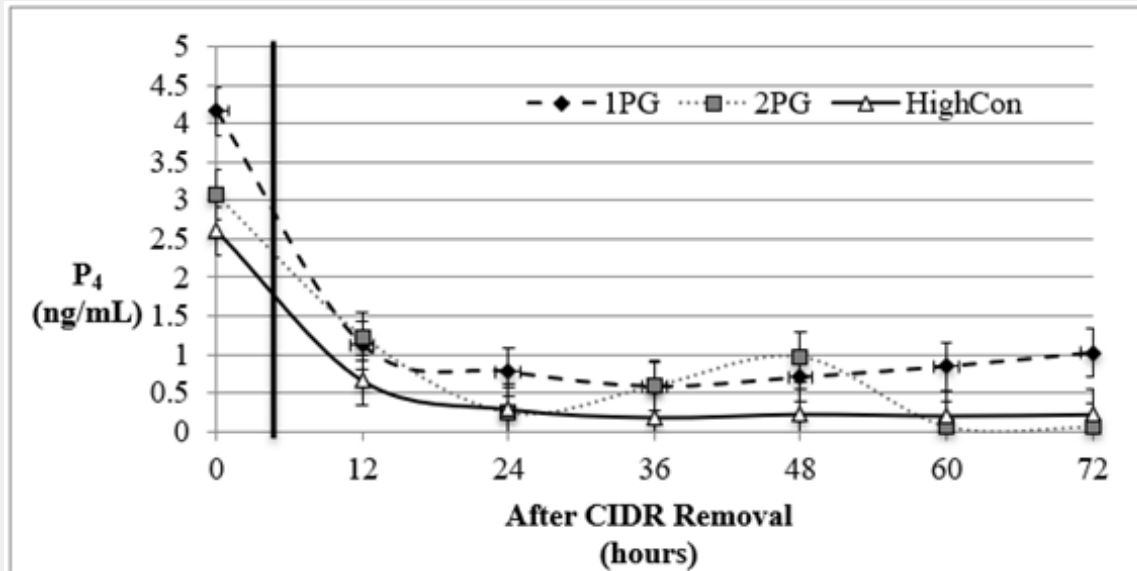


Figure 4: Serum progesterone (P4) concentrations between lactating dairy cows receiving one prostaglandin F_{2α} (PG) injection (1PG; n = 24), two PG injections 12 h apart (2PG; n = 22), or one high concentration PG (HighCon; n = 21) in a 5-day controlled internal drug release (CIDR)-Cosynch protocol. Time 0 is d5, 60 min after CIDR removal and before treatments were administered and was used as a covariate. Blood samples were collected every 12 hr for three days until d8 (72 hr) after treatment administration. Statistical inferences are not displayed on this graph as data was analyzed on a lognormal data transformation.

Table 1: Proportions of cows with progesterone (P4) concentrations ≤ 0.5 ng/mL at the time of AI and corpus luteum (CL) presence in lactating Holstein dairy cows subjected to one conventional prostaglandin F_{2α} (PG) injection (1PG), two conventional PG injections (2PG) administered 12 h apart, or one high concentration PG injection (HighCon) in a 5-day controlled-internal drug release (CIDR)-Cosynch protocol.

Variable	Treatments ¹			p-value
	1PG	2PG	HighCon	
Luteolysis ²				
60 h, % (no./no.)	54 (13/24) ^a	100 (22/22) ^b	90 (19/21) ^b	< 0.01
72 h, % (no./no.)	50 (12/24) ^a	100 (22/22) ^b	81 (17/21) ^b	< 0.01
Presence of CL ³				
d0, % (no./no.)	71 (17/24) ^{a,b}	89 (17/19) ^a	60 (12/20) ^b	=0.09
d8, % (no./no.)	52 (12/23) ^a	17 (3/18) ^b	30 (6/20) ^{a,b}	=0.04

¹In a 5-day CIDR-Cosynch protocol, cows were assigned randomly to receive one PG injection (1PG; n = 24), two PG injections given 12 h apart (2PG; n = 22), or one high-concentration PG (HighCon; n = 21) injection upon CIDR removal.

²P4 concentrations ≤ 0.5 ng/mL 60 h and 72 h after CIDR removal and initial treatment.

³Transrectal ultrasonography, d0 = existing CL. Total number of cows differ as a CL was not identified via ultrasonography but based on P4 concentrations (P4 > 1 ng/mL), a functional CL must have been present, therefore they were excluded this analysis.

^{a,b} Proportions with different superscripts within rows differ (p < 0.05).

Researchers have demonstrated that P/AI is improved or remains similar if the duration of CIDR treatment in a CIDR-Cosynch protocol is shortened from 7 to 5 days (interval between initial GnRH injection and CIDR insertion, to CIDR removal and

PG injection), and the interval from CIDR removal and PG to the second GnRH is extended from 48 h to 72 h [7-10, 23-25]. However, by shortening the interval between initial GnRH and PG injections, the success of pregnancy relies on the ability of exogenous PG to

successfully regress a GnRH-induced newly formed CL and reduce P4 concentrations to optimal concentrations ($P4 < 0.5$ ng/mL) by the time of AI [17, 20].

Recent studies in dairy and beef cows recommended administering two injections of PG to completely induce luteolysis by the time of AI when using timed-AI synchronization protocols [7-11, 19]. The results of the current study provided evidence for the first time that one injection of HighCon was as effective as 2PG in decreasing blood P4 and causing luteolysis by the time of AI in lactating dairy cows. To our knowledge there are only two studies in dairy cows that have examined the effectiveness of one or two PG injections in a 5-day Cosynch protocol, however these studies did not examine the effect of one injection of HighCon on luteolysis.

Santos et al. (2010) examined the effects of one or two conventional PG injections 24 h apart in a 5-day Cosynch protocol in lactating dairy cows. In this study, luteolysis was defined as $P4 > 1$ ng/mL at PG injection (d5) and $P4 < 1$ ng/mL at time of AI (d8). These authors concluded 2PG injections 24 h apart are needed to successfully lower P4 concentrations and cause luteal regression at the time of AI. These results are similar to the current study where 2PG P4 concentrations were less compared with 1PG at the time of AI. In contrast, our studies showed that one injection of HighCon PG was as successful as 2PG in lowering P4 (< 0.05 ng/mL). It should be noted that in the study by Santos et al., (2010), cows in the 2PG injections group still had P4 concentrations > 0.5 ng/mL at the time of AI and may not have been at optimal P4 concentrations to maximize pregnancy. Additionally, a CIDR was not included during the 5-day Cosynch protocol in the study by Santos et al. (2010), nor were P/AI between 1PG and 2PG evaluated.

Using a similar timed-AI protocols Ribeiro et al. (2012), examined the effects of conventional 1PG or 2PG injections 24 h apart on P/AI in a 5-day CIDR-Cosynch protocol in dairy cows. Like the current study, the incidence of luteolysis was numerically greater with 2PG compared with 1PG (95.7% vs. 82%). However, our study differs in that one injection of HighCon PG was as effective in causing luteolysis as 2PG. It should be noted that these researchers did not compare the effects of 1PG versus 2PG specifically in the 5-day CIDR-Cosynch protocol. Rather, they reported the luteolytic effects of 1PG versus 2PG injections by combining the results of two different protocols. Therefore, it cannot be determined if there were any differences between 1PG versus 2PG in a 5-day CIDR-Cosynch protocol on P4 concentrations, luteolysis, and P/AI.

Administration of 2PG injections 12 h apart, or one HighCon PG injection were effective in reducing P4 concentrations to ≤ 0.5 ng/mL by the time of AI and causing complete luteolysis in this study. Interestingly, in our study, mean serum P4 concentrations for the HighCon treatment (0.22 ng/mL) were also like the P4

concentrations observed for the cows that received 2PG injections in the Ribeiro et al. (2012) study. The fact that no difference in the proportion of cows with complete luteolysis was detected between the 2PG and HighCon treatments lends further support to our hypothesis that HighCon is as effective as 2PG in causing luteolysis in a 5-day CIDR-Cosynch protocol. However, given the limited number of observations we acknowledge the limited power of detecting differences.

If we were to redefine the definition of complete luteolysis from $P4 \leq 0.5$ ng/mL to $P4 < 1$ ng/mL, as defined by others [10,19], the proportion of cows with complete luteolysis would increase to 95% in HighCon (data not shown). Thus, for the first time we were able to demonstrate that one injection of HighCon (dinoprost tromethamine), a high concentration of Lutalyse (dinoprost tromethamine), is as effective as 2PG (12 h apart) injections in decreasing P4 concentrations (causing complete luteolysis) by the time of AI in lactating dairy cows subjected to a 5-day CIDR-Cosynch protocol.

Considering P4 concentrations and its relationship to fertility, the administration of only PG (HighCon) is more practical for synchronizing dairy cows, as it reduces animal handling, labor, and drug costs. Furthermore, the addition of a second PG treatment within the 5-day CIDR-Cosynch program increases labor associated with additional animal handlings, and may decrease protocol compliance, ultimately reducing the potential benefits of two PG injections in a 5-day CIDR-Cosynch protocol. Future research is needed to compare the effects of P4 profiles on P/AI to further support the use of one high concentrated PG injection in a 5-day CIDR-Cosynch protocol for lactating dairy cows.

Conclusion

An important factor that limits the success of any TAI program, such 5-day CIDR-Cosynch is inducing luteolysis of all luteal structures in response to PG. To improve greater odds of pregnancy, it is argued that the concentrations of P4 should reach to < 0.5 ng/mL at the time of AI. Our study provides evidence that one does of HighCon as effective as two doses of conventional PG in reducing P4 while reducing labor and compliancy associated with animal handling. Provided the small sample size in the current study, future research is needed to compare the effects of one injection of HighCon with two injections of PG on P/AI to further support the use of one high concentrated PG injection in a 5-day CIDR-Cosynch protocol for lactating dairy cows.

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