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Luteolytic effects of cloprostenol sodium in lactating dairy cows treated with G6G/Ovsynch

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ABSTRACT

The probability of a pregnancy decreases substantially in lactating dairy cows treated with Ovsynch if luteolysis is delayed or incomplete. Two $PGF_{2\alpha}$ products are currently approved in the United States for luteolysis in lactating dairy cattle, dinoprost tromethamine and cloprostenol sodium. Cloprostenol has a longer half-life compared with dinoprost, is more resistant to endogenous metabolism, and is maintained in circulation longer. We hypothesized that cloprostenol could reduce the time to complete luteolysis compared with dinoprost because of differences in half-life. Lactating dairy cows received the same presynchronization strategy (G6G; 25 mg of $PGF_{2\alpha}$ – 2 d – 100 μg of GnRH – 6 d – 100 μg of GnRH – 7 d – final $PGF_{2\alpha}$ treatment). At the time of the final $PGF_{2\alpha}$, cows (n = 35) were randomly assigned to receive either 500 μg of cloprostenol or 25 mg of dinoprost. Blood samples were collected daily before and serially after $PGF_{2\alpha}$ treatment to analyze circulating concentrations of progesterone (P_4) and estradiol (E_2). Ultrasound examinations of ovaries were performed to measure sizes of follicles and corpora lutea (CL) and determine time of ovulation. Considering only cows with complete luteolysis, mean circulating P_4 was lower for cows given cloprostenol than for those given dinoprost between 0 and 12 h postinjection, but not at 24, 36, or 48 h. A rapid decrease in P_4 was observed 1 h after $PGF_{2\alpha}$ (6.54 ± 0.27 to 3.77 ± 0.22 ng/mL) followed by a complete rebound 1 h later (3.77 ± 0.22 to 5.07 ± 0.31 ng/mL) followed by a steady decline in both treatment groups. Serum concentrations of E_2 were greater at 48 h posttreatment in cloprostenol-treated cows (2.74 ± 0.15 pg/mL) than in dinoprost-treated cows (2.37 ± 0.19 pg/mL). Cows that did not have complete luteolysis did not ovulate (0/7) during the 6-d period following treatment. Time to complete luteolysis and ovulation was 29.1 ± 1.1 versus 29.4 ± 1.7 and 101 versus 103 h posttreatment in cloprostenol compared with dinoprost. A negative relationship was observed between P_4 at 12 h posttreatment and concentrations of E_2 48 h posttreatment ($b = -0.6905$; $R^2 = 0.23$). In summary, cows treated with cloprostenol had lower concentrations of P_4 for the first 12 h following treatment and subsequently greater concentrations of E_2 compared with dinoprost, although no differences were observed in these 2 $PGF_{2\alpha}$ analogs for time to complete luteolysis or time to ovulation.

Key words: cloprostenol sodium, dinoprost tromethamine, luteolysis, Ovsynch

INTRODUCTION

Pharmacological control of luteolysis is a key component of Ovsynch programs in lactating dairy cows (GnRH – 7 d – $PGF_{2\alpha}$ – 2 d – GnRH – 16 h – AI; Pursley et al., 1995, 1997a,b). Two types of $PGF_{2\alpha}$ products are commercially available in the United States, dinoprost tromethamine, a tromethamine salt of the natural $PGF_{2\alpha}$, and cloprostenol sodium, a synthetic analog. Cloprostenol has a benzyl chlorine ring that makes this molecule more resistant to endogenous metabolism compared with dinoprost. Therefore, cloprostenol has approximately a 23-fold longer biological half-life compared with dinoprost (McCracken et al., 1999; approximately 3 h vs. 8 min).

Several studies (Pursley et al., 1997b; Moreira et al., 2000; Souza et al., 2007) using Ovsynch indicated that incomplete or delayed luteolysis following the final $PGF_{2\alpha}$ is a problem. At the time of $PGF_{2\alpha}$ of Ovsynch more than 1 corpus luteum (**CL**) could be present depending on the stage of the estrous cycle at which Ovsynch is initiated. For example, if Ovsynch is initiated following deviation of the dominant follicle from subordinates during the first follicular wave, the dominant follicle will likely ovulate to the GnRH-induced LH surge and a new CL and follicular wave may develop. Thus, at time of $PGF_{2\alpha}$ 7 d later, at least a 7-d and an older CL will be present in the ovaries.

Ovsynch induces ovulation with a final GnRH-induced LH surge 48 h after $PGF_{2\alpha}$ (Pursley et al., 1995) to allow for timed-artificial insemination 16 h after final GnRH. Serum concentrations of progesterone (**P_4**) following $PGF_{2\alpha}$ must decline to <0.5 ng/mL by 2 d after

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injection or the probability of pregnancy is diminished (Souza et al., 2007; Brusveen et al., 2009). Subnormal concentrations of P_4 at the time of AI may reduce uterine contractility given that P_4 decreases the number of oxytocin, angiotensin II, and estrogen receptors in the uterus and antagonizes estrogen induction of estrogen receptors in the uterus, especially in the myometrium (Graham and Clarke, 1997). Delayed regression (prolonged P_4 clearance) or incomplete regression may have a negative indirect effect on estradiol-17β (**E_2**) production (Bridges and Fortune, 2003).

The objective was to determine differences in dynamics of luteolysis between dinoprost tromethamine and cloprostenol sodium at the $PGF_{2\alpha}$ injection of the Ovsynch protocol without the final administration of GnRH. We hypothesized that circulating concentrations of P_4 would be lower following cloprostenol sodium compared with dinoprost tromethamine utilized at the $PGF_{2\alpha}$ of Ovsynch and in turn enhance subsequent concentrations of E_2 compared with dinoprost tromethamine.

MATERIALS AND METHODS

Management of Cows

This trial was conducted between May and October 2007 at the Michigan State University Dairy Teaching and Research facility (East Lansing). Four groups of lactating Holstein dairy cows between 45 and 85 DIM with milk production (mean ± SEM) during the week of treatment of 45.6 ± 0.32 kg/d were used. Cows were housed in a tie-stall barn with free access to water and fed twice daily with a TMR consisting primarily of corn and alfalfa silages and ground corn balanced to NRC (2001) recommendations. Cows were milked twice daily. The Institutional Animal Care and Use Committee at Michigan State University approved all animal-related procedures.

Calculation of Sample Size and Definition of Complete Luteolysis

Number of cows needed per treatment was calculated utilizing G*Power 3.0 (Faul et al., 2007). The "MANOVA Test for Repeated Measures Between Factors" power analysis indicated that 26 subjects were needed to detect a 0.5 ng/mL difference in P_4 concentrations between 2 treatments with $\alpha = 0.05$ and $\beta = 0.8$. To determine the effect of treatment on P_4 and E_2 following treatment, only cows that had complete luteolysis (defined by cows with serum P_4 concentrations <0.5 ng/mL 56 h following treatment) and had continued decrease of serum P_4 concentrations for the next 3 d were used. Effect of treatment on a percentage of cows with complete luteolysis was analyzed in a larger group of cows in a subsequent trial reported in the companion paper (Martins et al., 2011). Approximately 15% of cows were expected to have incomplete luteolysis; therefore, "n" was increased to account for this difference.

Treatment and Data Collection

Fifty-four cows received pre-Ovsynch treatments, which consisted of an injection of 500 μg of cloprostenol (**P1**; Estrumate, Schering-Plough Animal Health, Union, NJ), followed in 2 d with 100 μg of GnRH (**G1**; Ovacyst, IVX Animal Health Inc., St. Joseph, MO); 6 d later, the first GnRH of Ovsynch was initiated (100 μg of GnRH, **G2**; Figure 1). Only cows that had basal concentrations of P_4 after P1 and ovulation characterized by the disappearance of a dominant follicle and subsequent formation of a new CL by 48 h after G1 and G2 were included in the experiment. Cows (n = 35) that met these criteria were blocked by parity and randomly assigned within block to treatments of either 500 μg of cloprostenol (Estrumate; n = 17) or 25 mg of dinoprost (Lutalyse, Pfizer Animal Health, Kalamazoo, MI; n = 18) 7 d after G2. Retrospectively, cows with decreasing P_4 before treatment were excluded from analyses. Although Ovsynch normally includes GnRH approximately 2 d following $PGF_{2\alpha}$, the experiment did not include this final GnRH to assess the effect of treatment on follicular growth and peak concentrations of E_2. Thus, cows were allowed to manifest estrus and ovulation via endogenous mechanisms. All injections were administered i.m. with either an 18-gauge (injections of $PGF_{2\alpha}$) or a 20-gauge (injections of GnRH) 3.8-cm needle in the semimembranosus or semitendinosus muscles.

Blood samples were collected daily from P1 until treatment by coccygeal venipuncture before ultrasound examinations and before any injection of either GnRH or $PGF_{2\alpha}$. An indwelling catheter was placed in the jugular vein 2 or 3 d before treatment (0 h) to collect blood samples at frequent intervals. Blood samples were collected from jugular catheters from treatment (0 h) to ovulation or 144 h after treatment if cows did not ovulate. Blood collections were made every 60 min for the first 12 h after treatment, every 2 h between 12 and 24 h, every 4 h between 24 h and 72 h, and every 6 h between 72 h and ovulation or 144 h. Blood samples were collected using Vacutainer tubes without anticoagulant (BD Vacutainer, Preanalytical Solutions, Franklin Lakes, NJ) and refrigerated for 6 to 12 h. Se-

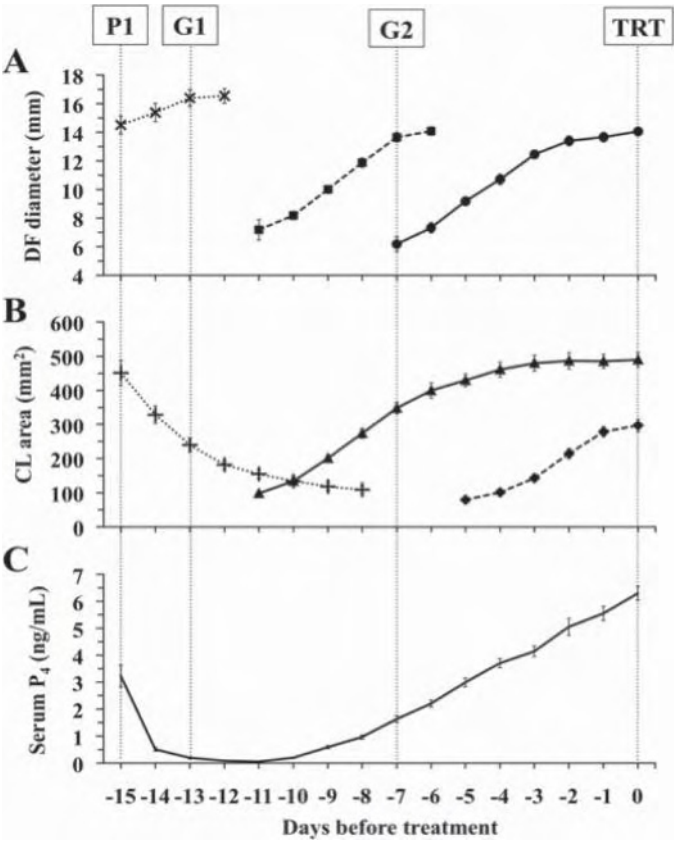

Figure 1. Description of follicle and corpora lutea (CL) development and progesterone (P_4) concentrations before treatment in lactating dairy cows. (A) Mean diameter (±SEM) of dominant follicles (DF; mm) in cows (n = 29) at first $PGF_{2\alpha}$ (P1; ···×···) and in cows that ovulated following the first (G1; ···×···) and second (G2; ---■---) injections of GnRH, and the final preovulatory follicle that spontaneously ovulated within 144 h following treatment (TRT; —●—). (B) Corpora lutea area (mm^2) in cows (n = 26) with a single functional CL at first $PGF_{2\alpha}$ (P1; ···+···) and with single ovulations (n = 29) to the first (G1; —▲—) and second (G2; ---♦---) injections of GnRH. (C) Serum concentrations of P_4 (ng/mL) are described in relation to each pretreatment injection of $PGF_{2\alpha}$ (P1) and GnRH (G1 and G2). Timing of pretreatment injections of $PGF_{2\alpha}$ and GnRH is designated with vertical dotted lines. Cows (n = 6) with multiple ovulations following G1 or G2 were not included although growth and development of follicles and CL from these cows had similar patterns to those of cows with single ovulations.

rum was separated by centrifugation at 2,000 × *g* for 20 min at 4°C and stored at −20°C for later hormonal analyses.

Transrectal ultrasound examinations were conducted daily from P1 until treatment, every 12 h for 3 d, and then every 6 h until detection of ovulation or 144 h posttreatment using a real-time, B-mode, Aloka SSD-500V ultrasound machine with a 7.5-MHz linear array probe (Aloka Co. Ltd., Wallingford, CT). Height and width of the largest size of CL and the antrum of each follicle >4 mm in diameter were measured with built-in calipers. Follicular and luteal measurements were recorded in an ovarian map for each cow with date and time of examinations. Mean follicular diameter (D) was calculated by the average of height (H) and width (W) of each follicle (D = H + W/2). If a fluid-filled central cavity was detected within the CL, a cross-section area of the cavity was determined in the same way as a cross-section area of the total CL. Total luteal area of each CL was calculated subtracting the cavity area from the total CL area. The following equation was used to calculate CL and cavity area: 0.5 H × 0.5 W × π (Kastelic et al., 1990). Cows at time of treatment (0 h) had a minimum of 2 and maximum of 4 CL.

Hormonal Assays

Concentrations of P_4 were quantified in serum via RIA (Coat-A-Count Progesterone, Siemens Diagnostics, Los Angeles, CA). Inter- and intraassay CV were 9.2 and 5.5%, respectively, for high P_4 quality controls, and 9.6 and 6.6%, respectively, for low P_4 quality controls. Sensitivity of the assay was 0.02 ng/mL. Estradiol concentrations were quantified in a subset group of serum samples, including samples 0 (time of treatment), 24, and 48 h, and every 12 h between 48 h and ovulation or until 144 h in cows that did not ovulate. Serum samples (500 μL) were ether extracted in duplicate and then measured using a modified version of a commercially available RIA MAIA kit (Polymedco Inc., Cortland Manor, NY; Prendiville et al., 1995). Inter- and intraassay CV were 8.9 and 13.5%, respectively. Sensitivity of the assay was 0.5 pg/mL.

Statistical Analyses

Data were tested for normality of residuals with the Shapiro-Wilk test or Studentized residual plots for each variable. Variables that did not fulfill assumptions for normality were transformed by natural log and reanalyzed. For clarity, actual means of the data are presented. Discrete variables were analyzed by least-squares ANOVA, using the GLM procedure of SPSS (version 16.0, SPSS Inc., Chicago, IL). Repeated-measures variables such as mean concentrations of P_4 and E_2 over time were analyzed using the MIXED procedure with the REPEATED statement with cows nested in treatment specified in the SUBJECT option of SAS (version 9.2, SAS Inst. Inc., Cary, NC). For the MIXED procedure, fit statistic parameters for unstructured, compound symmetry, first-order autoregressive, heterogeneous compound symmetry, and heterogeneous first-order autoregressive covariance structures were tested. The covariance structure with the lowest values for the Bayesian information criterion was used for the analyses. Only cows that had complete luteolysis and

ovulation by 144 h after treatment were analyzed for repeated measures. Serum P_4 concentrations over time were analyzed in the following periods: −24 h from treatment to 12 h after treatment, 12 to 24 h, 24 to 48 h, and 48 to 90 h after treatment. Parity, treatment, time, and their interactions were included in the original model. Interactions that were $P > 0.2$ were removed from the model. Only time, treatment, parity, treatment by time, and parity by time remained in the final model for effect of treatment on serum P_4 concentrations. Serum concentrations of E_2 were analyzed from 0 to 96 h after treatment. In addition, parity, treatment, time, and their interactions were included in the original model. Only treatment, time, and their interactions remained in the final model for serum E_2 analyses. Parity, treatment by parity, parity by time, and group by parity by time interactions were not significant ($P > 0.20$) and were removed from the final model. A one-tailed test was utilized for treatment effects because our hypothesis was that P_4 would be less and E_2 would be greater following treatment with cloprostenol.

Effects of ovulatory follicle diameter at time of G1 and G2 on P_4 and CL area at time of treatment were tested using regression analyses with the REG procedure of SAS. Effect of serum P_4 concentrations at 12 h after treatment on serum E_2 concentrations at 24 and 48 h after treatment were analyzed by the REG procedure of SAS. Effect of time from complete luteolysis to ovulation was analyzed by the REG procedure of SAS.

RESULTS

Pretreatment Validation

Figure 1 describes dominant follicle and CL growth before treatment. All cows (n = 35) had luteolysis following P1 and ovulation and a new follicular wave following G1 and G2. Thus, all cows had at least 1 d-13 and 1 d-7 CL and a preovulatory follicle 7 d from induction (G2) of emergence at time of treatment. Three cows had 2 d-13 and 1 d-7 CL, one cow had 1 d-13 and 2 d-7 CL, and 2 cows had 2 d-13 and 2 d-7 CL (4 cows were in the cloprostenol sodium group and 2 cows were in the dinoprost tromethamine group). The diameter of preovulatory follicles at time of treatment for cows with single ovulations (n = 26) did not differ ($P = 0.12$) between treatments or parities ($P = 0.15$) and averaged 13.3 ± 0.3 mm. Luteal areas of d-13 CL and d-7 CL were not different ($P = 0.6$ and $P = 0.6$) between treatments, with areas of 446.3 ± 17.8 mm^2 and 273.2 ± 17.1 mm^2, respectively. Serum P_4 concentrations 24 h before (5.4 ± 0.2 and 5.6 ± 0.3 ng/mL for cloprostenol sodium and dinoprost tromethamine; $P = 0.6$) and at time of treatment (6.0 ± 0.3 and 6.3 ± 0.4 ng/mL for cloprostenol sodium vs. dinoprost tromethamine; $P = 0.5$) were not different between treatments. Serum P_4 concentrations for treatments combined were 6.1 ± 0.2 ng/mL at time of treatment (Figure 1), ranging from 3.6 to 9.0 ng/mL in individual cows.

Follicle size at time of G1 and G2 had a direct effect on size and function of the resulting CL (Figure 2). In follicles that ovulated in response to G1 and G2, the sizes of follicles at time of each injection of GnRH were related to luteal area (G1, $P = 0.01$; G2, $P = 0.04$) and P_4 concentrations (G1, $P < 0.001$; G2, $P < 0.001$) at time of treatment.

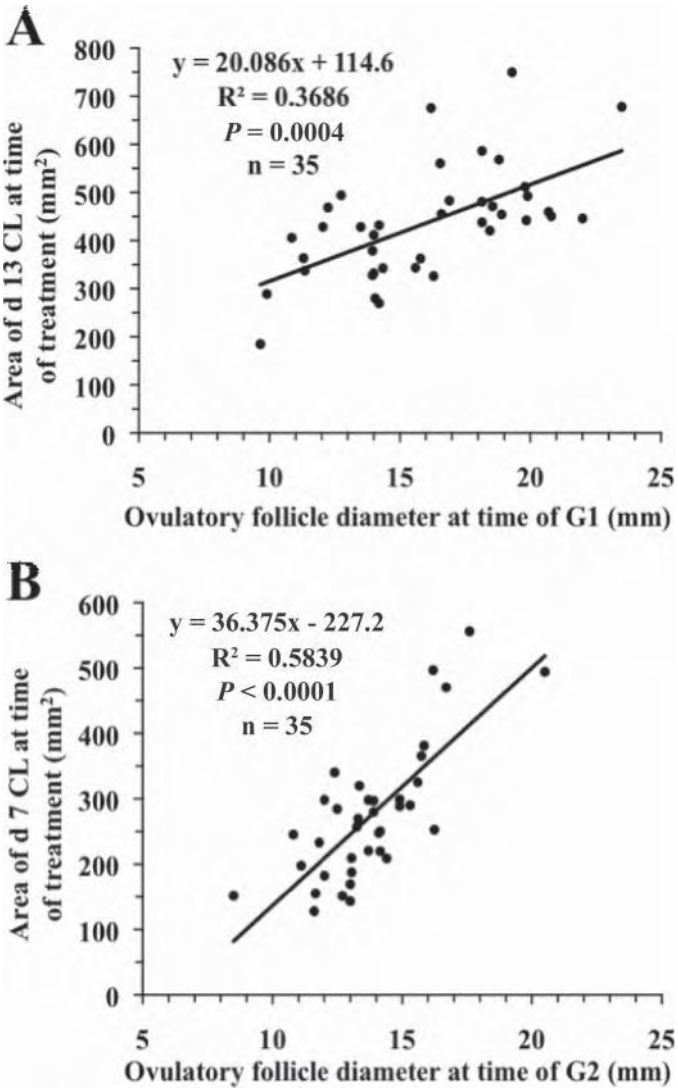

Figure 2. Regression analyses of the relationship between ovulatory follicle diameter (mm) at time of the first (G1; A) and second (G2; B) injections of GnRH and area of corpora lutea (CL; mm^2) that developed following ovulation of these ovulatory follicles measured at time of treatment with $PGF_{2\alpha}$ (n = 35) in lactating dairy cows.

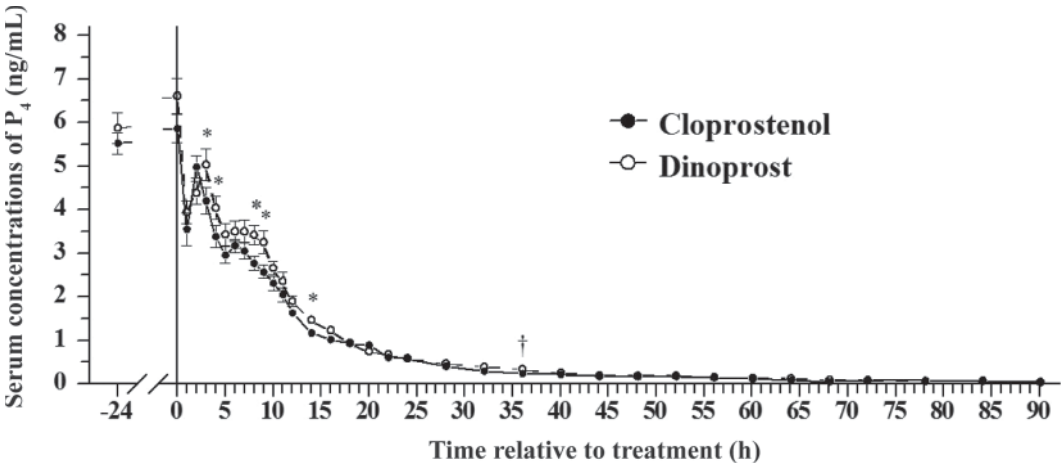

Figure 3. Mean (±SEM) serum progesterone (P_4) concentrations (ng/mL) from 1 d before to 90 h following treatment with cloprostenol sodium compared with dinoprost tromethamine in lactating dairy cows with at least one d-7 corpus luteum (CL) and one d-13 CL that had complete luteolysis by 56 h after $PGF_{2\alpha}$ and ovulated by 144 h after treatment (27/35). Concentrations of P_4 during this first 12-h period were different between treatments ($P = 0.025$) but not different from 12 to 24 ($P = 0.46$), 24 to 48 ($P = 0.16$), or 48 to 90 h ($P = 0.12$) after treatment. †$P < 0.10$ and *$P < 0.05$.

Milk production from 3 d before to 3 d after treatments was not different ($P = 0.3$) between treatments (44.0 ± 2.9 and 47.2 ± 2.5 kg/d for cloprostenol sodium and dinoprost tromethamine, respectively). Milk production was different ($P < 0.0001$) among first (n = 12; 35.4 ± 1.5 kg/d), second (n = 16; 47.0 ± 2.0 kg/d) and ≥third parities (n = 7; 60.0 ± 2.5 kg/d). Parity had no effect ($P = 0.9$) on treatment outcomes.

Effect of Treatment on Dynamics of Luteolysis and Ovulation

An effect of $PGF_{2\alpha}$ type on serum concentrations of P_4 was observed for the first 12 h following treatment ($P = 0.025$; Figure 3). Cows treated with cloprostenol sodium had a more rapid decrease in P_4 during this period compared with dinoprost tromethamine. Specifically, cows treated with cloprostenol sodium had lower serum P_4 concentrations 3 ($P = 0.021$), 4 ($P = 0.025$), 8 ($P = 0.015$), and 9 h ($P = 0.008$) after treatment. Mean serum P_4 concentrations were not different between types of $PGF_{2\alpha}$ from 12 to 24 h ($P = 0.46$; Figure 3). Cows treated with cloprostenol sodium had lower ($P = 0.02$) serum P_4 concentrations at 14 h after treatment compared with cows treated with dinoprost tromethamine. Treatment effects were not apparent ($P = 0.16$) 24 to 48 h posttreatment, and no effect of treatment ($P = 0.12$) was observed from 48 to 90 h posttreatment.

Mean time between treatment and complete luteolysis ranged from 18 to 40 h and was not different ($P = 0.8$) between cloprostenol sodium (29.1 ± 1.1 h) and dinoprost tromethamine groups (29.4 ± 1.7 h). Mean interval from treatment to ovulation ranged from 87 to 123 h and was not different ($P = 0.56$) between cloprostenol sodium (101 ± 2 h) and dinoprost tromethamine (103 ± 2 h) treatments. Interval from complete luteolysis to ovulation ranged from 55 to 93 h, with a mean interval of 72.5 ± 8.3 h. Parity did affect ($P = 0.04$) time to regression. Second-parity cows had a shorter time to complete luteolysis compared with first-parity cows (26.5 vs. 32.0 h; $P = 0.02$), with a tendency observed for the comparison with ≥third-parity cows (26.5 vs. 31.6 h; $P = 0.07$). The size of the preovulatory follicles at the final measurement before ovulation for cows with single ovulations was not different ($P = 0.39$) for cloprostenol sodium (18.0 ± 0.5 mm) and dinoprost tromethamine (18.6 ± 0.4 mm).

Seven cows did not have complete luteolysis. Two of the 7 cows that did not have complete luteolysis had delayed luteolysis at 90 and 114 h from treatment and ovulated 156 and 180 h after treatment, respectively, with intervals of 66 h between complete luteolysis and ovulation. Five of the 7 cows that did not have complete luteolysis had a decrease in serum P_4 concentrations to <1 ng/mL 36 h posttreatment. Four of these cows subsequently had an increase in serum P_4 concentrations to >1 ng/mL from 36 to 84 h after treatment, and 1 of these cows maintained subluteal concentrations (between 0.5 and 1 ng/mL) until 144 h posttreatment. Examples of cows that did not have complete luteolysis by 56 h posttreatment are in Figure 4.

Effect of Treatment on Subsequent Concentrations of E_2

Mean serum E_2 concentrations were greater ($P = 0.02$) at 48 h after treatment for cloprostenol sodium compared with dinoprost tromethamine (Figure 5).

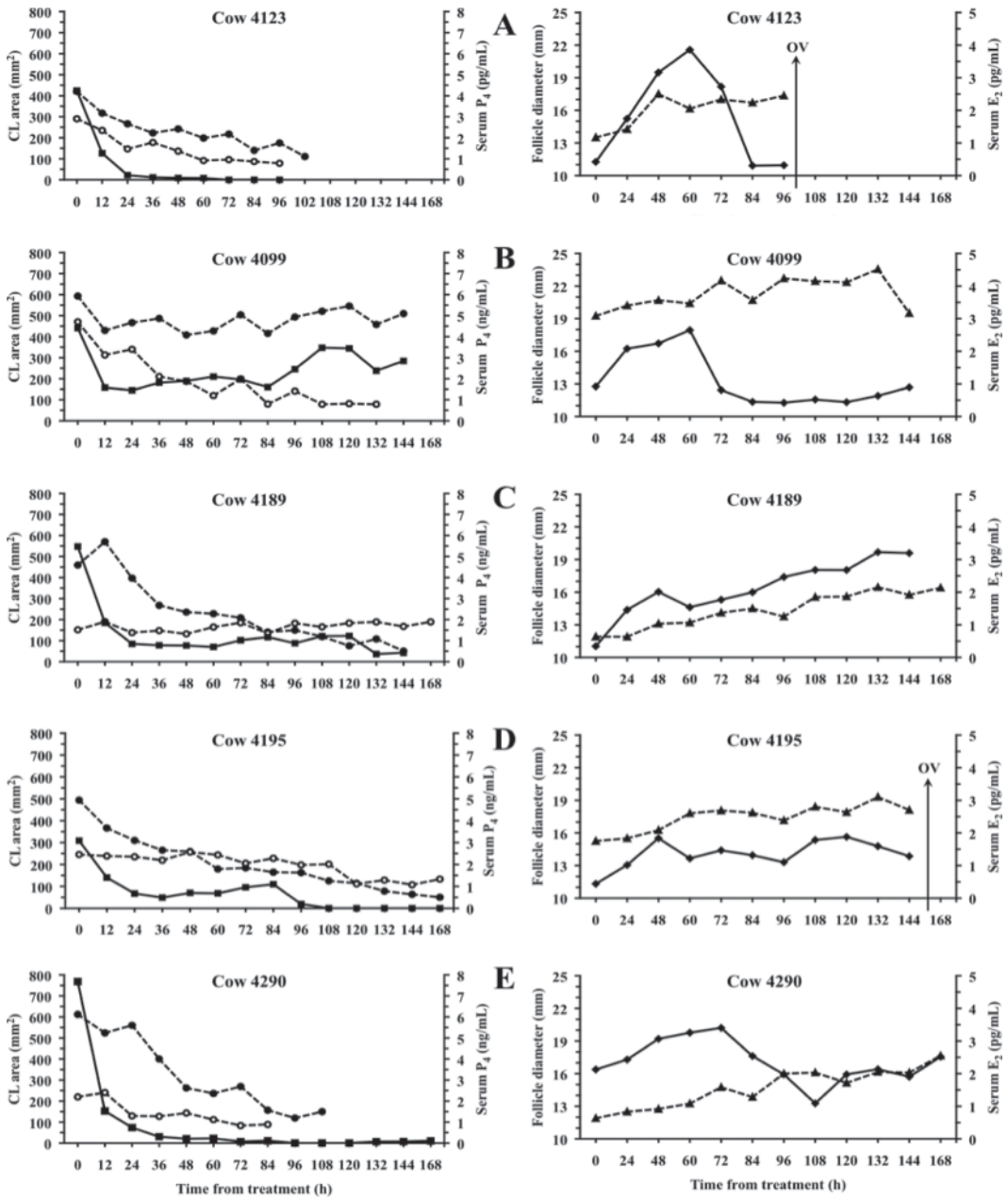

Figure 4. Individual profiles of lactating dairy cows that typified various outcomes following treatment with cloprostenol sodium compared with dinoprost tromethamine in lactating dairy cows. Graphs on left show corpora lutea (CL) areas (mm^2) of d 13 (---●---) and d 7 CL (---○---), and serum concentrations of P_4 (— ■ —) after treatment. Graphs on right show dominant follicle diameter (---▲---) and serum concentrations of estradiol (E_2; — ♦ —) after treatment. Panel A (cow # 4123) describes a cow that had complete luteolysis by 56 h posttreatment and ovulation (OV) before 144 h posttreatment (27/35); panel B (cow # 4099) describes a cow in which the d-13 CL did not regress and the dominant follicle did not ovulate (3/35); panel C (cow # 4189) describes a cow in which the d-7 CL did not regress and the dominant follicle did not ovulate (2/35); panel D (cow # 4195) describes a cow with delayed luteolysis after 56 h posttreatment and ovulation after 144 h posttreatment (2/35); and panel E (cow # 4290) describes the cow that underwent luteolysis before 56 h posttreatment, but did not ovulate (1/35).

Serum concentrations of E_2 were less ($P = 0.048$) at 84 and 96 h ($P = 0.05$) in cloprostenol sodium compared with dinoprost tromethamine after treatment. No association was found for peak concentrations of E_2 and time from treatment to ovulation ($P = 0.83$).

Dynamics of Luteolysis with Treatments Combined

Serum concentrations of P_4 decreased ($P < 0.0001$) from 6.54 ± 0.27 to 3.77 ± 0.22 ng/mL in the first hour after treatment. Subsequently, a significant increase (P

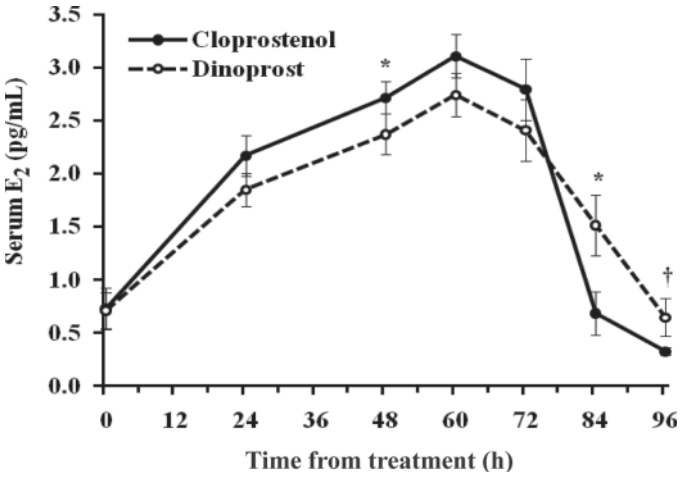

Figure 5. Mean (±SEM) serum concentrations of estradiol (E_2; pg/mL) normalized to $PGF_{2\alpha}$ treatment in lactating dairy cows with at least one d-7 and one d-13 corpora lutea (CL) and treated with cloprostenol sodium or dinoprost tromethamine that had complete luteolysis by 56 h after $PGF_{2\alpha}$ and ovulated by 144 h after treatment (27/35). Mean serum E_2 concentrations were greater ($P = 0.02$) at 48 h after treatment for cloprostenol sodium compared with dinoprost tromethamine. Serum concentrations of E_2 were less at 84 h ($P = 0.048$) and 96 h ($P = 0.05$) after treatment in cloprostenol sodium compared with dinoprost tromethamine. †$P = 0.05$ and *$P < 0.05$.

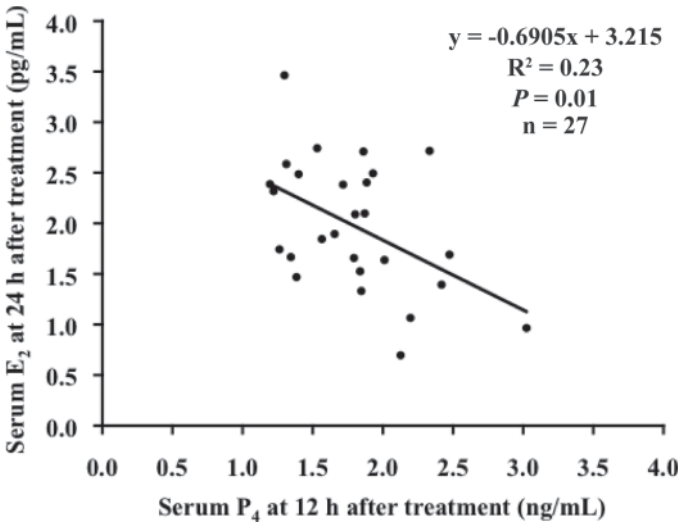

Figure 6. Regression analysis of the relationship between serum concentrations of progesterone (P_4) 12 h after treatment and serum concentrations of estradiol (E_2) 24 h after treatment in lactating dairy cows with treatments combined. Only cows with complete luteolysis and ovulation (n = 27) were included.

< 0.0001) in serum P_4 occurred in the second hour after treatment from 3.77 ± 0.22 to 5.07 ± 0.31 ng/mL. Regression analyses indicated that serum concentrations of E_2 at 24 h after PGF_{2a} injection were greater in cows when their serum concentrations of P_4 at 12 h after $PGF_{2\alpha}$ were lower ($P = 0.01$; Figure 6).

Time from treatment to ovulation was positively related ($P < 0.0001$) to time from treatment to complete luteolysis (Figure 7). The regression equation for prediction of time from treatment to ovulation was 76.2 h + (0.88 × time from treatment to complete luteolysis).

DISCUSSION

Results of the present study indicate that cloprostenol sodium had lower serum concentrations of P_4 following induced luteolysis for the initial 12-h period compared with dinoprost tromethamine. This difference might be due to the difference in resistance to endogenous metabolism between the products. Dinoprost tromethamine is metabolized in a similar mechanism to that of endogenous $PGF_{2\alpha}$ by 3 major steps. The initial metabolic step is the dehydrogenation of the OH group on C-15 and reduction of the double bond at carbons 13–14 by the enzymes 15-hydroxydehydrogenase and 13,14-reductase. This rapid, initial metabolic step is followed by β-oxidation and ω-oxidation (Oesterling et al., 1972; Bourne and Hathway, 1979; Bourne et al., 1980). Approximately 65% of dinoprost is metabolized in one passage through the lungs in the cow (McCracken et al., 1999). Cloprostenol has an oxyaryl moiety structure that blocks ω-oxidation and the action of the dehydrogenase and reductase enzymes and reduces β-oxidation (Bourne et al., 1980). Therefore, cloprostenol is more resistant to endogenous metabolism and as a result has a much longer biological half-life (~3 h; Reeves, 1978) than dinoprost tromethamine (~7–8 min; Kindahl et al., 1976). Thus, the added time that cloprostenol is

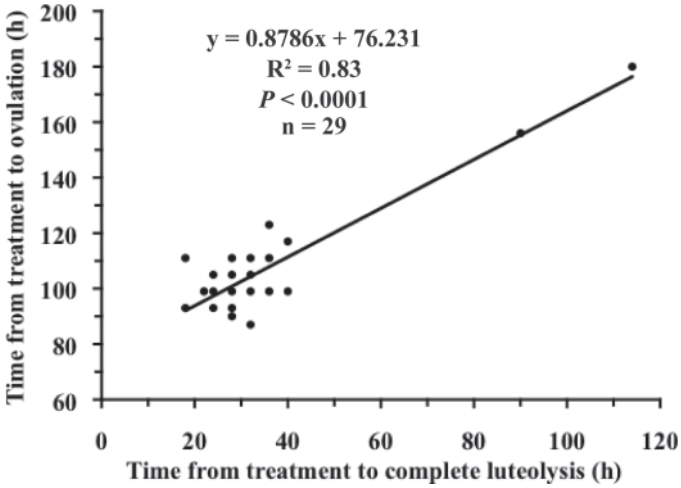

Figure 7. Regression analysis of the relationship between time from treatment to complete luteolysis and time from treatment to ovulation. All cows that had complete luteolysis (including delayed luteolysis) and ovulation (including ovulations after 144 h) were included in this analysis (n = 29).

in circulation may have affected concentrations of P_4 during the first 12 h posttreatment.

Subsequent concentrations of E_2 were enhanced following treatment with cloprostenol sodium compared with dinoprost tromethamine. This difference may be due to increased pulses of LH due to a greater decrease in P4 in the cloprostenol group in the first 12 h following treatment. A study by Bergfeld et al. (1996) indicated that a more rapid decrease in P_4 increased LH pulsatility, which in turn enhanced E_2 production of the dominant follicle. When treatments were combined, a significant relationship was observed between the level of P_4 in cows 12 h after treatment and subsequent concentrations of E_2 in these cows 24 h posttreatment.

Luteolysis is one of several key steps in Ovsynch that is limiting to fertility in cows that are timed-inseminated following Ovsynch. Previous data from our laboratory indicated that initiating Ovsynch on d 6 of the estrous cycle increased P_4 before $PGF_{2\alpha}$ of Ovsynch and more consistently controlled growth of the ovulatory follicle (Bello et al., 2006). In that study, both the level of P_4 at the time of $PGF_{2\alpha}$ of Ovsynch and the size of the ovulatory follicle at time of final GnRH of Ovsynch were key factors in predicting the probability of pregnancy. These data indicated that the greater the ovulation rate to the first GnRH of Ovsynch, the greater the percentage of cows with accessory CL and increased P_4. Three recently developed presynchronization strategies, Presynch-11(Galvão et al., 2007), G6G (Bello et al., 2006), and Double Ovsynch (Souza et al., 2008), increased the percentage of cows that had first-wave dominant follicles that could be responsive to the LH surge induced by the first injection of GnRH of Ovsynch compared with cows treated with Ovsynch at random stages of the estrous cycle. Thus, 7 d later at the time of the $PGF_{2\alpha}$ of Ovsynch, most cows that were presynchronized with these strategies would have a mature and an accessory CL that would have to undergo luteolysis to a single dose of $PGF_{2\alpha}$. In the current study, we chose to ensure that all cows had at least one d-13 and one d-7 CL at time of treatment to test potential differences between cloprostenol sodium and dinoprost tromethamine in the decrease of P_4 in cows in this scenario. Our findings indicate that most cows had complete luteolysis, by our definition, following a single dose of cloprostenol sodium or dinoprost tromethamine. The only differences detected between these 2 luteolysins were decreases in P_4 in the initial 12 h posttreatment and an increase in E_2 48 h posttreatment. Otherwise, no differences were uncovered in this study.

Because we used limited numbers of cows in this study to determine differences between treatments in percentages of cows that did not have complete luteolysis, we report the efficacy of induced CL regression with cloprostenol sodium compared with dinoprost tromethamine in much greater numbers of cows in the companion paper (Martins et al., 2011).

A significant decrease in P_4 levels was observed in the first hour after treatment followed by a rebound in the second hour for each cow. Ginther et al. (2007) reported a similar response in P_4 concentrations in dairy heifers. This may be due to an acute increase in oxytocin. Studies indicated that $PGF_{2\alpha}$ injection acutely stimulated the release of oxytocin in vivo (Shaw and Britt, 2000; Keator et al., 2008) approximately 10-fold within 15 min. In the current study, all cows had a rebound in serum P_4 concentrations in the second hour after treatment, and then all cows had a consistent decrease in P_4, with the exception of the few cows that did not have complete CL regression. Time to complete functional luteolysis had a highly positive association with time to ovulation. Peak concentrations of E_2 did not appear to influence time to ovulation, although all cows had an ovulatory follicle that was likely functional at time of treatment. The majority of cows had a 60- to 80-h interval between time that P_4 concentrations fell below 0.5 ng/mL and ovulation. Cows that did not have a decrease in P_4 concentration to <0.5 ng/mL 56 h posttreatment did not have ovulation of the dominant follicle. These follicles were most likely functional at time of $PGF_{2\alpha}$ due to the increase in E_2 in each of these cows following treatment and the lack of a new follicular wave in the days that followed. In Ovsynch programs, the final GnRH-induced LH surge will likely ovulate this dominant follicle. Yet, if luteolysis does not occur in a timely manner, as seen in some cows of this study, conception rates may be affected negatively.

CONCLUSIONS

Cloprostenol sodium induced a greater decrease in serum P_4 concentrations during the first 12 h following treatment compared with dinoprost tromethamine. No differences in the decrease in P_4 were observed from 12 h until 144 h after treatment. The initial difference in the decrease in P_4 during the first 12 h appeared to result in an increase in serum E_2 concentration in cows treated with cloprostenol sodium.

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