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# Effects of cloprostenol sodium at final prostaglandin $F_{2\alpha}$ of Ovsynch on complete luteolysis and pregnancy per artificial insemination in lactating dairy cows

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## ABSTRACT

Luteolysis is a key event in Ovsynch programs of lactating dairy cows. Studies indicate that as many as 20% of cows treated with a Presynch/Ovsynch program have delayed or incomplete luteolysis using dinoprost tromethamine. Cows must have complete luteolysis to have a chance to become pregnant. Dinoprost tromethamine has a short half-life of approximately 7 to 8 min. Cloprostenol sodium is more resistant to endogenous metabolism and is maintained in circulation for a longer time (half-life = 3 h). The objective was to determine if cloprostenol sodium could increase the percentage of cows with complete luteolysis and subsequent pregnancy per artificial insemination (P/AI) in lactating dairy cows compared with dinoprost tromethamine when administered within a presynchronization plus Ovsynch program for first artificial insemination (n = 652) and an Ovsynch resynchronization program for second or later AI (second+; n = 394). Blood samples were collected daily for 5 d beginning at the $PGF_{2\alpha}$ of Ovsynch in a subset of cows (n = 680) for first and second+ AI to measure circulating concentrations of progesterone ($P_4$) and estradiol ($E_2$). Complete luteolysis was defined as cows with functional corpus luteum (CL) at time of treatment and serum concentrations of $P_4$ <0.5 ng/mL at 56, 72, and 96 h after treatment. Percentage of cows with functional CL that had complete luteolysis after treatment was not greater for cloprostenol sodium compared with dinoprost tromethamine in first (79 vs. 80%, respectively) or second+ AI (70 vs. 72%, respectively). In addition, mean serum concentrations of $P_4$ were not less for cows treated with cloprostenol sodium following treatment. Pregnancy per AI of cows treated with cloprostenol sodium tended to be greater than dinoprost tromethamine for first (40 vs. 35%; respectively) but not second+ AI (23 vs. 21%, respectively). Cows with greater serum $P_4$ concentrations at time of $PGF_{2\alpha}$ of Ovsynch had a greater probability of undergoing complete luteolysis after $PGF_{2\alpha}$ of Ovsynch and pregnancy at 39 d after timed AI (i.e., 50% pregnant at 8 vs. 28% pregnant at 4 ng/mL $P_4$). Serum concentrations of $E_2$ at 56 h after $PGF_{2\alpha}$ of Ovsynch were a positive predictor of pregnancy at 39 d after timed AI. In summary, cloprostenol sodium tended to improve P/AI. Cows with greater serum concentrations of $P_4$ at time of $PGF_{2\alpha}$ of Ovsynch had a greater chance of luteolysis and pregnancy.
**Key words:** cloprostenol sodium, dinoprost tromethamine, luteolysis, Ovsynch

## INTRODUCTION

Insufficient luteolysis is a rate-limiting factor for successful pregnancy per AI (**P/AI**) following Ovsynch (Souza et al., 2007; Brusveen et al., 2009). Data from Souza et al. (2007) indicated that cows with progesterone (**$P_4$**) concentrations >0.5 ng/mL 2 d after $PGF_{2\alpha}$ injection of Ovsynch had a 50% decrease in P/AI compared with cows <0.5 ng/mL. The percentage of cows that do not have complete luteolysis following $PGF_{2\alpha}$ of Ovsynch varies among studies, ranging from 5 to 20% (Moreira et al., 2000; Gümen et al., 2003; Brusveen et al., 2009). These studies used only dinoprost tromethamine, a tromethamine salt of the natural $PGF_{2\alpha}$. In addition to the importance of complete luteolysis, the amount of time for $P_4$ to decrease to basal levels after $PGF_{2\alpha}$ injection may play a critical role in the probability of a pregnancy following Ovsynch. Cows with complete luteolysis that had a more rapid decrease in circulating $P_4$ concentrations were more fertile than those with a slower decline in $P_4$ levels (Brusveen et al., 2009). The mechanism(s) involved in how subluteal concentrations of $P_4$ (0.5 to 1.0 ng/mL) 2 d following the $PGF_{2\alpha}$ of Ovsynch decreases P/AI is not clear. It is likely not due to lack of ovulation because previous studies have shown that cows have a high ovulatory response (>90%) to the final GnRH of Ovsynch (Pursley et al., 1995), even under high concentrations of $P_4$ (Bello et al., 2006).

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Dinoprost has a short half-life (7 to 8 min; Kindahl et al., 1976) and is rapidly metabolized in a similar manner to endogenous $PGF_{2\alpha}$ metabolism (Bourne et al., 1980; McCracken et al., 1999). A second injection of dinoprost tromethamine 24 h after the first injection improved the percentage of cows that had complete luteolysis (Brusveen et al., 2009). Cloprostenol is a more potent synthetic analog of $PGF_{2\alpha}$ due to the amount of product (0.5 mg of cloprostenol vs. 25 mg of dinoprost) needed to induce luteolysis (Dukes et al., 1974) and is commercially available in the United States. Cloprostenol has an oxyaryl function that reduces rate of metabolism (Bourne et al., 1980); thus, it is more resistant to endogenous metabolism and has a much longer half-life (approximately 3 h; Reeves, 1978) compared with dinoprost. Studies have compared the differences in luteolysis and fertility between these drugs in lactating dairy cows with mixed results (Seguin et al., 1985; Martineau, 2003; Répási et al., 2005). Yet it is not clear whether differences exist between these luteolytic agents in Ovsynch programs such as G6G, Presynch-11, and Double-Ovsynch (Bello et al., 2006; Galvão et al., 2007; Souza et al., 2008) that create multiple corpora lutea (**CL**) at time of the final PGF of Ovsynch. These programs intend to presynchronize most cows to d 6 or 7 of the estrous cycle when the first injection of GnRH of Ovsynch is administered. Cows that were on d 6 of the estrous cycle at the time of GnRH had a 97% chance of ovulation (Bello et al., 2006). If cows respond to the GnRH-induced LH surge, they generally have at least 2 CL (more if double ovulations occur) at the time of the $PGF_{2\alpha}$ of Ovsynch. Greater numbers of CL at time of luteolysis increase $P_4$ (Bello et al., 2006; Stevenson et al., 2007) and cows with greater $P_4$ have a greater probability of pregnancy (Bello et al., 2006). Thus, at least 1 accessory CL 7 d old and an older CL (d 13 or 14) will require luteolysis (Bello et al., 2006; Stevenson et al., 2007) and more if double ovulations occur (Wiltbank et al., 2000).

The overall objectives were (1) to determine the effect of cloprostenol sodium on percentage of cows with complete luteolysis and P/AI in cows treated with a Presynch/Ovsynch program compared with dinoprost tromethamine, and (2) to determine the effect of $P_4$ concentrations at the time of and following treatment on luteolysis and fertility in lactating dairy cows. We hypothesized that cloprostenol would enhance luteolysis in cows with multiple CL and subsequently improve P/AI of lactating dairy cows.

## MATERIALS AND METHODS

This trial was conducted from January to August 2008 at Green Meadow Farms (Elsie, MI). Lactating Holstein cows (n = 862) with milk production between 11.8 and 71.2 kg/d received a total of n = 1,046 AI (n = 652 first AI, n = 394 second+ AI). Cows were housed in a freestall barn with free access to water and were fed a TMR 3 times daily. Cows were separated in pens (n = 4) by parity (first, second, and third or later parities, with 2 pens for the latter). The TMR consisted of corn and alfalfa silages and corn-soybean meal-based concentrates formulated to meet or exceed nutrient recommendations for lactating dairy cows (NRC, 2001). Cows were milked 3 times daily. All injections were administered with single-dose syringes in semimembranosus or semitendinosus muscles of cows by trained personnel from our laboratory with 18-gauge (injections of $PGF_{2\alpha}$) or 20-gauge (injections of GnRH) 3.8-cm needles. The Institutional Animal Care and Use Committee at Michigan State University approved all procedures.

### *Experimental Design Synchronization for First AI*

Lactating Holstein cows (n = 652) were blocked by parity then randomly assigned to treatment on a weekly basis. Controls received 25 mg of dinoprost (Lutalyse, Pfizer Animal Health, Kalamazoo, MI) and treated cows received 500 μg of cloprostenol (Estrumate, Schering Plough Animal Heath, Summit, NJ) for each $PGF_{2\alpha}$ injection of a presynchronization and Ovsynch program. Cows received 100 μg of gonadorelin diacetate tetrahydrate (Cystorelin, Merial Ltd., Duluth, GA) for each GnRH injection of the presynchronization and Ovsynch program. Cows were assigned randomly by parity to 1 of 3 presynchronization protocols beginning 33 to 41 DIM: (1) 2 injections of $PGF_{2\alpha}$ 14 d apart with the second injection 11 d (Galvão et al., 2007) before the first GnRH of Ovsynch (Galvão et al., 2007; Brusveen et al., 2008); (2) 2 injections of $PGF_{2\alpha}$ 14 d apart and an intravaginal progesterone releasing device (**CIDR**; Pfizer Animal Health) inserted at time of the second $PGF_{2\alpha}$ for 7 d, with GnRH administered upon CIDR removal, while the first GnRH of the Ovsynch protocol was administered 6 d later (Martins et al., 2009); and (3) 2 injections of $PGF_{2\alpha}$ 14 d apart and a CIDR inserted at time of the second $PGF_{2\alpha}$ for 5 d with GnRH administered 2 d after CIDR removal. Pregnancy per AI did not differ ($P > 0.4$) for the 3 presynchronization programs and was not considered in the analyses of treatment effects. The first GnRH of Ovsynch was administered 6 d later (Martins et al., 2009). All cows received an Ovsynch program that consisted of GnRH followed in 7 d with $PGF_{2\alpha}$ (controls vs. treatment) and then GnRH 56 h later (Brusveen et al., 2008). All cows received AI 16 h following the final GnRH of Ovsynch (Pursley et al., 1998) at 70 to

76 DIM. Cows detected with presence of mucopurulent vaginal discharge or other clinical signs of acute illness before AI were excluded from the experiment. Five AI technicians performed AI with commercial semen from multiple sires purchased by the farm; technicians were blind to treatments. Pregnancy diagnoses were performed by transrectal palpation 39 d after AI by farm veterinarians who were blind to treatments. A second pregnancy diagnosis was performed 99 ± 3 d after AI in cows determined pregnant at the initial diagnosis. All cows detected in estrus before first pregnancy diagnosis were considered not pregnant.

### Resynchronization of Nonpregnant Cows

All cows that received AI (first and greater) and not re-inseminated following detected estrus received 100 μg of gonadorelin diacetate tetrahydrate (Cystorelin, Merial Ltd.) 32 ± 3 d for the first GnRH of Ovsynch to initiate a resynchronization program. Cows diagnosed not pregnant 7 d later (n = 394) were randomly assigned by parity and service number to receive 25 mg of dinoprost tromethamine (control) or 500 μg of cloprostenol sodium (treated). Cows received an injection of 100 μg of gonadorelin diacetate tetrahydrate 56 h after $PGF_{2\alpha}$ (treatment) for the final GnRH of Ovsynch, and AI was performed approximately 16 h later. Cows received AI by the same technicians and service sires and were diagnosed for pregnancy as in first-AI cows.

### Analysis of Luteal Function

To determine the effect of $PGF_{2\alpha}$ type on percentage of cows with complete luteolysis and P/AI (%), a subset of cows with functional CL was considered in the analyses (n = 490). Cows with functional CL were defined as having decreasing concentrations of $P_4$ from treatment to 24 h after treatment, but maintaining concentrations ≥0.24 and 0.09 ng/mL 24 and 56 h after treatment, respectively. These thresholds were defined based on Martins et al. (2011) that had $P_4$ concentrations before and after treatment in cows with known functional CL. Cows with no $P_4$ on day of treatment were defined as noncycling (no functional CL). Only cows defined as having functional CL were included in the calculation for the effect of treatment on percentage of cows with complete luteolysis (n = 490). Complete induced luteolysis was defined as cows with declining $P_4$ concentrations to <0.5 ng/mL 56 h after $PGF_{2\alpha}$ with a continued decrease to <0.5 ng/mL 72 and 96 h after $PGF_{2\alpha}$ injection. Cows were estimated to have at least 1 d-7 and 1 d-13 CL at time of $PGF_{2\alpha}$ of Ovsynch if concentrations of $P_4$ were between 3.6 and 8.6 ng/mL based on calculations from Martins et al. (2011).

### $P_4$ and Estradiol Assays

Blood samples were collected by coccygeal venipuncture in a subset of first and second+ AI cows (n = 680) at final $PGF_{2\alpha}$ of Ovsynch and daily for 4 d to assess $P_4$ and estradiol (**$E_2$**) concentrations. Blood samples were taken using Vacutainer tubes without anticoagulant (BD Vacutainer, Preanalytical Solutions, Franklin Lakes, NJ) and refrigerated for 6 to 12 h. Serum was then separated by centrifugation at 2,000 × g for 20 min at 4°C and stored at −20°C for later hormonal analyses.

Concentrations of serum $P_4$ were quantified with RIA (Coat-A-Count Progesterone, Siemens Diagnostics, Los Angeles, CA). Intra- and interassay CV were 4.9 and 3.2%, respectively. Sensitivity of the assay was 0.02 ng/mL.

Concentrations of $E_2$ were quantified from blood collected on the day of $PGF_{2\alpha}$ and the next 3 d in a subset of first-AI cows that had functional CL and complete luteolysis (n = 192). Serum samples (500 μL) were ether extracted in duplicate and then measured using a modified version (Prendiville et al., 1995) of a commercially available RIA MAIA kit (Polymedco Inc., Cortland Manor, NY). Intra- and interassay CV were 13.9 and 11.5%, respectively. Sensitivity of the assay was 0.5 pg/mL. Each $P_4$ and $E_2$ assay contained equal numbers of cows by treatments (5 samples and 5 duplicates for $P_4$ and 4 samples and 4 duplicates for $E_2$) and were alternated within assay by treatment.

### Statistical Analyses

Binomial variables were analyzed using the MIXED procedure of SAS (version 9.2, SAS Inst. Inc., Cary, NC). A one-tailed test was used because the hypothesis was that cloprostenol could increase the rate of luteolysis and P/AI. The final model considered treatment, parity, and their interactions as fixed effects and cows as a random effect. No treatment by presynchronization program interaction ($P = 0.9$) was observed; thus, data from the 3 different presynchronization programs were combined.

Repeated-measures variables such as concentrations of $P_4$ and $E_2$ over time were analyzed using the MIXED procedure of SAS with the REPEATED statement and cows nested in treatment specified in the SUBJECT option. Fit statistic parameters were tested in the MIXED procedure. The covariance structure with lowest values for the Bayesian information criterion was used for the analyses. Predicted probabilities of pregnancy were computed using the LOGISTIC procedure of SAS.

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.

## RESULTS

### Effect of Treatment on Luteolysis and P/AI

Treatment did not affect overall mean serum concentrations of $P_4$ during the 5-d period of blood collection ($P = 0.46$) in cows with complete luteolysis following treatment (Figure 1). Treatment did not affect the percentage of cows with complete luteolysis ($P = 0.21$) or when divided into quartiles based on $P_4$ at time of treatment for first service (Table 1; $P > 0.10$). A trend ($P = 0.09$) was observed for cloprostenol sodium to increase P/AI in cows treated with Ovsynch preceded by a presynchronization program for first AI (Table 2) compared with dinoprost tromethamine, but no effect within first, second, or ≥third parities (Figure 2) or following an Ovsynch resynchronization program for second+ AI when diagnosed at 39 or 96 d post-AI (Table 2). The type of $PGF_{2\alpha}$ did not affect ($P = 0.44$) overall mean circulating concentrations of $E_2$ at 0 (0.35 ± 0.03 vs. 0.34 ± 0.03 pg/mL), 24 (1.17 ± 0.05 vs. 1.12 ± 0.05 pg/mL), 56 (1.75 ± 0.08 vs. 1.64 ± 0.08 pg/mL), or 72 (0.30 ± 0.01 vs. 0.31 ± 0.02 pg/mL) h after cloprostenol or dinoprost, respectively, in cows with functional CL at time of $PGF_{2\alpha}$ and with complete luteolysis.

### Effect of Parity and AI Number on Luteolysis and P/AI

First-parity cows (n = 253) had greater first service P/AI (47.8%) compared with second- and ≥third-parity cows (31.4%; n = 398; $P < 0.01$). A greater percentage ($P = 0.03$) of first-AI cows (79 vs. 71) had complete luteolysis following treatment compared with cows with second+ AI (n = 490). In first-AI cows that were estimated to have both a d-7 and a d-13 CL, first-parity cows (n = 180) had greater ($P = 0.03$) concentrations of $P_4$ (6.0 ± 0.16 vs. 5.54 ± 0.11 ng/mL) at time of treatment and a greater percentage of cows ($P < 0.01$) with complete luteolysis compared with second- and ≥third-parity cows (94 vs. 81%) following treatment.

### Relationships Between Concentrations of $P_4$ Pre- and Posttreatment with Luteolysis and Probability of Pregnancy

A positive relationship ($P < 0.001$) was observed between concentrations of $P_4$ at $PGF_{2\alpha}$ of Ovsynch and the predicted probability of cows with complete luteolysis (Figure 3) in cows with functional CL at time of treatment. A positive relationship ($P < 0.001$) was observed between concentrations of $P_4$ at time of treatment and the probability of pregnancy in cows with functional CL at time of treatment (Figure 4). In the same analysis, when considering only cows with complete luteolysis, a trend ($P = 0.08$) was observed for a positive relationship of $P_4$ at time of treatment with the probability of a pregnancy. A greater ($P < 0.001$) percentage of pregnant cows were classed as having >6 ng/mL of $P_4$ at time of treatment compared with nonpregnant cows (Table 3). A greater ($P < 0.01$) percentage of nonpregnant cows were classed as having between 1 and 2 ng/mL $P_4$ at time of treatment compared with pregnant cows (Table 3).

Table 4 describes the shift in percentage of cows that fall into 7 classes of $P_4$ concentrations following treatment. The percentage of cows within classes did not differ 24 h after treatment. Beginning 56 h after treatment and continuing at 72 and 96 h after treatment, significant shifts occurred in the percentage of cows that became pregnant and the cows that were diagnosed not pregnant. At 56 h posttreatment, only 8% of cows with $P_4$ levels between 0.5 and 1 ng/mL (n = 48) became pregnant. At 96 h posttreatment, 94% of pregnant cows had $P_4$ ≤0.3 ng/mL compared with 68% of cows diagnosed not pregnant. In addition, at 96 h posttreatment, 29% of nonpregnant cows had $P_4$

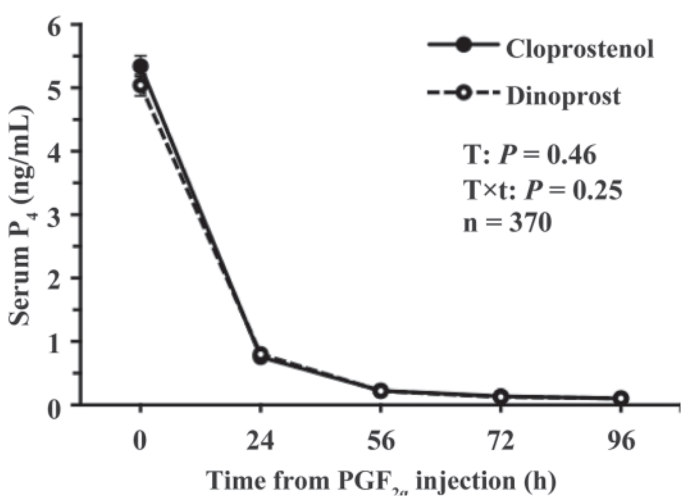


**Figure 1.** Effect of treatment with cloprostenol sodium compared with dinoprost tromethamine on clearance of serum concentrations of progesterone ($P_4$) in lactating dairy cows that received a Presynch/Ovsynch program for first or Ovsynch for second or later AI that had functional corpus luteum (CL; $P_4$ concentrations ≥0.24 ng/mL 24 h and ≥0.09 ng/mL 56 h after treatment) at time of treatment and had undergone complete luteolysis ($P_4$ <0.5 ng/mL 56, 72, and 96 h after $PGF_{2\alpha}$ injection). $P$-values for treatment main effect (T) and interaction of treatment by time (T × t) are shown.

**Table 1.** Effect of treatment with cloprostenol sodium (CLO) or dinoprost tromethamine (DINO) on percentage of cows with complete luteolysis and percentage pregnant in a subset of lactating dairy cows at first AI (n = 258) with a functional corpus luteum[1] at time of treatment within quartiles of concentrations of progesterone ($P_4$) on day of treatment

| Item | Quartile based on $P_4$ at day of $PGF_{2\alpha}$ for first service | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0–25% | | 25–50% | | 50–75% | | 75–100% | | Total | |
| | CLO | DINO | CLO | DINO | CLO | DINO | CLO | DINO | CLO | DINO |
| $P_4$ at day of $PGF_{2\alpha}$ (range; ng/mL) | 0.64–3.78 | 1.00–3.59 | 3.85–5.26 | 3.60–5.03 | 5.31–6.51 | 5.13–6.28 | 6.55–13.74 | 6.38–13.23 | 0.64–13.74 | 1.00–13.23 |
| $P_4$ at day of $PGF_{2\alpha}$ (ng/mL; mean ± SEM) | 2.54 ± 0.14 | 2.29 ± 0.15 | 4.50 ± 0.06 | 4.51 ± 0.07 | 5.91 ± 0.07 | 5.71 ± 0.06 | 8.09 ± 0.27 | 7.84 ± 0.28 | 5.24 ± 0.20 | 5.06 ± 0.20 |
| Cows with complete luteolysis,[2,3] % (n/n) | 52 (17/33) | 67 (22/33) | 91 (29/32) | 81 (26/33) | 81 (26/32) | 84 (26/31) | 94 (30/32) | 91 (29/32) | 79 (102/129) | 80 (103/129) |
| P/AI[4] 39–42 d,[3] % (n/n) | 21 (7/33) | 24 (8/33) | 50 (16/32) | 39 (13/33) | 31 (10/32) | 35 (11/31) | 66 (21/32) | 56 (18/32) | 42 (54/129) | 38 (50/129) |
| P/AI 39–42 d,[3] % of cows with complete luteolysis (n/n) | 41 (7/17) | 36 (8/22) | 55 (16/29) | 50 (13/26) | 38 (10/26) | 38 (10/26) | 70 (21/30) | 59 (17/29) | 53 (54/102) | 48 (48/103)[5] |
| Primiparous[3] (%) | 39 | 30 | 38 | 33 | 41 | 29 | 53 | 62 | 43 | 40 |

[1] $P_4$ concentrations $\geq$0.24 ng/mL 24 h and $\geq$0.09 ng/mL 56 h after treatment.

[2] Complete luteolysis = $P_4$ <0.5 ng/mL 56, 72, and 96 h after $PGF_{2\alpha}$ injection.

[3] No differences between treatments ($P > 0.05$).

[4] P/AI = pregnancy per artificial insemination.

[5] Two cows that did not have complete luteolysis by our definition conceived.

>0.5 ng/mL compared with 2% of pregnant cows ($P < 0.001$). The relationship between concentrations of $P_4$ 56 h after treatment with the predicted probability of pregnancy was significant ($P = 0.001$) among cows that were <1.0 ng/mL P4 at that time, but was not significant ($P = 0.80$) in cows that were <0.5 ng/mL at 56 h posttreatment.

### *Analysis of Cows with Incomplete Luteolysis*

Cows that did not have complete luteolysis (n = 119) were separated into 3 groups of $P_4$ dynamics based on concentrations of $P_4$ at 96 h posttreatment: delayed luteolysis ($P_4$ levels <0.5 ng/mL at 96 h after $PGF_{2\alpha}$ injection), incomplete luteolysis ($P_4$ levels between 0.5 and 1 ng/mL at 96 h after injection), or no luteolysis ($P_4$ levels >1.0 ng/mL at 96 h after injection), and were compared with cows with complete luteolysis (Figure 5) throughout the collection period. The number of cows that were pregnant at first pregnancy diagnosis was 150/370 (40.5%) for complete, 4/19 (21.1%) for delayed, 3/32 (9.3%) for incomplete, and 0/68 for the no luteolysis groups.

### *Effect of Serum $E_2$ Concentration at Time of Final GnRH of Ovsynch on Pregnancy Outcome*

A positive relationship was observed between concentrations of $E_2$ at final GnRH of Ovsynch and the predicted probability of pregnancy (Figure 6) among first-AI cows with functional CL at time of treatment and complete luteolysis following treatment. Pregnant cows had greater ($P = 0.02$) concentrations of $E_2$ 56 h after $PGF_{2\alpha}$ compared with nonpregnant cows (n = 192 total). Descriptively, 190/193 cows had an increase in $E_2$ from time of treatment until 48 h later. Ninety-eight percent of those cows (186/190) had at least a 50% decrease in $E_2$ 16 h following the final GnRH of Ovsynch.

## DISCUSSION

Results of the present study indicated that, compared with dinoprost tromethamine, cloprostenol sodium had only a tendency to increase the percentage of cows pregnant, and did not increase the percentage of cows with complete luteolysis or enhance the decrease in serum concentrations of $P_4$ after the injection of $PGF_{2\alpha}$ of Ovsynch. Thus, we must reject the hypothesis that cloprostenol would enhance luteolysis. Several studies during the past 30 yr have compared cloprostenol sodium and dinoprost tromethamine in cattle (Seguin et al., 1985; Martineau, 2003; Répási et al., 2005). In these studies, the effects of these $PGF_{2\alpha}$ analogs on luteolysis, estrous response, P/AI, and pregnancy rate

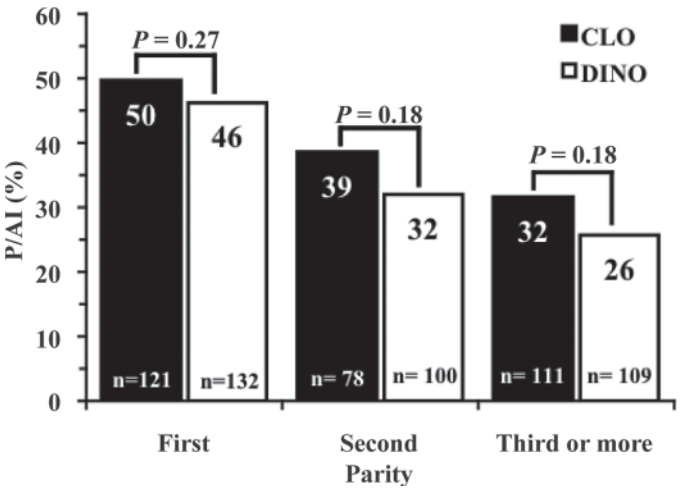


**Figure 2.** Effect of treatment with cloprostenol sodium (CLO) compared with dinoprost tromethamine (DINO) on pregnancy per AI (P/AI, %) in first-, second-, and ≥third-parity lactating dairy cows that received a Presynch/Ovsynch program for first AI and were diagnosed for pregnancy 39 ± 3 d after AI.

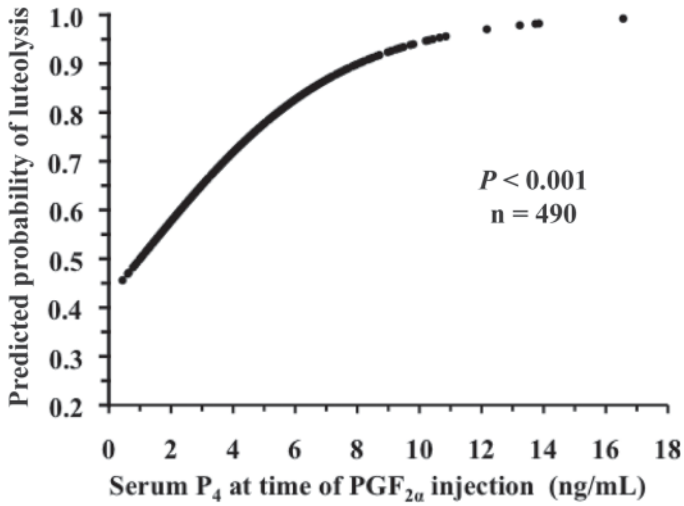


**Figure 3.** Predicted probability of complete luteolysis based on concentrations of progesterone ($P_4$ <0.5 ng/mL 56, 72, and 96 h after $PGF_{2\alpha}$ injection) at time of $PGF_{2\alpha}$ injection of Ovsynch in lactating dairy cows with functional corpus luteum (CL; $P_4$ concentrations ≥0.24 ng/mL 24 h and ≥0.09 ng/mL 56 h after treatment; n = 490) at time of treatment.

were inconsistent and ranged from increases in estrous expression in favor of cloprostenol to no differences in estrous expression or P/AI between products. In addition, before the start of the current study no studies had compared these 2 $PGF_{2\alpha}$ products in a Presynch/Ovsynch and resynchronization scheme with timed AI for lactating dairy cows. Since then, one study has been published that indicated no difference in P/AI but an advantage in percentage of cows with complete luteolysis for dinoprost tromethamine (Stevenson and Phatak, 2010) compared with cloprostenol sodium.

Our data indicated that the chance of complete luteolysis is ≤80%, regardless of type of $PGF_{2\alpha}$ product. Our definition of complete luteolysis was based on data from our laboratory (unpublished) and others (Souza et al., 2007; Brusveen et al., 2009) that indicated that cows with $P_4$ >0.5 ng/mL 2 d following $PGF_{2\alpha}$ had very limited chances of pregnancy. Current data agree that cows that did not have complete luteolysis had an approximately 5% chance of pregnancy. Ten percent of cows that had functional CL at time of treatment and had $P_4$ levels between 0.5 and 1 ng/mL 56 h following treatment had only an 8% chance of becoming pregnant. Cows that did become pregnant in this group were cows just above the 0.5 ng/mL $P_4$ threshold. These data and those of others (Souza et al., 2007; Brusveen et al., 2009) would argue that the threshold for luteolysis 2 d following treatment should be decreased to <0.5 ng/mL in laboratories utilizing the same RIA (Coat-a-Count) that was used in these studies. Thus, it is critical that luteolysis is maximized during the Ovsynch program and that circulating concentrations of $P_4$ fall <0.5 ng/mL within 2 d following $PGF_{2\alpha}$. In only 3% of cows with $P_4$ <0.5 ng/mL 2 d following $PGF_{2\alpha}$ did $P_4$ increase above that level 3 or 4 d following $PGF_{2\alpha}$.

Average serum concentrations of $P_4$ did not continue to decrease from 56 to 96 h after injection in cows defined to have incomplete luteolysis even though $P_4$ was similar in cows with complete and delayed luteolysis 24

**Table 2.** Effect of treatment with cloprostenol sodium (CLO) compared with dinoprost tromethamine (DINO) on pregnancy per AI (P/AI, %) and pregnancy loss percentage in lactating dairy cows between first and second pregnancy diagnosis for first and second and later AI

| | First AI | | | Second+ AI | | |
|---|---|---|---|---|---|---|
| Item | CLO | DINO | *P*-value | CLO | DINO | *P*-value |
| P/AI 39–42 d, % (n/n) | 40 (125/310) | 35 (121/341) | 0.09 | 23 (49/213) | 21 (38/181) | 0.32 |
| P/AI 96–103 d, % (n/n) | 37 (114/307) | 33 (112/340) | 0.12 | 20 (42/212) | 20 (35/179) | 0.95 |
| Pregnancy loss 39–42 and 96–104 d, % (n/n) | 7 (8/122) | 7 (8/120) | 0.95 | 13 (6/48) | 3 (1/36) | 0.11 |

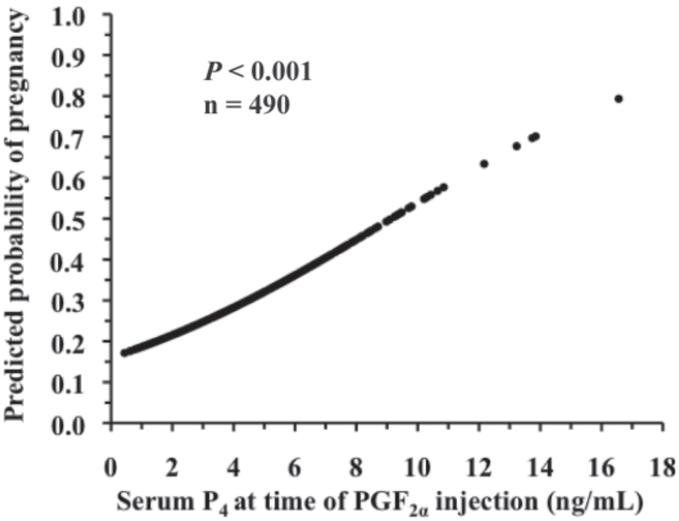


**Figure 4.** Predicted probability of pregnancy based on concentrations of progesterone ($P_4$) at time of $PGF_{2\alpha}$ of Ovsynch for cows with functional corpus luteum (CL; $P_4$ concentrations ≥0.24 ng/mL 24 h and ≥0.09 ng/mL 56 h after treatment; n = 490) at time of treatment.

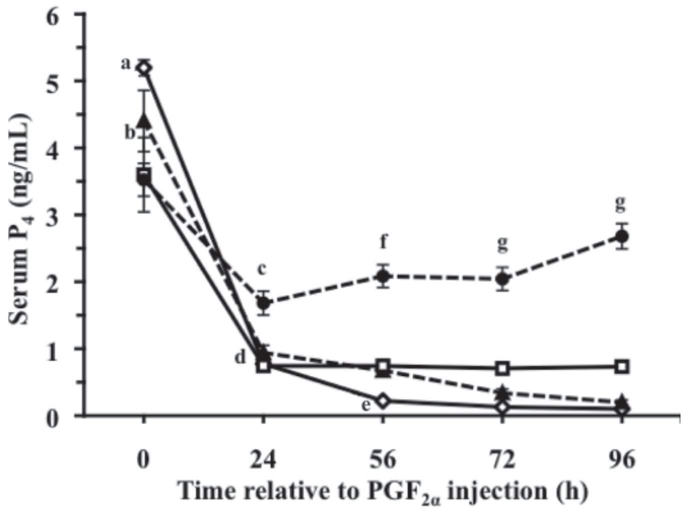


**Figure 5.** Daily concentrations of progesterone ($P_4$) from treatment (combined) to 96 h posttreatment in lactating dairy cows with functional corpus luteum (CL; $P_4$ concentrations ≥0.24 ng/mL 24 h and ≥0.09 ng/mL 56 h after treatment) that had complete luteolysis (C, —◇—, $P_4$ <0.5 ng/mL 56, 72, and 96 h after $PGF_{2\alpha}$ injection; n = 370), delayed luteolysis (D, ---▲---, $P_4$ <0.5 ng/mL 96 h after $PGF_{2\alpha}$ injection; n = 19), incomplete luteolysis (I, —□—, $P_4$ between 0.5 and 1 ng/mL 96 h after injection; n = 32), no luteolysis or recovery (N, ---●---, $P_4$ >1.0 ng/mL 96 h after injection; n = 68). [a]C different from I and N ($P < 0.001$); [b]D different from I and N ($P < 0.05$); [c]N different from C, D, and I ($P < 0.001$); [d]C different from D ($P < 0.05$); [e]C different from D, I, and N ($P < 0.001$); [f]N different than D and I ($P < 0.001$); [g]all groups at these times (72 and 96 h) were different from each other ($P < 0.02$).

h after treatment. As mentioned previously, cows that responded to the first GnRH of Ovsynch likely had a d-7 CL at time of treatment. It is likely that the d-7 CL in a portion of these cows may be somewhat refractory to $PGF_{2\alpha}$-induced luteolysis. Even though most of these cows have a decrease similar to cows that have complete CL regression by 24 h following treatment, they likely still have luteal cells that did not respond and continued to secrete $P_4$. It appears that an additional injection of $PGF_{2\alpha}$ may solve this problem of incomplete luteolysis in a Presynch/Ovsynch program. Brusveen et al. (2009) provided evidence that an additional injection of $PGF_{2\alpha}$ 24 h following the $PGF_{2\alpha}$ of Ovsynch reduced the percentage of cows that had incomplete luteolysis from 15 to 4%.

Répási et al. (2005) proposed that exogenous $PGF_{2\alpha}$ could affect the size of large luteal cells and luteal capillary cells without affecting small luteal cells. They believed the initial decline in $P_4$ production might have been due to temporary degenerative changes in the endothelial cells of luteal capillaries, which subsequently recovered. Then, small luteal cells would be able to respond to LH with an increase in $P_4$ secretion. Because small luteal cells are responsible for approximately 30% of the total $P_4$ production by the CL (Farin et al., 1989; Weems et al., 2006), in the case of incomplete luteolysis, a small fraction of small luteal cells may still be producing and secreting subluteal $P_4$ concentrations, making complete luteolysis during this time less probable.

A study from our laboratory indicated that serum concentrations of $P_4$ at time of $PGF_{2\alpha}$ of Ovsynch were a predictor for probability of pregnancy (Bello et al., 2006), although numbers of cows were limited. The present study, with much greater numbers, supports the finding that level of $P_4$ at time of $PGF_{2\alpha}$ is critical for

**Table 3.** Distribution (% of total) of pregnant and nonpregnant lactating dairy cows across ranges in progesterone ($P_4$) concentrations at time of treatment with cloprostenol sodium or dinoprost tromethamine (treatments combined)[1]

| Pregnancy status 39 ± 3 d after AI | $P_4$ range at time of $PGF_{2\alpha}$ injection (ng/mL) | | | | | | | Total | n |
|---|---|---|---|---|---|---|---|---|---|
| | 0–1 | 1–2 | 2–3 | 3–4 | 4–5 | 5–6 | >6 | | |
| n | 8 | 56 | 60 | 53 | 90 | 77 | 146 | | 490 |
| Not pregnant (% of total not pregnant) | 2.1 | 14.1 | 13.8 | 11.7 | 17.1 | 16.2 | 24.9 | 100 | 333 |
| Pregnant (% of total pregnant) | 0.6 | 5.7 | 8.9 | 8.9 | 21.0 | 14.6 | 40.1 | 100 | 157 |
| *P*-value | 0.23 | <0.01 | 0.12 | 0.34 | 0.31 | 0.64 | <0.001 | | 490 |

[1]Only cows with functional corpus luteum at time of treatment ($P_4$ concentrations ≥0.24 ng/mL 24 h and ≥0.09 ng/mL 56 h after treatment).

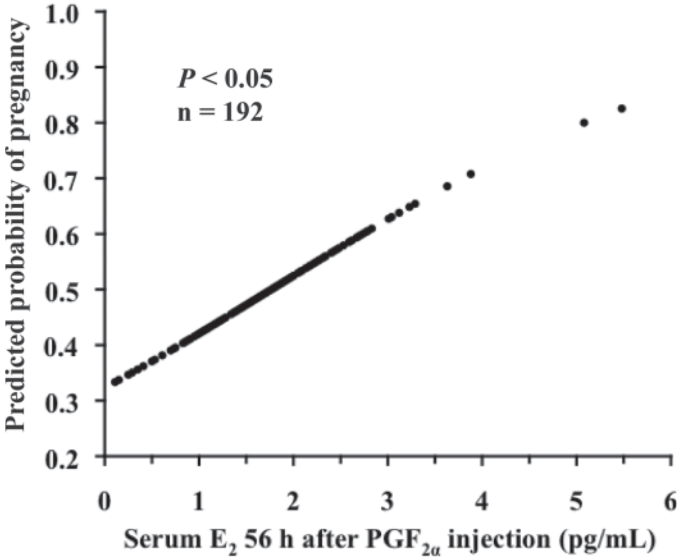


**Figure 6.** Predicted probability of pregnancy based on concentrations of estradiol ($E_2$) at time of final GnRH of Ovsynch for cows with functional corpus luteum (CL; $P_4$ concentrations $\geq$0.24 ng/mL 24 h and $\geq$0.09 ng/mL 56 h after treatment; n = 192) at time of treatment and subsequently had complete luteolysis ($P_4$ <0.5 ng/mL 56, 72, and 96 h after $PGF_{2\alpha}$ injection).

pregnancy success in lactating dairy cows. Level of $P_4$ was predictive of chances for complete luteolysis, thus explaining part of the reason for the positive relationship between $P_4$ concentrations at $PGF_{2\alpha}$ and P/AI. The positive relationship between $P_4$ and luteolysis may simply be due to fact that the greater the $P_4$, the more mature the CL. As CL become more mature, the likelihood of induced luteolysis should be greater. Fonseca et al. (1983) were the first to report that lactating dairy cows with relatively greater circulating concentrations of $P_4$ have a greater chance for pregnancy. Bisinotto et al. (2010) found a positive relationship between concentrations of $P_4$ and fertility. Cows that received the first GnRH of Ovsynch to start a new cycle had an ovulatory follicle that developed under low concentrations of $P_4$ versus cows that received the first GnRH of Ovsynch on d 7 of the cycle and a new accessory CL was initiated and a new ovulatory follicle developed under greater concentrations of $P_4$. Cows that had ovulatory follicles developing under higher concentrations of $P_4$ due to the accessory CL had greater conception rates.These data along with data from the current study would indicate that level of $P_4$ in circulation before luteolysis affects fertility.

Serum $E_2$ concentration at time of GnRH of Ovsynch was an indicator of fertility. Probability of pregnancy was greater in cows with higher $E_2$ at last GnRH of Ovsynch. In addition, pregnant cows diagnosed at 39 d after AI had greater $E_2$ concentrations at time of GnRH

**Table 4.** Distribution (% of total) of pregnant and nonpregnant lactating dairy cows across ranges in progesterone ($P_4$) concentrations 24, 56, 72, and 96 h following treatment with cloprostenol sodium or dinoprost tromethamine (treatments combined)[1]

| Time after $PGF_{2\alpha}$ (h) | Pregnancy status 39 ± 3 d after AI | Progesterone range (ng/mL) 0–0.1 | 0.1–0.2 | 0.2–0.3 | 0.3–0.4 | 0.4–0.5 | 0.5–1.0 | >1.0 | Total (%) | n[2] |
|---|---|---|---|---|---|---|---|---|---|---|
| 24 | n | 0 | 0 | 19 | 46 | 63 | 232 | 126 | | 486 |
| | Not pregnant (% of total not pregnant) | 0.0 | 0.0 | 3.6 | 9.4 | 11.5 | 47.4 | 28.2 | 100 | 329 |
| | Pregnant (% of total pregnant) | 0.0 | 0.0 | 4.5 | 9.6 | 15.9 | 48.4 | 21.7 | 100 | 157 |
| | *P*-value | — | — | 0.66 | 0.95 | 0.17 | 0.84 | 0.15 | | |
| 56 | n | 17 | 167 | 111 | 48 | 32 | 48 | 59 | | 482 |
| | Not pregnant (% of total not pregnant) | 3.3 | 29.5 | 20.1 | 9.4 | 6.4 | 13.4 | 17.9 | 100 | 328 |
| | Pregnant (% of total pregnant) | 3.9 | 45.5 | 29.2 | 11.0 | 7.1 | 2.6 | 0.6 | 100 | 154 |
| | *P*-value | 0.76 | <0.001 | 0.03 | 0.61 | 0.78 | <0.001 | <0.001 | | |
| 72 | n | 154 | 157 | 54 | 21 | 14 | 26 | 62 | | 488 |
| | Not pregnant (% of total not pregnant) | 27.5 | 28.4 | 9.0 | 5.1 | 4.2 | 7.2 | 18.6 | 100 | 333 |
| | Pregnant (% of total pregnant) | 40.0 | 40.0 | 15.5 | 2.6 | 0.0 | 1.3 | 0.6 | 100 | 155 |
| | *P*-value | <0.01 | 0.02 | 0.04 | 0.19 | <0.01 | <0.01 | <0.001 | | |
| 96 | n | 216 | 111 | 26 | 9 | 9 | 28 | 63 | | 462 |
| | Not pregnant (% of total not pregnant) | 39.5 | 22.5 | 5.8 | 2.3 | 1.3 | 8.1 | 20.6 | 100 | 310 |
| | Pregnant (% of total pregnant) | 61.2 | 27.0 | 5.3 | 1.3 | 3.3 | 2.0 | 0.0 | 100 | 152 |
| | *P*-value | <0.001 | 0.22 | 0.87 | 0.52 | 0.13 | 0.01 | <0.001 | | |

[1]Only cows with functional corpus luteum at time of treatment ($P_4$ concentrations $\geq$0.24 ng/mL 24 h and $\geq$0.09 ng/mL 56 h after treatment).

[2]Different n between times were due to missing samples.

than nonpregnant cows. This finding supports previously reported studies (Ireland and Roche, 1982; Bello et al., 2006). However, Bello et al. (2006) detected that the importance of $E_2$ levels as a predictor of fertility differed with size of preovulatory follicle. Supplementation of 1 mg of $E_2$ 8 h before the final GnRH of Ovsynch increased fertility in cows that ovulated follicles between 15 and 19 mm and in first-service cows (Souza et al., 2007). Although we did not collect ovulation data on these cows, 98% of a subset of cows that had an increase in $E_2$ from treatment to final GnRH of Ovsynch had at least a 50% decrease in $E_2$ 16 h following the final GnRH of Ovsynch. These data indicate not only that most cows likely had a functional dominant follicle(s) at time of the final GnRH of Ovsynch, but also a high likelihood of ovulation due to the final GnRH of Ovsynch.

## CONCLUSIONS

Cloprostenol sodium tended to improve P/AI compared with dinoprost tromethamine when used within Ovsynch preceded by a presynchronization program. More importantly, cows with greater serum concentrations of $P_4$ at time of $PGF_{2\alpha}$ of Ovsynch had a greater chance for luteolysis and pregnancy. In addition, cows with greater concentrations of $E_2$ at time of final GnRH of Ovsynch had a greater chance for pregnancy. Novel fertility programs that are able to enhance these 2 hormones during Ovsynch may help solve the problem of low fertility of lactating dairy cows.

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