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Compared to dinoprost tromethamine, cloprostenol sodium increased rates of estrus detection, conception and pregnancy in lactating dairy cows on a large commercial dairy

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Abstract

Using two $PGF_{2\alpha}$ treatments 14 days apart as a way to enhance estrus detection rate following the 2nd treatment is a reproductive management tool that continues to be used on large dairy farms. In one study, in cows with a functional CL and a dominant follicle, treatment with cloprostenol vs. dinoprost resulted in greater peripheral estradiol concentrations. The objective of the present study was to determine if cloprostenol could enhance pregnancy rates of cows in a large dairy herd using a $PGF_{2\alpha}$ program for 1st artificial insemination (AI). Lactating dairy cows (n = 4549) were randomly assigned to receive two treatments of either 500 μg cloprostenol or 25 mg dinoprost 14 days apart, with the 2nd treatment on the 1st day of the voluntary waiting period (57 DIM). Cows detected in estrus within 5 days after the 2nd treatment were inseminated. There was no effect of treatment on day of estrus detection, with 78% of cows inseminated on Days 3 or 4 following treatment. Cloprostenol increased ($P < 0.01$) estrus detection rates in 1st parity cows compared to dinoprost, 42.4 vs. 34.0%. In cows inseminated on Days 3 or 4 after treatment, cloprostenol increased ($P = 0.05$) conception rates compared to dinoprost, 38.3 vs. 34.4%. When treatments and parities were combined, conception rates increased ($P < 0.02$) with interval after treatment (27.0, 36.4, and 44.5% for Days 1 or 2, Days 3 or 4, and Day 5, respectively). Cloprostenol increased ($P = 0.02$) overall pregnancy rate compared to dinoprost, 14.4 vs. 12.2%. In summary, cloprostenol increased fertility in 1st parity cows inseminated on Days 3 or 4 following treatment and subsequently enhanced pregnancy rates of 1st parity lactating dairy cows compared to dinoprost. Fertility appeared greater in cows expected to have had a young antral ovarian follicle at treatment.

Keywords: Cloprostenol sodium; Dinoprost tromethamine; Estrus detection rate; Conception rate; Pregnancy rate; Lactating dairy cows

1. Introduction

Cloprostenol sodium is a potent synthetic analogue of $PGF_{2\alpha}$ that is resistant to endogenous metabolism because of an oxyaryl function [1] and thus has a long half-life ($T_{½} \sim 3$ h) [2]. Comparatively, dinoprost has a short half-life ($T_{½} \sim 8$ min) [3] and is rapidly metabolized similar to endogenous $PGF_{2\alpha}$ metabolism [1,4]. In recent studies [5,6], there were no differences in percentage of cows with, or time to, complete luteolysis following administration of $PGF_{2\alpha}$ in an Ovsynch program, either G6G/Ovsynch or Presynch-11/Ovsynch. However, cloprostenol enhanced peripheral estradiol concentrations in cows with a functional dominant follicle and CL treat-

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ment [5]. In that regard, circulating estradiol concentrations were greater 48 h after treatment with cloprostenol compared to dinoprost in cows with preovulatory follicles that were 7 days from a GnRH-induced new follicular wave [5]. This was attributed to a slightly more rapid decrease in progesterone (P4) during the first 12 h post-treatment in cows given cloprostenol vs. dinoprost [5]. Cows in which P4 decreased faster had greater LH pulsatility [7] and estradiol concentrations [5,7]. Increased estradiol concentrations at the onset of estrus may enhance the likelihood of detecting estrus [8] and perhaps fertility [9]. In that regard, the predicted probability of pregnancy increased when estradiol concentrations were greater at time of the final GnRH of Ovsynch [6,9].

The interval from $PGF_{2\alpha}$ to estrus is primarily dependent on antral age and maturity of the largest functional follicle [10] at time of treatment. Cows at random stages of the estrous cycle and follicular waves at time of $PGF_{2\alpha}$ treatment may have antral follicles at pre- or post-deviation stages of development. Cows with pre-deviation follicles ovulate later (>120 h) than those with post-deviation follicles [10]. Lactating dairy cows with a dominant preovulatory follicle at $PGF_{2\alpha}$-induced luteolysis ovulated 102 $\pm$ 2 h after $PGF_{2\alpha}$ treatment [5]. The interval from the LH surge to ovulation was approximately 29 h [11]; therefore, cows with a dominant preovulatory follicle when given $PGF_{2\alpha}$ would be expected to be in standing estrus approximately 72 h later. However, cows inseminated following estrus at 24 or 48 h after $PGF_{2\alpha}$ likely have already undergone luteolysis before treatment [5]. Cows inseminated $\geq$5 days following $PGF_{2\alpha}$ likely had luteolysis in response to the treatment, but had follicles at early stages of development at the time of treatment. Therefore, cloprostenol's beneficial effect of increased circulating estradiol concentrations would likely only be manifest in cows in estrus 3 to 4 days after treatment.

There is a paucity of reports that used large numbers of cows to determine the effect of cloprostenol on fertility of cows inseminated following a detected estrus on Days 3 or 4 post-treatment. The objectives of this study were to determine the effect of cloprostenol (synthetic $PGF_{2\alpha}$ analogue) compared to dinoprost (natural $PGF_{2\alpha}$) on percentage of cows detected in estrus, and rates of conception and pregnancy in cows inseminated on Days 3 or 4 post-treatment. We hypothesized that cloprostenol enhances fertility in lactating dairy cows because of differences in half-life, particularly in cows inseminated 3 or 4 days after treatment, as they were likely to have had a functional CL and a mature preovulatory follicle at treatment.

2. Materials and methods

2.1. General farm information

This trial was conducted from June 2009 to February 2010 on a large California dairy. Herd milk production during this interval averaged 33 kg/cow/d. Cows were housed in free stall barns, fed a total mixed ration twice daily, and had *ad libitum* access to water. Cows were segregated in pens (n = 10) by parity (1st = 4 pens, 2nd+ = 6 pens). The TMR consisted of corn, wheat 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 twice daily.

2.2. Experimental design

Healthy lactating Holstein cows (n = 4549) were assigned randomly to treatment using odd/even ear tag numbers. Cows with even ear tag numbers were assigned to receive 500 μg cloprostenol im (Estrumate, Merck Animal Health, Desoto, KS, USA) and cows with odd numbers were assigned to receive 25 mg dinoprost im (Lutalyse, Pfizer Animal Health, New York, NY, USA) at both 46 $\pm$ 3 and 60 $\pm$ 3 DIM on a weekly basis on a Wednesday morning from 600 to 1100 h. The voluntary waiting period was 57 days in milk, in accordance with standard operating procedures on the farm. Cows were monitored once daily for signs of estrus, with observation of tail head chalk as the primary method of detection. Cows were inseminated immediately after detection of estrus on Days 1–5 (Thursday through Monday, 600–1100 h) following treatment. All treatments were administered with 16 or 18-gauge 2.5 cm needles in the semimembranosus or semitendinosus muscles, by attending veterinarians or trained farm personnel under direct veterinary supervision. Cows with a mucopurulent vaginal discharge or other clinical signs of acute illness before AI were excluded from the experiment.

A crew of five AI technicians performed AI (n = 2123 cows) with commercial semen from multiple sires. Technicians were blind to treatments. This crew worked 5-day shifts following treatment for the experimental period. Because the crew responsible for detection of estrus and AI in this study did not work on Days 6 and 7 after treatment, those data were not collected, because of potential inconsistencies between

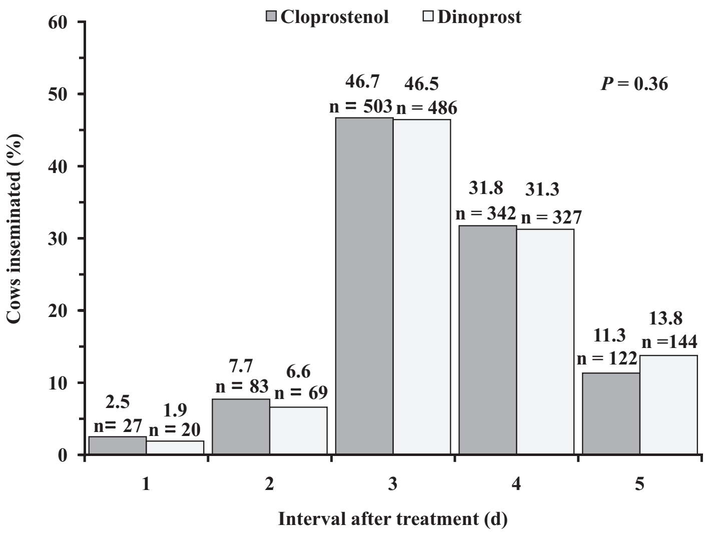

Fig. 1. Effect of treatment of 500 μg cloprostenol versus 25 mg dinoprost on the distribution of lactating dairy cows (%) that were inseminated during each day of the 5-d interval after treatment (n = 2123).

crews in determination of estrus and AI technique. Pregnancy diagnoses were performed by transrectal palpation 36 to 42 days post-AI by farm veterinarians, blind to treatment. Only cows that did not return to estrus in the period from AI to 36 to 42 days post-AI were subjected to pregnancy diagnosis; cows that returned to estrus during this period were considered not-pregnant. Conception rate was defined as the percentage of cows that received AI during the 5-day period, or specifically Days 3 and 4 following treatment, that were diagnosed pregnant at pregnancy diagnosis. Pregnancy rate was defined as the percentage of cows treated that became pregnant due to AI during either the 5-day period following treatment, or specifically Days 3 and 4 following treatment. Cows that left the herd before pregnancy diagnosis (n = 18) were not considered for conception or pregnancy rates.

2.3. Statistical analyses

To determine if there was an effect of variables (parity, service sire, AI technician, month of treatment, and days in milk) on treatment outcomes, binomial variables were analyzed using a generalized linear mixed model fitted with the GLIMMIX procedure of the statistical software SAS (version 9.2, SAS Institute, Inc., Cary, NC, USA). Variables were removed from the model in a stepwise fashion if there was not an effect ($P < 0.10$) on treatment outcomes. Because there was no effect of variables measured on treatment outcome, the final model considered only treatment. The Mantel-Hantzel χ^2 test for Independence was used to detect differences in estrus detection rate, conception rate, and pregnancy rate, because there was no effect of variables measured on treatment outcomes. A one-tailed test was used, because the hypothesis was that cloprostenol increases estrus detection, conception and pregnancy rate compared to dinoprost, because of differences in pharmacodynamics and previously published data [5]. Effect of treatment on the distribution of cows in estrus during the 5 days estrus detection period was analyzed using PROC FREQ (SAS, version 9.2, SAS Institute, Inc.).

3. Results and discussion

3.1. Effect of treatment on estrus detection rates

Of the 4549 cows treated with cloprostenol or dinoprost, 2123 (46.7%) were detected in estrus within 5 days of treatment and received AI. This estrus detection rate may appear low, but there are several reasons why cows may not exhibit estrus in the 5 days after $PGF_{2\alpha}$. Cows that are anovular or near 1st ovulation at time of 2nd $PGF_{2\alpha}$ will not respond; that could be as many as 15 to 20% of cows treated [12,13]. Other reasons are that small percentages of cows may have exhibited estrus after the detection period, had a silent estrus, lack of detection, or may not have had complete luteal regression. A previous study with daily estrus detection and AI after second $PGF_{2\alpha}$ of Presynch (two injections of $PGF_{2\alpha}$ 14 days apart) had a similar estrus detection rate response (48.7%) [14]. The distribution of cows

Table 1
Effect of 500 μg cloprostenol versus 25 mg dinoprost on rates of estrus detection, conception, and pregnancy in 1st, 2nd, and 3rd+ parity lactating dairy cows that were artificially inseminated on Days 3 or 4 during the 5-d interval after the second of two cloprostenol or dinoprost treatments 14 d apart, with a voluntary waiting period of 57 d in milk. Numbers of cows are displayed in parentheses.

Parity	Estrus detection rate (%)			Conception rate (%)			Pregnancy rate (%)		
	Cloprostenol	Dinoprost	P value	Cloprostenol	Dinoprost	P value	Cloprostenol	Dinoprost	P value
1	42.4 (825)	34.0 (830)	<0.01	41.1 (348)	38.5 (278)	0.25	17.4 (822)	13.0 (824)	<0.01
2	33.7 (531)	33.9 (531)	0.48	38.2 (178)	35.8 (179)	0.32	12.8 (530)	12.2 (530)	0.35
3+	35.2 (897)	37.5 (935)	0.15	35.2 (315)	30.5 (348)	0.10	12.4 (895)	11.4 (930)	0.25
Overall (n)	47.8 (2253)	45.6 (2296)	0.07	38.3 (841)	34.4 (805)	0.05	14.4 (2247)	12.2 (2284)	0.02

inseminated daily during the 5-day period following treatment was not different for cloprostenol vs. dinoprost ($P = 0.36$; Fig. 1). The small proportion of cows detected in estrus (9.4%) on Days 1 or 2 following treatment would be considered a normal percentage in estrus following endogenous luteolysis during a 21 d estrous cycle. It is unlikely that lactating dairy cows could undergo $PGF_{2\alpha}$-induced luteolysis and exhibit standing estrus within 48 h post-treatment [5,6,10]. Instead, these cows would likely have undergone, or were in the process of, an endogenously induced luteolysis before treatment [5,6,10]. There was a difference in the percentage of cows detected in estrus between Days 1 or 2 ($P < 0.01$) in all cows, regardless of treatment. The greater percentage of cows in estrus on Day 2 compared to Day 1 may represent cows in the process of natural luteolysis at time of injection that may have undergone complete luteolysis in a more rapid manner. Thus, Day 2 may represent both cows with natural estrus and cows with a more rapid decrease due in part to $PGF_{2\alpha}$ treatment. Martins, et al. 2011 [5] demonstrated that average interval to complete luteolysis in lactating dairy cows treated with $PGF_{2\alpha}$ was approximately 29 ± 1.0 h post-treatment and the interval from complete luteolysis to ovulation was 72.5 ± 8.3 h. The regression equation for prediction of time of $PGF_{2\alpha}$ treatment to ovulation was highly predictive in this study ($R = 0.83$; $P < 0.0001$). Time from induced LH surge (onset of estrus) to ovulation was 29 h in an earlier study [15]. Thus, the earliest that cows would exhibit standing estrus with a mature CL at time of $PGF_{2\alpha}$ would be approximately 72 h.

Most cows detected in estrus were inseminated 3 or 4 days following treatment (78% of total cows inseminated). This response was similar to Seguin, et al. 1985 [16] that had 76% of estrus responses on Days 3 or 4 after administration of $PGF_{2\alpha}$ on random days of diestrus. It is likely these cows had $PGF_{2\alpha}$-induced luteolysis from treatment and likely had mature dominant follicles treatment, because of the limited rate of growth of follicles. A greater percentage of 1st parity cows treated with cloprostenol were detected in estrus compared to dinoprost on Days 3 or 4 following treatment (Table 1). When considering the entire 5 days estrus detection period, there was an overall trend ($P = 0.07$) in favor of treatment with cloprostenol compared to dinoprost (47.8 vs. 45.6%; $n = 4549$) in the proportion of cows that received AI during the 5-day period following treatment. The reason for this trend was once again because of differences in 1st parity cows (Table 1). In multiparous cows, there were no treatment differences for percentage of cows detected in estrus. The likely reason for an increase in percentage of cows detected in estrus and inseminated because of treatment with cloprostenol may have been because of greater circulating estradiol concentrations following treatment compared to dinoprost. In a recent study by Martins, et al. 2011 [5], cows treated with cloprostenol had a greater decrease in progesterone during the 1st 12 h following treatment, and greater estradiol concentrations 48 h after treatment compared to those given dinoprost tromethamine. In that study, when treatments were combined, there was a strong relationship between the decrease in progesterone and a subsequent increase in estradiol. Kinder, et al. 1996 [7] demonstrated that greater decreases in progesterone increased pulses of LH and subsequent circulating estradiol concentrations.

The remainder of cows received AI on Day 5 (12.5%). These cows most likely had predeviation sized follicles at treatment [10], although some may have had delayed luteolysis that increased intervals estrus and ovulation [5]. If cloprostenol enhances estradiol production because of an accelerated decrease in progesterone, cows in this group would likely not have follicles mature enough to respond to increased LH pulsatility. Data on Days 6 or 7 were not collected, because of potential differences in the number of inseminators on duty those days and potential differences in compliance among inseminators.

It is not clear why only 1st parity cows were affected by treatment. Data from Sangsritavong, et al. 2002 [17] indicated that cows with greater feed intake may have lower circulating concentrations of estradiol. First parity cows clearly have lower dry matter intake than older cows [18]. Thus, 1st parity cows may have had greater circulating estradiol concentrations, because of less dry matter intake and subsequently may have had a greater chance of exhibiting behavioral signs of standing estrus. Cloprostenol may have a greater impact in 1st parity cows, because of greater opportunities to enhance circulating estradiol concentrations, as already discussed. Another potential reason for greater chances of detecting 1st parity cows in estrus may deal with having greater mobility than older cows, thus allowing for potential differences in treatment.

It was not apparent if the 1st $PGF_{2\alpha}$ treatment impacted stage of estrous cycle at time of 2nd $PGF_{2\alpha}$ (treatment), 14 d later. Mathematically, if cows responded to the 1st $PGF_{2\alpha}$, they should be at midcycle (~ 10–11 d) and near time of follicle wave turnover at the 2nd treatment. Thus, if a significant percentage of cows responded to the 1st PGF, it would seem logical that a significant percentage of cows would be at early stages of 2nd follicular wave development and require more than 4 d before standing estrus and an LH surge. Approximately 12% of the cows detected in estrus during the 5-d period were in estrus on Day 5. Our data collection ended on Day 5, so it was not known what percentage of cows would have been detected on Days 6 or 7. If there were substantial numbers of cows at very early stages of follicle development at treatment, it may have taken more than 5 d for those cows to have follicles develop and ovulate.

Previous studies that evaluated the difference in estrus expression following these two $PGF_{2\alpha}$ products had either too few numbers to interpret outcomes [19–21], were not different [22,16], or demonstrated an increase in estrus expression in cattle treated with cloprostenol compared to dinoprost [23].

3.2. *Effect of treatment on conception rates*

Cloprostenol increased (P = 0.05) conception rates in cows that received AI on Days 3 or 4 following treatment (Table 1). The best estimate of an effect of treatment on conception rates would likely be from cows that expressed estrus and received AI on Days 3 or 4 following treatment, as previously discussed. This may be due to a more rapid decrease in progesterone and increase in estradiol [5]. Bello, et al. 2006 [9] reported that the greater the circulating estradiol concentrations, the greater the probability for conception. Cows that expressed estrus and were inseminated on Days 1 and 2, or 5 following treatment would likely not benefit from treatment with cloprostenol, because of the lack of either a functional CL (Days 1 and 2; [5]) or dominant follicle (Day 5; [10]) at treatment.

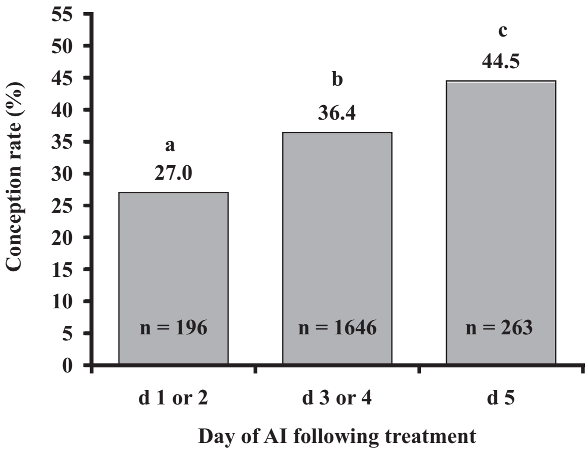

Fig. 2. Effect of day of AI following treatment with either 500 μg cloprostenol or 25 mg dinoprost on conception rate (%) of lactating dairy cows, combined for all parities. Conception rates (%) of cows inseminated on Days 3 or 4 were greater than those inseminated on Days 1 or 2 ($P < 0.01$). Conception rates of cows inseminated on Day 5 were greater than those inseminated on Days 3 or 4 ($P < 0.02$), or on Days 1 or 2 ($P < 0.001$).

Studies [24–28] that compared cloprostenol and dinoprost in beef cattle demonstrated no difference or had too few numbers to evaluate conception rate outcomes. A study from Martineau 2003 [22], demonstrated that cloprostenol increased conception rate and subsequent pregnancy rate in n = 402 lactating dairy cows inseminated following estrus. Stevenson and Phatak 2010 [29], demonstrated no differences in conception rates between the two products when utilized in Presynch or Resynch programs. Cairoli, et al. 2006 [30], found no differences in conception rates between cloprostenol and dinoprost in cows inseminated following an observed estrus.

There was no effect ($P > 0.10$) of parity, service sire, AI technician, month of AI, or days in milk at AI on treatment outcomes. When treatments were combined, there was an effect of month of AI (P = 0.02) and service sire (P = 0.03), but not AI technician or parity (P = 0.2) on conception rates. Cows inseminated in November and December had greater conception rates than cows inseminated in June through October.

Conception rates were greater ($P < 0.01$) in cows that received AI on Days 3 or 4 following treatment vs. Days 1 or 2 (36.0 vs. 27.0%). Also, conception rates were greater for cows inseminated on Day 5 (44.5%) vs. Days 3 or 4 ($P < 0.02$), or cows inseminated on Days 1 or 2 ($P < 0.001$; Fig. 2). Thus, cows that exhibited a spontaneous estrus (endogenously induced) were less fertile than cows that were induced with exogenous $PGF_{2\alpha}$. In addition, it appeared that cows with small antral follicles (predeviation) at time treatment were more fertile. It is likely these follicles were younger (i.e., antral age) at the time of the LH surge compared to both other groups (Days 1 or 2 or Days 3 or 4). In contrast, cows that were in estrus on Days 1 or 2 were likely late 2nd follicular wave and had a much older antral age follicle at luteolysis. The ovulatory follicle has key roles in reproductive success by coordinating hormonal interactions, final maturation of the oocyte, and ovulation. Several studies have reinforced a consistent association between physiological characteristics of the ovulatory follicle and fertility of lactating dairy cows, including follicular age, size, and function (E_2 production capacity) [9,31–33].

3.3. Effect of treatment on pregnancy rate

Cows given cloprostenol that were inseminated on Days 3 or 4 following treatment had greater pregnancy rates ($P = 0.02$; Table 1) than those given dinoprost; this was due to 1st parity cows that had greater pregnancy rates ($P < 0.01$) given cloprostenol compared to dinoprost (Table 1). There was no effect of treatment on pregnancy rates in 2nd and 3rd + parity cows that were in estrus on Days 3 or 4, or throughout the estrus detection period. Throughout the estrus detection period, 1st parity cows treated with cloprostenol had greater (21.4 vs. 17.7%; $P < 0.03$) pregnancy rates compared to those given dinoprost. However, multiparous cows were not affected by treatment (15.8 vs. 16.8%; $P = 0.35$, 2nd parity, and 15.1 vs. 14.9%; $P = 0.45$, 3rd+ parity, for cloprostenol vs. dinoprost, respectively).

3.4. Summary and conclusions

Pregnancy rate was enhanced with cloprostenol in 1st parity cows, especially in those that likely had functional dominant follicles and a CL at treatment. Cloprostenol increased rates of both estrus detection rates and conception in 1st parity cows, perhaps because of a faster decrease in progesterone that in turn increased estradiol production by dominant follicles.

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