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Rates of luteolysis and pregnancy in dairy cows after treatment with cloprostenol or dinoprost

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Abstract

Our objective was to determine whether rates of luteolysis or pregnancy differed in lactating dairy cows of known progesterone status and either known or unknown luteal status after either cloprostenol or dinoprost was injected as part of a timed-insemination program. In Experiment 1, 2358 lactating dairy cows in six herds were given two injections of $PGF_{2\alpha}$ 14 d apart (Presynch), with the second injection given 12 to 14 d before the onset of a timed AI protocol (Ovsynch). Cows (n = 1094) were inseminated when detected in estrus after the Presynch $PGF_{2\alpha}$ injections. Cows not inseminated (n = 1264) were enrolled in the Ovsynch protocol and assigned randomly to be treated with either cloprostenol or dinoprost as part of the timed-AI protocol. In cows having pretreatment concentrations of progesterone $\geq$ 1 ng/mL and potentially having a functional corpus luteum (CL) responsive to cloprostenol (n = 558) or dinoprost (n = 519), dinoprost increased ($P < 0.05$) luteal regression from 86.6 to 91.3%. Despite a significant increase in luteolysis, pregnancies per AI did not differ between luteolytic agents (dinoprost = 37.8% and cloprostenol = 36.7%). Fertility was improved in cows of both treatments having reduced concentrations of progesterone at 72 h and in cows showing signs of estrus. In Experiment 2, an ovulation-resynchronization program was initiated with GnRH or saline in 427 previously inseminated lactating dairy cows of unknown pregnancy status in one herd. Seven days later, pregnancy was diagnosed and nonpregnant cows were blocked by number of CL and assigned randomly to be treated with cloprostenol or dinoprost. Compared with cloprostenol, dinoprost increased ($P < 0.05$) luteal regression from 69.1 to 78.5%, regardless of the number of CL present or the total luteal volume per cow. Pregnancies per AI did not differ between dinoprost (32.8%) and cloprostenol (31.3%). Although dinoprost was more effective than cloprostenol at inducing luteolysis in lactating dairy cows exposed to an Ovsynch or ovulation-resynchronization protocol, resulting fertility did not differ between products.

Keywords: Corpus luteum; Dairy cattle; Luteolysis; Pregnancy per AI; Prostaglandin $F_{2\alpha}$

1. Introduction

Since the first $PGF_{2\alpha}$ product was introduced in the United States in 1979 (Lutalyse, The Upjohn Co., Kalamazoo, MI, USA), several agonists and generic $PGF_{2\alpha}$ products have become available by prescription. The major difference in available products is between those that are chemically the same as uterine-derived $PGF_{2\alpha}$ (dinoprost) [1] and its agonist (cloprostenol sodium) [2]. The half-life of elimination in blood of 0.5 mg of free acid ^{14}C-cloprostenol is 3 h [2] and is longer than the blood half-life of a few minutes for dinoprost [1], because a benzyl chlorine ring is substituted at position 17 of the fatty-acid structure of $PGF_{2\alpha}$. Whether this property makes the agonist

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cloprostenol more effective in lysing the corpus luteum (CL) is equivocal.

Different physiological responses of bovine females to administration of either cloprostenol or dinoprost have been reported for luteolysis [3], receptor binding [4], changes in intrauterine pressure [5], estrus expression [6–14], conception rates [7,8–10,12–16], and pregnancy rates [7,9,12–16]. An unpublished meta-analysis (A. L. Skidmore, personal communication) of some of these factors did not find significant differences ($\alpha > 0.05$) in conception rate, pregnancy rate, or overall differences in detected estrus. Odds ratios (OR), however, were consistently greater than 1.0, indicating only numerical trends in the combined studies that consistently favored cloprostenol over dinoprost.

Strict timed-AI programs are common place on dairy operations because they are reliable and not wholly dependent on visual or other means of detecting estrus [17]. The Ovsynch protocol (injection of GnRH 7 d before and 48 h or 72 h after treatment with $PGF_{2\alpha}$; timed AI at 72 h) synchronizes follicular maturation and luteal regression [18,19], resulting in approximately 20–30% of cows having at least two luteal structures at the time of $PGF_{2\alpha}$ injection [20]. A good test of luteolytic efficacy between product types (dinoprost vs. cloprostenol) is possible in lactating cows to which the Ovsynch protocol is applied, because a larger proportion of cows have more than one CL to regress at the time of $PGF_{2\alpha}$ injection.

We hypothesized that if one $PGF_{2\alpha}$ product was more effective than another as a luteolytic agent, lactating dairy cows having ancillary luteal structures would be an effective model for testing that difference. Therefore, the present study consisted of two experiments. The objective of the first experiment was to determine the efficacy of luteal regression in response to two chemically different luteolytic products (cloprostenol vs. dinoprost), as determined by changes in blood progesterone concentrations and subsequent pregnancy outcome of lactating dairy cows exposed to either of the two products before first postpartum AI. The objective of the second experiment was similar to that of the first, except the number of CL and total luteal tissue volume were quantified in previously inseminated nonpregnant dairy cows before treatment injections were given.

2. Materials and methods

The Kansas State University (Manhattan, KS) Institutional Animal Care and Use Committee approved all procedures involving cows in this study.

Table 1
Herd characteristics for lactating dairy cows enrolled in Experiments 1 and 2.

Traits	Experiment 1						Experiment 2
	Herd 1	Herd 2	Herd 3	Herd 4	Herd 5	Herd 6	
First and last AI dates	January 30, 2008 to May 13, 2008	January 24, 2008 to May 17, 2008	January 29, 2008 to May 13, 2008	January 28, 2008 to May 13, 2008	January 26, 2008 to May 18, 2008	February 13, 2008 to May 30, 2008	November 8, 2007 to July 10, 2008
Herd	California	California	California	California	California	California	Kansas
Herd size (n)	727	1,162	1,025	1,186	1,759	2,446	248
Milking frequency (times/d)	3	3	3	3	3	3	2
First pregnancy diagnosis (d)	36	36	35	36	36	32	30
Pregnancies per AI[a], % (n)	38.4 (86)	26.0 (181)	38.9 (113)	31.2 (185)	40.6 (192)	33.5 (337)	...
Cows enrolled in experiments (n)	112	163	100	110	257	488	306
Pregnancies per AI [b] (%)	42.0	27.6	26.0	31.8	38.5	42.2	33.3
	Mean ± SD						
Body condition score[c]	2.7 ± 0.2	2.7 ± 0.3	2.7 ± 0.3	2.8 ± 0.6	2.8 ± 0.4	2.7 ± 0.2	2.4 ± 0.5
Test-day milk[d] (kg)	51 ± 10	53 ± 10	54 ± 10	55 ± 10	55 ± 10	43 ± 11	44 ± 11
Days in milk at treatment AI	82 ± 2	83 ± 5	82 ± 2	83 ± 2	82 ± 2	86 ± 2	188 ± 93
Serum progesterone (ng/mL)	Mean ± SEM						
Before treatment	4.0 ± 0.30	3.6 ± 0.20	3.8 ± 0.30	4.5 ± 0.30	3.8 ± 0.20	4.0 ± 0.10	4.6 ± 0.15
48 h	0.6 ± 0.06	0.7 ± 0.07	0.7 ± 0.09	0.8 ± 0.08	0.9 ± 0.07	0.6 ± 0.02	...
72 h	0.6 ± 0.06	0.7 ± 0.06	0.6 ± 0.07	0.7 ± 0.07	1.0 ± 0.07	0.6 ± 0.04	1.0 ± 0.06

[a] Pregnancy outcome after inseminations made upon detected estrus during the Presynch period before enrollment in Experiment 1.
[b] Post-treatment outcome after first service (Experiment 1) or after a repeat service (Experiment 2).
[c] Assessed at treatment injection (1 = thin and 5 = fat).
[d] Average fat (3.5%)-corrected milk (Experiment 1) or energy-corrected milk (Experiment 2) of the test-day milk weight immediately before treatment injection.

2.1. Experiment 1

2.1.1. Experimental design

Lactating dairy cows (n = 2358) were pre-enrolled at multiple sites in Merced and Stanislaus counties in the Central Valley of California. Various characteristics of six herds are summarized in Table 1. Cows were enrolled in a Presynch protocol (two 25-mg $PGF_{2\alpha}$ injections IM; Lutalyse; Pfizer Animal Health, New York, NY, USA) given 14 d apart. Cows detected in estrus in response to the Presynch $PGF_{2\alpha}$ injections were inseminated. In Herds 1–6, 1094 cows were inseminated during the Presynch period. The residual cows (n = 1264) not inseminated during the Presynch preenrollment period were then enrolled in a Cosynch-72 timed AI program (GnRH injection given 7 d before and 72 h after treatment with $PGF_{2\alpha}$; timed AI at 72 h) that was initiated 12–14 d after the second Presynch injection (Fig. 1). Cows were assigned randomly to either cloprostenol (n = 650) or dinoprost (n = 614) as

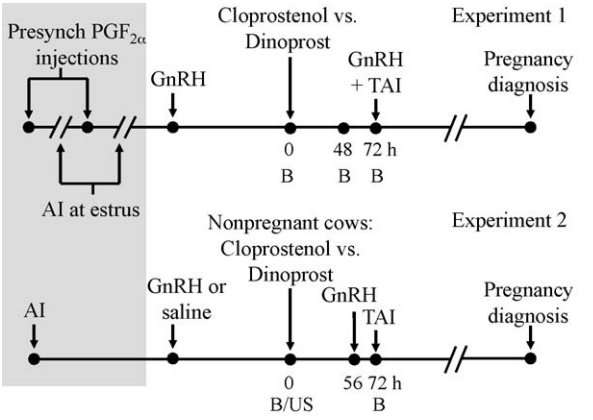

Fig. 1. *Experiment 1*. Lactating dairy cows (n = 2358) in six California dairy herds were preenrolled in a Presynch protocol (two $PGF_{2\alpha}$ injections given 14 d apart). Cows detected in estrus in response to the Presynch $PGF_{2\alpha}$ injections were inseminated (n = 1094), whereas the remaining cows (n = 1264) were treated with the Cosynch-72 timed AI protocol beginning 12 or 14 d after the second Presynch injection. Alternate cows were given 0.5 mg of cloprostenol (n = 650) or 25 mg of dinoprost (n = 614) before timed AI as part of the Cosynch-72 protocol. *Experiment 2*. In one Kansas herd, 427 cows of unknown pregnancy status were enrolled in an Ovsynch-Resynch procedure (GnRH [n = 333] or saline injection [n = 94] given 7 d before a not-pregnant diagnosis before treatment with cloprostenol or dinoprost, followed in 56 h by a GnRH injection and timed AI at 72 h). Ovarian structures were mapped and sized by transrectal ultrasonography (US) at the time of the not-pregnant diagnosis. Blood samples (B) were collected before treatment injection (0 h) and at 48 and 72 h (Experiment 1) or at 0 and 72 h (Experiment 2). Shaded area for Experiment 1 represents pretreatment Presynch injections. Cows not inseminated were then assigned randomly to treatments in Experiment 1. Shaded area for Experiment 2 represents pretreatment AI in which only resulting nonpregnant cows were enrolled in the experiment.

the treatment $PGF_{2\alpha}$ that preceded AI. Cows received 0.5 mg of cloprostenol (2 mL of Estrumate IM; Schering Plough Animal Health, Union, NJ, USA) or 25 mg of dinoprost (5 mL of Lutalyse IM) before AI as part of the Cosynch-72 procedure. Body condition scores (BCS; 1 = emaciated, 5 = obese) were assigned at treatment [21] in 1049 of 1264 (83.0%) cows studied. The GnRH injection given 7 d before treatment and at the scheduled AI, was either of two products (2 mL of Fertagyl, Intervet Inc., Millsboro, NJ, USA, or 2 mL of Cystorelin, Merial Ltd., Duluth, GA, USA).

Cows detected in estrus (377 of 1264) during the 3 d between the treatment injection and the scheduled AI were inseminated while restrained in feed line lockups. Detection of estrus included visual observation, but also relied on tail chalk removal when cows were examined each morning while restrained in feed line lockups. Inseminations made during the breeding week after treatment injections included those made after detected estrus and at 72 h post-treatment before the scheduled timed AI. A few cows in one herd detected in estrus after the timed AI were re-inseminated when still in estrus 12 h later. Breeding codes at the time of AI after treatment were: (1) timed AI-coded cows having no diagnosed signs of estrus before or at the time of AI; (2) estrus-coded cows inseminated before the scheduled timed AI (83%) or double inseminated (timed AI and then re-inseminated because of later estrus expression; 17%); and (3) timed AI + estrus-coded cows diagnosed in estrus at the timed AI. The remaining cows (887 of 1264) not inseminated at estrus received the scheduled timed AI at 72 h post-treatment, of which 34 cows received the treatment injection and were sampled for blood, but were culled before pregnancy diagnosis. Pregnancy was diagnosed weekly in five herds by transrectal palpation beginning at 35 d after AI; in the sixth herd, pregnancy was diagnosed by transrectal ultrasonography 32 d after AI.

2.1.2. Blood collection and radioimmunoassay

Blood samples were collected from all cows before treatment injection (0 h) and at 48 h and 72 h. Samples were stored on ice and transported to the laboratory for storage at 5 °C until serum was harvested by centrifugation (1,200 × g) the following morning. Serum samples were stored at −15 °C until they were shipped frozen each week on ice packs (< 48 h delivery) to our laboratory in Kansas. Progesterone was quantified in serum by radioimmunoassay [22]. Intra- and inter-assay CV for 31 assays were 6.6 and 5.8%, respectively, for a pooled serum sample that averaged 3.84 ± 0.04 ng/mL (n = 95).

2.1.3. Definitions

Because not all cows had a functional CL at the time of treatment injection, six progesterone response classifications were created on the basis of progesterone concentrations at 0, 48, and 72 h post-treatment. A cutoff concentration of 1 ng/mL was applied; concentrations $\geq$ 1 ng/mL defined a functional CL, whereas those <1 ng/mL indicated either a nonfunctional or regressing CL. The six progesterone responses were: (1) fast luteolysis occurred when blood concentrations of progesterone were $\geq$ 1 ng/mL at treatment (high; H) and < 1 ng/mL (low; L) at 48 h and 72 h after treatment (i.e., H-L-L); (2) slow luteolysis = H-H-L or L-H-L; (3) premature luteolysis = L-L-L; (4) partial or no luteolysis = H-H-H; (5) no possible luteolysis (cows in metestrus = L-H-H or L-L-H); and (6) rebound = H-L-H (Fig. 2). Luteal regression (success or failure) was assessed only in cows having progesterone concentration $\geq$ 1 ng/mL at 0 h (pretreatment). Cows having luteolysis must have had progesterone concentrations $\geq$ 1 ng/mL before treatment and < 1 ng/mL by 72 h post-treatment.

2.1.4. Statistical analyses

Changes in progesterone concentrations at 48 h and 72 h and proportional changes (P48 and P72 ratios) in progesterone relative to pretreatment progesterone concentration (0 h) were analyzed by ANOVA (procedure GLM, SAS Inst. Inc, Cary, NC, USA), using a model that included type of progesterone response classification (fast, slow, premature, partial, none possible, or rebound), treatment (cloprostenol vs. dinoprost), their interaction, lactation number (1, 2, 3, or 4+), herd (n = 6), and progesterone concentration at 0 h as a covariate.

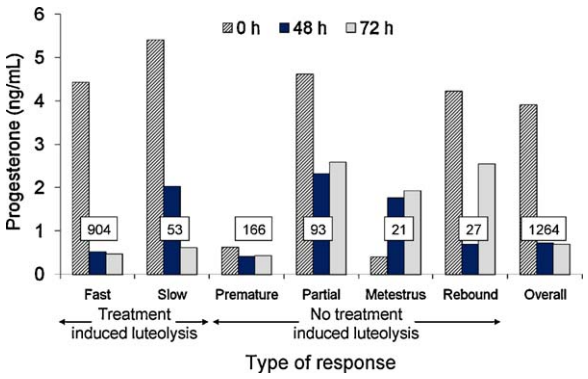

Fig. 2. Progesterone concentrations at 0, 48, and 72 h after injection of either cloprostenol or dinoprost in lactating dairy cows (Experiment 1). Type of response (luteolysis) was determined by the pattern of change in progesterone concentration. Concentrations at 0, 48, and 72 h were classified as high (H; $\geq$1 ng/mL) or low (< 1 ng/mL; L). Combinations of patterns of change were: fast luteolysis (H-L-L), slow luteolysis (H-H-L), premature luteolysis (L-L-L), partial or no luteolysis (H-H-H), no luteolysis (cows in metestrus: L-L-H and L-H-H), and partial luteolysis but rebound (H-L-H). Numbers of samples for each classification are shown for each triplet of means.

Successful luteolysis was assessed only in cows having pretreatment progesterone concentrations $\geq$ 1 ng/mL by logistic regression (procedure LOGISTIC, SAS Inst. Inc.). The initial model included treatment, lactation number, month of year (n = 4), herd, treatment by herd interaction, and progesterone concentration at 0 h as a covariate. The final model produced by backward stepwise selection of independent variables entered or retained in the model was based on a Wald statistic ($P < 0.10$) and consisted of treatment, month, and herd.

Pregnancies per AI (P/AI) were categorized on the basis of final progesterone concentration at 72 h post-treatment ($\geq$ 0.2 vs. <0.2 ng/mL, increasing by tenths to $\geq$ 0.8 vs. <0.8 ng/mL). Differences between treatments were determined by Chi-square using the Cochran-Mantel-Haenszel statistic (SAS Inst. Inc.). In addition, P/AI also were calculated from final progesterone concentrations in six ranges (< 0.314, 0.315–0.390, 0.391–0.459, 0.460–0.545, 0.546–0.747, and > 0.747 ng/mL); there were nearly equal numbers of cows in each concentration range. The logistic model included concentration range (n = 6), treatment, their interaction, herd, and lactation number. The final model excluded lactation number.

Pregnancies per AI were analyzed by logistic regression to determine the effects of treatment, type of progesterone response to treatment (fast, slow, premature, partial, none possible, or rebound). Likewise, P/AI were analyzed for cows in which luteolysis was possible (pretreatment progesterone concentrations $\geq$ 1 ng/mL), breeding code (timed AI, estrus, or timed AI + estrus), lactation number, herd, and sire within herd, AI technician within herd, fat (3.5%)-corrected milk (average test-day milk yield immediately before and after AI), month (n = 4), and BCS. The model including BCS represented a reduced number of cows, as BCS was not recorded in all cows. The final models produced by backward selection (described previously) consisted of type of progesterone response, herd, and breeding code. Unweighted percentages are reported with OR and their 95% confidence intervals.

2.2. Experiment 2

In one Kansas herd, 427 lactating dairy cows of unknown pregnancy status were enrolled in an

Ovsynch-Resynch procedure. An injection of GnRH [2 mL of Fertagyl, Intervet Inc.; n = 333] or saline [n = 94]) was given 7 d before a not-pregnant diagnosis preceding treatment with $PGF_{2\alpha}$ followed in 56 h by a GnRH injection and timed AI at 72 h; Fig. 1). Cows were enrolled randomly, with approximately every fourth cow assigned to be given saline rather than GnRH. Cows were eligible to be treated with either cloprostenol or dinoprost as in Experiment 1 when one or more CL was detected ultrsonographically before treatment.

Ovarian follicles and CL were mapped and sized by transrectal ultrasonography at the time of the not-pregnant diagnosis for purposes of counting of follicles $\geq$ 10 mm in diameter and the number of CL before treatment. All CL were assumed to be spherical. Diameter of structures was determined by averaging their largest cross-sectional width and height measured by ultrasound electronic calipers. Volume of the CL was calculated as follows:

$$\text{Volume} = 4/3 \times R^3 \times \pi$$

where W = largest width and H = largest height of the structure; R = radius (W/2 + H/2)/2, and π = 3.14159. When a CL contained a fluid-filled cavity, volume of the cavity was subtracted from the calculated CL volume.

Body condition scores were assigned at treatment as in Experiment 1. Blood samples were collected at 0 h and 72 h after treatment injection. Samples were stored on ice and transported to the laboratory for storage at 5 °C until serum was harvesting by centrifugation (1,200 × g). Serum samples were stored at −15 °C until they were assayed for progesterone concentration by radioimmunoassay [22]. Intra- and inter-assay coefficients of variation for eight assays were 8.3 and 6.0%, respectively, for a pooled serum sample that averaged 3.66 ± 0.09 ng/mL (n = 22). Successful luteolysis was assumed to have occurred when pretreatment progesterone concentrations were $\geq$1 ng/mL and then decreased to < 1 ng/mL by 72 h.

Pregnancy diagnosis via transrectal ultrasonography subsequent to AI occurred 32 to 39 d after timed AI. A positive pregnancy outcome required presence of anechoic uterine fluid and a large CL or anechoic uterine fluid and presence of an embryo.

2.2.1. Statistical analyses

Luteal regression was analyzed by logistic regression as described for Experiment 1. The initial model for assessing factors influencing possible luteal regression included treatment, number of CL (1 vs. >1), lactation number (1 vs. >1), most recent test-day energy-corrected milk yield preceding treatment ($\leq$43.1 vs. >43.1 kg [median]), administration of GnRH or saline 7 d before treatment, number of ovarian follicles at treatment ($\leq$1 vs. >1), days in milk ($\leq$161 vs. >161 [median]), season (hot = May to August; moderate = March, April, September, and October; cold = November to February), pretreatment progesterone concentration (covariable), and all two-way interactions of the preceding independent variables with treatment. The final model produced by backward stepwise selection of independent variables entered or retained in the model was based on a Wald statistic ($P < 0.10$) and consisted of treatment, number of CL, BCS, and season.

Pregnancies per AI were analyzed by logistic regression, as described previously for Experiment 1. The initial model included all variables described previously for luteal regression plus the effect of AI technician. The final model consisted of treatment, number of CL, GnRH, or saline injection, BCS, and technician. Unweighted percentages are reported with OR and their 95% confidence intervals.

Progesterone concentrations at 0 h and 72 h were analyzed using a general linear model in a split-plot configuration. Treatment was tested by the whole-plot error (cow within treatment). Least squares means and SEM were reported.

3. Results

Herd characteristics are summarized by herd for both experiments (Table 1).

3.1. Experiment 1

3.1.1. Serum progesterone responses

Progesterone concentrations in the six progesterone response classifications at 0 h, 48 h, and 72 h are shown (Fig. 2). The majority of cows fit the fast luteolytic response (71.5%), followed by premature luteolysis (13.1%), partial luteolysis (7.4%), slow luteolysis (4.2%), no luteolysis for cows in which progesterone rebounded after treatment (2.1%), and cows that were in metestrus at treatment (1.7%). Proportions of cows in each classification did not differ among treatments.

Progesterone concentrations at 0 h, 48 h, and 72 h after treatment are summarized by type of progesterone response classification (Table 2). Only one treatment difference was detected; progesterone concentrations were less ($P < 0.01$) in cows treated with cloprostenol than in cows treated with dinoprost at 48 h in the slow

Table 2
Changes in progesterone concentrations in lactating dairy cows at 0, 48, and 72 h after treatment with cloprostenol or dinoprost or proportional change from pretreatment (0 h) concentration (Experiment 1).

Response[a]	Hour	Concentration (ng/mL)		Proportion of 0 h concentration	
		Cloprostenol	Dinoprost	Cloprostenol	Dinoprost
Fast	0	4.58 ± 0.13 (455)[b]	4.57 ± 0.13 (449)	1.0	1.0
	48	0.50 ± 0.03	0.53 ± 0.03	0.16 ± 0.04	0.17 ± 0.04
	72	0.44 ± 0.03	0.44 ± 0.03	0.14 ± 0.03	0.15 ± 0.03
Slow	0	5.58 ± 0.50 (28)	5.28 ± 0.53 (25)	1.0	1.0
	48	1.73 ± 0.10**	2.35 ± 0.10	0.59 ± 0.15	0.81 ± 0.16
	72	0.61 ± 0.12	0.58 ± 0.12	0.17 ± 0.11	0.16 ± 0.12
Premature	0	0.69 ± 0.30 (81)	0.70 ± 0.29 (85)	1.0	1.0
	48	0.39 ± 0.06	0.40 ± 0.06	0.74 ± 0.10	0.74 ± 0.09
	72	0.39 ± 0.07	0.39 ± 0.07	0.82 ± 0.06	0.78 ± 0.06
Partial	0	4.23 ± 0.34 (62)	5.31 ± 0.48 (31)	1.0	1.0
	48	2.28 ± 0.07	2.38 ± 0.09	0.66 ± 0.11	0.57 ± 0.16
	72	2.47 ± 0.08	2.72 ± 0.11	0.74 ± 0.07	0.65 ± 0.10
Metestrus	0	0.50 ± 0.80 (11)	0.40 ± 0.83 (10)	1.0	1.0
	48	1.66 ± 0.16	1.83 ± 0.16	5.21 ± 0.24	4.85 ± 0.25
	72	1.99 ± 0.18	1.76 ± 0.19	5.73 ± 0.18*	4.27 ± 0.19
Rebound	0	4.21 ± 0.73 (13)	4.56 ± 0.71 (14)	1.0	1.0
	48	0.70 ± 0.14	0.69 ± 0.14	0.24 ± 0.24	0.23 ± 0.23
	72	2.37 ± 0.17	2.69 ± 0.17	0.77 ± 0.16	1.02 ± 0.15

* Different from dinoprost ($P < 0.01$) within hour.
[a] Fast luteolysis occurred when blood concentrations of progesterone were ≥1 ng/mL at treatment (high; H) and <1 ng/mL (low; L) at 48 and 72 h after treatment (i.e., H-L-L), slow luteolysis = H-H-L or L-H-L, premature luteolysis = L-L-L, partial incomplete luteolysis = H-H-H, no luteolysis possible (cows in metestrus = L-H-H or L-L-H), and rebound = H-L-H (see Fig. 2).
[b] Number of cows per treatment-hour.

response classification. When progesterone concentrations were expressed as a proportion of the pretreatment concentration (0 h), one treatment difference was detected at 72 h in the metestrous classification (Table 2).

When considering only cows having pretreatment progesterone concentrations ≥ 1 ng/mL and potentially eligible to respond to a luteolytic stimulus, the proportion of cows having successful luteolysis (progesterone < 1 ng/mL at 72 h) was greater ($P < 0.01$) in cows treated with dinoprost than with cloprostenol (Table 3). Odds ratio indicated that the probability for successful luteolysis were 96% greater (OR = 1.96; 95% confidence interval = 1.23 to 3.16; $P < 0.01$) for cows treated with dinoprost than for cows treated with cloprostenol. In the reduced set of cows for which BCS was measured, thinner cows (BCS ≤ 2.5) were 2.15 times (95% confidence interval = 1.31–3.54; $P < 0.05$) more likely to have luteolysis than cows having BCS >2.5 (93.7 vs. 86.8%, respectively).

3.1.2. Pregnancies per AI

Pregnancies per AI with regard to the progesterone response to treatment did not differ between treatments (Table 4). As expected, few pregnancies occurred in cows in the partial, none, and rebound luteolysis categories. The best conception occurred in cows having fast-type luteolysis (44.5%), followed by slow (29.1%) and premature (24.2%) luteolysis. Moreover,

Table 3
Proportion of lactating dairy cows having luteal regression in response to cloprostenol or dinoprost treatment (Experiment 1)[a].

Herd	Treatment		Overall
	Cloprostenol	Dinoprost	
	% (n)		
1	93.5 (46)	91.7 (48)	92.6 (94)
2	83.1 (65)	96.8 (62)	89.8 (127)
3	89.1 (46)	89.7 (39)	89.4 (85)
4	78.0 (50)	86.3 (51)	82.1 (101)
5	74.5 (98)	77.8 (99)	76.1 (197)
6	92.1 (253)	97.3 (220)	94.5 (473)
Total	86.6* (558)	91.3 (519)	89.1 (1077)

* Different from dinoprost ($P < 0.01$). Odds ratio = 1.96 (95% confidence interval = 1.23 to 3.16).
[a] Only cows having pretreatment progesterone concentrations ≥1 ng/mL were analyzed. Luteolysis was considered successful for fast (H-L-L) or slow (H-H-L) categories and failed for partial incomplete (H-H-H) or rebound (H-L-H) categories; other categories (premature [n = 166] and metestrus [n = 21]) were excluded entirely (see Fig. 2).

Table 4
Pregnancies per AI in lactating dairy cows after AI with regard to progesterone response to cloprostenol or dinoprost treatment (Experiment 1)[a].

Response	Treatment		
	Cloprostenol	Dinoprost	Overall[b]
	% (n)		
Fast	45.2 (442)	43.8 (434)	44.5[x] (876)
Slow	28.6 (28)	29.6 (27)	29.1[x,y] (55)
Premature	22.5 (80)	25.9 (85)	24.2[y] (165)
Partial	3.2 (62)	6.7 (30)	4.3[z] (92)
Metestrus	10.0 (10)	25.0 (8)	16.7[y] (18)
Rebound	30.8 (13)	9.1 (11)	20.8[y] (24)

[a]Fast luteolysis classification was defined as when blood concentrations of progesterone were ≥1 ng/mL at treatment (high; H) and <1 ng/mL (low; L) at 48 and 72 h after treatment (i.e., H-L-L); slow luteolysis = H-H-L or L-H-L; premature luteolysis = L-L-L; partial or no luteolysis = H-H-H; no luteolysis (cows in metestrus = L-H-H or L-L-H); and rebound = H-L-H (see Fig. 2).
[b]Excludes 34 cows that were culled before pregnancy diagnosis.
[x-z]Mean percentages having different superscript letters differ ($P \leq 0.05$).

P/AI did not differ between treatments in cows that had or did not have functional luteolysis (Table 5). As expected, P/AI was greater ($P < 0.05$) when functional luteolysis occurred than when it did not occur (Table 5). Progesterone concentration in cows with luteal regression (0.45 ± 0.03 ng/mL; n = 117) was less ($P < 0.001$) than that in cows having no luteal regression (2.59 ± 0.07 ng/mL; n = 955).

Pregnancies per AI were determined for cows on the basis of progesterone concentrations at 72 h after treatment. Number of pregnancies achieved did not differ between treatments when progesterone concentrations at 72 h post-treatment were varied from < 0.2 ng/mL to < 0.8 ng/mL. Combined P/AI for treatments were 51.5% (n = 33) when progesterone was < 0.2 ng/mL, 44.9% (n = 176) at < 0.3 ng/mL, 41.6% (n = 447) at < 0.4 ng/mL, 41.3% (n = 721) at < 0.5 ng/mL, 41.2% (n = 894) at < 0.6 ng/mL, 40.9% (n = 995) at < 0.7 ng/mL, and 41.2% (n = 1045) at < 0.8 ng/mL at 72 h post-treatment. When progesterone concentrations at 72 h after treatment were categorized in concentration groups with nearly equal numbers of cows per treatment-concentration range, P/AI also did not differ between treatments. Pregnancies per AI were 43.5% (n = 207) when concentrations were < 0.314 ng/mL at 72 h and numerically declined from there: 0.314 to 0.390 ng/mL (41.2%; n = 199), 0.391 to 0.459 ng/mL (41.5%; n = 205), 0.460 to 0.545 ng/mL (41.6%; n = 202), 0.546 to 0.747 ng/mL (37.2%; n = 207), and > 0.747 ng/mL (19.1%; n = 210). When progesterone concentrations at 72 h were > 0.747 ng/mL, P/AI was reduced ($P < 0.05$) to 19.1%; P/AI ranged from 37.2 to 43.5% when progesterone concentration was ≤ 0.747 ng/mL.

Table 5
Pregnancies per AI in lactating dairy cows in response to cloprostenol or dinoprost injection as part of the Cosynch-72 protocol (Experiment 1).

Item	Treatment[a]		
	Cloprostenol	Dinoprost	Overall
	% (n)		
Luteal regression[b]			
No	8.0 (75)	7.3 (41)	7.8[x] (116)
Yes	44.3 (469)	43.1 (459)	43.8[y] (928)
Body condition[c,d]			
≤2.5	38.0 (234)	34.1 (226)	36.1[x] (460)
>2.5	37.2 (293)	42.5 (266)	39.7[x] (559)
Herd[c]			
1	43.9 (57)	40.0 (55)	42.0[x,y] (112)
2	25.0 (80)	30.1 (83)	27.6[x] (163)
3	34.0 (53)	17.0 (47)	26.0[x] (100)
4	30.9 (55)	32.7 (55)	31.8[x,y] (110)
5	37.7 (130)	39.4 (127)	38.5[y] (257)
6	40.0 (260)	44.7 (228)	42.2[y] (488)
Breeding code[c,e]			
Timed AI	33.6 (453)	33.5 (400)	33.5[x] (853)
Estrus	38.3 (107)	43.1 (102)	40.7[x,y] (209)
Timed AI + estrus	53.3 (75)	50.5 (93)	51.8[y] (168)
Total	36.7 (635)	37.8 (595)	

[a]Treatment was applied 3 d before scheduled timed AI.
[b]Includes only 1044 cows eligible for luteal regression (progesterone concentrations ≥1 ng/mL before treatment) for which pregnancy outcome was known before culling.
[c]Includes all cows, regardless of whether luteal regression occurred.
[d]Body scores were assessed in 1019 cows for which pregnancy outcome was known before culling.
[e]Timed AI-coded cows had no diagnosed signs of estrus before AI; estrus-coded cows were inseminated after treatment injection but before the scheduled timed AI (83%) or double inseminated (17%; showed estrus after timed AI and were reinseminated); and timed AI + estrus-coded cows were in estrus at the timed AI.
[x,y]Mean percentages having different superscript letters differ ($P \leq 0.05$).

Differences in P/AI were detected among herds (Table 5). Although P/AI did not differ between treatments (Table 5), breeding codes indicated that cows in estrus at the timed AI had greater ($P < 0.05$) P/AI than those receiving timed AI without estrual symptoms. Cows inseminated post-treatment after detected estrus before the scheduled timed AI, or those double inseminated because estrus was detected after timed AI, had intermediate P/AI (Table 5). Most cows that were estrus-coded before AI expressed estrus (83%) during 1–3 d before the scheduled timed AI compared with 17% of cows reinseminated within 24 h

Table 6
Factors affecting luteal regression after treatment with cloprostenol and dinoprost in lactating dairy cows having one or more than one CL before treatment (Experiment 2).

Item	n[a]	Luteal regression (%)	Odds ratio	95% confidence limits	P value
Treatment					
Cloprostenol	191	69.1	Referent		0.001
Dinoprost	205	78.5	1.64	1.01–2.68	
No. of CL					
1	293	74.7	Referent		0.231
2+	103	71.8	1.41	0.80–2.49	
Body condition					
≤2.25	220	83.2	Referent		0.001
>2.25	176	62.5	2.72	1.66–4.46	
Season[b]					
Hot	73	57.5	Referent		0.001
Moderate	83	68.7	1.52	0.75–3.05	
Cold	240	80.8	3.10	1.68–5.71	

[a] Although a CL was visible, 31 of 427 cows that did not have pretreatment progesterone concentrations ≥1 ng/mL (not eligible for luteolysis) were excluded.
[b] Hot (May to August); moderate (March, April, September, and October); cold (November to February).

after the timed AI because of detected signs of estrus. Further, similar proportions of cows displayed estrus after cloprostenol and dinoprost (49.6 vs. 51.7%), respectively.

At five of six dairies, several characteristics of 757 cows inseminated before enrollment in the Experiment 1 in response to Presynch injections of $PGF_{2\alpha}$ were recorded. Fewer cows were inseminated after the first Presynch injection (10.1%) than after the second injection (89.9%). Cows were submitted for insemination on the basis of observations from an activated heatmount detector (3%; n = 23), chalk rubs (2.5%; n = 19), other secondary signs of estrus (0.4%; n = 3), and standing estrus (94.1%; n = 713). For those signs of estrus, P/AI averaged 13.0, 10.5, 33.3, and 35.9%, respectively. Interval to detected estrus after the first Presynch injection was shorter ($P < 0.001$) than after the second injection (4.5 ± 0.1 vs. 9.6 ± 0.3 d). Interval to estrus also differed among dairies and lactation numbers, with cows in their second or greater lactation having shorter ($P < 0.05$) intervals than first-lactation cows (6.7 ± 0.2 d vs. 7.8 ± 0.3 d).

3.2. Experiment 2

3.2.1. Luteal regression

The proportion of 427 cows having one, two, or three CL before treatment was 75.2% (n = 321), 22.7% (n = 97), and 2.1% (n = 9), respectively. Of those cows initiating the Ovsynch-Resynch protocol with either saline or GnRH, 17 of 94 cows (18%) treated with saline had more than one CL at treatment compared with 89 of 333 cows treated with GnRH (26.7%; P = 0.087). Among factors analyzed (treatment, number of CL, lactation number, energy-corrected milk yield, injection of GnRH 7 d before treatment, number of ovarian follicles ≥ 10 mm in diameter, BCS, days in milk, season, and pretreatment progesterone concentration in cows having either one or more than one CL), only treatment, BCS, and season were significant (Table 6). Luteal regression was 1.64 times more ($P < 0.001$) likely when using dinoprost than when using cloprostenol. Regression of CL in cows having one CL was 74.7% and did not differ from that of cows having more than one CL (71.2%). Corpora lutea in cows having greater body condition (BCS > 2.25) were 2.72 times less likely to regress (Table 6). The poorest CL regression occurred during summer (Table 6).

3.2.2. Pregnancies per AI

Among factors tested that may have influenced pregnancy outcome (treatment, number of CL, lactation number, energy-corrected milk yield, injection of GnRH 7 d before treatment, number of ovarian follicles ≥ 10 mm in diameter, BCS, days in milk, season, and pretreatment progesterone concentration in cows having either one or more than one CL), only GnRH injection 7 d before treatment (P = 0.059) and BCS (P = 0.09) tended to be significant (Table 7). Injecting GnRH 7 d before treatment tended (P = 0.059) to increase odds of P/AI by 1.71 times, and greater BCS tended (P = 0.09) to decrease P/AI (Table 7).

3.2.3. Progesterone and luteal characteristics

Pretreatment progesterone concentrations did not differ before treatments of dinoprost or cloprostenol

Table 7
Factors affecting pregnancies per AI (P/AI) after treatment with cloprostenol and dinoprost in lactating dairy cows having one or more than one CL before treatment (Experiment 2).

Item	n[a]	P/AI[b] (%)	Odds ratio	95% confidence limits	P value
Treatment					
Cloprostenol	198	31.3	Referent		0.707
Dinoprost	201	32.8	1.09	0.71–1.66	
No. of CL					
1	298	31.5	Referent		0.758
2+	101	33.7	1.08	0.66–1.76	
Upfront GnRH[c]					
No	87	23.0	Referent		0.059
Yes	312	34.6	1.71	0.98–3.00	
Body condition					
$\leq$ 2.25	229	35.8	Referent		0.090
$>$ 2.25	170	27.1	1.49	0.95–2.30	

[a] Excludes 28 of 427 cows for which pregnancy outcome was not known before culling.
[b] Includes all cows regardless of CL regression status.
[c] GnRH was given 7 d before treatment.

were applied (4.75 $\pm$ 0.2 vs. 4.57 $\pm$ 0.2 ng/mL). By 72 h post-treatment, concentrations were similar between dinoprost and cloprostenol treatments (0.89 $\pm$ 0.2 vs. 1.03 $\pm$ 0.2 ng/mL). Pretreatment progesterone concentrations in serum were greater ($P < 0.001$) for cows bearing more than one CL (5.92 $\pm$ 0.31 ng/mL; n = 106) than for cows having only one CL (4.22 $\pm$ 0.19 ng/mL; n = 321). Fewer cows having more than one CL had lesser progesterone concentrations and more cows had greater progesterone concentrations across the progesterone profile for all cows (Fig. 3; upper panel). This greater progesterone was related to greater total luteal volume in cows having more than one CL (Fig. 3; lower panel). Progesterone also tended ($P = 0.09$) to be greater in cows just before treatment when they were injected with GnRH 7 d earlier (5.37 $\pm$ 0.22 ng/mL; n = 333 vs. 4.77 $\pm$ 0.32 ng/mL; n = 94). Cows having only one follicle $\geq$ 10 mm had greater progesterone concentrations just before treatment than cows having two or more follicles $\geq$10 mm in diameter ($P < 0.05$; 5.38 $\pm$ 0.25 ng/mL; n = 208 vs. 4.76 $\pm$ 0.24 ng/mL; n = 219).

Diameter (25.1 $\pm$ 0.4 mm; n = 32 vs. 25.7 $\pm$ 0.7 mm; n = 106) and cavity-corrected volume (8.6 $\pm$ 0.4 cm^3 vs. 9.2 $\pm$ 0.7 cm^3) of the largest CL did not differ between cows having one or more than one CL. The proportion of single CL vs. multiple CL having a cavity also was not different (44.4%; n = 320 vs. 41.5%; n = 106, respectively), although the ancillary CL (20.4 $\pm$ 0.4 mm; n = 106) were smaller ($P < 0.01$) in multiple-CL cows, and the proportion of ancillary CL having a fluid-filled cavity was 37.7%. Total luteal volume in multiple-CL cows (14.2 $\pm$ 0.7 cm^3) was greater ($P < 0.001$) than that

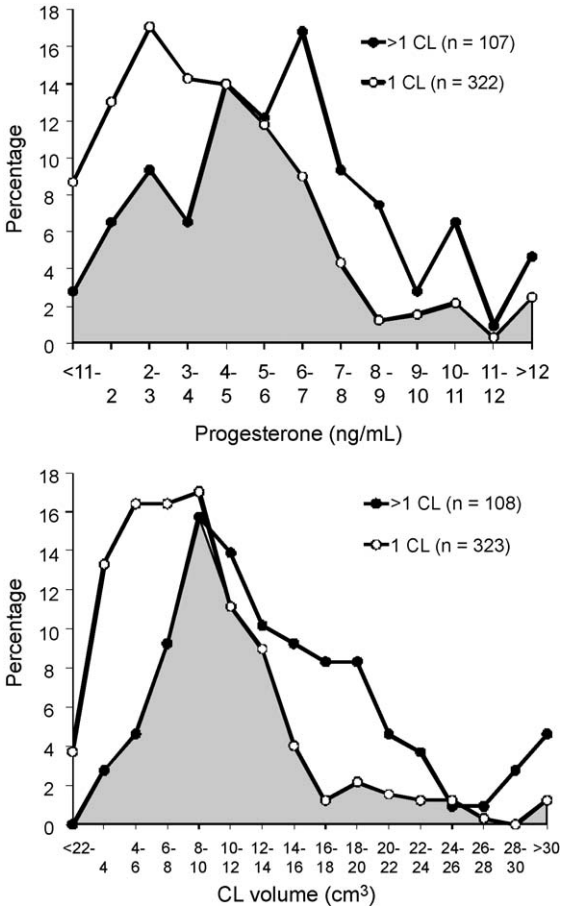

Fig. 3. Proportional frequency of lactating dairy cows having either one or more than one CL and the respective progesterone concentrations (upper panel) or respective total luteal volume (lower panel) before luteolysis. Shaded portions represent areas of overlap between one versus more than one CL (Experiment 2).

in cows having only one CL (8.6 ± 0.4 cm^3). Despite having more luteal tissue volume, multiple-CL cows had a lesser ($P = 0.052$) progesterone concentration (ng/mL) per cm^3 of luteal volume than cows with only one CL (0.49 ± 0.04 vs. 0.57 ± 0.02), suggesting possible differences in composition, secretion rate, or elimination rate of progesterone in cows having single vs. multiple CL.

4. Discussion

There are several reports in the literature comparing physiological responses of bovine females to administration of either cloprostenol or dinoprost. Of primary interest in the present study was the potential for increased luteolytic efficacy for cloprostenol because of its longer blood half-life (3 h versus a few minutes) [1,2] and greater affinity for the $PGF_{2\alpha}$ receptor [4] compared with the native $PGF_{2\alpha}$ structure or dinoprost. Clearance of $PGF_{2\alpha}$ or dinoprost ($PGF_{2\alpha}$ produced by chemical synthesis) is accomplished by one or two passages through the liver and/or lungs, and its residues do not accumulate in blood after repeated daily injection in cattle [1].

Cloprostenol is a synthetic racemic analog of $PGF_{2\alpha}$. Normally, a racemic mixture of isomers D-cloprostenol and L-cloprostenol is obtained by chemical synthesis [2]. Both cloprostenol and the pure D-cloprostenol are used in veterinary medicinal products; however, only the D-isomer of cloprostenol exhibits luteolytic activity [2]. Only D-cloprostenol binds to the $PGF_{2\alpha}$ receptor in bovine CL and myometrial cells [23].

In the present study, dinoprost was more effective than cloprostenol at reducing progesterone concentrations by 72 h in both experiments (Tables 3 and 6). When cows had more than one CL (1.65 times more luteal tissue volume in multiple-CL cows than in single-CL bearing cows) present before treatment, reduction in progesterone concentrations by 72 h post-treatment was similar between products (Table 6). Actual or proportional decrease in progesterone or rate of decline in progesterone concentrations in both experiments did not differ between treatments, as reported elsewhere for bovine blood [3,7] and milk [24]. Most importantly, the pregnancy outcomes of cows inseminated at first service or at repeat services did not differ, regardless of which luteolytic product was applied. This lack of difference was true for cows that had luteolysis (Table 5), as well as for those with partial or incomplete luteolysis, as defined by their progesterone response to treatment (Table 4).

The number of experimental units (cow) initially planned per treatment (630 cows per treatment) was expected to provide sufficient power to determine statistical significance when P/AI between treatments differed by 7.5% units and P/AI after first postpartum AI ranged from 30 to 40% ($\alpha = 0.05$; $\beta = 0.80$). Our inability to detect a difference in P/AI between treatments is, in effect, a finding of a true "no difference" between products used in these breeding management protocols to produce pregnancies.

An unpublished meta analysis (A. L. Skidmore, personal communication) of conception rates (21 studies), pregnancy rates (11 studies), and overall estrus-detection rates (15 studies) reported OR that exceeded 1.0. Mean ratios exceeding 1.0 indicate a relative advantage or greater probability of success for cows treated with cloprostenol vs. dinoprost. Mean OR for those traits were: conception rate (1.04; 95% CI = 0.82–1.38; $P > 0.20$); pregnancy rate 1.09; 95% CI = 0.92–1.31; $0.10 < P < 0.20$); and overall estrus-detection rate (1.04; 95% CI = 0.93–1.21; $P > 0.20$). Although numerically greater, the 95% confidence intervals failed to produce any $P \leq 0.05$.

In the present study, several factors contributed to improved conception, regardless of treatment. Cows having lesser basal progesterone concentrations at 72 h after treatment had significantly higher conception. Further, cows showing signs of estrus clearly had improved conception rates, whether that occurred post-treatment or at the time of AI (Table 5). Cows having greater progesterone concentrations at the $PGF_{2\alpha}$ injection of the Ovsynch protocol and greater concentrations of estradiol at 48 h after $PGF_{2\alpha}$ had greater probabilities of pregnancy assessed 35 d after timed AI [25]. Greater estradiol probably reflected a greater probability of a mature preovulatory follicle and potentially greater expression of estrus and improved fertility, consistent with our results.

Other researchers [26] measured both serum concentrations of progesterone and estradiol, which induces sexual behavior, and reported no differences among heifers treated with dinoprost, cloprostenol, or fenprostalene. In one large estrus-synchronization study of 1002 beef heifers, dinoprost and cloprostenol were equally efficacious for inducing a synchronous fertile estrus [12]. Similar results were reported between the two products in nonlactating Holstein cows [7] and Angus cows and heifers [9]. Interval to estrus after either dinoprost or cloprostenol was not different in dairy heifers or in previously superovulated heifers that had multiple CL [26].

It is unclear how body condition may influence luteolytic potency of either product. In both experiments, thinner cows were more likely to have luteolysis

than cows with greater BCS even though the median cut points were slightly different (2.50 vs. 2.25) between Experiments 1 and 2, respectively. Perhaps a less functional follicle ovulated in thinner cows and formed a CL that was more sensitive to the luteolytic effects of $PGF_{2\alpha}$. We could find no report in the literature corroborating the effect of BCS on luteolysis.

Further, season was a significant source of variation for luteolytic success in Experiment 2 but not in Experiment 1. Seasonal differences in luteolysis between experiments are likely explained by the timing of the experimental periods. All of the first experiment was conducted from January to May in California during a moderate season; the poorer results for luteal regression were observed during summer months in Kansas. In seasonally breeding mares, there were indications that changes in luteal function during the autumn transition were not the result of alterations in the ability of the uterus to produce $PGF_{2\alpha}$ or changed CL sensitivity to $PGF_{2\alpha}$ [27]. Because the lung is mostly responsible for clearance of $PGF_{2\alpha}$, perhaps increased respiratory rates associated with elevated temperature and humidity of summer are associated with more rapid clearance of $PGF_{2\alpha}$ and poorer luteolysis [28]. Delayed luteolysis in heat stressed versus thermoneutral lactating dairy cows may have been caused by failure of follicular estradiol to initiate the series of endocrine events leading to luteolysis that resulted from decreased synthesis of estradiol [29]. Wilson et al. [29] also concluded that heat stress inhibited ovarian follicular growth and dominance during the preovulatory period, resulting in abnormal ovarian function as manifested by a decreased proestrous rise in estradiol and changes in numbers of follicular waves per estrous cycle.

Cows in Experiment 2 were enrolled in an Ovsynch-Resynch program (average day from last AI = 36.5 $\pm$ 0.6 d; median day from last AI = 33) 7 d before pregnancy diagnosis were either treated with saline or GnRH. Cows treated with GnRH tended to have more than one CL, greater progesterone concentrations, and greater P/AI than saline-treated cows. Further, cows having only one follicle $\geq$ 10 mm compared with two or more follicles at treatment had greater progesterone concentrations possibly indicating better follicular wave synchronization after previous ovulation induced by GnRH. These results supported the concept that the upfront GnRH injection may be beneficial to outcomes from Ovsynch-Resynch program. It was earlier demonstrated in a larger study, however, that for cows enrolled in a similar Ovsynch-Resynch program before d 35 since last AI were not likely to benefit in pregnancy outcome from GnRH versus saline, compared with those enrolled after d 35 [30].

In conclusion, on the basis of our definition for luteolysis, which required progesterone concentrations to be $\geq$ 1 ng/mL before treatment and $<$ 1 ng/mL by 72 h after treatment, dinoprost was a more effective luteolytic product than cloprostenol. This was true in both experiments, including cows known to have more than one CL before treatment. However, despite this difference in luteolytic efficacy, no differences in pregnancy outcomes were detected between cloprostenol and dinoprost in either experiment. A novel finding was that luteolysis was less effective in cows with BCS exceeding 2.50 (Experiment 1) or 2.25 (Experiment 2) than in thinner cows. We concluded that both products were equally effective luteolysins and produced similar pregnancy outcomes in lactating dairy cows.

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