Luteolysis and Pregnancy Outcomes in Dairy Cows after Treatment with Estrumate or Lutalyse

J. S. Stevenson and A. P. Phatak[1]

Summary

In Experiment 1, lactating dairy cows (n = 1,230) in 6 herds were treated with 2 injections of prostaglandin $F_{2\alpha}$ (**$PGF_{2\alpha}$**) 14 days apart (**Presynch**), with the second injection administered 12 to 14 days before the onset of a timed AI protocol (**Ovsynch**). Cows were inseminated when detected in estrus after the Presynch $PGF_{2\alpha}$ injections. Cows not inseminated were enrolled in the Ovsynch protocol and were assigned randomly to be treated with either Estrumate or Lutalyse as part of a timed artificial insemination (**AI**) protocol. Blood samples were collected before treatment injection (0 hour) and 48 and 72 hours later. In cows having progesterone concentrations ≥1 ng/mL at 0 hour and potentially having a functional corpus luteum (**CL**) responsive to a luteolytic agent, Lutalyse increased ($P < 0.05$) luteal regression from 83.9 to 89.3%. Despite a significant increase in luteolysis, pregnancy rate per AI did not differ between treatments. Fertility was improved in both treatments in cows having reduced progesterone concentrations at 72 hours and in those showing signs of estrus. In Experiment 2, an ovulation resynchronization (**Ovsynch-Resynch**) program was initiated with gonadotropin-releasing hormone (**GnRH**) or saline in 427 previously inseminated lactating dairy cows of unknown pregnancy status in 1 herd. Seven days later, pregnancy was diagnosed and nonpregnant cows were blocked by number of CL and assigned randomly to receive Estrumate or Lutalyse. Diameter of each CL was recorded and blood samples were collected at 0 and 72 hours after treatment to assess serum progesterone. A fixed-time AI was given at 72 hours after treatment and approximately 16 hours after a GnRH injection to induce ovulation. Lutalyse 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 exposed to treatment. Pregnancy rate per AI did not differ between treatments. Although Lutalyse was slightly more effective than Estrumate in inducing luteolysis in lactating dairy cows exposed to an Ovsynch or Ovsynch-Resynch protocol, resulting pregnancy outcomes did not differ between products.

Introduction

Since the first prostaglandin $F_{2\alpha}$ (**$PGF_{2\alpha}$**) product (Lutalyse, The Upjohn Co., Kalamazoo, MI) was introduced in the United States in 1979, 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 similar to uterine-derived $PGF_{2\alpha}$ (Lutalyse, ProstaMate, and In Synch) and its agonists (Estrumate and estroPLAN).

Different physiological responses of bovine females to administration of either Estrumate or Lutalyse have been reported for luteolysis, receptor binding, intrauterine pressure, estrus expression, conception rates, and pregnancy rates. An unpublished meta-analysis of some of these factors did not find significant differences in conception rate and pregnancy rate or overall differences in detected estrus. Odds ratios, however, consistently were greater than 1.0, indicating trends in the combined studies that numerically, but not significantly, favored Estrumate over Lutalyse.

[1] Technical Services Veterinarian, Alta California, Hilmar, CA.

Strict timed artificial insemination (**AI**) programs have become common in dairy operations because they are reliable and not dependent on visual or other means of detecting estrus in cattle. The **Ovsynch** protocol (injection of gonadotropin-releasing hormone (**GnRH**) 7 days before and 48 or 72 hours after treatment with prostaglandin $PGF_{2\alpha}$; timed AI at 72 hours) synchronizes follicular maturation and luteal regression, resulting in about 20 to 30% of cows having at least 2 luteal structures at the time of $PGF_{2\alpha}$ injection. A good test of luteolytic efficacy between product types (Lutalyse vs. Estrumate) is possible in lactating cows to which the Ovsynch protocol is applied because a larger proportion of cows have more than 1 corpus luteum to regress at the time of the $PGF_{2\alpha}$ injection.

The present study consisted of 2 experiments. The objective of the first experiment was to determine the efficacy of luteal regression in response to 2 chemically different luteolytic products (Estrumate vs. Lutalyse) as determined by changes in progesterone concentrations in blood and subsequent pregnancy outcome of lactating dairy cows exposed to either of the 2 products before first postpartum AI. The objective of the second experiment was similar to that of the first, except the number of corpora lutea (**CL**) and total luteal tissue volume were quantified in previously inseminated nonpregnant dairy cows before treatment injections were administered.

Experimental Procedures

Experiment 1

Lactating dairy cows were enrolled at multiple sites in Merced and Stanislaus counties in the Central Valley of California. Cows were enrolled in a **Presynch** protocol (2 $PGF_{2\alpha}$ injections administered 14 days apart; Lutalyse; Pfizer Animal Health, New York, NY). Cows detected in estrus in response to the Presynch $PGF_{2\alpha}$ injections were inseminated, and the residual cows were then enrolled in a Cosynch-72 timed AI program (GnRH injection administered 7 days before and 72 hours after treatment with $PGF_{2\alpha}$; timed AI at 72 h) that was initiated 12 to 14 days after the second Presynch injection (Figure 1). Cows were assigned randomly to either of 2 luteolytic product injections as the treatment $PGF_{2\alpha}$ that preceded AI. Cows received i.m. 2 mL of Estrumate (0.5 mg of Cloprostenol, Schering Plough Animal Health, Union, NJ) or 5 mL of Lutalyse (Dinoprost, Pfizer Animal Health) before AI as part of the Cosynch-72 procedure. Body condition scores (**BCS**; 1 = emaciated, 5 = obese) were assigned at treatment in 1,019 of 1,230 (82.8%) cows studied.

Cows detected in estrus after the treatment injection and before the scheduled AI were inseminated while restrained in feed line lockups. Estrus detection 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 that followed treatment injections included those made after detected estrus and at 72 hours posttreatment by appointment. A small proportion of cows in 1 herd detected in estrus after AI were reinsminated when still in estrus 12 hours later. Breeding codes at the time of AI were as follows: (1) timed AI-coded cows had no diagnosed signs of estrus before or at the time of AI, (2) estrus-coded cows were inseminated before the scheduled timed AI (83%) or double inseminated (timed AI and then reinseminated because of estrus expression; 17%), and (3) timed AI + estrus-coded cows were diagnosed in estrus at the timed AI.

Pregnancy was diagnosed weekly in 5 herds by transrectal palpation of the uterus beginning at 35 days after AI; in the sixth herd, pregnancy was diagnosed by transrectal ultrasonography at

day 32 after AI. At all locations, blood samples were collected before treatment injection (0 hour) and at 48 and 72 hours. Progesterone was quantified in serum by radioimmunoassay.

Experiment 2

In our Kansas State University herd, 333 lactating dairy cows of unknown pregnancy status were enrolled in an ovulation resynchronization (**Ovsynch-Resynch**) procedure (GnRH injection administered 7 days before a not-pregnant diagnosis before treatment with $PGF_{2\alpha}$ followed in 56 hours by a GnRH injection and timed AI at 72 h; Figure 1). Cows were eligible to be treated randomly with either Estrumate or Lutalyse as in Experiment 1 when 1 or more CL was present upon transrectal ultrasonography before treatment. An additional 94 cows not preenrolled in an Ovsynch-Resynch protocol and having a CL at a not-pregnant diagnosis were administered randomly either Estrumate or Lutalyse followed in 56 hours by a GnRH injection and timed AI at 72 hours.

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 ≥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. 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 and 72 hours after treatment injection and later assayed for progesterone content. Pregnancy diagnosis subsequent to AI occurred 32 to 39 days after timed AI. A positive pregnancy outcome required presence of uterine fluid and a large CL or uterine fluid and presence of an embryo.

Results and Discussion

Experiment 1

When considering only cows having pretreatment progesterone concentrations ≥1 ng/mL and potentially eligible to respond to either Estrumate or Lutalyse, the proportion of cows having successful luteolysis (progesterone <1 ng/mL at 72 hours) was greater ($P < 0.05$) in cows treated with Lutalyse (Table 1). Further, in 5 of 6 herds, the proportion of cows having luteal regression was numerically greater after Lutalyse. The odds ratio indicated that the odds for successful luteolysis were 95% greater (odds ratio = 1.95; 95% confidence interval = 1.27 to 2.45; $P < 0.05$) for cows treated with Lutalyse than for cows treated with Estrumate. In the reduced set of cows with recorded BCS, thinner cows (BCS ≤2.5) were 2.15 times (95% confidence interval = 1.31 to 3.54; $P < 0.05$) more likely to have luteolysis than cows having BCS >2.25 (93.7 vs. 86.8%, respectively).

Pregnancy rate per AI (**PR/AI**) did not differ between treatments in cows with or without luteolysis or in cows with different BCS. Differences in PR/AI were detected among locations (Table 2). At 4 of 6 locations, PR/AI was numerically greater for cows treated with Lutalyse than for cows treated with Estrumate. Although PR/AI did not differ between treatments, breeding codes indicated that cows in estrus at the timed AI had greater ($P < 0.05$) PR/AI than those receiving timed AI without estrual symptoms. Cows inseminated after detected estrus before the scheduled timed AI or those double inseminated because estrus was detected after timed AI had intermediate PR/AI (Table 2). Most of the cows that were estrus coded before AI

expressed estrus (83%) during 1 to 3 days before the scheduled timed AI compared with 17% of cows reinseminated within 24 hours after the timed AI because of detected signs of estrus. Further, similar proportions of cows displayed estrus after Estrumate and Lutalyse (49.6 vs. 51.7%, respectively).

Experiment 2

The proportion of 427 cows having 1, 2, or 3 CL before treatment was 75.2% (n = 321), 22.7% (n = 97), and 2.1% (n = 9), respectively. Among factors analyzed (treatment, number of CL, lactation number, energy-corrected milk yield, injection of GnRH 7 days before treatment, number of ovarian follicles ≥10 mm in diameter, BCS, days in milk, season, and pretreatment progesterone concentration in cows having 1 or more than 1 CL), only treatment, BCS, and season were significant (Table 3). Luteal regression was 1.64 times more ($P < 0.001$) likely with Lutalyse than with Estrumate. Regression of CL in cows having 1 CL was 74.7% and did not differ from that for cows having more than 1 CL (71.2%). Corpora lutea in cows having greater BCS (>2.25 vs. ≤2.25) were 2.72 times less likely to regress (83.2 vs. 62.5%, respectively). The poorest CL regression occurred during summer (57.5%); the best occurred during winter (80.8%).

Among factors tested that may influence pregnancy outcome (treatment, number of CL, lactation number, energy-corrected milk yield, injection of GnRH 7 days before treatment, number of ovarian follicles ≥10 mm in diameter, BCS, days in milk, season, and pretreatment progesterone concentration in cows having 1 or more than 1 CL), only GnRH injection 7 days before treatment ($P = 0.059$) and BCS ($P = 0.09$) tended to be significant (Table 4). Pregnancy rate per AI varied little between Estrumate and Lutalyse treatments (31.3 vs. 32.8%, respectively). Likewise, no advantage for either product was detected for PR/AI whether cows had 1 or more than 1 CL (31.5 vs. 33.7%, respectively). Injecting GnRH 7 days before treatment tended ($P = 0.059$) to increase the odds of PR/AI (34.6 vs. 23%) by 1.71 times (for none vs. GnRH, respectively), and greater (>2.25 vs. ≤2.25) BCS tended ($P = 0.09$) to decrease PR/AI (27.1 vs. 35.8%, respectively).

Pretreatment progesterone concentrations did not differ before treatments of Lutalyse or Estrumate were applied (4.75 ± 0.2 vs. 4.57 ± 0.2 ng/mL, respectively). By 72 hours posttreatment, concentrations were similar between treatments (0.89 ± 0.2 vs. 1.03 ± 0.2 ng/mL, respectively). Pretreatment progesterone concentrations in serum were greater ($P < 0.001$) for cows having more than 1 CL (5.92 ± 0.31 ng/mL; n = 106) than for cows having only 1 CL (4.22 ± 0.19 ng/mL; n = 321).

On the basis of our definition for luteolysis, which required progesterone concentrations to be ≥1 ng/mL before treatment and <1 ng/mL by 72 hours after treatment, Lutalyse was slightly more effective as a luteolytic product than Estrumate. This was true in both experiments, including cows known to have 1 or more than 1 CL before treatment. Although a slight difference in luteolytic efficacy was observed, no differences in pregnancy outcome were detected in either experiment. In both experiments, luteolysis was less effective in cows with BCS exceeding 2.25 or 2.50 compared with thinner cows. We concluded that both products were equally effective luteolysins for producing similar pregnancy outcomes in lactating dairy cows.

Table 1. Proportion of cows having luteal regression in response to Estrumate or Lutalyse treatment (Experiment 1)[1]

	Treatment		
Location	Estrumate	Lutalyse	Overall
	% (n)		
1	93.5 (46)	91.7 (48)	92.6 (94)
2	82.8 (64)	96.7 (61)	89.6 (125)
3	88.1 (42)	92.1 (38)	90.0 (80)
4	78.0 (50)	88.0 (50)	83.0 (100)
5	74.5 (98)	77.6 (98)	76.0 (196)
6	92.1 (252)	98.2 (217)	94.9 (469)
Total	86.4** (552)	92.0 (512)	89.1 (1,064)

** Different from Lutalyse ($P < 0.01$). Odds ratio = 1.95 (95% confidence interval = 1.29 to 2.94).

[1] Only cows having luteolysis (pretreatment progesterone concentrations ≥1 ng/mL and 72-hour posttreatment concentrations <1 ng/mL) were analyzed.

Table 2. Pregnancies per artificial insemination (AI) in response to Estrumate or Lutalyse injection as part of the Cosynch-72 protocol (Experiment 1)

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

[ab] Mean percentages within column having different superscript letters differ ($P \leq 0.05$).

[1] Treatment was applied 3 days before scheduled timed AI.

[2] Includes only cows eligible for luteal regression (progesterone concentrations ≥1 ng/mL before treatment).

[3] Includes all cows regardless of whether luteal regression occurred.

[4] Body condition score was assessed in 1,019 of 1,230 (82.8%) cows.

[5] 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.

Table 3. Factors affecting luteal regression after treatment with Estrumate and Lutalyse in lactating dairy cows having 1 or more than 1 corpus luteum before treatment (Experiment 2)

Treatment	n[1]	Luteal regression (%)	Odds ratio	Confidence limits	*P* value
Estrumate	191	69.1	Referent		0.001
Lutalyse	205	78.5	1.64	1.01-2.68	

[1] Although a corpus luteum was visible, 31of 427 cows that did not have pretreatment progesterone concentrations ≥1 ng/mL (not eligible for luteolysis) were excluded.

Table 4. Factors affecting pregnancy rate per artificial insemination (PR/AI) after treatment with Estrumate and Lutalyse in dairy cows having 1 or more than 1 corpus luteum before treatment (Experiment 2)

Treatment	n[1]	PR/AI[2] (%)	Odds ratio	Confidence limits	*P* value
Estrumate	198	31.3	Referent		0.707
Lutalyse	201	32.8	1.09	0.71-1.66	

[1] Excludes 28 of 427 cows for which pregnancy outcome was not known before culling.
[2] Includes all cows regardless of luteal regression status.

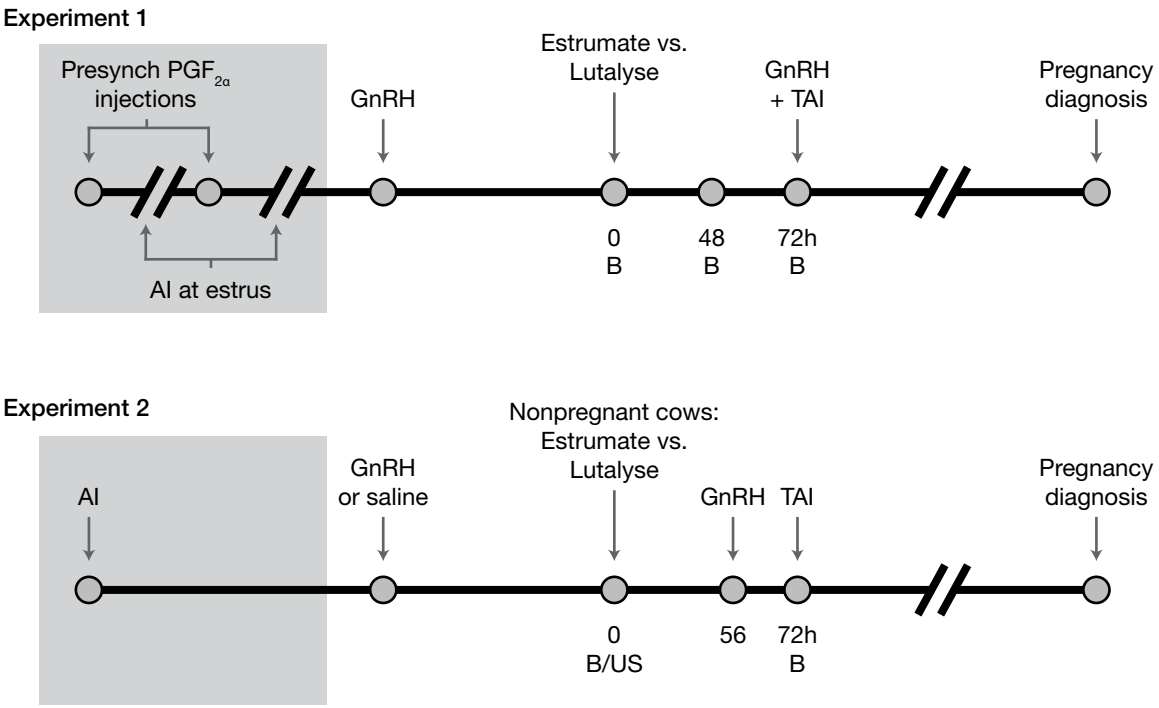

Figure 1. Experimental design of treatments.
Experiment 1. Lactating dairy cows in 6 California dairy herds were enrolled in a Presynch protocol (2 $PGF_{2\alpha}$ injections administered 14 days apart). Cows detected in estrus in response to the Presynch $PGF_{2\alpha}$ injections were inseminated, and the remaining cows were treated with the Cosynch-72 timed AI protocol beginning 12 or 14 days after the second Presynch injection. Alternate cows were administered 2 mL of Estrumate or 5 mL of Lutalyse before timed AI as part of Cosynch-72.

Experiment 2. At 1 Kansas location, cows of unknown pregnancy status were enrolled in an Ovsynch-Resynch procedure (GnRH injection or none administered 7 days before a not-pregnant diagnosis before treatment with Estrumate or Lutalyse followed in 56 hours by a GnRH injection and timed AI at 72 hours). Ovarian structures were mapped and sized by transrectal ultrasonography (US) at the time of the not-pregnant diagnosis.

At all locations, blood samples (B) were collected before treatment injection (0 hour) and at 48 and 72 hours (Experiment 1) or at 0 and 72 hours (Experiment 2). Shaded area for Experiment 1 represents pretreatment Presynch injections. Cows not inseminated were then assigned randomly to the experiment. Shaded area for Experiment 2 represents pretreatment AI in which only resulting nonpregnant cows were enrolled in the experiment.