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Abstract
Three experiments were conducted to determine if a larger dose of prostaglandin F2α (PG) administered on day 6 of a 5-day Ovsynch timed artificial insemination (AI) program would induce regression of the corpus luteum to facilitate AI and pregnancy outcomes similar to a traditional 5-day program with two doses of PG. When applying a 5-day program, cows that ovulate in response to the first GnRH injection have a new corpus luteum (CL) that is 2 days younger when PG is administered in a 5- versus 7-day program. To regress successfully the younger CL, a second injection of PG must be given 24 hours after the first PG injection to prevent reduced pregnancy rate after the timed AI. These experiments demonstrated that administering 50 mg PG (10 mL Lutalyse) on day 6 produced luteolysis as efficiently as 25 mg PG (5 mL Lutalyse) administered on days 5 and 6 when the cut point for progesterone was 1 ng/mL 72 hours after the first PG injection or 48 hours after the larger PG dose. In contrast, when the cut point was 0.5 ng/mL, the larger dose of PG was less effective. Pregnancy outcomes in cows did not differ between treatment doses except in one herd (Exp. 3). Although pregnancy outcomes were reduced only in one herd with the larger PG dose, this difference may be confounded with the earlier injection of the second GnRH injection 16 hours before timed AI, rather than failure of luteolysis in response to the larger dose of PG. Delaying the timing of AI, injection of the second GnRH, or both may be warranted to allow sufficient time for progesterone to decrease to basal concentrations in response to a larger dose of PG on day 6 to prevent a reduction in fertility.; Dairy Day, 2014, Kansas State University, Manhattan, KS, 2014; Dairy Research, 2014 is known as Dairy Day, 2014

Keywords
Dairy Day, 2014; Kansas Agricultural Experiment Station contribution; no. 15-156-S; Report of progress (Kansas Agricultural Experiment Station and Cooperative Extension Service); 1111; Luteal tissue; Luteolysis; Ovulation; Progesterone; Pregnancy rate

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Luteolysis and Pregnancy Outcomes after Change in Dose Delivery of Prostaglandin F$_{2\alpha}$ in a 5-day Timed Artificial Insemination Program in Dairy Cows

J. S. Stevenson, S. L. Pulley, and S. L. Hill

Summary
Three experiments were conducted to determine if a larger dose of prostaglandin F$_{2\alpha}$ (PG) administered on day 6 of a 5-day Ovsynch timed artificial insemination (AI) program would induce regression of the corpus luteum to facilitate AI and pregnancy outcomes similar to a traditional 5-day program with two doses of PG. When applying a 5-day program, cows that ovulate in response to the first GnRH injection have a new corpus luteum (CL) that is 2 days younger when PG is administered in a 5- versus 7-day program. To regress successfully the younger CL, a second injection of PG must be given 24 hours after the first PG injection to prevent reduced pregnancy rate after the timed AI. These experiments demonstrated that administering 50 mg PG (10 mL Lutalyse) on day 6 produced luteolysis as efficiently as 25 mg PG (5 mL Lutalyse) administered on days 5 and 6 when the cut point for progesterone was 1 ng/mL 72 hours after the first PG injection or 48 hours after the larger PG dose. In contrast, when the cut point was 0.5 ng/mL, the larger dose of PG was less effective. Pregnancy outcomes in cows did not differ between treatment doses except in one herd (Exp. 3). Although pregnancy outcomes were reduced only in one herd with the larger PG dose, this difference may be confounded with the earlier injection of the second GnRH injection 16 hours before timed AI, rather than failure of luteolysis in response to the larger dose of PG. Delaying the timing of AI, injection of the second GnRH, or both may be warranted to allow sufficient time for progesterone to decrease to basal concentrations in response to a larger dose of PG on day 6 to prevent a reduction in fertility.

Key words: luteal tissue, luteolysis, ovulation, progesterone, pregnancy rate

Introduction
Most timed AI (TAI) programs apply a combination of GnRH and prostaglandin F$_{2\alpha}$ (PG) to control follicular wave initiation, ovulation, and corpus luteum (CL) regression (luteolysis) in dairy herds before first or repeat AI. These programs generally consist of injecting GnRH (day 0), a standard 25-mg dose of PG on days 5 and 6 (5-day program) or a single dose of PG on day 7 (7-day program), GnRH at 56 hours (Ovsynch-56) or 72 hours (CO-Synch-72) after PG with TAI administered on day 8 (5-day program) or day 10 (7-day program). Unless PG is administered on days 5 and 6 in a 5-day program, luteolysis fails to occur in a proportion of cows that formed a new CL after GnRH administration on day 0.

A recent report in nonlactating cows modified the 5-day program by applying a larger dose (250% of normal) of a PG analog on day 6 and compared that with standard doses of PG on days 5 and 6. Results indicated that the larger dose of PG on day 6 (which required less animal handling) produced luteolytic outcomes and final preovulatory fol-
licle diameter similar to the standard doses of PG on days 5 and 6. In a study consisting of 2 experiments conducted in lactating dairy cows, estrous cycles were presynchronized before a 5-d Ovsynch program to test whether a single large dose (200% of control) of either Estrumate or cloprostenol (1 mg) or Lutalyse or dinoprost (50 mg) administered on day 5 would produce acceptable rates of luteolysis, pregnancy, or both compared with two standard split doses (0.5 or 25 mg, respectively) administered on days 5 and 6. In the first experiment, cows were treated with 0.5 mg cloprostenol on days 5 and 6 (d 0 = GnRH-1 as in Figure 1) compared with 1 mg cloprostenol (double dose) on day 5. More luteolytic failures occurred, and pregnancy outcomes were reduced by as much as one-third in cows receiving the 1-mg dose of cloprostenol in the first experiment. In the second experiment of similar design, a 50-mg dose of dinoprost (10 mL Lutalyse) administered on day 5 resulted in reduced pregnancy outcomes compared with the standard 25-mg doses administered on days 5 and 6. In contrast, increasing the dose of cloprostenol from 0.5 to 0.75 mg (2 to 3 mL) on day 7 of a 7-day program increased luteal regression in multiparous, but not in primiparous cows, resulting in improved pregnancy outcomes at 39 days after AI.

We hypothesized that administering 50 mg of PG to lactating dairy cows on day 6 would produce similar rates of luteolysis as measured by decreased CL tissue area and serum progesterone without compromising pregnancy outcomes. Our objectives were: (1) determine the effect of the standard control dose of PG on days 5 and 6 with a single larger (200% of control) dose of PG on day 6 in lactating dairy cows before first postpartum AI (Experiment 1) on luteal tissue area and progesterone concentrations and before repeat services on progesterone concentrations (Experiment 2); and (2) assess luteolysis (one herd) and pregnancy outcomes in two separate herds (Experiment 3).

Materials and Methods

Experiment 1

Estrous cycles were presynchronized (GnRH [2 mL Factrel, Pfizer Animal Health, Madison, NJ] 7 days before administration of 25 mg of PG [5 mL Lutalyse, Pfizer Animal Health]) in 61 lactating Holstein cows (18 primiparous and 43 multiparous). Eleven days later, cows were enrolled randomly within parity in a 5-day Ovsynch-72 program (62 to 71 DIM) and treatments were administered as illustrated in Figure 1 (control cows [25-mg dose of PG on days 5 and 6; n = 31] and treated cows [single 50-mg dose of PG on day 6; n = 30]).

On day 0, follicles and original CL were mapped and measured by transrectal ultrasonography (5.0 MHz linear-array transducer, Aloka 500V, Corometrics Medical Systems, Inc., Wallingford, CT). On days 5 through 9, ovarian follicles, new GnRH-induced CL, and original CL were measured. The largest ovarian follicle on day 8 was traced back to its first appearance to determine the putative preovulatory follicle diameter. Spherical cavity-free area of luteal structures was calculated. Luteolysis was defined to occur when concentrations of progesterone were ≥1 ng/mL on day 5 and <1 ng/mL on day 8. Blood serum was assayed by radioimmunoassay for progesterone in both experiments. Assay sensitivity was 1.9 ± 0.5 pg/mL. Inter- and intra-assay coefficients of variation for 4 assays were 6.5 and 7.9%, respectively.
Pregnancy was diagnosed by transrectal ultrasonography on day 32 after TAI. A positive pregnancy outcome required presence of anechoic uterine fluid and a CL ≥ 25 mm in diameter or anechoic uterine fluid and presence of an embryo with a heartbeat.

**Experiment 2**

Cows diagnosed not pregnant to a previous AI were treated with GnRH on day 0 and assigned randomly to the same two treatments as described in Exp. 1 (Figure 1). Blood was collected on days 0, 5, 6, and 8. Only data from 63 cows having serum progesterone ≥ 1 ng/mL on day 5 were analyzed. Concentrations of progesterone and occurrence of luteolysis were analyzed as in Exp. 1.

**Experiment 3**

Weekly clusters of lactating dairy cows were enrolled in two treatments (Figure 2) during an entire calendar year as part of a 5-day timed AI Resynch-Ovsynch program (GnRH 5 days before [day 0; GnRH-1] and 56 [p.m. on day 7; GnRH-2] or 72 hours [day 8; GnRH-2] after PG with timed AI on day 8). Enrollment occurred on the same day (day 0) as a negative pregnancy diagnosis (30 to 36 days after last AI in herd 1 or days 34 to 40 in herd 2). Control cows received a 25-mg dose of PG (5 mL Lutalyse) on days 5 and 6 (2 × 25), and treated cows received one single 50-mg dose of PG on day 6 (1 × 50; 10 mL Lutalyse). Cows in herd 1 were blocked by parity and assigned randomly to treatments: 2 × 25 (n = 142) or 1 × 50 (n = 140). In herd 2, even-tagged cows received the 2 × 25 (n = 422) treatment, and odd-tagged cows received 1 × 50 (n = 450) treatment. Body condition scores were assessed (1 = thin and 5 = obese) either weekly in herd 1 or monthly in herd 2.

In herd 1, ovaries were scanned by transrectal ultrasonography to determine the number of CL and number of ovarian follicles ≥ 10 mm in diameter on day 0. Subsequent to treatment and timed AI, pregnancy was diagnosed by transrectal ultrasonography 30 to 36 days after AI. In herd 2, pregnancy was determined by palpation per rectum of the uterus and its contents on days 34 to 40 after AI. In both herds, a second pregnancy confirmation was conducted between 60 and 70 days post-AI.

In herd 1, blood was collected on days 0, 5, 6, and 8. Blood serum was assayed for progesterone by radioimmunoassay. Assay sensitivity was 1.3 ± 0.5 pg/mL. Inter- and intra-assay coefficients of variation for 5 assays were 8.2 and 4.8%, respectively.

**Results and Discussion**

**Experiment 1**

On day 0, 51 of 61 cows had at least 1 CL and 15 had 2 or more CL, whereas 10 cows (5 cows per treatment) had no CL. On day 5, 34 of 61 cows had at least 1 new CL, and 5 cows had 2 or more new CL. Therefore, the ovulation response to GnRH on day 0 was 31 of 61 (50.8%). Numbers of cows with 1, 2, or ≥3 total CL (original plus new CL) on day 5 were as follows: 1 CL: 12 vs. 13; 2 CL: 13 vs. 14; and ≥3 or more CL: 6 vs. 3 for control and 50-mg cows, respectively.

Original luteal tissue area was similar on day 5 but differed between treatments on day 6 (P = 0.001) and day 7 (P = 0.009) and tended (P = 0.068) to be less on day 8 for the
control. In contrast, no differences were detected between treatments for GnRH-induced luteal tissue area.

Concentrations of progesterone differed \( (P = 0.001) \) only on day 6 between treatments (Figure 3; upper panel). Luteolysis occurred in all 31 controls but failed to occur in 2 of 30 (6.7%) 50-mg cows in which no CL were present on day 0, but 1 or 3 new GnRH-induced CL were present on day 5 in the 2 cows with luteolytic failure.

Pregnancy outcomes were 12 of 30 (40%) for control cows and 15 of 30 (50%) for 50-mg cows. One control cow was culled before pregnancy was determined.

**Experiment 2**

Concentrations of progesterone differed between treatments only on day 6 (Figure 3; lower panel). Luteolysis occurred in all 29, 50-mg cows but failed to occur in 2 of 34 (5.9%) controls. Pregnancy outcomes at day 32 after TAI were 17 of 33 (52%) for control cows and 13 of 29 (45%) for 50-mg cows. One control cow was culled before pregnancy diagnosis.

**Experiment 3**

**Progesterone.** Concentrations of progesterone differed \( (P < 0.01) \) between treatments on day 6 and 8. More \( (P < 0.05) \) 1 × 50 than 2 × 25 cows had concentrations of progesterone ≥1 ng/mL on day 6, but similar proportions of cows in each treatment had low \(<1\ ng/mL\) concentrations by day 8 (Figure 4). Progesterone also differed among cycle statuses on day 0 and 5, but not between treatments, which had not yet been administered. Both anestrous and new-CL cows in both treatments had low concentrations on day 0 and differed \( (P < 0.05) \) from those of early- and late-cycle cows, which differed \( (P < 0.05) \) from one another.

On day 5, concentrations of progesterone were near baseline in late-cycle cows, suggesting early spontaneous luteolysis before treatment on days 5 or 6, whereas concentrations in new CL cows increased \( (P < 0.01) \) by 7.4 to 10.3 times from day 0 to 5, indicating ovulation occurred after GnRH-1 on day 0. Concentrations of progesterone in early-cycle cows were elevated on both days 0 and 5 (Figure 4). Relative differences among cycle statuses for cows in both treatments on day 6 were consistent with what was observed on day 5, except on day 6, new-CL and early-cycle cows no longer differed from one another. By day 8, concentrations of progesterone did not differ among cycle statuses for cows in either treatment.

**Luteolysis.** Cows treated with 50 mg PG on day 6 were more \( (P = 0.003) \) likely to have incomplete luteolysis when the cut point on day 8 was <0.5 ng/mL. In contrast, when the cut point was <1 ng/mL, no difference was detected between treatments (Table 1). Luteolysis in cows most likely to fail to respond to PG (early-cycle and new-CL cows) did not differ between early-cycle and new-CL cows (86.5 vs. 83.0%) for the <0.5 ng/mL cut point or for the <1 ng/mL cut point (97.4 vs. 100%), respectively.

Cycle status within treatment reflected the overall treatment effects for luteolysis. Although treatment differences were not detected for early-cycle cows between treatments, luteolysis in the 2 × 25 vs. 1 × 50 cows reflected the overall treatment differences.
in Table 1 at the <0.5 ng/mL cut point (94.8 vs. 78.2%; \( P = 0.495 \)) and at the <1 ng/mL cut point (87.5 vs. 79.3%; \( P = 0.191 \)), respectively. Defined luteolysis was not affected by either BCS or number of follicles >10 mm assessed on day 0.

In response to treatment on day 5, concentrations of progesterone on day 6 decreased in 2 × 25 cows in response to the first of two 25-mg injections and differed (\( P = 0.001 \)) from those in the 50-mg treatment for early-cycle and new-CL cows only, whereas no treatment differences were detected in late-cycle and anestrous cows (Figure 4). Although concentrations of progesterone had decreased further by day 8, difference (\( P = 0.001 \)) between treatments existed only for early-cycle cows, indicating that early-cycle cows also may have had additional new luteal tissue in the form of a new CL that was resistant to the luteolytic effects of PG.

**Pregnancy Outcomes.** Pregnancy per AI at the first and second diagnosis period was reduced in anestrous and late-cycle cows compared with early-cycle and new-CL cows in both treatments for cows in herd 1. Pregnancy per AI (30 to 36 days post-AI) for cows in herd 1 with luteolysis defined at the cut point of <0.5 ng/mL did not differ between 2 × 25 vs. 1 × 50 treatments (48.5% [\( n = 101 \)] vs. 37.5% [\( n = 80 \)]) or at the cut point of <1 ng/mL (46.3% [\( n = 108 \)] vs. 41.0% [\( n = 100 \)]), respectively. Neither parity (\( P > 0.50 \)) nor BCS had any effect (\( P > 0.31 \)) on pregnancy per AI in herd 1.

Pregnancy outcomes at 30 to 40 day differed (\( P < 0.001 \)) among herds, but the 1 × 50 cows tended (\( P = 0.071 \)) to have lesser fertility only in herd 2 (Table 2). This treatment difference in herd 2 was confirmed (\( P = 0.036 \)) at the later pregnancy diagnosis in herd 2 (Table 2).

We conclude that one large dose (50 mg PG) administered on day 6 is luteolytic (regressed the CL) using a cut point of 1 ng/mL, which is consistent with earlier reports in non-lactating and lactating cows (Exp. 1 and 2), but not so when a more conservative cut point of 0.5 ng/mL was applied (Exp. 3). Although progesterone may eventually decrease to sufficient concentrations (complete functional luteolysis) with time, it may not achieve sufficiently basal concentrations in some cows to prevent reduced pregnancy outcomes, particularly those defined as early-cycle, having a new CL on day 0, or both. Although pregnancy outcomes were reduced only in herd 2 with 1 × 50 treatment compared with herd 1, this difference may be confounded with the earlier injection of GnRH-2 16 hours before timed AI in herd 2 rather than failure of luteolysis to the larger dose of PG. Delaying the timing of AI, injection of GnRH-2, or both, may be warranted to allow sufficient time for progesterone to decrease to basal concentrations in response to a larger dose of PG on day 6 to prevent a reduction in conception.
### Table 1. Luteolysis at 2 different cut points for cows treated with 2 × 25 or 1 × 50 mg of PGF₂α in herd 1 (Exp. 3)¹

<table>
<thead>
<tr>
<th>Treatment²</th>
<th>Luteolysis (%)</th>
<th>Odds ratio</th>
<th>95% confidence interval</th>
<th>P-value</th>
</tr>
</thead>
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<tr>
<td>2 x 25</td>
<td>93.3</td>
<td>Referent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 x 50</td>
<td>78.5</td>
<td>0.264</td>
<td>0.108 – 0.645</td>
<td>0.003</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment²</th>
<th>Luteolysis (%)</th>
<th>Odds ratio</th>
<th>95% confidence interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 x 25</td>
<td>100.0</td>
<td>Referent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 x 50</td>
<td>96.3</td>
<td>&lt; 0.1</td>
<td>. . .</td>
<td>0.947</td>
</tr>
</tbody>
</table>

¹ Progesterone ≥1 ng/mL on day 5 and either <0.5 or <1 ng/mL 72 hours later on day 8.
² Cows received either 25 mg PGF₂α (PG) on days 5 and 6 or a 50 mg PG on day 6 as part of a 5-day Resynch-Ovsynch program (d 0 = GnRH-1).

### Table 2. Pregnancy per AI in both herds at 30 to 40 and 60 to 70 days post-AI (Exp. 3)

<table>
<thead>
<tr>
<th>Stage of pregnancy, days</th>
<th>Treatment¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 × 25</td>
</tr>
<tr>
<td>30 to 40</td>
<td>% (n)</td>
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<tr>
<td>Herd 1</td>
<td>37.2 (139)</td>
</tr>
<tr>
<td>Herd 2</td>
<td>24.7 (422)</td>
</tr>
<tr>
<td>60 to 70</td>
<td></td>
</tr>
<tr>
<td>Herd 1</td>
<td>31.3 (139)</td>
</tr>
<tr>
<td>Herd 2</td>
<td>22.7 (422)</td>
</tr>
</tbody>
</table>

¹ Cows received either 25 mg PGF₂α (PG) on days 5 and 6 or a 50 mg PG on day 6 as part of a 5-day Resynch-Ovsynch program (d 0 = GnRH-1).
Figure 1. Scheme of treatments and measurements for Experiments 1 and 2. In Experiment 1, estrous cycles were presynchronized by injecting 100 μg GnRH and 25 mg of PG beginning between 62 and 71 days in milk. Cows were assigned randomly to receive either 25-mg doses of PG on days 5 and 6 or a 50-mg dose of PG on day 6. Ovarian structures were measured by transrectal ultrasonography (S) and mapped on day 0 and days 5 through 9. Both original CL on day 0 and GnRH-induced CL identified on day 5 were measured and monitored for diameter and luteal area (CL cavity area was deducted from total luteal area). Blood samples (B) were collected before each ovarian scan.

Figure 2. Experiment 3 treatment schemes in two herds. In herd 1, cows were assigned randomly to receive either 25 mg PGF₂₀ (PG) on days 5 and 6 or a 50-mg dose of PG on day 6. Blood samples were collected on days 0, 5, 6, and 8 to measure progesterone. In herd 2, even-tagged cows received 25 mg PG on days 5 and 6, and odd-tagged cows received 50 mg PG on day 6. The second GnRH (GnRH-2) injection was administered on the afternoon of day 7, and timed AI (TAI) occurred on the morning of day 8 in herd 2, but TAI occurred in herd 1 when GnRH-2 was administered on day 8 (72 hours after the first 25-mg PG treatment).
Figure 3. Concentrations of progesterone in control (25-mg doses of PG on days 5 and 6) and treated cows (50 mg of PG only on day 6) for cows in Exp. 1 (upper panel) and Exp. 2 (lower panel). Concentrations differed ($P = 0.001$) between treatments only on day 6 in both experiments.
Figure 4. Concentrations of progesterone on days 0, 5, 6, and 8 for cows treated with either 25 mg PGF$_{2α}$ (PG) on days 5 and 6 (2 × 25) or 50 mg PG on day 6 (1 × 50) in Exp. 3. Cows were classified by concentrations of progesterone on day 0 and 5: (1) anestrus (<1 ng/mL on both days); (2) early cycle (≥1 ng/mL on both days); (3) late cycle (≥1 ng/mL on day 0 and <1 ng/mL on day 50); and (4) new CL (<1 ng/mL on day 0 and ≥1 ng/mL on day 5). a,b Means within treatment differed (P < 0.05) among cycle statuses.