Pregnancy outcomes after change in dose delivery of prostaglandin $F_{2\alpha}$ and time of gonadotropin-releasing hormone injection in a 5-day timed artificial insemination program in lactating dairy cows¹

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ABSTRACT

We demonstrated that 50 mg of $PGF_{2\alpha}$ on d 6 successfully induced luteolysis in lactating dairy cows enrolled in a traditional 5-d Ovsynch-72 program [GnRH injection 5 d before (d 0; GnRH-1) and 56 (p.m. on d 7; GnRH-2) or 72 h (d 8; GnRH-2) after a 25-mg injection of $PGF_{2\alpha}$ (d 5 and 6 after GnRH injection); timed artificial insemination (AI) on d 8]. Our current objective was to determine pregnancy outcomes in lactating dairy cows after a 50-mg injection of $PGF_{2\alpha}$ on d 6 or a 25-mg injection of $PGF_{2\alpha}$ on d 5 and 6 in a 5-d Ovsynch program. Cows in herd 1 diagnosed not pregnant between 30 and 36 d since last AI were enrolled to receive either a 50-mg injection of $PGF_{2\alpha}$ on d 6 (1×50 ; $n = 134$) or a 25-mg injection of $PGF_{2\alpha}$ on d 5 and 6 (2×25 ; $n = 139$) after GnRH-1 (d 0), with GnRH-2 at 72 h after $PGF_{2\alpha}$ injection (d 5), concurrent with timed AI (d 8). Cows in herd 2 diagnosed not pregnant between 34 and 40 d were treated similarly: even-tagged cows received the 2×25 ($n = 422$) treatment, and odd-tagged cows received the 1×50 ($n = 450$) treatment, except that GnRH-2 was administered at 56 h. Blood collected from cows in herd 1 at d 0, 5, 6, and 8 was assayed for progesterone. Luteolysis was defined to occur when progesterone concentration was ≥ 1 ng/mL on d 5, and 72 h later (d 8) was either <0.5 ng/mL or <1 ng/mL. Progesterone concentrations did not differ between treatments on pretreatment d 0 and 5, but were greater in 1×50 than 2×25 cows on d 6 (4.7 ± 0.2 vs. 1.1 ± 0.2 ng/mL) and d 8 (0.43 ± 0.04 vs. 0.19 ± 0.04 ng/mL), respectively. Luteolysis was greater in the 2×25 versus 1×50 treatment when the cut point was 0.5 ng/mL, whereas no difference was detected when the cut point was <1 ng/mL on d 8. Lack of complete luteolysis was greater in cows classified as early cycle on d 0 or having a new corpus luteum after

d 0 because progesterone concentration was greater on d 5 and 6 than for cows classified as late cycle on d 0 or cows having low progesterone on d 0 and 5. Pregnancy per AI at 30 to 40 d did not differ between 2×25 and 1×50 cows having luteolysis by d 8 or in all cows (37.2 vs. 33.3%) in herd 1, respectively, but differed in herd 2 (24.7 vs. 19.5%; no treatment by herd interaction). We conclude that incomplete luteolysis by d 8 was greater in 1×50 cows using a cut point of <0.5 ng/mL at AI. The difference in pregnancy outcome in herd 2 may have resulted from insufficient time for complete luteolysis before GnRH-2 at 56 h compared with GnRH-2 at 72 h (at AI) in herd 1.

Key words: luteal tissue, luteolysis, ovulation, progesterone

INTRODUCTION

Most timed AI programs in the United States apply a combination of GnRH and $PGF_{2\alpha}$ to control follicular wave initiation, ovulation, and luteolysis in dairy cows before first and repeat AI. These programs generally consist of injecting GnRH (d 0), a standard 25-mg dose of $PGF_{2\alpha}$ on d 5 and 6 (5-d program) or a single dose of $PGF_{2\alpha}$ on d 7 (7-d program), and GnRH at 56 h (Ovsynch-56) or 72 h (Cosynch-72) after $PGF_{2\alpha}$ injection, with timed AI administered on d 8 (5-d program) or d 10 (7-d program). Unless $PGF_{2\alpha}$ is administered on d 5 and 6 in a 5-d program, luteolysis fails to occur in a proportion of cows that formed a new corpus luteum (CL) after GnRH administration on d 0 (Santos et al., 2010). Studies have demonstrated that a larger dose (200% of normal) of either $PGF_{2\alpha}$ (Ribeiro et al., 2012a) or a $PGF_{2\alpha}$ analog (Ribeiro et al., 2012a) given on d 5 failed to induce luteal regression equal to a standard dose of $PGF_{2\alpha}$ administered on d 5 and 6, thus compromising pregnancy per AI.

A recent report in nonlactating cows modified the 5-d program by applying a larger dose (250% of normal) of a $PGF_{2\alpha}$ analog on d 6 and compared that with standard doses of $PGF_{2\alpha}$ on d 5 and 6 (Valdecabres-Torres et al., 2013). Results indicated that the larger dose of $PGF_{2\alpha}$ on d 6 produced luteolytic outcomes

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and final preovulatory follicle diameters similar to the standard doses of PGF_{2α} on d 5 and 6. We confirmed the previous results in 2 experiments demonstrating similar luteal regression and decreased progesterone concentrations in lactating dairy cows treated with 50 mg of PGF_{2α} on d 6 compared with 25-mg doses on d 5 and 6 (Stevenson et al., 2013). Furthermore, with the 50-mg treatment scheme, handling of individual cows was reduced from 4 to 3 times before AI.

Based on the latter study, we hypothesized that the single larger dose of PGF_{2α} on d 6 would not compromise pregnancy outcomes in lactating dairy cows compared with 25 mg of PGF_{2α} on d 5 and 6. The current study examined the luteolytic success of the 50-mg dose in 1 herd and compared pregnancy outcomes in 2 herds after a standard control dose of PGF_{2α} on d 5 and 6 with a single larger (200% of control) dose of PGF_{2α} on d 6 in lactating dairy cows inseminated after a nonpregnant diagnosis.

MATERIALS AND METHODS

Experimental Treatments and Cows

Lactating dairy cows (>99% purebred Holstein) in 2 herds located in northeastern Kansas were housed in freestall barns bedded with sand and milked 3 times daily. Cows were fed a TMR diet consisting of alfalfa hay, straw, corn silage, soybean meal, whole cottonseed, corn or milo grain, corn gluten feed (herd 1 only), vitamins, and minerals balanced for 40 to 45 kg of 3.5% milk.

Weekly clusters of lactating dairy cows were enrolled in 2 treatments (Figure 1) during an entire calendar year as part of a 5-d timed AI Resynch-Ovsynch program [GnRH injection 5 d before (d 0; **GnRH-1**) and 56 (p.m. on d 7; **GnRH-2**) or 72 h (d 8; GnRH-2) after PGF_{2α} injection, with timed AI on d 8]. Enrollment occurred on the same day (d 0) as a negative pregnancy diagnosis (30 to 36 d after last AI in herd 1 or d 34 to 40 in herd 2). Control cows received a 25-mg dose of PGF_{2α} on d 5 and 6 (**2 × 25**), and treated cows received a single 50-mg dose of PGF_{2α} on d 6 (**1 × 50**). 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 the 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 d 0. Subsequent to treatment and timed AI, pregnancy

was diagnosed by transrectal ultrasonography 30 to 36 d after AI. A positive pregnancy outcome required the presence of anechoic uterine fluid and a CL or anechoic uterine fluid and the presence of an embryo with a heartbeat. In herd 2, pregnancy was determined by palpation per rectum of the uterus and its contents on d 34 to 40 after AI. In both herds, a second pregnancy confirmation was conducted between 60 and 70 d after AI.

In herd 1, blood was collected on d 0, 5, 6, and 8. Blood serum was assayed for progesterone by RIA (Stevenson et al., 2012). Assay sensitivity was 1.3 ± 0.5 pg/mL. Interassay and intraassay coefficients of variation for 5 assays were 8.2 and 4.8%, respectively.

Definitions

In herd 1, functional (different from structural) luteolysis was defined to occur when concentrations of progesterone were ≥1 ng/mL on d 5 and either <0.5 or <1 ng/mL (2 cut points) on d 8 (72 h after the first 25-mg PGF_{2α} injection of the 2 × 25 treatment). Not knowing when complete functional luteolysis occurred, using the 2 cut points allowed a measurement along the continuum of progesterone decline to complete functional luteolysis or to basally sustained concentrations of progesterone characteristic of proestrus and

Figure 1. Experimental treatment scheme in 2 herds. In herd 1, cows were assigned randomly to receive either 25 mg of PGF_{2α} (PG) on d 5 and 6 or a 50-mg dose of PG on d 6. Blood samples were collected on d 0, 5, 6, and 8 to measure progesterone. In herd 2, even-tagged cows received 25 mg of PG on d 5 and 6 and odd-tagged cows received 50 mg of PG on d 6. The second GnRH injection (GnRH-2) was administered on the afternoon of d 7 and timed AI (TAI) occurred on the morning of d 8 in herd 2, but in herd 1 TAI occurred when GnRH-2 was administered on d 8 (72 h after the first 25-mg PG treatment). Color version available in the online PDF.

estrus. For purposes of determining which cows failed to respond to PGF_{2α} treatments, cows were classified into 4 cycle statuses (as of d 0) on the basis of serum concentrations of progesterone on d 0 and 5:

- (1) anestrus cows = progesterone concentration was <1 ng/mL (low) on d 0 and 5;
- (2) late-cycle cows = progesterone concentration was high (≥ 1 ng/mL) on d 0, but low on d 5 (assumption: late-cycle cows were in late diestrus on d 0 and their CL spontaneously regressed before d 5);
- (3) early-cycle cows = progesterone concentration was high on both days;
- (4) new-CL cows = progesterone was low on d 0, but high on d 5 (assumption: these cows formed a new CL either spontaneously or in response to GnRH-1-induced LH release on d 0).

Cows in the early-cycle status may have had a new CL, but that could not be determined without an ovarian scan on d 5, which was not done. We hypothesized that luteolysis in early-cycle and new-CL cows would be more likely to be incomplete by d 8 or fail in response to the 1×50 treatment.

Statistical Analyses

The success of functional luteolysis at 2 cut points (<0.5 or <1 ng/mL on d 8) defined earlier was determined by applying PROC LOGISTIC of SAS (SAS Institute Inc., Cary, NC), using a model consisting of treatment (2×25 vs. 1×50), parity (primiparous vs. multiparous), treatment $\times$ parity interaction, median BCS (≤ 2.50 vs. > 2.50), number of CL, and number of ovarian follicles ≥ 10 mm on d 0. The final model produced by backward stepwise selection of independent variables entered or retained in the model was based on a Wald statistic ($P < 0.10$). Odds ratios (OR) and 95% confidence intervals are reported.

Changes in concentrations of progesterone on d 0, 5, 6, and 8 were analyzed by PROC MIXED in SAS. Effects of cycle status and treatment on pretreatment concentrations on d 0 and 5 were analyzed using a model consisting of treatment, cow within treatment (error term for testing treatment differences), cycle status (anestrus, early cycle, late cycle, and new CL), day (0 and 5), and cycle status $\times$ day interaction. Because blood samples on d 0 and 5 were collected before treatment, these progesterone concentrations were treated as covariates in the model consisting of treatment, day (6 and 8), cycle status, and appropriate interactions with treatment. An unstructured covariance structure was applied in the model (produced the smallest

Akaike information criterion statistic). Proportions of cows within treatment having progesterone concentrations ≥ 1 ng/mL on d 0, 5, 6, and 8 were determined by chi-squared using the Cochran-Mantel-Haenszel row mean scores test (PROC FREQ; SAS Institute Inc.). Pregnancy outcomes at 30 to 36 d after AI in herd 1 were analyzed using PROC GLIMMIX in SAS with a model consisting of treatment, parity, cycle status, and median BCS.

Preliminary analyses of pregnancy outcomes at 30 to 40 d and at 60 to 70 d after AI using a general linear model revealed a herd effect ($P < 0.001$), but no treatment $\times$ herd interaction ($P > 0.6$). The herd effect may have resulted from differences in the timing of GnRH-2 injection (i.e., administering GnRH too early before complete luteolysis) between herds (72 h in herd 1 and 56 h in herd 2, with AI occurring at 72 h in both herds), rather than a treatment difference. Therefore, the pregnancy outcomes from both herds were not combined but analyzed separately by PROC LOGISTIC in SAS to determine individual herd responses using a model consisting of treatment, season of AI (winter, spring, summer, and autumn), median BCS (≤ 2.50 vs. > 2.50), and median DIM ($148 < \text{herd 1} \leq 148$ vs. $151 < \text{herd 2} \leq 151$). In separate herd analyses, the final model was produced by a backward stepwise elimination based on the Wald statistic criterion when $P < 0.10$. The final model eliminated all variables except for treatment in both herds. Because no treatment differences were detected in herd 1 at both stages of pregnancy, pregnancy outcomes at both stages of pregnancy were not combined, but analyzed separately by herd using PROC GLIMMIX in SAS, using the same model described previously to produce least squares means.

RESULTS

Progesterone

Concentrations of progesterone differed ($P < 0.01$) between treatments on d 6 and 8 (Figure 2). More ($P < 0.05$) 1×50 than 2×25 cows had concentrations of progesterone ≥ 1 ng/mL on d 6, but similar proportions of cows in each treatment had low (<1 ng/mL) concentrations by d 8 (Figure 2). Progesterone concentration also differed among cycle statuses on d 0 and 5, but not between treatments, which had not yet occurred. Both anestrus and new-CL cows in both treatments had low concentrations on d 0 and differed ($P < 0.05$) from those of early- and late-cycle cows, which differed ($P < 0.05$) from one another (Figure 3).

On d 5, concentrations of progesterone were near baseline in late-cycle cows, suggesting early spontaneous luteolysis, whereas concentrations in new CL cows

Percentage of cows within treatment with high or low progesterone

High ≥ 1 ng/mL						Low < 1 ng/mL	
d 0		d 5		d 6		d 8	
2 x 25	1 x 50	2 x 25	1 x 50	2 x 25	1 x 50	2 x 25	1 x 50
72.5 ^a	66.4 ^a	72.5 ^a	66.4 ^a	34.5 ^a	82.1 ^b	98.6 ^a	94.3 ^a

^{a,b} Means within day differed ($P < 0.05$) between treatment.

Figure 2. Concentrations of progesterone on d 0, 5, 6, and 8 for cows treated with either 25 mg of PGF_{2α} (PG) on d 5 and 6 (2 × 25) or 50 mg of PG on d 6 (1 × 50). Error bars represent SEM. Color version available in the online PDF.

increased ($P < 0.01$) by 7.4 to 10.3 times from d 0 to 5, indicating that ovulation occurred after GnRH-1 on d 0. Concentrations of progesterone in early-cycle cows were elevated on both d 0 and 5. Relative differences among cycle statuses for cows in both treatments on d 6 were consistent with what was observed on d 5, except that on d 6, new-CL and early-cycle cows no longer differed from one another. By d 8, concentrations of progesterone did not differ among cycle statuses for cows in either treatment.

Luteolysis

Cows treated with 50 mg of PGF_{2α} on d 6 were more likely ($P = 0.003$) to have incomplete luteolysis when the cut point on d 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 PGF_{2α} (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 versus 1 × 50 cows reflected the overall treatment differences in Table 1 at the <0.5 ng/mL cut point (94.8 vs. 78.2%; $P = 0.50$) and at the <1 ng/mL cut point (87.5 vs. 79.3%; $P = 0.19$), respectively. Defined luteolysis was not affected by either BCS or number of follicles >10 mm assessed on d 0.

In response to treatment on d 5, concentrations of progesterone on d 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 anestrus cows (Figure 3). Although concentrations of progesterone had decreased further by d 8, differences ($P = 0.001$) between treatments existed only for some 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 PGF_{2α}.

Table 1. Luteolysis at 2 different cut points for cows treated with 2 × 25 or 1 × 50 mg of PGF_{2α} in herd 1¹

Treatment ²	Luteolysis (%)	Odds ratio	95% CI	P-value
<0.5 ng/mL				
2 × 25	93.3	Referent		
1 × 50	78.5	0.264	0.108 – 0.645	0.003
<1 ng/mL				
2 × 25	100.0	Referent		
1 × 50	96.3	<0.1	—	0.95

¹Progesterone concentration ≥1 ng/mL on d 5 and either <0.5 or <1 ng/mL 72 h later on d 8.

²Cows received either 25 mg of PGF_{2α} on d 5 and 6 or 50 mg of PGF_{2α} on d 6 as part of a 5-d Resynch-Ovsynch program (d 0 = GnRH-1).

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 (Table 2). Pregnancy per AI (30 to 36 d after AI) for cows in herd 1 with

luteolysis defined at the cut point of <0.5 ng/mL did not differ between 2 × 25 versus 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.

Figure 3. Concentrations of progesterone on d 0, 5, 6, and 8 for cows treated with either 25 mg of PGF_{2α} (PG) on d 5 and 6 (2 × 25) or 50 mg of PG on d 6 (1 × 50). Cows were classified by concentrations of progesterone on d 0 and 5: (1) anestrous (<1 ng/mL on both days), (2) early cycle (≥1 ng/mL on both days), (3) late cycle (≥1 ng/mL on d 0 and <1 ng/mL on d 5), and (4) new corpus luteum (CL; <1 ng/mL on d 0 and ≥1 ng/mL on d 5). Means within treatment with different letters (a–d) differed (*P* < 0.05) among cycle statuses. Error bars represent SEM. Color version available in the online PDF.

Table 2. Pregnancy per AI at 2 post-AI times in herd 1 based on treatment and cycle status

Treatment ¹	Cycle status ²			
	Anestrus	Late cycle	Early cycle	New CL
Cows, no.	24	32	158	59
d 30 to 36 (%)				
2 × 25	0.0	22.2	43.9	52.0
1 × 50	10.0	14.3	43.4	32.3
Cycle status mean	4.1 ^a	18.5 ^a	43.7 ^b	40.9 ^b
d 60 to 66 (%)				
2 × 25	0.0	16.7	36.6	52.0
1 × 50	10.0	7.1	36.8	29.4
Cycle status mean	4.0 ^a	12.1 ^a	36.3 ^b	39.1 ^b

^{a,b}Percentages within a row with different superscript letters differ ($P < 0.01$) among cycle statuses.

¹Cows received either 25 mg of PGF_{2α} on d 5 and 6 or 50 mg of PGF_{2α} on d 6 as part of a 5-d Resynch-Ovsynch program (d 0 = GnRH-1).

²Cows were classified by concentrations of progesterone on d 0 and 5: anestrus (<1 ng/mL on both days), early cycle (≥1 ng/mL on both days), late cycle (≥1 ng/mL on d 0 and <1 ng/mL on d 5), and new corpus luteum (CL; <1 ng/mL on d 0 and ≥1 ng/mL on d 5).

Pregnancy outcomes at 30 to 40 d differed ($P < 0.001$) among herds, but the 1 × 50 cows tended ($P = 0.071$) to have lesser fertility only in herd 2 (Table 3). This treatment difference in herd 2 was confirmed ($P = 0.036$) at the later pregnancy diagnosis in herd 2 (Table 3).

DISCUSSION

In the present study, progesterone concentrations in lactating dairy cows indicative of defined luteolysis resulting from the 50-mg dose of PGF_{2α} on d 6 was as effective as the standard split 25-mg doses on d 5 and 6 only at a cut point of <1 ng/mL on d 8, but not when the cut point was set at <0.5 ng/mL, indicating inadequate time for complete luteal regression by 48 h after the 50-mg dose of PGF_{2α}. At the less conservative cut point of 1 ng/mL, the present results corroborate earlier reports of successful luteolysis in nonlactating (Valdecabres-Torres et al., 2013) and lactating cows (Stevenson et al., 2013). Because the first of 2 × 25-mg doses was administered 24 h earlier than the single 50-mg dose, the 2 × 25 treatment had a time advantage in

reducing concentrations of progesterone before AI on d 8 compared with the single larger dose.

Problematic cows for incomplete or failed luteolysis at a more conservative cut point of 0.5 ng/mL in 5-d programs are those with multiple luteal structures, new luteal structure(s) resulting from GnRH-1-induced ovulation, or both. Results of the present study clearly demonstrate that late-cycle cows whose CL regressed spontaneously before treatment on d 5 are not at risk for luteolytic failure. Neither are the defined anestrus cows at risk because they had no luteal structures on d 0 or 5. Unfortunately, both anestrus and late-cycle cows are at risk for reduced conception compared with early-cycle cows and those bearing a new CL (Table 2). The latter 2 groups of cows have CL that must regress in a timely fashion to accommodate GnRH-2-induced LH surges and ovulation.

The remaining defined new-CL cows and early-cycle cows, which may well have included cows with GnRH-1-induced luteal structures, but could not be differentiated from our defined new-CL cows, were those at risk for luteolytic failure. Although the latter 2 cycle statuses of cows had concentrations of progesterone <1

Table 3. Pregnancy per AI in both herds at 30 to 40 and 60 to 70 d after AI

Stage of pregnancy	Treatment ¹		P-value
	2 × 25	1 × 50	
30 to 40 d [% (no.)]			
Herd 1	37.2 (139)	33.3 (134)	0.52
Herd 2	24.7 (422)	19.5 (450)	0.071
60 to 70 d [% (no.)]			
Herd 1	31.3 (139)	28.2 (134)	0.61
Herd 2	22.7 (422)	16.9 (450)	0.036

¹Cows received either 25 mg of PGF_{2α} on d 5 and 6 or 50 mg of PGF_{2α} on d 6 as part of a 5-d Resynch-Ovsynch program (d 0 = GnRH-1).

ng/mL on d 8 (Figure 3), their concentrations on d 8 were significantly elevated only in the early-cycle cows in the 1×50 compared with the 2×25 cows (0.48 ± 0.06 vs. 0.19 ± 0.06 ng/mL, respectively). Despite this small difference in progesterone concentrations between treatments, pregnancy outcomes were not negatively affected in herd 1. Rather, pregnancy outcomes at d 30 to 36 and d 60 to 66 for the early-cycle and new-CL cows were greater than those for anestrous and late-cycle cows in which luteolysis was not at risk. These differences in pregnancy outcomes in anestrous and late-cycle cows were more likely related to ill timing of AI, which is a testable hypothesis, rather than failure of luteolysis. At least in herd 1 in which blood sampling occurred, pregnancy outcome differed by only 3.9 percentage units and was not affected by treatment; however, one cannot ignore the potential of a type II error in this interpretation. Research results in 2,465 suckled beef cattle in 13 herds enrolled in a 5-d Cosynch + controlled internal drug release (**CIDR**) program demonstrated that one 50-mg injection of PGF_{2 α} at CIDR insert removal (d 5) produced pregnancy outcomes similar to two 25-mg doses of PGF_{2 α} administered on d 5 at 0 and 8 h after PGF_{2 α} and CIDR insert removal (51 vs. 55%), respectively (Bridges et al., 2012).

Overall pregnancy outcomes in herd 2 were less than in herd 1 for unknown reasons. Herd 2 was used during the previous full calendar year in another study (Pulley et al., 2013a) in which resynchronized pregnancy outcomes were similar to what was reported for herd 1 in the present study. This herd difference in pregnancy outcomes may have resulted from the difference in timing of GnRH-2 relative to AI (herd 1: GnRH-2 was administered at the timed AI at 72 h vs. herd 2: GnRH-2 was administered at 56 h after the first PGF_{2 α} injection on d 5, 16 h before timed AI).

In herd 2, administration of GnRH-2 occurred approximately 32 h after the 1×50 PGF_{2 α} treatment compared with similarly treated cows in herd 1 in which GnRH-2 was administered at 48 h after treatment. As a consequence, herd 2 cows probably had greater progesterone concentrations at GnRH-2 that may have resulted in attenuated LH responses to GnRH-2 (e.g., failure of LH surges or reduced LH concentrations). Peak LH concentrations and the area under the LH curve were greater in 12 cows with low (<1 ng/mL) progesterone concentrations compared with 12 cows having high (>1 ng/mL) progesterone concentrations (Giordano et al., 2012). Furthermore, we observed similar varying LH responses to GnRH in differing progesterone environments in more than 70 lactating dairy cows at 3 different GnRH-treatment times between 52 and 68 DIM (Pulley et al., 2013b).

A recent review of the effects of progesterone on reproduction in dairy cattle indicated that inadequate luteolysis results in elevated concentrations of progesterone at AI and reduced pregnancy outcomes (Wiltbank et al., 2014). Furthermore, an actual concentration threshold of progesterone at the time of AI may be in play. Results from 2 studies (Souza et al., 2007; Brusveen et al., 2009) indicated a decrease in pregnancy outcomes when progesterone exceeded 0.4 ng/mL near the time of GnRH-2 of Ovsynch (16 h before timed AI). Decreased pregnancy outcomes were evident even in cows known to have ovulated after GnRH-2 in the previous studies and also in cows inseminated after detected estrus when progesterone was slightly elevated at AI (De Silva et al., 1981).

Pregnancy outcomes were compromised in at least 6 reviewed studies (Wiltbank et al., 2014) in lactating dairy cows induced to ovulate after GnRH-2 in timed AI programs when progesterone was in the range of 0.3 to 0.5 ng/mL near the time of AI. Mechanisms whereby fertility may be compromised include reduced fertilization risk by altering of sperm or ova transport by progesterone attenuating oviductal or uterine contractility (Hunter, 2005) or blastocyst formation and subsequent embryo development (Silva and Knight, 2000).

Evidence is equivocal supporting lesser pregnancy outcomes in lactating dairy cows inseminated at 72 h after PGF_{2 α} injection in various 5-d timed AI programs when GnRH-2 is administered 56 to 58 h after PGF_{2 α} compared with GnRH-2 injected at 72 h, concurrent with the timed AI. In cows inseminated as part of a Presynch-Ovsynch (5-d Ovsynch program initiated 12 d after the second PGF_{2 α} of Presynch) program before first postpartum AI, no difference in pregnancy outcome was detected in freestall-housed cows administered GnRH-2 at 56 versus 72 h [AI at 72 h; 46.4 vs. 45.4% (d 32) or 40.7 vs. 38.6% (d 60), respectively; Bisinotto et al., 2010]. In contrast, in another experiment of grazing cows whose estrous cycles were presynchronized with Presynch-Ovsynch (5-d Ovsynch program initiated 10 d after the second PGF_{2 α} injection of Presynch), pregnancy outcome at d 30 tended to be reduced in cows administered GnRH-2 and inseminated at 58 versus 72 h (54.3 vs. 61.1%) and was reduced at d 65 (46.5 vs. 54.9%), respectively (Ribeiro et al., 2012b). Furthermore, in cows treated with a 7-d Ovsynch protocol (AI at 72 h) and cows receiving GnRH-2 at 56 versus 72 h after PGF_{2 α} , pregnancy outcomes differed from those of 5-d programs cited previously, in that after first (42.6 vs. 21.7%) timed AI, pregnancy per AI was less at d 52 to 54 after AI in the 72-h treatment with GnRH-2 concurrent with timed AI, but did not differ in Resynch cows (29.6 vs. 24.1%, respectively; Brusveen

et al., 2008). Age of the preovulatory follicle and period of follicular dominance differs in 5- and 7-d programs, particularly in cows in which ovulation occurs after GnRH-1 (Santos et al., 2010).

Effective 5-d programs have been reported in which ovulation was synchronized in lactating dairy cows inseminated at first service when housed in freestall confinement (Bisinotto et al., 2010) or grazing operations (Ribeiro et al., 2011). In those studies, timed AI occurred 72 h after PGF_{2α}, and GnRH-2 was administered 12 to 18 h before timed AI or at the time of AI. In a 5-d program, a second dose of PGF_{2α} was given on d 6 to ensure regression of any new luteal structure that formed after GnRH-1-induced ovulation. In 5-d programs, pregnancy outcomes were reduced if only a single 25-mg dose was given (Santos et al., 2010) or if 2 doses were administered on the same day (5 d after GnRH-1) compared with giving one dose on d 5 and a second dose on d 6 (Ribeiro et al., 2012a).

In a study consisting of 2 experiments conducted in lactating dairy cows (Ribeiro et al., 2012a), estrous cycles were presynchronized before a 5-d Ovsynch program to test whether a single large dose (200% of control) of either cloprostenol (1 mg) or dinoprost (50 mg) administered on d 5 would produce acceptable rates of luteolysis, pregnancy, or both, compared with 2 standard split doses (0.5 or 25 mg, respectively) administered on d 5 and 6. In the first experiment, cows were treated with 0.5 mg of cloprostenol on d 5 and 6 (d 0 = GnRH-1, as in Figure 1) compared with 1 mg of cloprostenol (double dose) on d 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 (Ribeiro et al., 2012a), a 50-mg dose of dinoprost administered on d 5 resulted in reduced pregnancy outcomes compared with the standard 25-mg doses administered on d 5 and 6. In contrast, increasing the dose of cloprostenol from 0.5 to 0.75 mg on d 7 of a 7-d program increased luteal regression in multiparous, but not in primiparous cows, resulting in improved pregnancy outcomes at 39 d after AI (Giordano et al., 2013).

We conclude that one large dose (50 mg of PGF_{2α}) administered on d 6 was luteolytic using a cut point of 1 ng/mL, consistent with earlier reports in nonlactating (Valdecabres-Torres et al., 2013) and lactating cows (Stevenson et al., 2013), but not so when a more conservative cut point of 0.5 ng/mL was applied. 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 d 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 h before timed AI in herd 2 rather than failure of luteolysis to the larger dose of PGF_{2α}. 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 PGF_{2α} on d 6 to prevent a reduction in conception.

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