Pregnancy per AI after Presynchronizing Estrous Cycles with Presynch-10 or PG-3-G before Ovsynch-56 in Four Dairy Herds of Lactating Dairy Cows

J. S. Stevenson and S. L. Pulley

Summary

The objective was to determine the effect of 2 presynchronization treatments on first-service pregnancy rate in 4 dairy herds during warm and cool seasons of the year. Cows with ear tags ending with even digits at calving were enrolled in **Presynch-10** with 2, 25-mg injections of prostaglandin $F_{2\alpha}$ (i.e., **PG-1** and **PG-2**) 14 days apart. Cows with ear tags ending with odd digits were enrolled in **PG-3-G** comprising 1, 25-mg injection of PG (**Pre-PG**) 3 days before 100 µg gonadotropin-releasing hormone (**Pre-GnRH**), with the Pre-PG injection administered at the same time as PG-2 in the Presynch-10 treatment in the Presynch-10 treatment. Ten days after PG-2 or Pre-PG, all cows were enrolled in a timed artificial insemination (**TAI**) protocol (Ovsynch-56; injection of GnRH 7 days before [GnRH-1] and 56 hours after [GnRH-2] PG with AI 16 to 18 hours after GnRH-2). Median days in milk (**DIM**) at scheduled TAI were 75 days, which did not differ among herds. Cows detected in estrus before the scheduled TAI were inseminated early (early bred; **EB**). Pregnancy was diagnosed at days 32 to 38 and at days 60 to 66 after TAI by transrectal ultrasonography or transrectal palpation. Data were analyzed with herd as a random effect and with fixed effects of treatment (EB, Presynch-10, PG-3-G), parity (primiparous vs. multiparous), season (hot [June through September] vs. cool-cold [October through May]), DIM, estrus at TAI (0 vs. 1), and all 2-way interactions with treatment. The pregnancy rate at days 32 to 38 for EB (n = 472), Presynch-10 (n = 1,247), and PG-3-G (n = 1,286) were 31.4, 35.0, and 41.2%, respectively; pregnancy rate at days 60 to 66 was 29.8, 32.2, and 37.3%, respectively. Season significantly influenced pregnancy rate at days 32 to 38 and days 60 to 66, but a treatment by season interaction was not detected. The pregnancy rate for PG-3-G and Presynch-10 treatments did not differ during cool-cold weather (d 32 to 38: 46.8 vs. 44.3%; days 60 to 66: 41.6 vs. 41.1%, respectively), but PG-3-G and Presynch-10 produced a higher pregnancy rate than EB at days 32 to 38. During summer, pregnancy rate in PG-3-G was greater than in Presynch-10 (days 32 to 38: 35.9 vs. 26.7% or days 60 to 66: 33.2 vs. 24.4%, respectively), and pregnancy rate in EB cows did not differ from that of Presynch-10 cows. Although pregnancy loss did not differ for EB, Presynch-10, and PG-3-G treatments (4.0, 6.7, and 9.3%, respectively), pregnancy loss from days 32 to 38 and days 60 to 66 was 2-fold greater in thinner cows (<2.5 vs. ≥2.5; 9.0 vs. 4.4%). We concluded that presynchronizing estrous cycles with PG-3-G produced more pregnancies than inseminating cows at estrus during cooler weather and was superior to Presynch-10 during summer.

Key words: Presynch-10, PG-3-G, pregnancy rate per AI

Introduction

Timed AI (**TAI**) programs facilitate control of estrous cycles in lactating dairy cattle and provide viable options to AI programs based solely on detection of estrus. The most commonly used TAI programs are variations of the original Ovsynch protocol (injection of gonadotropin-releasing hormone or GnRH 7 days before [**GnRH-1**] and 48 hours after [**GnRH-2**] prostaglandin F_{2a} (**PG**) with TAI administered 16 hours after GnRH-2), which is used widely in the U.S. dairy industry.

When estrous cycles of lactating dairy cows are presynchronized to days 5 through 12 of the cycle before enrolling cows in the Ovsynch protocol, pregnancy rate per AI is further augmented. Standard presynchronization programs in which two injections of PG administered 14 days apart (**Presynch**) with the Ovsynch protocol initiated 14 days (**Presynch-14)**, 12 days (**Presynch-12**), 11 days (**Presynch-11**), or 10 days later (**Presynch-10)** have been tested in lactating dairy cows. The Presynch programs generally improve pregnancy rate compared with cows randomly allocated to Ovsynch alone. Presynch programs with shorter intervals of 11 days between the second Presynch PG injection (i.e., Presynch-11) and onset of Ovsynch improved pregnancy rate compared with programs with longer intervals (Presynch-14).

Other presynchronization schemes tested included those in which PG is injected first, then GnRH is injected either 2 days (**G-6-G)** or 3 days later (**PG-3-G**), followed by enrollment in the Ovsynch protocol in 6 or 7 days, respectively, tended to improve pregnancy rate. In addition, use of an Ovsynch protocol to presynchronize cows (i.e., non-breeding Ovsynch) before the TAI Ovsynch program (**Double Ovsynch)** resulted in improved pregnancy rate compared with Presynch-12 in primiparous cows, but not in multiparous cows.

Most studies reported in the literature have excluded presynchronization and TAI treatments during summer. Dairy cows whose estrous cycles were presynchronized with Presynch-12 or a progesterone insert-GnRH combination before a TAI program and who were exposed to heat stress (temperature-humidity index [THI] > 72) were 5.8 times more likely to have a poorer pregnancy rate than those not exposed to heat stress. Furthermore, cows in that study were 7.4 times more likely to abort an established pregnancy between 28 and 56 days of gestation. Compared with cooler conditions, chronic seasonal heat stress or hyperthermia alters follicular steroidogenesis, which leads to formation of suboptimal corpus luteum (**CL**) and reduced progesterone; factors that likely reduce synchronization efficiency and subsequent fertility.

We recently demonstrated that the PG-3-G presynchronization program (PG followed in 3 days by GnRH) followed by the Ovsynch protocol 7 days after GnRH) produced more cows with CL, more CL per cow, greater progesterone, and greater ovulatory response to GnRH-1 than cows whose estrous cycles were presynchronized with Presynch-10 before applying the Ovsynch program. The objectives of the current study were to determine the effect of these 2 presynchronization treatments on first service pregnancy rate in four dairy herds during hot and cool-cold seasons of the year and to validate our preliminary report (Dairy Research 2011, Report of Progress 1057, p. 31–35) that suggested the superiority of the PG-3-G treatment for achieving greater pregnancy rate.

Experimental Procedures

Lactating dairy cows from four herds in northeast Kansas were enrolled in the study. Three herds comprised cows calving from September 2010 through September 2011, with cows from the remaining herd calving from September 2009 through September 2011. All herds included cows that were milked thrice daily and fed diets consisting of alfalfa hay, corn silage, soybean meal, whole cottonseed, corn or milo grain, corn gluten feed, vitamins, and minerals.

At calving, 3,285 dairy cows (>95% were Holsteins with the residual representing crosses of Holstein with either Jersey, Brown Swiss, or Scandinavian Red) were clustered into breeding groups on a weekly (Herds 2, 3, and 4) or biweekly (Herd 1) basis. Characteristics of herds used in the experiment are summarized in Table 1. Enrollment in the study began at a median 42 days in milk **(DIM)** (41 ± 0.1 d; mean ± SE). Cows with ear tags ending with even digits were enrolled in Presynch-10: 2, 25-mg injections of $PGF_{2\alpha}$ (i.e., **PG-1** and **PG-2**; 5 mL Lutalyse, Pfizer Animal Health, Madison, NJ) administered 14 days apart (Figure 1). Cows with ear tags ending with odd digits were enrolled in PG-3-G: 1, 25-mg injection of PG (Pre-PG; 5 mL Lutalyse, Pfizer Animal Health) 3 days before 100 µg GnRH (Pre-GnRH; 2 mL Fertagyl, Merck Animal Health, Whitehouse Station, NJ), with the Pre-PG injection administered at the same time as PG-2 in the Presynch-10 treatment (Figure 1). Cows subsequently were enrolled in a TAI protocol (Ovsynch-56; injection of GnRH 7 days before [GnRH-1] and 56 hours after [GnRH-2] PG with AI 16 to 18 hours after GnRH-2) 10 days after PG-2 or Pre-PG injections. Treatment injections were staggered within each cluster so all cows were inseminated on the same day of the week. Cows were at a median of 75 DIM (74 ± 0.1 days) when inseminated at TAI. Treatment assignments were hand-delivered weekly to each dairy farm. At each weekly herd visit, cows scheduled for TAI that week were given body condition scores (**BCS**; 1= thin, 5 = fat).

Of the 3,285 cows originally enrolled in the study, 280 cows were dropped from the study because of culling (n = 207), death (n = 30), failure to inseminate (n = 36), or insemination after the scheduled TAI date (n = 7). Furthermore, 472 cows identified in estrus at any time after PG-1, including the day before scheduled TAI, were inseminated early before completing the entire experimental protocol (early bred; **EB**) and did not receive further scheduled injections. These 472 early inseminations occurred a median of 58 DIM (61 ± 0.5 days). The EB cows included those identified by rubbed tail chalk or tail paint, or by vaginal mucus. Final numbers of cows included in statistical analyses included 1,483 cows in the Presynch-10 treatment, 1,522 cows in the PG-3-G treatment, and 472 EB cows.

At each insemination, date, sire, technician, and breeding codes (chalk or tail paint rub, or mucus) was entered in DC305 (Herd 4) or PC-DART (Herds 1, 2, and 3) software. Full access to herd data was provided by dairy cooperators with weekly herd downloads. For purposes of determining when these EB cows were inseminated relative to treatment injections, proportions of EB cows inseminated between PG-1 and PG-2 (or Pre-PG), PG-2 (or Pre-PG) and GnRH-1, GnRH-1 and PG, and PG and GnRH-2 were determined.

Pregnancy diagnosis was conducted weekly by transrectal ultrasonography in Herds 1 and 4 and by transrectal palpation in Herds 2 and 3. Cows presented for pregnancy diagnosis were from 32 to 38 days since TAI. A second confirmatory diagnosis occurred 4 weeks later (60 to 66 days after TAI) and was performed by palpation per rectum at Herds 2, 3, and 4. The same veterinary clinic serviced Herds 2, 3, and 4, and 1 veterinary practitioner performed nearly 100% of the pregnancy diagnoses at Herds 2 and 3. A positive pregnancy outcome by ultrasonography 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. Positive pregnancy diagnosis by palpation was made by membrane slip or palpation of the amniotic vesicle.

Date of first repeat insemination was recorded for all cows after the initial AI. These cows were considered not pregnant unless a subsequent pregnancy diagnosis confirmed the pregnancy to be established earlier at first service based on size of the fetus. Return intervals to a second AI were categorized as early (<18 d), normal (18 to 25 d), or late (>25 d) for purposes of analysis.

Results and Discussion

Early bred cows

Of the 3,005 cows that completed the study, 472 (15.7%) were inseminated early (Table 2). Proportional distribution of cows inseminated early according to their assigned treatment did not differ regardless of when AI occurred after PG-1, PG-2 (or Pre-PG), GnRH-1, or PG (before scheduled day of TAI).

Pregnancy outcomes

Factors that significantly influenced pregnancy rate at days 32 to 38 and days 60 to 66 are summarized in Table 3. No significant interactions were detected between treatment and season or treatment and parity. Although treatment only tended ($0.071 < P < 0.107$) to affect pregnancy rate at both pregnancy diagnoses, parity ($P = 0.037$), season ($P < 0.001$), and occurrence of estrus at TAI ($P < 0.001$) accounted for significant variation in pregnancy outcomes.

All cows inseminated during the cool-cold months of the year had greater ($P < 0.001$) pregnancy rates than those inseminated during summer (Table 3). Cows identified in estrus on the day of TAI had a greater ($P < 0.001$) pregnancy rate at days 32 to 38 than those not detected in estrus in both treatments: PG-3-G cows, 50.5% (n = 99) vs. 39.3% (n = 1,187), and Presynch-10 cows, 47.9% (n = 117) vs. 33.8% (n = 1,130).

Primiparous cows had greater ($P < 0.05$) pregnancy rate than multiparous cows, but only at days 32 to 38. Slightly more pregnancy loss in primiparous cows after days 32 to 38 seemed to preclude the difference in pregnancy rate for cows at days 60 to 66 (Table 3). Pregnancy loss did not differ for EB, Presynch-10, and PG-3-G treatments (5.1, 7.0, and 9.2%, respectively). Pregnancy loss between pregnancy diagnoses, however, was affected by BCS. Cows with BCS < 2.5 (n = 501) had more ($P = 0.002$) than twice as many pregnancy losses (9.0 vs. 4.4%) than cows (n = 524) with BCS ≥ 2.5.

Contrasts of PG-3-G vs. EB for pregnancy rate showed increased pregnancy rate for PG-3-G at days 32 to 38 ($P < 0.05$) and at days 60 to 66 ($P < 0.05$). During summer, pregnancy rate at days 32 to 38 was greater ($P < 0.05$) for PG-3-G than for Presynch-10 (Figure 2). Results for pregnancy rate at days 32 to 38 during cool-cold weather did not differ between PG-3-G and Presynch-10, except both PG-3-G ($P < 0.05$) and Presynch-10 ($P = 0.053$) differed from EB cows (Figure 2). Treatment differences in pregnancy rate at days 60 to 66 during summer followed the same pattern as differences at days 32 to 38 (data not shown). In contrast, during cool-cold months, only PG-3-G tended ($P = 0.115$) to have greater pregnancy rate than EB cows, and PG-3-G did not differ from Presynch-10.

Returns to insemination

Proportions of EB cows that returned to estrus and were reinseminated <18 days after AI were greater ($P < 0.05$) than those in PG-3-G and Presynch-10 that completed the TAI protocol (Table 4). Fewer ($P < 0.05$) Presynch-10 and PG-3-G cows returned to estrus in the normal 18- to 25-day interval compared with EB cows. More ($P < 0.05$) Presynch-10 and PG-3-G cows were reinseminated after day 25.

In summary, cows treated with the PG-3-G treatment had greater pregnancy rate per AI than those treated with Presynch-10 during summer. Based on our previous study with the same treatments, we expect that PG-3-G is a more effective presynchronization treatment than other Presynch (14, 12, 11, or 10) treatments because of the Pre-GnRH injection and its ability to induce ovulation in anovular cows, although we did not examine this hypothesis in the present study. Evidence for that hypothesis was reported in our earlier study. Furthermore, because we detected no differences in the timing or distribution of early inseminations in the EB cows, both treatments effectively induced estrus for early inseminations. Given the potential advantages to anovular cows of the Pre-GnRH injection in the PG-3-G treatment documented with the PG-3-G treatment in our earlier study and its superior pregnancy outcome response during summer, PG-3-G may be a better presynchronization treatment to employ than Presynch-10.

Table 1. Characteristics of herds in which the experiment was conducted

	Herd			
Item	1[1]	2[2]	3[2]	4[2]
Milking cows, n	235	1,528	648	1,049
Rolling herd average milk, lb	29,689	25,567	24,529	31,696
Test-day average milk, lb	93	77	77	90
Days to first service	70	77	75	67
Calving interval, months	13.8	13.3	14.5	13.1

[1] Covered, sand-bedded, 2-row free stalls with overhead sprinklers in the feed alley and shade cloth covering the feeding area and feed bunk during summer.
[2] Curtain-sided, confined 2- or 4-row barns equipped with fans (above feed lines, sand-bedded free stalls, or both), sprinklers above the feed lines, and grooved concrete floors.

Table 2. Distribution of 472 cows inseminated early according pre-assigned treatment

	Pre-assigned treatment[1] before early artificial insemination	
When early inseminated[1]	Presynch-10	PG-3-G
	--------------------------- % (no.) ---------------------------	
PG-1 until PG-2 (or Pre-PG)	6.2 (16)	8.0 (17)
PG-2 (or Pre-PG) until GnRH-1	56.4 (146)	53.0 (113)
GnRH-1 until PG	6.5 (17)	8.0 (17)
PG until GnRH-2	30.9 (80)	31.0 (66)
Total	100 (259)	100 (213)

[1] See Figure 1 for description of treatment programs.

Table 3. Factors included in the logistic models that significantly influenced pregnancy rate per artificial insemination (AI) at days 32 to 38 or at days 60 to 66 after AI

		Pregnancy rate per AI, day of diagnosis	
Factor	Cows, n	32 to 38	60 to 66
		%	
Treatment[1]			
Early bred	472	31.4[a]	29.8[a]
Presynch-10	1,247	35.0[ab]	32.2[ab]
PG-3-G	1,286	41.2[b]	37.3[b]
Parity			
1	1,185	38.1[a]	35.2[a]
2+	1,820	33.6[b]	30.9[a]
Season			
Cool-cold	2,034	42.2[a]	38.9[a]
Hot	971	29.8[b]	27.6[b]
Estrus at AI			
Yes	688	39.3[a]	35.6[a]
No	2,317	32.4[b]	30.6[a]

[a,b] Contrasts within column and factor with different superscript letters differ ($P \leq 0.05$).
[1] See Figure 1 for description of treatment programs.

Table 4. Distribution of cows according to reinsemination intervals after artificial insemination (AI) at first service

	Treatment[1]		
Days from timed AI	Early bred	Presynch-10	PG-3-G
	% (n)		
<18	13.2[a] (40)	9.2[b] (73)	6.9[b] (52)
18 to 25	38.3[a] (116)	28.6[b] (227)	27.2[b] (205)
>25	48.5[a] (147)	62.2[b] (494)	65.9[b] (496)

[a,b] Proportions within row having different superscript letters differ ($P < 0.05$).
[1] See Figure 1 for description of treatment programs.

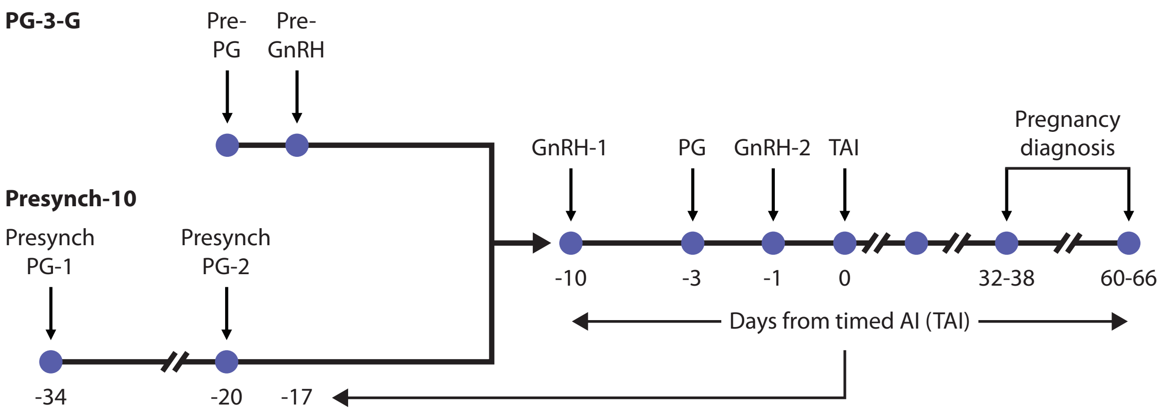

Figure 1. Experimental design. At calving, lactating dairy cows were assigned randomly to 2 treatments according to ending ear tag number (odd or even): PG-3-G or Presynch-10. Cows received 100 μg GnRH at Pre-GnRH, GnRH-1, or GnRH-2; 25 mg of $PGF_{2\alpha}$ at Pre-PG, Presynch PG-1, Presynch PG-2, and PG. Some cows were inseminated early at any time after PG-1 upon detection of estrus.

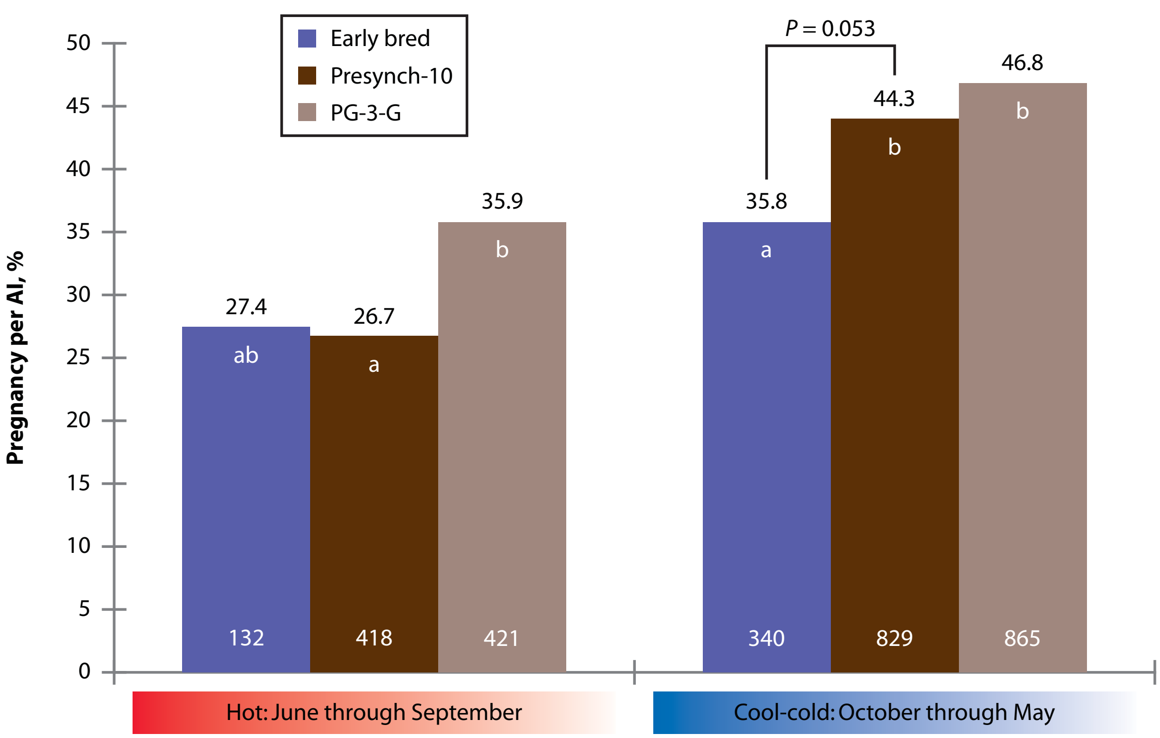

Figure 2. Pregnancy rate per artificial insemination (AI) (measured at days 32 to 38 after timed AI) for early bred, Presynch-10, and PG-3-G cows (treatments are defined in Figure 1) during June through September and October through May.

[a,b] Proportions within season having different letters differ ($P < 0.05$).