Pregnancy per artificial insemination after presynchronizing estrous cycles with the Presynch-10 protocol or prostaglandin F_{2α} injection followed by gonadotropin-releasing hormone before Ovsynch-56 in 4 dairy herds of lactating dairy cows¹

J. S. Stevenson² and S. L. Pulley

Department of Animal Sciences and Industry, Kansas State University, Manhattan 66506-0201

ABSTRACT

The objective was to determine the effect of 2 presynchronization treatments on first-service pregnancy per artificial insemination (P/AI) 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 (Presynch-10): two 25-mg injections of PGF_{2α} (i.e., PG-1 and PG-2) 14 d apart. Cows with ear tags ending with odd digits were enrolled in PG-3-G: one 25-mg injection of PG (Pre-PG) 3 d before injection of 100 µg of GnRH (Pre-GnRH), with the Pre-PG injection administered at the same time as PG-2 in the Presynch-10 treatment. Ten days after PG-2 or Pre-PG, all cows were enrolled in a timed AI protocol (Ovsynch-56; injection of GnRH 7 d before GnRH-1 and 56 h after GnRH-2 PG with AI 16 to 18 h after GnRH-2). Median days in milk (DIM) at scheduled timed AI were 75 d, which did not differ among herds. Cows detected in estrus before the scheduled timed AI were inseminated early (early bred, EB). Pregnancy was diagnosed at d 32 to 38 and at d 60 to 66 after timed AI by transrectal ultrasonography or transrectal palpation. Data were analyzed with herd as a random effect and with fixed effects of treatment (EB, Presynch-10, or PG-3-G), parity (primiparous vs. multiparous), season [hot (June through September) vs. cool-cold (October through May)], DIM, estrus at timed AI (0 vs. 1), and all 2-way interactions with treatment. The P/AI at d 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; P/AI at d 60 to 66 was 29.8, 32.2, and 37.3%, respectively. Season significantly influenced P/AI at d 32 to 38 and d 60 to 66, but a treatment by season interaction was not detected. The P/AI for PG-3-G and Presynch-10

treatments did not differ during cool-cold weather (d 32 to 38: 46.8 vs. 44.3%; d 60 to 66: 41.6 vs. 41.1%, respectively), but PG-3-G and Presynch-10 produced more P/AI than EB at d 32 to 38. During the summer, P/AI in PG-3-G was greater than in Presynch-10 (d 32 to 38: 35.9 vs. 26.7% and d 60 to 66: 33.2 vs. 24.4%, respectively), and P/AI 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 d 32 to 38 and d 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 P/AI than inseminating cows at estrus during cooler weather and was superior to Presynch-10 during the summer.

Key words: Presynch-10, prostaglandin F_{2α}-gonadotropin-releasing hormone-Ovsynch protocol, pregnancy per artificial insemination

INTRODUCTION

Timed AI programs facilitate control of estrous cycles in lactating dairy cattle and provide viable options to AI programs solely based on detection of estrus. The most commonly used timed AI programs are variations of the original Ovsynch protocol [Pursley et al., 1998; injection of GnRH 7 d before (**GnRH-1**) and 48 h after (**GnRH-2**) PGF_{2α} (**PG**), with timed AI administered 16 h after GnRH-2], which is used widely in the US dairy industry (Caraviello et al., 2006; Moeller et al., 2010).

When estrous cycles of lactating dairy cows are presynchronized to d 5 through 12 of the cycle before enrolling cows in the Ovsynch protocol, pregnancy per AI (**P/AI**) is further augmented (Vasconcelos et al., 1999). Standard presynchronization programs in which 2 injections of PG administered 14 d apart (**Presynch**) with the Ovsynch protocol initiated 14 d (**Presynch-14**; Navanukraw et al., 2004), 12 d (**Presynch-12**; Moreira et al., 2001; El-Zarkouny et al.,

Received May 9, 2012.

Accepted July 15, 2012.

¹Contribution number 12-412-J from the Kansas Agricultural Experiment Station, Manhattan.

²Corresponding author: jss@k-state.edu

2004), 11 d (**Presynch-11**; Galvão et al., 2007), or 10 d later (**Presynch-10**; Stevenson et al., 2011; Stevenson et al., 2012) have been tested in lactating dairy cows. The Presynch programs generally improve P/AI compared with cows randomly allocated to Ovsynch alone (Moreira et al., 2001; El-Zarkouny et al., 2004; Navanukraw et al., 2004). Moreover, Presynch programs with shorter intervals of 11 d between the second Presynch PG injection (i.e., Presynch-11) and onset of Ovsynch improved P/AI compared with programs with longer intervals (Presynch-14; Galvão et al., 2007). One reason for improved P/AI with shorter Presynch intervals is greater ovulatory response to GnRH-1 (Vasconcelos et al., 1999; Galvão et al., 2007).

Other presynchronization schemes tested by first injecting PG and then GnRH 2 d (**G-6-G**; Bello et al., 2006) or 3 d later (**PG-3-G**; Peters and Pursley, 2002; Stevenson et al., 2012), followed in 6 or 7 d, respectively, by enrollment in the Ovsynch protocol, tended to improve P/AI. In addition, use of an Ovsynch protocol to presynchronize cows (i.e., nonbreeding Ovsynch) before the timed AI Ovsynch program (**Double Ovsynch**; Souza et al., 2008) resulted in improved P/AI compared with Presynch-12 in primiparous, but not in multiparous, cows. Regardless of presynchronization scheme, improved P/AI in timed AI programs is associated with (1) greater ovulation response to GnRH-1 of Ovsynch (Vasconcelos et al., 1999; Bello et al., 2006; Chebel et al., 2006; Stevenson et al., 2012), (2) more cows with a functional corpus luteum (**CL**; Galvão and Santos, 2010; Stevenson et al., 2012) or more CL per cow at GnRH-1 (Stevenson et al., 2012), (3) greater progesterone at GnRH-1 (Stevenson et al., 2012), and (4) greater ovulatory response to GnRH-1 and GnRH-2 injections of Ovsynch (Rutigliano et al., 2008; Galvão and Santos, 2010).

Most studies reported in the literature have excluded presynchronization and timed AI treatments during the summer. Dairy cows, whose estrous cycles were presynchronized with Presynch-12 or a progesterone insert-GnRH combination before a timed AI program and exposed to heat stress [temperature-humidity index (**THI**) >72; NOAA, 1976], were 5.8 times more likely to have poorer P/AI than those not exposed to heat stress (Rutigliano et al., 2008). Furthermore, cows in that study were 7.4 times more likely to abort an established pregnancy between 28 and 56 d of gestation. Compared with cooler conditions, chronic seasonal heat stress or hyperthermia alters follicular steroidogenesis, which leads to formation of suboptimal CL and reduced progesterone (Wolfenson et al., 2000); factors that likely reduce synchronization efficiency and subsequent fertility.

We recently demonstrated that the PG-3-G presynchronization program [PG followed in 3 d by GnRH (Stevenson et al., 2012), followed by the Ovsynch protocol 7 d 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 P/AI in 4 dairy herds during hot and cool-cold seasons of the year and to validate our preliminary report (Stevenson et al., 2012) that suggested the superiority of the PG-3-G treatment for achieving greater P/AI.

MATERIALS AND METHODS

Experimental Cows

Lactating dairy cows from 4 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 three times daily and fed diets consisting of alfalfa hay, corn silage, soybean meal, whole cottonseed, corn or milo grain, corn gluten feed, vitamins, and minerals.

Experimental Design

At calving, 3,285 dairy cows (>95% were Holsteins, with the residual representing crosses of Holstein with Jersey, Brown Swiss, or Norwegian Red) were clustered into breeding groups weekly (herds 2, 3, and 4) or every 2 wk (herd 1). Characteristics of herds used in the experiment are summarized in Table 1. Enrollment in the study began at a median 42 DIM (41 ± 0.1 d; mean $\pm$ SE). Cows with ear tags ending with even digits were enrolled in Presynch-10: two 25-mg injections of PG (i.e., **PG-1** and **PG-2**; 5 mL of Lutalyse; Pfizer Animal Health, Madison, NJ) were administered 14 d apart (Figure 1). Cows with ear tags ending with odd digits were enrolled in PG-3-G: one 25-mg injection of PG (Pre-PG; 5 mL of Lutalyse; Pfizer Animal Health) 3 d before injection of 100 μ g of GnRH (Pre-GnRH; 2 mL of 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 timed AI protocol [Ovsynch-56; Brusveen et al., 2008; injection of GnRH 7 d before (GnRH-1) and 56 h after (GnRH-2) PG with AI 16 to 18 h after GnRH-2] 10

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 [injection of PGF_{2α} (PG) and then GnRH 3 d later, followed by enrollment in the Ovsynch protocol 7 d later] or Presynch-10 (2 injections of PG administered 14 d apart, with the Ovsynch protocol initiated 10 d later). Cows received 100 µg of GnRH at Pre-GnRH (3 d before GnRH-1 injection), GnRH-1 (GnRH injection 7 d before PG injection), or GnRH-2 (GnRH injection 56 h after PG injection) and 25 mg of PGF_{2α} at Pre-PG (3 d before Pre-GnRH injection), Presynch PG-1, Presynch PG-2, and PG injection. Some cows were inseminated early (early bred) at any time after PG-1 upon detection of estrus. Color version available in the online PDF.

d 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 d; mean $\pm$ SE) when inseminated at timed AI. Treatment assignments were hand delivered weekly to each dairy farm. At each weekly herd visit, cows scheduled for timed AI that week were body condition scored (1 = thin and 5 = fat; Ferguson et al., 1994). All injections and inseminations were made by onsite trained farm personnel with whom the corresponding author has conducted designed studies for more than 10 yr.

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 timed AI date ($n = 7$). Furthermore, 472 cows identified in estrus at any time after PG-1, including the day before scheduled

timed AI, were inseminated early (early bred, **EB**) before completing the entire experimental protocol and did not receive further scheduled injections. These 472 early inseminations occurred a median of 58 DIM (61 ± 0.5 d; mean $\pm$ SE). 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 DairyCOMP 305 (Valley Agriculture Software, Tulare, CA; herd 4) or PC-DART (Dairy Records Management Systems, Raleigh, NC; 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

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

Item	Herd			
	1 ¹	2 ²	3 ²	4 ²
Milking cows (n)	235	1,528	648	1,049
Rolling herd average milk (kg)	13,467	11,597	11,126	14,377
Test-day average milk (kg)	42	35	35	41
Days to first service	70	77	75	67
Calving interval (mo)	13.8	13.3	14.5	13.1

¹Covered, sand-bedded, 2-row freestalls with overhead sprinklers in the feed alley, and shade cloth covering the feeding area and feed bunk during the summer.

²Curtain-sided, confined 2- or 4-row barns equipped with fans (above feed lines, sand-bedded freestalls, or both), sprinklers above the feed lines, and grooved concrete floors.

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 (5.0 MHz linear-array transducer, Aloka 500V; Corometrics Medical Systems Inc., Wallingford, CT) and 4 (Easi-Scan; BCF Technology USA Ltd. LLC, Rochester, MN) and by transrectal palpation in herds 2 and 3. Cows presented for pregnancy diagnosis were from 32 to 38 d since timed AI. A second confirmatory diagnosis occurring 4 wk later (60 to 66 d after timed AI) was performed by palpation per rectum in herds 2, 3, and 4. The same veterinary clinic serviced herds 2, 3, and 4, and 1 veterinary practitioner performed almost 100% of the pregnancy diagnoses in herds 2 and 3. A positive pregnancy outcome by ultrasonography required the presence of anechoic uterine fluid and a CL ≥ 25 mm in diameter or anechoic uterine fluid and the presence of an embryo with a heartbeat. Positive pregnancy diagnosis by palpation was made by membrane slip or palpation of the amniotic vesicle.

The 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 the 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.

Hourly temperature and relative humidity data were obtained from the Kansas State University Weather Library for the duration of data collection (November 2009 through October 2011). The official weather station is located at 39.2°N latitude, 96.6°W longitude, and at elevation of 336.6 m on the Kansas State University Agronomy Farm <2 km south of where the experimental cows were housed (herd 1). The heat index (THI) was calculated using a standard formula (NOAA, 1976). For each 24-h period, average daily THI values were determined. This weather station was located approximately 95 to 105 km south of herds 2, 3, and 4, where temperatures tend to be slightly cooler year round.

Statistical Analyses

Data for P/AI at d 32 to 38, d 60 to 66, and pregnancy loss between the 2 diagnoses were analyzed using the GLIMMIX procedure (SAS Institute Inc., Cary, NC), with herd as a random effect and with fixed effects of treatment (EB, Presynch-10, or PG-3-G), parity (primiparous vs. multiparous), season [hot (June

through September) vs. cool-cold (October through May)], DIM (median days <74 vs. median days ≥ 74), estrus at timed AI (0 vs. 1; evidence of estrus included rubbed tail chalk or tail paint), BCS (<2.5 vs. ≥ 2.5), and all 2-way interactions with treatment. Selected contrasts of treatment were made within season in the GLIMMIX procedure.

Proportions of cows returning to AI after the timed AI at 3 intervals (<18 , 18 to 25, or >25 d) and the proportion of cows inseminated early [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 analyzed by chi-squared test (SAS Institute Inc.) using the row mean scores of the Cochran-Mantel-Haenszel statistics (PROC FREQ; SAS Institute Inc.) for combinations of 2×2 contingency tables.

RESULTS

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 timed AI).

Pregnancy Outcomes

Factors that significantly influenced P/AI at d 32 to 38 and d 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 ($0.071 < P < 0.11$) to affect P/

Table 2. Distribution of 472 cows inseminated early according to preassigned treatment¹

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

¹Cows were enrolled in Presynch-10: two 25-mg injections of PGF_{2α} (i.e., PG-1 and PG-2) 14 d apart or in PG-3-G: one 25-mg injection of PG (Pre-PG) 3 d before injection of 100 µg of GnRH (Pre-GnRH), with the Pre-PG injection administered at the same time as PG-2 in the Presynch-10 treatment. Cows subsequently were enrolled in a timed AI protocol [Ovsynch-56; injection of GnRH 7 d before (GnRH-1) and 56 h after (GnRH-2) PG with AI 16 to 18 h after GnRH-2] 10 d after PG-2 or Pre-PG injections.

Table 3. Factors included in the logistic models that significantly influenced pregnancy per AI at d 32 to 38 or at d 60 to 66 after AI

Factor	Cows (n)	Pregnancy per AI (%)	
		d 32 to 38	d 60 to 66
Treatment ¹			
Early bred ²	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 a column and factor with different superscript letters differ ($P \leq 0.05$).

¹Cows were enrolled in Presynch-10: Two 25-mg injections of PGF_{2α} (i.e., PG-1 and PG-2) 14 d apart or in PG-3-G: one 25-mg injection of PG (Pre-PG) 3 d before injection of 100 µg of GnRH (Pre-GnRH), with the Pre-PG injection administered at the same time as PG-2 in the Presynch-10 treatment. Cows subsequently were enrolled in a timed AI protocol [Ovsynch-56; injection of GnRH 7 d before (GnRH-1) and 56 h after (GnRH-2) PG with AI 16 to 18 h after GnRH-2] 10 d after PG-2 or Pre-PG injections.

²Cows inseminated early at first service upon detection of estrus before scheduled timed AI.

AI at both pregnancy diagnoses, parity ($P = 0.037$), season ($P < 0.001$), and occurrence of estrus at timed AI ($P < 0.001$) accounted for significant variation in pregnancy outcomes.

All cows inseminated during the cool-cold months of the year had greater ($P < 0.001$) P/AI than those inseminated during the summer (Table 3). Cows identified in estrus on the day of timed AI had greater ($P < 0.001$) P/AI at d 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 ($P < 0.05$) P/AI than multiparous cows, but only at d 32 to 38. Slightly more pregnancy loss in primiparous cows after d 32 to 38 seemed to preclude the difference in P/AI for cows at d 60 to 66 (Table 3). Pregnancy loss did not differ for EB, Presynch-10, and PG-3-G treatments (4.0, 6.8, and 9.3%, 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 versus EB for P/AI showed increased P/AI for PG-3-G at d 32 to 38 ($P < 0.05$)

and at d 60 to 66 ($P < 0.05$). During the summer, P/AI at d 32 to 38 was greater ($P < 0.05$) for PG-3-G than for Presynch-10 (Figure 2). Results for P/AI at d 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 P/AI at d 60 to 66 during the summer followed the same pattern as differences at d 32 to 38 (Figure 3). In contrast, during cool-cold months, only PG-3-G tended ($P = 0.12$) to have greater P/AI 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 d after AI were greater ($P < 0.05$) than those in PG-3-G and Presynch-10 that completed the timed AI protocol (Table 4). Fewer ($P < 0.05$) Presynch-10 and PG-3-G cows returned to estrus in the normal 18- to 25-d interval compared with EB cows. More ($P < 0.05$) Presynch-10 and PG-3-G cows were reinseminated after d 25.

Patterns of percentage distribution of cows returning to estrus during 30 d after first AI were similar between EB cows and those receiving the timed AI (Figure 4). With the exception of d 21 after AI, the proportions of reinseminations between d 20 and 25 were similar between EB and timed AI cows.

Figure 2. Pregnancy per AI (measured at d 32 to 38 after timed AI) for early bred, Presynch-10 [2 injections of PGF_{2α} (PG) administered 14 d apart, with the Ovsynch protocol initiated 10 d later], and PG-3-G (injection of PG and then GnRH 3 d later, followed by enrollment in the Ovsynch protocol 7 d later) cows (treatments are defined in Figure 1) during June through September and October through May. Proportions within season having different letters (a and b) differ ($P < 0.05$). Color version available in the online PDF.

Figure 3. Pregnancy per AI (measured at d 60 to 66 after timed AI) for early bred, Presynch-10 [2 injections of PGF_{2α} (PG) administered 14 d apart, with the Ovsynch protocol initiated 10 d later], and PG-3-G (injection of PG and then GnRH 3 d later, followed by enrollment in the Ovsynch protocol 7 d later) cows (treatments are defined in Figure 1) during June through September and October through May. Proportions within season having different letters (a and b) differ ($P < 0.05$). Color version available in the online PDF.

DISCUSSION

One of our objectives was to determine whether the PG-3-G presynchronization treatment was superior to Presynch-10 as indicated by our previous observations (Stevenson et al., 2012). The major difference between the 2 treatments was the addition of a GnRH injection

Table 4. Distribution of cows according to reinsemination intervals after AI at first service

Days from timed AI	Treatment ¹ [% (n)]		
	Early bred ²	Presynch-10	PG-3-G
<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 a row having different superscript letters differ ($P < 0.05$).

¹Cows were enrolled in Presynch-10: two 25-mg injections of PGF_{2α} (i.e., PG-1 and PG-2) 14 d apart or in PG-3-G: one 25-mg injection of PG (Pre-PG) 3 d before injection of 100 μg of GnRH (Pre-GnRH), with the Pre-PG injection administered at the same time as PG-2 in the Presynch-10 treatment. Cows subsequently were enrolled in a timed AI protocol [Ovsynch-56; injection of GnRH 7 d before (GnRH-1) and 56 h after (GnRH-2) PG with AI 16 to 18 h after GnRH-2] 10 d after PG-2 or Pre-PG injections.

²Cows inseminated early at first service upon detection of estrus before scheduled timed AI.

as part of the presynchronization. Furthermore, determining which of these presynchronization treatments was more effective during the summer was an important objective of the study. Clearly, the PG-3-G treatment was effective in producing more P/AI than EB cows, averaged for all seasons, and greater for PG-3-G than for Presynch-10 cows during the summer. Because this study was carried out in 4 herds and herd was treated as a random effect in the model, these results are significant for their application to most US dairy herds. We did not detect any interaction of treatment with

Figure 4. Percentage distribution of cows in estrus during 30 d after first AI did not differ between early bred cows and those receiving the timed AI treatments {PG-3-G [injection of PGF_{2α} (PG) and then GnRH 3 d later, followed by enrollment in the Ovsynch protocol 7 d later] and Presynch-10 (2 injections of PG administered 14 d apart, with the Ovsynch protocol initiated 10 d later)}. Color version available in the online PDF.

either season or parity, contrary to results reported for another similar presynchronization method (Double Ovsynch; Souza et al., 2008) that included GnRH. In that study, the Double Ovsynch treatment was effective in primiparous cows, but not in multiparous cows, when compared with Presynch-12. Why these differences should exist is not clear, because the only difference between PG-3-G and Double Ovsynch is the omission of a GnRH injection in the PG-3-G treatment that is administered 7 d before the Pre-PG and Pre-GnRH injections in Double Ovsynch.

An earlier report (Peters and Pursley, 2002) failed to detect an increase in P/AI for PG-3-G + Ovsynch cows compared with cows enrolled in a standard Ovsynch protocol; however, in primiparous cows, P/AI was 53% (39/73) in PG-3-G cows compared with 44% (30/69) in Ovsynch cows (similar to the effects of Double Ovsynch vs. Presynch-12 in primiparous cows: 65.2 vs. 45.2%; Souza et al., 2008). Overall, the P/AI of 41.5% (87/209) in PG-3-G cows did not differ from 38.3% (84/219) in Ovsynch-treated cows (Peters and Pursley, 2002). In contrast, we did not observe any differences in treatment effects on P/AI in primiparous or multiparous cows in the present study.

Except for the differences in P/AI detected during the summer in the present study between PG-3-G and Presynch-10, our P/AI during non-summer months corroborates a recent report (Ribeiro et al., 2011) of grazing Holstein (31 kg/d) and Jersey (22 kg/d) cows producing less milk than cows in the present study. In that study, no difference was detected in P/AI at d 30 or 65 for cows in Presynch-11 compared with G-6-G (similar to PG-3-G, in which PG is injected 2 d before GnRH but followed by Ovsynch in 6 d rather than 7 d in PG-3-G). The timed AI program used in this study (Ribeiro et al., 2011) was a 5-d Cosynch, in which an additional PG injection was given 24 h after the single PG during the breeding week, which was applied 5 d after GnRH-1, and GnRH-2 was injected at the time of AI (72 h after the first PG during the breeding week).

Another report also detected no difference in P/AI at d 30 in grazing dairy cows treated with Double Ovsynch compared with Presynch-10 when cows were treated with a 5-d, 58- or 72-h Cosynch timed AI protocol (timed AI + GnRH-2 injection administered at either 58 or 72 h after PG with timed AI at 72 h; Ribeiro et al., 2012). At the d-65 pregnancy confirmation in the latter study, however, in cows whose estrous cycles were presynchronized with Presynch-10, those inseminated and receiving GnRH-2 at 58 h had reduced P/AI compared with those receiving GnRH-2 and inseminated at 72 h. As pointed out previously, the PG-3-G treatment in our study may produce similar results to Double

Ovsynch because they share the single or double use of GnRH, respectively, in the presynchronization portion of the ovulation-synchronization programs.

Superiority of P/AI in the PG-3-G treatment compared with Presynch-10 during the summer is especially encouraging because of the expectation that Presynch-10 and Presynch-11 are likely better presynchronization alternatives to Presynch-14. Pregnancy per AI for cows treated with Presynch-11 before Ovsynch has been established as superior to that of Presynch-14 (Galvão et al., 2007). On the basis of studies in which GnRH was given at various stages of the first follicular wave, the intervals of 10 or 11 d (d 5 to 11 of the estrous cycle) between the presynchronization PG-2 injection and GnRH-1 of Ovsynch should facilitate the greatest ovulatory responses to GnRH-1 (Vasconcelos et al., 1999; Galvão et al., 2007) and greater P/AI (Chebel et al., 2006; Stevenson et al., 2007; Rutigliano et al., 2008).

Compared with Presynch-10, PG-3-G, similar to other presynchronization treatments that include GnRH, can induce ovulation before the onset of the timed AI program (Bello et al., 2006; Souza et al., 2008), particularly in anovular cows. The previous studies described treatments G-6-G and Double Ovsynch, respectively, which included a pre-GnRH injection that was preceded 2 or 3 d earlier by a PG injection. In theory, most ovular cows to which G-6-G, Double Ovsynch, or PG-3-G is applied should be between d 4 and 6 of the cycle at GnRH-1 and have an increased incidence of ovulation after a GnRH injection (Vasconcelos et al., 1999). In anovular cows, the Pre-GnRH injection provides at least one more opportunity to induce ovulation before the Ovsynch protocol is applied 6 (Bello et al., 2006) to 7 d (Souza et al., 2008; Stevenson et al., 2012) later.

In our previous study (Stevenson et al., 2012), the same treatments were used as in the present study. In that study, several factors in the PG-3-G treatment that were superior to Presynch-10 included (1) more PG-3-G cows ovulated (80%) after the Pre-GnRH injection than spontaneously ovulated after PG-2 of Presynch-10 (53%); (2) PG-3-G cows had more CL per cow, more cows with at least 1 CL, and more cows with progesterone ≥ 1 ng/mL than Presynch-10 cows at the time of GnRH-1 injection; (3) increased progesterone in PG-3-G than in Presynch-10 cows (5.9 vs. 5.0 ng/mL) before GnRH-1 injection; and (4) multiple positive responses were detected in anovular cows after PG-3-G compared with Presynch-10 (Stevenson et al., 2012). No further differences in ovarian function, however, were detected after the Ovsynch PG injection, indicating that the PG-3-G presynchronization effects were highly effective in synchronizing ovarian events to

maximize pregnancy outcomes after timed AI. We can only assume that these same differences were produced by the same PG-3-G treatment in the present study.

Fertility after a timed AI program is enhanced when (1) more cows have a CL before PG injection (Chebel et al., 2006); (2) cows have greater concentrations of progesterone at GnRH-1 (Stevenson et al., 2012) and at PG injection (Bello et al., 2006); and (3) a greater proportion of cows ovulate after GnRH-1 and GnRH-2 injection (Vasconcelos et al., 1999; Galvão and Santos, 2010). We suggest that some of these factors, including potential benefits to anovular cows resulting from the PG-3-G treatment in the present and former studies (Stevenson et al., 2012), may explain the improved P/AI at both pregnancy diagnoses during the summer compared with Presynch-10.

Why PG-3-G would produce more P/AI than Presynch-10 only during the summer is unclear. Relatively few controlled timed AI studies have been conducted during the summer. The P/AI in the current study during months of heat stress (Figures 2 and 3) were reasonably good compared with a previous study that also included summer breedings (<10%; Rutigliano et al., 2008). In that study, the mean THI of 72 (a THI of 72 is when heat stress begins in cattle; mild = 72 to 79; moderate = 80 to 89; severe = 90 to 98; danger >98; NOAA, 1976) was exceeded from mid June through early September. Cows exposed to heat stress in that study were 5.8 times more likely to have poorer P/AI than those not exposed to heat stress, and cows were 7.4 times more likely to abort an established pregnancy between 28 and 56 d of gestation (Rutigliano et al., 2008). In our study, the mean THI exceeded 72 during a similar period (May through October). Compared with cooler conditions, chronic seasonal heat stress or hyperthermia alters follicular steroidogenesis, which leads to formation of suboptimal CL and reduced progesterone (Wolfenson et al. 2000); factors that likely reduce synchronization efficiency and subsequent fertility. That hypothesis was substantiated experimentally when supplementing cooled cows during the summer with a progesterone insert for 13 d starting at d 5 postestrus resulted in improved P/AI (Friedman et al., 2012).

Only 15.7% of cows were inseminated early in the present study and more than half of those EB cows were inseminated during the 10-d period between the PG-2 (or Pre-PG) injection and the GnRH-1 injection. In another study in which cows were designed to be inseminated early after either Presynch-12 or Presynch-14, 48.7% of cows were inseminated during the 12 to 14 d after PG-2 (Chebel et al., 2010). The treatment similarity in the proportion of cows inseminated early (Table 2) is somewhat surprising given that the

PG-3-G cows did not receive the Presynch PG-1 injection (Figure 1). This result may be partly explained by the fact that Presynch-10 cows expressed estrus after PG-1 had not reached the end of the voluntary waiting period and were electively not inseminated. Despite the expectation that the Pre-GnRH injection administered after the Pre-PG injection would reduce expression of estrus in cows that received the Pre-PG injection, the proportion of cows inseminated during this interval did not differ between treatments. This finding was quite surprising, because when cows or heifers were treated with PG, expression of estrus was rare (<5%) during the subsequent 48 to 50 h when GnRH was administered 30 to 32 h after PG injection compared with controls given only PG (56.3%; Stevenson et al., 1996).

When dairy managers apply these timed AI programs, they should consider whether cows identified as in estrus should be inseminated early. Evidence for not doing so is supported by our results (Figures 2 and 3), in which during the cool-cold months of the year, but not during the summer, PG-3-G cows had better (11% units at d 32 to 38) or tended (7.3% units at d 60 to 66) to have better P/AI than EB cows. Although not significantly different, PG-3-G cows had numerically (8.5% units at d 32 to 38 and 7.5% units at d 60 to 66) greater P/AI than EB cows during the summer. Upon examining these differences in the 4 herds, 1 herd had similar pregnancy outcomes for EB and timed AI cows, but in the other herds, the P/AI for EB cows were less than P/AI for cows that completed the entire timed AI program. These results indicate that managers should be cautious in engaging in so-called cherry-picking of cows that express estrus during the timed AI program. Generally, fertility is better after detected standing estrus than after submissions to AI based on rubbed tail chalk or tail paint, vaginal mucus, or other detectors of estrual activity. Depending on the interpretation of more subtle signs of estrus, fertility of EB cows might be more or less than those completing the timed AI program. It is important to note that the recorded expression of estrus in the current study was assessed mostly by chalk or paint rubs or mucus.

Why differences occurred in return to insemination intervals (Table 4) for EB cows and cows that completed both timed AI programs is unclear. Fewer shorter returns (<18 d) in timed AI cows are consistent with our observations that less than 5% of cows returned to estrus during this interval (Stevenson and Pulley, 2012) after a timed AI at first service. In that study, approximately one-third returned to estrus in the normal 18- to 25-d period, which is consistent with the present results. Furthermore, we reported that 25% of cows that retained their original CL to d 28 after timed

AI at first service were diagnosed as not pregnant at d 32 (Stevenson and Pulley, 2012). These differences in return intervals could exist partly because EB cows are almost 100% synchronized compared with a lesser percentage of timed AI cows and a result of pregnancy failure in any cow after pregnancy recognition but before first pregnancy diagnosis.

CONCLUSIONS

Cows treated with the PG-3-G treatment had greater P/AI than those treated with Presynch-10 during the summer. Based on our previous study with the same treatments (Stevenson et al., 2012), 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 (Stevenson et al., 2012). 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 (Stevenson et al., 2012) and its superior pregnancy outcome response during the summer, PG-3-G may be a better presynchronization treatment to use than Presynch-10.

ACKNOWLEDGMENTS

We thank the owners and operators of Meier Dairy (Palmer, KS), Ohlde's Dairy (Linn, KS), and Linn Wilcox Creek Dairy (Linn, KS) for allowing this study to be conducted at their operations. We thank M. V. Scheffel and others at the Kansas State University Dairy Teaching and Research Center (Manhattan) for care of cows enrolled in this study. Partial funding was provided by the Kansas Dairy Commission (Muscotah, KS).

REFERENCES

- Bello, N. M., J. P. Steibel, and J. R. Pursley. 2006. Optimizing ovulation to first GnRH improved outcomes to each hormonal injection of Ovsynch in lactating dairy cows. *J. Dairy Sci.* 89:3413–3424.
- Brusveen, D. J., A. P. Cunha, C. D. Silva, P. M. Cunha, R. A. Sterry, E. P. Silva, J. N. Guenther, and M. C. Wiltbank. 2008. Altering the time of the second gonadotropin-releasing hormone injection and artificial insemination (AI) during Ovsynch affects pregnancies per AI in lactating dairy cows. *J. Dairy Sci.* 91:1044–1052.
- Caraviello, D. Z., K. A. Weigel, P. M. Fricke, M. C. Wiltbank, M. J. Florent, N. B. Cook, K. V. Nordlund, N. R. Zwald, and C. L. Rawson. 2006. Survey of management practices on reproductive performance of dairy cattle on large US commercial farms. *J. Dairy Sci.* 89:4723–4735.
- Chebel, R. C., M. J. Al-Hassan, P. M. Fricke, J. E. P. Santos, J. R. Lima, C. A. Martel, J. S. Stevenson, R. Garcia, and R. L. Ax. 2010. Supplementation of progesterone via controlled internal drug release inserts during ovulation synchronization protocols in lactating dairy cows. *J. Dairy Sci.* 93:922–931.
- Chebel, R. C., J. E. P. Santos, R. L. A. Cerri, H. M. Rutigliano, and R. G. S. Bruno. 2006. Reproduction in dairy cows following progesterone insert presynchronization and resynchronization protocols. *J. Dairy Sci.* 89:4205–4219.
- El-Zarkouny, S. Z., J. A. Cartmill, B. A. Hensley, and J. S. Stevenson. 2004. Pregnancy in dairy cows after synchronized ovulation regimens with or without presynchronization and progesterone. *J. Dairy Sci.* 87:1024–1037.
- Ferguson, J. D., D. T. Galligan, and N. Thomsen. 1994. Principal descriptors of body condition score in Holstein cows. *J. Dairy Sci.* 77:2695–2703.
- Friedman, E., Z. Roth, H. Voet, Y. Lavon, and D. Wolfenson. 2012. Progesterone supplementation postinsemination improves fertility of cooled dairy cows during the summer. *J. Dairy Sci.* 95:3092–3099.
- Galvão, K. N., M. F. Sá Filho, and J. E. P. Santos. 2007. Reducing the interval from presynchronization to initiation of timed artificial insemination improves fertility in dairy cows. *J. Dairy Sci.* 90:4212–4218.
- Galvão, K. N., and J. E. P. Santos. 2010. Factors affecting synchronization and conception rate after the Ovsynch protocol in lactating Holstein cows. *Reprod. Domest. Anim.* 45:439–446.
- Moeller, L. M., N. A. Michael, J. C. Dalton, and G. C. Lamb. 2010. Evaluating reproductive outcomes in United States Holstein dairies. *J. Dairy Sci.* 93(E-Suppl. 1):408. (Abstr.)
- Moreira, F., C. Orlandi, C. A. Risco, R. Mattos, F. Lopes, and W. W. Thatcher. 2001. Effects of presynchronization and bovine somatotropin on pregnancy rates to a timed artificial insemination protocol in lactating dairy cows. *J. Dairy Sci.* 84:1646–1659.
- Navanukraw, C., D. A. Redmer, L. P. Reynolds, J. D. Kirsch, A. T. Graul-Bilska, and P. M. Fricke. 2004. A modified presynchronization protocol improves fertility to timed artificial insemination in lactating dairy cows. *J. Dairy Sci.* 87:1551–1557.
- NOAA (National Oceanic and Atmospheric Administration). 1976. Livestock hot weather stress. Pages 31–37 in US Dept. Commerce, Natl. Weather Serv. Central Reg., Reg. Operations Manual Lett. NOAA, Washington, DC.
- Peters, M. W., and J. R. Pursley. 2002. Fertility of lactating dairy cows treated with Ovsynch after presynchronization injections of PGF_{2α} and GnRH. *J. Dairy Sci.* 85:2403–2406.
- Pursley, J. R., R. W. Silcox, and M. C. Wiltbank. 1998. Effect of time of artificial insemination on pregnancy rates, calving rates, pregnancy loss, and gender ratio after synchronization of ovulation in lactating dairy cows. *J. Dairy Sci.* 81:2139–2144.
- Ribeiro, E. S., R. L. A. Cerri, R. S. Bisinotto, F. S. Lima, F. T. Silvestre, L. F. Greco, W. W. Thatcher, and J. E. P. Santos. 2011. Reproductive performance of grazing dairy cows following presynchronization and resynchronization protocols. *J. Dairy Sci.* 94:4984–4996.
- Ribeiro, E. S., A. P. A. Monteiro, F. S. Lima, H. Ayres, R. S. Bisinotto, M. Favoreto, L. F. Greco, R. S. Marsola, W. W. Thatcher, and J. E. P. Santos. 2012. Effects of presynchronization and length of proestrus on fertility of grazing dairy cows subjected to a 5-day timed artificial insemination protocol. *J. Dairy Sci.* 95:2513–2522.
- Rutigliano, H. M., F. S. Lima, R. L. Cerri, L. F. Greco, J. M. Vilela, V. Magalhães, F. T. Silvestre, W. W. Thatcher, and J. E. Santos. 2008. Effects of method of presynchronization and source of selenium on uterine health and reproduction in dairy cows. *J. Dairy Sci.* 91:3323–3336.
- Souza, A. H., H. Ayres, R. M. Ferreira, and M. C. Wiltbank. 2008. A new presynchronization system (Double-Ovsynch) increases fertility at first postpartum timed AI in lactating dairy cows. *Theriogenology* 70:208–215.
- Stevenson, J. S. 2011. Alternative programs to presynchronize estrous cycles in dairy cattle before a timed artificial insemination program. *J. Dairy Sci.* 94:205–217.

- Stevenson, J. S., Y. Kobayashi, M. P. Shipka, and K. C. Rauchholz. 1996. Altering conception of dairy cattle by gonadotropin-releasing hormone preceding luteolysis induced by prostaglandin $F_{2\alpha}$. *J. Dairy Sci.* 79:402–410.
- Stevenson, J. S., M. A. Portaluppi, and D. E. Tenhouse. 2007. Factors influencing upfront single and multiple-ovulation incidence, progesterone, and luteolysis before a timed insemination resynchronization protocol. *J. Dairy Sci.* 90:5542–5551.
- Stevenson, J. S., and S. L. Pulley. 2012. Characteristics and retention of luteal structures, extended postinsemination cycle, progesterone, and pregnancy-specific protein B in serum after human chorionic gonadotropin treatment of dairy cows. *J. Dairy Sci.* 95:4396–4409.
- Stevenson, J. S., S. L. Pulley, and H. I. Mellieon Jr. 2012. Prostaglandin $F_{2\alpha}$ and gonadotropin-releasing hormone GnRH administration improve progesterone status, luteal number, and proportion of ovular and anovular dairy cows with corpora lutea before a timed AI program. *J. Dairy Sci.* 95:1831–1844.
- Vasconcelos, J. L. M., R. W. Silcox, G. J. Rosa, J. R. Pursley, and M. C. Wiltbank. 1999. Synchronization rate, size of the ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the estrous cycle in lactating dairy cows. *Theriogenology* 52:1067–1078.
- Wolfenson, D., Z. Roth, and R. Meidan. 2000. Impaired reproduction in heat-stressed cattle: Basic and applied aspects. *Anim. Reprod. Sci.* 60–61:535–547.