The effect of presynchronization with prostaglandin $F_{2\alpha}$ and gonadotropin-releasing hormone simultaneously, 7 d before Ovsynch, compared with Presynch-10/Ovsynch on luteal function and first-service pregnancies per artificial insemination

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ABSTRACT

Pregnancy per artificial insemination (P/AI) following Ovsynch is optimized when cows ovulate to the first GnRH of Ovsynch. Fertility programs are designed to presynchronize cows to d 6 or 7 of the estrous cycle to increase the chances of ovulation of a first-wave dominant follicle to the first GnRH of Ovsynch. The hypothesis of this experiment was that simplification of a presynchronization program through the combination of PGF_{2 α} and GnRH on the same day, 7 d before Ovsynch, would allow for similar P/AI compared with Presynch-10. Lactating dairy cows ($n = 432$) 41 to 47 d in milk (DIM) were randomly assigned to 2 treatments within parities for first service. Control cows received Presynch-10/Ovsynch consisting of the following: PGF_{2 α} -14 d-PGF_{2 α} -10 d-GnRH-7 d-PGF_{2 α} -56 h-GnRH-16 h-AI. Treated cows received PGF_{2 α} and GnRH-7 d-GnRH-7 d-PGF_{2 α} -56 h-GnRH-16 h-AI. All cows received a supplemental injection of PGF_{2 α} 24 h after the PGF_{2 α} of Ovsynch to enhance complete luteolysis. All cows received timed AI between 75 and 81 DIM. Blood was collected to assess circulating concentrations of progesterone (P4), and the number and size of corpora lutea (CL) were recorded using ultrasonography on day of PGF_{2 α} of Ovsynch. The administration of PGF_{2 α} simultaneously with GnRH and 7 d before Ovsynch (PG+G) had similar P/AI at 28 (46 vs. 48%), 35 (43 vs. 43%), 49 (39 vs. 39%), and 77 d post-AI (38 vs. 39%) compared with Presynch-10. No differences were observed in P/AI in primiparous versus multiparous cows at 28 (52 vs. 45%), 35 (48 vs. 41%), 49 (45 vs. 37%), and 77 d post-AI (43 vs. 36%). No difference existed between treatments in percentage of cows with

functional CL at PGF_{2 α} of Ovsynch, total luteal area (mm²), or serum concentrations of P4 at time of PGF_{2 α} of Ovsynch, regardless of parity. Number of CL had a tendency to be greater for multiparous PG+G vs. Presynch-10 cows (2.34 ± 0.09 vs. 2.15 ± 0.08) but not in primiparous cows (1.95 ± 0.10 vs. 1.98 ± 0.11). In summary, administering both PGF_{2 α} and GnRH on the same day, 7 d before the start of Ovsynch, appears to be a simple and effective alternative to Presynch-10 Ovsynch.

Key words: presynchronization, Ovsynch, progesterone, fertility, lactating dairy cow

INTRODUCTION

Effective synchronization of ovulation programs ensure luteolysis and subsequent ovulation of a mature dominant follicle in an 8-h period to allow for a successful timed AI. Progress has been made in the past decade in improving fertility of lactating dairy cows through manipulating ovarian dynamics before AI (Wiltbank and Pursley, 2014). The improvement in fertility is considered to be due to greater control of the hormone milieu, primarily progesterone (P4), and antral age of the ovulatory follicle (Pursley and Martins, 2011), which is regulated mainly by ovulation to the first GnRH of Ovsynch (Vasconcelos et al., 1999) and the development of an accessory corpus luteum (CL; Bello et al., 2006). Cows that ovulated to this injection had greater fertility after timed AI than cows that did not ovulate (Bello et al., 2006; Chebel et al., 2006; Keskin et al., 2010).

Initiating Ovsynch on d 6 or 7 of the estrous cycle maximizes ovulatory response following the first GnRH of Ovsynch, controls follicle and CL development, and increases pregnancy outcome following timed AI (Pursley and Martins, 2011). Three presynchronization strategies that increase the percentage of cows on d 6 or 7 of the estrous cycle at the first GnRH of Ovsynch are G6G (Bello et al., 2006; Astiz and Fargas, 2013;

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Dirandeh et al., 2015), Double-Ovsynch (Souza et al., 2008; Herlihy et al., 2012; Ayres et al., 2013), and Presynch-10 (Stevenson, 2011; Stevenson et al., 2012; Stevenson and Pulley, 2012) or Presynch-11 (Galvão et al., 2007). Presynch-10/11, for example, is designed to synchronize cows to d 6 to 8 of the cycle because most cycling cows will be in estrus 3 to 4 d following the second PGF_{2α} if they respond to the second injection of PGF_{2α}. These programs successfully improve reproductive performance of lactating dairy cows following first service compared with cows inseminated following estrus (Strickland et al., 2010; Gumen et al., 2012; Jimenez-Krassel et al., 2012) and might be referred to as “fertility programs for lactating dairy cows” (Wiltbank and Pursley, 2014; Fricke, 2016). Yet, fertility programs can be logistically challenging because of the number of injections and the possibility for compliance problems. Dairy operations that are limited to administering presynchronization injections on the same day of the week as Ovsynch are restricted to use only Presynch-14 or -12 (Moreira et al., 2001; Navanukraw et al., 2004). These programs limit fertility success following timed AI because they presynchronize cows to d 8 to 10 of the estrous cycle, when first wave dominant follicles begin to become atretic, P4 is increasing, and chances of ovulation to the first GnRH of Ovsynch begins to be reduced compared with d 6 or 7. Thus, fewer cows are likely to have a new accessory CL formed and a new follicular wave initiated in response to a GnRH-induced LH surge.

Creating a simpler presynchronization program for first service with fertility outcomes similar to G6G, Double Ovsynch, and Presynch-10/11 may be beneficial for dairy producers. The G6G program uses the administration of PGF_{2α} and GnRH 2 d apart to induce a new cycle 6 d before Ovsynch. However, it is not clear if combining the administration of PGF_{2α} simultaneously with GnRH and 7 d before Ovsynch (**PG+G**) would induce a new cycle and allow for cows to be on d 7 of the estrous cycle at time of the first GnRH of Ovsynch. The administration of PGF_{2α} and GnRH concurrently in cows on d 8 or 10 of the estrous cycle did not affect luteolytic actions of PGF_{2α} compared with PGF_{2α} and saline (Stevens et al., 1993).

The aim of this study was to determine differences in pregnancy per AI (**P/AI**) between PG+G and Presynch-10 in first service lactating dairy cows. In addition, we determined differences between the treatments' luteal and follicle parameters and serum concentrations of P4 at the time of the PGF_{2α} of Ovsynch. Presynch-10 was chosen as the control in this study because synchronized cows can be near the same day of the cycle at the start of Ovsynch as cows synchronized with PG+G. We hypothesized that presynchronization

with PG+G would have similar P/AI compared with Presynch-10 in first service lactating dairy cows.

MATERIALS AND METHODS

This experiment was conducted from May 2012 to January 2013 on a commercial dairy farm (Nobis Dairy Farm, St. Johns, MI) with approximately 900 lactating dairy cows that were milked 3 times daily. Rolling herd average milk production during this period was approximately 40 kg/cow per day. Cows were housed in freestall barns, fed a TMR once daily, and had free access to feed and water. The TMR consisted of corn, wheat, and alfalfa silages and corn- and soybean meal-based concentrates formulated to meet or exceed nutrient recommendations for lactating dairy cows (NRC, 2001). Trained personnel from our laboratory administered all injections of PGF_{2α} (0.5 mg of cloprostenol, 2 mL of Estrumate, Merck Animal Health, Madison, NJ) and GnRH (86 µg of gonadorelin, 2 mL of Fertagyl, Merck Animal Health) with single-dose syringes in semimembranosus or semitendinosus muscles of cows. The Institutional Animal Care and Use Committee at Michigan State University approved all procedures described in this manuscript.

Experimental Design

Weekly cohorts of lactating dairy cows ($n = 432$) between 41 and 47 DIM were blocked by parity and randomly assigned to 2 treatments for first service. Control cows ($n = 214$) received Presynch-10 consisting of 2 injections of PGF_{2α} 14 d apart with the second injection 10 d before the first GnRH of Ovsynch (GnRH-7 d-PGF_{2α}-24 h-PGF_{2α}-32 h-GnRH-16 h-AI; Figure 1). Treated cows (PG+G; $n = 218$) received PGF_{2α} and GnRH in different sites a few seconds apart 7 d before the first GnRH of Ovsynch. A second PGF_{2α} was administered 24 h following the first PGF_{2α} of Ovsynch to ensure luteolysis (Brusveen et al., 2009). Timing of final GnRH of Ovsynch remained at 56 h following the first PGF_{2α}. All cows received timed AI 16 h after the final GnRH of Ovsynch at 75 to 81 DIM. Three AI technicians blind to treatments performed artificial insemination. Commercial semen from multiple sires purchased by the farm was used. Only one sire was used to inseminate weekly cohorts of cows; therefore, treatments were not confounded by an effect of service sire.

Ovarian Ultrasonography and Pregnancy Diagnoses

Ovaries were mapped in a random subset of cows on day of first PGF_{2α} of Ovsynch ($n = 375$; Figure 1) by transrectal ultrasonography as previously described

Figure 1. Schematic illustration of experimental design with time of hormone injections (PGF_{2α} and GnRH), blood collection (B), and ultrasonography examination of ovaries (US) for each treatment, Presynch-10 (control) versus PG+G. PG = PGF_{2α}; PG+G = administration of PGF_{2α} simultaneously with GnRH and 7 d before Ovsynch.

by Martins et al. (2011a) using a Sonosite MicroMaxx ultrasound with a 7.5-MHz multifrequency linear array probe with a range of 5 to 10 MHz (Sonosite, Bothell, WA). Height (**H**) and width (**W**) of the largest size of each follicle with average diameter >7 mm and CL with average diameter >12 mm were measured with build-in calipers. Fluid-filled cavities in CL were measured in the same fashion. The area of each CL and each cavity (if present) was calculated with the following equation: $0.5 H \times 0.5 W \times \pi$. The luteal area of each CL was calculated by subtracting the cavity area from the total CL area. Total luteal area was the sum of the luteal area of all CL. Diameter of each follicle was calculated by the average of H and W of each follicle. Follicles ≥ 25 mm were considered cysts based on previous data (Jou et al., 1999; Dobson and Nanda, 1992; Borromeo et al., 1996) and were not considered in the analysis of largest follicle size.

Trained personnel from our laboratory blind to treatment performed pregnancy diagnoses on d 28 post-AI using the SonoSite MicroMaxx ultrasound already described. Farm veterinarians blind to treatment performed pregnancy diagnoses on d 35, 49, and 77 after AI using an Ibex Pro ultrasound machine (E.I. Medical Imaging, Loveland, CO). Pregnancy was confirmed by the heartbeat of an embryo.

Blood Collection, Hormone Assays, and Analysis of Luteal Function

Blood samples were collected from the coccygeal vein or artery of a subset of cows at time of first PGF_{2α} of Ovsynch (n = 404) and 28 d post-AI (n = 327) using Vacutainer tubes without anticoagulant (BD Vacutainer, Franklin Lakes, NJ) to measure serum concentrations of P4. Following collection, samples were refrigerated, transported to our laboratory, and maintained at 4°C

overnight. Serum was separated within 24 h after collection by centrifugation at $2,000 \times g$ for 20 min at 4°C and stored at -20°C for later P4 analyses.

Serum concentrations of P4 samples in duplicates were quantified with RIA (Coat-a-Count, Siemens Diagnostic, Los Angeles, CA). Intra- and interassay coefficients of variation were 9.1 and 6.0%, respectively. Cows with serum concentrations of P4 ≥ 1.00 ng/mL were considered to have a functional CL.

Statistical Analyses

Binomial variables were analyzed using logistic regression with a generalized linear mixed model implemented with the GLIMMIX procedure of SAS (version 9.4, SAS Inst. Inc., Cary, NC). The model considered treatment (Presynch-10 or PG+G) and parity category (primiparous or multiparous) as fixed effects and week as a random effect. Two-way interaction of treatment and parity category was only considered in the model if $P < 0.20$. Difference between treatments in continuous variables was analyzed using a linear mixed linear model applying the MIXED procedure of SAS with the same fixed effects and random effect as described for the GLIMMIX procedure. Treatment and parity number interaction was only considered in the model if $P < 0.20$. Normal distribution of the residuals was tested with studentized residual plots for each variable. Residuals were considered normally distributed for all variables. Distribution of cows across different ranges of serum P4 and follicle diameter were analyzed by chi-squared test using the FREQ procedure of SAS. Effects of total luteal area and serum concentrations of P4 at time of PGF_{2α} of Ovsynch were tested using regression analyses with the REG procedure of SAS. All analyses used a 2-tailed test and all values were considered different when $P \leq 0.05$.

Figure 2. Distribution (% of total) of cows within treatments, Presynch-10 (control; n = 200) versus PG+G (n = 204), across ranges of progesterone (P4) concentrations (ng/mL) at time of PGF_{2α} of Ovsynch. PG+G = administration of PGF_{2α} simultaneously with GnRH and 7 d before Ovsynch.

RESULTS

Percentage of cows with functional CL, total luteal area, and concentrations of serum P4 at time of first PGF_{2α} of Ovsynch did not differ between treatments (Table 1). No interaction of treatment and parity was observed in luteal measurements ($P = 0.51$). Figure 2 describes the differences in distribution of cows (%) within treatment across ranges of P4 at first PGF_{2α} of Ovsynch. A shift occurred in the percentage of cows in the medium low (3 to 4.99 ng/mL) and high P4 ranges (≥ 7 ng/mL) between treatments. A relationship existed between total luteal area and serum concentration of P4 at first PGF_{2α} (Figure 3). No effect of treatment on the percentage of cows with 1, 2, or 3+ CL at the time of PGF_{2α} of Ovsynch was observed. The size of the largest follicle at first PGF_{2α} of Ovsynch

had a negative relationship with CL number and luteal volume (Table 2).

The majority of cows in the study had follicles ≥ 9 mm (98%) at PGF_{2α} of Ovsynch. The number of follicles ≥ 9 mm at PGF_{2α} of Ovsynch was not different between treatments (Table 3). The average diameter of the largest follicle at PGF_{2α} of Ovsynch was greater for multiparous cows treated with Presynch-10 compared with PG+G (Table 3). When cows were divided into 8 categories based on largest follicle diameter (Figure 4), a greater percentage of multiparous cows treated with Presynch-10 had the largest follicle diameter ≥ 21 and < 25 mm compared with PG+G. In addition, a smaller percentage of multiparous cows treated with Presynch-10 had the largest follicle diameter between 11 and 13 mm compared with PG+G (Figure 4). The mean diameter of the largest follicle at time of PGF_{2α} of Ovsynch and the distribution of cows in different ranges of follicle diameter did not differ between treatments in primiparous cows (Table 3 and Figure 4). The percentage of cows within treatments that had a cyst (≥ 25 mm) did not differ between treatments (Table 3).

Treatment did not affect P/AI at 28, 35, 49, and 77 d post-AI (Figure 5) compared to controls. No difference between primiparous and multiparous was observed on P/AI at 28 ($P = 0.16$; 52 vs. 45%, respectively), 35 ($P = 0.17$; 48 vs. 41%, respectively), 49 ($P = 0.11$, 45 vs. 37%, respectively), and 77 d post-AI ($P = 0.13$, 43 vs. 36%, respectively). Interaction of treatment and parity was not significant ($P \geq 0.59$) for P/AI at any day post-AI. The percentage of cows with pregnancy loss between 28 and 35, 35 and 49, and 49 and 77 d post-AI was not different between treatments (Figure 6). No relationship ($P \geq 0.46$) was observed between parity and pregnancy loss in any of the periods evaluated.

Table 1. Effect of treatment, Presynch-10 (control) versus PG+G,¹ in a subset of first-service lactating dairy cows with functional corpora lutea (CL) at time of PGF_{2α} of Ovsynch on luteal function indices

Item	Presynch-10 (control)	PG+G	P-value
Cows with functional CL ² [% (no./total)]	94 (187/200)	91 (186/204)	0.38
Primiparous	94 (64/68)	92 (67/73)	0.59
Multiparous	93 (123/132)	91 (119/131)	0.49
Number of CL ³ [$\pm$ SEM (n)]	2.09 $\pm$ 0.07 (169)	2.20 $\pm$ 0.07 (173)	0.18
Primiparous	1.98 $\pm$ 0.11 (57)	1.95 $\pm$ 0.10 (62)	0.90
Multiparous	2.15 $\pm$ 0.08 (112)	2.34 $\pm$ 0.09 (111)	0.08
Total luteal area [mm ² $\pm$ SEM (n)]	712 $\pm$ 21 (169)	716 $\pm$ 22 (173)	0.91
Primiparous	635 $\pm$ 32 (57)	613 $\pm$ 33 (62)	0.67
Multiparous	752 $\pm$ 27 (112)	774 $\pm$ 27 (111)	0.45
Serum P4 ⁴ at PGF _{2α} of Ovsynch ³ [ng/mL $\pm$ SEM]	6.14 $\pm$ 0.22	5.57 $\pm$ 0.19	0.07
Primiparous	6.09 $\pm$ 0.34	5.33 $\pm$ 0.30	0.11
Multiparous	6.17 $\pm$ 0.29	5.70 $\pm$ 0.25	0.24

¹PG+G = administration of PGF_{2α} simultaneously with GnRH and 7 d before Ovsynch.

²Cows with functional CL = serum P4 ≥ 1.00 ng/mL.

³Only cows with functional CL were used in these analyses.

⁴P4 = progesterone.

Table 2. Relationships between number of corpora lutea (CL), total luteal area, circulating concentrations of progesterone (P4), and largest follicle diameter at PGF_{2α} of Ovsynch in a subset of first-service lactating dairy cows with functional CL (serum P4 ≥1.00 ng/mL) at time of PGF_{2α} of Ovsynch (n = 342)

CL number	No.	Total luteal area (mm ² ± SEM)	Serum P4 (ng/mL ± SEM)	Largest follicle diameter ¹ (mm ± SEM)	Cows within treatments		P-value
					Presynch-10 (control; %)	PG+G ² (%)	
1	79	398 ± 15 ^a	4.18 ± 0.26 ^a	15.8 ± 0.4 ^a	25 ^a	21 ^{a*}	0.45
2	166	713 ± 15 ^b	5.89 ± 0.20 ^b	14.3 ± 0.2 ^b	50 ^b	47 ^b	0.52
≥3	97	974 ± 23 ^c	7.12 ± 0.31 ^c	13.4 ± 0.3 ^c	25 ^a	32 ^{a*}	0.15

^{a-c}Means and percentages within a column with different letters differ ($P < 0.05$).

¹Only follicles with diameter ≥9 and <25 mm were used in this analysis.

²PG+G = administration of PGF_{2α} simultaneously with GnRH and 7 d before Ovsynch.

*Percentages within a column tended to differ ($P = 0.06$).

Cows with pregnancy loss between 28 to 77 d post-AI had similar largest follicle diameters ($P = 0.60$; 14.1 ± 0.6 vs. 14.4 ± 0.2 mm) and serum P4 at time of first PGF_{2α} of Ovsynch ($P = 0.15$; 6.33 ± 0.53 vs. 5.54 ± 0.23 ng/mL) and 28 d post-AI ($P = 0.15$; 7.27 ± 0.52 vs. 7.40 ± 0.20 ng/mL) compared with cows without pregnancy loss during the same period.

DISCUSSION

The overarching objective of this study was to develop a simpler and shorter synchronization program for dairy operations that are unable to use programs such as G6G, Presynch-10, or Double Ovsynch because of limitations of labor or other logistical constraints. These 3 fertility programs increase P/AI compared with AI following estrus (Gumen et al., 2012) and other synchronization programs (Bello et al., 2006; Galvão et al., 2007; Souza et al., 2008) primarily because they synchronize a greater percentage of cows on d 6 or 7 of

the estrous cycle to increase the chances of ovulation to the first GnRH of Ovsynch (Vasconcelos et al., 1999; Bello et al., 2006). Cows that ovulate to the first GnRH of Ovsynch have a greater chance for pregnancy (Bello et al., 2006; Chebel et al., 2006; Keskin et al., 2010). The present study tested the simultaneous administration of PGF_{2α} and GnRH 7 d before Ovsynch as a presynchronization strategy for first service lactating dairy cows.

Pharmacological manipulation of ovarian function to synchronize cows to d 6 or 7 of the estrous cycle requires the synchronous induction of a new estrous cycle. Presynchrony administration of PGF_{2α} must induce luteolysis, and GnRH must induce ovulation. Fertility programs such as Double Ovsynch, G6G, and Presynch-10 or 11 were designed to maximize the percentage of cows that start Ovsynch on d 6 or 7 of the cycle. No follicle and CL data at time of presynchronization treatments or at first GnRH of Ovsynch were collected in this study. But, P4 and CL number and size were collected at PGF_{2α} of Ovsynch. Level of P4 and number of CL at PGF_{2α} of Ovsynch are key indicators of ovulation to first GnRH of Ovsynch and are primarily driven from presynchronization outcomes. Bisinotto et al. (2015) indicated that cows supplemented with P4 or with a CL at time of first GnRH of Ovsynch have a greater chance for pregnancy. Treatment had no effect on number of CL, luteal area, or serum concentrations of P4 at time of PGF_{2α} of Ovsynch compared within parities. Cows with a functional CL at time of PGF_{2α} of Ovsynch had greater chances of pregnancy compared with cows with no functional CL. This finding is in agreement with a previous study (Chebel et al., 2006). Both treatments had a high percentage of cows with functional CL at time of PGF_{2α} of Ovsynch (>90%). Previous studies that used fertility treatments also reported a high percentage of cows with functional CL, including Presynch-10 [87.8% (Stevenson, 2011); 85.7% (Stevenson et al., 2012)], Double-Ovsynch [84.4%

Figure 3. Relationship between total luteal area (mm²) and serum concentrations of progesterone (P4, ng/mL) at time of first PGF_{2α} of Ovsynch in cows with functional corpus luteum (P4 ≥1.00 ng/mL).

Table 3. Effect of treatment (Presynch-10 vs. PG+G¹) on number and size of follicles at PGF_{2α} of Ovsynch

Item	Presynch-10 (control)	PG+G	P-value
Cows with follicles ≥ 9 mm [% (no./total)]	98 (180/183)	97 (187/192)	0.72
Number of follicles ≥ 9 mm [$\pm$ SEM ² (n)]	1.83 $\pm$ 0.07 (180)	1.76 $\pm$ 0.06 (187)	0.47
Mean of largest follicle diameter ² (mm $\pm$ SEM)	14.9 $\pm$ 0.2	14.0 $\pm$ 0.2	0.04
Primiparous (mm $\pm$ SEM)	13.9 $\pm$ 0.4	13.8 $\pm$ 0.4	0.77
Multiparous (mm $\pm$ SEM)	15.3 $\pm$ 0.3	14.1 $\pm$ 0.3	0.002
Cows with cyst ³ [% (no./total)]	9 (19/183)	7 (14/192)	0.64

¹PG+G = administration of PGF_{2α} simultaneously with GnRH and 7 d before Ovsynch.

²Only follicles with diameter ≥ 9 and < 25 mm were used in this analysis.

³Follicles with diameter ≥ 25 mm were considered cyst.

(Souza et al., 2008), 88% (Ayres et al., 2013)], and G7G [93.3% (Stevenson et al., 2012)]. Treatment affected the percentage of cows with concentrations of P4 in the 3.0 to 4.9 ng/mL range and > 7.0 ng/mL. Presynch-10

shifted a greater percentage of cows from 3.0 to 4.9 ng/mL to > 7.0 ng/mL of P4 relative to PG+G.

Presynchronization programs that used only PGF_{2α} have been tested to improve fertility of cows treated

Figure 4. Distribution (% of total) of cows within treatments, Presynch-10 (control) versus PG+G, across different ranges of largest follicle diameter (mm) at time of PGF_{2α} of Ovsynch for primiparous and multiparous cows. PG+G = administration of PGF_{2α} simultaneously with GnRH and 7 d before Ovsynch.

Figure 5. Effect of treatment, Presynch-10 (control) versus PG+G, on pregnancy/AI on d 28, 35, 49, and 77 post-AI. PG+G = administration of PGF_{2α} simultaneously with GnRH and 7 d before Ovsynch.

with Ovsynch by increasing ovulation to the first GnRH of this program. Simple presynchronization programs that only used a single injection of PGF_{2α} 3 d (Meyer et al., 2007), 10 d (LeBlanc and Leslie, 2003), and 12 d (Cartmill et al., 2001) before Ovsynch did not improve P/AI following timed AI compared with Ovsynch alone. However, programs that used 2 PGF_{2α} injections 14 d apart and 14 (Navanukraw et al., 2004) and 12 d (Moreira et al., 2001; El-Zarkouny et al., 2004) before Ovsynch were able to improve P/AI compared with Ovsynch without any presynchronization. Later, a shorter interval of 11 d between the second PGF_{2α} of the presynchronization and Ovsynch (Presynch-11) improved P/AI compared with an interval of 14 d (Presynch-14; Galvão et al., 2007). The increase in P/AI for Presynch-11 was attributed to

the greater ovulatory response to the first GnRH of Ovsynch for Presynch-11 compared with Presynch-14 (Galvão et al., 2007). Previous data indicated that the majority of cows that receive 2 injections of PGF_{2α} 14 d apart are observed in estrus 3, 4, and 5 d following the last PGF_{2α} injection (Chebel et al., 2006; Pursley et al., 2012). Vasconcelos et al. (1999) showed that the first GnRH of Ovsynch has a greater probability of ovulation when it is administered between d 5 and 9 of the estrous cycle. Moreover, data from our laboratory showed that d 6 and 7 of the estrous cycle are the best days of the estrous cycle to induce ovulation following the first GnRH of Ovsynch (Bello et al., 2006; Bello and Pursley, 2007). Therefore, the optimal interval between 2 PGF_{2α} injections of Ovsynch seems to be 10 (Presynch-10) or 11 d (Presynch-11). For this reason and because of the logistics of the reproductive management of the farm, we decided to use Presynch-10 as a control in our experiment to compare with PG+G.

Ribeiro et al. (2012) compared Presynch-10 vs. Double-Ovsynch in combination with 2 different 5 d Cosynch programs (GnRH-5 d-PGF_{2α}-1 d-PGF_{2α}-1.5 or 2 d-GnRH + AI) for first service of grazing Holsteins, Jersey, and crossbred cows (n = 1,754). No differences were observed between Presynch-10 and Double-Ovsynch on P/AI at 30 (59.1 vs. 56.8%, respectively) and 65 d post-AI (52.4 vs. 52.5%, respectively). The use of grazing cows with low production (~6,000 kg/lactation) and a large number of crossbred cows might explain the greater P/AI achieved in that study (Ribeiro et al., 2012) compared with our present study. Stevenson et al. (2012) compared Presynch-10 to a variation of the G7G protocol (PGF_{2α}-3 d-GnRH-7 d-Ovsynch) in 3,005 dairy cows in 4 different herds and did not find differences in P/AI during cooler weather (October through May). However, G7G had higher P/AI compared with Presynch-10 during the hot season (June through September) (Stevenson and Pulley, 2012). In the present study, PG+G had a 7-d interval between presynchronization GnRH and the initiation of Ovsynch as G7G. We found that P/AI did not differ between PG+G and Presynch-10, but the numbers of cows per treatment were limited, resulting in a high probability of a Type II error.

Stevenson (2011) compared Presynch-10 to 4 presynchronization programs including Presynch-14 and Presynch-12 in combination with a Co-synch program (GnRH-7 d-PGF_{2α}-72 h-GnRH + AI). In that study, Presynch-10 had similar P/AI at 32 d (35%) and 60 d post-AI (31%) compared with Presynch-14 (32 and 29%) and Presynch-12 (37 and 34%). In the present study, P/AI of Presynch-10 appears to be comparatively greater. This difference might be due to the second injection of PGF_{2α} 1 d after the first PGF_{2α} of Ovsynch

Figure 6. Effect of treatment, Presynch-10 (control) versus PG+G, on percentage of cows with pregnancy loss in different periods of gestation. PG+G = administration of PGF_{2α} simultaneously with GnRH and 7 d before Ovsynch.

(Brusveen et al., 2009) and the 16-h interval between the final GnRH of Ovsynch and timed AI (Pursley et al., 1998) that we used in the present study. Cows that do not have complete luteolysis following the PGF_{2α} of Ovsynch have significantly reduced chances of pregnancy following timed AI (Martins et al., 2011b). The use of an additional PGF_{2α} injection in Ovsynch enhanced the percentage of cows with complete luteolysis (Brusveen et al., 2009). Regarding timing of AI relative to the GnRH-induced LH surge, timed AI 16 h following the last GnRH of Ovsynch is clearly the optimal time to achieve higher P/AI (Pursley et al., 1998). Thus, the combination of an extra injection of PGF_{2α} 24 h after the PGF_{2α} of Ovsynch and timed AI 16 h after the final GnRH of Ovsynch used in our experimental design might optimize P/AI of the Ovsynch program for both treatments in our experiment. Ensuring luteolysis appears to be critical in any program that uses presynchrony before Ovsynch. In addition, treatment did not change serum concentrations of P4 at the time of first PGF_{2α}. Progesterone at time of PGF_{2α} of Ovsynch appears to be a good marker for high fertility. Several studies have shown a positive relationship with P4 at time of PGF_{2α} of Ovsynch and the probability of pregnancy (Bello et al., 2006; Bisinotto et al., 2010; Martins et al., 2011b). The greater the P4 at PGF_{2α} of Ovsynch, the greater the chance for pregnancy, and PG+G performed well in this regard.

A recent study tested the use of a single injection of GnRH 7 d before Ovsynch (**GGPG**) as a simpler presynchronization strategy for first service cows compared with Double-Ovsynch (Carvalho et al., 2014). When used for the resynchronization of nonpregnant cows for second or greater services, GGPG improved P/AI compared with Ovsynch (Dewey et al., 2010; Mendonça et al., 2012; Lopes et al., 2013). However, first service cows treated with GGPG had reduced P/AI compared with Double-Ovsynch (Carvalho et al., 2014). One of the problems of GGPG for first service presynchronization was attributed to the higher percentage of cows in the late stages of the estrous cycle at the beginning of the Ovsynch (Carvalho et al., 2014). A higher percentage of cows treated with GGPG had serum P4 <2.00 ng/mL at time of PGF_{2α} of Ovsynch. These cows might have started the endogenous luteolysis process before the administration of PGF_{2α} of Ovsynch, most likely resulting in an LH surge before the final GnRH of Ovsynch and ovulation before the timed AI (Vasconcelos et al., 1999; Moreira et al., 2000). Cows ovulating before timed AI had reduced P/AI (Pursley et al., 1998). The difference between GGPG and PG+G used in the present study is the administration of PGF_{2α} simultaneously with the first GnRH of GGPG, 7 d before Ovsynch. It seems important to initiate a new cycle with any presynchro-

nization program, in this case using PGF_{2α}, to avoid initiating Ovsynch too late in the estrous cycle.

Overall, PG+G could be advantageous because it only adds one more day of animal handling to the Ovsynch program and it does not add any extra weekdays for injections compared with Ovsynch. The implementation of PG+G might reduce final cost of the presynchronization program compared with Presynch-10. Although GnRH products are usually slightly more expensive than PGF_{2α} products, reduction of labor cost with one less weekday to administer injections would reduce the final cost of the program. In addition to the economic advantage, PG+G reduced the length of the program compared with Presynch programs. Further, PG+G may also offer a reasonable alternative to more complex fertility programs to enhance P/AI for first service. This program may be a good strategy for farms that want to limit the number of days in the week that cows are handled. Additional studies with larger number of cows are necessary to determine whether PG+G can be considered a fertility treatment or whether a Type II error influenced our results.

CONCLUSIONS

Results from the present study indicate that administering both PGF_{2α} and GnRH on the same day, 7 d before the start of Ovsynch (PG+G), achieves similar P/AI compared with Presynch-10. The percentage of cows with functional CL, serum concentrations of P4, and the number and size of CL and follicles at time of PGF_{2α} of Ovsynch appear to be similar to those in other fertility programs.

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