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A Modified Presynchronization Protocol Improves Fertility to Timed Artificial Insemination in Lactating Dairy Cows

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ABSTRACT

To compare 2 hormonal protocols for submission of lactating dairy cows for timed artificial insemination (TAI), nonpregnant lactating Holstein cows (n = 269) >60 d in milk were randomly assigned to each of 2 treatments to receive TAI (TAI = d 0). Cows assigned to the first treatment (Ovsynch, n = 134) received 50 μg of GnRH (d –10), 25 mg of $PGF_{2\alpha}$ (d –3), and 50 μg of GnRH (d –1) beginning at a random stage of the estrous cycle. Cows assigned to the second treatment (Presynch, n = 135) received Ovsynch but with the addition of 2 $PGF_{2\alpha}$ (25 mg) injections administered 14 d apart beginning 28 d (d –38 and –24) before initiation of Ovsynch. All cows received TAI 16 to 18 h after the second GnRH injection. Ovulatory response after each GnRH injection for a subset of cows (n = 109) and pregnancy status 42 d after TAI for all cows were assessed using transrectal ultrasonography. Based on serum progesterone (P_4) profiles determined for a subset of cows (n = 109), P_4 concentrations decreased for Presynch cows after the first 2 $PGF_{2\alpha}$ injections, and Presynch cows had greater P_4 concentrations at the $PGF_{2\alpha}$ injection on d –3 compared with Ovsynch cows. Although the proportion of cows ovulating after the first and second GnRH injections did not differ statistically between treatments (41.1 and 69.6% vs. 35.9 and 81.1% for Ovsynch vs. Presynch, respectively), pregnancy rate per artificial insemination (PR/AI) at 42 d post TAI was greater for Presynch than for Ovsynch cows (49.6 vs. 37.3%). Parity, DIM, and body condition score (BCS) at TAI did not affect PR/AI to TAI. These data support use of this presynchronization protocol to increase PR/AI of lactating dairy cows receiving TAI compared with Ovsynch.
(**Key words:** Ovsynch, Presynch, TAI, GnRH, $PGF_{2\alpha}$)

Abbreviation key: Ovsynch = synchronization regimen using sequential injections of GnRH and $PGF_{2\alpha}$ to control ovulation for timed insemination, **Presynch** = postpartum regimen using 2 injections of $PGF_{2\alpha}$ to prepare cows for Ovsynch, **PR/AI** = pregnancy rate per artificial insemination, $\mathbf{P_4}$ = progesterone, **TAI** = timed artificial insemination.

INTRODUCTION

The development of hormonal synchronization protocols that allow for timed artificial insemination (**TAI**) have provided a management tool for initiating first postpartum AI and thereby precisely controlling the voluntary waiting period in lactating dairy cows. A hormonal protocol for synchronizing ovulation in lactating dairy cows (i.e., Ovsynch) uses injections of GnRH and $PGF_{2\alpha}$ (Pursley et al., 1995; Burke et al., 1996; Pursley et al., 1997a) and is an effective method for hormonally programming cows to receive TAI. A presynchronization strategy in which cows receive 2 injections of $PGF_{2\alpha}$ administered 14 d apart beginning 26 d before initiation of Ovsynch (i.e., Presynch) increased pregnancy rate per artificial insemination (**PR/AI**) to TAI in lactating dairy cows compared with Ovsynch (Moreira et al., 2001; El-Zarkouny et al., 2002). The reported increase in PR/AI due to Presynch may justify the added labor and management required to administer the additional $PGF_{2\alpha}$ injections and is the primary advantage of implementing Presynch vs. Ovsynch for programming cows to receive first postpartum TAI.

A common question regarding practical implementation of Presynch in on-farm situations pertains to the importance of the 12-d interval between the second $PGF_{2\alpha}$ injection of Presynch and the first GnRH injection of Ovsynch. If this interval could be extended to 14 rather than 12 d, the first 4 injections of the protocol could be scheduled for the same day of the week during successive weeks. This becomes important for compliance by dairy producers that assign groups of cows to initiate the protocol on a weekly basis so that administration schedules do not get confused among groups of cows (Fricke et al., 2003). Although many dairy producers have implemented this modified Presynch protocol to better fit their reproductive management scheme,

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empirical data to establish that this modified protocol improves PR/AI compared with Ovsynch have not been published.

The objective of this experiment was to compare PR/AI to TAI after synchronization of ovulation using Ovsynch or a modified Presynch protocol. We did not directly compare the modified Presynch protocol described in the present study with the Presynch protocol reported by Moreira et al. (2001). Our hypothesis was that the modified Presynch protocol would increase PR/AI to TAI compared with Ovsynch. In addition, we wanted to characterize follicular and luteal responses to the various hormone injections of the protocols and assess the effect of parity, DIM, and BCS at TAI on PR/AI.

MATERIALS AND METHODS

Animals and Treatments

Primiparous and multiparous lactating Holstein dairy cows were housed in free-stall barns located at the NDSU Dairy Research Unit in Fargo and were milked twice daily. Cows were fed a TMR twice daily throughout the experiment for ad libitum consumption to meet or exceed requirements for a lactating dairy cow weighing 650 kg and producing 45 kg of milk with 3.5% fat (NRC, 2001). Average milk production for cows assigned to this trial was 39 ± 1.2 kg/d during the study period.

Nonpregnant Holstein cows (n = 269) were allocated to breeding groups consisting of 8 to 18 cows so they could be managed together to receive first postpartum TAI at 6-wk intervals between groups during the experiment. Breeding groups included cows submitted for first postpartum TAI that were past the voluntary waiting period (60 DIM) as well as later lactation nonpregnant cows submitted for second or greater postpartum TAI within the herd. Within a breeding group, cows were blocked by parity (primiparous vs. multiparous) and DIM at TAI (60 to 100 vs. >100 DIM) as part of the randomization procedure to minimize confounding of these variables between treatments. Cows were randomly assigned within a breeding group to each of 2 treatments (Ovsynch vs. Presynch). Mean DIM at initiation of treatment was 99.6 ± 6.3 (range 22 to 443) and 89.5 ± 5.8 (range 22 to 365) for the Ovsynch and Presynch groups, respectively.

Cows in the Ovsynch group (n = 134) received i.m. injections of vehicle as control (0.9% normal saline), 50 μg of GnRH (Cystorelin; Merial, Ltd., Duluth, GA) and 25 mg of $PGF_{2\alpha}$ (Lutalyse; Pharmacia Animal Health, Kalamazoo, MI) as follows: d –38, vehicle; d –24, vehicle; d –10, GnRH; d –3, $PGF_{2\alpha}$; d –1, GnRH. This is a modified Ovsynch protocol using 50 μg of GnRH per injection that was shown to result in similar reproductive performance when compared to using 100 μg of GnRH per injection in lactating dairy cows in a confinement-based dairy (Fricke et al., 1998). Cows in the Presynch group (n = 135) received the modified Ovsynch protocol described for cows in the Ovsynch group but whose estrous cycles were presynchronized using 2 $PGF_{2\alpha}$ injections administered 14 d apart on d –38 and d –24 with initiation of the modified Ovsynch protocol 14 d after the second $PGF_{2\alpha}$ injection. This modified Presynch protocol differs from that reported by Moreira et al. (2001) by using a 14 d (rather than a 12 d) interval between the second Presynch $PGF_{2\alpha}$ injection and the first GnRH injection of Ovsynch.

Cows in both treatment groups received TAI 16 to 18 h after the second GnRH injection. Semen from 6 proven sires was used randomly across treatments, with a similar number of units of semen from each sire used per treatment for TAI. All TAI services were performed by the NDSU dairy herd manager throughout the trial. Cows diagnosed nonpregnant after TAI were reassigned to the experiment during the next available breeding group.

Data Collection Periods

Data for this study was collected during 2 periods. Period 1 comprised cows (n = 109) enrolled into the trial from August 1999 to August 2000, and period 2 comprised cows (n = 160) enrolled into the trial from September 2000 to April 2002. Pregnancy status was determined 42 d after TAI for all cows during both periods using an ultrasound machine equipped with a transrectal 7.5 MHz linear-array transducer (Scanner 200 VET; Classic Medical Supply, Inc., Tequesta, FL). Visualization of a fluid-filled uterine horn and the presence of a conceptus were used as positive indicators of pregnancy, and PR/AI was calculated as the number of cows diagnosed pregnant at 42 d after TAI expressed as a percentage of cows receiving the synchronization protocol.

Additional data were collected during period 1 to determine follicular and luteal responses to the various injections of the synchronization protocols. To determine luteal status and luteal response to $PGF_{2\alpha}$ administration, blood samples were collected by venipuncture of the median caudal vein immediately before and 24 h after vehicle (Ovsynch cows) or $PGF_{2\alpha}$ (Presynch cows) administration on d –38 and –24 and immediately before and 24 h after $PGF_{2\alpha}$ administration on d –3 (all cows). Ovulatory response to GnRH injections administered on d –10 and –1 was conducted for all cows during period 1 as described previously (Fricke et al., 1998). Briefly, ovarian structures (antral follicles > 8 mm in diameter and CL) were monitored using the ultrasound machine and transducer described above. Follicle diameter was measured using the digital calipers of the ultrasound machine, and ovulatory follicle diameter was calculated as the mean of the vertical and horizontal diameter measurements (mm) for each follicle. Synchronized ovulation rate was calculated as the number of cows that ovulated a follicle within 48 h of the first and second GnRH injection, expressed as a percentage of the total number of cows receiving the synchronization protocol (Fricke et al., 1998).

P_4 Radioimmunoassay

Blood samples were allowed to clot for 24 h at 4°C, centrifuged (3000 × *g* for 20 min), and serum was collected and stored at –20°C until assayed for progesterone (**P_4**) concentrations using a previously validated radioimmunoassay (Redmer et al., 1991). Antiserum (GDN-337) was made in a rabbit by using a progesterone-11-BSA conjugate. Samples were analyzed in 19 assays. Interassay and intraassay coefficients of variation were 6.49 and 4.57%, respectively, and assay sensitivity was 0.025 ng/mL.

Body Condition Scoring

Body condition scores were assessed by the herd manager at TAI to determine the effect of BCS on response variables. Body condition score was assigned to each cow using a quarter-point scale from 1 to 5, where 1 = emaciated and 5 = obese (Ferguson et al., 1994). Cows were categorized into 2 groups according to BCS at TAI: those with less than average BCS and those with greater than or equal to average BCS.

Statistical Analyses

Categorical data (ovulatory responses to GnRH and PR/AI) were analyzed using the LOGISTIC procedure of SAS (SAS, 2000). The statistical model included treatment and parity (primiparous vs. multiparous), with DIM and BCS at TAI as regression variables and all 2-way interactions with treatment. The effect of AI sire was not included in this model but sires were distributed evenly between treatments. To further analyze the data, continuous variables (DIM and BCS) were converted to categorical variables by grouping cows by DIM at TAI (60 to 100 vs. >100 d) and BCS at TAI (< mean BCS vs. ≥ mean BCS). Treatment interactions with parity primiparous vs. multiparous), DIM, and BCS were then reanalyzed using chi-square analysis (Cochran-Mantel-Haenszel statistic) of SAS. Continuous data were analyzed using procedure GLM of SAS.

Table 1. Proportion of cows ovulating after the first and second GnRH injections and pregnancy rate per artificial insemination (PR/AI) to TAI for lactating dairy cows receiving Ovsynch or a modified Presynch protocol.

Item	Treatment[1]	
	Ovsynch	Presynch
Synchronized ovulation rate		
Ovulation after first GnRH (%)	41.1	35.8
(no./no.)	(23/56)	(19/53)
Ovulation after second GnRH (%)	69.6	81.1
(no./no.)	(39/56)	(43/53)
PR/AI (%)	37.3[a]	49.6[b]
(no./no.)	(50/134)	(67/135)

[a,b]Proportions differ ($P < 0.05$).

[1]See Materials and Methods for a detailed description of the synchronization protocols.

Serum P_4 concentrations were analyzed with a nested analysis of variance with treatment, animal (treatment), and day included in the model, and differences between specific means were evaluated by using the Tukey's Studentized Range (HSD) and Bonferroni *t*-tests (Sokal and Rohlf, 1995).

RESULTS AND DISCUSSION

Hormonal protocols that allow for TAI and minimize visual detection of estrus must be practical to implement within the day-to-day operation of a dairy farm or the protocol will fail due to lack of compliance (Fricke et al., 2003). The modified Presynch protocol assessed in the present study differs from that reported by Moreira et al. (2001) by using a 14-d rather than a 12-d interval between the second Presynch $PGF_{2\alpha}$ injection and the first GnRH injection of Ovsynch. Although this modified Presynch protocol has been adopted by many dairy producers and incorporated into the design of several recent field trials (Pancarci et al., 2002; Fricke et al., 2003), a direct comparison of this modified Presynch protocol with Ovsynch has not been reported. We did not directly compare the modified Presynch protocol described in the present study with the Presynch protocol reported by Moreira et al. (2001) but chose to directly compare the modified Presynch protocol with Ovsynch to determine whether this modified protocol would increase PR/AI compared with Ovsynch. Results from this experiment are important because they provide empirical data by which dairy managers and their consultants can make sound decisions regarding implementation of reproductive management protocols.

In agreement with our hypothesis, PR/AI of cows receiving the modified Presynch protocol was greater ($P < 0.05$) than that of cows receiving Ovsynch (Table 1). This observation supports and extends previous reports in which presynchronization using 2 injections of $PGF_{2\alpha}$

14 d apart beginning 26 d before initiation of Ovsynch increased PR/AI to TAI in lactating dairy cows compared with Ovsynch (Moreira et al., 2001; El-Zarkouny et al., 2002). Although these studies have reported PR/AI increases due to Presynch using 2 injections of $PGF_{2\alpha}$, specific mechanisms by which presynchronization improves PR/AI remain to be determined.

One possible mechanism by which Presynch may improve PR/AI is by presynchronizing the estrous cycle so that cows initiate the first injection of Ovsynch at a specific stage of the cycle. Initiation of Ovsynch on d 5 to 9 of the estrous cycle in lactating dairy cows resulted in a greater synchronization rate and PR/AI compared with other stages of the cycle (Vasconcelos et al., 1999). Administration of the first GnRH injection of Ovsynch on d 5 to 10 of the estrous cycle may increase the probability of ovulating the dominant follicle of the first follicular wave of the estrous cycle, thereby improving synchrony of emergence of a new wave and synchronized ovulation rate to the second GnRH injection of Ovsynch. In the present study, the proportion of cows ovulating after the first GnRH injection did not differ between treatments and, although the proportion of cows ovulating after the second GnRH injection was numerically greater for Presynch cows, synchronized ovulation rate to the second GnRH injection did not differ statistically between treatments (Table 1). Although synchronized ovulation rates to Ovsynch and Presynch in the present study are lower than those reported previously for Ovsynch (Fricke et al., 1998; Vasconcelos et al., 1999), PR/AI to TAI are similar to those reported in studies comparing Ovsynch and Presynch (Moreira et al., 2001; El-Zarkouny et al., 2002).

Size of the ovulatory follicle, which is related to the stage of the estrous cycle and follicular wave development, may also play a role in fertility. Lactating dairy cows in early (d 1 to 4) and late (d 17 to 21) stages of the estrous cycle exhibited larger follicles at ovulation and lower PR/AI to TAI compared with cows initiating Ovsynch on d 5 to 9 of the estrous cycle (Vasconcelos et al., 1999). In the present study, however, mean diameter of follicles that ovulated after the first and second GnRH injections of the synchronization protocols did not differ between treatments. Mean diameters of ovulatory follicles were 11.4 ± 0.6 and 12.0 ± 0.6 mm for Ovsynch cows at the first and second GnRH injections, respectively, and was 12.1 ± 0.8 and 12.6 ± 0.5 mm for Presynch cows at the first and second GnRH injections, respectively.

The possibility of Type II errors (declaring no difference between groups when a difference does exist) must be considered when interpreting data from the present study. The likelihood of detecting treatment effects in synchronization rate was limited in the present study because only the first 109 cows enrolled into the study were assessed for synchronization rate and because of the small difference in synchronization rate observed between treatment groups. Had a statistical difference in synchronization rate been detected, the 11-percentage-unit increase in synchronization rate due to Presynch would not fully account for the 12-percentage-unit increase in PR/AI. At a PR/AI of 50%, synchronization rate would have to be double the increase in PR/AI to fully account for the observed percentage increase. Thus, although Presynch may have increased PR/AI by increasing synchronization rate in the present study, other mechanisms must also contribute to the Presynch effect.

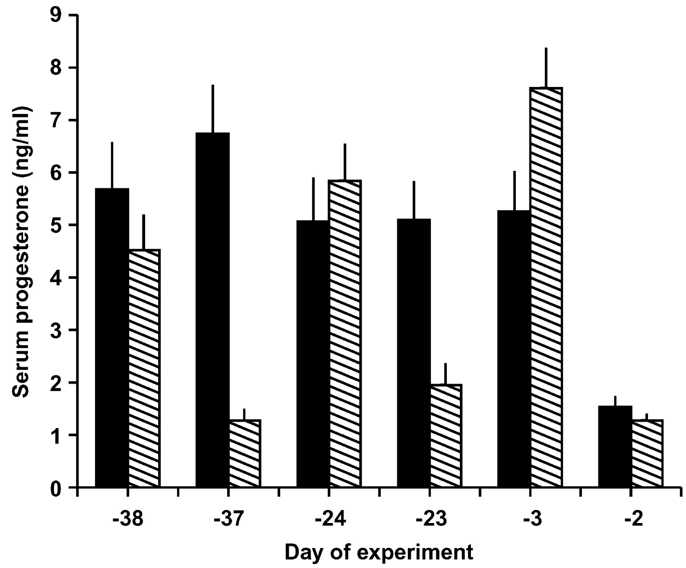

Figure 1. Mean (± SEM) serum progesterone concentrations (ng/mL) of lactating dairy cows receiving Ovsynch (n = 56; black bars) or Presynch (n = 53; hatched bars). Blood samples were collected immediately before the first 2 $PGF_{2\alpha}$ injections (Presynch cows) or vehicle injections (Ovsynch cows) and 24 h later (i.e., d –38, –37, –24, –23) and immediately before and 24 h after the $PGF_{2\alpha}$ injection on d –3 (i.e., d –3, and –2) during period 1 (d 0 = timed AI). Mean serum P_4 concentrations were greater ($P < 0.05$) for Ovsynch than for Presynch cows on d –37 and –23 and greater ($P < 0.05$) for Presynch than for Ovsynch cows on d –3. When combined across treatments, serum P_4 concentrations were greater ($P < 0.01$) on d –3 than on d –2.

The stage of the cycle at administration of the first GnRH injection of Ovsynch on d 5 to 10 of the estrous cycle may provide a more favorable P_4 environment during development of the ovulatory follicle, which may affect PR/AI. Based on serum P_4 profiles of cows collected during period 1 immediately before and 24 h after the first two $PGF_{2\alpha}$ (Presynch cows) or vehicle (Ovsynch cows) injections, Presynch cows as a group responded to the first 2 $PGF_{2\alpha}$ injections as reflected by a decrease in serum P_4 (Figure 1). Furthermore, serum P_4 concentrations were greater ($P < 0.01$) for

Table 2. Effect of DIM, parity, and BCS at timed AI on pregnancy rate per artificial insemination (PR/AI) for lactating cows receiving Ovsynch or a modified Presynch protocol.

	PR/AI (%)		
Item	Ovsynch (no./no.)	Presynch (no./no.)	Total
DIM			
60 to 100	31.9 (22/69)	47.8 (33/69)	39.9 (55/138)
>100	43.1 (28/65)	51.5 (34/66)	47.3 (62/131)
P-value	0.18	0.67	0.22
Parity			
Primiparous	40.0 (18/45)	43.4 (23/53)	41.8 (41/98)
Multiparous	36.0 (32/89)	53.7 (44/82)	44.4 (76/171)
P-value	0.65	0.24	0.68
BCS[1]			
< Mean BCS	31.5 (23/73)	49.4 (38/77)	40.7 (61/150)
≥ Mean BCS	44.3 (27/61)	50.0 (29/58)	47.1 (56/119)
P-value	0.13	0.94	0.29

[1]Mean BCS for all cows = 3.10 ± 0.02.

Presynch cows on d –3 compared with Ovsynch cows (Figure 1). Although we did not assess serum P_4 concentrations at initiation of Ovsynch, our data suggest that a greater proportion of Presynch cows had high P_4 concentrations at initiation of Ovsynch based on the higher P_4 concentrations on d –3 and the similar ovulatory response between treatments after the first GnRH injection (Table 1).

Although the modified Presynch protocol resulted in higher P_4 concentrations on d –3, data supporting a positive effect of high P_4 at initiation of Ovsynch on PR/AI are equivocal. A single injection of $PGF_{2\alpha}$ administered 10 d before initiation of Ovsynch to lactating cows in 5 commercial herds in Ontario, Canada, did not improve conception rate compared with Ovsynch alone (LeBlanc and Leslie, 2003). Administration of $PGF_{2\alpha}$ to lactating cows 12 d before the first GnRH injection of Ovsynch shifted cows into the early luteal phase of the estrous cycle at the time of the first GnRH injection, but no improvement in reproductive performance was observed compared with Ovsynch (Cordoba and Fricke, 2001). By contrast, a single injection of $PGF_{2\alpha}$ administered 12 d before Ovsynch shifted cows to early diestrus at the first GnRH injection but increased PR/AI only in multiparous but not primiparous cows compared with Ovsynch (Cartmill et al., 2001). When lactating cows were presynchronized with $PGF_{2\alpha}$ 10 d and GnRH 7 d before initiation of Ovsynch, the increase in the proportion of luteal-phase cows at initiation of Ovsynch did not affect PR/AI to TAI (Peters and Pursley, 2002).

One possible explanation for the increase in PR/AI using 2 injections of $PGF_{2\alpha}$ compared with other presynchronization strategies is that 2 successive injections of $PGF_{2\alpha}$ may exert a positive effect on the uterine environment. In lactating dairy cows, PR/AI increased with increasing number of estruses occurring during the postpartum period (Thatcher and Wilcox, 1972), and a positive effect of $PGF_{2\alpha}$ administration on PR/AI has been reported in cattle (Roche, 1976; Macmillan and Day, 1982). Cyclic cows should express estrus once if not twice before TAI when subjected to a Presynch protocol using 2 injections of $PGF_{2\alpha}$ administered 12 or 14 d apart. In support of this idea, cows that were cyclic at initiation of presynchronization using 2 injections of $PGF_{2\alpha}$ exhibited increased PR/AI, but when anovular cows were included in the analysis, no effect of presynchronization was detected (Moreira et al., 2001). Exogenous $PGF_{2\alpha}$ may also act directly through upregulation of a uterine immune response as demonstrated in sows (Wulster-Radcliffe et al., 2001) and ewes (Lewis and Wulster-Radcliffe, 2001). Further research is needed to fully understand the mechanisms by which presynchronization increases PR/AI in lactating dairy cows.

Based on logistic regression analysis, parity, DIM, and BCS at TAI did not affect PR/AI in the present study. These results are in contrast to studies that have shown these variables to affect PR/AI in lactating cows. First-parity cows receiving TAI exhibited a higher PR/AI than second- or third- and greater parity cows (Peters and Pursley, 2002), and primiparous cows exhibited a higher PR/AI to TAI than multiparous cows (Pancarci et al., 2002). By contrast, second-parity cows exhibited a higher PR/AI than either first or third or greater parity cows in another study (Pursley et al., 1997a). These 3 studies included observations from 427, 371, and 732 cows, respectively, and it is impossible to discern whether these effects are biological or are due to random variation in a binomial variable such as PR/AI. Several studies have shown that cows receiving TAI early postpartum (<75 DIM) had a lower PR/AI than cows receiving TAI later during lactation (Pursley et al., 1997b, 1998). Furthermore, PR/AI were 37, 42, and 48% at first, second, and third TAI, suggesting a positive relationship between DIM at TAI and PR/AI (Pursley et al., 1997a). Finally, cows with a BCS ≥ 2.5 (on a scale of 1 to 5) had greater PR/AI than cows with a BCS < 2.5 (Moreira et al., 2000). The lack of an effect of BCS in the present study may have occurred due to the relatively good BCS of the cows enrolled in this study and the uniformity in BCS among cows, with few cows having excessively high or low BCS at TAI.

When data from the present study were reanalyzed using chi-square analysis, Ovsynch cows receiving TAI later during lactation tended ($P = 0.18$) to have a greater PR/AI than Ovsynch cows receiving TAI earlier during lactation, but this trend was not detected for Presynch cows (Table 2). The trend for a treatment interaction with DIM at TAI on PR/AI may be confounded in the present study because, although cows were blocked by DIM as part of the randomization procedure, Presynch cows initiated treatment approximately 10 d earlier in lactation on average compared with Ovsynch cows (99.6 ± 6.3 vs. 89.5 ± 5.8 DIM). Similarly, when cows were grouped based on the average BCS for all cows assigned to the study (3.10 ± 0.02), Ovsynch cows that had greater than or equal to average BCS tended ($P = 0.13$) to exhibit greater PR/AI than cows with less than average BCS, but this trend was not detected for Presynch cows (Table 2). Results in Table 2 pertaining to the effects of DIM, parity, and BCS at TAI on PR/AI should be interpreted with caution because of the possibility of Type II errors discussed previously, especially when cows assigned to each treatment were allocated to subgroups within each treatment for analysis.

CONCLUSIONS

Results from this experiment support use of this modified Presynch protocol to increase PR/AI of lactating dairy cows receiving TAI compared with Ovsynch. On-farm implementation of this modified Presynch protocol allows for administration of the first 4 injections of the protocol on the same day of the week and administration of all hormone injections to be restricted to 2 d of the week. Such considerations are important for farms choosing to implement systematic synchronization and resynchronization systems with minimal labor required for hormone administration, TAI, and estrus detection (Fricke et al., 2003). Further research is needed to understand the mechanisms by which presynchronization using 2 injections of $PGF_{2\alpha}$ increases PR/AI in lactating dairy cows.

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