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Evaluation of Two Hormonal Protocols for Synchronization of Ovulation and Timed Artificial Insemination in Dairy Cows Managed in Grazing-Based Dairies

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ABSTRACT

To evaluate the efficacy of two hormonal protocols for synchronization of ovulation and timed artificial insemination (TAI) in dairy cows managed in grazing-based dairies, lactating dairy cows (n = 142) from two grazing-based dairies were randomly assigned to one of three treatment groups. Cows in the first group (Ovsynch) received 50 μg of GnRH (d –10); 25 mg of $PGF_{2\alpha}$ (d –3), and 50 μg of GnRH (d –1) followed by timed AI on d 0. Cows in the second group (PGF + Ovsynch) received a modified Ovsynch and timed AI similar to Ovsynch but with the addition of 25 mg of $PGF_{2\alpha}$ 12 d (d –22) before initiation of Ovsynch. Cows in the third group (control) received standard reproductive management in place on each farm. Luteolysis occurred in 90.5% of cows exhibiting luteal function on d –22 in the PGF + Ovsynch treatment group, whereas none of the cows in the Ovsynch group underwent luteolysis on d –22. Synchronization rate (i.e., ovulatory response at 48 h after the second GnRH injection), conception rates at TAI and pregnancy rates after 35 d of breeding were similar for cows in the Ovsynch and PGF + Ovsynch groups. The proportion of anovular cows at the first GnRH injection of the synchronization protocols (d –10) was similar for cows receiving Ovsynch (28.0%) and PGF + Ovsynch (30.7%), and conception rate at TAI was similar for cycling (45.8%) and anovular (30.0%) cows. The cumulative pregnancy rate was greater for cows receiving TAI compared with control cows after 7 d of breeding (41.2 vs. 20.0%) but did not differ at 35 d of breeding (54.9 vs. 60.0%). Administration of $PGF_{2\alpha}$ 12 d before initiation of Ovsynch did not improve synchronization, conception, or pregnancy rate compared with the standard Ovsynch protocol. Synchronization of ovulation to initiate timed AI at the onset of the breeding season resulted in earlier establishment of pregnancy compared with standard reproductive management.

(**Key words:** Ovsynch, timed AI, dairy cattle, grazing)

Abbreviation key: $\mathbf{P_4}$ = progesterone, **TAI** = timed AI.

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INTRODUCTION

To achieve and maintain a 12-mo calving interval in which calving, lactation, and dry periods are relatively synchronous within a grazing-based herd, the majority of cows must resume cyclicity and establish pregnancy within 90 d after calving (McDougall et al., 1998). Thus, for grazing-based dairies in Wisconsin, the majority of cows must conceive within a 2- to 3-mo breeding period during the summer (June, July, and August) so that calving and the onset of lactation coincide with the onset of pasture growth during the spring (March, April, and May). Reproductive efficiency of grazing-based dairies during summer months in Wisconsin is further limited due to negative effects of heat stress on reproduction in both cows and natural service bulls (Hansen et al., 1992; Barth and Bowman, 1994). Cows that fail to conceive during the breeding period in a grazing-based dairy are problematic because they must either be culled from the herd and replaced with another animal or supplemented with extra feed after pasture forages diminish in the fall. Either option is economically prohibitive when reproductive efficiency is poor and a significant proportion of cows within the herd fail to conceive during the breeding period. Thus, maximizing reproductive efficiency during the summer breeding period is essential for achieving and maintaining a grazing-based dairy system in Wisconsin. Reproductive management strategies that improve AI-service rates during the breeding period may increase the percentage of cows that conceive, thereby allowing for seasonally calved herds.

Progress toward improving reproductive efficiency in lactating dairy cows was realized by combining timed AI (**TAI**) with a protocol for synchronization of ovulation that can be initiated at a random stage of the estrous cycle (Pursley et al., 1995). This protocol, commonly called Ovsynch, synchronizes follicular development, luteal regression, and time of ovulation, thereby allowing for TAI after the second GnRH injection and improving the AI service rate (Pursley et al., 1995). Because TAI can be initiated on a predetermined date, first AI service can be scheduled to occur for all cows in a grazing-based dairy on the first day of the breeding season, thereby achieving a 100% AI service rate. Furthermore, although Ovsynch can be initiated at a random stage of the estrous cycle, initiation of Ovsynch on d 5 to 9 of the estrous cycle results in greater synchronization and conception rates compared with other stages (Vasconcelos et al., 1999). Presynchronization of cows to initiate Ovsynch on d 5 to 9 of the estrous cycle by administration of $PGF_{2\alpha}$ 12 d before the first GnRH injection of the Ovsynch protocol may further improve synchronization and conception rate compared with the standard Ovsynch protocol.

In confinement-based dairies, virgin heifers respond poorly to Ovsynch and TAI compared with AI to a detected estrus (Pursley et al., 1997b), possibly due to physiologic factors associated with milk production (Wiltbank et al., 2001). Although Ovsynch is an effective method for improving reproductive efficiency in confinement-based dairies (Burke et al., 1996; Pursley et al., 1997a,b; Britt and Gaska, 1998), it has not been evaluated as a strategy for tightening the breeding period for cows managed in grazing-based dairies in the United States. Because lactating cows managed in grazing-based dairies often produce less milk compared with cows in confinement-based dairies, it is possible that ovarian responses to hormonal protocols for synchronizing ovulation may differ among lactating dairy cows managed in various systems.

The primary objective of this study was to determine the effect of a single injection of $PGF_{2\alpha}$ administered 12 d before initiation of the Ovsynch protocol as a potential presynchronization strategy compared with the standard Ovsynch protocol. A secondary objective was to compare standard reproductive management with management using synchronization of ovulation and TAI in grazing-based dairies. Our hypotheses were 1) administration of $PGF_{2\alpha}$ 12 d before initiation of Ovsynch would shift cows to the early luteal stage of the estrous cycle when Ovsynch is initiated, thereby resulting in greater synchronization and conception rates compared with the standard Ovsynch protocol; and 2) initiation of TAI at the onset of the breeding season would increase cumulative pregnancy rate during the breeding season compared with standard reproductive management.

MATERIALS AND METHODS

Farms and Animals

This field trial was conducted from May 10 to July 19, 1999, on two semiseasonal, spring-calving, grazing-based dairies located in south central Wisconsin. Because these dairies were semiseasonal, only cows that had calved and had a minimum of a 50 d postpartum interval at the onset of the designated breeding period were available for the trial. For farm 1, 120 of a total of about 500 lactating cows were allocated to this study; for farm 2, 45 of a total of about 130 lactating cows were allocated to this study. Of the 165 cows originally assigned to the experiment, 23 cows (n = 8 from the Ovsynch group; n = 9 from the $PGF_{2\alpha}$ treatment group; and n = 6 from the control group) were excluded from the analysis because they had one or more missing data points. Missing data occurred due to unavailability of cows during on-farm blood sampling and ultrasound scanning periods and cows that were sold or became ill during the course of the experiment. Thus, lactating dairy cows (n = 142) assigned to this study included purebred Holstein (n = 14) and crossbred cows (n = 128) with various percentages of Holstein and Jersey genetics. Cows were in their first (n = 41) or later (n = 101) lactation (mean ± SEM lactation number = 2.4 ± 1.4).

For both farms, all cows were milked twice daily and, in addition to grazing, cows received a supplemental TMR that was offered twice per day, before or after each milking. Average daily milk production for both farms was between 27 to 33 kg per cow during the study period.

Treatment Groups

Cows were blocked by lactation number and DIM and were randomly but unequally assigned to one of three treatment groups. Cows (n = 50) in the first group (Ovsynch) received a hormonal protocol for synchronization of ovulation initiated at a random stage of the estrous cycle as described previously (Fricke et al., 1998) using i.m. injections of GnRH (Cystorelin; Merial, Ltd., Iselin, NJ) and $PGF_{2\alpha}$ (Lutalyse; The Pharmacia-Upjohn Co., Kalamazoo, MI) as follows: d –10, 50 μg of GnRH; d –3, 25 mg of $PGF_{2\alpha}$; d –1, 50 μg of GnRH. Cows (n = 52) in the second group ($PGF_{2\alpha}$ + Ovsynch) received a modified Ovsynch protocol but with the addition of 25 mg of $PGF_{2\alpha}$ 12 d (d –22) before initiation of the first GnRH injection of Ovsynch. Cows (n = 40) in the third group (control) received standard reproductive management in place on each farm, which consisted of pasture mating using natural service bulls (farm 1; cow:bull ratio = 25:1) or AI after detection of estrus aided by the use of pressure-activated heat mount detectors (Kamar, Inc., Steamboat Springs, CO; farm 2).

Cows allotted to the study were randomly assigned to treatment groups until 46 cows were assigned to each group; the remaining cows were then randomly assigned to the Ovsynch and $PGF_{2\alpha}$ + Ovsynch groups. More cows were assigned to the Ovsynch and $PGF_{2\alpha}$ + Ovsynch groups because this comparison constituted the primary hypothesis of this study. Inclusion of a control group with fewer cows was necessary for testing the secondary hypothesis of this study.

For both farms, all cows in the Ovsynch and $PGF_{2\alpha}$ + Ovsynch groups received TAI 12 to 18 h after the second GnRH injection at the onset of the AI breeding season (d 0; mean ± SEM DIM at TAI = 66 ± 14). Each herd manager was blind to treatment and chose AI service sires for each mating as part of the farm's standard program of reproduction and genetics. Thus, we assumed that any sire effects were distributed randomly among treatment groups. For each farm, AI was conducted by one to three experienced herd personnel who also were blind to treatment. For farm 1, natural service bulls were introduced on d 1 of the breeding season approximately 24 h after TAI. For farm 2, AI during the breeding season was conducted based on visual detection of estrus aided by the use of pressure-activated heat mount detectors (Kamar, Inc., Steamboat Springs, CO).

To assess nutritional energy balance and weight loss during the prebreeding period, BCS was assessed by the same individual on d –30 and 0, and a BCS was assigned to each cow based on a scale from 1 to 5, where 1 = thin and 5 = obese (Wildman et al., 1982).

Progesterone ELISA

Blood samples were collected from all cows via coccygeal venipuncture on d –22 (i.e., day of the first $PGF_{2\alpha}$ injection for the $PGF_{2\alpha}$ + Ovsynch group), d –21, and d –10 (i.e., day of the first GnRH injection for cows in the Ovsynch and $PGF_{2\alpha}$ + Ovsynch treatment groups). Blood samples were allowed to clot for 24 h at 4°C, centrifuged (3000 × *g* for 20 min), and serum was harvested and stored at –20°C until assayed for progesterone concentrations by ELISA (Rasmussen et al., 1996). Interassay and intraassay coefficients of variation were 5.5 and 4.3%, respectively.

Assessment of Reproductive Cyclicity

Serum samples were classified based on serum progesterone (**P_4**) concentrations as either low (≤1 ng/ml) or high (>1 ng/ml), and cows were grouped into P_4 classes based on blood samples collected on d –22 and –10 (e.g., 12 d apart). This system allowed cows to be classified based on reproductive status (anovular vs. cycling) immediately before initiation of the first GnRH injection of the hormonal synchronization protocols. Cows with two consecutive low serum P_4 samples (LL; n = 42); were classified as anovular, whereas cows with two consecutive High serum P_4 samples (HH; n = 24) or one High and one Low serum P_4 sample (HL or LH; n = 76) were classified as cycling.

Assessment of Luteal Function

For cows in the Ovsynch and $PGF_{2\alpha}$ + Ovsynch treatment groups, luteal function and response to $PGF_{2\alpha}$ administration on d –22 ($PGF_{2\alpha}$ + Ovsynch) were assessed based on blood samples collected on d –22 and –21. Serum samples were classified based on P_4 concentrations as either low (≤1 ng/ml) or high (>1 ng/ml). Luteolysis was defined as having occurred when serum P_4 classification changed from high P_4 on d –22 to low P_4 on d –21. Similarly, luteal function was assessed for cows in the Ovsynch and $PGF_{2\alpha}$ + Ovsynch treatment groups at initiation of the first GnRH injection of each synchronization protocol on d –10. Cows with high serum P_4 concentrations were defined as having luteal function (i.e., a functional CL), whereas cows with low serum P_4 concentrations were defined as having no luteal function.

Synchronization Rate

Synchronization rate (i.e., ovulatory response to the second GnRH injection) for cows in the Ovsynch and $PGF_{2\alpha}$ + Ovsynch treatment groups was evaluated by using transrectal ultrasound as described previously (Fricke et al., 1998). Briefly, ovarian structures (antral follicles >8 mm in diameter and CL) were monitored with an ultrasound machine equipped with a transrectal 7.5 MHz linear-array transducer (Aloka 500V; Corometrics Medical Systems, Inc., Wallingford, CT). Synchronization rate was determined by the presence of a large antral follicle (≥ 10 mm in diameter) at the time of the second GnRH injection and absence of that follicle at an ultrasound examination conducted 48 h later. Synchronization rate was calculated as the number of cows that ovulated at least one follicle within 48 h of the second GnRH injection expressed as a percentage of the total number of cows receiving the hormonal synchronization protocol.

Conception and Cumulative Pregnancy Rates

Pregnancy status was determined with the ultrasound machine and transducer described for determining synchronization rate. Visualization of a fluid-filled uterine horn and the presence of a conceptus were used as positive indicators of pregnancy as early as 25 d after breeding(Fricke et al., 1998). Pregnancy status was assessed for all cows on d 32 (~7 d of breeding) and again on d 60 (~35 d of breeding) after start of breeding. For cows in the Ovsynch and $PGF_{2\alpha}$ + Ovsynch treatment groups, conception rate at 32 d post-TAI was calculated in two ways as described previously (Fricke et al., 1998). The number of cows diagnosed pregnant expressed as a percentage of cows receiving the synchronization protocol was defined as the overall conception rate. The number of cows diagnosed pregnant expressed as a percentage of cows that ovulated at least one follicle within 48 h of the second GnRH injection of the Ovsynch protocol was defined as the synchronized conception rate. Cumulative pregnancy rate was calculated based on results from pregnancy diagnosis conducted for both 7 and 35 d of the breeding period and were defined as the total number of pregnant cows expressed as a percentage of the total number of cows within each treatment group.

Because breeding after d 1 of the breeding period was managed with natural service bulls on farm 1 as part of their standard reproductive management, and because cows were first assessed for pregnancy status on d 32 of the breeding period, cows in the Ovsynch and $PGF_{2\alpha}$ + Ovsynch treatment groups diagnosed pregnant on d 32 after TAI and in which ovulation was synchronized were classified as unequivocally pregnant to TAI rather than to natural service bulls. Because some of the cows receiving TAI that failed to synchronize an ovulation (n = 18) could have returned to estrus and been breed by natural service within the first 7 d of the breeding season, cumulative pregnancy rate at d 32 may slightly overestimate true conception rate to TAI. Despite this possibility, ultrasound operators subjectively confirmed that the amount of fluid and fetal tissue mass was representative of a 32 d rather than an earlier pregnancy.

Statistical Analyses

Preliminary data analyses found no effects of farm on synchronization, conception, or pregnancy rates when tested by chi-square analysis using the Cochran-Mantel-Haenszel statistic of SAS (1989); therefore, data from both farms were combined for all subsequent analyses. Differences between the two hormonal synchronization systems (Ovsynch and $PGF_{2\alpha}$ + Ovsynch) on categorical variables (synchronization rate, synchronized conception rate, cumulative pregnancy rate, luteal function, anovulation, and luteolysis) were tested by chi-square analysis using the Cochran-Mantel-Haenszel statistic of SAS (1989). Because conception rate at d 32 and cumulative pregnancy rate at d 60 did not differ between the two hormonal synchronization systems, data for cows receiving TAI were combined. Differences between cows receiving TAI versus standard reproductive management (TAI vs. control) on categorical variables (cumulative pregnancy rate at d 32 and 60) were tested by chi-square analysis using the Cochran-Mantel-Haenszel statistic of SAS (1989). Serum progesterone concentrations were analyzed with a repeated measures design using the PROC MIXED procedure of SAS (1989). Fixed effects in the model included treatment, day, and treatment × day interaction. Animal within treatment was considered a random effect.

RESULTS

Body Condition Score

Mean body condition score at d −30 and 0 and BCS loss from d −30 to 0 did not differ among treatments. Mean BCS for all cows was 2.75 ± 0.03 at d −30 and decreased ($P < 0.01$) to 2.50 ± 0.03 at d 0. To assess the effect of BCS loss on conception rate at d 32, cows receiving TAI (n = 102) were divided into two groups based on loss of <0.25 or >0.25 score points. First-service conception rate was 40.8% (29/71) for cows with a BCS loss of <0.25, which did not differ from that of 41.9% (13/31) for cows with a BCS loss of >0.25.

Reproductive Status, Luteal Function, and Luteolysis

For cows receiving TAI, the overall incidence of anovulation was 29.4% (30/102) and did not differ between treatment groups (Table 1). The incidence of anovulation for control cows was 30% (12/40) and did not differ from that of cows receiving TAI. The proportion of all cows and the proportion of cycling cows exhibiting luteal function on d −22 did not differ between treatment groups (Table 1). Luteolysis occurred in 90.5% (19/21) of cows exhibiting luteal function on d −22 in the $PGF_{2\alpha}$ + Ovsynch treatment group, whereas none of the cows in the Ovsynch group underwent luteolysis on d −22 (Table 1). Although the proportion of all cows exhibiting luteal function on d −10 only tended to be greater ($P < 0.07$) for cows in the $PGF_{2\alpha}$ + Ovsynch group compared with cows in the Ovsynch group, the proportion of cycling cows exhibiting luteal function on d −10 was greater ($P < 0.05$) for cows in the $PGF_{2\alpha}$ + Ovsynch group compared with cows in the Ovsynch group (Table 1).

There was a treatment × day interaction ($P < 0.01$) in serum P_4 concentrations in blood samples collected

Table 1. Reproductive status, luteal function, and luteolysis for cows receiving Ovsynch or $PGF_{2\alpha}$ + Ovsynch.

	Treatment group			
	Ovsynch		$PGF_{2\alpha}$ + Ovsynch	
Item	%	(no./no.)	%	(no./no.)
Anovular cows[1]	28.0	(14/50)	30.8	(16/52)
$PGF_{2\alpha}$ on d –22				
Luteal function, all cows	38.0	(19/50)	40.4	(21/52)
Luteal function, cyclic cows	52.8	(19/36)	58.3	(21/36)
Luteolysis, cows with luteal function	0.0[a]	(0/19)	90.5[b]	(19/21)
GnRH on d –10				
Luteal function, all cows	40.0[c]	(20/50)	57.7[d]	(30/52)
Luteal function, cycling cows	55.6[a]	(20/36)	80.6[b]	(29/36)

[a,b]Within a row, percentages with different superscripts differ ($P < 0.05$).
[c,d]Within a row, percentages with different superscripts tended to differ ($P < 0.07$).
[1]Number of cows with plasma progesterone concentrations <1 ng/ml on d –22 and –10.

from cycling cows in the Ovsynch and $PGF_{2\alpha}$ + Ovsynch treatment groups from d –22, –21, and –10 (Figure 1). Serum P_4 decreased from 1.73 ng/ml on d –22 to 0.48 ng/ml on d –21 in response to $PGF_{2\alpha}$ for cows in the $PGF_{2\alpha}$ + Ovsynch group.

Synchronization, Conception, and Cumulative Pregnancy Rates for Cows Receiving TAI

Synchronization rates, synchronized conception rates, and cumulative pregnancy rates at TAI or after 35 d of breeding did not differ between cows in the Ovsynch and $PGF_{2\alpha}$ + Ovsynch treatment groups (Table 2). The overall synchronization rate and synchronized conception rate for all cows receiving TAI was 82.4 (84/102) and 50.0% (42/84), respectively. In addition, conception rate did not differ based on reproductive status (cycling vs. anovular) within or across treatment groups (Table 3).

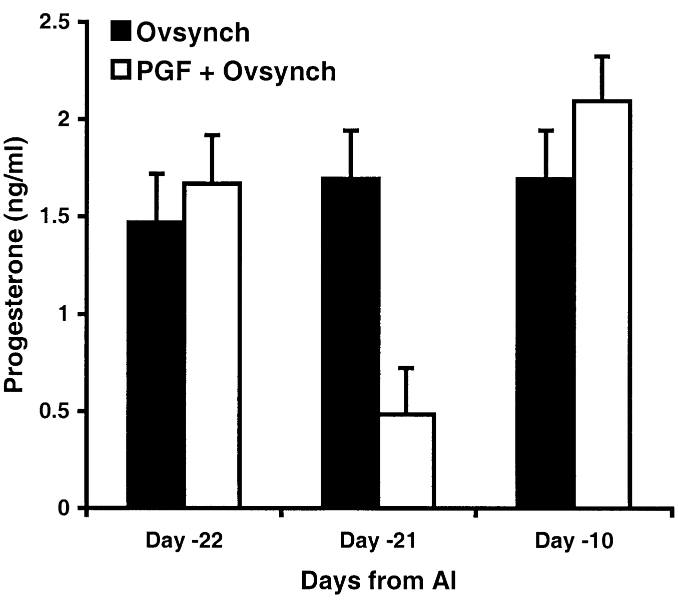

Figure 1. Serum progesterone concentrations (mean ± SEM) of cycling cows on d –22, –21, and –10 of the experiment (d 0 = breeding season onset). Cows in the $PGF_{2\alpha}$ + Ovsynch group received an i.m. injection of $PGF_{2\alpha}$ after blood sample collection on d –22. Serum P_4 decreased ($P < 0.01$) from 1.73 ng/ml on d –22 to 0.48 ng/ml on d –21 in response to $PGF_{2\alpha}$ for cows in the $PGF_{2\alpha}$ + Ovsynch group.

Standard Reproductive Management vs. TAI

Cumulative pregnancy rate after 7 d of breeding season was greater ($P < 0.01$) for cows receiving TAI compared with cows receiving standard reproductive management (Table 4). In contrast, cumulative pregnancy rate after 35 d of breeding did not differ for cows receiving TAI compared with cows receiving standard reproductive management (Table 4).

DISCUSSION

In the present study, administration of a luteolytic dose of $PGF_{2\alpha}$ 12 d before the first GnRH injection of the Ovsynch protocol in lactating dairy cows managed in grazing-based dairy systems did not improve synchronization rate or conception rate after TAI compared with the standard Ovsynch protocol. The rationale behind this "presynchronization" strategy was based on a previous study (Vasconcelos et al., 1999) in which day of the estrous cycle when Ovsynch was initiated affected synchronization and conception rate. 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 conception rates after TAI compared with cows initiating Ovsynch on d 5 to 9 of the estrous cycle (Vasconcelos et al., 1999). Furthermore, cows in the first half of the estrous cycle

Table 2. Effect of treatment on synchronization rate, synchronized conception rate, and cumulative pregnancy rate for cows receiving Ovsynch or $PGF_{2\alpha}$ + Ovsynch.

	Treatment group[1]			
	Ovsynch		$PGF_{2\alpha}$ + Ovsynch	
Item	%	(no./no.)	%	(no./no.)
Synchronization rate[2]	86.0	(43/50)	78.8	(41/52)
Synchronized conception rate[3]	51.2	(22/43)	48.8	(20/41)
Cumulative pregnancy rate[4]				
At Timed AI	44.0	(22/50)	38.5	(20/52)
Day 35 of breeding	60.0	(30/50)	50.0	(26/52)

[1]For each item, no statistical difference between treatment groups was detected using chi-square analysis.

[2]Number of cows that ovulated a follicle within 48 h of the second GnRH injection, expressed as a percentage of cows receiving the hormonal protocol.

[3]Number of cows diagnosed pregnant expressed as a percentage of cows ovulating a follicle within 48 h of the second GnRH injection of the hormonal protocol.

[4]Number of cows diagnosed pregnant to timed AI (ultrasonography 32 d after breeding) or after 35 d of breeding (ultrasonography at 60 d after breeding) as expressed as a percentage of cows within respective treatment groups.

(d 1 to 12) exhibited a greater synchronization rate in response to Ovsynch than cows in the second half of the estrous cycle (d 13 to 21; Vasconcelos et al., 1999). In a similar study conducted with dairy heifers, initiation of Ovsynch during the early to midluteal phase of the estrous cycle (d 5 to 10) resulted in optimal pregnancy rates compared with other stages of the cycle (Moriera et al., 2000a). Thus, strategies to presynchronize cows so that initiation of Ovsynch occurs during the early luteal phase of the estrous cycle may improve reproductive performance of cows receiving Ovsynch and TAI (Moriera et al., 2000a).

We hypothesized that administration of a luteolytic dose of $PGF_{2\alpha}$ at a random stage of the estrous cycle beginning 12 d before initiation of Ovsynch would induce luteal regression in those cows with a responsive CL, thereby increasing the proportion of cows that initiate Ovsynch on d 5 to 9 of the estrous cycle and decreasing the proportion of cows early in the estrous cycle (d 1 to 4) or during the later portion of the estrous cycle (d 10 to 21). In the present study, 40.4% of cows receiving $PGF_{2\alpha}$ 12 d before initiation of Ovsynch had a functional CL (as assessed by progesterone concentrations ≥1 ng/ml), and 90.5% of the cows exhibiting luteal function underwent luteolysis within 24 h after $PGF_{2\alpha}$ administration. Furthermore, although mean serum progesterone concentration did not differ between cows receiving $PGF_{2\alpha}$ + Ovsynch and Ovsynch at initiation of the first GnRH injection, the proportion of cycling cows exhibiting luteal function was greater for cows receiving $PGF_{2\alpha}$ + Ovsynch compared with cows receiving Ovsynch.

Based on these observations, administration of $PGF_{2\alpha}$ 12 d before the first GnRH injection of Ovsynch shifted some cows into the early luteal phase of the estrous cycle at the time of the first GnRH injection that would have been in the later stages of the estrous cycle had they not received $PGF_{2\alpha}$. Despite this shifting of cows to earlier stages of the estrous cycle at initiation of the first GnRH injection in the $PGF_{2\alpha}$ + Ovsynch group, reproductive performance did not improve compared with the standard Ovsynch protocol in the present study. The synchronization rates of 86 and 78.8% reported in this study are similar to the previously reported rates of 87 (Vasconcelos et al.,

Table 3. Conception rates after timed AI based on reproductive status.

	Reproductive status[1]			
	Cycling		Anovular	
Item	%	(no./no.)	%	(no./no.)
Ovsynch	47.2	(17/36)	35.7	(5/14)
$PGF_{2\alpha}$ + Ovsynch	44.4	(16/36)	25.0	(4/16)
Overall	45.8	(33/72)	30.0	(9/30)

[1]For each item, no statistical difference between reproductive status groups was detected using chi-square analysis.

Table 4. Cumulative pregnancy rate in untreated lactating dairy cows (Control) or lactating cows receiving TAI after synchronization of ovulation (Ovsynch and $PGF_{2\alpha}$ + Ovsynch).

	Treatment group			
	TAI		Control	
Item	%	(no./no.)	%	(no./no.)
Cumulative pregnancy rate[1]				
Day 7 of breeding	41.2[a]	(42/102)	20.0[b]	(8/40)
Day 35 of breeding	54.9	(56/102)	60.0	(24/40)

[a,b]Within a row, percentages with different or superscripts differ ($P < 0.01$).

[1]Number of cows diagnosed pregnant after timed AI or 7 d of breeding (ultrasonolgraphy 32 d after breeding) or after 35 d of breeding (ultrasonography at 60 d after breeding) as expressed as a percentage of cows within respective treatment groups.

1999) and 84% (Fricke et al., 1998) for lactating dairy cows managed in confinement-based dairies.

Similarly, the overall conception rates of 44.0 and 38.5% to TAI are similar to that previously reported using Ovsynch and timed AI in lactating dairy cows in confinement-based dairies (Pursley et al., 1997a, 1977b; Fricke et al., 1998). Although administration of a single injection of $PGF_{2\alpha}$ 12 d before initiation of Ovsynch has previously been reported to increase conception rate in lactating dairy cows (Cartmill et al., 2000), this approach may not have presynchronized enough cows to affect synchronization or conception rate in the present study. Indeed, a presynchronization strategy in which two injections of $PGF_{2\alpha}$ administered 14 d apart preceded initiation of Ovsynch by 12 d improved conception rate in lactating dairy cows in confinement-based dairies (Moriera et al., 2000b).

Overall, 28.0% of cows receiving Ovsynch and 30.7% of cows receiving $PGF_{2\alpha}$ + Ovsynch had low (<1 ng/ml) serum progesterone concentrations at two samples collected 12 d apart before the first GnRH injection of the synchronization protocols, suggesting a high incidence of anovulation in the present study. Although a previous study reported that only 3% of lactating dairy cows were anovular between 56 and 70 DIM (Stevenson et al., 1999), a recent study that included lactating dairy cows from six Midwest US states reported a 28.8% incidence of anovulation (Pursley et al., 2001). In the present study, numerical differences of 11.5 to 19.4 percentage units in conception rates for anovular versus cycling cows receiving either of the synchronization protocols were not significant. These results demonstrate the effectiveness of GnRH for inducing ovulation in anovular dairy cows similar to that reported previously (Archbald et al., 1990; Stevenson et al., 1999). In support of this observation, anovular lactating dairy cows in confinement-based dairies (Pursley et al., 2001) and postpartum beef cows (Geary et al., 1998) conceived to a TAI after Ovsynch. Further research is needed to evaluate strategies for inducing ovulation and establishing pregnancy in anovular dairy cows in grazing-based dairy systems.

To compare the effectiveness of synchronization of ovulation and TAI with standard reproductive management in these grazing-based dairies, data from cows receiving both TAI protocols were pooled and compared to control cows that were not synchronized. For cows receiving TAI, the cumulative pregnancy rate as diagnosed at the present study, cows receiving TAI had a greater cumulative pregnancy rate early in the breeding season compared with control cows. In contrast, cumulative pregnancy rates after 35 d of breeding did not differ between groups, although cumulative pregnancy rates were low for this stage of the breeding season.

High ambient temperatures after TAI and throughout the breeding period may have affected overall pregnancy rates in the present study. We retrospectively collected official temperature data (Midwestern Climate Center, Champaign, IL) reported at a research station located within 40 miles of both farms (Arlington Experimental Farm, Arlington, WI; Station ID: 470308). The average number of days when the temperature was ≥90°F was 1 for June 1999 and 9 for July 1999, and high maximum temperatures during the trial were 85, 90, 98, and 87°F for the months of May, June, July, and August 1999, respectively. Heat stress affects reproductive performance in females by affecting both oocyte quality during the periovulatory period and increasing early embryonic loss (Hansen et al., 1992). Heat stress also impairs bull fertility by decreasing sperm concentration, lowering sperm motility, and increasing the percentage of morphologically abnormal sperm in an ejaculate (Barth and Bowman, 1994). This effect of heat stress on bull fertility interacts with reduced conception rates of cows experiencing heat stress to further decrease herd fertility and may have affected reproductive performance, especially on farm 1, which used natural service bulls for breeding cows during this period of heat stress.

The rate at which cows become pregnant is determined by an interaction between the conception rate and the service rate. The greater pregnancy rate for cows receiving TAI compared with control cows likely occurred because 100% of cows in the TAI groups were serviced at the onset of the breeding season. Although some of the control cows may have received an artificial or natural service shortly after the onset of the breeding season, most would not have been far enough along in gestation to be diagnosed pregnant by ultrasonography at d 32. Conception rate was not directly determined for cows in the control group, and we cannot clearly assess the effect of conception rate on cumulative pregnancy rates in the present study. Ultrasonography at d 60 corresponds with measuring pregnancy results from approximately the first 35 d (d 60 – 25 d = 35 d) of the breeding season. Most synchronized cyclic cows would have had two opportunities to conceive, while control cows would have had but 1.7 cycle lengths in which to conceive. Therefore, the similar cumulative pregnancy rate (35 d of breeding) of control cows to cows receiving TAI would suggest that conception was greater for control cows bred at spontaneous estruses. However, synchronization of ovulation to initiate AI at the onset of the breeding season likely resulted in earlier establishment of pregnancy compared with standard reproductive management.

Finally, it is possible that true differences in conception and synchronization rates between treatment groups were not statistically detected in the present study. The possibility of type II errors (declaring no difference between groups when a difference does exist) must be considered whenever small numbers of experimental units are assigned to treatment groups. In the present study, the likelihood of detecting treatment effects was limited by the number of cows that were enrolled in the study and the small differences in synchronization and conception rates observed between treatment groups. Further studies with larger numbers of experimental units per treatment to adequately compare conception rates of lactating dairy cows in grazing-based dairies under various management strategies are warranted.

CONCLUSION

Administration of $PGF_{2\alpha}$ 12 d before initiation of Ovsynch did not improve synchronization, conception, or pregnancy rate compared with the standard Ovsynch protocol. However, synchronization of ovulation to initiate AI at the onset of the breeding season resulted in earlier establishment of pregnancy compared with standard reproductive management. Further research is needed to fully assess the efficacy of hormonal protocols for synchronization of ovulation and TAI for reproductive management of lactating dairy cows in grazing based dairy systems.

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