Reproductive Management - How to Conceive and Maintain a Pregnancy in High Genetic Merit/Liveweight Cows on Low Input Systems.

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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 two to three month 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 artificial insemination (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 (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. 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 for lactating dairy cows managed in grazing-based dairies in the United States. This paper is an overview of two field trials conducted on grazing-based dairies in Wisconsin in 1999 (Cordoba and Fricke, 2001) and 2000 (Cordoba and Fricke, 2002) to assess the efficacy of using hormonal protocols for fixed-time artificial insemination to improve reproductive efficiency in grazing-based dairy systems in Wisconsin.

Study 1 - Evaluation of Two Hormonal Protocols for Synchronization of Ovulation and Timed AI in Dairy Cows Managed in Grazing-Based Dairies (J. Dairy Sci. 84:2700-2708)

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 GnRH (Day –10); 25 mg $PGF_{2\alpha}$ (Day –3) and 50 µg GnRH (Day –1) followed by timed AI on Day 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 $PGF_{2\alpha}$ 12 d (Day –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 Day –22 in the PGF + Ovsynch treatment group, whereas none of the cows in the Ovsynch group underwent luteolysis on Day –22. Synchronization rate (i.e., ovulatory response at 48 h after the second GnRH injection), conception rate at Day 32 and pregnancy rate at Day 60 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 (Day –10) was similar for cows receiving Ovsynch (28.0%) and PGF + Ovsynch (30.7%), and conception rate at Day 32 was similar for cycling (45.8%) and anovular (30.0%) cows receiving TAI. Cumulative pregnancy rate was greater for cows receiving TAI compared to control cows at Day 32 (41.2% vs. 20.0%) but did not differ at Day 60 (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.

Introduction

Although Ovsynch can be initiated at a random stage of the estrous cycle, initiation of Ovsynch on Days 5 to 9 of the estrous cycle results in greater synchronization and conception rates compared to other stages (Vasconcelos et al., 1999). Presynchronization of cows to initiate Ovsynch on Days 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.

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 on synchronization and conception rate compared to 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 result in greater synchronization and conception rates compared to 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 to standard reproductive management.

Materials and Methods

This field trial was conducted from May 10 to July 19, 1999 on two semi-seasonal, spring-calving, grazing-based dairies located in South-central Wisconsin. Because these dairies were semi-seasonal, 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. 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 total-mixed ration 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.

Cows were blocked by lactation number and days in milk 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: Day –10, 50 µg GnRH; Day –3, 25 mg $PGF_{2\alpha}$; Day –1, 50 µg GnRH. Cows (n = 52) in the second group ($PGF_{2\alpha}$ + Ovsynch) received a modified Ovsynch protocol but with the addition of 25 mg $PGF_{2\alpha}$ 12 d (Day –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 (Day 0; mean ± SEM days-in-milk 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 Day 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).

Results

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 Day –22 did not differ between treatment groups (Table 1). Luteolysis occurred in 90.5% (19/21) of cows exhibiting luteal function on Day –22 in the $PGF_{2\alpha}$ + Ovsynch treatment group, whereas none of the cows in the Ovsynch group underwent luteolysis on Day –22 (Table 1). Although, the proportion of all cows exhibiting luteal function on Day –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 Day –10 was greater ($P < 0.05$) for cows in the $PGF_{2\alpha}$ + Ovsynch group compared with cows in the Ovsynch group (Table 1).

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)	30.7	(16/52)
$PGF_{2\alpha}$ on Day -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 Day -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)

[1]Number of cows with plasma progesterone concentrations <1ng/ml on days -22 and -10.
[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 ($P < 0.07$).

Synchronization rate, synchronized conception rate and cumulative pregnancy rate at Day 32 and Day 60 of the breeding season 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).

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]				
Day 32	44.0	(22/50)	38.5	(20/52)
Day 60	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 hours of the second GnRH injection of the hormonal protocol.

[4]Number of cows diagnosed pregnant at 32 and 60 d after the first day of the breeding season expressed as a percentage of cows within that treatment group.

Table 3. Conception rates after TAI 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.

Cumulative pregnancy rate at Day 32 of the breeding season was greater ($P < 0.01$) for cows receiving TAI compared with cows receiving standard reproductive management (Table 4). By contrast, cumulative pregnancy rate on Day 60 of the breeding season did not differ for cows receiving TAI compared with cows receiving standard reproductive management (Table 4).

Table 4. Cumulative pregnancy rate in untreated lactating dairy cows (Control) or lactating cows receiving TAI after synchronization of ovulation (Ovsynch and PGF2α + Ovsynch).

	Treatment group			
	TAI		Control	
Item	%	(no./no.)	%	(no./no.)
Cumulative Pregnancy Rate[1]				
Day 32	41.2[a]	(42/102)	20.0[b]	(8/40)
Day 60	54.9	(56/102)	60.0	(24/40)

[1]Number of cows diagnosed pregnant at 32 and 60 d after the first day of the breeding season expressed as a percentage of cows within that treatment group.

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

Conclusions

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. Synchronization and conception rates to Ovsynch in this study were similar to that reported for cows housed in confinement dairies (Fricke et al., 1998). 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.

Study 2 - Initiation of the Breeding Season in a Grazing-Based Dairy using Synchronization of Ovulation (J. Dairy Sci. 85:1752-1763)

Abstract

Lactating dairy cows (n = 228) in a semi-seasonal, grazing-based dairy were subjected to artificial insemination (AI) to start the 23-d breeding season (d 0 to 22) followed by natural service (d 23 to 120). Cows were randomly assigned to: 1) Ovsynch (GnRH, d –10; $PGF_{2\alpha}$, d -3; GnRH, d -1; timed AI, d 0) followed by AI at estrus (tail paint removal) on d 1 to 22 (Ovsynch; n = 114); or 2) AI at estrus (tail paint removal) throughout 23 d of AI breeding (Tail Paint; n = 114). Days to first AI service were greater and the 23-d AI service rate was less for Tail Paint vs. Ovsynch cows (12.0 ± 0.6 d vs. 0 d; and 84.2% vs. 100%, respectively). However, conception to first AI was greater for Tail Paint vs. Ovsynch cows (47.3% vs. 27.3%, respectively). Cows in the Tail Paint group received only one AI during 23 d of AI but 46.4% of Ovsynch cows received a second AI, with similar conception (43.1%) to that of Tail Paint cows at first AI (47.3%). Based on serum progesterone, incomplete luteal regression after $PGF_{2\alpha}$, and poor ovulatory responses to GnRH contributed to lower conception to timed AI in the Ovsynch group. Cumulative pregnancy rates for Tail Paint and Ovsynch cows did not differ after 23 d of AI breeding (47.3% vs. 46.3%, respectively) nor after 120 d of AI/natural service breeding (80.5% vs. 83.3%, respectively). Ovsynch failed to synchronize lactating cows in a grazing-based dairy resulting in reduced conception to timed AI compared with AI after tail paint removal.

Introduction

We previously assessed synchronization and conception rates for lactating dairy cows on two grazing-based dairies that received TAI after either Ovsynch or a modified Ovsynch protocol (Cordoba and Fricke, 2001). Observed synchronization rates (e.g., ovulation of a follicle within 48 h of the second GnRH injection) of 84% and 78% were similar to previously reported rates of 87% (Vasconcelos et al., 2001) and 84% (Fricke et al., 1998) for lactating dairy cows managed in confinement-based dairies. Similarly, the overall conception rates to TAI of 43.4% and 41.0% (assessed using ultrasonography at d 32 post TAI) were similar to that previously reported using Ovsynch and TAI in lactating dairy cows in confinement-based dairies (Pursley et al., 1997a, b; Fricke et al., 1998). However, because conception rate was not directly determined for control cows that were bred to a standing estrus in that study, we could not clearly assess the effect of conception rate on cumulative pregnancy rates. Furthermore, the similar cumulative pregnancy rate after 35 d of breeding of control cows and cows receiving TAI indicated that conception rate might have been greater for control cows bred at a spontaneous estrus (Cordoba and Fricke, 2001). Based on these results, we wanted to further assess use of Ovsynch to induce TAI on the first day of the AI breeding period in grazing-based dairies, especially with regard to conception rate after TAI versus AI to a spontaneous estrus.

We hypothesized that initiation of AI breeding on the first day of the breeding season using Ovsynch followed by AI after removal of tail paint would result in improved reproductive performance compared with AI after removal of tail paint alone. Our objective was to compare use of Ovsynch to initiate AI at the onset of the breeding season versus AI after removal of tail paint in a grazing-based dairy system in Wisconsin on interval to first service and on first service conception rate, and to characterize ovarian responses of cows receiving Ovsynch.

Materials and Methods

This field trial was conducted during the summer of 2000 in a grazing-based dairy located in south-central WI. This farm comprised approximately 1200 mature dairy cows, 228 of which were assigned to this study. This farm was managed so that a portion of the herd calved seasonally and the remainder of the herd calved throughout the year and, therefore, was classified as a semi-seasonal rather than a seasonal dairy. Cows within this herd were chosen for this study based on the farm managers desire to achieve a tight conception pattern for seasonal calving and because these cows were grazed and milked together throughout the trial as a single group. Cows assigned to this study were milked twice daily and were rotationally grazed on improved grass paddocks throughout the trial. Cows also received supplemental concentrate that was offered twice daily during each milking. Average milk production for cows assigned to this trial was 20 kg/d during the study period.

Primiparous (n = 24) and multiparous (n = 204) Holstein x Brown Swiss and Brown Swiss x Holstein crossbred dairy cows (n = 228) were subjected to a 23-d AI breeding period (d 0 to 22) beginning at the onset of the breeding season (d 0). Mean days in milk and lactation number at the onset of the breeding season for cows assigned to this study were 150.5 ± 5.6 (range = 50 to 290) and 3.3 ± 0.1 (range = 1 to 6), respectively. Ten days before the onset of the breeding season, cows were assigned randomly to each of two treatment groups. Cows were blocked by lactation number and days in milk as part of the randomization procedure to minimize confounding of these variables between treatments.

Cows (n = 114) 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; Pharmacia Animal Health, Kalamazoo, MI) as follows: d –10, 50 µg GnRH; d –3, 25 mg $PGF_{2\alpha}$; d –1, 50 µg GnRH. This is a modified Ovsynch protocol using a reduced dosage of GnRH (50 vs. 100 µg) per injection that was evaluated in lactating dairy cows in a confinement-based dairy (Fricke et al., 1998). First AI service for cows in the Ovsynch group was conducted as a TAI 12 to 18 h after the second GnRH injection immediately after the morning milking of the first day of the AI breeding period (d 0; May 19, 2000). Cows (n = 114) in the second group (Tail Paint) received AI during the AI breeding period based on removed tail paint. In addition, cows in the Ovsynch group that were detected in estrus after the initial TAI, received a second AI service after removed tail paint throughout the AI breeding period.

To monitor estrous activity, all cows received tail paint (Detail Estrus-Detection Tail Paint, New AgriTech, Inc, Little York, NY) after the morning milking at the onset of the AI breeding period (d 0). Briefly, tail paint was applied in a strip 5 cm wide by 20 cm long over the coccygeal vertebrae of the tail head following the manufacturer's instructions. Each cow was evaluated for evidence of tail paint removal at the afternoon milking. Eleven days after the initial tail painting, existing paint was touched up to ensure accurate detection of estrus throughout the remainder of the AI breeding period. Cows in which tail paint was removed were considered in estrus and received AI and a fresh application of tail paint after within 1 h after completion of the afternoon milking. Natural service sires were introduced to this group of cows at the end of the AI breeding period (June 10, 2000) and remained with the herd for 14 weeks (September 16, 2000). A minimum of six and a maximum of eight natural service sires grazed with this group of cows at any given time during the natural service breeding period. Natural service matings for individual cows and bulls were not recorded during the natural service breeding period.

Semen from multiple AI sires was used and assigned to cows by the herdsman before the start of the AI breeding period. At the time of mating assignment, the herdsman was unaware of the treatment assigned to each cow so that AI sires were randomly distributed between treatments. One professional AI technician from a local AI stud with over 15 yr experience conducted all TAI and AI services throughout the trial. For Ovsynch cows, TAI was conducted immediately after milking on the morning of d 0 over a 3-h period. All cows were caught in a palpation rail in groups of 12 immediately after exiting the parlor. Two people in addition to the herdsman assisted the AI technician by determining the correct mating for each cow, locating and thawing straws of semen, and loading and retrieving AI guns. Artificial insemination based on tail paint removal began at the afternoon milking of d 0. At each afternoon milking throughout the AI breeding period, cows from both treatment groups in which tail paint was removed were separated from the rest of the herd and placed in a holding pen with ad libitum access to fresh water. The professional AI technician arrived at the farm within 1 h after the afternoon milking and inseminated cows in which tail paint was removed during the previous 24-h period.

Results and Discussion

In cattle, body condition score is a subjective estimate of the amount of subcutaneous fat (Edmonson et al., 1989), and cows with a low BCS at calving exhibit poor reproductive performance (Markusfeld et al., 1997). In the present study, mean BCS for all cows was 2.89 ± 0.03 at d –20 and decreased ($P < 0.01$) to 2.63 ± 0.03 at d 0. Although cows experiencing a BCS loss of more than 1 point after parturition experience reduced reproductive performance (Butler and Smith, 1989; Ruegg and Milton, 1995), BCS loss for all cows from d –20 to d 0 in the present study was moderate at 0.28 ± 0.02. Anovular cows in the present study experienced greater ($P < 0.05$) BCS loss from d –20 to 0 and had 37 fewer ($P < 0.05$) days in milk at the onset of the AI breeding period compared with cycling cows (Table 1). In a previous study, anestrous cows lost more body weight resulting in a more negative energy status than cycling cows, and differences in energy balance among cows was greatest during the first 3 wk postpartum (Staples et al., 1990). Thus, anovular cows in the present study had a shorter period from calving to the onset of the AI breeding period in which to undergo first ovulation and resume postpartum cyclicity and were likely in a more negative energy status than herdmates with a greater postpartum interval between calving and the onset of the AI breeding period. Although conception rate after Ovsynch is greater for cows with a BCS ≥ 2.5 (scale 1 to 5) than for cows with a BCS < 2.5 (Moreira et al., 2000b), no effect of BCS on conception rate to TAI was observed in the present study, possibly due to the relatively good BCS of the cows in this study, the modest BCS losses among cows, and the low incidence of anovular cows in the Ovsynch group.

Table 1. Body condition score (BCS) loss and days in milk at the onset of the AI breeding period for anovular and cycling cows in the Ovsynch and Tail Paint treatment groups.

			Treatment group[1]			
			Ovsynch		Tail Paint	
Reproductive status[2]	BCS loss[3]	Days in milk[4]	%	(no./no.)	%	(no./no.)
Anovular	0.44 ± 0.06[a]	116.2 ± 14.8[a]	13.1	(13/99)	17.2	(17/99)
Cycling	0.28 ± 0.02[b]	152.8 ± 6.0[b]	86.9	(86/99)	82.8	(82/99)

[a,b]Within a column, means with different superscripts differ ($P < 0.05$).

[1]No treatment difference was detected.

[2]Serum samples collected on d –20 and –10 were classified based on progesterone concentrations as either low (≤1.0 ng/ml) or high (>1.0 ng/ml). Cows with two consecutive low samples were classified as anovular; cows with two consecutive high samples or one high and one low sample were classified as cycling.

[3]Body condition score loss (mean ± SEM) was calculated based on BCS assessed on a five-point scale (1=emaciated, 5=obese) on d –20 and 0 of the experiment.

[4]Average days in milk (mean ± SEM) at the onset of the AI breeding period (d 0).

Submission rate to first AI service during the AI breeding period was greater (P < 0.01) for cows receiving Ovsynch compared with cows receiving tail paint (Table 2). By design, mean (± SEM) d of the AI breeding period at first AI service for Ovsynch cows was 0.0 ± 0.0 d compared with 12.0 ± 0.6 d for Tail Paint cows. Conception rate to first AI service for Tail Paint cows receiving AI after removed tail paint was greater (P < 0.01) than that of Ovsynch cows receiving TAI (Table 2). Poor conception rates to Ovsynch were reported in a study conducted on seasonally calving lactating dairy cows in southern Australia in which cows received either Ovsynch or AI to a standing estrus induced using $PGF_{2\alpha}$. Pooled conception rate for cows receiving Ovsynch was 38.1% compared with 65.9% for cows receiving AI to an induced estrus (Jemmeson, 2000). These results are contrary to initial reports in which conception rates of lactating dairy cows managed in confinement-based dairies receiving Ovsynch were similar to that of cows receiving AI after a standing estrus (Pursley et al., 1997a,b). However, other studies have reported that Ovsynch results in lower conception rates compared with AI after estrus (Jobst et al., 2000; Stevenson et al., 1999). Factors explaining the variation in conception rate to TAI among herds are unknown at this time but may include the proportion of anovular cows in the herd, the follicular dynamics of individual cows within the herds, or the ability of farm personnel to implement Ovsynch in their herds.

No Tail Paint cows received a second AI service during the AI breeding period, whereas 46.4% of Ovsynch cows returned to service during the AI breeding period (Table 2). Conception rate to second AI service after removed tail paint for Ovsynch cows was 43.1%, which did not differ from that of Tail Paint cows at first AI service after removed tail paint (Table 2). Of the 80 Ovsynch cows diagnosed nonpregnant to TAI, 51 returned to service during the AI breeding period resulting in a 63.8% service rate to second AI service. The disparity in the AI submission rate of 84.2% to first AI service for cows in the Tail Paint group and the 63.8% return service rate among non pregnant cows to second AI service in the Ovsynch group may have occurred because the Ovsynch cows were returning to service after TAI, whereas the Tail Paint cows had not previously received AI. Lactating dairy cows exhibit high rates of embryonic loss after AI

(Fricke et al., 1998; Smith and Stevenson, 1995; Vasconcelos et al., 1997). Although speculative, it is possible that some of the Ovsynch cows conceived to TAI and subsequently lost those pregnancies near or after the end of the AI breeding period, thereby reducing the number of cows returning to service during the AI breeding period. Also, because 30 pregnant cows are eliminated from among potential Ovsynch cows returning to estrus, the remaining cows may include a biased proportion of cows that are less sexually active.

Table 2. Effect of treatment on reproductive performance of lactating dairy cows during the AI breeding period.

	Treatment group[1]	
Item	Ovsynch	Tail Paint
First AI service		
Method of AI	Timed AI	AI at estrus
AI submission rate (%)	100.0[a]	84.2
(no./no.)	(114/114)	(96/114)
Mean (± SEM) Day of AI breeding period	0.0 ± 0.0[a]	12.0 ± 0.6
Conception rate (%)	27.3[a]	47.3
(no./no.)	(30/110)	(43/91)
Second AI service		
Method of AI	AI at estrus	AI at estrus
AI submission rate (%)	46.4[a]	0.0
(no./no.)	(51/110)	(0/114)
Mean (± SEM) Day of AI breeding period	17.0 ± 5.8	-
Conception rate (%)	43.1[b]	-
(no./no.)	(22/51)	-

[1]Ovsynch cows were managed using synchronization of ovulation (50 µg GnRH, d –10; 25 mg $PGF_{2\alpha}$, d –3; 50 µg GnRH, d –1) and fixed-time AI (d 0) followed by estrous detection and AI after removed tail paint for the remainder of the AI breeding period; Tail Paint cows were managed using estrous detection and AI after removed tail paint for the duration of the AI breeding period.

[a]Different ($P < 0.01$) from Tail Paint.

[b]Conception rate for Ovsynch cows receiving AI after removed tail paint (second AI service) did not differ from that of Tail Paint cows receiving AI after removed tail paint (first AI service).

Successful synchronization to the Ovsynch protocol involves synchronizing growth of a new follicular wave at the onset of the protocol (controlled by the first GnRH injection on d –10), synchronization of luteal regression (induced by $PGF_{2\alpha}$ on d –3), and synchronization of ovulation (induced by GnRH on d –1). The stage of the estrous cycle when Ovsynch is initiated also affects synchronization and conception rate to the Ovsynch protocol in both lactating dairy cows (Vasconcelos et al., 2001) and dairy heifers (Moreira et al., 2000a). Cows receiving Ovsynch in the present study were assigned to progesterone (P_4) classes and were separated and summed as two groups: those P_4 classes with high P_4 at d –1 (HHH, LHH, HLH, and LLH) and those with low P_4 at d –1 (LLL, HLL, LHL, and HHL). Only 91.8% (101/110) of the Ovsynch cows with a known pregnancy status at d 35 were assigned to P_4 classes because one or more serum samples from 9 cows receiving Ovsynch were missing on d –10, –3, and/or –1 (Table 3).

The P_4 classes were used to estimate the stage of the cycle at initiation of Ovsynch, response to the first GnRH injection, and luteolysis in response to $PGF_{2\alpha}$ for cows receiving the Ovsynch protocol similar to the method described by Moreira et al., 2001 but with some modifications. Unfortunately, these estimates are speculative because multiple ultrasound scans and estrus expression before initiation of treatments were not evaluated in the present study. In addition, the day of the AI breeding period at return AI service for Ovsynch cows returning to service was used to further analyze the response to Ovsynch. Of the 51 Ovsynch cows returning to estrus during the AI breeding period (Table 2), one cow returning to estrus on d 15 and one cow returning to estrus on d 18 were not assigned to a P_4 class due to missing serum samples (Table 3). The 49 cows returning to estrus that were assigned to a P_4 class were grouped into one of three return periods (d 0 to 13, 14 to 17, or 18 to 22; Table 3) based on the two peak periods of return service activity exhibited by Ovsynch cows from d 14 to 17 and d 18 to 22 and those Ovsynch cows receiving AI service before d 14.

Table 3. Frequency distribution, anovular cows, conception rate to TAI, and cows returning to AI service during the AI breeding period for cows receiving Ovsynch based on serum P_4 concentrations collected on d –10, –3, and –1 of the experiment (d 0 = TAI).

P_4-class[1]	n	Anovular cows[2] % (no.)	Conception rate[3] % (no.)	Cows returning to service[4] % (no.)	Day of AI breeding period at AI (no.)		
					0-13	14-17	18-22
High P_4 at d –1							
HHH	4	0.0 (0)	0.0 (0)	100.0 (4)	0	2	2
LHH	5	0.0 (0)	0.0 (0)	60.0 (3)	1	2	0
HLH	3	0.0 (0)	0.0 (0)	100.0 (3)	0	3	0
LLH	0	0.0 (0)	0.0 (0)	0.0 (0)	0	0	0
Σ	12	0.0 (0)	0.0 (0)	83.3 (10)	1	7	2
Low P_4 at d –1							
LLL	5	80.0 (4)	0.0 (0)	40.0 (2)	1	1	0
HLL	28	0.0 (0)	14.3[a] (4)	83.3 (20)	3	6	11
LHL	33	24.2 (8)	39.4[b] (13)	45.0 (9)	2	0	7
HHL	23	0.0 (0)	43.5[b] (10)	53.8 (7)	3	0	4
Σ	89	13.5 (12)	30.3 (27)	61.3 (38)	9	7	22
Overall	101	11.9 (12)	26.7 (27)	64.9 (48)	10	14	24

[a,b]Within a column, different superscripts denote significant contrasts ($P < 0.05$). Due to small cell sizes, cows classified as HHH, LHH, HLH, LLH, and LLL were excluded from the statistical analysis for conception rate to TAI.

[1]Combinations of highs (H; > 1 ng/ml) and lows (L; ≤ 1 ng/ml) represent serum progesterone (P_4) concentrations at d –10 (first GnRH injection), –3 ($PGF_{2\alpha}$ injection), and –1 (second GnRH injection) of Ovsynch.

[2]Serum samples collected on d –20 and –10 were classified based on P_4 concentration as either low (≤1.0 ng/ml) or high (>1.0 ng/ml). Cows with two consecutive low samples were

classified as anovular; cows with two consecutive high samples or one high and one low sample were classified as cyclic.

[3]Proportion of cows diagnosed pregnant to TAI (ultrasonography at 35 d post TAI).
[4]Cows returning to service during the AI breeding period (percentage of non-pregnant cows).

Ovsynch cows with High P_4 at d –1 failed to synchronize luteal function in response to the Ovsynch protocol and, therefore, failed to conceive to the Ovsynch protocol. Of the 101 Ovsynch cows assigned to a P4 class, 12.0% had high P_4 at d –1 with 4 HHH cows, 5 LHH cows, 3 HLH cows, and 0 LLH cows. None of these cows were anovular, none were diagnosed pregnant at d 35, and 83.3% returned to service during the AI breeding period (Table 3). Of the 10 cows returning to service, most (n = 7) returned on d 14 to 17, consistent with failed luteal regression; one cow returned on d 6 and the other two cows returned on d 18 and 22 after TAI. Ovsynch cows with low P_4 at d –1 had no functional CL at the second GnRH injection, and these P_4 classes comprised all anovular cows, and those cows that were successfully synchronized by the Ovsynch protocol. Of the 101 Ovsynch cows assigned to a P_4 class, 88.1% had low P_4 at d –1, 13.5% of these cows were anovular, 30.3% of these cows were diagnosed pregnant at d 35, and 43.8% returned to service during the AI breeding period (Table 3). Of the 39 cows returning to service, 10 cows returned on d 0 to 13, 7 cows returned on d 14 to 17, and 22 cows returned on d 18 to 22. Cows with low P4 at d –1 comprised 5 LLL cows, 28 HLL cows, 33 LHL cows, and 23 HHL cows.

Cumulative pregnancy rate for Ovsynch and Tail Paint cows was similar after 23 d of AI breeding (ultrasonography at d 49) and after 120 d of AI/natural service breeding (rectal palpation at d 179; Table 4). Two cows in the Ovsynch group that were diagnosed pregnant at the ultrasound examination on d 35 (e.g., to TAI) were diagnosed nonpregnant at the ultrasound examination on d 49 (e.g., after 23 d of AI breeding). In contrast to Ovsynch, the tail paint system used in the present study resulted in excellent reproductive performance. Submission rate to first AI service during the AI breeding period was 85.7%, with a first AI service conception rate of 47.3%. In a previous study in New Zealand, 94.5% of synchronized dairy heifers were detected in estrus using a tail paint system (Macmillan et al., 1988). Tail paint resulted in similar cumulative pregnancy rates after 24 and 120 d of breeding compared with use of Ovsynch to initiate TAI at the onset of the AI breeding period. Results from the present study indicate that Ovsynch is not an effective reproductive management tool for lactating dairy cows managed in grazing-based dairies due to the poor conception rate to TAI. Further research is needed to develop protocols that effectively synchronize ovulation in lactating dairy cows that respond poorly to Ovsynch.

Finally, although cumulative pregnancy rate after 120 d of breeding did not differ between treatment groups, the cumulative pregnancy rate of 82% across both treatment groups was low for this stage of the breeding season. By comparison, cumulative pregnancy rate after only 56 d of breeding was nearly 82% for dairy cows in Australia (Jemmeson, 2000). Introduction of natural service sires after the 23-d AI breeding period in the present study increased the number of pregnancies in this group of cows by only 35%. Although factors responsible for the poor reproductive performance observed in the present study are not known, heat stress can affect reproductive performance in cows by affecting both oocyte quality during the periovulatory period and increasing early embryonic loss (Hansen et al., 1992). Heat stress also impairs fertility of natural service sires by decreasing sperm concentration, lowering sperm motility, and increasing the percentage of morphologically abnormal sperm in an ejaculate (Barth and Bowman, 1994). We have previously implicated heat stress as a factor for poor reproductive performance in grazing-based dairies during natural service breeding in a previous field trial in Wisconsin during the summer breeding period (Cordoba and Fricke, 2001). Official temperature data (Midwestern Climate Center, Champaign, IL) was collected from a research station located within 10 miles of this farm (Dodgeville, WI; Station ID: 472173). Although the mean maximum daily temperature was relatively cool during the AI breeding period (69.4 °F), reported high maximum temperatures during the trial were 82, 86, 84, 88, and 92 °F for the months of May, June, July, August, and September 2000, respectively.

Table 4. Effect of treatment on cumulative pregnancy rate after 23 d of AI breeding and after 120 d of AI/natural service breeding.

	Treatment group[1]			
	Ovsynch		Tail Paint	
Cumulative pregnancy rate[2]	%	(no./no.)	%	(no./no.)
After 23 d of AI breeding	46.3	(50/108)	47.3	(43/91)
After 120 d of AI/natural service breeding	83.3	(80/96)	80.5	(66/82)

[1]No treatment differences were detected.

[2]Number of cows diagnosed pregnant after 23 d of AI breeding (ultrasonography 49 d after the first day of the AI breeding period) or after 120 d of breeding (rectal palpation 179 d after the first day of the AI breeding period) expressed as a percentage of cows within that treatment group.

Conclusions

Conception rate to TAI of lactating dairy cows in this grazing-based dairy receiving Ovsynch at first service was lower than that of cows receiving AI after removed tail paint at first service. Progesterone profiles of Ovsynch cows indicated that incomplete luteal regression after $PGF_{2\alpha}$ and poor ovulatory responses to GnRH related to the stage of the cycle at initiation of Ovsynch contributed to the poor reproductive performance of cows in this grazing-based dairy receiving Ovsynch. Furthermore, despite the improved AI submission rate during the AI breeding period, Ovsynch did not improve cumulative pregnancy rate after 23 d of AI breeding or after 120 d of AI/natural service breeding compared with use of tail paint to initiate AI breeding. Results from the present study indicate that Ovsynch is not an effective reproductive management tool for lactating dairy cows managed in grazing-based dairies. However, variation in the response to Ovsynch may occur across herds under various management systems and physiologic scenarios. Further research is needed to understand the underlying causes for variation among herds in responsiveness to Ovsynch and to develop protocols that effectively synchronize ovarian function in lactating dairy cows that respond poorly to Ovsynch.

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