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Fertility of Dairy Cows after Resynchronization of Ovulation at Three Intervals Following First Timed Insemination

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ABSTRACT

Lactating Holstein cows (n = 711) on a commercial dairy farm in Wisconsin received a hormonal synchronization protocol to initiate first timed artificial insemination (TAI) on the following postpartum schedule: two injections of 25 mg $PGF_{2\alpha}$ at 32 ± 3 d and 46 ± 3 d (Presynch); 100 μg GnRH at 60 ± 3 d; 25 mg $PGF_{2\alpha}$ at 67 ± 3 d; and 100 μg GnRH + TAI at 69 ± 3 d (Ovsynch). At first TAI, cows were randomly assigned to initiate the first GnRH injection of a hormonal protocol for resynchronization of ovulation (Resynch; 100 μg GnRH, d 0, 25 mg $PGF_{2\alpha}$, d 7, 100 μg GnRH + TAI, d 9) at 19 (D19), 26 (D26), or 33 d (D33) after first TAI to set up a second TAI service for cows failing to conceive to Ovsynch. Overall pregnancy rate per artificial insemination (PR/AI) to Ovsynch assessed 68 d after TAI was 31% and did not differ among treatment groups. For Resynch, PR/AI was assessed 26 d after TAI for D19 and D26 cows and 33 d after TAI for D33 cows. Overall PR/AI to Resynch was 32%. However, the PR/AI for D26 (34%) and D33 (38%) cows to Resynch was greater than for D19 cows (23%). Cows with a CL at the $PGF_{2\alpha}$ injection (D19 cows) or at the first GnRH injection (D26 + D33 cows) of Resynch exhibited greater PR/AI to Resynch compared with cows without a CL. Survival analysis (failure time) of cows in the D26 and D33 treatment groups across the first three TAI services did not differ statistically. Although administration of GnRH to pregnant cows 19 d after first TAI service did not appear to induce iatrogenic embryonic loss, initiation of Resynch 19 d after first TAI service resulted in a lower PR/AI compared with initiation of Resynch 26 or 33 d after first TAI service.
(**Key words:** synchronization, resynchronization, dairy cow, timed artificial insemination)

Abbreviation key: Cosynch = timed insemination coincides with last injection of GnRH in Ovsynch, **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, **Resynch** = synchronization regimen using GnRH and $PGF_{2\alpha}$ to resynchronize ovulation in cows after first insemination, **TAI** = timed AI.

INTRODUCTION

The development of hormonal synchronization protocols that allow for timed AI (**TAI**) have provided a management tool for initiating first-postpartum AI and thereby precisely controlling the voluntary waiting period in lactating dairy cows. A common hormonal protocol for synchronizing ovulation in lactating dairy cows uses injections of GnRH and $PGF_{2\alpha}$ (**Ovsynch**; Pursley et al., 1995; Burke et al., 1996; Pursley et al., 1997a, 1997b) and is an effective method for hormonally programming cows to receive TAI. A presynchronization strategy in which cows receive two injections of $PGF_{2\alpha}$ administered 14 d apart beginning 26 to 28 d before initiation of Ovsynch (**Presynch**) has been reported to increase conception rate to TAI in lactating dairy cows compared with Ovsynch alone (Moreira et al., 2001; El-Zarkouny et al., 2002; Navanukraw et al., 2002). Many dairy farms in Wisconsin have incorporated synchronization protocols as the primary strategy for submitting cows for first TAI service.

Although reliance on synchronization of ovulation and TAI for improving service rate to first AI reduces the impact of poor estrus detection efficiency, the high AI submission rate to first TAI often is followed by a time lag that can exceed 60 d before cows failing to conceive are reinseminated. Because AI conception rates of high producing lactating dairy cows are reported to be 40% or less (Pursley et al., 1997a, 1997b; Fricke et al., 1998; Jobst et al., 2000), 60% or more of the cows that receive TAI will fail to conceive and, therefore, require a resynchronization strategy for aggressively initiating subsequent AI services. Coupling a nonpregnancy diagnosis with a management decision to rapidly reinitiate AI service improves reproductive efficiency and pregnancy rate by decreasing the interval

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between AI services, thereby increasing AI service rate (Fricke, 2002).

Fricke (2002) proposed an aggressive resynchronization protocol in which groups of cows past the voluntary waiting period receive their first postpartum TAI after synchronization of ovulation. On d 18 after TAI, all cows would receive an injection of GnRH regardless of their pregnancy status. Nonpregnant cows would receive a $PGF_{2\alpha}$ injection on d 25 after TAI based on a nonpregnancy diagnosis using ultrasound and would continue resynchronization with Ovsynch, whereas pregnant cows would discontinue the resynchronization protocol. A field trial conducted in Florida in which cows received GnRH on d 20 after TAI followed by transrectal ultrasound and $PGF_{2\alpha}$ administration to nonpregnant cows on d 27 was reported by Moreira et al. (2000b). These authors reported an interaction for cows resynchronized with GnRH on d 20 after TAI in which embryonic loss from d 20 to 27 after TAI was increased for bST-treated pregnant cows receiving GnRH but not for non-bST-treated pregnant cows. Because of this observation, this resynchronization strategy was discontinued (Moreira et al., 2000b). Hormonal resynchronization systems that program nonpregnant cows to receive subsequent AI services need to be developed and further assessed so that systematic reproductive management programs can be implemented to aggressively manage reproduction in lactating dairy cows on commercial dairies (Fricke, 2002).

The objective of this study was to evaluate pregnancy rate per AI (**PR/AI**), and early embryonic loss after initiation of a resynchronization of ovulation protocol initiated at three intervals after first postpartum TAI on a commercial dairy farm in Wisconsin.

MATERIALS AND METHODS

Farm Description and Data Collection

Lactating Holstein dairy cows on a commercial dairy farm comprising approximately 1100 lactating cows located in north-central Wisconsin (Athens) were enrolled in this study beginning on May 10, 2001, and ending on May 30, 2002. Cows were housed in free-stall barns and were fed a TMR once daily with ad libitum access to feed and water. Cows were milked 3 times daily at approximately 8-h intervals, and average milk production per cow was 36.3 kg/d during the study period. Synchronization of ovulation for first TAI service and resynchronization of ovulation for second and greater TAI services was performed using i.m. injections of GnRH (100 μg of Cystorelin; Merial, Ltd., Duluth, GA) and $PGF_{2\alpha}$ (25 mg of Lutalyse; Pharmacia Animal Health, Kalamazoo, MI).

Cow lists for injection schedules, pregnancy examinations, and reproductive events for individual cows were generated, tracked, and recorded using a commercial on-farm computer software program (Dairy Comp 305, Valley Agricultural Software, Tulare, CA). Cows assigned to the study were coded by treatment, and the “cowfile” was archived and saved every 3 to 5 wk throughout the study to capture individual cow data throughout the study period. Data from cowfile archives were transferred into a computer spreadsheet program (Microsoft Excel 2002, Microsoft Corporation, Redmond, WA) for organization and manipulation of data before statistical analysis using SAS (SAS Inst. Inc., Cary, NC).

Submission of Cows for First Postpartum TAI Service: Presynch + Ovsynch

Lactating cows (n = 711) were allocated weekly to breeding groups, each of which included cows that had calved within a given calendar week (12.7 ± 0.7 cows/group; range = 3 to 23 cows/group). In this way, cows were managed in groups to receive hormone injections and first postpartum TAI on 2 preselected days of the week (Tuesdays and Thursdays). All cows received a hormonal synchronization protocol (Presynch + Ovsynch) to receive first postpartum TAI as follows: 25 mg of $PGF_{2\alpha}$ (d 32 ± 3 and 46 ± 3; Presynch), 100 μg GnRH (d 60 ± 3), 25 mg $PGF_{2\alpha}$ (d 67 ± 3), and 100 μg GnRH (d 69 ± 3) postpartum (Ovsynch). All cows received TAI immediately after administration of the second GnRH injection of the Ovsynch protocol (d 0). Thus, the average DIM at first postpartum TAI for all cows enrolled in this experiment was 69 ± 3 d.

Submission of Cows for Second Postpartum TAI Service: Resynch

At first TAI service, cows were randomly assigned to each of three treatment groups for resynchronization of ovulation [100 μg of GnRH (d -9); 25 mg of $PGF_{2\alpha}$ (d -2) and 100 μg of GnRH + TAI (d -0)] to induce a second TAI for cows failing to conceive to Ovsynch (i.e., Resynch). Within a breeding group, cows were blocked by parity (primiparous vs. multiparous) as part of the randomization procedure to minimize confounding of this variable among treatment groups. All cows (n = 235) in the first group (D19) received a GnRH injection (100 μg) 19 d after Ovsynch TAI, a $PGF_{2\alpha}$ injection (25 mg) at a nonpregnancy diagnosis using transrectal ultrasound 26 d after Ovsynch TAI, and a second GnRH injection (100 μg) and Resynch TAI 28 d after Ovsynch TAI. Cows (n = 240) in the second group (D26) received a GnRH injection (100 μg) at a nonpregnancy diagnosis using transrectal ultrasound 26 d after Ovsynch TAI, a $PGF_{2\alpha}$ injection (25 mg) 33 d after Ovsynch TAI, and a second GnRH injection (100 μg) and Resynch TAI 35 d after Ovsynch TAI. Cows (n = 236) in the third group (D33) received a GnRH injection (100 μg) at a nonpregnancy diagnosis using transrectal ultrasound 33 d after Ovsynch TAI, a $PGF_{2\alpha}$ injection (25 mg) 40 d after Ovsynch TAI, and a second GnRH injection (100 μg) and Resynch TAI 42 d after Ovsynch TAI.

Submission of Cows for Third and Greater Postpartum TAI Service

Throughout the study, cows that failed to conceive to a given TAI service were resynchronized until they became pregnant or were culled from the herd. Because of the potential detrimental effects of GnRH administered to pregnant cows receiving bST reported by Moreira et al. (2000b), cows in the D19 group that failed to conceive to second TAI service (e.g., Resynch) were resynchronized using the D26 Resynch schedule. Thus, D19 cows did not receive a GnRH injection 19 d after the first Resynch TAI, but received an injection of GnRH after a nonpregnancy diagnosis 26 d after the first Resynch TAI. For the third TAI service, cows in the D26 and D33 groups were resynchronized according to the resynchronization schedule for each treatment group. After third TAI service nonpregnant cows in all 3 treatment groups were resynchronized for subsequent TAI services similar to the D26 treatment group. As time progressed, treatment intervals after the first Resynch TAI service were unintentionally extended due to missed injections and failure to locate specific cows on injection days, or intentionally due to injury or illness.

Transrectal Ultrasonography

All ultrasound examinations and hormone injections were conducted by the herd veterinarian, who is also an ownership partner of the dairy, and who had managed reproduction in dairy cattle using transrectal ultrasonography for the previous 7 yr. Pregnancy examinations and hormone injections were conducted immediately after milking by restraining cows in a palpation rail located in the return alley exiting the milking parlor. Visualization of a fluid-filled uterine horn and the presence of a conceptus were used as positive indicators of pregnancy 26 d after TAI using an ultrasound machine equipped with a transrectal 7.5 MHz linear-array transducer (Aloka 500V; Corometrics Medical Systems, Inc., Wallingford, CT; Fricke et al., 1998). The number of cows diagnosed pregnant to TAI expressed as a percentage of cows within that treatment group receiving TAI was defined as the PR/AI. In addition, both ovaries of each cow were visualized at each pregnancy exam using ultrasound and the presence or absence of a CL was recorded. A cow was recorded as having a CL when the CL diameter was estimated to be ≥10 mm. This definition of a CL was adopted because it allowed for rapid and accurate evaluation of CL size using the 10-mm hash marks on the ultrasound screen without repeated freezing of the ultrasound image during weekly herd checks.

Pregnancy loss was assessed for cows that conceived to first TAI service during the data collection period. Cows diagnosed pregnant were scheduled for a pregnancy recheck using transrectal ultrasound on d 68 of gestation. A cow that was pregnant at the first pregnancy exam but in which the fetus was dead (assessed by lack of a fetal heartbeat) was considered pregnant and the loss was recorded as occurring on that day. Cows with questionable fetal viability were rechecked 1 wk later. Pregnancy loss for cows diagnosed pregnant to Ovsynch was calculated as the number of pregnant cows that lost a pregnancy by d 68 of gestation expressed as a percentage of cows diagnosed pregnant at 26 (D19 and D26 cows) or 33 d (D33 cows) after Ovsynch TAI.

Temperature Data

Official temperature data (Midwestern Climate Center, Champaign, IL) reported at a research station located within 20 km of the farm (Medford, WI; Station ID: 475255; latitude: 45° 07′, longitude: 90° 20′) were collected retrospectively. Temperature data were collected for days that TAI was performed for each breeding group throughout the experiment. The high and low temperatures on the day of TAI were recorded, and the high temperature was used to evaluate the effect of temperature at TAI on conception rate for cows within a weekly breeding group.

Statistical Analyses

Procedure LOGISTIC of SAS (SAS Inst. Inc.) was used to analyze the effect of treatment on PR/AI. The model included treatment, parity, and the two-way interaction, with high temperature at TAI as a regression variable. The effect of AI sire was not included in this model, but sires were distributed evenly among treatments. Within each treatment group, the effect of the presence or absence of a CL on PR/AI was tested by chi-square analysis using the Cochran-Mantel-Haenszel statistic of SAS.

Survival analysis (i.e., failure time analysis), of cows in the D26 and D33 groups was compared across the first three TAI services using a Weibull proportional hazards model. Number of days to pregnancy across the first three TAI services was calculated using the hazard function. This function reflects the instantaneous probability (risk) of an individual cow becoming pregnant at time t and was modeled as:

$$h_{ijk}(\mathrm{t}) = h_0(t, \gamma, \rho)\exp[T_i \cdot L_j \cdot S_k(\mathrm{t})]$$

where

$h_{ijk}(\mathrm{t})$ = hazard function of a given cow at time t;
$h_0(t, \gamma, \rho)$ = Weibull baseline hazard function with scale parameter γ and shape parameter ρ (these parameters were estimated from the data);
T_i = time-independent effect of treatment;
L_j = time-independent effect of parity;
$S_k(\mathrm{t})$ = time-dependent effect of season at TAI, assumed to be piecewise constant with change points at each TAI for a given cow.

Four seasons per year were defined, and their change points occurred on March 21, June 21, September 21, and December 21 of each calendar year during the experiment. Observations from cows that became pregnant during the study period were considered as uncensored. Observations from cows that were sold before becoming pregnant, cows that failed to become pregnant by the end of the study period, and cows that experienced embryonic loss after a positive pregnancy diagnosis were considered as censored. For cows that experienced embryonic loss, the date of the last TAI service was used as the time of censoring. The Survival Kit Version 3.12, a set of Fortran programs written by Ducrocq and Sölkner (1998), was used for the survival analysis. Details regarding the algorithms for estimation are given by Ducrocq (1994), and theoretical aspects are discussed by Ducrocq and Casella (1996).

RESULTS AND DISCUSSION

Resynchronization Strategy

Reproductive management protocols that allow for TAI and minimize or eliminate visual estrus detection 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. In the present study, submission of cows for first postpartum TAI service was scheduled so that the first 4 injections of the Presynch plus Ovsynch protocol occurred on Tuesdays followed by the second GnRH injection and TAI occurring on Thursdays. This TAI schedule for both the Ovsynch and Resynch protocols used in this study represent a variation in which the TAI is performed during the same cow-handling period as the second GnRH (**Cosynch**), thereby eliminating one handling period compared with the first reported Ovsynch protocol (Pursley et al., 1995). Although the timing of insemination in a Cosynch strategy may not maximize conception rate to TAI (Pursley et al, 1998; Dalton et al., 2001), use of Cosynch allows for cows to be handled at the same time of the day on different days, thereby allowing for cows to be restrained in self-locking head gates or a palpation rail after a specified milking in 3× milking systems in which cow-handling periods are dictated by the milking routine rather than by preselected protocol intervals.

Initiation times for Resynch for each of the 3 treatment groups in this study were chosen to occur on Tuesdays so that injection schedules would remain consistent for all cows assigned to weekly breeding groups at any given time. To adhere to the Tuesday/Thursday schedule, all pregnancy examinations were conducted on Tuesdays. To fit the reproductive management system, the first pregnancy examination using transrectal ultrasound was conducted 26 d after TAI for the D19 and D26 cows, and 33 d after TAI for the D33 cows. Under most on-farm conditions, pregnancy diagnosis can be rapidly and accurately diagnosed using transrectal ultrasound as early as 26 d post AI (Kastelic et al., 1991; Filteau and DesCôteaux, 1998). Sensitivity and specificity of pregnancy diagnosis using ultrasound was 45 and 82%, respectively, when conducted between 21 and 25 d post AI but increased to 98 and 88%, respectively, when conducted between 26 and 33 d post AI (Pieterse et al., 1990a). Thus, the reproductive management systems assessed in this trial allow for administration of all hormone injections, Ovsynch and Resynch TAI services, and pregnancy examinations to be restricted regularly to either Tuesdays or Thursdays.

Pregnancy Rate per Artificial Insemination After First TAI Service: Ovsynch

Inherent to the experimental design, first assessment of pregnancy status was not conducted at the same interval after the Ovsynch TAI among the three treatment groups. Pregnancy status after the Ovsynch TAI was first assessed 26 d after TAI for cows in the D19 and D26 groups, whereas pregnancy status was assessed 33 d post Ovsynch TAI for cows in the D33 group (Table 1). Overall PR/AI to Ovsynch was 40% (286/711) and was greater for D19 and D26 cows than for D33 cows (Table 1). This difference is likely due to a greater period in which embryonic loss can occur in the D33 cows due to the increased interval from TAI to pregnancy diagnosis (26 vs. 33 d). When pregnancy status was reassessed for all treatment groups at 68 d after Ov-

Table 1. Pregnancy rate per artificial insemination (PR/AI) and pregnancy loss after timed artificial insemination (TAI) to Ovsynch.

	Treatment group			
Item	D19	D26	D33	Overall
Interval from Ovsynch TAI to 1st pregnancy exam (d)	26	26	33	—
PR/AI at 1st pregnancy exam, %	46[a]	42[a]	33[b]	40
(no./no.)	(108/235)	(101/240)	(77/236)	(286/711)
Interval from Ovsynch TAI to 2nd pregnancy exam (d)	68	68	68	—
PR/AI at 2nd pregnancy exam, %	33	30	29	31
(no./no.)	(78/235)	(73/240)	(68/236)	(219/711)
Interval between pregnancy exams (d)	42	42	35	—
Pregnancy loss, %	28[a]	28[a]	12[b]	23
(no./no.)	(30/108)	(28/101)	(9/77)	(67/286)

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

synch TAI, overall PR/AI to Ovsynch was 31% (219/711) and did not differ among treatments (Table 1). Thus, differences in PR/AI at the first pregnancy exam and pregnancy losses between the first and second pregnancy exams among treatment groups likely represent an artifact of time of assessment of pregnancy status after TAI inherent to the experimental design rather than to treatment differences.

For Ovsynch, PR/AI for the D19 and D26 cows in the present study is similar to that reported previously using either a 12-d (Moreira et al., 2001; El-Zarkouny et al., 2002) or a 14-d (Navanukraw et al., 2002) interval between the second $PGF_{2\alpha}$ injection of Presynch and the first GnRH injection of the Ovsynch protocol. Although this study was not designed to evaluate the effect of presynchronization on PR/AI, the 8-percentage-unit difference in overall PR/AI to the Presynch/Ovsynch versus first Resynch TAI (Table 1 vs. Table 3) in the present study may reflect the increased PR/AI to TAI due to presynchronization reported previously (Moreira et al., 2001; El-Zarkouny et al., 2002; Navanukraw et al., 2002).

Pregnancy loss contributes to reproductive inefficiency because fertility assessed at any point during pregnancy is a function of both conception rate and pregnancy loss (Fricke, 2002). For cows diagnosed pregnant to Ovsynch, overall pregnancy loss occurring between the first pregnancy exam and 68 d of gestation was 23% (67/286) and was greater for D19 and D26 cows than for D33 cows (Table 1). Although not a direct comparison, the numbers of pregnancies lost in each treatment group almost fully accounted for differences in PR/AI to the first pregnancy exam after the Ovsynch TAI among treatment groups (Table 1). Because cows diagnosed pregnant at an early ultrasound exam have a greater risk of early embryonic loss, these cows must undergo subsequent pregnancy examinations to identify and rebreed cows that experience such loss (Fricke, 2002). If left unidentified, cows experiencing embryonic loss after an early pregnancy diagnosis would actually reduce reproductive efficiency by extending intervals from calving to conception.

Of cows diagnosed pregnant at 28 d post TAI, 10 to 16% experience embryonic loss by 56 d after TAI (Mee et al., 1994; Vasconcelos et al., 1997; Fricke et al., 1998). Although the magnitude of embryonic loss in this study is greater that that reported in previous studies, the period over which loss was assessed beginning earlier in gestation (26 to 33 d). In a previous study (Vasconcelos et al., 1997), pregnancy loss in lactating dairy cows was 11% from 28 to 42 d, 6% from 42 to 56 d, and 2% from 56 to 98 d post AI, suggesting that losses are highest early and subsequently decrease as gestation ensues. In the present study, at least 46% of cows in the D19 group were pregnant at the time of GnRH administration (Table 1), whereas GnRH was administered only to cows after a nonpregnancy diagnosis in the D26 group. Both PR/AI and embryonic losses were similar between these treatment groups (Table 1), suggesting that no iatrogenic embryonic loss occurred in pregnant cows receiving GnRH in the D19 group from d 26 to 68 of gestation. Although the present study does not include the appropriate control groups to substantiate or refute the observation of Moreiera et al. (2000b), a study reported during the course of this experiment in which GnRH was administered to cows 21 d after AI before a pregnancy examination 28 d after AI showed no negative effect of GnRH on conception rate or embryonic losses compared to untreated cows (Chebel et al., 2002). Collectively, these data support that GnRH does not induce iatrogenic embryonic loss when administered to pregnant cows.

Pregnancy Rate per Artificial Insemination After Second TAI Service: Resynch

A total of 41 cows diagnosed nonpregnant to Ovsynch were not enrolled for Resynch for second TAI service

Table 2. Outcomes for cows diagnosed nonpregnant to Ovsynch that were not enrolled for Resynch for second TAI service.

	Treatment group			
Outcome	Day 19 (n = 127) n (%)	Day 26 (n = 139 n (%)	Day 33 (n = 159) n (%)	Overall (n = 425) n (%)
Died	0 (0.0)	1 (0.7)	0 (0.0)	1 (0.2)
AI to a detected estrus	5 (3.9)	10 (7.2)	9 (5.7)	24 (5.6)
Marked as "do not breed"	1 (0.8)	7 (5.0)	6 (3.8)	14 (3.3)
Sold	1 (0.8)	0 (0.0)	1 (0.6)	2 (0.5)
Total	7 (5.5)	18 (12.9)	16 (10.1)	41 (9.6)

for various reasons (Table 2). Only 5.6% of cows failing to conceive to Ovsynch were visually detected in estrus and inseminated (Table 2), underscoring the reliance of this farm on TAI for inseminating cows. Overall PR/AI to Resynch was 32% and was greater for D26 and D33 cows than for D19 cows (Table 3). Although PR/AI to TAI protocols can vary widely among farms, PR/AI after Resynch for the D26 and D33 cows in the present study is similar to conception rate after Ovsynch reported previously (Pursley et al., 1995; Fricke et al., 1998; Jobst et al., 2000).

Specific reasons for the poor Resynch PR/AI for D19 cows in the present study are unknown. Success of the Ovsynch protocol in lactating dairy cows varies depending on cyclicity status and stage of the estrous cycle at initiation of Ovsynch (Moreira et al., 2001). Stage of the estrous cycle at initiation of Ovsynch affects synchronized ovulation rate to the first GnRH injection and subsequent conception rate to TAI (Vasconcelos et al., 1999; Moreira et al., 2000a). In addition, cows initiating the Ovsynch protocol during early to mid diestrus (d 5 to 12 of the estrous cycle) when serum progesterone concentrations were high, ovulated smaller follicles and had greater conception rates than cows initiating Ovsynch during metestrus, late diestrus, or proestrus (Vasconcelos et al, 1999). Assuming an average estrous cycle duration of 23 d (Savio et al., 1990; Pursley et al., 1993; Sartori, 2002) for cows failing to conceive to Ovsynch, initiation of Resynch in the present study would approximately coincide with three stages of the estrous cycle: proestrus (D19), metestrus (D26), and diestrus (D33). Under this scenario and based on previous reports (Vasconcelos et al., 1999; Moreira et al., 2000a), initiation of Resynch 33 d after TAI should yield the highest PR/AI. In the present study, PR/AI to Resynch was numerically greatest (38%) for the D33 group but not statistically greater than the D26 group (Table 3). This occurred in spite of the longer interval from Resynch TAI to pregnancy diagnosis in the D33 group (33.7 d) compared to the D26 group (26.6 d) and in addition to the expected increased incidence of embryonic loss among treatment groups similar to that observed for PR/AI to Ovsynch.

Progesterone status or the presence or absence of a CL at Resynch initiation, both of which are related to stage of the estrous cycle also can affect PR/AI. Although progesterone status during the Resynch protocol was not assessed in the present study, presence or absence of a CL at pregnancy examinations was recorded. Although these data are limited in the present study because prostaglandin responsiveness and presence or absence of ovarian follicles was not known, these data are pertinent because this information can be collected via transrectal ultrasonography or rectal palpation by a bovine practitioner in a commercial setting. In the present study, PR/AI was nearly triple ($P < 0.05$) for D19 cows with a CL (28%) than for cows without a CL (10%) at the $PGF_{2\alpha}$ injection of Resynch (Table 4). Nearly one-fourth of D19 cows lacked a CL at $PGF_{2\alpha}$ administration suggesting that induction of ovulation

Table 3. Pregnancy rate per artificial insemination (PR/AI) after timed artificial insemination (TAI) to Resynch beginning 19, 26, or 33 d after first TAI.

	Treatment group			
Item	D19	D26	D33	Overall
Mean (± SEM) interval (d) from Resynch TAI to pregnancy exam (range)	27.1 ± 0.4 (26 to 54)	26.6 ± 0.2 (26 to 40)	33.7 ± 0.4 (26 to 75)	—
PR/AI, % (no./no.)	23[a] (28/120)	34[b] (41/121)	38[b] (54/143)	32 (123/384)

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

Table 4. Effect of the presence or absence of a corpus luteum (CL) at the first GnRH injection (d 19) or $PGF_{2\alpha}$ injection (d 26 and 33) on pregnancy rate per artificial insemination (PR/AI) after Resynch.

		Presence of a CL[1] at:				
		First GnRH injection		$PGF_{2\alpha}$ injection		
Treatment	Item	Yes	No	Yes	No	*P*-value
	n	—	—	91	29	
Day 19	% of cows	—	—	76	24	
	PR/AI (%)	—	—	28	10	0.04
	n	82	39	—	—	
Day 26	% of cows	68	32	—	—	
	PR/AI (%)	35	31	—	—	0.39
	n	112	31	—	—	
Day 33	% of cows	78	22	—	—	
	PR/AI (%)	41	26	—	—	0.09
	n	194	70	—	—	
Day 26 + 33	% of cows	74	27	—	—	
	PR/AI (%)	39	29	—	—	0.09

[1]A cow was recorded as having a CL present when the CL diameter was estimated to be = 10 mm at the ultrasound examination.

by the first GnRH injection of Resynch may have been poor, because all of those cows received GnRH 7 d before the pregnancy diagnosis. Similarly, PR/AI to Resynch tended to be greater ($P = 0.09$) for D26 + D33 cows with a CL (39%) than for cows without a CL (29%) at the first GnRH injection of Resynch (Table 4). Based on previous reports (Vasconcelos et al., 1999; Moreira et al., 2000a), these cows may have been at an unfavorable stage of the estrous cycle when Resynch was initiated. Interestingly, presence or absence of a CL at Resynch initiation did not affect PR/AI in the D26 group. Perhaps ovulatory response to the first GnRH injection was high in this group because they were in the early stage of the estrous cycle when a dominant follicle with ovulatory capacity is present (Vasconcelos et al., 1999). Further experiments are required to define the physiology that determines PR/AI after initiation of Resynch at various intervals post TAI.

Survival Analysis

Survival analysis is a powerful methodology for evaluation of reproductive data, because it allows the inclusion of time-dependent covariates, accommodates skewed or unknown distributions of time to pregnancy, and properly handles censored (cows failing to become pregnant) records (Smith and Quaas, 1984; Ducrocq and Solkner, 1998; Vukasinovic, 1999). Time from first TAI to pregnancy in the D26 and D33 groups was compared across the first three TAI services using a Weibull proportional hazards model. Cows in the D19 group were excluded from this analysis because these cows initiated Resynch after the Ovsynch TAI using the D19 treatment schedule but were shifted to the D26 Resynch schedule for third TAI service. For cows that experienced embryonic loss, the date of the last TAI service was used as time of censoring. Censoring of cows that experienced embryonic loss in this way adjusted for differences in PR/AI observed between the D26 and D33 treatment groups that were artifacts of the difference in length of the TAI to pregnancy diagnosis interval between treatments. Although cows in the D33 group had a 5% greater risk of pregnancy across the first three TAI services than cows in the D26 group (Table 5), this difference was not statistically significant. Thus, the 7-d advantage of the D26 group over the D33 group was negated by the increased rate of embryonic loss observed in the D26 group due to the earlier pregnancy exam and the longer period in which embryonic loss could occur.

High ambient temperatures at TAI affected PR/AI in the present study. Heat stress affects reproductive performance in dairy cows by affecting both oocyte quality during the periovulatory period and increasing early embryonic loss (Hansen et al., 1992; Sartori et al., 2002). Cows had a 41% greater ($P = 0.05$) risk of pregnancy during fall 2001 and a 71% greater ($P < 0.01$) risk of pregnancy during winter 2001 to 2002 compared with summer 2001 (Table 5). In addition, retrospective analysis of official temperature data showed a relationship between monthly PR/AI to Ovsynch and Resynch TAI versus mean high daily temperature at TAI for the breeding groups (Table 6 and Figure 1). For Ovsynch, a 1°C increase in mean daily high temperature at TAI resulted in a 2-percentage-unit decrease in PR/AI, whereas for the first Resynch, a 1°C increase in mean daily high temperature at TAI resulted in a 3-percentage-unit decrease in PR/AI to TAI (Table 6). Although heat stress has been well documented in Southern US states (Hansen et al., 1992), these observations high-

Table 5. Estimated risk of pregnancy from the Weibull model analysis of time from first timed artificial insemination to pregnancy. Risk ratios were constrained to 1.0 for fourth parity, Summer 2001, and treatment D26 (class with the lowest risk of pregnancy).

Covariate	Class	Uncensored failures	Risk ratio	*P*-value[1]
Parity	1	91	1.08	0.80
	2	154	1.05	0.88
	3	25	1.04	0.91
	4	11	1.00	—
Season of TAI	Spring 2001	26	1.16	0.51
	Summer 2001	63	1.00	—
	Fall 2001	71	1.41	0.05
	Winter 2001-2002	79	1.71	<0.01
	Spring 2002	44	1.23	0.30
Treatment	D26	133	1.00	—
	D33	150	1.05	0.69

[1]Probability of a significant difference from the class that was constrained to 1.0.

light the impact of heat stress on PR/AI in lactating cows in Northern US states during seasons and months when ambient temperatures are high.

CONCLUSIONS

Results from this study argue against the D19 group as a viable resynchronization strategy based on the poor PR/AI after the Resynch TAI. Because time to pregnancy did not differ significantly between the D26 and D33 groups in this study, several resynchronization strategies might be considered. A veterinarian who can accurately determine pregnancy status via rectal palpation 33 d post TAI could incorporate the D33 Resynch strategy without reliance on transrectal ultrasound for early pregnancy diagnosis. Assuming that administration of GnRH to pregnant cows 33 d after TAI does not induce iatrogenic embryonic loss, all cows could be administered GnRH at 33 d after TAI. Cows would then receive $PGF_{2\alpha}$ at a nonpregnancy diagnosis via rectal palpation conducted 1 wk later. Alternatively, veterinarians who have incorporated transrectal ultrasound for reproductive management could adopt the D26 Resynch strategy as reported herein, or alternatively, could administer GnRH to all cows regardless of their pregnancy status 26 d post TAI. Cows would then receive $PGF_{2\alpha}$ at a nonpregnancy diagnosis via transrectal ultrasound conducted 1 wk later.

Table 6. Mean high and low temperature at timed artificial insemination (TAI) and pregnancy rate per artificial insemination (PR/AI) to TAI by month.

	Temperature[1] (C)		PR/AI		
Month	Low	High	Ovsynch TAI % (no./no.)	Resynch TAI % (no./no.)	All TAI % (no./no.)
May, 2001	7.1	19.8	42 (24/57)	—	42 (24/57)
Jun, 2001	11.8	23.1	36 (21/59)	21 (5/24)	31 (26/83)
Jul, 2001	13.9	26.8	23 (13/56)	30 (10/33)	26 (23/89)
Aug, 2001	14.6	26.8	15 (8/52)	16 (7/44)	16 (15/96)
Sep, 2001	7.7	19.4	43 (21/49)	26 (9/35)	36 (30/84)
Oct, 2001	0.7	12.6	50 (17/34)	39 (12/31)	45 (29/65)
Nov, 2001	0.3	9.6	39 (24/62)	22 (6/27)	34 (30/89)
Dec, 2001	−8.1	−0.2	43 (26/61)	36 (8/22)	41 (34/83)
Jan, 2002	−10.9	−1.9	40 (30/75)	45 (19/42)	42 (49/117)
Feb, 2002	−10.1	0.7	57 (37/65)	46 (13/28)	54 (50/93)
Mar, 2002	−10.7	−0.1	52 (22/42)	50 (15/30)	51 (37/72)
Apr, 2002	−0.5	10.0	42 (24/57)	26 (5/19)	38 (29/76)
May, 2002	3.5	15.6	45 (19/42)	22 (7/32)	35 (26/74)
Jun, 2002	13.2	24.1	—	46 (6/13)	46 (6/13)
Jul, 2002	16.4	28.2	—	25 (1/4)	25 (1/4)
Overall	3.3	14.3	40 (286/711)	32 (123/384)	37 (409/1095)

[1]Official temperature data (Midwestern Climate Center, Champaign, IL) reported at a research station located within 12 miles of the farm. For Ovsynch, a 1°C increase in mean daily high temperature at TAI resulted in a 2-percentage-unit decrease in PR/AI, whereas for the first Resynch, a 1°C increase in mean daily high temperature at TAI resulted in a 3-percentage-unit decrease in PR/AI to TAI.

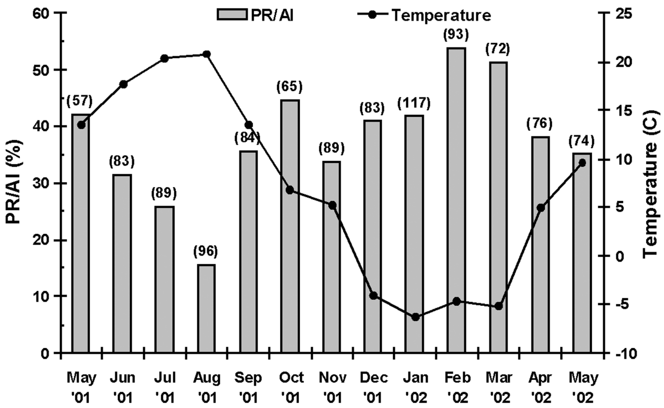

Figure 1. Effect of mean high ambient temperature on pregnancy rates to timed AI (TAI) by month. Pregnancy rate per artificial insemination (PR/AI) represents all Ovsynch and Resynch TAI services during each respective month. Numbers above bars are the total number of TAI services for each month. Temperature data represents the mean high daily temperature at the time of TAI for all TAI services occurring each month. Cows had greater ($P = 0.05$) pregnancy rates during fall and winter months compared to summer months.

Adoption of systematic Ovsynch/Resynch strategies by large dairy operations may provide a research model to investigate important aspects of reproductive biology in lactating dairy cows. For example, systematic resynchrony effectively eliminates the vagaries of low estrous detection rates when trying to identify factors associated with differences between cows that influence success or failure to initiate pregnancy. Using records from large herds adopting systematic resynchrony systems, individual cows that lose or retain pregnancies could be coupled with health, production, and environmental information obtained for the same cows at resynchrony preceding subsequent inseminations. Future studies may be developed to answer these as well as other questions regarding reproductive events in dairy cattle in herds using systematic synchronization and resynchronization systems.

Although use of ultrasound is not required to implement either the D26 or D33 Resynch protocols, use of ultrasound for reproductive management of lactating dairy cows is not without benefit (Fricke, 2002). The observation that cows without a CL at initiation of Resynch had poor conception rates provides a management opportunity to detect such cows and employ an alternative treatment strategy. Because specificity and sensitivity of detection of ovarian structures is greater for ultrasound than for rectal palpation (Pieterse et al., 1990b), use of ultrasound may be beneficial if such a differential management strategy is developed. Finally, it is possible that the optimal resynchronization strategy for this herd may not perform optimally in other herds. A difference in populations with varying proportions of cows exhibiting two or three follicular waves per cycle has been suggested to impact conception rate to Ovsynch (Cordoba and Fricke, 2002). Further research is needed to further develop successful resynchronization strategies for managing reproduction in lactating dairy cows.

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