J. Dairy Sci. 92:3643–3650
doi:10.3168/jds.2008-1704

Effect of interval to resynchronization of ovulation on fertility of lactating Holstein cows when using transrectal ultrasonography or a pregnancy-associated glycoprotein enzyme-linked immunosorbent assay to diagnose pregnancy status

E. Silva,* R. A. Sterry,* D. Kolb,† N. Mathialagan,‡ M. F. McGrath,§ J. M. Ballam,§ and P. M. Fricke*[1]
*Department of Dairy Science, University of Wisconsin, Madison 53706
†Lodi Veterinary Clinic, Lodi, WI 53597
‡Pfizer Pharmaceutical Company, St. Louis, MO 63167
§Monsanto Agricultural Company, St. Louis, MO 63167

ABSTRACT

The objective of this study was to compare 2 strategies for resynchronization of ovulation based on nonpregnant diagnoses using transrectal ultrasonography or a pregnancy-associated glycoprotein (PAG) ELISA. Lactating Holstein cows (n = 1,038) were submitted for first postpartum timed artificial insemination (TAI) using a Presynch + Ovsynch protocol. After the initial breeding, cows were randomly assigned to initiate resynchronization 25 d (D25) or 32 d (D32) later. Pregnancy status of cows initiating Resynch 25 d after TAI was determined 27 d after TAI by using a PAG ELISA, whereas pregnancy status of cows initiating Resynch 32 d after TAI was determined 39 d after TAI using transrectal ultrasonography. Cows diagnosed as not pregnant continued the Resynch protocol by receiving an injection of $PGF_{2\alpha}$ 7 d after the initial GnRH injection and a second GnRH injection 54 h after the $PGF_{2\alpha}$ injection. Cows in both treatments were inseminated approximately 16 h after the second GnRH injection. Blood samples for analysis of progesterone (P_4) were collected at the first GnRH injection of each Resynch protocol. Pregnancies per AI (P/AI) of nonpregnant cows initiating Resynch 25 vs. 32 d after first postpartum TAI did not differ 39 d after TAI and were 28.3 vs. 30.9% for D25 vs. D32 cows, respectively. Mean P_4 at the first GnRH injection of Resynch was greater for D32 than for D25 cows (3.67 ± 0.22 vs. 2.83 ± 0.22 ng/mL), indicating that the Resynch treatments were initiated at different stages of the estrous cycle. After blocking P_4 concentration into low (<1.0 ng/mL) or high (≥1.0 ng/mL) classes, P_4 class was not found to affect P/AI 39 d after TAI. Early resynchronization was not found to affect P/AI 39 d after TAI; however, early resynchronization did decrease days between inseminations and the interval from the initial nonpregnant diagnosis to conception. Earlier detection of nonpregnant cows using the PAG ELISA in conjunction with a TAI resynchronization program may improve the rate at which cows become pregnant in a dairy herd compared with transrectal ultrasonography conducted at a later stage after TAI.

Key words: resynchronization, transrectal ultrasonography, pregnancy-associated glycoprotein

INTRODUCTION

Protocols for resynchronization of ovulation (Resynch) can increase the effective AI service rate and reduce the interval between AI services (Fricke, 2002). More efficient reproductive management is achieved if nonpregnant cows are accurately identified early and then enrolled in a resynchronization protocol that results in acceptable fertility. The measurement of a protein originating from the binucleate cells of the trophoblast in the blood of the dam has been studied as a potential early pregnancy test in cattle (Sasser et al., 1986; Zoli et al., 1992; Green et al., 2005; Silva et al., 2007a). One such test is the determination of pregnancy-associated glycoprotein (**PAG**) concentrations by ELISA, which is thought to be accurate for determining pregnancy status in cattle as early as 27 d after insemination (Green et al., 2005). This test could supplement or replace the use of transrectal ultrasonography for early pregnancy diagnoses. In a previous study (Silva et al., 2007a), the ability of a PAG ELISA to identify pregnant cows (sensitivity) varied from 93.5 to 96.3%, whereas the ability to identify nonpregnant cows (specificity) ranged from 91.7 to 96.8%.

Cows diagnosed as not pregnant after Presynch + Ovsynch and timed AI (**TAI**) need to be resynchronized early. Because exposure of pregnant cows to GnRH does not cause iatrogenic pregnancy loss (Fricke et al., 2003;

Received September 11, 2008.
Accepted March 26, 2009.
[1] Corresponding author: pmfricke@wisc.edu

Chebel et al., 2004), initiating the first GnRH injection of Resynch 1 wk before pregnancy diagnosis can further reduce the interval between TAI. The optimal time to initiate synchronization using Ovsynch for first TAI occurs between d 5 and 10 of the estrous cycle (Vasconcelos et al., 1999; Moreira et al., 2000; Galvão et al., 2007). One approach to Resynch strategies is to reduce the interval between TAI by choosing the optimal stage after TAI to initiate Resynch (Fricke et al., 2003; Sterry et al., 2006). Fricke et al. (2003) reported lower fertility for cows with Resynch initiated 19 d after TAI (23%) compared with cows with Resynch initiated 26 d (34%) or 33 d (38%) after TAI. This was probably due to variable stages of the estrous cycle within each treatment when the protocol was initiated. Further information is needed to establish the optimal timing of initiation of Resynch to achieve maximum fertility and pregnancy rates.

The objectives of this study were 1) to compare the fertility of cows with Resynch initiated 25 vs. 32 d after a previous TAI based on nonpregnant diagnoses using a PAG ELISA (**D25**) or transrectal ultrasonography (**D32**) and 2) to estimate the impact of early, nonpregnant diagnoses on days between inseminations and interval from initial insemination to conception. Our hypothesis was that fertility (risk of conceiving) would be greater for cows in which Resynch was initiated 32 d after a previous TAI compared with Resynch initiated 25 d after a previous TAI.

MATERIALS AND METHODS

Animals and Management

Approximately 1,100 lactating Holstein cows on a commercial dairy farm located in south-central Wisconsin (De Forest, WI) were initially enrolled in this study from December 2004 to August 2005. Cows were housed in free-stall barns and were fed a TMR with ad libitum access to feed and water. Cows were milked three times daily and the protocol dictated that all cows receive bST (Posilac, 500 mg, Monsanto Co., St. Louis, MO) beginning 57 to 70 d postpartum and continuing every 14 d throughout the study.

Lists for scheduled injections and pregnancy examinations for individual cows were generated weekly using a commercial on-farm computer software program (Dairy Comp 305, Valley Agricultural Software, Tulare, CA). This program also was used to track and record treatment groups, reproductive outcomes, individual cow events, and monthly milk production records for each cow enrolled in the experiment. Cows assigned to the study were coded by treatment at the second $PGF_{2\alpha}$ injection of Presynch. Data from archives were transferred into a computer spreadsheet program (Microsoft Excel 2002, Microsoft Corporation, Redmond, WA) for organization and manipulation of data before statistical analysis with SAS (SAS Institute, 2004).

Submission of Cows for First Postpartum TAI

Lactating Holstein cows (n = 1079) were allocated weekly to treatment groups based on their date of calving. Cows were managed in groups to receive hormonal injections on 2 preselected days of the week (Tuesdays and Thursdays) with TAI on Fridays (Figure 1). All cows were submitted to an initial synchronization protocol (Presynch + Ovsynch) using i.m. injections of GnRH (100 μg of gonadorelin diacetate tetrahydrate; 2 mL of Cystorelin; Merial Ltd., Duluth, GA) and $PGF_{2\alpha}$ (25 mg of dinoprost tromethamine sterile solution; 5 mL of Lutalyse; Pfizer Animal Health, New York, NY) before the first postpartum TAI. Injections were scheduled as follows: $PGF_{2\alpha}$ (d 39 ± 3 and d 53 ± 3), GnRH (d 65 ± 3), $PGF_{2\alpha}$ (d 72 ± 3), GnRH 54 h after the third $PGF_{2\alpha}$, followed by TAI approximately16 h later (d 75 ± 3 postpartum). A total of 1,079 cows were initially enrolled in the study; however, 37 cows were excluded from the complete analysis because they were sold, died, or failed to complete the correct hormonal injection sequence during the Presynch + Ovsynch or Resynch protocol. Four cows were excluded on the day of pregnancy diagnosis because of veterinary health observations. Thus, a total of 1,038 cows were available for the initial pregnancy diagnosis, whereas 907 were used in the analysis.

Submission of Cows to Resynch Groups

Cows were randomized to resynchronization groups beginning at 53 ± 3 d postpartum on the day of the second $PGF_{2\alpha}$ injection of the Presynch + Ovsynch protocol. Cows were blocked by week postpartum and parity (primiparous or multiparous) as part of the randomization procedure. Cows with a BCS $\leq$2.0 (Wildman et al., 1982) were not enrolled in the study because of breeding criteria established by the farm manager (n = 13). Cows were assigned randomly to 1 of 2 treatments for resynchronization of ovulation using Ovsynch (i.e., Resynch) initiated either 25 d (D25) or 32 d (D32) after their previous TAI. Pregnancy diagnoses for D25 cows were performed by determining the concentration of PAG by ELISA, whereas transrectal ultrasonography was used to determine pregnancy status for D32 cows. Although the 2 methods of pregnancy diagnosis could confound the efficacy of resynchronization, these methods were shown to be essentially equivalent (Silva et al., 2007a). Resynchronization protocols and blood sample collection schedules are summarized in Figure 1.

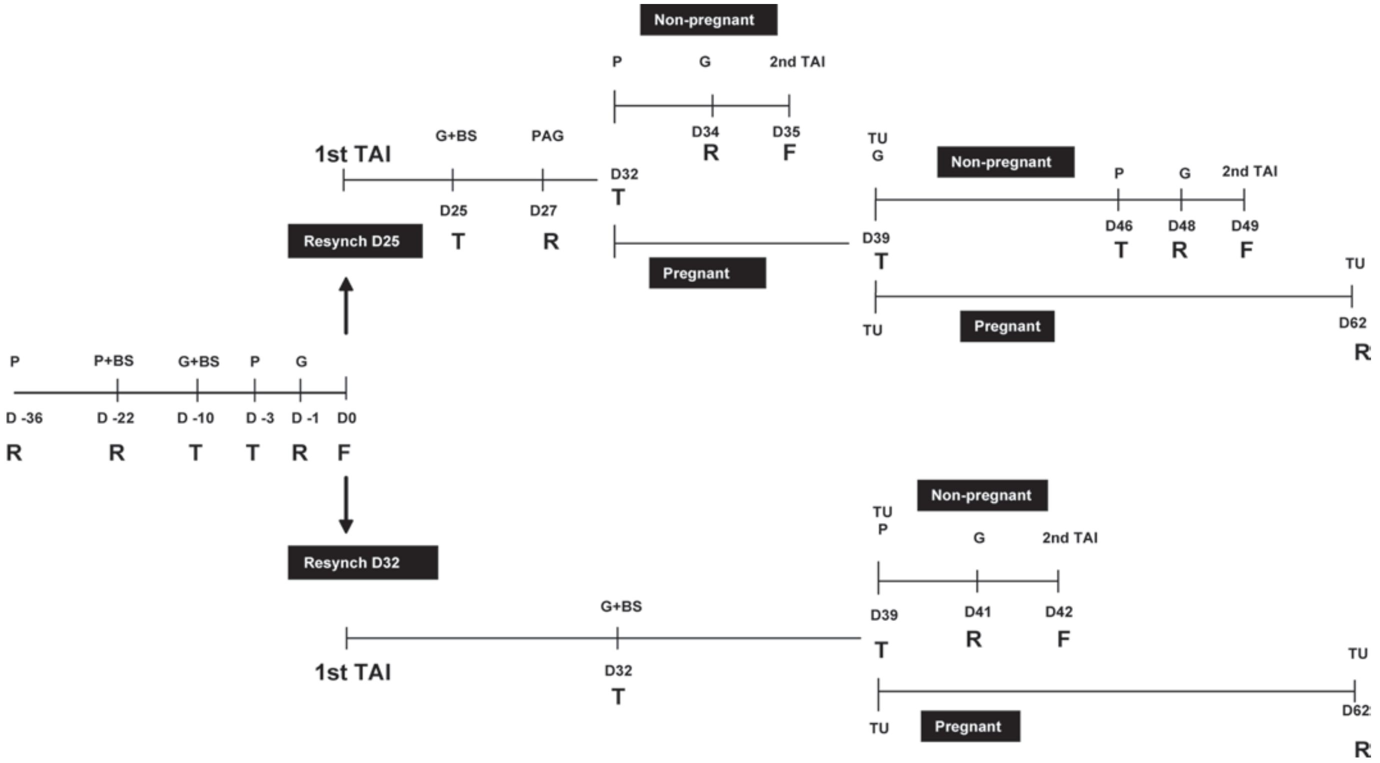

Figure 1. Protocol for blood sample collection and ultrasound examination for determination of pregnancy status in each Resynch treatment. Cows were enrolled in the study at the second $PGF_{2\alpha}$ injection of the Presynch + Ovsynch protocol (d −22). Nonpregnant cows after the first timed AI (TAI) were randomized to receive 1 of 2 resynchronization protocols beginning 25 (D25 Resynch) or 32 (D32 Resynch) d after TAI. Cows in the D25 Resynch treatment received the first GnRH injection of the Resynch protocol 25 d after TAI before assessment of pregnancy diagnosis. At 27 d after TAI, pregnancy diagnoses were determined by pregnancy-associated glycoprotein (PAG) ELISA assay, and nonpregnant cows continued the Resynch treatment with a $PGF_{2\alpha}$ injection at 32 d and a second GnRH injection 54 h after the $PGF_{2\alpha}$ injection. Cows in the D32 Resynch treatment received the first GnRH injection 32 d after TAI 7 d before pregnancy diagnosis, and pregnancy status was established 7 d later by transrectal ultrasonography examination at 39 d. Cows diagnosed as not pregnant continued the Resynch treatment with a $PGF_{2\alpha}$ injection immediately after transrectal ultrasonography examination and a GnRH injection 54 h after the $PGF_{2\alpha}$ injection. Cows in both Resynch treatments received TAI approximately 16 h after the second GnRH injection. If cows failed to conceive after the first Resynch treatment, they remained in the same treatment for a second Resynch and TAI. A second pregnancy diagnosis by transrectal ultrasonography occurred 62 d after TAI to reevaluate pregnant cows from the D25 and D32 Resynch treatments after the transrectal ultrasonography evaluation at 39 d. Blood samples were collected at the initiation of each Resynch protocol to determine serum progesterone concentration. P = 25 mg of $PGF_{2\alpha}$; G = 100 μg of GnRH; BS = blood sample for determination of progesterone concentration; PAG = blood sample collection for determination of PAG concentration; TU = transrectal ultrasonography; D = day; T = Tuesday; R = Thursday; F = Friday.

D25 Group Treatment. Cows (n = 528) enrolled in the D25 group received the first GnRH injection of Resynch 25 d after their previous TAI but before their pregnancy status had been established. Pregnancy status for D25 cows was determined from a PAG ELISA based on a plasma blood sample collected 27 d after TAI (Thursday). Cows in the D25 group diagnosed as nonpregnant based on the PAG ELISA continued with the Resynch protocol and received $PGF_{2\alpha}$ 5 d after the pregnancy examination (Tuesday). The second GnRH injection of the Resynch protocol was given 54 h later (Thursday). All cows received TAI approximately 16 h after the second GnRH injection of the resynchronization protocols. Cows diagnosed as pregnant 27 d after TAI based on the concentration of PAG were reexamined 39 d after TAI using transrectal ultrasonography to determine pregnancy loss. Cows diagnosed as pregnant at 39 d were reexamined 62 d after TAI. If cows were diagnosed as not pregnant 39 d after TAI, they received a GnRH injection immediately after transrectal ultrasonography to initiate resynchronization for a subsequent TAI.

If the PAG ELISA outcome was missing for a cow, the pregnancy diagnosis 27 d after TAI was based on a transrectal ultrasonography examination performed 27 d after TAI. The transrectal ultrasonography examination was performed at the same time as blood collection so that cows with missing PAG outcomes could continue the Resynch protocol.

D32 Resynch Group. All cows enrolled in the D32 group (n = 510) received a GnRH injection 32 d after TAI, and pregnancy status was assessed 7 d later at 39 d after TAI by using an ultrasound machine equipped with a 5- to 10-MHz linear-array transducer (Easi-Scan, BCF Technology, Livingston, Scotland, UK). Nonpregnant cows continued Resynch by receiving an i.m. injection of 25 mg of $PGF_{2\alpha}$ after pregnancy status was determined by transrectal ultrasonography, followed by a second GnRH injection of the Resynch protocol 54 h after the $PGF_{2\alpha}$ injection. All cows received TAI approximately 16 h after the second GnRH injection of the resynchronization protocols. A second pregnancy diagnosis based on transrectal ultrasonography was conducted 62 d after TAI. If a given cow had a missed transrectal ultrasonography outcome 39 d after TAI, this cow was excluded from the analysis because the cow did not receive the $PGF_{2\alpha}$ injection of the Resynch protocol.

Blood Sample Collection for the PAG ELISA

Blood samples used for the PAG ELISA were collected 27 d (Thursday) after TAI throughout the trial using 3-mL plasma dipotassium (K_2) EDTA evacuated tubes (Vacutainer, BD, Franklin Lakes, NJ). Samples were collected via venipuncture of the median caudal vein or artery. Immediately after collection, samples were placed on ice and were shipped as whole blood on ice packs in Styrofoam shipping containers from the University of Wisconsin-Madison to the Monsanto Company in St. Louis, Missouri, using an overnight shipping courier (FedEx Corporation, Memphis, TN).

Blood samples were analyzed in the laboratory at the Monsanto Company for PAG concentration using an enzyme-linked immuno-sandwich assay (Harlow and Lane, 1998) as described by Green et al. (2005), with slight modifications. Briefly, 96-well ELISA plates were coated with rabbit anti-PAG polyclonal antibodies in coating buffer (0.1 M Na_2CO_3 buffer, pH 9.35) and allowed to incubate overnight at 4°C. The plates were then washed 4 times (200 μL/well for each wash) with wash buffer (PBS, pH 7.4, containing 0.05% Tween 20) using an automatic 96-well plate washer (ELx405, BioTek, Winooski, VT). Blocking solution (200 μL/well) was then added to each well and the plates were incubated for 1 h at 37°C. After the 1 h incubation, the blocking solution was removed and the wells were washed 4 times with 300 μL of wash buffer using the plate washer. After the last wash, either 100 μL of a bovine plasma sample or a PAG standard prediluted in blocking buffer was added to duplicate wells. Blocking buffer was also used as the blank. The plates were then incubated at 37°C for 1 h. After this incubation, plates were washed 4 times with 300 μL of wash buffer using the plate washer. Biotin-labeled PAG antibody (100 μL/well) diluted in blocking buffer was added to each well and incubated for 1 h at 37°C. After incubation, the plates were again washed 4 times with 300 μL of wash buffer. Streptavidin-horseradish peroxidase (100 μL/well, diluted in blocking buffer) was added to each well and incubated for 1 h at 37°C, and then the plates were again washed 4 times with 300 μL of wash buffer. After washing, horseradish peroxidase substrate solution (100 μL/well) was added to each well and incubated at room temperature (approximately 25°C) for 15 min with shaking to allow color development. Color development was stopped by adding 1 M hydrochloric acid (HCl, 100 μL/well). A SpectraMax Plus Microplate Reader (MDS Analytical Technologies, Sunnyvale, CA) was used to measure the absorbance. SoftMax Pro (MDS Analytical Technologies) was used to estimate PAG concentration in each well by using the standard curve and a nonlinear regression plot. A standard curve was included on every ELISA plate.

Plasma samples from cows with a PAG concentration greater than a preestablished cutoff value were identified as pregnant. Preestablished cutoff values were determined by Monsanto laboratory personnel and remained unknown to laboratory personnel at the University of Wisconsin-Madison throughout the trial. Pregnancy outcomes based on the PAG ELISA were delivered to the laboratory personnel at the University of Wisconsin-Madison via e-mail and were subsequently delivered to the farm. Overall time from blood sample collection to the return of the pregnancy outcomes to the farm was approximately 36 h. This allowed D25 cows diagnosed as not pregnant based on the PAG ELISA to receive $PGF_{2\alpha}$ and continue the Resynch protocol at 5 d after blood sample collection.

Determination of Pregnancies per AI for the Resynch Analysis

Pregnancies per AI (**P/AI**) 27 d after TAI were calculated only for D25 cows because the D32 Resynch pregnancy status was determined at 39 d after TAI. Pregnancies per AI were calculated as the number of pregnant cows divided by the total number of cows that were evaluated. Cows diagnosed as not pregnant 27 d after TAI in the D25 group were considered not pregnant 39 d after TAI for calculation of P/AI. Pregnancy loss was calculated between 39 and 62 d after TAI, and was calculated as the number of cows diagnosed as pregnant 39 d after TAI that were diagnosed as not pregnant 62 d after TAI based on transrectal ultrasonography. Cows failing to conceive after the first Resynch treatment (first Resynch) remained in the same group for a second Resynch and TAI (second Resynch).

Blood Sample Collection for Progesterone RIA

To determine the cyclic status of cows before the first TAI after Presynch + Ovsynch, blood samples were collected at the second $PGF_{2\alpha}$ injection of Presynch and at the first GnRH injection of Ovsynch from 1,025 of the 1,038 cows (i.e., 98.7% of the cows completing the initial Presynch + Ovsynch protocol). Cows with serum progesterone (**P_4**) $\geq$1.0 ng/mL in one or both of these serum samples were classified as cyclic, whereas cows with serum P_4 <1.0 ng/mL in both samples were classified as anovular as described previously (Moreira et al., 2001; Stevenson et al., 2006; Silva et al., 2007a).

To determine luteal status at the first GnRH injection of Resynch, blood samples were collected at the first GnRH injection of the Resynch protocols from a subgroup of cows for first Resynch (n = 190 D25 cows and 177 D32 cows) and second Resynch (n = 109 D25 cows and 97 D32 cows) to determine P_4 concentration. Cows with serum P_4 concentrations $\geq$1 ng/mL at the time of the first GnRH injection of Resynch were considered to have a functional corpus luteum (**CL**), whereas cows with serum P_4 concentrations <1 ng/mL were considered to lack a functional CL (Rivera et al., 2004).

Blood samples for P_4 evaluation were collected via venipuncture of the median caudal vein or artery into evacuated 10-mL tubes (Vacutainer, BD). Blood samples were allowed to clot for 24 h at 4°C and then centrifuged (1,935 × *g* for 15 min, 4°C), and serum was collected and stored at −20°C until assayed for P_4 by using a solid-phase, no-extraction RIA (Coat-a-Count Progesterone, Diagnostic Products Corporation, Los Angeles, CA). Interassay and intraassay coefficients of variation were 10.8 and 2.9%, respectively.

Statistical Analyses

Days open was calculated as the time from first TAI until conception for pregnant cows and from the first TAI until the time when a third resynchronization could have been initiated for nonpregnant cows. Nonpregnant cows that were removed from the study (n = 30 for D25, n = 11 for D32) before receiving their second resynchronization were assigned a days open value equal to the days when they could have received a third resynchronization.

The ANOVA for linear mixed effects models was used to analyze the continuous variables of BCS, days open, days between inseminations, DIM at TAI, and P_4 concentration. Common to all models were the fixed effects of treatment, parity, and treatment × parity interaction and the random effect of start block. Resynch number (first Resynch vs. second Resynch) and interactions between the fixed effects of treatment and parity with resynchronization number were included in the analysis of P_4 concentration and DIM at TAI. Repeated measures on individual cows were accounted for in the models of P_4 concentration and DIM at TAI by using a first-order autoregressive error structure. Days in milk at the first TAI was included as a covariable in the analysis of DIM.

A generalized linear mixed effects model was used to analyze the binomial responses of pregnancy (pregnant or open), pregnancy loss (maintained or loss), and P_4 class (<1.0 or $\geq$1.0 ng/mL). The data were fit as having a binomial error with a logit link. Treatment, parity, resynchronization number, and all 2-factor interactions with treatment were considered as fixed effects in the models, and start block was entered as a random effect.

The data were analyzed by SAS software (SAS Institute, 2004) using PROC MIXED for the linear mixed models and PROC GLIMMIX for the generalized linear mixed models. Least squares means are reported.

RESULTS AND DISCUSSION

P/AI After Presynch + Ovsynch

The number of cows included in each phase of the study is summarized in Table 1. A total of 58 D25 cows and 73 D32 cows were excluded from the analyses after the first TAI because of malfunction of the 96-well plate washer. These cows are not included in the numbers reported in Table 1. Another 21 and 25 nonpregnant cows in the D25 and D32 groups were excluded from the analysis. Reasons for exclusion were 1) marked "do not breed," 2) died or sold, and 3) failed to complete the correct injection schedule. A total of 262 D25 cows and 226 D32 cows remained in the study for the first resynchronization. Twenty-eight D25 cows were outside the 70-d breeding window at the time of their second resynchronization because of a lost pregnancy between 27 and 39 d in 1 of their first 2 TAI. These cows were excluded from the second resynchronization. For the second resynchronization, a total of 146 and 141 cows were included in the analysis for D25 and D32 groups, respectively.

The percentage of anovular cows based on P_4 concentration at the second $PGF_{2\alpha}$ injection of Presynch and the first GnRH injection of Ovsynch was similar between treatments (D25 = 23.2% and D32 = 26.3%; $P > 0.10$). In addition, the mean value for BCS did not differ ($P > 0.10$) between D25 and D32 cows (2.89 ± 0.04 vs. 2.91 ± 0.04, respectively). Pregnancies per AI after Presynch + Ovsynch 27 d after TAI were 49.1% (231/470) for D25 cows. Pregnancies per AI at approxi-

Table 1. Number of cows included in the study by treatment and timed AI (TAI) number

Item	Treatment[1] D25	D32
Cows receiving pregnancy diagnosis after first TAI[2]	470	437
Pregnant 39 d after first TAI	187	186
Cows that did not continue for first Resynch[3]	21	25
Total number of cows available for first Resynch	262	226
Pregnant 39 d after first Resynch	86	74
Cows that did not continue for second Resynch[3]	2	11
Cows outside the 70-d breeding window[4]	28	0
Total number of cows available for second Resynch	146	141

[1] Cows in the D25 group received the first GnRH injection of Resynch 25 d after TAI. Cows in the D32 group received the first GnRH injection of Resynch 32 d after TAI.

[2] Pregnancy status 27 d after TAI for D25 Resynch cows was based on pregnancy-associated glycoprotein (PAG) by ELISA. Cows that had a pregnancy status determined during the period of malfunction of the 96-well plate washer were excluded from the study. The corresponding cows from the same start block were also excluded from the analyses.

[3] Cows not continuing resynchronization protocols because they were sold, died, failed to complete the correct hormonal injection, or were marked as "do not breed."

[4] D25 Resynch cows that were outside the 2-cycle, 70-d breeding window because of a lost pregnancy between 27 and 39 d for a previous TAI.

mately 27 d reported by other studies using transrectal ultrasonography ranged from 34.9 to 46.0% (Santos et al., 2001; Fricke et al., 2003; Galvão et al., 2004). When pregnancy status was determined using ultrasound 39 d after TAI, P/AI was 40.0% (187/468) for D25 cows and 43.0% (186/433) for D32 cows. Pregnancy loss for D25 cows between 27 and 39 d after first TAI was 19.2% and after resynchronization pregnancy loss was 8.5 and 20.9% for first and second Resynch (Table 2).

Pregnancy loss in lactating dairy cows varies widely among studies. Santos et al. (2004) summarized the data from 14 studies and reported a rate of pregnancy loss of 12.8% between 30 and 45 d of pregnancy. The rate of pregnancy loss after pregnancy diagnosis based on concentration of PAG by ELISA (i.e., 19.2% from 27 to 39 d) was similar to the pregnancy losses in another study when the pregnancy examination was based on transrectal ultrasonography (Galvão et al., 2004). Pregnancy loss is greater before d 35 of gestation (Santos et al., 2004); consequently, greater pregnancy loss was expected when pregnancy examination was performed 27 d after TAI. Use of this PAG ELISA for determination of pregnancy status 27 d after TAI does not appear to increase rate of pregnancy loss between pregnancy examinations compared with transrectal ultrasonography.

Fertility and Breeding After Resynchronization

Cows in the D25 group had fewer ($P < 0.0001$) DIM at TAI than D32 cows for the first (113.8 ± 0.23 vs. 119.2 ± 0.25) and second resynchronizations (147.2 ± 0.31 vs. 161.2 ± 0.31). The D25 cows also had fewer ($P < 0.001$) total days open after the initial TAI (77.4 ± 0.9; pregnant + open cows) compared with D32 cows (91.9 ± 1.0). By design, there were fewer days between TAI for D25 than D32 cows (37.0 ± 0.24 vs. 42.0 ± 0.00). Pregnancies per AI at 27 d for D25 cows were 35.9 and 29.4% for the first and second Resynch, respectively (Table 2). Effect of treatment on P/AI was based on pregnancy examinations conducted 39 and 62 d after TAI (Table 3).

Variables and P-values analyzed in the model for P/AI are summarized in Table 3. For fertility 39 and 62 d after TAI, no effect of treatment was found on P/AI. Parity, Resynch number, and the interactions between treatment and parity and between treatment and Resynch number were not significant. Fricke et al. (2003) did not report a difference in fertility between Resynch initiated at 26 or 33 d after TAI, but the interval for pregnancy diagnosis was longer for D33 cows (33.7 vs. 26.6 d) and did not allow for a direct comparison between Resynch treatments. Another study comparing D26 with D33 Resynch treatments concluded that Resynch initiated 33 d after TAI increased P/AI at 33 and 40 d after TAI for primiparous, but not multiparous, cows (Sterry et al., 2006).

P_4 Analysis

A difference between treatments ($P < 0.01$) was observed for mean P_4 concentration at the first GnRH injection, with lower P_4 concentration for the D25 Resynch treatment (2.83 ± 0.22 vs. 3.67 ± 0.22). After assigning P_4 concentrations into high vs. low categories, a greater ($P < 0.001$) percentage of D32 cows had high P_4 (74.4%) compared with D25 cows (54.3%). The difference in P_4 concentration at initiation of the resynchroni-

Table 2. Pregnancies per AI (P/AI) 27 d after timed AI (TAI) and pregnancy loss from 27 to 39 d for d 25 resynchronization (D25) cows after the first TAI and after the first and second resynchronization

Item	First TAI	First Resynch	Second Resynch
P/AI 27 d after TAI[1]			
%	49.1	35.9	29.4
n/n	231/470	94/262	43/146
Pregnancy losses (27 to 39 d)			
%	19.2	8.5	20.9
n/n	44/229	8/94	9/43

[1] The first pregnancy examination was based on concentrations of pregnancy-associated glycoprotein (PAG) by ELISA, and pregnant cows were reevaluated 12 d later (39 d after TAI) using transrectal ultrasonography to determine pregnancy loss.

Table 3. Effect of treatment (Trt),[1] parity, and Resynch number (RES no.)[2] on pregnancies per AI (P/AI) and pregnancy loss[3] after timed AI

	Treatment									
	D25		D32			*P*-value				
Item	First Resynch	Second Resynch	First Resynch	Second Resynch	Overall (early vs. late)	Trt	Parity	RES no.	Trt × Parity	Trt × RES
P/AI (39 d)										
%	32.8	23.3	32.7	29.1	28.3 vs. 30.9	0.487	0.299	0.094	0.672	0.218
n/n	86/262	34/146	74/226	41/141						
LSM	33.8	23.5	31.7	30.0						
P/AI (62 d)										
%	27.7	21.7	31.0	25.7	25.3 vs. 28.7	0.350	0.183	0.107	0.666	0.602
n/n	69/249	31/143	70/226	36/140						
LSM	29.2	21.9	30.8	26.7						
Pregnancy loss (39 to 62 d)										
%	5.5	0.0	5.4	10.0						
n/n	4/73	0/31	4/74	4/40						

[1] Cows in the D25 group received the first GnRH injection of Resynch 25 d after timed AI (TAI). The first pregnancy diagnosis was performed 27 d after TAI and was based on pregnancy-associated glycoprotein (PAG) concentrations assessed by ELISA; nonpregnant D25 cows continued the Resynch schedule and did not receive a pregnancy diagnosis at 39 d after TAI. These cows were considered not pregnant at 39 and 62 d to calculate pregnancies per AI (P/AI). Cows in the D32 group received the first GnRH injection of Resynch 32 d after TAI. The first pregnancy diagnosis was performed 39 d after TAI using transrectal ultrasonography.

[2] Cows diagnosed as not pregnant after Presynch-Ovsynch TAI received a first Resynch. Cows diagnosed as not pregnant to the second TAI received a second Resynch treatment following the same Resynch group.

[3] Statistical analysis of pregnancy loss is not reported because of lack of convergence with low number of observations.

zation protocols is likely due to the stage of the estrous cycle at the onset of Resynch. Assuming an average estrous cycle length of 22 d, cows initiating Resynch 25 and 32 d after TAI are expected to be around 3 and 10 d of the estrous cycle, respectively. Therefore, D32 cows should have greater P_4 concentration at initiation of Resynch. Despite this logic, 16 to 22% of cows lacked a CL 33 d after TAI (Fricke et al., 2003; Sterry et al., 2006), suggesting that there is significant "biological drift" among a group of cows at various times after synchronization when using Presynch + Ovsynch and TAI. In light of this observation, resynchronization of cows given $PGF_{2\alpha}$ 12 d before initiation of resynchronization improved fertility to TAI compared with cows not presynchronized and submitted to resynchronization of ovulation 32 d after a prior TAI (Silva et al., 2007b).

Despite the difference in P_4 concentration between treatments, P_4 concentration did not ($P > 0.10$) affect P/AI 39 and 62 d after TAI in the present study. Overall, P/AI 39 d after TAI was 25.7 and 30.6%, and 62 d after TAI was 23.9 and 28.9% for the low and high P_4 classes, respectively. These data are in contrast to many resynchronization studies published to date, which support the view that fertility to TAI is greater when resynchronization is initiated when cows have high compared with low P_4. In the present study, cows with high P_4 at the initiation of resynchronization had a 4 to 5 percentage point greater P/AI compared with cows with low P_4, and our declaration of no difference could represent a type II statistical error. Another possible explanation for the similar P/AI between Resynch D25 and D32 cows is that both the D25 and D32 Resynch protocols might have been initiated during the early stages of the estrous cycle, thereby avoiding initiation of the protocol during the later stages of the cycle, which can result in premature expression of estrus and failure to synchronize an ovulation to the second GnRH injection (Vasconcelos et al., 1999; Moreira et al., 2000; Galvão et al., 2007). Similar results were reported by Fricke et al. (2003) based on the presence of a CL at initiation of Resynch, in which no difference in P/AI occurred between cows with or without a CL when Resynch was initiated 26 d after TAI. Moreover, there was only a tendency for P/AI to be different between cows with or without a CL when Resynch was initiated 33 d after TAI. Data regarding ovulation after GnRH injection and P_4 concentration at the $PGF_{2\alpha}$ injection were not collected in the present study, to clarify the reasons P/AI was similar between Resynch groups in the present study. Finally, the variation in synchrony among cows initiating either of the Resynch treatments in the present study may have been too great to detect significant differences in either fertility or stage of the cycle, which supports further exploration of protocols that presynchronize cows before the initiation of resynchronization (Silva et al., 2007b).

CONCLUSIONS

Initiation of resynchronization 25 d after an initial TAI resulted in fertility similar to initiation 32 d after TAI in lactating Holstein cows. Early resynchronization reduced DIM at TAI and total days open after the initial TAI.

ACKNOWLEDGMENTS

The authors thank Blue Star Dairy (De Forest, WI) and their staff for the use of their cows and facilities for this study.

REFERENCES

Chebel, R. C., J. E. P. Santos, J. P. Reynolds, R. L. A. Cerri, S. O. Juchem, and M. Overton. 2004. Factors affecting conception rate after artificial insemination and pregnancy loss in lactating dairy cows. Anim. Reprod. Sci. 84:239–255.

Fricke, P. M. 2002. Scanning the future—Ultrasonography as a reproductive management tool for dairy cattle. J. Dairy Sci. 85:1918–1926.

Fricke, P. M., D. Z. Caraviello, K. A. Weigel, and M. L. Welle. 2003. Fertility of dairy cows after resynchronization of ovulation at three intervals following first timed insemination. J. Dairy Sci. 86:3941–3950.

Galvão, K. N., J. E. P. Santos, S. O. Juchem, R. L. A. Cerri, A. C. Coscioni, and M. Villaseñor. 2004. Effect of addition of a progesterone intravaginal insert to a timed insemination protocol using estradiol cypionate on ovulation rate, pregnancy rate, and late embryonic loss in lactating dairy cows. J. Anim. Sci. 82:3508–3517.

Galvão, K. N., M. F. Sá Filho, and J. E. P. Santos. 2007. Reducing the interval from presynchrronization to initiation of timed artificial insemination improves fertility in dairy cows. J. Dairy Sci. 90:4212–4218.

Green, J. A., T. E. Parks, M. P. Avalle, B. P. Telugu, A. L. McLain, A. J. Peterson, W. McMillan, N. Mathialagan, R. R. Hook, S. Xie, and R. M. Roberts. 2005. The establishment of an ELISA for the detection of pregnancy-associated glycoproteins (PAGs) in the serum of pregnant cows and heifers. Theriogenology 63:1481–1503.

Harlow, E., and D. Lane. 1998. Immunoassays. Pages 553–612 in Antibodies: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Moreira, F., R. L. de la Sota, T. Diaz, and W. W. Thatcher. 2000. Effect of day of estrous cycle at the initiation of a timed artificial insemination protocol on reproductive responses in dairy heifers. J. Anim. Sci. 78:1568–1576.

Moreira, F., C. Orlandi, C. A. Risco, R. Mattos, F. Lopes, and W. W. Thatcher. 2001. Effect of presynchronization and bovine somatotropin on pregnancy rates to a timed artificial insemination protocol in lactating dairy cows. J. Dairy Sci. 84:1646–1659.

Rivera, H., H. Lopez, and P. M. Fricke. 2004. Fertility of Holstein dairy heifers after synchronization of ovulation and timed AI or AI after removed tail chalk. J. Dairy Sci. 87:2051–2061.

Santos, J. E. P., W. W. Thatcher, R. C. Chebel, R. L. A. Cerri, and K. N. Galvão. 2004. The effect of embryonic death rates in cattle on the efficacy of estrus synchronization programs. Anim. Reprod. Sci. 82–83:513–535.

Santos, J. E., W. W. Thatcher, L. Pool, and M. W. Overton. 2001. Effect of human chorionic gonadotropin on luteal function and reproductive performance of high-producing lactating Holstein dairy cows. J. Anim. Sci. 79:2881–2894.

SAS Institute. 2004. SAS Online Document 9.13. SAS Inst. Inc., Cary, NC.

Sasser, R. G., C. A. Ruder, K. A. Ivani, J. E. Butler, and W. C. Hamilton. 1986. Detection of pregnancy by radioimmunoassay of a novel pregnancy-specific protein in serum of cows and a profile of serum concentrations during gestation. Biol. Reprod. 35:936–942.

Silva, E., R. A. Sterry, D. Kolb, N. Mathialagan, M. F. McGrath, J. M. Ballam, and P. M. Fricke. 2007a. Accuracy of a pregnancy-associated glycoprotein ELISA to determine pregnancy status of lactating dairy cows twenty-seven days after timed artificial insemination. J. Dairy Sci. 90:4612–4622.

Silva, E., R. A. Sterry, D. Kolb, M. C. Wiltbank, and P. M. Fricke. 2007b. Effect of pretreatment with prostaglandin $F_{2\alpha}$ before resynchronization of ovulation on fertility of lactating Holstein cows. J. Dairy Sci. 90:5509–5517.

Sterry, R. A., E. Silva, D. Kolb, and P. M. Fricke. 2009. Strategic treatment of anovular dairy cows with GnRH. Theriogenology 71:534–542.

Sterry, R. A., M. L. Welle, and P. M. Fricke. 2006. Effect of interval from timed AI to initiation of resynchronization of ovulation on fertility of lactating dairy cows. J. Dairy Sci. 89:2099–2109.

Stevenson, J. S., J. R. Pursley, P. M. Fricke, H. A. Garverick, D. J. Kesler, J. S. Ottobre, and M. C. Wiltbank. 2006. Treatment of cycling and noncycling lactating dairy cows with progesterone during Ovsynch. J. Dairy Sci. 89:2567–2578.

Szenci, O., G. Gyulai, P. Nagy, and L. Kovács, JVarga, , and M. A. M. Taverne. 1995. Effect of uterus position relative to the pelvic inlet on the accuracy of early bovine pregnancy diagnosis by means of ultrasonography. Vet. Q. 17:37–39.

Vasconcelos, J. L. M., R. W. Silcox, G. J. M. Rosa, J. R. Pursley, and M. C. Wiltbank. 1999. Synchronization rate, size of ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the estrous cycle in lactating dairy cows. Theriogenology 52:1067–1078.

Wildman, E. E., G. M. Jones, P. E. Wagner, R. L. Boman, H. F. Troutt, and T. N. Lesch. 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. J. Dairy Sci. 65:495–501.

Zoli, A. P., L. A. Guilbault, P. Delahaut, W. B. Ortiz, and J. Beckers. 1992. Radioimmunoassay of a bovine pregnancy-associated glycoprotein in serum: Its application for pregnancy diagnosis. Biol. Reprod. 46:83–92.