

Treatment with Gonadotropin-Releasing Hormone After First Timed Artificial Insemination Improves Fertility in Noncycling Lactating Dairy Cows

R. A. Sterry,* M. L. Welle,† and P. M. Fricke¹*

*Department of Dairy Science, University of Wisconsin, Madison 53706

†Miltrim Farms, Inc., Athens, WI 54411

ABSTRACT

Lactating Holstein cows were assigned randomly to treatments to improve fertility after first postpartum timed artificial insemination (TAI). In Experiment 1, cows received no treatment (control; $n = 9$), a controlled internal drug releasing (CIDR) insert from 5 to 12 d after TAI (CIDR; $n = 9$), or 100 μg of GnRH 5 d after TAI (G5; $n = 7$). Although treatments did not affect circulating progesterone (P_4) concentrations from 5 to 19 d after TAI, there was a tendency for CIDR cows to have greater P_4 compared with control or G5 cows within 24 h after treatment. In 2 field trials, cows received either control ($n = 223$), CIDR ($n = 218$), or G5 ($n = 227$) treatments (Experiment 2), or control ($n = 160$), G5 ($n = 159$), or treatment with 100 μg of GnRH 7 d after TAI (G7; $n = 163$; Experiment 3). Treatment did not affect pregnancies per AI (P/AI) in Experiments 2 or 3; however, when data were combined to compare control ($n = 383$) and G5 ($n = 386$) treatments, P/AI tended to be greater for G5 (49.1%) than for control (45.8%) cows. This effect resulted from a GnRH treatment \times cyclicity status interaction in which P/AI for noncycling cows receiving G5 was greater than for noncycling control cows (45.5 vs. 31.1%). In conclusion, treatment with CIDR inserts after TAI had no effect on P/AI, whereas treatment with GnRH 5 d after TAI improved P/AI for noncycling, but not for cycling cows. **Key words:** cyclicity, dairy cow, controlled internal drug releasing insert, gonadotropin-releasing hormone

INTRODUCTION

Protocols that allow for fixed time insemination, such as **Ovsynch** (synchronization regimen using sequential injections of GnRH and $\text{PGF}_{2\alpha}$ to control ovulation for timed AI; Pursley et al., 1995), help overcome the pitfalls of poor detection and expression of estrus in

lactating cows. Farms adopting synchronization exclusively for first postpartum timed AI (TAI) and subsequent AI services have the opportunity to precisely determine the duration of the voluntary waiting period and improve AI service rate over time (Fricke et al., 2003). Because the 21-d pregnancy rate is a function of service risk and conception risk, the only strategy to increase pregnancy rate over a 21-d period using this management scheme is to improve fertility to TAI.

In the decade since the first published report describing Ovsynch, much attention has been devoted to optimizing synchronization and the timing of TAI, whereas less is known about improving fertility after TAI. Fertilization rate during winter in lactating dairy cows was 87%, yet only 52.8% of the resulting embryos were grades 1, 2, or 3 at 5 d after ovulation, suggesting that early embryonic mortality, rather than fertilization failure, is the primary obstacle to achieving reproductive success (Sartori et al., 2002). Thus, strategies that mitigate pregnancy loss may significantly improve reproductive efficiency.

Several studies have investigated the effect of treatment with GnRH or human chorionic gonadotropin (**hCG**) after insemination to improve fertility by inducing ovulation and forming an accessory corpus luteum (**CL**; Santos et al., 2001; Bartolome et al., 2005; Howard et al., 2005). Results from these studies have been mixed as demonstrated by the meta-analysis conducted by Peters et al. (2000). Although treatment with GnRH from 11 to 14 d after AI improved fertility in only 5 of 19 studies, GnRH treatment improved fertility when the data set was limited to 6 trials with 5 common variables. Progesterone (P_4) releasing intravaginal devices (**PRID**) or controlled internal drug releasing (**CIDR**) inserts (containing 1.38 g of P_4) also have been used as a postinsemination treatment in a limited number of studies, again with inconsistent results (Stevenson and Mee, 1991; Lopez-Gatiús et al., 2004; Hanlon et al., 2005a).

Although correlation alone does not support causality, a positive relationship between maternal P_4 after insemination and fertility has been reported (Mann and

Received January 27, 2006.

Accepted May 18, 2006.

¹Corresponding author: pmfricke@wisc.edu

Lamming, 2001; Gümen et al., 2003). Moreover, results to date do not provide conclusive evidence regarding the efficacy of post-AI treatments or cow factors that may affect treatment response. To further investigate the efficacy of postinsemination treatments to improve fertility, we examined 2 treatments: 1) treatment with GnRH 5 or 7 d after TAI to produce accessory CL, or 2) treatment with a new (previously unused) CIDR insert from 5 to 12 d after TAI.

The primary objective of this study was to evaluate pregnancies per AI (P/AI) and pregnancy loss after first postpartum TAI for cows treated with CIDR inserts or GnRH after TAI. A secondary objective was to determine the effect of treatments on circulating P_4 . Our hypothesis was that hormonal intervention after TAI would improve P/AI, possibly through a reduction in pregnancy loss.

MATERIALS AND METHODS

Experiment 1

Lactating Holstein cows ($n = 30$) located at the University of Wisconsin Dairy Cattle Research Center were enrolled in Experiment 1 beginning September 8, 2004, and ending October 27, 2004. Cows were housed in a tie stall facility, milked twice daily, and received a TMR ad libitum. Cows were allocated weekly to breeding groups, each of which included cows that had calved within a given calendar week, but had not yet received a first postpartum AI. All cows received a hormonal ovulation synchronization protocol (**Presynch + Ovsynch**; postpartum regimen using 2 injections of $PGF_{2\alpha}$ to synchronize estrous cycles prior to initiating Ovsynch) without detection of estrus before first postpartum TAI as follows: 25 mg of $PGF_{2\alpha}$ (d 34 ± 3 and d 48 ± 3 ; 5 mL of Lutalyse; Pfizer Animal Health, New York, NY), 100 μ g of GnRH (d 62 ± 3 ; 2 mL of Cystorelin; Merial, Ltd., Duluth, GA), 25 mg of $PGF_{2\alpha}$ (d 69 ± 3), and 100 μ g of GnRH (d 71 ± 3) postpartum, with TAI immediately after the second GnRH of Ovsynch (i.e., Cosynch). At TAI, cows ($n = 30$) were randomized to each of 3 treatments: 1) no further treatment (control; $n = 10$); 2) CIDR (Eazi-Breed CIDR, Pfizer Animal Health) from 5 to 12 d after TAI (CIDR, $n = 10$); or 3) 100 μ g of GnRH (2 mL Cystorelin; Merial, Ltd.) 5 d after TAI (G5, $n = 10$).

Ovarian structures in all cows in Experiment 1 were monitored by using an ultrasound machine equipped with a transrectal 7.5-MHz linear-array transducer (Aloka 500V; Corometrics Medical Systems, Inc., Wallingford, CT) to determine ovulatory response to Ovsynch and postinsemination treatments. Cows were considered to have synchronized an ovulation after the second GnRH injection of Ovsynch when 1 or more folli-

cles ≥ 10 mm were present at TAI and were absent at an ultrasound examination conducted 2 d later. Ovulatory response to the G5 treatment was determined by the presence of 1 or more follicles ≥ 10 mm 5 d after TAI and absence of 1 or more follicles 7 d after TAI. Only cows in which ovulation was synchronized after Ovsynch were eligible for enrollment into Experiment 1. Pregnancy status was determined about 30 d after TAI using the ultrasound machine and transrectal probe described above. Visualization of a CL, a fluid-filled uterine horn, and the presence of a conceptus with a heartbeat were positive indicators of pregnancy.

Blood samples were collected via venipuncture of the median caudal vein or artery into evacuated tubes (Vacutainer; BD Biosciences, Franklin Lakes, NJ) daily from 5 to 19 d after TAI for later analysis of serum P_4 , with the first sample collected immediately before administration of treatments. Blood samples were allowed to clot for 24 h at 4°C, centrifuged ($1,935 \times g$ for 15 min), and serum was harvested and stored at -20°C until assayed for P_4 using a solid-phase, no-extraction radioimmunoassay (Coat-a-Count Progesterone, Diagnostic Products Corp., Los Angeles, CA). Mean assay sensitivity was 0.1 ng/mL, and intra- and interassay coefficients of variation were 7.3 and 7.4%, respectively.

Experiments 2 and 3

For Experiments 2 and 3, lactating Holstein dairy cows on a commercial dairy farm of approximately 1,100 lactating cows located in north-central Wisconsin were enrolled at TAI into study from October 2, 2003, to June 17, 2004, for Experiment 2 and from June 24, 2004, to November 18, 2004, for Experiment 3. Cows were housed in free-stall barns and fed a TMR once daily with ad libitum access to feed and water. Cows were milked thrice daily at approximately 8-h intervals, and average milk production per cow at the monthly production test nearest the date of TAI was 41.1 ± 0.8 kg/d for Experiment 2, and 40.5 ± 1.2 kg/d for Experiment 3.

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 experiments. Cows assigned to the study were coded by treatment, and the cow file 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 Institute Inc., Cary, NC).

Lactating cows in Experiments 2 and 3 were allocated weekly to breeding groups as described in Experiment 1. In this way, cows were managed as groups to receive hormone injections and TAI on 2 preselected days of the week (Tuesdays and Thursdays). All cows received a hormonal synchronization protocol (Presynch + Ovsynch) using i.m. injections of 100 µg of GnRH (Cystorelin; Merial, Ltd.) and 25 mg of PGF_{2α} (5 mL Prostamate; IVX Animal Health, Inc., St. Joseph, MO) before first postpartum TAI as follows: PGF_{2α} (d 32 ± 3 and d 46 ± 3), GnRH (d 60 ± 3), PGF_{2α} (d 67 ± 3), and GnRH + TAI (d 69 ± 3) postpartum. Cows were not inseminated after detected estrus during the synchronization program. In Experiment 2, cows were randomized at TAI to each of the 3 treatments described in Experiment 1: 1) no further treatment (control, n = 223); 2) a CIDR insert from 5 to 12 d after TAI (CIDR, n = 218); or 3) 100 µg of GnRH 5 d after TAI (G5, n = 227). For Experiment 3, cows were randomized to each of 3 treatments to receive control (n = 160) or G5 (n = 159), or 100 or µg GnRH 7 d after TAI (G7, n = 163).

Assessment of Pregnancy Status and Luteal Status at Initiation of Ovsynch

Ultrasound examinations, hormone injections, and body condition scoring were conducted immediately after milking by the herd veterinarian and herd personnel while cows were restrained in a palpation rail located in the breezeway exiting the milking parlor. At the first GnRH of Ovsynch, cows were classified based on presence or absence of a CL (CL+ or CL-, respectively) using transrectal ultrasonography as described previously (Fricke et al., 2003). In the rare circumstance that a cow had a small amount of luteal tissue (e.g., <10 mm in diameter) but lacked a midcycle CL (i.e., ≥10 mm in diameter), these cows were classified as CL-. This criterion 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 (Fricke et al., 2003). Briefly, both ovaries of each cow were visualized using an ultrasound machine equipped with a transrectal 5.0-MHz linear-array transducer (Aloka 500V; Corometrics Medical Systems, Inc.) and the presence or absence of a CL was recorded. As part of the routine reproductive management on this dairy, all CL- cows at the first GnRH of Ovsynch received a CIDR insert between the first GnRH and the PGF_{2α} injections of Ovsynch. A BCS measured on a scale of 1 to 5, with 1 being emaciated and 5 being obese (Wildman et al., 1982), was assigned to all cows by the

herd veterinarian at the PGF_{2α} injection of Ovsynch. For pregnancy diagnosis, visualization of a CL, fluid-filled uterine horn, and presence of a conceptus were positive indicators of pregnancy 33 and 61 d after TAI using ultrasound as described previously (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 P/AI. Cows diagnosed pregnant 33 d after TAI were scheduled for a pregnancy recheck using transrectal ultrasound 61 d after TAI, and pregnancy loss was assessed between 33 and 61 d after TAI.

Experimental Design and Statistical Analyses

All experiments were conducted using a randomized complete block design (Morris, 1999). Each week at the initial TAI, cows within a weekly breeding group were blocked according to parity (primiparous vs. multiparous). Within each block, cows were assigned randomly to each of 3 treatments after first postpartum TAI.

To evaluate the effect of treatment on serum P₄ concentration, continuous data from Experiment 1 were analyzed using PROC MIXED for repeated measures (SAS Institute, Inc.). Variables included in the model were treatment, day, pregnancy status, and the treatment × day interaction.

Results from Experiments 2 and 3 were initially analyzed separately, after which data for control and G5 were combined from both experiments. Dichotomous data were analyzed using PROC LOGISTIC (SAS Institute Inc.). A multivariate logistical regression model was developed to analyze the effects of the categorical variables treatment, parity (primiparous vs. multiparous), season (spring, summer, winter, or fall) and luteal status (CL+ or CL-) at the first GnRH of Ovsynch, along with the continuous variable BCS at the PGF_{2α} of Ovsynch. In addition, all 2-way interactions of the explanatory variables with treatment on P/AI and pregnancy loss also were included. For the model analyzing the combined data (Experiment 2 vs. 3), experiment and the treatment × experiment interaction were added to the model. The effect of AI sire was not included in the model, but sires were distributed evenly among treatments.

All multivariate logistical regression models were constructed using a backward selection procedure with treatment retained as a fixed factor in each of the models. A Wald statistic criterion of $P < 0.15$ was set for including variables in the model. Odds ratios and 95% confidence intervals were calculated for significant main effects remaining in the final models. Data are presented as percentages and proportions with P -val-

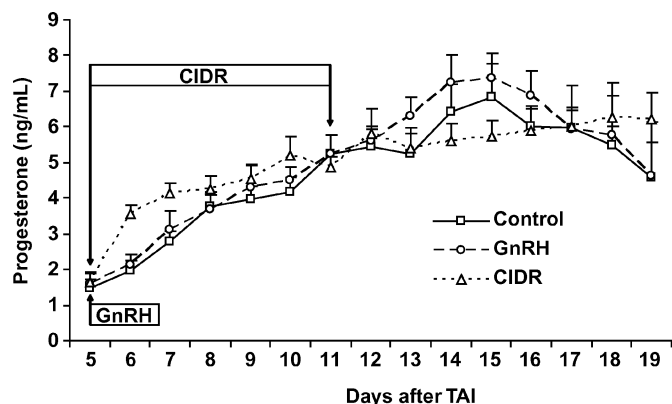


Figure 1. Progesterone profiles (ng/mL) after timed AI (TAI) for lactating Holstein cows in Experiment 1 receiving no treatment (control), GnRH 5 d after TAI (GnRH), or a CIDR insert from 5 to 12 d after TAI (CIDR). Cows received their first postpartum TAI after synchronization of ovulation using Presynch + Ovsynch [25 mg of PGF_{2α} (d 34 ± 3 and d 48 ± 3), 100 μg of GnRH (d 62 ± 3), 25 mg of PGF_{2α} (d 69 ± 3), and 100 μg of GnRH (d 71 ± 3) postpartum, with TAI immediately following the second GnRH of Ovsynch]. CIDR = controlled internal drug releasing insert containing 1.38 g of progesterone administered 5 to 12 d after TAI; GnRH = 100 μg of GnRH administered 5 d after TAI.

ues for main effects and interactions derived from the multivariate logistical regression analysis.

RESULTS AND DISCUSSION

Experiment 1

Initially, 10 cows were enrolled to each of the control, CIDR, and G5 treatments. One control cow, however, had P₄ concentrations that were <1 ng/mL throughout the study period; 1 CIDR cow underwent luteolysis by 12 d after TAI; and 3 cows failing to ovulate to G5 were excluded. These cows were subsequently removed from all analyses resulting in 9 control, 9 CIDR, and 7 G5 cows for analyses. Overall, treatments did not affect ($P > 0.56$) circulating P₄ concentrations; however, there tended ($P = 0.09$) to be a day × treatment interaction, and P₄ concentration differed ($P < 0.01$) by day. When data were analyzed based on day after TAI, CIDR cows tended ($P = 0.08$) to have greater circulating P₄ than control cows 6 d after TAI (Figure 1). There were no further treatment differences until 13 d after TAI when G5 cows tended ($P = 0.07$) to have greater P₄ than control cows. At 14 d after TAI, G5 cows had greater ($P < 0.03$) P₄ than CIDR cows, and at 15 d after TAI, G5 cows had ($P = 0.03$) greater circulating P₄ than CIDR cows.

Failure of the G5 treatment to increase P₄ in cows with an accessory CL when compared with control cows was unexpected. In previous studies in which GnRH

was administered to lactating dairy cows 5 d after TAI (Willard et al., 2003; Howard et al., 2006), circulating P₄ was greater in treated cows by 11 d after AI. Moreover, injection of hCG 6 d after estrus in beef cows (Fricke et al., 1993) or 5 d after estrus in lactating dairy cows (Santos et al., 2001) resulted in greater P₄ compared with untreated controls. Furthermore, treating previously anovular cows with hCG 5 d after insemination also resulted in greater P₄ 12 d after AI compared with no treatment (Hanlon et al., 2005b).

Less is known about the effect of CIDR inserts on circulating P₄ concentrations after insemination in lactating dairy cows. For cows synchronized with GnRH on d -7 and PGF_{2α} on d -2, inserting a new CIDR insert containing 1.38 g of P₄, or a previously used and autoclaved CIDR insert on d 0 for a 7-d period resulted in 81.2 and 83.2% of cows in which P₄ did not exceed 1.0 ng/mL (Cerri et al., 2005). The high proportion of cows with P₄ <1.0 ng/mL indicates the CIDR insert's limited effectiveness in increasing P₄ concentration in lactating cows (Cerri et al., 2005). Alternatively, treatment with a PRID of unspecified P₄ concentration 5 d after estrus increased P₄ for the first 3 d after insertion (Walton et al., 1990).

It is important to note that the distribution of pregnant and nonpregnant cows within treatments in Experiment 1 was not equal, and this may have affected serum P₄ concentrations. Although pregnancy status was included in the initial statistical model, this variable was removed during the backward selection procedure and was not included in the final logistical regression model. For the cows submitted to pregnancy diagnosis (1 control cow was sold before diagnosis), P/AI was 77.8% (7/9) for CIDR cows, 50.0% (4/8) for control cows, and 14.3% (1/7) for G5 cows. Although the number of cows per treatment in Experiment 1 is not adequate to compare P/AI, the disproportionate ratio of pregnant to nonpregnant cows among treatments may have differentially affected circulating P₄ concentrations based on the correlation between pregnancy status and increased P₄ early after TAI as previously reported (Gümen et al., 2003). Thus, the small proportion of pregnant G5 cows may explain the lack of a treatment effect on circulating P₄ when compared with control cows.

Experiment 2

Retention rate of CIDR inserts was 96.2% (154/160) for CL- cows treated with CIDR inserts during Presynch + Ovsynch, and 96.5% (220/228) for cows treated with CIDR inserts after TAI. These retention rates are similar to the 97.3% CIDR retention rates during a 7-d period after insertion reported by Chenault et al. (2003). For our study, the 8 cows receiving a CIDR

Table 1. Effect of luteal status at the first GnRH injection of Presynch + Ovsynch on pregnancies per AI (P/AI) and pregnancy loss for lactating Holstein cows in Experiment 2^{1,2}

Item	Treatment						P-value		
	Control ³		G5 ⁴		CIDR ⁵		Treatment	CL +/-	Trt × CL +/- ⁶
	CL-	CL+	CL-	CL+	CL-	CL+			
P/AI, % (no./no.)									
33 d	37.3 (22/59)	54.3 (89/164)	51.0 (26/51)	56.8 (100/176)	30.0 (12/40)	50.6 (90/178)	0.20	<0.01	—
61 d	32.2 (19/59)	50.3 (81/161)	44.0 (22/50)	52.8 (93/176)	22.5 (9/40)	46.9 (83/177)	0.18	<0.01	—
Pregnancy loss, % (no./no.)	13.6 (3/22)	5.8 (5/86)	12.0 (3/25)	7.0 (7/100)	25.0 (3/12)	6.7 (6/89)	0.14	0.01	—

¹Cows were classified as having a corpus luteum (CL) at the first GnRH of Ovsynch, following Presynch, when CL diameter was estimated to be ≥ 10 mm using transrectal ultrasonography. Cows with a CL estimated to be ≥ 10 mm at this time were classified as CL+ and those lacking a CL were classified as CL-.

²Cows received their first postpartum timed AI (TAI) at 69 ± 3 DIM after Presynch (PGF_{2 α} 32 \pm 3 and 46 \pm 3 DIM) followed by Ovsynch (GnRH, d 0; PGF_{2 α} , d 7; GnRH + TAI, d 9) initiated 14 d after the second Presynch injection.

³The control cows received no further treatment after AI.

⁴Cows received 100 μ g of GnRH 5 d (G5) after TAI.

⁵Cows received a controlled internal drug releasing (CIDR) insert containing 1.38 mg of progesterone from 5 to 12 d after TAI.

⁶The interaction of treatment \times CL status (CL +/-) was removed from the statistical model during the backward stepwise selection procedure for the analysis of P/AI and pregnancy loss.

insert after TAI that did not have a CIDR present at the scheduled day of removal were excluded from further analysis.

Percentages and proportions for P/AI and pregnancy loss for Experiment 2 are presented in Table 1. Variables remaining in the logistical regression model for P/AI at 33 and 61 d after the backwards selection procedure included treatment, season, CL status at the first GnRH of Ovsynch, BCS, and the season \times treatment interaction. For analysis of pregnancy loss, variables retained in the model included treatment, parity, BCS, CL status at the first GnRH of Ovsynch, and the BCS \times treatment interaction. Overall, P/AI 33 d after TAI did not differ ($P = 0.20$) among treatments and was 49.8% (111/223) for control, 55.5% (126/227) for G5, and 46.8% (102/218) for CIDR cows, respectively. Similarly, P/AI 61 d after TAI did not differ ($P = 0.18$) among treatments and was 45.5% (100/220) for control, 50.9% (115/226) for G5, and 42.4% (92/217) for CIDR cows, respectively. Pregnancy loss from 33 to 61 d after TAI did not differ ($P = 0.14$) among treatments and was 7.4% (8/108) for control, 8.0% (10/125) for G5, and 8.9% (9/101) for CIDR cows, respectively.

Because estrous cycles were presynchronized using 2 injections of PGF_{2 α} administered 14 d apart with the second PGF_{2 α} injection administered 14 d before the first GnRH injection of Ovsynch, we expected that cycling cows should have a midcycle CL at the first GnRH of Presynch + Ovsynch, whereas cows lacking a CL at the first GnRH injection of Presynch + Ovsynch most likely had a delayed resumption of postpartum cyclicity (i.e., were noncycling). In another experiment (Silva et

al., 2006), presence or absence of a CL at the first GnRH injection of Presynch + Ovsynch was compared with a previously published method for identifying anestrus cows using combinations of high (>1 ng/mL) and low (≤ 1 ng/mL) serum P₄ collected at the second PGF_{2 α} injection and the first GnRH injection of Presynch + Ovsynch (Moreira et al., 2001). Based on 863 cows, sensitivity, specificity, positive predictive value, and negative predictive value of using transrectal ultrasonography to identify cyclicity status were 85.7, 87.7, 64.7, 95.9%, respectively (Silva et al., 2006). Disagreements between a single ultrasound and 2 serum P₄ samples occurred because 9.0% of all cows had serum P₄ ≥ 1 ng/mL at the second PGF_{2 α} injection of Presynch and <1 ng/mL at the first GnRH injection of Ovsynch. Furthermore, 6.3% of all cows were classified as cycling because of the presence of a CL using ultrasound but had serum P₄ <1.0 ng/mL at the first GnRH injection of Ovsynch. Thus, the majority of CL- cows at the first GnRH injection of Presynch + Ovsynch were considered to be noncycling for the purposes of discussion in the present study.

In agreement with the present study, others (Bartolome et al., 2005; Howard et al., 2006) failed to observe a treatment effect when GnRH was administered 5 d after TAI. In contrast, Santos et al. (2001) reported an increase in pregnancy rate for lactating dairy cows receiving hCG 5 d after AI to estrus, particularly for those cows losing body condition between the time of AI and pregnancy diagnosis. Insertion of a PRID containing 1.5 g of P₄ from 5 to 13 d after first postpartum insemination failed to affect fertility in a limited number of cows (Stevenson and Mee, 1991). Treatment of

anestrous cows with a PRID beginning 4 or 5 d after AI also failed to improve fertility (Hanlon et al., 2005a). Use of a CIDR insert from 14 to 21 d after AI to resynchronize return to estrus for cows failing to conceive at first AI had a slight but significant negative effect on pregnancy rate to the previous insemination (32.7 vs. 36.7%; Chenault et al., 2003). In the present study, the minimal duration for which the CIDR insert increased circulating P₄ compared with untreated cows in Experiment 1 makes it difficult to distinguish if supplemental P₄ failed to improve P/AI or if the short duration of increased circulating P₄ was insufficient to increase P/AI in Experiment 2.

The CL+ cows had greater ($P < 0.01$) P/AI and less ($P < 0.01$) pregnancy loss than CL- cows (Table 1). The CL+ cows had P/AI of 53.9% (279/518) compared with 40.0% (60/150) for CL- cows 33 d after TAI (adjusted odds ratio (AOR) = 1.8; 95% CI = 1.2 to 2.6). By 61 d after TAI, CL+ cows had P/AI of 50.0% (257/514) vs. 33.6% (50/149) for CL- cows (AOR = 2.0; 95% CI = 1.4 to 3.0). Finally, fewer pregnancy losses were observed from 33 to 61 d after TAI for CL+ (6.5%, 18/275) compared with CL- cows (15.3%, 9/59; AOR = 0.3; 95% CI = 0.1 to 0.8).

Cows with a greater BCS during Presynch + Ovsynch had greater ($P < 0.01$) P/AI than cows with a lesser BCS (≥ 2.5). At 33 d after TAI, cows with a greater BCS had P/AI of 54.2% (264/487), compared with 41.6% (74/178) for cows with a lesser BCS (AOR = 1.6; 95% CI = 1.2 to 2.2). In addition, at 61 d after TAI, cows with a greater BCS had P/AI of 49.2% (238/484) vs. 38.6% (68/176) for cows with a lesser BCS (AOR = 1.6; 95% CI = 1.1 to 2.1). No differences ($P = 0.99$), however, occurred in pregnancy losses (5.5%, 4/72 vs. 8.8%, 23/261) based on BCS category.

Parity was removed from the model for P/AI during the backward selection procedure of the logistical regression analysis, but tended to affect pregnancy loss ($P < 0.07$; AOR = 1.6; 95% CI = 1.2 to 2.2). Primiparous cows had P/AI of 49.8% (113/227) 33 d after TAI compared with 51.2% (226/441) for multiparous cows. At 61 d after TAI, primiparous cows had P/AI of 47.6% (108/227) compared with 45.6% (199/436) for multiparous cows, respectively. Primiparous cows lost 4.4% (5/113) of their pregnancies from 33 to 61 d after TAI compared with 10.0% (22/221) for multiparous cows.

Season had no effect on P/AI or pregnancy loss; however, a tendency ($P < 0.09$) was detected for a treatment \times season interaction on P/AI 33 and 61 d after TAI, in which G5 and CIDR cows had greater P/AI during spring than control cows. Willard et al. (2003) reported a tendency for cows treated with GnRH 5 or 11 d after AI to have greater fertility during mild heat stress. Although heat stress has been identified as a factor

affecting response to GnRH treatment, high ambient temperatures in north-central Wisconsin in the present study were minimal during the study period compared with warmer climates (range: -21 to 25°C). Further, statistical interactions observed in the present study are relatively weak.

Experiment 3

Results from Experiment 2 showed no benefit of treating cows with CIDR inserts from 5 to 12 d after TAI but a nonstatistical trend for a benefit of treating cows with GnRH 5 d after TAI (Table 1). We speculated that the lack of a significant effect of the G5 treatment may have involved a Type II error (i.e., declaring no difference between the G5 and control treatments when a difference does exist). Thus, Experiment 3 was initiated immediately after Experiment 2 to continue the control and G5 treatments to further increase the statistical power of the comparison between the control and G5 treatments. Because of the lack of a treatment effect and the expense and labor involved at the cooperating dairy with the CIDR insert treatment in Experiment 2, the CIDR treatment was replaced by treatment with GnRH 7 d after TAI (G7). This design exposed two-thirds of the cows in the herd to the risk of a favorable treatment (e.g., GnRH post-TAI) while eliminating exposure of one-third of the cows to a costly and labor-intensive treatment that showed no benefit in Experiment 2. Because less than 160 cows were included in each treatment in Experiment 3, the possibility of Type II errors must be considered when interpreting these data.

As described previously, CL- cows received a CIDR insert during the Presynch + Ovsynch protocol and CIDR insert retention rate during this period was 96.2% (102/106). Variables remaining in the logistical regression model after the backwards selection procedure for P/AI 33 d after TAI included treatment, CL status at the first GnRH of Ovsynch, BCS, and the treatment \times CL status at the first GnRH of Ovsynch interaction. For the analysis of P/AI 61 d after TAI, the same variables remained in the model with the addition of parity. Finally, for the analysis of pregnancy loss, variables retained in the final model were treatment, parity, BCS, and the treatment \times BCS interaction. No differences were detected for P/AI 33 d ($P > 0.10$; control = 51.3%, 82/160; G5 = 49.7%, 79/159; G7 = 52.1%, 85/163), or 61 d after TAI (control = 46.3%, 74/160; G5 = 46.5%, 73/157; G7 = 46.9%, 76/162). A tendency ($P = 0.09$) was detected, however, for a treatment effect on pregnancy loss from 33 to 61 d after TAI (control = 9.8%, 8/82; G5 = 5.2%, 4/77; G7 = 9.5%, 8/84).

Table 2. Effect of the presence or absence of a corpus luteum (CL) at the first GnRH injection of Presynch + Ovsynch and pregnancies per artificial insemination (P/AI) and pregnancy loss for lactating Holstein cows in Experiments 2 and 3^{1,2}

Item	Treatment				P-value		
	Control ³		G5 ⁴		Treatment	CL +/-	Trt × CL +/- ⁵
	CL-	CL+	CL-	CL+			
P/AI, % (no./no.)							
33 d	37.7 (40/106)	55.2 (153/277)	51.1 (46/90)	54.3 (159/293)	0.08	0.02	0.08
61 d	31.1 (33/106)	51.5 (141/274)	45.5 (40/88)	50.7 (148/292)	0.11	<0.01	0.06
Pregnancy loss, % (no./no.)	17.6 (7/40)	6.0 (9/150)	9.1 (4/44)	6.3 (10/158)	0.15	0.02	—

¹Cows were classified as having a CL at the first GnRH of Ovsynch, following Presynch, when CL diameter was estimated to be ≥ 10 mm using transrectal ultrasonography. Cows with a CL estimated to be ≥ 10 mm at this time were classified as CL+ and those lacking a CL were classified as CL-.

²Cows received their first postpartum timed AI (TAI) at 69 ± 3 DIM after Presynch (PGF_{2 α} , 32 ± 3 and 46 ± 3 DIM) followed by Ovsynch (GnRH, d 0; PGF_{2 α} , d 7; GnRH + TAI, d 9) initiated 14 d after the second Presynch injection.

³The control cows received no further treatment after TAI.

⁴Cows received 100 μ g of GnRH 5 d (G5) after TAI.

⁵The interaction of treatment \times CL status (CL +/-) was removed from the statistical model during the backward stepwise selection procedure for the analysis of pregnancy loss.

Analysis of G5 and Control Treatments from Experiments 2 and 3

Data from Experiments 2 and 3 were combined and analyzed to compare G5 and control treatments, and percentages and proportions for P/AI and pregnancy loss for the analysis of the combined data are presented in Table 2. Variables remaining in the logistical regression model for P/AI 33 d after TAI in the backwards selection procedure included treatment, CL status at the first GnRH injection of Presynch + Ovsynch, BCS, the interaction of luteal status \times treatment, and the BCS \times treatment interaction. These same variables without the BCS \times treatment interaction were retained for the analysis of P/AI 61 d after TAI. For the analysis of pregnancy loss, variables retained in the model included treatment, parity, BCS, CL status, and the interaction of treatment \times BCS.

Overall, G5 cows tended ($P = 0.08$) to have more P/AI 33 d after TAI (53.1%, 205/386) compared with control cows (50.4%, 193/383). However, the P/AI 61 d after TAI did not differ ($P = 0.11$) between treatments (49.1%, 188/383 vs. 45.8%, 174/380 for G5 and control cows, respectively). Pregnancy loss from 33 to 61 d after TAI was not affected ($P = 0.15$) by treatment, and was 6.9% (14/202) for G5 cows and 8.4% (16/190) for control cows.

Although parity was removed from the final model during the backward selection procedure for the analysis of P/AI, parity did affect ($P < 0.01$) pregnancy loss. Primiparous cows had a P/AI 33 d after TAI of 53.1% (178/335) compared with 50.7% (220/434) for multiparous cows. At 61 d, primiparous cows had a P/AI of

50.9% (170/334) compared with 44.8% (192/429) for multiparous cows. Primiparous cows lost fewer pregnancies (4.0%, 7/177) compared with multiparous cows (10.7%, 23/215; AOR = 0.3; 95% CI = 0.1 to 0.7).

GnRH Treatment \times CL Status Interaction

When results from G5 and control cows from Experiments 1 and 2 were combined and analyzed, there was a tendency for a GnRH treatment \times CL status interaction in which CL- G5 cows had more P/AI than CL- control cows (Table 2). At 33 d after TAI, CL- G5 cows tended ($P = 0.08$) to have more P/AI (51.1%, 46/90), than CL- control cows (37.7%, 40/106). By 61 d after TAI, there was still a tendency ($P < 0.06$) for the GnRH treatment \times CL status interaction with CL- G5 cows having more P/AI than CL- control cows (45.5%, 40/88 vs. 31.1%, 33/106, respectively). Pregnancy loss from 33 to 61 d after TAI, however, did not differ between treatments (9.1%, 4/44 vs. 17.6%, 7/40 for G5 and control cows, respectively).

It is important to evaluate the type of synchronization protocol imposed before insemination when comparing reported responses to GnRH treatments administered after AI among studies. In the present study and in Bartolome et al. (2005), all cows were enrolled into the experiment after synchronization of ovulation using Presynch + Ovsynch. Although cyclicity status was not assessed in their study, Bartolome et al. (2005) also failed to detect a treatment effect on P/AI (47.7 vs. 44.4%; $P = 0.11$) for cows treated with GnRH 5 d after TAI. Hanlon et al. (2005b) treated cows with hCG 5 d

after AI (no palpable CL and were not observed in estrus before AI) with no subsequent effect on fertility. In that study, however, estrus was synchronized using a CIDR insert and estradiol benzoate, and only cows expressing estrus within the first 2 d of the breeding period were enrolled into the study. Similarly, Santos et al. (2001) limited enrollment to cows that displayed estrus after treatment with GnRH and PGF_{2α} resulting in 72% of eligible cows receiving insemination after a detected estrus before treatment with hCG. Because cows in the latter 2 studies were inseminated after detected estrus, noncycling cows were either excluded from enrollment, or the synchronization protocol imposed before treatment may have resolved the anestrus condition, thereby confounding the comparison of the effectiveness of GnRH administered after breeding among these studies with the present results.

Specific explanations for the GnRH treatment × luteal status interaction observed in the present study are not easily given at this time. More anovular cows inseminated as part of Ovsynch exhibited a short luteal phase (23%) compared with 6% of cyclic cows at the start of Ovsynch (Gümen et al., 2003). Premature luteal regression is caused by release of PGF_{2α} from the uterus, which is induced by oxytocin release about 5 d after estrus (Garverick et al., 1992; Inskeep, 2004). Increased circulating concentrations of estradiol from ovarian follicles may play a role in the release of luteal oxytocin. Hughes et al. (1987) demonstrated that electrocautery of preovulatory ovarian follicles prolonged luteal life span in heifers. Moreover, cows treated with GnRH 12 d after estrus had lesser concentrations of estradiol 13 to 16 d after estrus than nontreated cows (Mann et al., 1995; Mann and Picton, 1995). Thus, treatment of anestrus cows with GnRH after TAI may alter follicular dynamics and decrease estradiol concentrations at a time critical to avoid early release of PGF_{2α}, subsequent luteolysis, and termination of pregnancy.

Effect of BCS on Reproductive Endpoints from Experiments 2 and 3

Cows with a greater BCS during Presynch + Ovsynch had more ($P < 0.01$) P/AI than cows with a lesser BCS. At 33 d after TAI, cows with a greater BCS (>2.5) had P/AI of 55.2% (334/605) compared with 39.0% (64/164) for cows with a lesser BCS (≤ 2.5 ; AOR = 1.9; 95% CI = 1.3 to 2.7). At 61 d after TAI, cows with a greater BCS had more P/AI: 50.4% (303/601) vs. 36.4% (59/162) for cows with a lesser BCS (AOR = 1.5; 95% CI = 1.1 to 2.1). Pregnancy loss did not differ among cows with greater or lesser BCS.

Results from Moreira et al. (2000) agree with the present study in that cows classified as having poor

BCS had poorer fertility at 27 (18.1 vs. 33.8%) and 45 d (11.1 vs. 25.6%) after TAI. Overall, 32% (51/162) of cows with BCS ≤ 2.5 were CL⁻ compared with 24% for BCS ≥ 2.75 (145/604). Although cows with a lesser BCS were more likely to be identified as CL⁻, more cows with a greater BCS were identified as CL⁻ because of a greater proportion of the herd with greater BCS. Lopez et al. (2005) reported a linear relationship between BCS and the incidence of anestrus, with cows having a lesser BCS resulting in a greater likelihood of being noncycling. Overall, however, 63.1% of noncycling cows had a BCS ≥ 2.75 , because few cows had a lesser BCS. Thus, mechanisms other than poor BCS likely play a role in the cause of delayed postpartum cyclicity for the majority of noncycling cows (Lopez et al., 2005).

Overall, CL⁻ cows had fewer ($P < 0.05$) P/AI and more ($P < 0.05$) pregnancy loss compared with CL⁺ cows. In addition, P/AI for CL⁻ cows was 43.9% (86/196), compared with 54.7% (312/570) for CL⁺ cows 33 d after TAI (AOR = 0.5; 95% CI = 0.5 to 0.6). At 61 d after TAI, CL⁻ cows had fewer ($P < 0.01$) P/AI of 33.6% (73/194) vs. 50.0% (289/566) than CL⁺ cows (AOR = 0.6; 95% CI = 0.5 to 0.6). Finally, less pregnancy loss was observed for CL⁺ compared with CL⁻ cows (6.2%, 19/308 vs. 13.1%, 11/84; AOR = 0.4; 95% CI = 0.2 to 0.9). Similar to the present study, others (Moreira et al., 2001) reported that noncycling cows submitted to Ovsynch had reduced fertility compared with cycling herd mates.

CONCLUSIONS

Overall, treatment with CIDR inserts from d 5 to 12 or GnRH 7 d after TAI failed to improve fertility or reduce pregnancy loss in lactating dairy cows. When data from Experiments 2 and 3 were combined, treatment with GnRH 5 d after TAI tended to increase P/AI. The increase in P/AI for the G5 treatment occurred because of a GnRH treatment × cyclicity status interaction in which noncycling cows treated with GnRH 5 d after TAI tended to have more P/AI than untreated noncycling cows. Further research is needed to develop practical methods for identifying cows with delayed postpartum cyclicity and to identify the mechanisms by which the G5 treatment may improve fertility in lactating dairy cows.

ACKNOWLEDGMENTS

The authors thank Merial, Ltd. (Duluth, GA) for donating Cystorelin, Am Tech Group Inc. (St. Joseph, MO) for donating Prostagmate, and Pfizer Animal Health (New York, NY) for donating CIDR inserts for this experiment. We also thank Miltrim Farms, Inc. for use of their cows and facilities. This research was supported by Hatch project WIS04995 to P.M.F.

REFERENCES

- Bartolome, J. A., P. Melendez, D. Kelbert, K. Swift, J. McHale, J. Hernandez, F. Silvestre, C. A. Risco, A. C. M. Arteché, W. W. Thatcher, and L. F. Archbald. 2005. Strategic use of gonadotropin-releasing hormone (GnRH) to increase pregnancy rate and reduce pregnancy loss in lactating dairy cows subjected to synchronization of ovulation and timed insemination. *Theriogenology* 63:1026–1037.
- Cerri, R. L. A., H. M. Rutigliano, R. G. S. Bruno, and J. E. P. Santos. 2005. Progesterone (P4) concentrations and ovarian response after insertion of a new or a 7 d used intravaginal P4 insert (IPI) in proestrus lactating cows. *J. Dairy Sci.* 88(Suppl. 1):37. (Abstr.)
- Chenault, J. R., J. F. Boucher, K. J. Dame, J. A. Meyer, and S. L. Wood-Follis. 2003. Intravaginal progesterone insert to synchronize return to estrus of previously inseminated dairy cows. *J. Dairy Sci.* 86:2039–2049.
- 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.
- Fricke, P. M., J. N. Guenther, and M. C. Wiltbank. 1998. Efficacy of decreasing the dose of GnRH used in a protocol for synchronization of ovulation and timed AI in lactating dairy cows. *Theriogenology* 50:1275–1284.
- Fricke, P. M., L. P. Reynolds, and D. A. Redmer. 1993. Effect of human chorionic gonadotropin administered early in the estrous cycle on ovulation and subsequent luteal function in cows. *J. Anim. Sci.* 71:1242–1246.
- Garverick, H. A., W. G. Zollers, Jr., and M. F. Smith. 1992. Mechanisms associated with corpus luteum lifespan in animals having normal or subnormal luteal function. *Anim. Reprod. Sci.* 28:111–124.
- Gümen, A., J. N. Guenther, and M. C. Wiltbank. 2003. Follicular size and response to Ovsynch versus detection of estrus in anovulatory and ovular lactating dairy cows. *J. Dairy Sci.* 86:3184–3194.
- Hanlon, D. W., P. J. Davidson, A. R. Hittmann, and A. K. Joe. 2005a. Supplementing previously treated anestrous dairy cows with progesterone does not increase first-service conception rate. *Theriogenology* 63:239–245.
- Hanlon, D. W., G. M. Jarratt, P. J. Davidson, A. J. Millar, and V. L. Douglas. 2005b. The effect of hCG administration five days after insemination on the first service conception rate of anestrous dairy cows. *Theriogenology* 63:1938–1945.
- Howard, J. M., R. Manzo, J. C. Dalton, F. Frago, and A. Ahmadzadeh. 2006. Conception rates and serum progesterone concentration in dairy cattle administered gonadotropin releasing hormone five days after artificial insemination. *Anim. Reprod. Sci.* 95(3–4):224–233.
- Hughes, T. L., A. Villa-Godoy, J. S. Kesner, and R. L. Fogwell. 1987. Destruction of bovine ovarian follicles: Effects on the pulsatile release of luteinizing hormone and prostaglandin $F_{2\alpha}$ induced luteal regression. *Biol. Reprod.* 36:523–529.
- Inskeep, E. K. 2004. Preovulatory, postovulatory, and postmaternal recognition effects of concentrations of progesterone on embryonic survival in the cow. *J. Anim. Sci.* 82(E. Suppl.):E24–E39.
- Lopez, H., D. Z. Caraviello, L. D. Satter, P. M. Fricke, and M. C. Wiltbank. 2005. Relationship between level of milk production and multiple ovulations in lactating dairy cows. *J. Dairy Sci.* 88:2783–2793.
- Lopez-Gatius, F., P. Santolaria, J. L. Yaniz, and R. H. Hunter. 2004. Progesterone supplementation during the early fetal period reduces pregnancy loss in high-yielding dairy cattle. *Theriogenology* 62:1529–1535.
- Mann, G. E., and G. E. Lamming. 2001. Relationship between maternal endocrine environment, early embryo development and inhibition of the luteolytic mechanism in cows. *Reproduction* 121:175–180.
- Mann, G. E., G. E. Lamming, and M. D. Fray. 1995. Plasma oestradiol and progesterone during early pregnancy in the cow and the effects of treatment with buserelin. *Anim. Reprod. Sci.* 37:121–131.
- Mann, G. E., and H. M. Picton. 1995. Ovarian and uterine effects of a single buserelin injection on day 12 of the oestrous cycle in the cow. *J. Reprod. Fertil. Abstr. Ser.* 15:23 (Abstr. no. 61).
- Moreira, F., C. Orlandi, C. A. Risco, R. Mattos, F. Lopes, and W. W. Thatcher. 2001. Effects of presynchronization and bovine somatotropin on pregnancy rates to a timed artificial insemination protocol in lactating dairy cows. *J. Dairy Sci.* 84:1646–1659.
- Moreira, F., C. Risco, M. F. Pires, J. D. Ambrose, M. Drost, M. DeLorenzo, and W. W. Thatcher. 2000. Effect of body condition on reproductive efficiency of lactating dairy cows receiving a timed insemination. *Theriogenology* 53:1305–1319.
- Morris, T. R. 1999. Experimental design and analysis in animal sciences. CABI Publishing, New York, NY.
- Peters, A. R., T. A. Martinez, and A. J. Cook. 2000. A meta-analysis of the effect of GnRH 11–14 days after insemination on pregnancy rates in cattle. *Theriogenology* 54:1317–1326.
- Pursley, J. R., M. O. Mee, and M. C. Wiltbank. 1995. Synchronization of ovulation in dairy cows using PGF_{2α} and GnRH. *Theriogenology* 44:915–923.
- Santos, J. E. P., 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.
- Sartori, R., R. Sarto-Bergfeldt, S. A. Mertens, J. N. Guenther, J. J. Parrish, and M. C. Wiltbank. 2002. Fertilization and early embryonic development in heifers and lactating cows in summer and lactating and dry cows in winter. *J. Dairy Sci.* 85:2803–2812.
- Silva, E. P. B., R. A. Sterry, and P. M. Fricke. 2006. Assessment of a practical method for identifying anovular lactating dairy cows. *J. Dairy Sci.* 89(Suppl. 1):205. (Abstr.)
- Stevenson, J. S., and M. O. Mee. 1991. Pregnancy rates of Holstein cows after postinsemination treatment with a progesterone-releasing intravaginal device. *J. Dairy Sci.* 74:3849–3856.
- Walton, J. S., G. W. Halbert, N. A. Robinson, and K. E. Leslie. 1990. Effects of progesterone and human chorionic gonadotropin administration five days postinsemination on plasma and milk concentrations of progesterone and pregnancy rates of normal and repeat breeder dairy cows. *Can. J. Vet. Res.* 54:305–308.
- 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.
- Willard, S., S. Gandy, S. Bowers, K. Graves, A. Elias, and C. Whisnant. 2003. The effects of GnRH administration postinsemination on serum concentrations of progesterone and pregnancy rates in dairy cattle exposed to mild summer heat stress. *Theriogenology* 59:1799–1810.