Timed Artificial Insemination of Two Consecutive Services in Dairy Cows Using Prostaglandin $F_{2\alpha}$ and Gonadotropin-Releasing Hormone

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ABSTRACT

Timed artificial insemination (TAI) protocols use $PGF_{2\alpha}$ and GnRH injections to synchronize ovulation. The objective was to evaluate the PGPG protocol (d 0, $PGF_{2\alpha}$; d 3, GnRH; d 11, $PGF_{2\alpha}$; d 13, GnRH and TAI) for first TAI and also examine methods for second TAI in nonpregnant cows. A factorial test of the first $PGF_{2\alpha}$ and first GnRH injections within the PGPG protocol was performed (the last $PGF_{2\alpha}$ and GnRH injections were deemed essential to the TAI). Lactating dairy cows ($n = 804$) in a commercial herd were assigned to 1 of 5 first-TAI treatments, which were PGPG, GPG (d 0, no treatment; d 3, GnRH; d 11, $PGF_{2\alpha}$; d 13, GnRH and TAI), PPG (d 0, $PGF_{2\alpha}$; d 3, no treatment; d 11, $PGF_{2\alpha}$; d 13, GnRH and TAI), and PG (d 0, no treatment; d 3, no treatment; d 11, $PGF_{2\alpha}$; d 13, GnRH and TAI); the Ovsynch protocol (GnRH, 7 d, $PGF_{2\alpha}$, 2 d, GnRH and TAI) was the positive control. For resynchronization, cows received either GnRH or the control (no injection) on d 22 after TAI. Nonpregnant cows on d 28 were then treated with $PGF_{2\alpha}$ on d 29, GnRH on d 31, and TAI [i.e., resynchronization treatments of ReGPG (received GnRH on d 22) and RePG (did not receive GnRH on d 22)]. Pregnancy rates for PGPG, GPG, PPG, PG, and Ovsynch were similar at d 28 after first TAI. Analyses of multiple explanatory factors by logistic regression detected an effect of uterine or ovarian abnormality on the d-28 pregnancy rate (normal more likely to be pregnant). Day-42 pregnancy rates were affected by uterine or ovarian abnormality (normal more likely to be pregnant), postpartum disease occurrence (healthy cows more likely to be pregnant), milk production, and days in milk. Treatment was not significant for the d-42 pregnancy rate. Effects of postpartum disease, milk production, and days in milk on the d-42 pregnancy rate were apparently manifested through their effects on embryonic loss be-

tween d 28 and 42 of pregnancy. High-producing cows that received TAI early postpartum were most likely to experience embryonic loss. Day-42 pregnancy rates after the resynchronization treatment were affected by an interaction of the first synchronization treatment with the resynchronization treatment. We concluded that although PGPG can be used for TAI, a simpler TAI protocol that includes the last 2 injections ($PGF_{2\alpha}$, 2 d; GnRH and TAI) would be equally effective.

Key words: dairy cow, reproduction, estrus, synchronization

INTRODUCTION

Timed AI (TAI) protocols are a popular alternative to insemination after observed estrus (Nebel and Jobst, 1998; Lucy et al., 2004; Moore and Thatcher, 2006). The majority of the TAI protocols use $PGF_{2\alpha}$ and GnRH given as a series of injections. The first injection of GnRH regresses or ovulates the dominant follicle and initiates a new follicular wave in the majority of cows (Thatcher et al., 1996). Prostaglandin $F_{2\alpha}$ (generally given 7 d after GnRH) causes regression of the corpus luteum (CL). A second injection of GnRH (2 d after $PGF_{2\alpha}$) causes an LH surge that initiates the physiological events leading to ovulation (Thatcher et al., 2002).

Kojima et al. (2000) combined a $PGF_{2\alpha}$ -GnRH- $PGF_{2\alpha}$ protocol with melengestrol acetate (MGA) in an effort to obtain a more synchronous pattern of estrus in beef cattle. Beef cows were fed MGA for 7 d and were then treated with $PGF_{2\alpha}$ (d 0; MGA withdrawal), a GnRH injection on d 4, and a $PGF_{2\alpha}$ injection on d 11 (7-11 Synch). Melengestrol acetate is not an approved feed additive for dairy cattle. Therefore, Borman et al. (2003) removed the MGA but retained the sequence of $PGF_{2\alpha}$ and GnRH injections with an 8-d interval between the GnRH and the second $PGF_{2\alpha}$ injection. To increase the synchrony of ovulation, Borman et al. (2003) also added an estradiol cypionate injection 1 d after the second $PGF_{2\alpha}$ injection. Scheer-Oelrichs (2003) replaced the estradiol cypionate injection with GnRH and found that nearly 90% of treated postpartum dairy cows had ovulated on d 14 of the protocol.

The protocol initially tested by Scheer-Oelrichs (2003) was a series of $PGF_{2\alpha}$ and GnRH injections that was

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followed by TAI (d 0, PGF_{2α}; d 3, GnRH; d 11, PGF_{2α}; d 13, GnRH and TAI; **PGPG** protocol). The PGPG protocol is similar to Ovsynch (d 0, GnRH; d 7, PGF_{2α}; d 9, GnRH and TAI; Pursley et al., 1997) because PGF_{2α} and GnRH are given in series. The protocol differs from Ovsynch in that the PGPG method is initiated by using a single PGF_{2α} injection. The PGF_{2α} injection theoretically acts as a presynchronization treatment that groups the cows that regress their CL into the first third of the estrous cycle (first follicular wave; Mihm et al., 2002). The interval between the first GnRH and PGF_{2α} is also different, being 8 d for PGPG compared with 7 d for Ovsynch.

A second TAI for cows diagnosed as nonpregnant is possible if pregnancy detection is conducted and a resynchronization treatment involving PGF_{2α} and GnRH is applied. Nonpregnant cows may be treated with Ovsynch that is initiated after pregnancy diagnosis (Pursley et al., 1997). Alternatively, the first GnRH injection in the Ovsynch protocol can be administered 7 d before the pregnancy exam so that the PGF_{2α} injection of Ovsynch can be given immediately after a not-pregnant diagnosis (Chebel et al., 2003; Fricke et al., 2003; Bartolome et al., 2005). Cows diagnosed as not pregnant on d 28 to 30 after first TAI are in the early stage of an estrous cycle (assuming cows were not pregnant and experienced an undetected return to estrus). In these cows, pretreating with GnRH 1 wk before the pregnancy exam may be ineffective because the GnRH injection is scheduled for a time when a cow would otherwise be in estrus or be in the early phase of the estrous cycle (21 to 23 d after first TAI).

The first objective was to test the effect of the first PGF_{2α} and first GnRH injections within the PGPG protocol (the last PGF_{2α} and GnRH injections were deemed essential to the TAI). The test was conducted by systematically removing the first PGF_{2α} injection, the first GnRH injection, or both and examining pregnancy rates after first TAI. Cows subjected to first TAI were then randomly assigned to either GnRH or no treatment on d 22 after first TAI. This facilitated our second objective, which was to test the utility of the first GnRH injection within an Ovsynch-like resynchronization protocol that was initiated on d 22 after first TAI.

MATERIALS AND METHODS

First TAI

The experiment was conducted on a commercial dairy farm in northern Missouri during the months of March through June using lactating Holstein dairy cows (n = 804). Housing was in a standard free-stall barn with grooved concrete floors. Cows were milked 3 times daily and fed a TMR (primarily alfalfa silage, alfalfa hay, corn silage, and concentrates). Cows enrolled in the experi-

Figure 1. Diagrammatic representation of the sequence of injections administered to cows assigned to the PGPG (PGF_{2α}, 3 d; GnRH, 8 d; PGF_{2α}, 2 d; GnRH and TAI), GPG (GnRH, 8 d; PGF_{2α}, 2 d; GnRH and TAI), PPG (PGF_{2α}, 11 d; PGF_{2α}, 2 d; GnRH and TAI), PG (PGF_{2α}, 2 d; GnRH and TAI), and Ovsynch (GnRH, 7 d; PGF_{2α}, 2 d; GnRH and TAI) protocols. Cows were injected with either PGF_{2α} [5 mL of Lutalyse sterile solution (Pfizer Animal Health, Kalamazoo, MI)] or GnRH [2 mL of Cystorelin (Merial, Iselin, NJ)] on the day of the experiment (indicated on the timeline, bottom). TAI = timed artificial insemination (2 to 6 h after the final GnRH injection).

ment were typical of commercial dairy cows found in the midwestern United States. Four sequential breeding groups of approximately 200 cows per group (2 groups with first inseminations in April and 2 groups with first inseminations in May) were enrolled in the experiment. Cows with gross reproductive tract abnormalities (adhesions, tumors, etc.) were omitted from the study. Cows with enlarged ovaries (possibly cystic) or with evidence of an off-color uterine discharge were treated according to the study protocol, but their condition was noted and included as an explanatory variable in the data analyses (uterus or ovary abnormal; about 9% of study cows). For the purposes of this study, “first” and “second” inseminations refer to the first and second inseminations of the study and not the first and second postpartum inseminations for the individual cow (cows may have had one or more inseminations before being enrolled).

Cows were randomly assigned to 1 of 5 TAI treatments (Figure 1). A factorial design of treatments was applied in which the first factor was the first PGF_{2α} injection and the second factor was the first GnRH injection. The PGPG cows (n = 140) were treated with PGF_{2α} (d 0), GnRH (d 3), PGF_{2α} (d 11), and GnRH (d 13), followed by TAI (d 13). Other treatments were **GPG** (d 0, no treatment; d 3, GnRH; d 11, PGF_{2α}; d 13, GnRH and TAI; n = 169), **PPG** (d 0, PGF_{2α}; d 3, no treatment; d

11, PGF_{2α}; d 13, GnRH and TAI; n = 165), and **PG** (d 0, no treatment; d 3, no treatment; d 11, PGF_{2α}; d 13, GnRH and TAI; n = 178). The Ovsynch protocol (GnRH, 7 d; PGF_{2α}, 2 d; GnRH and TAI; n = 152) was the positive control.

Treatments were balanced for lactation number, breeding group (n = 4), insemination technician (n = 5), and insemination sire (n = 3). The PGF_{2α} injections were 5 mL of Lutalyse sterile solution (Pfizer Animal Health, Kalamazoo, MI) and the GnRH injections were 2 mL of Cystorelin (Merial, Iselin, NJ). Injections were given i.m. in the flank. All injections were given while cows were being milked in a rotary platform milking parlor. Injection records were kept. Cows that did not receive the correct sequence of injections were dropped from the study. Cows were subjected to TAI after they had exited the milking parlor, returned to their pens, and been restrained in headlocks. The time of TAI averaged 3 h after the last GnRH injection, and all TAI were completed within 6 h of the last cow leaving the milking parlor. Employees of the University of Missouri administered injections and performed inseminations. Detection of estrus was not conducted during the study.

Resynchronization of Second TAI and Pregnancy Diagnosis

Cows in breeding groups 1 to 3 were randomly assigned to receive either GnRH or the control (no injection) on d 22 after first TAI. The randomly assigned treatment of GnRH on d 22 created 2 resynchronization treatments (**ReGPG** and **RePG**) for cows diagnosed as not pregnant on d 28. The ReGPG treatment was GnRH on d 22, 7 d; PGF_{2α}, 2 d; GnRH and TAI. The RePG treatment was no treatment on d 22, 7 d; PGF_{2α}, 2 d; GnRH and TAI. The pregnancy diagnosis and PGF_{2α} injection were not done on the same day because of the large number of cows receiving the pregnancy exam and because the goal was to reduce the variance for time of the PGF_{2α} injection. Cows in the fourth breeding group were not resynchronized because their second insemination would have been in late June, a time of potentially severe heat stress. The pregnancy diagnosis was conducted transrectally on d 28 by using an Aloka 500-V ultrasound scanner with either a 5- or 7.5-MHz transducer (Corometrics Medical Systems, Wallingford, CT). The presence or absence of a CL (regardless of diameter) was recorded at the pregnancy diagnosis. Diagnosis of pregnancy and detection of the CL were accomplished by employees of the University of Missouri. Second-insemination cows were subjected to a single pregnancy diagnosis on d 42 after the second insemination.

Statistical Analysis

Milk production (kg/d) and DIM were determined for each cow when her respective breeding group started on the trial (first injection). Pregnancy rate was defined as the number of pregnant cows divided by the number of cows that received TAI. For statistical analyses, pregnancy on d 28 and 42 after insemination was coded as 0 (not pregnant) or 1 (pregnant). The presence of a CL at the d-28 pregnancy exam (after first insemination) was coded as 0 (not present) or 1 (present). Embryonic loss was calculated for cows that were pregnant on d 28. Cows that were pregnant on d 28 and pregnant on d 42 were coded as 0, no embryonic loss. Cows that were pregnant on d 28 and not pregnant on d 42 were coded as 1, having embryonic loss. Embryonic loss was not examined after the second insemination because the pregnancy diagnosis was done only on d 42 after the second insemination. A final pregnancy rate was calculated for cows in groups 1 to 3 (collective outcome for the first and second inseminations that was based on d-42 pregnancy examinations; 0 = not pregnant to both inseminations, 1 = pregnant to either insemination).

Pregnancy and embryonic loss rates were analyzed by using the logistic regression procedure of SAS (SAS Institute, 1998; PROC LOGISTIC). Explanatory variables in the statistical model were fit as discrete variables [first AI treatment (n = 5), resynchronization treatment (n = 2), first AI treatment by resynchronization treatment, breeding group (n = 4), uterine or ovarian abnormality (0 = normal; 1 = abnormal), postpartum disease occurrence (mastitis, milk fever, ketosis, lameness, etc.; 0 = none, 1 = occurrence), sire used for AI (n = 3), inseminator (n = 5), lactation number (1, 2, or ≥3), and number of previous inseminations (0, 1, 2, or ≥3)] were fit as continuous variables. Continuous variables in the model (milk production and DIM) were fit as both first order (milk and DIM) and second order (milk × milk and DIM × DIM). Backward elimination was used to create the final statistical model. Explanatory variables remained in the model when $P < 0.15$. Probabilities for the d-42 pregnancy rate and embryonic loss rate were predicted using logistic regression estimates obtained from the model and an inverse link function according to the method described by Mesa et al. (2006). Statistical significance was inferred at $P < 0.05$ unless stated otherwise.

RESULTS

First Insemination

Cows assigned to different synchronization treatments were similar for lactation number (2.0 ± 0.1), DIM (156 ± 4), daily milk production (35 ± 1 kg), and number

of previous inseminations (1.7 ± 0.1). Logistic regression analyses detected an effect ($P < 0.001$) of uterine or ovarian abnormality on the d-28 pregnancy rate. Cows with a normal uterus or ovary were 4.8 times more likely (odds-ratio estimate) to be pregnant when compared with cows with an abnormal uterus or ovary [95% Wald confidence limits of 2.0 and 11.3; actual pregnancy rates were 6/67 (9%) and 216/674 (32%) for abnormal and normal, respectively]. All other effects (including treatment) were deemed not significant and were removed from the model by backward elimination. Day-42 pregnancy rates were affected by uterine or ovarian abnormality [$P < 0.001$; normal cows 26.3 times more likely to be pregnant; 95% Wald confidence limits of 3.6 and 191.1; actual pregnancy rates were 1/71 (1%) and 188/726 (26%) for abnormal and normal, respectively] and postpartum disease occurrence [$P < 0.05$; healthy cows 1.5 times more likely to be pregnant; 95% Wald confidence limits of 1.0 and 2.2; actual pregnancy rates were 51/239 (21%) and 138/558 (25%) for unhealthy and healthy cows, respectively]. There was a quadratic effect ($P < 0.05$) of milk production, and linear ($P < 0.01$) and quadratic ($P < 0.05$) effects of DIM on the d-42 pregnancy rate (Figure 2A). All other effects (including treatment; Table 1) were deemed not significant and were removed from the model by backward elimination. The rate of embryonic loss (d 28 to 42; Table 1) was affected by uterine or ovarian abnormality [$P < 0.001$; abnormal cows 71.4 times more likely to experience embryonic loss; 95% Wald confidence limits of 5.6 and 1,000; actual embryonic loss rates were 5/6 (83%) and 39/209 (19%) for abnormal and normal, respectively] and postpartum disease occurrence [$P < 0.06$; unhealthy cows were 2.3 times more likely to experience embryonic loss; 95% Wald confidence limits of 1.0 and 5.3; actual embryonic loss rates were 14/57 (25%) and 30/158 (19%) for unhealthy and healthy, respectively]. There was a linear effect ($P < 0.05$) of milk production, and linear ($P < 0.01$) and quadratic ($P < 0.07$) effects of DIM on the rate of embryonic loss (Figure 2B). No effect of synchronization treatment was detected for the rate of embryonic loss (Table 1).

Second Insemination (Resynchronization)

A total of 627 cows in breeding groups 1 to 3 were examined by ultrasound on d 28 as part of the resynchronization treatment. Eighty-five percent ($n = 531$ cows) had a CL visible by ultrasound. There was an effect ($P < 0.001$) of uterine or ovarian abnormality on the presence of a CL at the d-28 pregnancy exam after first TAI. Cows with a normal uterus or ovary were 5.5 times more likely (odds-ratio estimate) to have a CL when compared with cows with an abnormal uterus or ovary [95% Wald

confidence limits of 3.1 and 9.9; actual CL rates were 33/58 (57%) and 498/569 (88%) for abnormal and normal, respectively]. There was a tendency ($P < 0.10$) for a linear effect of DIM and a quadratic ($P < 0.05$) effect of DIM on the presence of a CL at d 28 (Figure 3). All other effects on d-28 CL rates (including resynchronization treatment) were deemed not significant and were removed from the model by backward elimination. Day-42 pregnancy rates for the resynchronization were affected by an interaction ($P < 0.05$) of first synchronization treatment with second resynchronization treatment (Table 2). The interaction was apparently caused by specific first-insemination groups that had superior resynchronization pregnancy rates when placed on either RePG or ReGPG. This was particularly evident for the PPG and PG first-insemination groups. All other effects were not significant and were removed from the model by backward elimination. Final pregnancy rates to synchronization and resynchronization were affected by uterine or ovarian abnormality [cows with a normal uterus or ovary were 4.9 times more likely to be pregnant, with 95% Wald confidence limits of 2.3 and 10.4; actual pregnancy rates were 9/53 (17%) and 220/492 (45%) for abnormal and normal, respectively] and the interaction ($P < 0.05$) of first synchronization treatment with second resynchronization treatment (Table 2).

DISCUSSION

The PGPG method is initiated by using a single $\text{PGF}_{2\alpha}$ injection that acts as a presynchronization step. The presynchronization groups cattle into the first third of the estrous cycle (first follicular wave) for the final $\text{PGF}_{2\alpha}$ and GnRH injections. The last 2 injections ($\text{PGF}_{2\alpha}$ and GnRH) of PGPG are common to many TAI protocols and reflect the need for luteolysis and an LH surge before TAI. Our belief was that the last 2 injections represented the minimal requirement to synchronize ovulation. In this experiment we tested each initial injection of the PGPG protocol (i.e., the first $\text{PGF}_{2\alpha}$ and the first GnRH) relative to this minimal requirement. We also tested Ovsynch as a positive control. We found that d-28 pregnancy rates were numerically greatest for Ovsynch (37.8%) and lowest for PGPG (26.0%; Table 1). Other treatments (GPG, PPG, and PG) were intermediate between PGPG and Ovsynch. By d 42, the numeric trend for a difference between PGPG (lowest pregnancy rate) and Ovsynch (highest pregnancy rate) still existed.

The results were unexpected because we found that PGPG (our theoretically optimized program) was no better than the other protocols that involved fewer injections. Initial work suggested an advantage of PGPG compared with $\text{PGF}_{2\alpha}$ alone or Cosynch (Meyer et al., 2003). The advantage of PGPG, compared with $\text{PGF}_{2\alpha}$ alone,

Figure 2. Three-dimensional response surface depicting the d-42 pregnancy probability (A) and the embryonic loss probability (B) for cows with different DIM and daily milk production. The results are for the pregnancy exams after first timed AI. Embryonic loss was calculated for cows that were pregnant on d 28. Cows that were pregnant on d 28 and pregnant on d 42 were defined as having no embryonic loss. Cows that were pregnant on d 28 and not pregnant on d 42 were defined as having embryonic loss. Milk production and DIM were recorded for each cow when her respective breeding group started on the trial (first injection). There were 103, 397, and 230 cows with <20, 21 to 40, and >40 kg of milk production, respectively. There were 268, 114, 115, 89, 61, and 132 cows with 40 to 80, 81 to 120, 121 to 160, 161 to 200, 201 to 240, and >240 DIM, respectively.

may be explained by the inherent advantages of TAI over insemination after estrus in herds with poorer estrus-detection rates (Nebel and Jobst, 1998). When the PGPG protocol was compared with Cosynch (Meyer et al.,

2003), cows were inseminated if they displayed estrus during the protocol or were continued on the protocol and received TAI if estrus was not observed. In the previous work of Meyer et al. (2003), about 25% of the PGPG

Table 1. Number of cows inseminated and percentage pregnant at d 28 and 42 after insemination, and number and percentage of pregnant cows with embryonic loss for cows treated with 1 of 5 timed AI (TAI) protocols

Treatment ¹	Pregnancy exam day				Embryonic loss ²	
	28		42			
	No.	%	No.	%	No.	%
PGPG	131	26.0	139	20.1	33	21.1
GPG	158	30.4	168	25.0	47	19.1
PPG	152	28.9	164	22.6	43	16.2
PG	165	27.3	175	21.1	42	23.8
Ovsynch	135	37.8	151	29.8	50	22.0
Total ³	741	30.0	797	23.7	215	20.5

¹PMPG (PGF_{2α}, 3 d; GnRH, 8 d; PGF_{2α}, 2 d; GnRH and TAI), GPG (GnRH, 8 d; PGF_{2α}, 2 d; GnRH and TAI), PPG (PGF_{2α}, 11 d; PGF_{2α}, 2 d; GnRH and TAI), PG (PGF_{2α}, 2 d; GnRH and TAI), Ovsynch (GnRH, 7 d; PGF_{2α}, 2 d; GnRH and TAI). PGF_{2α} = 5 mL of Lutalyse sterile solution (Pfizer Animal Health, Kalamazoo, MI); GnRH = 2 mL of Cystorelin (Merial, Iselin, NJ).

²Embryonic loss was calculated for cows that were pregnant on d 28. Cows that were pregnant on d 28 and pregnant on d 42 were coded as 0, no embryonic loss. Cows that were pregnant on d 28 and not pregnant on d 42 were coded as 1, having embryonic loss.

³There were 804 cows in the trial. Sixty-three cows and 7 cows were not pregnancy-diagnosed on d 28 and 42, respectively.

cows were inseminated after the first injection of PGF_{2α} and there was no evidence for lesser TAI pregnancy rates in PMPG cows compared with Cosynch cows. The cows in estrus within 3 d after the first PGF_{2α} would be on d 8 to 10 of the estrous cycle at the final GnRH if they were not inseminated and continued on the program. The first-wave dominant follicle on d 8 to 10 of the estrous cycle would be near the end of its expected life span (Roche et al., 1999; Mihm et al., 2002). Aged follicles and oocytes having poorer fertility may therefore be sys-

Figure 3. Quadratic relationship between DIM and the percentage of cows with a corpus luteum at the d-28 pregnancy exam. The results are for the pregnancy exam after first timed AI. The DIM were recorded for each cow when her respective breeding group started on the trial (first injection). There were 268, 114, 115, 89, 61, and 132 cows with 40 to 80, 81 to 120, 121 to 160, 161 to 200, 201 to 240, and >240 DIM, respectively.

Table 2. Number inseminated and percentage pregnant at d 42 after insemination for cows that were initially treated with 1 of 5 first-insemination protocols and then treated with 1 of 2 resynchronization protocols

First TAI ¹	Second TAI ²	Pregnancy rate, d 42			
		Second TAI		First and second TAI ³	
		N	%	N	%
PMPG	ReGPG	31	19.4	44	34.1
PMPG	RePG	43	14.0	58	32.8
GPG	ReGPG	44	25.0	64	42.2
GPG	RePG	35	31.4	56	48.2
PPG	ReGPG	39	17.9	50	32.0
PPG	RePG	34	41.2	57	57.9
PG	ReGPG	33	36.4	51	51.0
PG	RePG	51	15.7	69	31.9
Ovsynch	ReGPG	28	14.3	48	39.6
Ovsynch	RePG	22	18.2	48	52.1
Total ⁴		360	23.1	545	42.0

¹PMPG [PGF_{2α}, 3 d; GnRH, 8 d; PGF_{2α}, 2 d; GnRH and timed AI (TAI)], GPG (GnRH, 8 d; PGF_{2α}, 2 d; GnRH and TAI), PPG (PGF_{2α}, 11 d; PGF_{2α}, 2 d; GnRH and TAI), PG (PGF_{2α}, 2 d; GnRH and TAI), Ovsynch (GnRH, 7 d; PGF_{2α}, 2 d; GnRH and TAI). PGF_{2α} = 5 mL of Lutalyse sterile solution (Pfizer Animal Health, Kalamazoo, MI); GnRH = 2 mL of Cystorelin (Merial, Iselin, NJ).

²Second TAI treatments (resynchronization) were applied to cows diagnosed as nonpregnant on d 28 after first TAI. The ReGPG treatment was GnRH on d 22, 7 d; PGF_{2α}, 2 d; GnRH and TAI. The RePG treatment was no treatment on d 22, 7 d; PGF_{2α}, 2 d; GnRH and TAI. Cows in the fourth breeding group were not resynchronized.

³Cows in breeding groups 1 to 3 (subjected to both first TAI and second resynchronized TAI) that were confirmed pregnant at 42 d to either the first or second TAI.

⁴There were 804 cows in the trial. Breeding groups 1 to 3 consisted of 675 cows that were eligible for both synchronization and resynchronization. The 360 cows comprised the 438 cows that were diagnosed as not pregnant at d 28 (candidates for resynchronization) minus 55 of the nonpregnant cows that did not continue on the resynchronization minus 23 cows that were treated but for which a final pregnancy diagnosis was not done.

tematically created by PMPG when estrual cows are not inseminated during the protocol. Although not tested in the present study, the implication is that PMPG could be used if estrus were detected after the first PGF_{2α}. This approach, which needs to be tested in a large field trial, would decrease cow handling and drug expense because cows inseminated after estrus are not treated with subsequent injections for TAI.

Although the results were unexpected and suggest a need to continue studying PMPG in its present form, a few specific points about this study can be made. By performing a carefully designed study, we were able to show that a fairly simple protocol (PG) could be used for TAI if a 21% pregnancy rate (d 42) were considered acceptable. By using a factorial design, it was also possible to measure the advantage conferred by the first injection of GnRH within the Ovsynch protocol (about a 9 percentage point increase in pregnancy rate over PG alone). Systematic evaluation of synchronization proto-

cols, as done in the present study, may be the only way to truly evaluate the efficacy of each injection, eliminate needless injections, and reduce the complexity of TAI programs.

A variety of explanatory variables were examined for their effects on d-28 pregnancy status, d-42 pregnancy status, and embryonic loss (d 28 to 42). The only factor found significant for the d-28 pregnancy rate was uterine or ovarian status; cows with an abnormal uterus or ovary (about 9% of the study cows) were less likely to be pregnant. The compromised state of the reproductive tract likely caused the lower pregnancy rates in cows with an abnormal uterus or ovary. Whereas only one explanatory factor was significant for the d-28 pregnancy rate, a variety of explanatory variables (postpartum disease, milk production, and DIM) were significant for the d-42 pregnancy rate. The fact that specific explanatory variables were not significant at d 28, but were significant at d 42, implies that the variables affecting the d-42 pregnancy rate might be acting in part through an effect on embryonic loss between d 28 and 42 (Table 1). This relationship was born out by our statistical analysis, in which postpartum disease, milk, and DIM (variables that were significant for the d-42 pregnancy rate) were significant explanatory variables for embryonic loss between d 28 and 42. An effect of postpartum disease on embryonic loss has been identified and a series of studies was reviewed (Santos et al., 2004). Clinical mastitis after breeding, for example, doubled the rate of embryonic loss (Moore et al., 2005).

The response surface for the probability of d-42 pregnancy relative to DIM and milk production showed an increasing probability of pregnancy with increasing DIM at TAI (Figure 2A). The surface for embryonic loss showed the opposite trend, in which embryonic loss decreased with DIM (Figure 2B). The collective interpretation is that d-42 pregnancy rates increase with DIM in part because embryonic loss between d 28 and d 42 is lower for cows with greater DIM. A greater rate of embryonic loss in cows that were inseminated early postpartum also was found in a large study of New Zealand dairy cows (McDougall et al., 2005). Cows having fewer DIM were more likely to be anestrus (Rhodes et al., 2003) and to have a clinical or subclinical uterine infection (Lewis, 1997; Sheldon, 2004; Gilbert et al., 2005). These 2 factors may in themselves lead to the greater rate of embryonic loss in early postpartum dairy cattle (Santos et al., 2004). Cows having fewer DIM also may have less complete uterine involution, a condition associated with low pregnancy rates (Sheldon, 2004). Others have found no relationship between DIM and embryonic loss (Chebel et al., 2004), and this may reflect inherent differences in the reproductive management systems used in the respective studies.

Cows can be resynchronized for TAI by placing nonpregnant cows on Ovsynch once their status (nonpregnant) has been diagnosed. This approach was initially applied after d 32 to 38 pregnancy diagnosis (Pursley et al., 1997) and was later applied earlier to nonpregnant cows (Chebel et al., 2003; Fricke et al., 2003; Bartolome et al., 2005). We tested the utility of the initial GnRH injection by randomly assigning cows to receive an injection of GnRH or no treatment at 22 d after first AI. This created 2 resynchronization groups (ReGPG and RePG). When examined across all treatments, the ReGPG treatment did not increase the number of cows with a CL at the d-28 pregnancy exam (87 vs. 83%, ReGPG vs. RePG). There was a quadratic relationship for DIM and the percentage of cows with a CL (Figure 3). This relationship may reflect an improved metabolic status of later postpartum cows and the positive effect of improved metabolic status on the follicle and its capacity to ovulate (Lucy, 2003). The GnRH injection did not increase pregnancy rates to the resynchronization (23 vs. 23%, ReGPG vs. RePG) and had no effect on the overall pregnancy rates to first TAI [either the d-28 pregnancy exam (30 vs. 30%), the d-42 pregnancy exam (22 vs. 24%), or embryonic loss (23 vs. 19%), ReGPG vs. RePG, respectively]. The latter results (no effect of GnRH treatment on pregnancy) are in agreement with those of others who administered GnRH on d 21 (Chebel et al., 2003). Final pregnancy rates across all treatments for the first TAI (23.7%; Table 1) and the second resynchronized TAI (23.1%; Table 2) were nearly identical.

We detected an interaction effect of the first TAI treatment with the resynchronization treatment on the pregnancy rate in response to resynchronization and also for the overall pregnancy rate (entire trial; Table 2). This interaction was unexpected and was not anticipated in the initial trial design. Thus, the sample sizes for each treatment combination were small. The interaction of the first TAI treatment and the second TAI treatment was apparently caused by individual combinations of treatments that were superior in terms of pregnancy rate. For example, cows initially synchronized with PG and then resynchronized with ReGPG had a final pregnancy rate of greater than 51%. Cows initially synchronized with PG and then resynchronized with RePG had a final pregnancy rate of 32%, nearly 20 percentage points less. This may be explained by the advantage of placing cows with relatively poor follicular synchrony (PG program) back on an Ovsynch-style resynchronization program (ReGPG). In contrast, cows initially placed on PPG had numerically greater final pregnancy rates when resynchronized with the RePG program. As mentioned, the individual cell sizes were small for this analysis. Nonetheless, the results are intriguing in that they imply an interaction of synchronization systems when dif-

ferent systems are used for first and second TAI. Programs for resynchronization may need to be evaluated in the context of the entire postpartum reproductive program.

CONCLUSIONS

The PGPG TAI protocol was tested. Initial studies suggested an advantage for PGPG relative to other synchronization programs. In the current study, however, PGPG was found to have no advantage relative to other TAI programs and yielded pregnancy rates that were numerically lower than those with Ovsynch. Surprisingly, cows treated with a simple protocol (PGF_{2α}, 48 h; GnRH and TAI) had pregnancy rates that were equivalent to PGPG. Early-lactation and high-milk-producing cows had high rates of embryonic loss between d 28 and 42 of pregnancy. Pregnancy rates for resynchronized second-insemination TAI on d 31 were not improved by treating cows with GnRH 7 d before the PGF_{2α} injection.

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