Reducing the Interval from Presynchronization to Initiation of Timed Artificial Insemination Improves Fertility in Dairy Cows

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ABSTRACT

The objective was to determine if reducing the interval from presynchronization to the first GnRH injection (G1) of a timed artificial insemination (AI) protocol improves pregnancy per AI. One thousand two hundred fourteen Holstein cows, at 37 ± 3 d in milk (DIM), were stratified by parity, DIM, and milk yield in the first month postpartum and randomly assigned to control ($n = 412$), 2 injections of PGF_{2 α} at 37 ± 3 and 51 ± 3 DIM, then enrolled in a timed AI protocol 14 d later; PShort ($n = 410$), 2 injections of PGF_{2 α} at 40 ± 3 and 54 ± 3 DIM, then enrolled in a timed AI protocol 11 d later; or PShortG ($n = 392$), same as PShort, but with an injection of GnRH 7 d before G1. All cows received the same timed AI protocol (d 65, G1; d 72, PGF_{2 α} ; d 73, 1 mg of estradiol cypionate; d 75, AI). A subset of 1,000 cows had their ovaries examined by ultrasonography at G1 and 7 d later when PGF_{2 α} of the timed AI was given to determine presence of corpus luteum (CL) and ovulation to G1. Pregnancy was diagnosed on d 38 after timed AI, and pregnant cows were reevaluated for pregnancy 4 wk later. Altering the interval between presynchronization and G1 did not affect the proportion of cows with a CL at G1, but GnRH 7 d before G1 increased the proportion of cows with a CL. Ovulation to G1 was greater for 11 compared with the 14 d interval, but GnRH did not improve ovulation. The increased ovulation to G1 when the interval was reduced from 14 to 11 d was observed only in cows with a CL at G1, but treatment did not affect ovulation in cows without a CL at G1. Treatment affected the pregnancy per AI on d 38 and 66 after insemination, and they were greater for the 11 compared with 14-d interval, but addition of GnRH did not improve pregnancy per AI. Cows ovulating to G1 had greater pregnancy per AI regardless of whether or not they had a CL at G1. Reducing the interval from presynchronization to initiation of the

timed AI protocol from 14 to 11 d increased ovulation to G1 and pregnancy per AI in lactating dairy cows.

Key words: dairy cow, presynchronization, reproduction, timed artificial insemination

INTRODUCTION

Because of the challenging aspect of estrus detection, insemination protocols that use GnRH and PGF_{2 α} to synchronize emergence of follicle wave, corpus luteum (CL) regression, and ovulation (e.g., Ovsynch; Pursley et al., 1995) have become an integral portion of reproductive management in dairy herds (Caraviello et al., 2006). After the advent of the Ovsynch protocol for timed insemination of cows (Pursley et al., 1995), a protocol for presynchronization of estrus with 2 injections of PGF_{2 α} administered 14 d apart with initiation of the timed AI protocol 12 (Moreira et al., 2001) or 14 d later (Navanukraw et al., 2004) was developed and increased pregnancy per AI in cyclic cows (Moreira et al., 2001) and all cows (Navanukraw et al., 2004).

The improvement in pregnancy per AI for cows submitted to timed AI protocols after presynchronization is believed to be caused by an increased proportion of cows in early to mid diestrus when the first GnRH injection (G1) of the timed AI protocol is administered, thereby improving synchronization of follicle wave emergence and preventing spontaneous luteolysis before completion of the protocol (Vasconcelos et al., 1999; Moreira et al., 2000). Although initiating the Ovsynch 12 or 14 d after the presynchronization increased pregnancy per AI compared with no presynchronization, it is unknown if it altered ovulation to G1, which is critical for enhancing pregnancy per AI in timed AI protocols (Chebel et al., 2006) because ovulation to G1 enhanced embryo quality in lactating dairy cows (Cerri et al., 2005).

Chebel et al. (2006) observed that most cows were in estrus between 3 and 6 d after the second PGF_{2 α} of the presynchronization protocol when no progesterone insert was utilized during presynchronization. Therefore, if estrous response after the second PGF_{2 α} of the presynchronization follows a similar pattern to that observed by Chebel et al. (2006), it is plausible to specu-

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late that the best day to initiate the timed AI protocol with GnRH would be 11 d later because cows would be on estrous cycle d 5 to 8, when ovulatory response to G1 should be optimized. Furthermore, at the time of the PGF_{2α} of the timed AI, cows should be on estrous cycle d 12 to 15, which precedes spontaneous luteolysis, thereby minimizing asynchrony of ovulation when the program is completed.

Although presynchronization with PGF_{2α} significantly improved response to the first timed AI, PGF_{2α} has no effect on the estrous cycle of anovular cows, thereby limiting the efficacy of presynchronization programs based solely on PGF_{2α}. Recent data from Bello et al. (2006) demonstrated that cows receiving GnRH 2 d after induced luteolysis had greater response to G1 when the Ovsynch protocol was initiated 6 d later. Furthermore, their study demonstrated that synchronization of ovulation to the Ovsynch protocol was improved when G1 was administered 8 d after induced luteolysis or 6 d after a previous GnRH injection (Bello et al., 2006). We speculated that administering GnRH 4 d after induced luteolysis and 7 d before G1 would increase the proportion of cows with a CL at the time of G1 and maximize ovulation to G1. Furthermore, administration of GnRH 7 d before G1 was expected to induce cyclicity in anovular cows, which might improve fertility in dairy cows.

The hypotheses of the current study were that reducing the interval from presynchronization to initiation of the timed AI protocol from 14 to 11 d would increase ovulation to G1 in cows with a CL at G1 and, consequently, improve pregnancy per AI. It was further hypothesized that inclusion of a GnRH injection 7 d before initiation of the timed AI would increase the number of cows with CL and improve incidence of ovulation to G1 and pregnancy per AI in cows receiving the treatment with an 11-d interval between presynchronization and G1. Therefore, the objectives of the current study were to compare incidence of ovulation, pregnancy per AI, and pregnancy loss of lactating dairy cows subjected to 2 different intervals between presynchronization with PGF_{2α} and initiation of timed AI (11 vs. 14 d). An additional objective was to evaluate if administration of GnRH 7 d before initiation of the timed AI protocol would further improve reproductive responses to a shorter interval between presynchronization and G1.

MATERIALS AND METHODS

Animals, Housing, and Feeding

The University of California Davis Institutional Animal Care and Use Committee approved all procedures involving animals used for this study. Lactating Holstein cows, 913 primiparous and 301 multiparous from

a commercial dairy farm with 5,800 dairy cows in the San Joaquin Valley of California were used in the study. The 3.5% FCM rolling herd average for the year of 2006 was 10,600 kg/cow, with average ($\pm$ SE) daily milk yield for the enrolled primiparous and multiparous cows during the study period of 29.1 ± 0.2 and 41.7 ± 0.35 kg/d, respectively. Cows were fed the same diet as TMR, once daily, formulated using the CPM-Dairy software (version 3.0.8) and evaluated using the NRC (2001) software. Diets met or exceeded the dietary requirements for a lactating cow weighing 680 kg and producing 45 kg of 3.5% FCM when consuming 25 kg/d of DM (NRC, 2001). The chemical analysis of the TMR resulted in 92.2% OM, 17.1% CP, 29.8% NDF, 19.5% ADF, 4.1% lignin, 4.8% crude fat, 0.64% Ca, 0.40% P, 0.38% Mg, 1.31% K, 0.30% S, 0.32% Na, and 0.28% Cl, with estimated NE_L of 1.70 Mcal/kg and RDP of 9.3% of DM.

Cows were housed in freestall barns equipped with fans and sprinklers that were activated when environmental temperature exceeded 24°C. Primiparous and multiparous cows were housed in separate pens throughout the study. Cows were milked 3 times daily, and yields of milk were measured and recorded automatically for individual cows for each milking. Average milk yield during the first 8 wk postpartum was utilized to determine the effect of milk yield on reproductive responses. All cows had their body condition scored using a 5-point (1 = thin to 5 = fat) system (Ferguson et al., 1994) on the day of G1.

Treatments and Artificial Insemination

Weekly, a cohort of 41 to 120 cows at 37 ± 3 DIM were stratified by parity, DIM, and milk yield in the first month of lactation, and randomly assigned to 1 of 3 treatments: control ($n = 412$), 2 injections of PGF_{2α} at 37 ± 3 and 51 ± 3 DIM, then enrolled in a timed AI protocol 14 d later; **PShort** ($n = 410$), 2 injections of PGF_{2α} at 40 ± 3 and 54 ± 3 DIM, then enrolled in a timed AI protocol 11 d later; **PShortG** ($n = 392$), same as PShort, but with an injection of GnRH 7 d before G1 (Figure 1).

The timed AI protocol consisted of a 100-μg injection of GnRH i.m. (Cystorelin, 50 μg/mL of gonadorelin diacetate tetrahydrate, Merial Ltd., Iselin, NJ) on d 65, followed 7 d later by an s.c. injection of 25 mg of PGF_{2α} (Lutalyse, 5 mg of dinoprost tromethamine/mL, Pfizer Animal Health, New York, NY), and 24 h later by an i.m. injection of 1 mg of estradiol cypionate (**ECP**, 2 mg/mL estradiol cypionate, Pfizer Animal Health, New York, NY), with timed AI performed 48 h after the ECP injection (Pancarci et al., 2002). Cows were observed for signs of estrus once daily, in the afternoon, by removal of tail chalk applied daily using paintsticks (All-

Figure 1. Diagram of activities during the study and description of treatments indicated as control, PShort, and PShortG. ECP = injection of 1 mg of estradiol cypionate; GnRH = injection 100 μ g of gonadotropin releasing hormone; PGF = injection of 25 mg of prostaglandin $F_{2\alpha}$; US = ultrasonographic examination of the ovaries.

weather Paintstik, LA-CO Industries, Chicago, IL), and cows observed in estrus after the injection of ECP were inseminated the same morning or timed AI as indicated previously. Proportion of cows inseminated in estrus the day after ECP injection was similar ($P = 0.83$) for all treatments and averaged 20%, with the remainder 80% inseminated on the day of timed AI.

Ovarian Ultrasonography and Ovulatory Responses

Ultrasonographic examination of the ovaries was performed using a 7.5-MHz linear transducer (Sonovet 2000, Universal Medical System, Bedford Hills, NY) in a subset of 1,000 cows at 65 and 72 DIM, which coincided with the injections of G1 and PGF $_{2\alpha}$ of the timed AI protocol. Data from ultrasonography were used to determine presence of CL at G1 and PGF $_{2\alpha}$ of the timed AI protocol and to evaluate ovulation to G1, which was characterized by appearance of a new CL in either ovary on d 72 (Figure 1).

Pregnancy Diagnosis and Pregnancy Loss

All cows were examined for pregnancy by palpation of the uterus per rectum on d 38 ± 1 after AI, and pregnant cows were reevaluated for pregnancy 4 wk later, on d 66 ± 1 after AI. The detection of an embryonic vesicle was used as indicator of pregnancy. Pregnancy per AI was defined as the number of pregnant cows divided by the total number of AI in each treatment,

which corresponded to the total number of cows in each treatment because all animals were inseminated. Cows diagnosed as pregnant on d 38 and found to be nonpregnant on d 66 were considered to have experienced pregnancy loss.

Statistical Analyses

The experimental design was a completely randomized within weekly cohorts, after stratification by parity, DIM, and milk yield in the first month postpartum. Dichotomous outcomes such as presence of a CL at G1 or at the PGF $_{2\alpha}$ of the timed AI protocol, incidence of ovulation after G1, pregnancy per AI, and pregnancy loss were analyzed by logistic regression using the LOGISTIC procedure of SAS (SAS Inst. Inc., Cary, NC). A backward stepwise regression model was used and variables were continuously removed from the model by the Wald statistic criterion if $P > 0.15$. Explanatory variables considered for inclusion in the models were treatment, parity (primiparous vs. multiparous), BCS categorized as <2.75 or ≥ 2.75 , milk production in the first 8 wk postpartum categorized as above or below the mean for primiparous and multiparous, and month when AI was performed. Presence of a CL at G1 and ovulation to G1 were analyzed for the subset of 1,000 cows in which ultrasonography was performed. In addition, preplanned orthogonal contrasts were performed to determine the effect of interval between presynchronization and G1 for 14 vs. 11 d (control vs. PShort +

Table 1. Effect of presynchronization treatment on ovarian dynamics of dairy cows

Item	Presynchronization treatment ¹			P-value ²		
	Control	PShort	PShortG	TRT	C1	C2
	[% (no./no.)]					
Corpus luteum (CL) at first GnRH of timed AI	80.6 (274/340)	74.2 (250/337)	88.2 (285/323)	<0.001	0.95	<0.001
Ovulation to first GnRH of timed AI						
Overall	44.7 (152/340)	61.4 (207/337)	62.2 (201/323)	<0.001	<0.001	0.28
Cows with CL at first GnRH	37.2 (102/274)	54.4 (136/250)	59.7 (170/285)	<0.001	<0.001	0.29
Cows without CL at first GnRH	75.8 (50/66)	81.6 (71/87)	81.6 (31/38)	0.63	0.34	0.99
CL at PGF _{2α} of timed AI	90.9 (309/340)	93.5 (315/337)	94.7 (306/323)	0.16	0.07	0.49

¹Control = 2 injections of PGF_{2α} at 37 and 51 DIM, then enrolled in the timed AI 14 d later; PShort = 2 injections of PGF_{2α} at 40 and 54 DIM, then enrolled in the timed AI 11 d later; PShortG = same as PShort, but with an injection of GnRH 7 d before the first GnRH of the timed AI.

²TRT = Effect of treatment; C1 = preplanned orthogonal contrast for the effect of 14 vs. 11 d interval (control vs. PShort + PShortG); C2 = preplanned orthogonal contrast for the effect of GnRH 7 d before initiation of timed AI (PShort vs. PShortG).

PShortG) and the effect of inclusion of GnRH 7 d before G1 (PShort vs. PShortG) on all reproductive outcomes.

Treatment differences with $P \leq 0.05$ were considered significant, and $0.05 < P \leq 0.10$ were considered a tendency toward difference.

RESULTS

Effect of Presynchronization Protocol on Ovarian Dynamics

Treatment affected ($P < 0.001$) the presence of a CL at G1, primarily because of inclusion of GnRH 7 d before G1 (Table 1). Interval between presynchronization and G1 had no influence on the proportion of cows with a CL at G1, but more ($P < 0.001$) cows receiving GnRH 7 d before G1 had a CL at the initiation of the timed AI protocol. Presence of a CL at G1 was also affected by BCS and parity. Proportion of cows having a CL was greater ($P < 0.001$) for cows with BCS ≥ 2.75 than for those with BCS < 2.75 (88.6 vs. 75.7%) and for multiparous than primiparous cows (86.5 vs. 79.4%). Ovulation to G1 was affected ($P < 0.001$) by treatment and was greater ($P < 0.001$) for cows receiving the presynchronization with 11 compared with 14 d interval to G1; however, addition of GnRH 7 d before G1 had no influence on ovulatory response. The increased incidence of ovulation to G1 for PShort and PShortG was primarily because of a greater ($P < 0.001$) ovulatory response in cows with a CL at G1 because ovulation incidence was not affected by treatment in cows without a CL at G1 (Table 1). Incidence of ovulation after G1 was also affected by parity and presence of a CL at G1. Multiparous cows had greater ($P = 0.003$) ovulation incidence than primiparous cows (63.1 vs. 54.1%), and cows with no CL at G1 had greater ($P < 0.001$) ovulation incidence than cows with a CL (79.6 vs. 50.4%). Reducing the interval from presynchronization to G1 tended to in-

crease ($P = 0.07$) the proportion of cows with a CL at the day of induced luteolysis in the timed AI, but addition of GnRH 7 d before initiating the timed AI had no influence on presence of CL the day of PGF_{2α} injection of the timed AI.

Effect of Ovulation to G1 and Presence of a CL at G1 on Pregnancy per AI and Pregnancy Loss

Cows ovulating to G1 had greater ($P < 0.05$) pregnancy per AI on d 38 and 66 after AI regardless of the presence of a CL at G1, but ovulation did not affect pregnancy loss (Table 2). Cows with a CL at G1 had greater ($P < 0.01$) pregnancy per AI on d 38 and 66 after AI and smaller ($P = 0.04$) pregnancy loss compared with cows without a CL at G1. No interaction ($P > 0.15$) was observed between presynchronization treatment and presence of CL or incidence of ovulation to G1 on the reproductive responses evaluated.

Effect of Presynchronization Protocol on Pregnancy per AI and Pregnancy Loss

Reducing the interval between presynchronization and G1 from 14 to 11 d increased pregnancy per AI on d 38 ($P = 0.02$) and 66 ($P = 0.04$) after timed AI (Table 3). However, injection of GnRH 7 d before G1 did not further improve pregnancy per AI of cows subjected to the 11-d interval between presynchronization and initiation of the timed AI protocol. Parity, BCS at G1, and month when cows were inseminated also affected ($P < 0.01$) pregnancy per AI, and they were smaller on d 38 after timed AI for multiparous than primiparous cows (30.6 vs. 40.3%), in cows with BCS < 2.75 than those with BCS ≥ 2.75 (31.8 vs. 45.0%), and for cows inseminated in the month of July than other months (30.4 vs. 41.6%). Similar effects were observed on d 66 after timed AI. Pregnancy loss between 38 and 66 d

Table 2. Effect of presence of a corpus luteum (CL) at the moment of the first GnRH (G1) of the timed AI protocol and ovulation to G1 on pregnancy per AI and pregnancy loss in dairy cows

	CL at G1		No CL at G1		P-value ¹		
	Ovulation	No ovulation	Ovulation	No ovulation	CL	Ovulation	CL × ovulation
Pregnant ²	[% (no./no.)]						
d 38	42.2 (172/408)	37.7 (151/401)	27.6 (42/152)	15.4 (6/39)	<0.01	0.05	0.30
d 66	39.6 (161/407)	34.2 (137/401)	22.0 (33/150)	15.4 (6/39)	<0.01	0.02	0.80
Pregnancy loss ³							
d 38 to 66	5.9 (10/171)	9.3 (14/151)	17.5 (7/40)	0.0 (0/6)	0.04	0.18	—

¹CL × ovulation = interaction between CL and ovulation.

²There were 2 and 1 cows diagnosed pregnant on d 38 but culled before pregnancy diagnosis on d 66 for cows without a CL and cows with a CL, respectively.

³The interaction between presence of CL at the first GnRH of the timed AI and ovulation to the first GnRH was not included in the logistic regression model due to quasi-complete separation of data points.

of gestation was not influenced by interval between presynchronization and G1, or by administration of GnRH 7 d before G1 (Table 3). The only predictors of pregnancy loss were parity and absence of CL at G1, and multiparous cows experienced greater ($P = 0.01$) pregnancy loss than primiparous cows (16.3 vs. 7.4%), and cows without a CL at G1 had greater pregnancy loss than those with a CL. No significant interaction was observed between treatment and other variables included in the models for any of the outcomes evaluated.

DISCUSSION

To the best knowledge of the authors this is the first time that different intervals between presynchronization with PGF_{2α} and initiation of the timed AI protocol have been compared for their effects on fertility in lactating dairy cows. The improvement in pregnancy per AI was likely the consequence of increased ovulatory response to G1 in cows with CL and reduced spontaneous luteolysis before the PGF_{2α} of the timed AI protocol.

Reducing the interval from 14 to 11 d tended to increase the proportion of cows with a CL at the PGF_{2α}, which was probably consequent to increased ovulation to G1, but possibly reduced spontaneous luteolysis as the cows that responded to the presynchronization were expected to be 3 d earlier in their estrous cycle in PShort and PShortG compared with control.

Cows that ovulated to G1 had greater pregnancy per AI to the Ovsynch protocol (Chebel et al., 2006). Moreover, ovulation to G1 improved embryo quality in lactating dairy cows (Cerri et al., 2005), which might partially explain the benefits to fertility of dairy cows. Addition of a GnRH injection 7 d before G1 in cows subjected to the 11-d interval treatment did not further increase pregnancy per AI, which suggests that GnRH was not required to optimize presynchronization. The lack of fertility response to the additional GnRH was likely caused by the inability of that treatment to improve ovulation to G1 in cows with 11-d interval. Administration of GnRH 2 d after a single PGF_{2α} injection, and initiating the Ovsynch protocol 6 d later has recently been shown to improve synchronization rate and tended

Table 3. Effect of presynchronization treatment on pregnancy per AI and pregnancy loss in dairy cows

	Presynchronization treatment ¹			P-value ²		
	Control	PShort	PShortG	TRT	C1	C2
Pregnant ³	[% (no./no.)]					
d 38	33.5 (138/412)	40.5 (166/410)	39.8 (156/392)	0.06	0.02	0.60
d 66	30.2 (124/410)	36.4 (149/409)	36.2 (142/392)	0.09	0.04	0.70
Pregnancy loss						
d 38 to 66	8.8 (12/136)	9.7 (16/165)	9.0 (14/156)	0.96	0.88	0.85

¹Control = 2 injections of PGF_{2α} at 37 and 51 DIM, then enrolled in the timed AI 14 d later; PShort = 2 injections of PGF_{2α} at 40 and 54 DIM, then enrolled in the timed AI 11 d later; PShortG = same as PShort, but with an injection of GnRH 7 d before the first GnRH of the timed AI.

²TRT = Effect of treatment; C1 = preplanned orthogonal contrast for the effect of 14 vs. 11 d interval (control vs. PShort + PShortG); C2 = preplanned orthogonal contrast for the effect of GnRH 7 d before initiation of timed AI (PShort vs. PShortG).

³There were 1 and 2 cows diagnosed pregnant on d 38 after AI, but culled before pregnancy diagnosis on d 68 of gestation from treatments PShort and control, respectively.

to improve pregnancy per AI when compared with initiating the Ovsynch at random stages of the estrous cycle (Bello et al., 2006). A single injection of $\text{PGF}_{2\alpha}$ given at random stages of the estrous cycle would likely induce estrus in no more than 70% of the cyclic cows, assuming that cows before d 4 of the estrous cycle would not respond, approximately 40% would respond on d 4, 80 to 90% on d 5 and 6, and more than 90% after d 6 of the estrous cycle. Therefore, when a single injection of $\text{PGF}_{2\alpha}$ is given, it is likely that synchronizing ovulation with GnRH 2 d later would benefit responses to the timed AI protocol initiated 8 d after the $\text{PGF}_{2\alpha}$. However, our results demonstrated that in programs in which presynchronization is composed of 2 $\text{PGF}_{2\alpha}$ injections, addition of GnRH 7 d before the initiation of the timed AI protocol did not improve pregnancy per AI in lactating dairy cows.

Multiparous cows, cows with $\text{BCS} < 2.75$, and cows inseminated during periods of heat stress have all been shown to experience reduced pregnancy per AI (Chebel et al., 2004; Rutigliano and Santos, 2005). Multiparous cows had reduced pregnancy per AI compared with primiparous cows in part because of increased pregnancy losses (Rutigliano and Santos, 2005; García-Ispuerto et al., 2006). Reduced BCS at AI were associated with increased anestrus (Rutigliano and Santos, 2005), and heat stress reduced fertilization and embryo quality in lactating cows (Sartori et al., 2002).

Cows without a CL at G1 experienced greater pregnancy loss from 38 to 66 d of gestation. Previously, it was suggested that anovular cows were at greater risk for pregnancy loss than cyclic cows (Santos et al., 2004), which has since been confirmed by Galvão et al. (2004). Recently, it was demonstrated that anovular cows were 1.3 times more likely to experience pregnancy loss between 30 and 58 d of gestation than cyclic cows (Rutigliano and Santos, 2005). It is possible that the increased pregnancy loss observed for cows with no CL at G1 was related to prevalence of anovular animals in that group of cows because most cows with no CL 11 to 14 d after presynchronization with 2 $\text{PGF}_{2\alpha}$ are likely not to be cycling. Most cyclic cows were expected to be between d 5 and 10 of the estrous cycle when the Ovsynch was initiated 12 d after presynchronization (Moreira et al., 2001).

As expected, injection of GnRH 7 d before G1 increased the proportion of cows with a detectable CL at G1 in cows subjected to the 11-d interval. This effect was probably because of induced ovulation in anovular cows and increased synchrony of ovulation after the GnRH injection 7 d before initiation of the timed AI protocol. In addition, the reduced proportion of cows with a CL for the PShort treatment might have been caused by animals that displayed estrus on d 8, 9, and

10 after the presynchronization. Chebel et al. (2006) observed that approximately 15% of the cows displayed estrus between d 8 and 10 after the presynchronization; therefore, performing the ultrasonography 11 d after the second $\text{PGF}_{2\alpha}$ instead of 14 d may not have allowed detection of a CL in all the cyclic cows in PShort.

Shortening the interval from presynchronization to G1 from 14 to 11 d was effective in increasing the ovulatory response to G1, and this effect was observed primarily in cows with a CL at G1. Cows in PShort and PShortG were likely between d 5 and 8 of the estrous cycle at G1 given that the majority of cows displayed estrus 3 to 6 d after the presynchronization (Chebel et al., 2006), and administration of GnRH between d 5 and 9 of the estrous cycle increased ovulatory response (Vasconcelos et al., 1999). Cows with a CL at G1 were cyclic and, therefore, should have responded to the presynchronization with $\text{PGF}_{2\alpha}$ and had their estrous cycle synchronized (Moreira et al., 2001). Conversely, cows without a CL at G1 were anovular or in proestrus, and these cows are expected to have high incidence of ovulation to GnRH but, if anovular, to not have responded to the presynchronization with $\text{PGF}_{2\alpha}$ (Moreira et al., 2001; Gümen et al., 2003). Another possibility is that these cows were in metestrus, but without a visible CL. From the pattern of estrous expression after presynchronization with $\text{PGF}_{2\alpha}$, approximately 20% of the cows observed in estrus were detected on d 9 to 13 after the second $\text{PGF}_{2\alpha}$ (Chebel et al., 2006). Therefore, it is possible that a small proportion of cows diagnosed as ovulating to G1 might be cows ovulating 1 to 4 d before G1.

It is not completely clear why multiparous cows had greater ovulatory response to G1 compared with primiparous cows. Previously, it was demonstrated that incidence of ovulation to GnRH given at random stages of the estrous cycle was reduced for nulliparous compared with lactating dairy cows (Pursley et al., 1995), but to our knowledge no previous studies have observed a difference in incidence of ovulation between primiparous and multiparous cows.

Ovulation to G1 was positively associated with increased pregnancy per AI on d 38 and 66 after insemination, which has also been observed by others (Chebel et al., 2006). Despite no evaluation of ovulation at completion of the timed AI protocol in the current study, cows that ovulated to G1 were more likely to ovulate to the final GnRH of the Ovsynch protocol, which leads to improved synchronization of ovulation and, consequently, increased pregnancy per AI (Vasconcelos et al., 1999; Bello et al., 2006). Although ECP and not GnRH was used to synchronize ovulation at completion of the timed AI protocol in the current study, it is expected that ovulation in response to estradiol would

also be improved in cows that ovulated at the beginning of the protocol after G1. Furthermore, cows that ovulated to G1 had improved embryo quality (Cerri et al., 2005), which is expected to favor pregnancy.

CONCLUSIONS

Reducing the interval from presynchronization to G1 increased the proportion of cows ovulating to G1, and this response was observed in cows with a CL at the day of G1. Compared with 14 d, lactating dairy cows presynchronized 11 d before G1 experienced increased pregnancy per AI. The benefits of reducing the interval from presynchronization to the timed AI protocol from 14 to 11 d were likely the result of improved ovulatory response to G1 because cows that ovulated had increased pregnancy per AI. Administration of GnRH 7 d before G1 did not further increase ovulatory response and pregnancy per AI of lactating dairy cows.

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