Resynchronization strategies to improve fertility in lactating dairy cows utilizing a presynchronization injection of GnRH or supplemental progesterone: I. Pregnancy rates and ovarian responses

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ABSTRACT

Objectives were to evaluate 3 resynchronization protocols for lactating dairy cows. At 32 ± 3 d after pre-enrollment artificial insemination (AI; study d -7), 1 wk before pregnancy diagnosis, cows from 2 farms were enrolled and randomly assigned to 1 of 3 resynchronization protocols after balancing for parity, days in milk, and number of previous AI. All cows were examined for pregnancy at 39 ± 3 d after pre-enrollment AI (study d 0). Cows enrolled as controls ($n = 386$) diagnosed not pregnant were submitted to a resynchronization protocol (d 0-GnRH, d 7-PGF_{2α}, and d 10-GnRH and AI) on the same day. Cows enrolled in the GGPG (GnRH-GnRH-PGF_{2α}-GnRH) treatment ($n = 357$) received a GnRH injection at enrollment (d -7) and if diagnosed not pregnant were submitted to the resynchronization protocol for control cows on d 0. Cows enrolled in CIDR treatment ($n = 316$) diagnosed not pregnant received the resynchronization protocol described for control cows with addition of a controlled internal drug release (CIDR) insert containing progesterone (P₄) from d 0 to 7. In a subgroup of cows, ovaries were scanned and blood was sampled for P₄ concentration on d 0 and 7. After resynchronized AI, cows were diagnosed for pregnancy at 39 ± 3 and 67 ± 3 d (California herds) or 120 ± 3 d (Arizona herds). Cows in the GGPG treatment had more corpora lutea than CIDR and control cows on d 0 (1.30 ± 0.11 , 1.05 ± 0.11 , and 1.05 ± 0.11 , respectively) and d 7 (1.41 ± 0.14 , 0.97 ± 0.13 , and 1.03 ± 0.14 , respectively). A greater percentage of GGPG cows ovulated to GnRH given on d 0 compared with CIDR and control cows (48.4, 29.6, and 36.6%,

respectively), but CIDR and control did not differ. At 39 ± 3 d after resynchronized AI, pregnancy per AI (P/AI) was increased in GGPG (33.6%) and CIDR (31.3%) cows compared with control (24.6%) cows. At 67 or 120 ± 3 d after resynchronized AI, P/AI of GGPG and CIDR cows was increased compared with control cows (31.2, 29.5, and 22.1%, respectively). Presynchronizing the estrous cycle of lactating dairy cows with a GnRH 7 d before the start of the resynchronization protocol or use of a CIDR insert within the resynchronization protocol resulted in greater P/AI after resynchronized AI compared with control cows.

Key words: resynchronization, GnRH, progesterone, dairy cow

INTRODUCTION

Reduced pregnancies per AI (P/AI) and estrous expression in lactating dairy cows in confinement have led to dramatic reproductive inefficiency in the dairy industry. After the initial postpartum AI, approximately 60% of lactating dairy cows do not conceive (Cerri et al., 2004; Chebel et al., 2006; Galvão et al., 2007). In addition, the percentage of cows that are reinseminated before being presented for pregnancy diagnosis is usually <60%; thus, cows that do not become pregnant may have intervals between AI longer than the duration of 2 estrous cycles if nonpregnancy diagnosis is not performed and resynchronization protocols are not implemented in a timely manner. Therefore, to avoid extended inter-insemination intervals, extended DIM, and reduced milk yield, it is critical that an efficient protocol for resynchronization and re-AI of nonpregnant cows is implemented.

These inefficiencies have led to research in synchronization of the estrous cycle and ovulation of lactating dairy cows allowing for timed AI. Synchronization of ovulation can be achieved by administration of GnRH, followed 7 d later by a PGF_{2α} injection, and 2 d later a second GnRH injection, resulting in ovulation approximately 30 h later, allowing for timed AI 12 to 16

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h after the second GnRH injection (Ovsynch; Pursley et al., 1995). Cows treated with GnRH between d 5 and 9 of the estrous cycle more likely ovulated, formed a new corpus luteum (CL), and developed a new follicular wave resulting in synchronized ovulation of a newly formed dominant follicle at the end of the synchronization protocol (Vasconcelos et al., 1999; Moreira et al., 2000). If cows fail to ovulate to the first GnRH injection, the follicle induced to ovulate at the end of the synchronization protocol may have undergone prolonged dominance, which leads to the ovulation of an older oocyte and consequently reduced embryo quality (Cerri et al., 2009) and decreased P/AI (Chebel et al., 2006). Pregnancies per AI can be reduced when synchronization protocols are initiated at random stages of the estrous cycle, because 10 to 30% of cows fail to have synchronized ovulation (Vasconcelos et al., 1999).

Improved ovulation to the first GnRH injection, synchronization of the estrous cycle, and P/AI can be achieved by presynchronizing the estrous cycle of cows with PGF_{2α} before the start of the synchronization protocols (Moreira et al., 2001; Galvão et al., 2007; Silva et al., 2007), but cows of unknown pregnancy status cannot be treated with PGF_{2α} until diagnosed not pregnant. Presynchronizing the estrous cycle of heifers with GnRH 6 d before the start of a synchronization protocol resulted in more heifers ovulating to the first GnRH injection of the synchronization protocol (Stevenson et al., 2008b). Because treatment of lactating dairy cows with GnRH did not pose a risk for maintenance of pregnancy (Chebel et al., 2003), its use as a presynchronization strategy for cows of unknown pregnancy status seems promising.

Treatment of lactating dairy cows with intravaginal progesterone (P₄) inserts induces cyclicity in anovular cows and heifers (Rhodes et al., 2003), tightens return to estrus in nonpregnant cows (Chenault et al., 2003), and improves synchronization of the estrous cycle when used during a synchronization protocol (Lima et al., 2009). Although in a few studies, use of an intravaginal P₄ insert within a synchronization protocol did not affect P/AI (El-Zarkouny et al., 2004; Galvão et al., 2004; Stevenson et al., 2006), in other studies, cows treated with intravaginal P₄ inserts during a synchronization protocol had increased P/AI after first postpartum AI (Stevenson et al., 2008a; Chebel et al., 2010), but it is not known whether the treatment of cows with intravaginal P₄ inserts during a resynchronization protocol could increase P/AI.

The hypotheses were that use of a presynchronization injection of GnRH 7 d before the start of a resynchronization protocol would result in improved ovulation to the first GnRH injection of the resynchronization protocol and improved P/AI. Furthermore, we hypoth-

esized that treating cows with an intravaginal P₄ insert during the resynchronization protocol would improve P/AI by improving synchronization of the estrous cycle before reinsemination.

Therefore, the objectives were to evaluate the effects of administering a GnRH injection 7 d before the start of a resynchronization protocol or treating cows with an intravaginal P₄ insert during the resynchronization protocol on ovarian responses, P₄ concentrations, P/AI, and pregnancy loss.

MATERIALS AND METHODS

Farm Description and Data Collection

The University of Arizona and University of California–Davis Institutional Animal Care and Use Committees approved all procedures. Lactating Holstein cows ($n = 1,059$) from 2 dairies, 1 in California (CA) and 1 in Arizona (AZ), were enrolled from January to June 2008. In CA, cows were housed in free-stall barns, whereas in AZ cows were housed in open dry-lot facilities. Primiparous and multiparous cows were housed separately and milked thrice daily at 8-h intervals throughout. At both sites, cows were fed a TMR, twice daily in AZ and once daily in CA, to meet or exceed the dietary requirements for lactating Holstein cows weighing 680 kg and producing 45 kg of 3.5% FCM (NRC, 2001). All cows received bST (Posilac, 500 mg, Monsanto Co., St. Louis, MO) at 11-d intervals in CA and at 14-d intervals in AZ, beginning 60 ± 3 d postpartum.

All cow lists including injection schedules, reproductive events, pregnancy examination, health events, and milk yield data on the test date closest to the date of reinsemination were generated, tracked, and recorded using a commercial on-farm computer software program (DHI Plus, DHI Computing Service, Provo, UT, or DairyComp 305, Valley Agricultural Software, Tulare, CA). To ensure accuracy and compliance, radio-frequency identification was used on both farms in accordance with the respective software.

Resynchronization Treatments

One day before enrollment, data from all eligible cows (≥ 1 previous AI) were extracted from the on-farm software and entered into a computer spreadsheet (Excel 2003, Microsoft Corp., Redmond, WA), and cows were randomly assigned to 1 of 3 resynchronization protocols after balancing for parity, DIM, and number of previous AI (Figure 1). The treatments were as follows: 1) control ($n = 386$): cows were diagnosed not pregnant (39 ± 3 d; study d 0) after pre-enrollment AI and sub-

BCS and Milk Yield

On d 0 all cows were evaluated for BCS (1 = emaciated, 5 = obese) as described by Ferguson et al. (1994). Milk yield data on the test date closest to the date of re-AI were divided into quartiles within parity (primiparous and multiparous) for purposes of statistical analysis.

Ovarian Ultrasonography

Ovaries from a subsample of cows ($n = 395$) in the CA herd were examined with ultrasound (Sonosite Titan, SonoSite Inc., or Sonovet 2000, Universal Ultrasound Inc., Bedford Hills, NY) equipped with a 5-MHz linear-array transducer on d 0 and 7. Presence, location, and size of CL and follicles >8 mm in diameter were recorded. Cows were considered to have ovulated to the GnRH injection given on d 0 if they had follicles ≥ 10 mm in diameter on d 0 and a new CL on d 7 on the same ovary. Cows that did not have follicles ≥ 10 mm in diameter on d 0 but had a new CL on d 7 were considered to have ovulated before the GnRH injection given on d 0.

Blood Sampling and Progesterone Analysis

Blood was collected (7 mL) from a subsample of cows via coccygeal venipuncture into evacuated heparinized tubes (Vacutainer, Becton Dickinson, East Rutherford, NJ) with Vacutainer collection needles (20 G; Becton Dickinson) on d 0 (AZ = 163 cows and CA = 189 cows) and d 7 (AZ = 146 cows and CA = 189 cows) for determination of plasma P_4 concentration. Upon collection, samples were immediately placed in ice and transferred to the laboratory within 8 h of collection. Samples were centrifuged at 4°C for 15 min at $2,000 \times g$ for plasma separation. Plasma was harvested and stored at -20°C until assayed for P_4 concentration. Assays were completed using a solid-phase no-extraction RIA (Coat-a-Count, Diagnostic Products Corp., Los Angeles, CA). Intra- and interassay coefficients of variation were 5.9 and 8.1%, respectively.

Cows with P_4 concentrations <1 ng/mL were considered without a functional CL and cows with $P_4 \geq 1$ ng/mL were considered to have a functional CL. Cows with P_4 concentration ≥ 1 ng/mL on d 0 and P_4 concentration <1 ng/mL on d 7 were considered to have had spontaneous luteolysis during the resynchronization protocol.

Statistical Analysis

Binomially distributed data were analyzed by logistic regression using the LOGISTIC procedure of SAS (SAS

Institute Inc., Cary, NC). All models included treatment (control, GGPG, or CIDR), parity (primiparous or multiparous), herd (AZ or CA), BCS, number of AI before enrollment, quartile of milk yield, and the interactions between treatment and parity, treatment and herd, and treatment and BCS. The final logistic regression model removed variables by a stepwise backward elimination based on the Wald statistic criterion when $P > 0.10$. Considering that the interval from resynchronized AI to the second pregnancy diagnosis was different between herds (67 ± 3 d after resynchronized AI in CA or at 120 ± 3 d after resynchronized AI in AZ), data were analyzed as P/AI at the second pregnancy diagnosis and pregnancy loss between the first and second pregnancy diagnosis separately for the AZ and CA herds. In addition, P/AI at the second pregnancy diagnosis and pregnancy loss between the first and second pregnancy diagnoses were analyzed by combining data from AZ and CA herds to determine whether the interaction between treatment and herd affected any of these outcomes.

Ordinal data were analyzed by Kruskal-Wallis using the GENMOD procedure of SAS. Concentration of P_4 and size of the largest follicle on d 0 and 7 were analyzed by ANOVA using the GLM procedure of SAS. Treatment, parity, herd, and their respective interactions were included in all models. Body condition score and milk yield quartiles were initially included in the models, but if they had $P > 0.10$, they were removed from the final analysis.

RESULTS

Parity Distribution, Interval from Pre-enrollment AI to Enrollment, DIM, Number of Previous AI, BCS, and Milk Yield

The percentage of primiparous cows enrolled (d -7) in each treatment was not ($P = 0.16$) different (control = 28.6, GGPG = 34.3, CIDR = 34.1%), but a greater ($P < 0.01$) percentage of primiparous cows were enrolled in the AZ herd than the CA herd (Table 1).

Interval from pre-enrollment AI to enrollment was not ($P = 0.90$) different among treatments (control = 32 ± 1 , GGPG = 32 ± 1 , CIDR = 32 ± 1 d), but cows in the AZ herd had shorter ($P < 0.01$) intervals from pre-enrollment AI to enrollment than those in the CA herd (Table 1).

Average DIM at enrollment was different ($P = 0.05$) among treatments. Control cows had greater ($P = 0.02$) DIM than GGPG cows at enrollment (146 ± 3 vs. 137 ± 3 d), whereas average DIM at enrollment of CIDR cows (141 ± 3 d) was not different from control ($P = 0.23$) or GGPG ($P = 0.26$) cows.

Table 1. Comparison of herds regarding percentage of first-lactation cows enrolled, interval between previous AI and enrollment, interval between previous AI and nonpregnancy diagnosis, DIM, number of previous AI, BCS at enrollment, milk yield on the test-day closest to enrollment, and projected 305-d milk yield

Item	Herd		P-value
	Arizona	California	
Number of cows enrolled	1,258	2,590	
Percentage pregnant to pre-enrollment AI, %	70.4	73.2	0.68
Percentage of first-lactation cows, %	38.8	28.2	<0.01
Interval between previous AI and enrollment, d ($\pm$ SEM)	31.2 $\pm$ 0.1	32.5 $\pm$ 0.1	<0.01
Interval between previous AI and pregnancy diagnosis, d ($\pm$ SEM)	38.2 $\pm$ 0.1	39.5 $\pm$ 0.1	<0.01
DIM at enrollment ($\pm$ SEM)	133 $\pm$ 3	149 $\pm$ 2	<0.01
Number of AI at enrollment			<0.01
Mean ($\pm$ SEM)	1.53 $\pm$ 0.04	2.37 $\pm$ 0.03	
Median	1.0	2.0	
BCS at enrollment			<0.01
Mean ($\pm$ SEM)	2.88 $\pm$ 0.02	2.79 $\pm$ 0.01	
Median	2.75	2.75	
Milk yield on the test-day closest to enrollment, kg/d ($\pm$ SEM)	44.1 $\pm$ 0.4	41.4 $\pm$ 0.3	<0.01
Projected 305-d milk yield, kg ($\pm$ SEM)	13,899 $\pm$ 86.2	11,385 $\pm$ 70.0	<0.01

No differences ($P = 0.36$) were detected among treatments regarding number of previous AI at enrollment (control = 1.99 ± 0.04 , GGPG = 1.84 ± 0.04 , and CIDR = 1.89 ± 0.05), but cows in the AZ herd had fewer AI at enrollment than cows in the CA herd (Table 1).

Body condition score at enrollment was not ($P = 0.37$) different among treatments (control = 2.86 ± 0.02 , GGPG = 2.82 ± 0.02 , CIDR = 2.83 ± 0.02). Cows in the AZ herd had ($P < 0.01$) greater BCS than those in the CA herd (Table 1). Multiparous cows had ($P = 0.05$) greater BCS at enrollment than primiparous cows (2.86 ± 0.01 vs. 2.81 ± 0.02).

Average daily milk yield according to the test-day closest to enrollment was not ($P = 0.38$) different among treatments (control = 42 ± 1 , GGPG = 43 ± 1 , CIDR = 43 ± 1 kg/d); however, average daily milk yield of cows in the AZ herd was greater ($P < 0.01$) than that of cows in the CA herd (Table 1). Similarly, projected 305-d milk yield was not ($P = 0.98$) different among treatments (control = $12,627 \pm 97$, GGPG = $12,649 \pm 95$, and CIDR = $12,650 \pm 104$ kg). Average projected 305-d milk yield was greater ($P < 0.01$) for cows in the AZ herd than for those in the CA herd (Table 1).

Ovarian Responses and Progesterone Concentration

Number of CL on d 0 was affected ($P < 0.01$) by treatment: GGPG cows had more CL than control and CIDR cows (Table 2). Although the concentration of P_4 on d 0 was not ($P = 0.62$) different among treatments, there was a tendency ($P = 0.09$) for treatment to affect percentage of cows with $P_4 \geq 1$ ng/mL on d 0 (Table 2). Body condition score did not affect the P_4 concentration ($P = 0.96$) or the percentage of cows with $P_4 \geq 1$ ng/mL ($P = 0.94$) on d 0.

Diameter of the largest follicle on d 0 was similar ($P = 0.91$) among treatments (Table 2). Percentage of cows with follicles ≥ 10 mm in diameter on d 0 was ($P = 0.03$) affected by treatment because control cows were less ($P = 0.01$) likely to have follicles ≥ 10 mm in diameter than CIDR cows; however, there were no differences between control and GGPG cows or GGPG and CIDR cows (Table 2). Diameter of the largest follicles of primiparous cows was smaller ($P = 0.02$) than that of multiparous cows (15.8 ± 1.2 vs. 17.7 ± 1.0 mm).

The percentage of cows that ovulated in response to the GnRH injection given on d 0 was affected ($P < 0.01$) by treatment, because GGPG cows were more likely to ovulate than control and CIDR cows (Table 2). There was no effect of treatment on the percentage of cows that ovulated before the GnRH injection given on d 0 ($P = 0.40$) or on the percentage of cows that had double ovulations after the GnRH injection given on d 0 ($P = 0.14$; Table 2). Consequently, GGPG cows had a greater ($P < 0.01$) number of CL on d 7 than did control and CIDR cows (Table 2). In addition, multiparous cows had ($P = 0.02$) more CL than primiparous cows (1.25 ± 0.1 vs. 1.03 ± 0.1). Progesterone concentration ($P = 0.46$) and percentage of cows with $P_4 \geq 1$ ng/mL ($P = 0.68$) were not different among treatments on d 7. The percentage of cows that had luteolysis between d 0 and 7 was not ($P = 0.58$) affected by treatment (Table 2). There was no ($P = 0.11$) effect of BCS on P_4 concentration on d 7. In addition, the percentage of cows with $P_4 \geq 1$ ng/mL on d 7 was not affected by BCS ($P = 0.37$) or by the interaction between treatment and BCS ($P = 0.99$). Although size of the largest follicle on d 7 was not ($P = 0.90$) different among treatments, GGPG and CIDR cows were ($P < 0.01$) more likely to

Table 2. Ovarian responses to the different resynchronization protocols

Item	Treatment ¹			P-value
	Control	GGPG	CIDR	
Day of GnRH injection given on d 0				
Number of corpora lutea (CL) ($\pm$ SEM)	1.05 $\pm$ 0.11 ^a	1.30 $\pm$ 0.11 ^b	1.05 $\pm$ 0.11 ^a	<0.01
Progesterone (P ₄) concentration, ng/mL ($\pm$ SEM)	4.5 $\pm$ 0.4	4.1 $\pm$ 0.3	4.0 $\pm$ 0.4	0.62
Cows with P ₄ $\geq$ 1.0 ng/mL, %	72.0 ^{AB}	84.7 ^A	66.1 ^B	0.09
Diameter of the largest follicle, mm ($\pm$ SEM)	16.6 $\pm$ 1.2	17.0 $\pm$ 1.2	16.6 $\pm$ 1.2	0.91
Cows with follicles $\geq$ 10 mm at GnRH, %	83.1 ^a	89.8 ^{ab}	93.6 ^b	0.03
Cows that ovulated before the GnRH given on d 0, %	2.1	4.7	1.6	0.40
Cows that ovulated to the GnRH given on d 0, %	36.6 ^a	48.4 ^b	29.6 ^a	<0.01
Cows that had double ovulation to the GnRH given on d 0, %	0.7	3.9	0.8	0.14
Day of PGF _{2α}				
Number of CL ($\pm$ SEM)	1.03 $\pm$ 0.14 ^a	1.41 $\pm$ 0.14 ^b	0.97 $\pm$ 0.13 ^a	<0.01
P ₄ concentration, ng/mL ($\pm$ SEM)	2.8 $\pm$ 0.3	3.1 $\pm$ 0.3	2.8 $\pm$ 0.3	0.46
Cows with P ₄ $\geq$ 1 ng/mL, %	71.0	74.4	75.6	0.68
Diameter of the largest follicle, mm ($\pm$ SEM)	17.6 $\pm$ 1.2	17.3 $\pm$ 1.3	17.3 $\pm$ 1.2	0.90
Cows with follicles $\geq$ 10 mm, %	81.0 ^a	89.8 ^b	94.4 ^b	<0.01
Cows with luteolysis from the day of GnRH to the day of PGF _{2α} , %	26.8	28.4	25.3	0.58

^{a,b}Values within a row with different superscripts differ ($P < 0.05$).

^{A,B}Values within a row with different superscripts differ ($P < 0.10$).

¹Treatments: control = cows submitted to the resynchronization protocol (GnRH, 7 d later PGF_{2 α} , and 3 d later GnRH concurrent with timed AI) if diagnosed not pregnant at 39 $\pm$ 3 d after pre-enrollment AI; GGPG = estrous cycles of cows were presynchronized with an injection of GnRH at enrollment (32 $\pm$ 3 d after pre-enrollment AI) and if diagnosed not pregnant at 39 $\pm$ 3 d after pre-enrollment AI were submitted to the resynchronization protocol described for control cows; and CIDR = cows diagnosed not pregnant at 39 $\pm$ 3 d after pre-enrollment AI were submitted to the resynchronization protocol described for control cows with the addition of a CIDR insert from the GnRH to the PGF_{2 α} injection.

have follicles $\geq$ 10 mm in diameter (Table 2) than were controls.

Percentage of Cows Pregnant and Prevalence of Pregnancy Loss

Treatment affected ($P = 0.03$) the percentage of cows diagnosed pregnant at 39 $\pm$ 3 d after resynchronized AI. A greater ($P < 0.05$) percentage of GGPG (33.6%) and CIDR (31.3%) cows were pregnant at 39 $\pm$ 3 d after resynchronized AI than control cows (24.6%; Table 3). When analyzing AZ and CA data combined, treatment affected ($P = 0.02$) the percentage of cows pregnant at the second pregnancy exam (67 $\pm$ 3 d after resynchronized AI in CA and 120 $\pm$ 3 d after resynchronized AI in AZ), because control cows (22.1%) were ($P = 0.02$) less likely pregnant than GGPG (31.2%) and CIDR (29.5%) cows (Table 3). When only data from AZ were analyzed, treatment tended ($P = 0.09$) to affect the percentage of cows pregnant at 120 $\pm$ 3 d after resynchronized AI. When only CA data were analyzed, treatment did not ($P = 0.16$) affect percentage of cows pregnant at 67 $\pm$ 3 d after resynchronized AI. Percentage of cows that had pregnancy loss between 39 and 67 or 120 $\pm$ 3 d after resynchronized AI was not ($P = 0.34$) affected by treatment when data from AZ and CA were analyzed together (Table 3). Similarly, when data from AZ ($P = 0.23$) and CA ($P = 0.90$) were analyzed

separately, treatment had no effect on percentage of cows that had pregnancy loss between the first and second pregnancy diagnosis (Table 3).

Cows in the CA herd were more ($P = 0.01$) likely diagnosed pregnant at 39 $\pm$ 3 d after resynchronized AI than cows in the AZ herd (32.2 vs. 25.0%). The percentage of cows diagnosed pregnant at 67 $\pm$ 3 d after resynchronized AI in the CA herd was greater ($P < 0.01$) than the percentage of cows diagnosed pregnant at 120 $\pm$ 3 d after resynchronized AI in the AZ herd (30.4 vs. 21.7%). As expected, prevalence of pregnancy loss was smaller ($P = 0.05$) between 39 $\pm$ 3 and 67 $\pm$ 3 d after resynchronized AI in the CA herd than between 39 $\pm$ 3 and 120 $\pm$ 3 d after resynchronized AI in the AZ herd (5.0 vs. 10.6%, respectively). The interaction between treatment and herd had no effect on P/AI at 39 $\pm$ 3 ($P = 0.88$) and 67 or 120 $\pm$ 3 ($P = 0.55$) d after resynchronized AI or on the prevalence of pregnancy loss between 39 $\pm$ 3 and 67 or 120 $\pm$ 3 d after resynchronized AI ($P = 0.40$; Table 3).

Body condition score had no effect on percentage of cows diagnosed pregnant at 39 ($P = 0.61$) and 67 or 120 $\pm$ 3 ($P = 0.48$) d after resynchronized AI or on the percentage of cows that had pregnancy loss between 39 and 67 or 120 $\pm$ 3 d after resynchronized AI ($P = 0.78$). Similarly, the interaction between treatment and BCS had no effect on percentage of cows diagnosed pregnant at 39 ($P = 0.43$) and 67 or 120 $\pm$ 3 ($P = 0.49$) d after

Table 3. Effect of treatment (Trt) and herd on pregnancy per AI (P/AI) at 39 ± 3 d and 67 or 120 ± 3 d after AI and pregnancy loss

Item	Treatment ¹			P-value		
	Control	GGPG	CIDR	Trt	Herd	Trt $\times$ Herd
Overall	(n = 386)	(n = 357)	(n = 316)			
P/AI on d 39 ± 3 , %	24.6 ^a	33.6 ^b	31.3 ^b	0.03	0.01	0.88
P/AI on d 67 or 120 ± 3 , %	22.1 ^a	31.2 ^b	29.5 ^b	0.02	<0.01	0.55
Pregnancy loss, %	9.8	5.3	5.1	0.34	0.05	0.40
Arizona	(n = 142)	(n = 124)	(n = 106)			
P/AI on d 39 ± 3 , %	19.7	30.7	25.5	NA ²	NA	NA
P/AI on d 120 ± 3 , %	15.6 ^{a,A}	27.9 ^b	22.9 ^B	0.09	NA	NA
Pregnancy loss, %	20	5.9	7.7	0.23	NA	NA
California	(n = 244)	(n = 233)	(n = 210)			
P/AI on d 39 ± 3 , %	27.5	35.2	34.3	NA	NA	NA
P/AI on d 67 ± 3 , %	25.8	32.9	32.9	0.16	NA	NA
Pregnancy loss, %	6.0	5.0	4.2	0.90	NA	NA

^{a,b}Values within a row with different superscripts differ ($P < 0.05$).

^{A,B}Values within a row with different superscripts differ ($P < 0.10$).

¹Treatments: control = cows submitted to the resynchronization protocol (GnRH, 7 d later PGF_{2 α} , and 3 d later GnRH concurrent with timed AI) if diagnosed not pregnant at 39 ± 3 d after pre-enrollment AI; GGPG = estrous cycles of cows were presynchronized with an injection of GnRH at enrollment (32 ± 3 d after pre-enrollment AI) and if diagnosed not pregnant at 39 ± 3 d after pre-enrollment AI were submitted to the resynchronization protocol described for control cows; and CIDR = cows diagnosed not pregnant at 39 ± 3 d after pre-enrollment AI were submitted to the resynchronization protocol described for control cows with the addition of a CIDR insert from the GnRH to the PGF_{2 α} injection.

²NA = not applicable.

resynchronized AI or on the percentage of cows that had pregnancy loss between 39 and 67 or 120 ± 3 d after resynchronized AI ($P = 0.98$).

DISCUSSION

Resynchronization programs are frequently used as a reproductive management strategy to reduce the interval between AI and improve the overall rate at which cows become pregnant. Although a large body of research exists on methods of resynchronization of lactating dairy cows, P/AI after resynchronized AI is often significantly lower than re-AI based on signs of estrus. Resynchronization protocols currently implemented in dairy herds are timed AI protocols designed for first postpartum AI, and the interval between previous AI and the start of such resynchronization protocols is dependent on the interval from AI to pregnancy diagnosis. As such, one of the most limiting factors to the success of such resynchronization protocol is the percentage of cows that ovulate in response to the first GnRH injection. Chebel et al. (2006) demonstrated that cows that ovulated to the first GnRH injection of a timed AI protocol had P/AI approximately 10% greater than those that did not ovulate. Such a difference in P/AI was probably because cows that do not ovulate to the GnRH injection have a prolonged period of dominance of the ovulatory follicle and embryos of reduced quality (Cerri et al., 2009). Vasconcelos et al. (1999) and Moreira et al. (2000) demonstrated that ovulation in response to the first GnRH injection of timed AI

protocols increased in cows and heifers that start such protocols between d 5 and 9 of the estrous cycle. Although one may suggest that starting resynchronization protocols between 27 and 31 d after previous AI would improve P/AI because the length of the estrous cycle of the lactating dairy cow is approximately 22 d (Sartori et al., 2004), this is unlikely to be a successful strategy because only 10% of lactating dairy cows returned to estrus 22 d after previous AI and <45% of them returned to estrus between 20 and 24 d after previous AI. Several factors affect the pattern of return to estrus of lactating dairy cows such as synchrony of the estrous cycle after submission to a timed AI protocol, percentage of cows truly in estrus when AI is based on signs of estrus, percentage of cows with short luteal phase, and percentage of cows that have early embryonic loss. Therefore, methods to presynchronize the estrous cycle before the start of resynchronization protocols must be developed and implemented. Cows that have never been AI or those that have been diagnosed not pregnant to a previous AI can have their estrous cycle presynchronized with sequential injections of PGF_{2 α} to maximize the percentage of them that start the timed AI protocol between d 5 and 9 of the estrous cycle (Moreira et al., 2001; Galvão et al., 2007; Silva et al., 2007). The use of PGF_{2 α} -based presynchronization protocols in cows of unknown pregnancy status would result in iatrogenic abortions, and implementation of such protocols only after cows are diagnosed not pregnant would result in extended interval between inseminations (Silva et al., 2007).

In the current study, the use of a presynchronizing injection of GnRH 1 wk before pregnancy diagnosis resulted in improvements in P/AI compared with cows that did not have their estrous cycles presynchronized. In a subsample of cows examined by ultrasonography, we demonstrated that cows that had their estrous cycles presynchronized with a GnRH injection had more CL than cows that did not have their estrous cycles presynchronized. Furthermore, presynchronizing the estrous cycle of cows with GnRH resulted in a greater percentage of cows ovulating in response to the GnRH injection given on d 0, which resulted in greater percentage of cows bearing a CL on d 7. It is likely that the presynchronizing injection of GnRH given 7 d before the start of the resynchronization protocol induced ovulation in cows bearing follicles ≥ 10 mm in diameter that resulted in the formation of a new CL and the recruitment of a new follicular wave approximately 36 to 60 h later (Ryan et al., 1991; Pursley et al., 1995). This would have resulted in GGPG cows starting the resynchronization protocol at approximately d 7 of the estrous cycle.

Bello et al. (2006) demonstrated that noninseminated lactating dairy cows that had their estrous cycles presynchronized with a PGF_{2 α} injection followed 2 d later by a GnRH injection, given 6 d before the start of a timed AI protocol, more likely ovulated to the first GnRH injection of the timed AI protocol than cows that were not presynchronized and those that received the presynchronizing GnRH injection 4 and 5 d before the start of the timed AI protocol. In the current study, concentrations of P₄ on d 0 and 7 were not affected by treatment, and the concentration of P₄ on d 0 was greater than the concentration of P₄ on d 7. We expected that GGPG cows would have greater P₄ concentrations on d 0 because of accessory CL formed after the presynchronizing injection of GnRH given 7 d earlier and the greater number of CL observed on d 0, but this did not occur. The decrease in P₄ from d 0 to 7 was probably a consequence of some cows having spontaneous luteolysis during the resynchronization protocol. In fact, in the subsample of cows from which P₄ concentrations were determined on d 0 and 7, 27.2% of cows had luteolysis during the resynchronization protocol.

In the current study, cows treated with a CIDR insert during resynchronization had greater P/AI than control cows. In general, CIDR treatment had no effect on ovarian responses during the resynchronization protocol compared with control cows, aside from diameter of the largest follicles. On d 0, CIDR cows more likely had follicles ≥ 10 mm in diameter compared with control cows. Considering that neither control nor CIDR

cows had received any treatments before the start of the resynchronization protocol, this difference can only be accounted for by random chance. On d 7, CIDR cows more likely had follicles ≥ 10 mm in diameter than control cows. This is likely a consequence of CIDR cows having luteolysis during the resynchronization protocol preventing them from ovulating because of CIDR treatment and the reduced P₄ concentration to which they were exposed, which may have resulted in hastened follicular growth because of greater endogenous pulsatile release of GnRH and LH (Calder et al., 1999; Nation et al., 2000; Inskeep, 2004). Actually, only 0.3% of CIDR cows were re-AI between d 0 and d 7, whereas 10.7 and 6.9% of control and GGPG cows, respectively, were re-AI during this interval (our unpublished data). Although conflicting results were reported regarding the effects of treatment of cows that did not display estrus following PGF_{2 α} -based presynchronization protocols with CIDR inserts during timed AI protocols on P/AI (El-Zarkouny et al., 2004; Stevenson et al., 2006, 2008a), a recent study demonstrated that one can expect improvements of approximately 5 percentage units in P/AI when cows are treated with CIDR insert during timed AI protocols (Chebel et al., 2006). Studies evaluating the use of CIDR inserts during the resynchronization protocol as described in the current study have not been reported. As mentioned above, CIDR treatment prevents cows from displaying estrus and ovulating even if luteolysis takes place during treatment because the low concentrations of P₄ during CIDR treatment inhibit pulsatile release of GnRH and LH, preventing follicles from growing and ovulating. Consequently, after CIDR removal, estrus and ovulation are more tightly synchronized (Chebel et al., 2006; Lima et al., 2009), which likely improves P/AI after fixed-time AI. Considering that, regardless of the interval between previous AI and the start of resynchronization protocols, estrous cycles that have not been presynchronized are at random stages at the start of resynchronization protocols, treatment with CIDR inserts likely improves P/AI by improving synchrony of the estrous cycle following the resynchronization protocol.

Although the ultimate goals of resynchronization strategies are to improve P/AI and reproductive efficiency, the cost effectiveness of such protocols must be evaluated. Economic models demonstrated that implementation of the GGPG or CIDR treatments throughout lactation would result in economic returns \$15 to \$30 greater than the implementation of the control protocol, even though the cost of the control resynchronization protocol used in the current study was the smallest of all 3 protocols (our unpublished data).

CONCLUSIONS

Presynchronizing the estrous cycle of lactating dairy cows with a GnRH injection given 7 d before pregnancy diagnosis and the start of a resynchronization protocol resulted in greater P/AI after resynchronized AI compared with control cows. This was likely the consequence of a greater percentage of GGPG cows ovulating to the presynchronizing GnRH injection, as observed by the greater number of CL and the greater percentage of GGPG cows ovulating to the GnRH injection given on d 0. Although CIDR treatment during the resynchronization protocol had little effect on ovarian responses, it increased P/AI after resynchronized AI compared with control cows. This improvement was likely a consequence of the improved synchrony of the estrous cycle after the resynchronization protocol as observed by the reduced percentage of CIDR cows re-AI in estrus from d 0 to 7. We conclude that improvements in P/AI of resynchronized cows are expected when the estrous cycles of cows are presynchronized with GnRH 7 d before the start of the resynchronization or when cows are treated with CIDR inserts during the resynchronization protocol. More studies are warranted to determine if there are additive effects of presynchronization with GnRH and treatment with CIDR during the resynchronization protocol on P/AI.

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