Supplemental progesterone and timing of resynchronization on pregnancy outcomes in lactating dairy cows

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ABSTRACT

The objective was to determine the effect of exogenous progesterone (P_4) in a timed artificial insemination (TAI) protocol initiated at 2 different times post-AI on pregnancies per AI (P/AI) in lactating dairy cows. Cows ($n = 1,982$) in 5 dairy herds were assigned randomly at a nonpregnancy diagnosis 32 ± 3 d post-AI to 1 of 4 resynchronization (RES) treatments arranged in a 2×2 factorial design using the Ovsynch-56 (GnRH, 7 d later PGF_{2 α} , 56 h later GnRH, 16 h later TAI) protocol. Treatments were as follows: cows initiating RES 32 ± 3 d after AI with no supplemental P_4 (d 32 RES-CON; $n = 516$); same as d 32 RES-CON plus a controlled internal drug release (CIDR) insert containing P_4 at the onset of Ovsynch-56 (d 32 RES-CIDR; $n = 503$); cows initiating RES 39 ± 3 d after AI (d 39 RES-CON; $n = 494$); and same as d 39 RES-CON plus a CIDR (d 39 RES-CIDR; $n = 491$). Cows were inseminated if observed in estrus before TAI. The P/AI was determined 32 and 60 d after TAI. In a subgroup of cows ($n = 1,152$), blood samples were collected and ovarian structures examined by ultrasonography on the days of the first GnRH (G1) and PGF_{2 α} of Ovsynch-56. Percentage of cows with a corpus luteum (CL) at G1 was unaffected by timing of treatments, but percentage of cows with a CL at PGF_{2 α} was greater for d 32 than for d 39 cows (87.9 vs. 79.4%). In addition, percentage of cows with $P_4 \geq 1$ ng/mL at G1 was unaffected by timing of treatments, but was increased for d 32 compared with d 39 RES cows on the day of the PGF_{2 α} of the RES protocols (86.5 vs. 74.3%). Treatment did not affect ovulation to G1 or P/AI 32 d after RES TAI (d 32 RES-CON = 30.1%, d 32 RES-CIDR = 28.8%, d 39 RES-CON = 27.5%, d 39 RES-CIDR = 30.5%). A greater percentage of d 39 RES cows underwent prema-

ture luteolysis during the RES protocol compared with d 32 RES cows. An interaction was detected between day of RES initiation and CIDR treatment, in which the CIDR increased P/AI 60 d after TAI for d 39 (CON = 23.7% vs. CIDR = 28.0%), but not for d 32 (CON = 26.9% and CIDR = 24.2%) cows. Pregnancy loss was unaffected by treatment. In addition, cows had improved P/AI 60 d after TAI when they received a CIDR and did not have a CL (CON-CL = 28.2%, CON-No CL = 19.2%, CIDR-CL = 27.0%, and CIDR-No CL = 26.5%) or had $P_4 < 1$ ng/mL (CON-High P_4 = 27.8%, CON-Low P_4 = 15.0%, CIDR-High P_4 = 25.0%, and CIDR-Low P_4 = 29.4%) at G1, but not if a CL was present or P_4 was ≥ 1 ng/mL at G1. In conclusion, addition of a CIDR insert to supplement P_4 during the RES protocol increased P/AI for cows initiating RES 39 ± 3 d after AI but not 32 ± 3 d after AI.

Key words: controlled internal drug release insert, dairy cow, resynchronization

INTRODUCTION

Dairy herd managers continue to be challenged by inadequate detection or expression of estrus in lactating dairy cows previously inseminated, and resynchronization (RES) protocols before timed AI (TAI) are often recommended for re-insemination of nonpregnant lactating dairy cows to increase the AI service rate. The limitation of RES protocols has traditionally been poor pregnancies per AI (P/AI) compared with TAI after first service (Bruno et al., 2013). Poor P/AI seems to result from the inability to properly synchronize the estrous cycle before or after nonpregnancy diagnosis (NPD) when cows start the RES protocol at random stages of the estrous cycle.

A controlled internal drug release (CIDR) insert containing 1.38 g of progesterone (P_4) induces cyclicity in anovular cows and heifers (Rhodes et al., 2003), concentrates the return to estrus in nonpregnant cows (Chenault et al., 2003), and improves synchronization

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of the estrous cycle when used during a TAI protocol (Lima et al., 2009). Some studies have shown variable results when including a CIDR insert as part of synchronization protocol for first AI (Stevenson et al., 2006; Chebel et al., 2010). These studies, however, did not evaluate the value of a CIDR in a RES protocol for subsequent AI. Recently, a 7-percentage-point increase in P/AI was observed when a CIDR was incorporated in an Ovsynch protocol for RES beginning at 39 ± 3 d after AI (Dewey et al., 2010).

Resynchronization programs are frequently used as a reproductive management strategy to reduce the interval between AI and increase the overall rate at which cows become pregnant. The interval between AI and the start of RES protocols depends on the interval from AI to NPD. The day of the estrous cycle on which the RES protocol is initiated is critical in determining the probability of ovulation to the first GnRH injection of the Ovsynch protocol, timing of luteolysis during the protocol, duration of dominance of the ovulatory follicle, and ultimately synchrony of the estrous cycle (Moreira et al., 2001; Fricke et al., 2003). Vasconcelos et al. (1999) observed that initiating the Ovsynch protocol between d 5 and 9 of the estrous cycle increased the percentage of cows that ovulated after the first GnRH (>90%) injection. In addition, a new follicular wave is recruited 24 to 48 h after the GnRH injection and a corpus luteum (CL) is present at the time of the prostaglandin (PG) $F_{2\alpha}$ injection administered on d 7 of the protocol. These orchestrated responses result in a properly timed luteolysis when synchronized ovulation is induced with the second GnRH administered 48 to 56 h after PGF $_{2\alpha}$ (Vasconcelos et al., 1999). Considering that duration of the estrous cycle of lactating dairy cows is approximately 23 d (Sartori et al., 2004) and the optimal time to begin a synchronization protocol is from d 5 to 9 of the estrous cycle (Vasconcelos et al., 1999), it may be advantageous to initiate RES protocols between 28 and 32 d after AI. Initiation of a RES protocol 19 to 21 d after AI resulted in reduced P/AI (Fricke et al., 2003), whereas P/AI was similar when RES was initiated 25 to 28 d after AI compared with starting the RES 32 to 33 d after AI (Fricke et al., 2003; Chebel et al., 2003; Sterry et al., 2006).

The hypothesis of the current experiment was that cows resynchronized with an Ovsynch protocol including a CIDR insert would have increased P/AI when RES began at either 32 or 39 ± 3 d post-AI. We also hypothesized that initiating the RES protocol 32 ± 3 d post-AI would improve P/AI because most cows would begin the RES protocol between d 5 and 12 of their estrous cycle, assuming that an undetected estrus occurred 20 to 27 d after the previous AI. Therefore, the objectives of the current experiment were to determine

the effects of P $_4$ supplementation via a CIDR insert during the Ovsynch protocol on P/AI of dairy cows initiating the RES program at different intervals after AI. A second objective was to determine if timing of initiation of RES after AI (d 32 vs. d 39) influences P/AI in lactating dairy cows.

MATERIALS AND METHODS

Cows and Facilities

The experiment was conducted from October 2009 through February 2010 at 5 dairy farms located in 5 states (Minnesota, Kansas, Wisconsin, Texas, and Florida). Lactating Jersey (Minnesota; $n = 417$), Holstein $\times$ Jersey (Texas; $n = 418$), and Holstein (Kansas, Wisconsin, and Florida; $n = 1,169$) cows previously inseminated were enrolled in the experiment 32 ± 3 d after last AI. Cows in Kansas and Florida were housed in naturally ventilated freestall barns, Texas cows were in an open dry-lot, and Minnesota and Wisconsin cows were housed in cross-ventilated freestall barns.

Treatments

Within each site, all cows were enrolled in the experiment 32 ± 3 d after AI and assigned randomly to 1 of 4 treatments arranged in a 2×2 factorial design (Figure 1). Treatments were initiation of the RES protocols either 32 or 39 ± 3 d post-AI, with or without inclusion of a CIDR insert (1.38 g of P $_4$; EaziBreed CIDR, Zoetis, Madison, NJ) on the day of the first GnRH injection of the RES protocol. Nonpregnancy was diagnosed by ultrasonography at 32 ± 3 d after AI for all cows regardless of when RES treatments were initiated. At NPD, cows were submitted to an Ovsynch-56 protocol [GnRH, followed 7 d later by PGF $_{2\alpha}$ (25 mg of dinoprost tromethamine; Lutalyse, Zoetis), 56 h later a second GnRH injection was administered, and TAI occurred 12 to 16 h later] initiated 32 d after AI or initiated 1 wk later for d 39 cows. The CIDR was inserted concurrent with the first GnRH (100 μ g of gonadorelin diacetate tetrahydrate; Fertagyl, Merck Animal Health, De Soto, KS) injection of the Ovsynch-56 protocol and was removed 7 d later at the PGF $_{2\alpha}$ injection. Therefore, the 4 treatments (Figure 1) were (1) cows examined for NPD 32 ± 3 d after AI and initiating the Ovsynch-56 protocol at NPD (**d 32 RES-CON**; $n = 516$); (2) cows examined for NPD 32 ± 3 d after AI and initiating the Ovsynch-56 protocol with a CIDR at NPD (**d 32 RES-CIDR**; $n = 503$); (3) cows examined for NPD 39 ± 3 d after AI and initiating the Ovsynch-56 protocol at NPD (**d 39 RES-CON**; $n = 494$); and (4) cows examined for NPD 39 ± 3 d after AI and initiating

Figure 1. Outline of resynchronization (RES) protocols used in the study. On day of enrollment (32 ± 3 d after AI), all cows were assigned randomly to 1 of 4 treatments in a 2×2 factorial design: RES protocol initiation and nonpregnancy diagnosis (NPD) on either d 32 or d 39 ± 3 post-AI, and insertion of a controlled internal drug release (CIDR) insert or not on day of NPD at first GnRH injection of the RES protocol. Pregnancy was diagnosed by ultrasonography (US) at 32 ± 3 or 39 ± 3 d after AI for cows in both d 32 and 39 treatments, respectively. At NPD, all cows received the Ovsynch-56 protocol [GnRH, followed 7 d later by PGF_{2α} (25 mg of dinoprost tromethamine), 56 h later a second GnRH injection, and timed AI (TAI) occurring 12 to 16 h after the last GnRH]. Cows in the CIDR treatment received a CIDR insert concurrent with the first GnRH (100 µg of gonadorelin diacetate tetrahydrate) injection of the Ovsynch-56 and removed 7 d later. Body condition score was assessed in all cows at NPD. In addition, blood samples (BS) were collected and ovaries were examined by US at the first GnRH and PGF_{2α} injection.

the Ovsynch-56 protocol with a CIDR at NPD (d 39 RES-CIDR; $n = 491$). In 4 of the 5 dairy farms, cows enrolled in the experiment and diagnosed not pregnant to the resynchronized TAI were reassigned randomly to treatment for subsequent AI.

Estrous Detection and AI

At all locations, detection of estrus was performed based on removal of tail paint or tail chalk that was applied daily to all cows. In addition, all cows were inseminated once daily in the morning by trained technicians distributed randomly across treatments. If cows were identified in estrus at any time throughout the study, they were inseminated and removed from any further treatment. Cows inseminated in estrus after the PGF_{2α} injection of the TAI protocol were considered to have completed the TAI protocol.

BCS and Milk Yield

Cows were scored for body condition (1 = emaciated, 5 = obese) at the time of nonpregnancy diagnosis using a 5-point scale (Ferguson et al., 1994). Cows were milked 2 or 3 times daily. Milk yields were recorded for individual cows once monthly and projected 305-d mature-equivalent milk yield was used for statistical analysis.

Ultrasonography of Ovaries and Blood Samples

Ovaries from a subgroup of randomly selected cows within each site (d 32 RES-CIDR = 303, d 32 RES-CON = 305, d 39 RES-CON = 271, d 39 RES-CIDR = 273) were examined by ultrasound at NPD, when cows received the first GnRH injection of the RES protocol, and again 7 d later to assess presence and location of CL and follicles ≥ 10 mm in diameter. Cows were con-

sidered to have ovulated in response to the first GnRH injection of the RES protocol if they had a follicle ≥ 10 mm in diameter on the day of the first GnRH injection and a new CL in the same ovary 7 d later.

Blood was sampled and analyzed for concentrations of P_4 on the day of the first GnRH injection of the TAI protocol (d 32 RES-CIDR = 296, d 32 RES-CON = 295, d 39 RES-CIDR = 257, d 39 RES-CON = 264) and on the day of the PGF_{2 α} injection of the TAI protocol (d 32 RES-CIDR = 295, d 32 RES-CON = 288, d 39 RES-CON = 254, d 39 RES-CIDR = 265). Blood samples were collected from the median coccygeal blood vessels using evacuated tubes. Upon collection, samples were placed on ice and transported to the laboratory where plasma or serum was harvested by centrifugation. Plasma or serum was stored at -20°C and later analyzed for P_4 by RIA. Samples were analyzed in duplicate in multiple assays. Intraassay CV ranged from 4.6 to 7.8%, and interassay CV ranged from 7.7 to 13.1%. Cows were classified as having either low (< 1 ng/mL) or high (> 1 ng/mL) P_4 concentration resulting in 4 permutations of P_4 at the first GnRH and the PGF_{2 α} injections of the Ovsynch-56 protocol, low-low, low-high, high-low, and high-high.

Pregnancy Diagnosis

At all locations, pregnancy status was diagnosed using ultrasonography 32 ± 3 d after AI. Pregnancy was characterized by the presence of an amniotic vesicle with an embryo with a heartbeat. Cows diagnosed pregnant were re-examined 60 ± 3 d after AI. Cows that displayed estrus and were re-inseminated before the first NPD were considered to be not pregnant to the AI. Pregnant cows re-inseminated between the first and second NPD were considered to have undergone pregnancy loss.

Pregnancies per AI were calculated by dividing the number of cows diagnosed pregnant at 32 ± 3 d or 60 ± 3 d after AI by the number of cows receiving AI in each treatment. Pregnancies per AI also were calculated for cows detected in estrus and AI. The proportion of cows having pregnancy loss was calculated as the number of cows diagnosed as not pregnant at 60 ± 3 d after AI plus the number of cows re-inseminated before re-examination divided by the number of cows diagnosed pregnant 32 ± 3 d after AI.

Statistical Analyses

The experiment was a completely randomized design with a 2×2 factorial arrangement of 4 treatments, with cows balanced for parity and number of previous AI. Cow was the experimental unit.

Ordinal data were analyzed by Kruskal-Wallis using the GENMOD procedure of SAS (version 9.2, SAS Institute Inc., Cary, NC). The P_4 concentrations and size of the largest follicle were analyzed by ANOVA using the GLM procedure of SAS. Fixed effects included in the models were day of protocol initiation (d 32 RES vs. d 39 RES), P_4 treatment (CON vs. CIDR), parity (primiparous vs. multiparous), BCS, milk yield on test-day closest to enrollment, DIM at enrollment, and interactions between treatments and the other independent variables. Fixed effects with $P > 0.10$ were removed until only fixed effects with $P \leq 0.10$ remained in the model. Day, P_4 treatment, and parity were forced into all models. The GLIMMIX procedure of SAS was used to analyze dichotomous outcomes by logistic regression using the binary distribution function, whereas continuous outcomes were analyzed by ANOVA fitting a normal distribution. Fixed effects included in the models were day of protocol initiation (d 32 RES vs. d 39 RES), P_4 treatment (CON vs. CIDR), parity (primiparous vs. multiparous), and DIM at enrollment. Fixed effects with $P > 0.10$ were removed in a stepwise backward elimination process until only fixed effects with $P \leq 0.10$ remained in the model. Day, P_4 treatment, and parity were forced into all models. Location was included in the model as a random effect, and cows were nested within location. Statistical significance was defined as $P \leq 0.05$ and statistical tendencies as $0.05 < P \leq 0.10$.

RESULTS

Table 1 depicts the comparisons between locations. As expected, differences were observed among locations for several independent variables (Table 2). No differences were detected among treatments regarding parity, interval between previous AI and enrollment in the study, DIM, number of inseminations, BCS, milk yield on test-day closest to enrollment, and projected 305-d mature-equivalent milk yield (Table 2).

Estrous Detection, Pregnancy per AI, and Pregnancy Loss

Estrus was observed daily and AI was performed on all cows at 4 (Kansas, Minnesota, Texas, and Wisconsin) of the 5 locations during the entire study. A total of 173 cows were observed in estrus and inseminated. Proportion of cows receiving AI after estrus from enrollment to the PGF_{2 α} injection differed ($P < 0.01$) among herds [Kansas: 10.9% (45/413); Minnesota: 4.8% (20/417); Texas: 17.0% (71/418); Wisconsin: 9.9% (37/375)]. In addition, the proportion of cows receiving AI after estrus decreased ($P = 0.03$) when RES was

Table 1. Comparison among locations for number of cows enrolled, breed composition, lactation number, interval between previous AI and enrollment, DIM, number of inseminations, BCS, milk yield on test day closest to enrollment, and projected 305-d mature-equivalent milk yield (305ME)

Item	Location of cooperating dairy farms ¹				
	FL	KS	MN	TX	WI
Cows, no.	381	413	417	418	375
Breed composition	Holstein (H)	H	Jersey (J)	H × J	H
First lactation, %	17.9 ^a	42.9 ^b	51.3 ^c	38.5 ^d	44.5 ^e
Interval between previous AI and enrollment, d	32.0 ± 0.1 ^a	32.1 ± 0.1 ^a	31.7 ± 0.1 ^b	31.4 ± 0.1 ^c	31.7 ± 0.1 ^b
DIM	151 ± 4 ^a	122 ± 4 ^b	176 ± 4 ^c	141 ± 4 ^d	186 ± 4 ^e
No. of inseminations	2.8 ± 0.1 ^a	2.2 ± 0.1 ^b	2.7 ± 0.1 ^a	2.0 ± 0.1 ^b	2.3 ± 0.1 ^b
BCS	2.9 ± 0.1 ^a	2.5 ± 0.1 ^b	2.8 ± 0.1 ^a	2.9 ± 0.1 ^a	2.8 ± 0.1 ^a
Milk yield on test-day closest to enrollment, kg/d	38.8 ± 0.5 ^a	47.2 ± 0.5 ^b	27.9 ± 0.5 ^c	33.0 ± 0.5 ^d	44.8 ± 0.5 ^e
Projected 305ME, kg	11,549 ± 118 ^a	12,694 ± 120 ^b	6,691 ± 127 ^c	9,431 ± 123 ^d	12,976 ± 126 ^e

^{a-e}Least squares means in the same row with different superscripts differ ($P < 0.05$).

¹Dairy herds located in Florida (freestall, FL), Kansas (freestall, KS), Minnesota (cross-ventilated freestall, MN), Texas (open dry lot, TX), and Wisconsin (cross-ventilated freestall, WI).

initiated 32 versus 39 d after AI or when a CIDR insert was applied ($P = 0.09$). No difference ($P = 0.31$) was detected in the proportion of cows observed in estrus between primiparous [9.4% (74/787)] and multiparous cows [8.1% (99/1,217)].

Overall, P/AI did not differ between cows receiving AI at estrus versus TAI 32 d [$P = 0.22$; 30.4% (52/171) vs. 29.3% (530/1811), respectively] or 60 d [$P = 0.15$; 27.5% (47/171) vs. 25.7% (462/1,797), respectively] after AI. In addition, pregnancy loss did not differ ($P = 0.59$) between cows inseminated after estrus versus TAI [9.6% (5/52) vs. 10.6% (55/517), respectively].

Neither CIDR treatment nor day of initiation of RES affected P/AI 32 d after TAI or pregnancy loss for estrus detected, TAI, or all cows (Table 3). An interaction ($P = 0.04$) between day of RES initiation and CIDR treatment, however, was detected for P/AI 60 d after TAI, with cows receiving CIDR inserts having increased P/AI for both TAI and all cows when inserted 39 d but not 32 d after AI (Table 3). No effect of day of RES initiation or CIDR treatment was detected when only cows inseminated after estrus were analyzed (Table 3). In addition, pregnancy loss was unaffected by day of RES initiation or CIDR treatment whether cows received AI after estrus versus TAI (Table 3). Parity did not affect P/AI 32 d after TAI ($P = 0.48$; primiparous = 29.9% vs. multiparous = 29.1%), 60 d after TAI ($P = 0.33$; primiparous = 26.6% vs. multiparous = 25.4%), or pregnancy loss ($P = 0.49$; primiparous = 9.3% vs. multiparous = 11.4%).

Pretreatment Luteal Effects on Pregnancy Outcomes

Among all cows ($n = 1,831$), except for those inseminated after estrus before the PGF_{2α} injection ($n = 173$), 73.6 and 73.9% had both CL and $P_4 \geq 1$ ng/mL on the day of first GnRH injection of the RES

protocol, respectively. An interaction occurred between CIDR treatment and presence of a CL on the day of the first GnRH injection for P/AI at both 32 and 60 d after TAI (Table 4). The CIDR treatment tended to improve P/AI at both 32 d ($P = 0.06$) and 60 d ($P = 0.07$) for cows without a CL compared with CON cows without a CL (Table 4). Pregnancy loss for cows with or without a CL was unaffected by CIDR treatment (Table 4). In addition, day of RES initiation did not affect P/AI for cows with or without a CL and treated with a CIDR or not when pregnancy was diagnosed 32 d after TAI ($P = 0.43$; d 32 RES-No CL = 24.9%; d 32 RES-CL = 31.9%; d 39 RES-No CL = 29.0%; d 39 RES-CL = 30.9%) or d 60 after AI ($P = 0.78$; d 32 RES-No CL = 22.0%; d 32 RES-CL = 27.2%; d 39 RES-No CL = 23.9%; d 39 RES-CL = 28.9%).

Consequently, when P/AI at both 32 and 60 d after TAI was evaluated for cows with $P_4 < \text{or} \geq 1$ ng/mL and having a CL or not on the day of the first GnRH injection of RES, an interaction occurred between CIDR treatment and presence of a CL (Table 5). For cows with $P_4 < 1$ ng/mL (low P_4), inclusion of a CIDR in the RES protocol increased P/AI at both 32 ($P < 0.01$) and 60 ($P < 0.01$) d after TAI compared with CON cows (Table 5). In contrast, for cows with $P_4 < \text{or} \geq 1$ mg/mL, pregnancy loss was unaffected by CIDR treatment (Table 5). Day of RES initiation did not affect P/AI for cows with $P_4 < \text{or} \geq 1$ mg/mL and treated with a CIDR 32 d after AI ($P = 0.78$; d 32 RES-low P_4 = 25.7%; d 32 RES-high P_4 = 29.9%; d 39 RES-low P_4 = 27.3%; d 39 RES-CL = 31.0%) or d 60 ($P = 0.74$; d 32 RES-low P_4 = 20.3%; d 32 RES-high P_4 = 25.6%; d 39 RES-low P_4 = 24.2%; d 39 RES-CL = 27.3%).

Ovarian and Progesterone Responses

The percentage of cows that had a CL and the number of CL on day of the first GnRH injection did not

Table 2. Comparison between resynchronization (RES) treatments for interval between previous AI and enrollment, DIM, number of inseminations, BCS, milk yield on test-day closest to enrollment, and projected 305-d mature-equivalent milk yield (305ME)

Item	Treatment ¹				P-value		
	d 32 RES		d 39 RES		Day	CIDR	Day × CIDR
	CON	CIDR	CON	CIDR			
Interval between previous AI and enrollment, d	31.7 ± 0.1	31.6 ± 0.1	31.7 ± 0.1	31.8 ± 0.1	0.19	0.82	0.15
DIM	158 ± 3	161 ± 3	160 ± 3	155 ± 3	0.44	0.83	0.17
No. of inseminations	2.4 ± 0.05	2.5 ± 0.05	2.4 ± 0.05	2.4 ± 0.05	0.84	0.30	0.16
BCS	2.8 ± 0.02	2.8 ± 0.02	2.8 ± 0.02	2.8 ± 0.02	0.95	0.52	0.91
Milk yield on test-day closest to enrollment, kg/d	30.4 ± 0.5	30.0 ± 0.5	30.7 ± 0.5	30.9 ± 0.5	0.23	0.91	0.47
Projected 305ME, kg	10,368 ± 101	10,321 ± 101	10,238 ± 103	10,276 ± 101	0.22	0.93	0.56

¹Treatments: d 32 RES = initiating the Ovsynch-56 RES protocol (GnRH, 7 d later PGF_{2α}, 56 h GnRH, and 16 h later timed AI) on d 32 post-AI; d 39 RES = initiating the Ovsynch-56 RES protocol on d 39 post-AI; CON (control) = no insertion of an intravaginal controlled internal drug release (CIDR) insert containing 1.38 g of progesterone (P₄) at the first injection of GnRH of the Ovsynch-56 protocol and removal at PGF_{2α}; CIDR = insertion of an intravaginal CIDR insert containing 1.38 g of P₄ at the first injection of GnRH of the Ovsynch-56 protocol and removal at PGF_{2α}.

Table 3. Effect of resynchronization (RES) treatment on estrus detection (ED) and AI between enrollment and PGF_{2α}, pregnancy per AI (P/AI) after re-insemination, and pregnancy loss

Item	Treatment ¹				P-value		
	d 32 RES		d 39 RES		Day	CIDR	Day × CIDR
	CON	CIDR	CON	CIDR			
ED/AI between enrollment and PGF _{2α} , %	6.0 (31/516)	4.4 (22/503)	14.0 (69/494)	10.4 (51/491)	0.04	0.10	0.83
P/AI for ED							
32 ± 3 d	35.5 (11/31)	31.8 (7/22)	29.8 (20/67)	27.4 (14/51)	0.62	0.82	0.86
60 ± 3 d	32.3 (10/31)	31.8 (7/22)	26.9 (18/67)	23.5 (12/51)	0.56	0.82	0.79
Pregnancy loss	9.1 (1/11)	0 (0/7)	10.0 (2/20)	14.3 (2/14)	0.94	0.96	0.99
P/AI for timed AI							
32 ± 3 d	30.1 (144/478)	28.8 (138/479)	27.5 (115/418)	30.5 (133/436)	0.80	0.83	0.19
60 ± 3 d	26.9 (128/476)	24.2 (115/476)	23.7 (98/413)	28.0 (121/432)	0.87	0.86	0.04
Pregnancy loss	9.9 (14/142)	14.8 (20/135)	10.9 (12/110)	6.9 (9/130)	0.35	0.90	0.24
P/AI for both timed AI and ED							
32 ± 3 d	30.4 (155/509)	28.9 (145/501)	27.8 (135/485)	30.2 (147/487)	0.74	0.93	0.19
60 ± 3 d	27.2 (138/507)	24.5 (122/498)	24.2 (116/480)	27.5 (133/483)	0.96	0.86	0.04
Pregnancy loss	9.8 (15/153)	14.1 (20/142)	10.8 (14/130)	7.6 (11/144)	0.31	0.67	0.14

¹Treatments: d 32 RES = initiating the Ovsynch-56 RES protocol (GnRH, 7 d later PGF_{2α}, 56 h GnRH, and 16 h later timed AI) on d 32 post-AI; d 39 RES = initiating the Ovsynch-56 RES protocol on d 39 post-AI; CON (control) = no insertion of an intravaginal controlled internal drug release (CIDR) insert containing 1.38 g of progesterone (P₄) at the first injection of GnRH of the Ovsynch-56 protocol and removal at PGF_{2α}; CIDR = insertion of an intravaginal CIDR insert containing 1.38 g of P₄ at the first injection of GnRH of the Ovsynch-56 protocol and removal at PGF_{2α}.

Table 4. Effect of controlled internal drug release (CIDR) insert treatment within a resynchronization protocol for cows with or without a corpus luteum (CL) at first GnRH injection on pregnancy per AI (P/AI) on d 32 and 60 after re-insemination, and pregnancy loss for cows receiving timed AI (TAI)

Item	Treatment ¹				P-value		
	CON		CIDR		CL	CIDR	CL × CIDR
	CL	No CL	CL	No CL			
Proportion, % (no./total)	73.1 (427/584)	26.8 (157/584)	74.1 (432/583)	25.9 (151/583)	<0.01	0.87	0.73
P/AI for TAI							
32 ± 3 d	32.7 (138/422)	22.9 (36/157)	30.1 (129/428)	30.5 (46/151)	0.15	0.65	0.06
60 ± 3 d	28.2 (118/419)	19.2 (30/156)	27.0 (115/426)	26.5 (40/151)	0.10	0.51	0.07
Pregnancy loss	12.6 (17/135)	14.3 (5/35)	9.5 (12/127)	14.9 (7/47)	0.57	0.34	0.57

¹Treatments: CON (control) = no insertion of an intravaginal CIDR insert containing 1.38 g of progesterone (P₄) at the first injection of GnRH of the Ovsynch-56 protocol (GnRH, 7 d later PGF_{2α}, 56 h GnRH and 16 h later timed AI) and removal at PGF_{2α}; CIDR = insertion of an intravaginal CIDR insert containing 1.38 g of P₄ at the first injection of GnRH of the Ovsynch-56 protocol and removal at PGF_{2α}.

differ for either day of RES initiation or CIDR treatment (Table 6). In addition, no difference was detected in the percentage of cows that had P₄ concentration ≥1 ng/mL on the day of the first GnRH injection (Table 6). In contrast, P₄ concentration was greater ($P = 0.02$) on the day of the first GnRH injection when RES was initiated 39 d compared with 32 d after AI (Table 6), but without effect ($P = 0.24$) of CIDR treatment (Table 6). An interaction ($P = 0.03$) between day of RES initiation and CIDR treatment was detected for the percentage of cows with follicles ≥10 mm in which the CIDR treatment reduced ($P = 0.01$) the percentage of cows with follicles ≥10 mm when the CIDR treatment was initiated 39 but not 32 d after AI (Table 6). Diameter of the largest follicle was greater ($P = 0.02$) for cows that initiated the RES protocol 32 d after AI (Table 6). The percentage of cows that had single or double ovulations after the first GnRH injection was unaffected by either day of RES initiation or CIDR treatment (Table 6).

On the day of PGF_{2α} injection, both the number of CL ($P < 0.01$) and the percentage of cows with a CL (P

= 0.03) increased when the RES protocol was initiated 32 d after AI (Table 6). Consequently, both P₄ concentration and percentage of cows with P₄ ≥1 ng/mL also increased ($P < 0.01$) when RES protocols were initiated 32 d after AI (Table 6). In addition, when RES was initiated 32 d after AI, percentage of cows with early luteolysis between the first GnRH injection and the day of the PGF_{2α} injection was less ($P < 0.01$) compared with cows initiating RES 39 d after AI (Table 6).

DISCUSSION

For cows initiating RES 39 d after AI, inclusion of a CIDR insert in the RES protocol increased P/AI. In addition, for cows treated with a CIDR insert that did not have a CL or had P₄ <1 ng/mL at the first GnRH injection of RES, P/AI was greater at both 32 and 60 d after TAI, regardless of day of RES initiation. Several studies have evaluated use of a CIDR insert as part of a synchronization protocol for first AI in lactating dairy cows with variable results on P/AI (Stevenson et al., 2006; Chebel et al., 2010). In contrast, few studies

Table 5. Effect of controlled internal drug release (CIDR) insert treatment within a resynchronization protocol for cows with high (≥1 ng/mL) or low (<1 ng/mL) progesterone (P₄) at first GnRH injection on pregnancy per AI (P/AI) on d 32 and 60 after re-insemination, and pregnancy loss for cows receiving timed AI (TAI)

Item	Treatment ¹				P-value		
	CON		CIDR		P ₄	CIDR	P ₄ × CIDR
	High P ₄	Low P ₄	High P ₄	Low P ₄			
Proportion, % (no./total)	73.6 (394/535)	26.4 (141/535)	74.2 (393/530)	25.9 (137/530)	<0.01	0.89	0.93
P/AI for TAI							
32 ± 3 d	32.4 (126/389)	19.3 (27/140)	28.5 (111/390)	33.8 (46/136)	0.20	0.24	<0.01
60 ± 3 d	27.8 (107/385)	15.0 (21/140)	25.0 (97/388)	29.4 (40/136)	0.11	0.13	<0.01
Pregnancy loss	12.3 (15/122)	22.2 (6/27)	11.9 (13/110)	13.0 (6/46)	0.55	0.48	0.47

¹Treatments: CON (control) = no insertion of an intravaginal CIDR insert containing 1.38 g of P₄ at the first injection of GnRH of the Ovsynch-56 protocol (GnRH, 7 d later PGF_{2α}, 56 h GnRH, and 16 h later timed AI) and removal at PGF_{2α}; CIDR = insertion of an intravaginal CIDR insert containing 1.38 g of P₄ at the first injection of GnRH of the Ovsynch-56 protocol and removal at PGF_{2α}.

Table 6. Effect of resynchronization (RES) treatment on ovarian responses and progesterone (P₄) concentration

Item	Treatment ¹				P-value	
	d 32 RES		d 39 RES		Day	Day × CIDR
	CON	CIDR	CON	CIDR		
Day of first GnRH						
No. of corpora lutea (CL)	0.7 ± 0.03	0.7 ± 0.03	0.7 ± 0.03	0.8 ± 0.03	0.16	0.70
Cows with CL, %	72.1	71.3	74.4	77.3	0.17	0.66
P ₄ concentration, ng/mL	4.1 ± 0.2	4.0 ± 0.2	4.9 ± 0.2	4.6 ± 0.2	0.02	0.52
Cows with P ₄ ≥ 1 ng/mL, %	75.3	73.0	71.8	75.5	0.57	0.87
Diameter of the largest follicle, mm	18.0 ± 0.4	17.9 ± 0.4	17.4 ± 0.4	16.8 ± 0.4	0.02	0.53
Cows with follicles ≥ 10 mm at GnRH, %	92.0	94.5	96.3	90.1	0.01	0.20
Cows that ovulated to the GnRH, %	49.7	49.0	44.9	41.3	0.14	0.61
Cows that had double ovulation to the GnRH given on d 0, %	6.8	6.5	7.1	3.9	0.36	0.32
Day of PGF _{2α}						
No. of CL	0.9 ± 0.02	0.9 ± 0.02	0.8 ± 0.02	0.8 ± 0.02	<0.01	0.80
Cows with CL, %	88.9	87.0	78.9	79.9	0.03	0.61
P ₄ concentration, ng/mL	4.7 ± 0.2	4.7 ± 0.2	3.6 ± 0.2	3.6 ± 0.2	<0.01	0.80
Cows with P ₄ ≥ 1 ng/mL, %	85.6	87.3	73.1	75.4	<0.01	0.09
Cows with luteolysis from day of GnRH to day of PGF _{2α} , %	13.6	12.0	22.7	21.3	<0.01	0.83

¹Treatments: d 32 RES = initiating the Ovsynch-56 RES protocol (GnRH, 7 d later PGF_{2α}, 56 h GnRH, and 16 h later timed AI) on d 32 post-AI; d 39 RES = initiating the Ovsynch-56 RES protocol on d 39 post-AI; CON (control) = no insertion of an intravaginal controlled internal drug release (CIDR) insert containing 1.38 g of P₄ at the first injection of GnRH of the Ovsynch-56 protocol and removal at PGF_{2α}; CIDR = insertion of an intravaginal CIDR insert containing 1.38 g of P₄ at the first injection of GnRH of the Ovsynch-56 protocol and removal at PGF_{2α}.

have evaluated the use of a CIDR insert as part of a RES protocol in lactating dairy cows (Dewey et al., 2010; Chebel et al., 2013). The first study to evaluate a CIDR insert as part of a RES protocol also reported an increase in P/AI (Dewey et al., 2010). The RES protocol was initiated 39 d after AI with or without a CIDR inserted at first GnRH injection and removed 7 d later at the PGF_{2α} injection, or a GnRH injection was administered 7 d before RES initiation. An increase in P/AI occurred when cows received a CIDR insert during the RES protocol or a GnRH injection 7 d before RES compared with RES cows (Dewey et al., 2010). Furthermore, no difference was detected between the CIDR treatment or when GnRH was administered 7 d before RES was initiated (Dewey et al., 2010). Recently, Chebel et al. (2013) did not observe an increase in P/AI when a CIDR insert was included in a RES protocol in lactating dairy cows. The authors attributed the lack of CIDR effect to treatment with GnRH or PGF_{2α} as an estrous cycle presynchronization strategy either 7 or 11 d, respectively, before initiation of the RES protocol. In addition, when the RES program was initiated 32 d post-AI in that study (Chebel et al., 2013), as well as in the present study, CIDR treatment did not increase pregnancy outcome.

One plausible explanation for the differential effect of inclusion of a CIDR insert based on day of initiation of RES is a more tightly synchronized return to estrus and ovulation after CIDR removal (Chebel et al., 2006; Lima et al., 2009). In the current experiment, a reduction in the number of cows displaying estrus and AI tended to occur when a CIDR insert was used. Use of CIDR inserts prevented cows from displaying estrus and ovulating even when luteolysis occurred during the CIDR insertion period (Chenault et al., 2003; El-Zarkouny and Stevenson, 2004; Dewey et al., 2010). Occurrence of premature luteolysis during treatment with a CIDR insert results in concentrations of circulating P₄ sufficient to inhibit GnRH and LH surges, preventing ovulation from occurring (Calder et al., 1999; Nation et al., 2000; Inskeep, 2004) until the CIDR insert is removed. This may improve synchrony of the estrous cycle of CIDR-treated cows. Bisinotto et al. (2010), however, initiated RES in lactating dairy cows 34 d after AI with or without a CIDR insert and observed an increase in P/AI. The improvement in P/AI with supplemental P₄ was observed only in cows that had a CL when the TAI was initiated, and the benefits were observed despite major changes in synchronization of the estrous cycle after AI. It appears that, in some cases, the effect of a CIDR on P/AI may occur from both improved estrous synchrony and increased P₄ in cows with a CL, as shown herein and in other studies (Bisinotto and Santos, 2011). It is

possible that the typical increase in P_4 from a single CIDR in high-producing cows, approximately 1 ng/mL (Bisinotto et al., 2013), improves preovulation follicle and oocyte quality in cows that have suboptimal systemic P_4 concentrations (Bisinotto and Santos, 2011).

Another reason for the differential effect of inclusion of a CIDR insert based on day of initiation of RES may be explained by cows initiating the RES protocol 39 d after AI at a more random stage of the estrous cycle. As mentioned above, more cows initiating RES 39 d after AI displayed estrus compared with cows initiating RES 32 d after AI, illustrating that cows were at a later stage of their estrous cycle when initiating the RES protocol 39 d after AI. Proper timing of RES initiation at different intervals after AI to improve P/AI has been evaluated. Fricke et al. (2003) observed that starting the RES protocol 19 to 21 d after AI reduced fertility; however, fertility was similar when RES began at 25 to 28 d after AI compared with starting the RES 32 to 33 d after AI (Fricke et al., 2003; Chebel et al., 2003; Sterry et al., 2006). A recent study reported no effect on fertility when RES was initiated either 32 or 39 d after AI (Lopes et al., 2013). Herein, when day of RES initiation was analyzed as a main effect, no differences were observed on P/AI. Vasconcelos et al. (1999) observed that when beginning the Ovsynch protocol at earlier stages of the estrous cycle (i.e., 5 to 9 d after estrus), more cows ovulated after the first GnRH (>90%) injection of an Ovsynch protocol. When estrous cycles of cows were presynchronized to allow more cows to start the Ovsynch protocol between 5 and 12 d after estrus, P/AI were increased compared with cows that started the Ovsynch protocol at random stages of the estrous cycle (Moreira et al., 2001). Further, when more cows were synchronized to earlier stages of the estrous cycle before starting Ovsynch (by reducing the time between presynchronization $PGF_{2\alpha}$ injections from 14 to 11), P/AI increased (Galvão et al., 2007).

Considering that the duration of the estrous cycle of high-producing lactating dairy cows is approximately 23 d (Sartori et al., 2004) and the interovulatory duration has increased from 20.2 to 22.3 d in a UK study (Royal et al., 2000), the majority of cows initiating the RES protocol 32 d after would have been around d 9 of their estrous cycle compared with cows initiating the RES protocol 39 d after AI, which would have been around d 16 of their estrous cycle. Although the percentage of cows that ovulated to the first GnRH injection of the RES protocol did not differ for either timing of treatment or inclusion of the CIDR insert, the proportion of cows that were detected in estrus and inseminated, number and proportion of cows with a CL, P_4 concentration at both the first GnRH and $PGF_{2\alpha}$ injections, proportion of cows with $P_4 \geq 1$ ng/

mL at $PGF_{2\alpha}$, and proportion of cows with luteolysis occurring between the first GnRH injection and the $PGF_{2\alpha}$ injection were all affected by timing of initiation of RES. These differences in responses illustrate that the average stage of the estrous cycle varied between cows initiating RES 32 versus 39 d after AI and emphasizes the need for appropriate strategies to improve synchrony of the estrous cycle before RES initiation, during treatment (i.e., CIDR insert inclusion), or both (Silva et al., 2007; Lopes et al., 2013).

Use of a CIDR insert increased P/AI for cows without a CL or when $P_4 < 1$ ng/mL at the first GnRH injection of the RES protocol. In studies that applied a CIDR insert in which cows were classified by cyclic status at the beginning of the TAI protocol for first AI, the interaction between CIDR treatment and cyclicity status had no effect on P/AI (El-Zarkouny and Stevenson, 2004; Stevenson et al., 2006; Chebel et al., 2010). In those studies, however, a greater proportion of cows were identified as anovular (range: 26.7 to 55.9%) compared with the current study, in which only 6% of the subsample of cows evaluated were anovular. Differences in prevalence of anovulation are most likely attributable to the design of the current study, in which cows were inseminated at greater DIM when fewer cows were anovular. Although the present experiment was not designed to test cyclicity status, potentially anovular or follicular-phase cows that did not have a CL or had $P_4 < 1$ ng/mL at first GnRH injection of the RES protocol had greater fertility, which, as mentioned previously, may be attributed to better synchrony of ovulation before TAI and improvement of P_4 concentration in cows without a CL. A benefit to including a CIDR insert was reported for cows without a CL that were selectively treated with a CIDR insert as part of a TAI protocol before first AI (Stevenson et al., 2008). In that study, cows without a CL and treated with a CIDR insert at the beginning of the TAI protocol had greater P/AI than those without a CL and not treated with a CIDR insert; however, the former had similar P/AI compared with cows having a CL and not treated with a CIDR insert. Treatment of cows with a CIDR insert when no CL is observed or when $P_4 < 1$ ng/mL at the initiation of the RES protocol probably improved P/AI by suppressing premature estrus and ovulation until TAI, or by exposing growing follicles and the uterus to greater P_4 concentrations, or both. Lactating dairy cows that did not ovulate in response to the first GnRH injection of the TAI protocol had smaller follicles at the end of the TAI protocol when treated with a CIDR compared with untreated controls (Lima et al., 2009). Inskeep (2004) noted that follicles maturing in the presence of marginally low concentrations of P_4 may be exposed to an increased frequency of pulsatile LH release, result-

ing in premature follicle and oocyte maturation and subsequent early embryo death or poor embryo quality.

In a recent study by Bisinotto et al. (2013), lactating Holstein cows were subjected to the 5-d TAI program and received 2 CIDR inserts if no CL was present, remained untreated if no CL was present (control), or were considered diestrus if ovaries had a CL. Progesterone supplementation with 2 CIDR inserts increased plasma P_4 to 2.65 ng/mL and restored fertility in lactating dairy cows lacking a CL at the initiation of the TAI protocol similar to that of cows in diestrus (Bisinotto et al., 2013). The authors concluded that supplementing P_4 with 2 CIDR inserts for cows without a CL increased P_4 concentrations and restored fertility similar to that of cows in diestrus, which is typically not observed when a single insert is used in high-producing lactating dairy cows (Bisinotto and Santos, 2011). This illustrates the P_4 deficiency experienced in lactating dairy cows and the opportunity to improve fertility with P_4 supplementation.

CONCLUSIONS

Results reported herein and in recent studies illustrate that CIDR inserts reduce the incidence of early ovulation before $PGF_{2\alpha}$ and increase synchronization of the estrous cycle, thereby resulting in improved synchrony of ovulation before TAI. In addition, inclusion of a CIDR insert within a RES protocol improved P/AI only when cows were not at optimal stages of the estrous cycle. Although the CIDR treatment can improve P/AI in cows without a CL or when $P_4 < 1$ ng/mL at the beginning of a RES protocol, initiation of RES at 32 compared with 39 d after AI did not affect P/AI.

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