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Supplementation of progesterone via controlled internal drug release inserts during ovulation synchronization protocols in lactating dairy cows[1]

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ABSTRACT

Our objective was to determine the effect of exogenous progesterone (P4) during a timed artificial insemination (TAI) protocol on pregnancies per AI (P/AI) in dairy cows not previously detected in estrus. Lactating cows (n = 3,248) from 7 commercial dairy herds were submitted to a presynchronization protocol (2 injections of $PGF_{2\alpha}$ 14 d apart; Presynch), and cows in estrus after the second $PGF_{2\alpha}$ received AI (EDAI; n = 1,583). Cows not inseminated by 12 to 14 d after the second $PGF_{2\alpha}$ injection were submitted to a TAI protocol (GnRH on d 0, $PGF_{2\alpha}$ on d 7, and GnRH + TAI 72 h after $PGF_{2\alpha}$). At onset of the TAI protocol, cows were balanced by parity and days in milk and assigned randomly to receive no exogenous P4 (control, n = 803) or a controlled internal drug release (CIDR) insert containing 1.38 g of P4 from d 0 to 7 (CIDR, n = 862). Blood samples were collected at the second $PGF_{2\alpha}$ injection of the Presynch and on the day of the first GnRH injection of the TAI protocol for P4 determination. When P4 in both samples was <1 ng/mL, cows were classified as anovular, whereas cows having at least 1 sample ≥1 ng/mL were classified as cyclic. Concentration of P4 at 11 to 14 d after AI was determined in a subgroup of cows (n = 453) from 2 herds. Pregnancy was diagnosed at 40 ± 5 and 65 ± 5 d after AI. Proportion of cows inseminated on estrus after the second $PGF_{2\alpha}$ injection of the Presynch protocol differed among herds (range = 26.7 to 59.8%). Overall P/AI for EDAI cows at 40 ± 5 and 65 ± 5 d were 36.2 and 33.7%, respectively, and pregnancy loss was 8.8%. Proportion of cyclic cows at the onset of the TAI protocol differed among herds (range from 66.5 to 86.3%), but did not differ between treatments (control = 72.4%, CIDR = 74.1%). Treatment affected P/AI at 40 ± 5 (control = 33.3%, CIDR = 38.1%) and 65 ± 5 (control = 30.0%, CIDR = 35.1%) d after AI but did not affect pregnancy loss (8.6%). Cyclic cows had greater P/AI at 40 ± 5 (38.2 vs. 29.3%) and 65 ± 5 d (35.1 vs. 26.1%) after AI, but cyclic status had no effect on pregnancy loss. Treatment affected P4 concentration after AI, with more CIDR cows having P4 ≥1 ng/mL (94.4 vs. 86.9%) and P4 ≥3.2 ng/mL (81.8 vs. 68.0%) at 11 to 14 d after AI compared with control cows. Treatment of cows not previously detected in estrus with a CIDR insert during a TAI protocol increased proportion of cows with functional CL after AI and P/AI.

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

INTRODUCTION

In recent years, dairy farms have relied heavily on estrus or ovulation synchronization protocols to submit cows for first postpartum AI, in particular the Ovsynch (Pursley et al., 1997) and Presynch + Ovsynch (Moreira et al., 2001; Navanukraw et al., 2004) protocols. These protocols allow for fixed-time AI (**TAI**) thus allowing managers to precisely control the DIM for first postpartum AI. This has minimized the limiting effects of reduced efficiency and accuracy of estrous detection on reproductive efficiency of lactating dairy cows. The proportion of pregnant cows 35 to 45 d after first postpartum AI, however, remains small and ranges from 20 to 45% (Moreira et al., 2001; El-Zarkouny et al., 2004; Sterry et al., 2007). This small proportion can be explained, in part, by the presence of relatively large proportion of anovular cows during the first 50 to 60 d postpartum, which has been reported to be between 20 and 50% (Moreira et al., 2001; El-Zarkouny et al., 2004; Santos et al., 2004).

Lack of exposure to progesterone (**P4**) before spontaneous or GnRH-induced ovulation results in greater

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risk for short luteal phases, which is characterized by luteolysis around d 10 of the estrous cycle (Rhodes et al., 2003; Inskeep, 2004), a time when the embryo does not produce sufficient IFN-τ to block the luteolytic cascade, resulting in reduced proportion of pregnant cows. Treatment of anovular cows with the Ovsynch protocol (Pursley et al., 1997) resulted in 51 to 91% of cows ovulating after the last GnRH injection (Fricke and Wiltbank, 1999; Cartmill et al., 2001; Gümen et al., 2003), but the concentrations of P4 to which cows are exposed to before ovulation and AI are low. Anovular cows treated with intravaginal controlled internal drug release (**CIDR**) inserts containing 1.38 g of P4 were more likely to resume cyclicity compared with untreated cows (Gümen and Wiltbank, 2005; Chebel et al., 2006; Cerri et al., 2009), and CIDR treatment provided priming with P4 that reduced the incidence of short luteal phases after AI (Rhodes et al., 2003; Cerri et al., 2009).

In many dairy farms, cows that display estrus after the second $PGF_{2\alpha}$ injection of the Presynch protocol are inseminated. Insemination of cows in estrus would likely increase the proportion of anovular cows submitted to TAI protocols (i.e., Ovsynch), because anovular cows are less likely to display estrus (Cerri et al., 2004; Chebel et al., 2006). This has been one of the motives for the recommendation to use CIDR inserts during the TAI protocol in cows not previously detected in estrus. In 3 of 6 experiments reported in 5 articles, inclusion of CIDR inserts to TAI protocols improved P/AI (El-Zarkouny et al., 2004, Exp. 1; Melendez et al., 2006; Stevenson et al., 2008), whereas in 3 experiments CIDR inserts had no effect (El-Zarkouny et al., 2004 , Exp. 2; Galvão et al., 2004; Stevenson et al., 2006]. Furthermore, response to treatment with CIDR inserts seems to be farm dependent (Stevenson et al., 2006) and, although CIDR-treated cows had greater P4 concentrations after AI in one study (Melendez et al., 2006), the reasons why the treatment with CIDR inserts during the TAI protocol improves pregnancies per AI (**P/AI**) are unknown.

The hypothesis of the current study was that P4 treatment (CIDR insert) of lactating dairy cows not previously detected in estrus as part of a TAI protocol would increase the proportion of cows becoming pregnant after TAI by promoting resumption of ovarian cycles in anovular cows and by improving synchronization of ovulation. Therefore, the objectives of this study were to evaluate P4 concentrations 11 to 14 d after AI, P/AI, and pregnancy loss of lactating dairy cows that were not detected in estrus after $PGF_{2\alpha}$-based presynchronization protocol and that were submitted to TAI protocols with the addition of supplemental P4 via a CIDR insert.

MATERIALS AND METHODS

Animals and Treatments

The study was conducted in 7 commercial dairy herds located in Arizona (one open dry lot dairy, **AZ**), California (one open dry lot dairy, **CA1**; and 2 free-stall dairies; **CA2** and **CA3**), Kansas (2 free-stall dairies, **KS1** and **KS2**), and Wisconsin (one free-stall dairy, **WI**). Weekly, lactating dairy cows were submitted to a presynchronization protocol that consisted of 2 injections of $PGF_{2\alpha}$ (25 mg of dinoprost as tromethamine salt; 5 mL, Lutalyse Sterile Solution, Pfizer Animal Health, New York, NY) given 14 d apart (Presynch protocol), with the last injection given at the end of the voluntary waiting period (**VWP**). The VWP among herds ranged from 47 ± 3 to 54 ± 3 DIM. Cows detected in estrus after the last $PGF_{2\alpha}$ injection of the presynchronization protocol were inseminated (**EDAI**), whereas those not detected in estrus were submitted to TAI protocols 12 d (CA1, CA2, CA3, KS1, and K2 herds) or 14 d (AZ and WI herds) after the end of the VWP.

At the beginning of the TAI protocol, cows were balanced according to parity and DIM and were assigned randomly to 1 of 2 treatments: control or CIDR. The TAI protocol consisted of an injection of GnRH (100 μg of gonadorelin diacetate tetrahydrate; 2 mL, Cystorelin; Merial Ltd., Iselin, NJ), followed 7 d later by an injection of $PGF_{2\alpha}$, and a second injection of GnRH + TAI 72 h later. Cows in the CIDR treatment received a CIDR (Eazi-Breed CIDR Cattle Insert; Pfizer Animal Health) insert containing 1.38 g of P4 from the first injection of GnRH to the $PGF_{2\alpha}$ injection of the TAI protocol.

Blood Samples, Progesterone Assay, and Characterization of Anovular Condition and Presence of Functional Corpus Luteum

Blood samples for determination of P4 concentration were collected from the median coccygeal blood vessels using evacuated tubes containing either K_2 EDTA or no anticoagulant. Upon collection samples were placed on ice and transported to the laboratory. Samples were either stored at 5°C for 24 h until plasma or serum was harvested or, upon arrival in the laboratory, plasma or serum was harvested immediately. Plasma or serum samples were frozen at −25°C and later analyzed for P4 by ELISA or RIA. Samples were analyzed in duplicate in the same assay. Intraassay CV ranged from 5.0 to 7.7%, and interassay CV ranged from 7.6 and 11.7%.

Samples collected on the day of the last $PGF_{2\alpha}$ injection of the Presynch protocol and at the time of the first GnRH injection of the TAI protocol were used to determine cyclic status. Cows having P4 <1 ng/mL in both samples were classified as anovular, whereas cows with at least one sample in which P4 ≥1 ng/mL were classified as cyclic. Based on concentrations of P4, cyclic cows were further classified as having a functional corpus luteum (**CL**) at the beginning of the TAI protocol. Therefore, if the blood sample collected at the time of the first GnRH injection of the TAI protocol had P4 ≥1.0 ng/mL cows were considered to have a functional CL, otherwise cows were considered to have no functional CL when P4 <1 ng/mL.

In a subgroup of cows (control = 222 and CIDR = 231) from CA and KS herds, blood also was sampled at 11 to 14 d after TAI. Cows were then classified as not having a functional CL when P4 <1.0 ng/mL or as having a functional CL when P4 ≥1 ng/mL at 11 to 14 d after AI. Furthermore, according to the receiver operating characteristic (ROC) analysis, the best sensitivity and specificity for predicting pregnancy status at 40 ± 5 d after AI was observed when P4 = 3.2 ng/mL. Therefore, cows were also classified as having P4 <3.2 ng/mL or P4 ≥3.2 ng/mL at 11 to 14 d after AI.

Estrous Detection and AI

Cows were observed daily in the morning for signs of estrus and those in estrus were inseminated on the same morning. Estrus was characterized by the following symptoms: removal of tail paint or tail chalk, vaginal mucous discharge, bellowing, increased nervousness and activity, walking fence line, swelling and reddening of the vulva, mounting other cows, lower milk yield, or standing estrus.

BCS and Milk Yield

In 6 of the 7 herds, cows were scored for body condition (1 = emaciated, 5 = obese; Ferguson et al., 1994) at enrollment. For purpose of statistical analysis, cows were classified according to BCS as low (BCS ≤2.75) or moderate (BCS ≥3.0). Cows were milked 2 or 3 times daily. Milk yields were recorded for individual cows once monthly and average yield of 3.5% FCM in the first 3 mo of lactation was recorded for individual cows to determine the effect of milk yield on P/AI and pregnancy loss.

Pregnancy Diagnosis and Reproductive Outcomes

Pregnancy was diagnosed by palpation per rectum of the uterus and its contents at 40 ± 5 d after AI. Cows diagnosed pregnant were re-examined by palpation per rectum of uterine contents at 65 ± 5 d after AI. In 1 herd, EDAI cows diagnosed pregnant 40 ± 5 d after AI were not re-examined at 65 ± 5 d after AI. Cows that displayed estrus and were re-inseminated before the first pregnancy diagnosis were considered to be not pregnant to the AI. Pregnant cows re-inseminated between the first and second pregnancy diagnosis were considered to have had pregnancy loss.

Pregnancies per AI were calculated by dividing the number of cows diagnosed pregnant at 40 ± 5 d or 65 ± 5 d after AI by the number of cows receiving AI in each treatment (control or CIDR). Pregnancies per AI also were calculated for EDAI cows. Proportion of cows having pregnancy loss was calculated as the number of cows diagnosed as not pregnant at 65 ± 5 d after AI plus the number of cows re-inseminated before re-examination divided by the number of cows diagnosed pregnant at 40 ± 5 d after AI.

Study Design and Statistical Analyses

The experiment was a completely randomized design with cows balanced by parity and DIM at enrollment. The number of experimental units (cow) initially planned per treatment (800 cows) was expected to provide sufficient power to determine statistical significance when P/AI between treatments differed in 4.8 percentage units and P/AI after first postpartum AI ranges from 30 to 40% ($\alpha = 0.05$; $\beta = 0.80$). Furthermore, the number of experimental units was expected to provide enough pregnant cows at 40 ± 5 d after AI so that a 6.2-percentage-unit difference in pregnancy loss between treatments would be statistically significant when pregnancy loss between 40 ± 5 and 65 ± 5 d after AI ranges from 10 to 21% ($\alpha = 0.05$; $\beta = 0.80$).

Dichotomous outcomes were analyzed by logistic regression using the LOGISTIC procedure of SAS (SAS Institute Inc., Cary, NC). Proportion of cows classified as cyclic was evaluated using a model that included treatment (control vs. CIDR), herd, parity (primiparous vs. multiparous), FCM, and interactions between treatment and the other independent variables. The initial model to evaluate P/AI and proportion of cows having pregnancy loss included treatment (control vs. CIDR), herd, parity (primiparous vs. multiparous), cyclic status (anovular vs. cyclic), FCM, and interactions between treatment and the other independent variables. To evaluate the effect of the interaction between treatment and BCS on P/AI and pregnancy loss, a second statistical analysis was conducted excluding cows without BCS data. The model was similar to that described previously, but it also included BCS (low vs. moderate) and the interaction between treatment and BCS. To evaluate the effect of treatment on P/AI and pregnancy loss of cyclic cows according to presence or absence of a functional CL at the beginning of the TAI protocol, only cyclic cows were included in the analysis and the statistical model used included treatment (control vs. CIDR), herd, parity (primiparous vs. multiparous), presence of functional CL at the beginning of the TAI protocol (CL vs. no CL), FCM, and interactions between treatment and the other independent variables. The models used to evaluate the proportion of cows having a functional CL (P4 $\geq$1 ng/mL) and the proportion of cows with P4 $\geq$3.2 ng/mL at 11 to 14 d after AI included treatment (control vs. CIDR), herd, parity (primiparous vs. multiparous), BCS, cyclic status, FCM, and interactions between treatment and the other independent variables. The logistic regression models removed variables by a backward elimination based on the Wald's statistic criterion when $P > 0.15$.

Progesterone concentrations 11 to 14 d after AI were analyzed by ANOVA using the GLM procedure of SAS. The initial model included treatment (control vs. CIDR), herd, parity (primiparous vs. multiparous), cyclic status, BCS, FCM, pregnancy status at 40 ± 5 d after AI, and the interactions between treatment and the remaining independent variables. In a second model, to evaluate the effect of treatment on P4 concentration at 11 to 14 d after AI according to presence of a functional CL at 11 to 14 d after AI (P4 $\geq$1 ng/mL), independent variables included were treatment (control vs. CIDR), herd, parity (primiparous vs. multiparous), cyclic status, BCS, FCM, pregnancy status at 40 ± 5 d after AI, presence of CL at 11 to 14 d after AI (CL vs. no CL), and the interactions between treatment and the remaining independent variables.

RESULTS

A total of 3,248 lactating dairy cows were submitted to the Presynch protocol. Of these cows, 48.7% were inseminated after detected estrus (EDAI = 1,583) before enrollment in the TAI protocol. A smaller ($P < 0.01$) proportion of primiparous compared with multiparous cows was inseminated after detected estrus (44.1 vs. 51.6%, respectively). The proportion of cows inseminated after detected estrus differed ($P < 0.01$) among herds and tended ($P = 0.10$) to be affected by the interaction between parity and herd (Table 1).

The average DIM at first postpartum AI was 65.8 ± 0.2 d and DIM at first AI ranged from 47 to 80. Average DIM at first AI differed ($P < 0.01$) among herds (range 63.2 ± 0.4 to 70.4 ± 0.4 d), and although parity ($P = 0.67$) did not affect DIM at first AI, the interaction between parity and herd did affect ($P = 0.01$) it (Table 1). Average yield of 3.5% FCM during the first 3 mo of lactation was 33.4 ± 0.2 and 43.5 ± 0.2 kg/d for primiparous and multiparous cows, respectively. Parity, herd, and the interaction between parity and herd affected ($P < 0.01$) FCM (Table 1).

Overall P/AI at 40 ± 5 d after AI for all cows (EDAI, control, and CIDR) was 36.2%. Parity ($P = 0.09$) and herd ($P = 0.09$) tended to affect P/AI at 40 ± 5 d after AI and the interaction between parity and herd affected ($P < 0.01$) P/AI (Table 1). At 65 ± 5 d after AI, P/AI for all cows (EDAI, control, and CIDR) was 33.7%, and was not affected by parity ($P = 0.16$) or herd ($P = 0.79$). The interaction between parity and herd, however, affected ($P = 0.02$) P/AI at 65 ± 5 d after AI (Table 1). Pregnancy loss from 40 ± 5 to 65 ± 5 d after AI was 8.8% for all cows (EDAI, control, and CIDR), and neither parity ($P = 0.79$), nor herd ($P = 0.59$), nor the interaction between parity and herd ($P = 0.79$) affected the proportion of cows having pregnancy loss (Table 1).

Among EDAI cows, the P/AI at 40 ± 5 d after AI was 36.6%, and it ranged from 25.3 to 41.2% among herds. At 65 ± 5 d after AI, EDAI cows had P/AI of 35.1% with a range from 29.9 to 38.6%. The increased minimum value for the range of P/AI at 65 ± 5 d after AI compared with the range observed at 40 ± 5 d after AI was a consequence of the nonexamination of EDAI cows from the herd having the smallest P/AI for EDAI cows at 40 ± 5 d after AI. Pregnancy loss from 40 ± 5 to 65 ± 5 d after AI was 9.0% for EDAI cows with a range from 3.5 to 12.5% among herds.

There were 803 and 862 cows in the control and CIDR treatments, respectively. The proportion of control and CIDR cows classified as cyclic at the beginning of the TAI protocol was similar ($P = 0.27$), but more ($P < 0.01$) multiparous than primiparous cows were cyclic (81.3 vs. 62.0%; Table 2). Herd affected ($P < 0.01$) the proportion of cows classified as cyclic and ranged from 66.5 to 86.3%.

Among cows from which BCS data were recorded, no difference in average BCS (2.6 ± 0.02; Table 2) was detected between treatments ($P = 0.71$) or parities ($P = 0.44$). Descriptive data regarding DIM at first AI and FCM for EDAI, control, and CIDR cows are presented in Table 2.

Pregnancy per AI and Pregnancy Loss for Control and CIDR cows

Cows in the CIDR treatment had ($P = 0.05$) greater P/AI at 40 ± 5 d after AI than control cows (38.1 vs. 33.3%; Table 3). Cyclic status affected ($P < 0.01$) P/AI at 40 ± 5 d after AI (Table 3) because a greater proportion of cyclic cows was diagnosed pregnant compared with anovular cows (38.2 vs. 29.3%). Neither parity ($P = 0.77$), nor herd ($P = 0.38$), nor FCM ($P = 0.63$)

Table 1. Number of cows, proportion of cows inseminated at detected estrus after the presynchronization protocol, average yield of 3.5% FCM in the first 3 mo of lactation, pregnancy per AI (P/AI), and pregnancy loss for all cows submitted to the presynchronization protocol according to herd and parity

Herd and parity[1]	Cows, n	Insemination after detected estrus,[2] %	DIM at first AI,[3] d (±SEM)	FCM,[4] kg/d (±SEM)	P/AI 40 ± 5 d after AI[6]	P/AI 65 ± 5 d after AI[7]	Pregnancy loss[5]
AZ							
Primiparous	141	51.1	73.8 ± 0.3	35.3 ± 0.7	34.8	34.8[8]	7.7
Multiparous	296	59.8	72.1 ± 0.3	39.4 ± 0.5	26.7	32.8[8]	0.0
CA1							
Primiparous	39	46.2	69.7 ± 0.7	31.6 ± 1.3	46.2	43.6	5.6
Multiparous	215	62.3	70.3 ± 0.3	46.4 ± 0.6	33.0	29.3	11.3
CA2							
Primiparous	279	43.4	66.1 ± 0.3	33.0 ± 0.5	35.5	31.9	10.1
Multiparous	595	51.1	66.1 ± 0.2	43.4 ± 0.3	40.7	37.9[9]	6.6
CA3							
Primiparous	62	24.2	65.0 ± 0.5	29.8 ± 1.0	40.3	37.1	8.0
Multiparous	276	50.4	65.0 ± 0.3	41.1 ± 0.5	40.6	35.9	11.6
KS1							
Primiparous	76	40.8	69.8 ± 0.5	30.7 ± 0.9	39.5	35.5	10.0
Multiparous	117	39.3	70.2 ± 0.4	36.9 ± 0.8	28.2	26.5	6.1
KS2							
Primiparous	124	22.6	64.0 ± 0.4	32.8 ± 0.8	36.3	33.9	6.7
Multiparous	164	29.9	64.0 ± 0.4	51.1 ± 0.7	31.1	27.6[9]	10.0
WI							
Primiparous	521	50.5	69.7 ± 0.2	34.1 ± 0.4	33.6	30.1[9]	9.3
Multiparous	343	54.2	69.6 ± 0.2	46.2 ± 0.5	42.6	37.9	11.0

[1]Commercial dairy herds located in Arizona (one open dry lot dairy, AZ), California (one open dry lot dairy, CA1; and 2 free-stall dairies, CA2 and CA3), Kansas (2 free-stall dairies, KS1 and KS2), and Wisconsin (one free-stall dairy, WI).

[2]Effect of parity ($P < 0.01$), herd ($P < 0.01$), parity by herd interaction ($P = 0.10$).

[3]Effect of parity ($P = 0.67$), herd ($P < 0.01$), parity by herd interaction ($P = 0.01$).

[4]Effect of parity ($P < 0.01$), herd ($P < 0.01$), parity by herd interaction ($P < 0.01$).

[5]Effect of parity ($P = 0.79$), herd ($P = 0.59$), parity by herd interaction ($P = 0.79$).

[6]Effect of parity ($P = 0.08$), herd ($P = 0.09$), parity by herd interaction ($P < 0.01$).

[7]Effect of parity ($P = 0.16$), herd ($P = 0.79$), parity by herd interaction ($P = 0.02$).

[8]Cows inseminated at detected estrus after the second $PGF_{2\alpha}$ injection of the presynchronization that were diagnosed pregnant at 40 ± 5 d after AI were not re-examined. Therefore, the number of primiparous and multiparous cows diagnosed pregnant at 40 ± 5 d after AI that were re-examined at 65 ± 5 d after AI was 71 and 124, respectively.

[9]A few cows diagnosed pregnant at 40 ± 5 d after AI were sold before re-examination at 65 ± 5 d after AI (CA2 = 1, KS2 = 1, WI = 3).

affected P/AI at 40 ± 5 d after AI. The interactions between treatment and cyclic status ($P = 0.75$; Table 3) and between treatment and herd ($P = 0.21$) did not affect P/AI at 40 ± 5 d after AI. The range of P/AI at 40 ± 5 d after TAI among herds was from 22.4 to 46.9% for control cows and from 32.7 to 41.4% for CIDR cows.

Pregnancies per AI at 65 ± 5 d after AI were greater ($P = 0.04$) for CIDR cows compared with control cows (35.1 vs. 30.0%; Table 3). Cyclic status affected ($P < 0.01$) P/AI at 65 ± 5 d after AI (cyclic = 35.1, anovular = 26.1%; Table 3), whereas parity ($P = 0.66$), herd ($P = 0.62$), and FCM ($P = 0.39$) had no effect on P/AI at 65 ± 5 d after AI. The interactions between treatment and cyclic status ($P = 1.00$; Table 3) and between treatment and herd ($P = 0.20$) also had no effect on P/AI at 65 ± 5 d after AI. Among herds, the range of P/AI at 65 ± 5 d after AI was 20.4 to 40.6% for control cows and 28.9 to 39.7% for CIDR cows.

Proportion of cows having pregnancy loss from 40 ± 5 to 65 ± 5 d after TAI did not differ ($P = 0.38$) between treatments (control = 9.7%, CIDR = 7.7%; Table 3). Cyclic status ($P = 0.30$; Table 3), parity ($P = 0.78$), herd ($P = 0.60$), and FCM ($P = 0.37$) also had no effect on incidence of pregnancy loss. Pregnancy loss among herds ranged from 0 to 16.7% for control cows and from 4.2 to 11.8% for CIDR cows.

Effect of Presence of a Functional CL at the Beginning of the TAI Protocol on P/AI and Pregnancy Loss of Cyclic Cows

Among cyclic cows (n = 1,199), 93.4% had P4 ≥1 ng/mL at the beginning of the TAI protocol and were considered to have a functional CL. Progesterone concentrations at the beginning of the TAI protocol were 3.9 ± 0.1 and 0.4 ± 0.3 ng/mL for cows classified as having or not having a functional CL, respectively. No difference ($P = 0.27$) was detected in the proportion of cows with a functional CL between treatments (Table 4), but CIDR cows had greater ($P = 0.01$) P4 concentrations at the beginning of the TAI protocol than control cows (3.9 ± 0.1 vs. 3.5 ± 0.1 ng/mL).

When only cyclic cows were included in the statistical model to evaluate P/AI and pregnancy loss, treatment tended ($P = 0.09$) to affect P/AI 40 ± 5 d after AI but presence of a functional CL at the beginning of the TAI protocol ($P = 0.46$) had no effect (Table 4). Furthermore, the interaction between treatment and presence of a functional CL at the beginning of the TAI protocol did not affect ($P = 0.36$) P/AI at 40 ± 5 d after AI (Table 4). Similarly, P/AI at 65 ± 5 d after AI also tended ($P = 0.09$) to be affected by treatment but presence of a functional CL at the beginning of the TAI protocol ($P = 0.38$), or the interaction between treatment and presence of a functional CL ($P = 0.30$; Table 4). Among cyclic cows, treatment ($P = 0.60$), presence of a functional CL ($P = 0.98$), and the interaction between treatment and presence of a functional CL ($P = 0.61$) did not affect the proportion of cows with pregnancy loss (Table 4).

P4 Concentration 11 to 14 d after TAI

In the subgroup of cows from which blood samples were collected at 11 to 14 d after TAI, P4 concentrations were affected ($P = 0.04$) by treatment (control = 5.0 ± 0.3 vs. CIDR = 5.7 ± 0.3 ng/mL; Table 5). Cyclic status (cyclic = 5.4 ± 0.2 vs. anovular = 5.3 ± 0.3 ng/mL; $P = 0.58$) and the interaction between treatment and cyclic status ($P = 0.93$) had no effect on P4 concentrations 11 to 14 d after AI (Table 5). Herd affected ($P < 0.01$) P4 concentrations after TAI (range from 4.6 ± 0.3 to 5.7 ± 0.3 ng/mL). Primiparous cows tended ($P = 0.08$) to have greater P4 concentrations after TAI than multiparous cows (5.6 ± 0.3 vs. 5.1 ± 0.2 ng/mL). Progesterone concentrations at 11 to 14 d after AI were affected ($P = 0.05$) by FCM, as cows with smallest milk yield had the smallest P4 concentration.

Proportion of cows having a functional CL (P4 ≥1 ng/mL) at 11 to 14 d after AI was affected ($P < 0.01$) by treatment (Table 5). A tendency ($P = 0.06$) was detected for cyclic status to affect the proportion of cows with P4 ≥ 1 ng/mL after AI (Table 5). The interaction between treatment and cyclic status, however, had no ($P = 0.70$) effect on the proportion of cows having P4 ≥1 ng/mL at 11 to 14 d after AI (Table 5). Parity ($P = 0.56$), herd ($P = 0.36$), BCS ($P = 0.52$), and FCM ($P = 0.53$) had no effect on proportion of cows with P4 ≥1 ng/mL 11 to 14 d after AI.

A greater ($P < 0.01$) proportion of CIDR cows had P4 ≥3.2 ng/mL at 11 to 14 d after AI (Table 5). Cyclic

Table 2. Descriptive statistics of DIM at first AI and average yield of 3.5% FCM during the first 3 mo of lactation for cows inseminated at detected estrus after the presynchronization (EDAI) or cows submitted to the timed AI protocol (control and CIDR)

Item	EDAI[1]		Control[2]		CIDR[3]		*P*-value		
	Primiparous	Multiparous	Primiparous	Multiparous	Primiparous	Multiparous	Trt[4]	Parity	Trt × Parity
Cows, n	548	1,035	330	473	364	498	—	—	—
Cyclic,[5] %	—	—	62.9	79.1	61.2	83.5	0.27	<0.01	0.08
BCS[6] (± SEM)	—	—	2.53 ± 0.03	2.50 ± 0.02	2.53 ± 0.03	2.52 ± 0.02	0.71	0.44	0.57
DIM at first AI, d (±SEM)	58.3 ± 0.3	58.0 ± 0.2	73.2 ± 0.3	73.3 ± 0.2	73.3 ± 0.3	73.3 ± 0.2	<0.01	0.67	0.60
FCM,[7] kg/d (±SEM)	32.3 ± 0.5	43.3 ± 0.3	32.3 ± 0.5	43.7 ± 0.4	32.7 ± 0.5	43.5 ± 0.4	0.77	<0.01	0.78

[1]EDAI = cows inseminated at detected estrus after the second injection of PGF of the presynchronization protocol.

[2]Control = cows not observed in estrus and submitted to a timed AI protocol (GnRH on d 0, $PGF_{2\alpha}$ on d 7, GnRH 48 to 72 h after PGF, and fixed time AI 0 to 24 h after the second GnRH). GnRH = 100 μg of gonadorelin diacetate tetrahydrate (2 mL, Cystorelin; Merial Ltd., Iselin, NJ); and $PGF_{2\alpha}$ = 25 mg of dinoprost as tromethamine salt (5 mL Lutalyse Sterile Solution; Pfizer Animal Health, New York, NY).

[3]CIDR = cows not observed in estrus and submitted to a timed AI protocol described for control cows, with the addition of a CIDR insert from d 0 to 7. CIDR insert = intravaginal controlled internal drug release (Eazi-Breed CIDR Cattle Insert; Pfizer Animal Health) insert containing 1.38 g of progesterone.

[4]Trt = treatment.

[5]Cows in which progesterone ≥1 ng/mL in at least 1 of 2 blood samples collected at the time of the second injection of PGF of the presynchronization and at the beginning of the timed AI protocol.

[6]Body condition scored at the beginning of the timed AI protocol.

[7]Average yield of 3.5% FCM during the first 3 mo of lactation.

Table 3. Effect of treatment (Trt) and cyclic status (CS) on pregnancy per AI (P/AI) at 40 ± 5 and 65 ± 5 d after AI and on pregnancy loss for cows submitted to timed AI

Item, % (n)	Control[1] Cyclic[3]	Control[1] Anovular[4]	CIDR[2] Cyclic[3]	CIDR[2] Anovular[4]	*P*-value Trt	*P*-value CS	*P*-value Trt × CS
P/AI at 40 ± 5 d after AI	35.6 (581)	27.2 (221)	40.5 (637)	31.4 (223)	0.05	<0.01	0.75
P/AI at 65 ± 5 d after AI	32.5 (581)	23.5 (221)	37.4 (636)[5]	28.7 (223)	0.04	<0.01	1.00
Pregnancy loss	8.7 (207)	13.3 (60)	7.4 (257)[5]	8.6 (70)	0.38	0.30	0.49

[1]Control = cows not observed in estrus and submitted to a timed AI protocol (GnRH on d 0, $PGF_{2\alpha}$ on d 7, GnRH 48 to 72 h after PGF, and fixed time AI 0 to 24 h after the second GnRH). GnRH = 100 µg of gonadorelin diacetate tetrahydrate (2 mL, Cystorelin; Merial Ltd., Iselin, NJ); and $PGF_{2\alpha}$ = 25 mg of dinoprost as tromethamine salt (5 mL Lutalyse Sterile Solution; Pfizer Animal Health, New York, NY).

[2]CIDR = cows not observed in estrus and submitted to a timed AI protocol described for control cows, with the addition of a CIDR insert from d 0 to 7. CIDR insert = intravaginal controlled internal drug release (Eazi-Breed CIDR Cattle Insert; Pfizer Animal Health) insert containing 1.38 g of progesterone.

[3]Cows in which progesterone ≥1 ng/mL in at least 1 of 2 blood samples collected at the time of the second injection of PGF of the presynchronization and at the beginning of the timed AI protocol.

[4]Cows in which progesterone <1 ng/mL in both blood samples collected at the time of the second injection of PGF of the presynchronization and at the beginning of the timed AI protocol.

[5]One cow diagnosed pregnant at 40 ± 5 d after AI was sold before re-examination at 65 ± 5 d after AI.

status (P = 0.16) and the interaction between treatment and cyclic status (P = 0.40) did not affect the proportion of cows with P4 ≥3.2 ng/mL (Table 5). Although parity (P = 0.13), BCS (P = 0.26), and FCM (P = 0.16) had no effect on proportion of cows with P4 ≥3.2 ng/mL at 11 to 14 d after AI, herd was a source ($P < 0.01$) of variation (range 67.1 to 84.8%).

Cows classified as having a functional CL at 11 to 14 d after AI had greater ($P < 0.01$) concentrations of P4 (5.7 ± 0.2 ng/mL) than those classified as not having a functional CL (1.1 ± 0.4 ng/mL). When presence of a functional CL was included in the model to evaluate P4 concentrations 11 to 14 d after AI, treatment (P = 0.61), cyclic status (P = 0.88), and the interaction between treatment and cyclic status (P = 0.97) had no effect on P4 concentrations. Similarly, the interaction between treatment and presence of a functional CL 11 to 14 d after AI had no effect (P = 0.62) on P4 concentrations.

DISCUSSION

The proportion of cows inseminated after detected estrus following the Presynch protocol was similar to that described in previous studies (Stevenson and Phatak, 2005; Chebel et al., 2006). Among cows not detected in estrus and submitted to the TAI protocol, 73.3% were classified as cyclic at the beginning of the TAI protocol. The range among herds was from 66.5 to 86.3%, which is within the range of previously published studies (Moreira et al., 2001; El-Zarkouny et al., 2004; Galvão et al., 2004).

Table 4. Effect of treatment (Trt) and presence of a functional corpus luteum (CL) at the beginning of the timed AI (TAI) protocol (CL or no CL) on pregnancy per AI (P/AI) at 40 ± 5 and 60 ± 5 d after AI and on pregnancy loss of cows classified as cyclic before the start of the TAI protocol

Item, % (n)	Control[1] CL[3]	Control[1] No CL[4]	CIDR[2] CL[3]	CIDR[2] No CL[4]	*P*-value Trt	*P*-value CL	*P*-value Trt × CL
Proportion	94.4 (534)	5.7 (32)	92.6 (586)	7.4 (47)	0.19	—	—
P/AI at 40 ± 5 d after AI	36.0 (534)	25.0 (32)	40.4 (586)	38.3 (47)	0.09	0.46	0.36
P/AI at 65 ± 5 d after AI	33.0 (534)	21.9 (32)	37.3 (585)[5]	36.2 (47)	0.09	0.38	0.30
Pregnancy loss	8.3 (192)	12.5 (8)	7.6 (236)[5]	5.6 (18)	0.70	0.98	0.61

[1]Control = cows not observed in estrus and submitted to a TAI protocol (GnRH on d 0, $PGF_{2\alpha}$ on d 7, GnRH 48 to 72 h after PGF, and fixed TAI 0 to 24 h after the second GnRH). GnRH = 100 µg of gonadorelin diacetate tetrahydrate (2 mL, Cystorelin; Merial Ltd., Iselin, NJ); and $PGF_{2\alpha}$ = 25 mg of dinoprost as tromethamine salt (5 mL Lutalyse Sterile Solution; Pfizer Animal Health, New York, NY).

[2]CIDR = cows not observed in estrus and submitted to a TAI protocol described for control cows, with the addition of a CIDR insert from d 0 to 7. CIDR insert = intravaginal controlled internal drug release (Eazi-Breed CIDR Cattle Insert; Pfizer Animal Health) insert containing 1.38 g of progesterone.

[3]Cows in which progesterone ≥1 ng/mL in the blood sample collected at the beginning of the TAI protocol.

[4]Cows in which progesterone <1 ng/mL in the blood sample collected at the beginning of the TAI protocol.

[5]One cow diagnosed pregnant at 40 ± 5 d after AI was sold before re-examination at 65 ± 5 d after AI.

Table 5. Effect of treatment (Trt) and cyclic status (CS) on progesterone (P4) concentration at 11 to 14 d after AI and proportion of cows with P4 ≥1 ng/mL or P4 ≥3.2 ng/mL at 11 to 14 d after timed AI

	Control[1]		CIDR[2]		*P*-value		
Item	Cyclic[3]	Anovular[4]	Cyclic[3]	Anovular[4]	Trt	CS	Trt × CS
P4, ng/mL	5.1 ± 0.3	4.9 ± 0.4	5.7 ± 0.3	5.6 ± 0.4	0.04	0.58	0.93
P4 ≥1 ng/mL, % (n)	88.8 (160)	82.3 (62)	95.4 (174)	91.2 (57)	<0.01	0.06	0.70
P4 ≥3.2 ng/mL, % (n)	70.6 (160)	61.3 (62)	83.9 (174)	75.4 (57)	<0.01	0.16	0.40

[1]Control = cows not observed in estrus and submitted to a timed AI protocol (GnRH on d 0, $PGF_{2\alpha}$ on d 7, GnRH 48 to 72 h after PGF, and fixed time AI 0 to 24 h after the second GnRH). GnRH = 100 μg of gonadorelin diacetate tetrahydrate (2 mL, Cystorelin; Merial Ltd., Iselin, NJ); and $PGF_{2\alpha}$ = 25 mg of dinoprost as tromethamine salt (5 mL, Lutalyse Sterile Solution; Pfizer Animal Health, New York, NY).

[2]CIDR = cows not observed in estrus and submitted to a timed AI protocol described for control cows, with the addition of a CIDR insert from d 0 to 7. CIDR insert = intravaginal controlled internal drug release (Eazi-Breed CIDR Cattle Insert; Pfizer Animal Health) insert containing 1.38 g of progesterone.

[3]Cows in which progesterone ≥1 ng/mL in at least 1 of 2 blood samples collected on the day of the second injection of $PGF_{2\alpha}$ of the presynchronization and at the beginning of the timed AI protocol.

[4]Cows in which progesterone <1 ng/mL in both blood samples collected on the day of the second injection of $PGF_{2\alpha}$ of the presynchronization and at the beginning of the timed AI protocol.

In the present study, cows not detected in estrus after the Presynch protocol and submitted to a TAI protocol with CIDR inserts had greater P/AI at 40 ± 5 and 65 ± 5 d after AI than those not treated with CIDR inserts. This improvement in P/AI, however, was independent of herd and cyclic status at the beginning of the TAI protocol, because cyclic and anovular cows treated with CIDR inserts had greater P/AI than their counterparts that did not receive a CIDR. Although the results of adding a CIDR insert to TAI protocols on reproductive performance of lactating dairy cows have been inconsistent, the lack of effect of the interaction between treatment with CIDR insert and cyclic status at the beginning of the TAI protocol on P/AI has been well documented. As mentioned above, 3 experiments reported increases in P/AI in cows submitted to TAI protocols and treated with CIDR inserts (El-Zarkouny et al., 2004, Exp. 1; Melendez et al., 2006; Stevenson et al., 2008), whereas 3 experiments reported no improvements in P/AI (El-Zarkouny et al., 2004, Exp. 2; Galvão et al., 2004; Stevenson et al., 2006). The reasons for such variation in response to CIDR treatment are difficult to identify. Four different designs were used in those experiments: 1) estrous cycles presynchronized with $PGF_{2\alpha}$ (El-Zarkouny et al., 2004, Exp. 2; Galvão et al., 2004; Melendez et al., 2006; Stevenson et al., 2008); 2) estrous cycles not presynchronized with $PGF_{2\alpha}$ (El-Zarkouny et al., 2004, Exp. 1; Stevenson et al., 2006); 3) only cows not detected in estrus were submitted to a TAI program and were eligible to receive CIDR treatment (Melendez et al., 2006); or 4) all cows were submitted to a TAI program and were eligible to receive CIDR treatment (El-Zarkouny et al., 2004, Exp. 1 and 2); Galvão et al., 2004; Stevenson et al., 2006, 2008). Further, synchronization of ovulation at the end of the TAI protocol was induced with GnRH (El-Zarkouny et al., 2004, Exp. 1 and 2; Melendez et al., 2006; Stevenson et al., 2006, 2008) or estradiol cypionate (Galvão et al., 2004). Nonetheless, in those experiments in which cows were classified according to cyclic status at the beginning of the TAI protocol, the interaction between CIDR treatment and cyclic status had no effect on P/AI (El-Zarkouny et al., 2004; Galvão et al., 2004; Stevenson et al., 2006). One of the reasons why inclusion of a CIDR insert during GnRH-based TAI protocols does not further improve reproductive performance of anovular cows may be because GnRH alone is capable of inducing ovulation in approximately 52 to 83% of anovular cows (Stevenson et al., 2008), whereas progesterone via the CIDR insert induced cyclicity in approximately 50% of anovular cows (Chebel et al., 2006; Cerri et al., 2009).

In the present study, cows classified as cyclic that had P4 <1 ng/mL on the day of initiation of the TAI protocol did not benefit from the treatment with a CIDR insert, even though, numerically, P/AI of cyclic cows with P4 <1 ng/mL and treated with a CIDR was almost equal to that of cyclic cows with P4 ≥1 ng/mL. Further, P/AI of cyclic cows that did not receive a CIDR and had P4 <1 ng/mL was numerically smaller than those with P4 ≥1 ng/mL. Positive effect of selective use of CIDR insert in cows without a CL diagnosed by ultrasonography at the beginning of the TAI protocol has been reported (Stevenson et al., 2008). In the experiment by Stevenson et al. (2008), cows not bearing a CL at the beginning of the TAI protocol treated with CIDR had greater P/AI than those not bearing a CL and not treated with CIDR, but the former had similar P/AI compared with cows bearing a CL and not treated with CIDR. It is likely that treatment of cows that had no CL or P4 <1.0 ng/mL at the beginning of the TAI protocol with progesterone via the CIDR insert may improve P/AI by suppressing premature estrus and ovulation until the end of the TAI protocol. It is also possible that treatment of cows with a CIDR insert when no CL is present or P4 <1 ng/mL at the beginning of the TAI protocol may improve reproductive performance by exposing growing follicles and the uterus to higher P4 concentrations. Lima et al. (2008) demonstrated that 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 CIDR inserts compared with those not treated. Small concentrations of P4 during follicular growth may result in increased pulsatile release of LH, which may cause premature follicle and oocyte maturation and reduced embryo quality and P/AI (Inskeep, 2004).

Cows treated with a CIDR insert during the TAI protocol were more likely to have P4 $\geq$1 ng/mL or P4 $\geq$3.2 ng/mL at 11 to 14 d after AI, despite the fact that proportion of anovular cows at the beginning of the TAI protocol did not differ between treatments, indicating that more CIDR-treated cows had a functional CL and were properly synchronized. Lima et al. (2008) demonstrated that cows treated with CIDR inserts had improved estrous cycle synchrony after a TAI protocol. Considering that proportion of cows with P4 $\geq$1 ng/mL at 11 to 14 d after AI was not affected by the interaction between treatment and cyclic status at the beginning of the TAI protocol, it is suggested that CIDR treatment during the TAI protocol did not increase the proportion of anovular cows resuming cyclicity after TAI. As mentioned earlier submission of anovular cows to GnRH-based TAI protocols resulted in 52 to 83% of them resuming cyclicity (Stevenson et al., 2008), which may limit additional positive effects of progesterone treatment on resumption of cyclicity.

Treatment with CIDR increased P4 concentrations 11 to 14 d after AI, but among cows bearing a functional CL, as determined by P4 $\geq$1 ng/mL 11 to 14 d later, no difference in P4 concentrations 11 to 14 d after AI were observed. This lack of difference indicates that CIDR inserts did not affect production of P4, but probably improved synchrony of the estrous cycle after AI because it increased the proportion of cows bearing a functional CL 11 to 14 d after AI. It has been demonstrated that CIDR-treated cows had greater P4 concentrations 12 to 14 d after AI compared with nontreated cows, regardless of pregnancy status (Melendez et al., 2006; Stevenson et al., 2008). Because no categorization of cows according to presence of CL 14 d after AI was made, however, it was not possible to determine if the increased P4 concentrations observed was a consequence of more CIDR-treated cows bearing a CL or CIDR treatment increasing P4 secretion. The observed increase in P4 concentration following AI is likely the result of improved synchronization of the estrous cycle (Lima et al., 2008), because during CIDR treatment, estrus and ovulation are inhibited even in the event of occurrence of spontaneous luteolysis.

CONCLUSIONS

On the basis of the present study and previous literature regarding the effects of progesterone treatment via the CIDR insert during TAI protocol, we conclude that the benefits resulting from treatment are most likely a result of reduced incidence of early ovulation before the $PGF_{2\alpha}$ injection of the TAI protocol and improved synchronization of the estrous cycle after AI. It is also evident from these studies that addition of a CIDR insert during the TAI protocol does not benefit anovular cows more than it does cyclic cows, and the expected improvement in P/AI should be approximately 5 percentage units.

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