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DETECTION OF NONCYCLING COWS BY HEATMOUNT DETECTORS AND ULTRASOUND BEFORE TREATMENT WITH PROGESTERONE

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Summary

Our objective was to determine accuracy of identifying anovulatory lactating dairy cows before the application of a timed AI protocol [with or without progesterone supplementation via a controlled internal drug release (CIDR) insert and 2 different timings of AI] by using heatmount detectors and a single ovarian ultrasound examination. At 6 Midwest locations, 1,072 cows were enrolled in a Presynch protocol (2 injections of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) 14 days apart) with the second injection administered 14 days before initiating the Ovsynch protocol (injection of gonadotropin releasing hormone (GnRH) 7 days before and 48 hours after $PGF_{2\alpha}$ injection, with timed AI at 0 or 24 hours after the second GnRH injection). Heatmount detectors were applied to cows at the time of the first Presynch injection, assessed 14 days later at the second Presynch injection and again at initiation of the Ovsynch protocol, and ovaries were examined for presence of a visible corpus luteum (CL) by ultrasound before initiation of treatment. Treatments were assigned to cows based on presence or absence of a visible CL: 1) anovulatory (no CL + CIDR insert for 7 d); 2) anovulatory (no CL + no CIDR); and 3) cycling (CL present). Further, every other cow in the 3 treatments was assigned to be inseminated concurrent with the second GnRH injection of Ovsynch (0 hour) or 24 hours later. Pregnancy was diagnosed at 33 and 61 days after the second GnRH injection. Heatmount detectors and a single ultrasound examination both underestimated proportions of cows classified as anovulatory or having no prior luteal activity compared with those classifications determined by concentrations of progesterone in blood serum. Overall accuracy of heatmount detectors and ultrasound was 71 and 84%, respectively. Application of progesterone to cows without a CL at the time of the first injection of GnRH reduced incidence of ovulation but improved pregnancy rates at day 33 or 61 compared with nontreated cows without a CL at the onset of the Ovsynch protocol. Pregnancy rates and pregnancy survival did not differ for cows having a CL before treatment compared with those not having a CL but treated with progesterone. Pregnancy rates were 1.5-fold greater for cows ovulating in response to the first GnRH injection. Timing of AI at 0 or 24 hours after the second GnRH injection did not alter pregnancy rates, but cows having prior luteal activity before treatment had improved pregnancy rates compared with anovulatory cows. We conclude that identifying anovulatory cows by ultrasound was more accurate than by heatmount detectors. Subsequent treatment of

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potential anovulatory cows with progesterone failed to improve fertility but had benefit for cows with prior estrous cycles at the onset of the timed AI (TAI) protocol, regardless of luteal status before the final luteolytic injection of $PGF_{2\alpha}$.

(Key words: anovulation, controlled internal drug release, Ovsynch, pregnancy rates.)

Introduction

Because fertility of lactating dairy cows is poor and has decreased more than 50% since 1970, improving fertility of lactating dairy cows is economically important to dairy producers. A previous study examined effects of supplemental progesterone via a progesterone-releasing, intravaginal CIDR insert in various experimental designs. Conception rates were greater for cows treated with progesterone during the Ovsynch protocol (injection of GnRH 7 days before and 48 hours after $PGF_{2\alpha}$ injection, with TAI between 0 and 24 hours after the second GnRH injection) at 28 and 56 days after TAI, respectively. In that study, conception rates were more positive for both cycling and noncycling or anovulatory cows treated with the CIDR insert compared with no CIDR treatment but only at 4 of the 6 locations at 28 days and at 3 of 6 locations at 56 days after TAI. This inconsistent response is corroborated by other large-scale studies.

Estrus-detection aids including tail paint, chalk, and heatmount detectors are inexpensive tools that may aid in the detection of noncycling cows before first AI. Likewise, examination of ovaries by transrectal palpation or ultrasonography is a means of identifying anovulatory cows that may benefit from progesterone supplementation as part of a TAI protocol.

Pregnancy rates are maximized when the TAI of the Ovsynch protocol is administered at 16 hours after the second GnRH injection. In practice, this timing is somewhat inconvenient and does not correspond to other management activities (e.g., AI, pregnancy diagnosis, vaccinations, and other treatments) that often occur while dairy cows are locked up at the feed line after morning milkings. Further, AI-pregnancy rates were not too different when AI occurred concurrent with the second GnRH injection or 24 hours later.

The objectives of the present experiment were to determine: 1) accuracy of detecting anovulatory cows (no prior estrous cycles) by using heatmount detectors applied to cows during the 28 days before initiating the Ovsynch protocol or by a single ovarian examination employing transrectal ultrasonography at the onset of the Ovsynch protocol; 2) benefit of applying progesterone (via a CIDR insert) during the first 7 days of the Ovsynch protocol; and 3) pregnancy rates after timing of AI occurred concurrent with the second GnRH injection of the Ovsynch protocol or 24 hours later.

Procedures

Experimental Locations

This study was a collaborative project of the North Central Regional Research Project 1006 of the Cooperative States Research, Education, and Extension Service (CSREES). Similar treatments were applied to lactating Holstein cows at 6 locations (Indiana, Kansas, Michigan, Minnesota, Missouri, and Wisconsin) where co-authors were located. A total of 1,072 cows were enrolled between April 2003 and October 2005. A similar experimental design was used at each location. New cows were enrolled weekly or biweekly into breeding clusters.

Experimental Protocol

Sampling, procedures, and design of treatments are illustrated (Figure 1). Cows were enrolled in a Presynch + Ovsynch protocol. Two, 25-mg injections of $PGF_{2\alpha}$ (5 mL of Lutalyse, Pfizer, New York, NY or 5 mL of Prostamate, Phoenix Scientific, Inc., St. Joseph, MO) were given i.m. 14 days apart, and the Ovsynch protocol was initiated 14 days after the second Presynch injection with the first injection of GnRH. Prostaglandin $F_{2\alpha}$ (25 mg) was administered 7 days later and followed in 48 hours by a second injection of GnRH. All injections of GnRH (100 μg) were administered i.m., consisting of 2 mL of Ova-Cyst (Phoenix Scientific, Inc.).

Heatmount detectors (Kamar Inc., Steamboat Springs, CO) were affixed midline to the rump of each cow between the tail head and the tuber coxae (hook bones). Detectors were placed on all cows before the first Presynch injection and were assessed before the second Presynch and the first GnRH injection of Ovsynch. Condition of heatmount detector was recorded as red, absent, white, or partially red (leaky). A new detector was applied before each injection if the detector was activated or absent. For purposes of this experiment, sexual behavior associated with estrus was assumed to have occurred when the heatmount detector was activated or absent (ovulatory or cycling cows). Leaky or nonactivated detectors were assumed to be associated with no prior estrual activity (anovulatory or noncycling cows).

Treatments

At the onset of the Ovsynch protocol, ovaries were examined by using transrectal ultrasonography. Ultrasonography was conducted by using a transrectal 5.0 or 7.5 MHz linear-array transducer (Aloka 500V; Corometrics Medical Systems, Inc., Wallingford, CT). Follicles and luteal structures were mapped, and follicles were sized by using internal calipers. Treatments were assigned to cows based on presence of a visible CL (CL absent vs. CL present): 1) anovulatory (no CL + CIDR insert for 7 d); 2) anovulatory (no CL + no CIDR); and 3) cycling (CL present). Further, every other cow within the 3 treatments was assigned to be inseminated concurrent with the second GnRH injection of Ovsynch (0 hour) or 24 hours later. Pregnancy was diagnosed at 33 and 61 days after the second GnRH injection. Presence of fluid in the uterus, a CL, and a viable embryo were evidence of pregnancy. For cows that were pregnant at the first diagnosis (day 33), a subsequent ultrasound examination at day 61 was used to determine pregnancy loss.

At 3 locations (KS, MI, and MI), follicles also were mapped and sized at further ovarian exams performed by ultrasound before the $PGF_{2\alpha}$ and second GnRH injections of Ovsynch and at 5 days after the second GnRH injection to determine if ovulation occurred in response to the second GnRH injection. Blood was collected at all locations before each hormonal injection and again at 5 and 12 days after the second GnRH injection. Concentrations of progesterone were measured to determine prior luteal activity before and after treatments.

Results and Discussion

Location Characteristics

Various outcomes of lactating dairy cows treated at each of 6 locations are summarized in Table 1. A total of 1,072 cows were enrolled in the study in 6 Midwest states. Nearly all traits in Table 1 differed among locations, except for pregnancy rate at day 33. Calving difficulty scores averaged 1.3 ± 0.48 (SD) and ranged from 1.2 to 1.7. Average body condition score after calving and before the first Presynch injection (37 ± 7 DIM) were 2.8 ± 0.48 (SD) and 2.6 ± 0.44 (SD), respectively.

Days in milk (DIM) at TAI averaged 75 ± 7 (SD), with all cows first inseminated between 61 and 107 days (90% were inseminated between 61 and 82 DIM). Pregnancy rates at days 33 and 61 averaged in the low to mid 30% range, with pregnancy loss averaging 10.2%. Cows having prior luteal activity before treatment (65 ± 7 DIM), as assessed by concentrations of progesterone in 3 blood samples collected during 28 days before treatment, averaged 83.7%. Regression of the CL in response to the $PGF_{2\alpha}$ of the Ovsynch protocol averaged 88.5% across locations.

Detection of Anovulatory Cows

Our first objective was to determine the accuracy of detecting anovulatory cows by means of heatmount detectors or a single ultrasound examination of ovarian structures in comparison with blood serum concentrations of progesterone. Both assessments were made at all 6 locations. Overall, relative to 3 blood serum concentrations assessed during 28 days before treatment, heatmount detectors and a single ultrasound exam of ovarian structures underestimated both anovulation and previous luteal or cycling activity. Relative to serum progesterone patterns, however, overall accuracy of heatmount detectors was 71.4% and that of a single ultrasound examination was 84%. Ultrasound differed (P < 0.001) from heatmount detectors in every category except for apparent prevalence, including being more (P < 0.001) sensitive and specific than heatmount detectors, with the Kappa coefficient (0.52) in the good-agreement range compared with detectors (0.11). Heatmount detectors had greater (P < 0.001) rates of false positives (identifying incorrectly prior estrual activity and subsequent luteal activity) and false negatives (missing prior estrual activity and subsequent luteal activity).

Ovarian Characteristics in Response to Progesterone

Our second objective was to determine the effects of progesterone (CIDR insert) in cows identified with or without a CL at the onset of the Ovsynch protocol. This assessment was made by a single ultrasound examination of ovaries at all 6 locations, and as cited earlier, some cows were not correctly categorized by the single examination. Incidence of ovulation in response to the first GnRH injection was less (P < 0.05) in cows having no CL and treated with progesterone (CIDR insert) than in no CL cows not receiving a CIDR (Table 2). Average incidence of ovulation was less (P < 0.001) for cows having a CL than for those without a CL (Table 2). Concentrations of progesterone for cows in the 3 previous classifications at onset of treatment were 0.7 ± 0.2, 0.6 ± 0.2, and 4.0 ± 0.1 ng/mL, respectively.

Diameter of the ovulatory follicle, assessed before the second GnRH injection, was smaller (P < 0.001) in cows with a CL present than for those with no CL, regardless of CIDR treatment (Table 2). Incidence of ovulation of that follicle, assessed 5 days later (after TAI), did not differ among cows having or not having a CL (Table 2) at the onset of treatment.

Fertility in Response to Progesterone

Pregnancy rates at days 33 and 61 and pregnancy loss in 1,068 cows (4 were culled before pregnancy diagnosis) during that interval are summarized in Table 2 for all 6 locations. Cows having a CL present at initiation of treatment had greater (P < 0.05) pregnancy rates at days 33 and 61 than those not having a CL . However, pregnancy rate at day 33 in CL absent cows treated with a CIDR did not differ from that in cows having a CL before treatment but was greater than for CL absent cows not treated with a CIDR. Cows requiring no calving assistance (calving difficulty score [CDS] = 1) subsequently had greater

($P < 0.05$) pregnancy rates than those requiring some (CDS > 1) assistance (42.7%; n = 693 vs. 34.3%; n = 375), respectively.

Pregnancy rates at day 61 followed the same pattern as those at day 33 (Table 2). Overall pregnancy loss was 10.4% and tended ($P < 0.10$) to be less in cows having a CL at the onset of treatment compared with cows not having a CL (Table 2).

Time of Insemination

Our third objective was to determine whether time of AI relative to the second GnRH injection (0 vs. 24 hours) altered pregnancy rates (Table 3). The largest difference (5.4 percentage points in 24 hours) in pregnancy rate at day 33 for timing of AI was observed in CL present cows. Otherwise, time of AI had no little effect on pregnancy outcome measured at day 33. Cows having a CL present at treatment initiation had greater ($P < 0.05$) pregnancy rates than CL absent cows not treated with a CIDR when inseminated at 24 hours, but not 0 hours after the second GnRH injection.

A CIDR treatment × location interaction tended ($P = 0.085$) to occur (Table 3) for pregnancy rates at day 33. Pregnancy rates for cows treated with a CIDR insert were greater at 4 locations than non-CIDR treated cows, but were less than non-CIDR treated cows at the other 2 locations. An interaction of location × time of AI tended ($P = 0.12$) to occur, in which pregnancy rates for cows inseminated at 0 hour were numerically greater at 3 locations, but lesser at 3 other locations. Overall, pregnancy rates at 24 hours were numerically greater than those made at 0 hour (37.4%, n = 530 vs. 34%, n = 538), respectively.

Concentrations of Progesterone

The relationship of pretreatment cycling status and presence of high vs. low concentrations of progesterone before the before injection of $PGF_{2\alpha}$ injection of Ovsynch is examined further in Table 4. Because of the inaccuracy of identifying cycling status by ultrasound relative to blood concentrations of progesterone in these cows, 55.5% of 155 CL absent cows were truly not cycling and treated with a CIDR. Further, 44% of 116 CL absent cows were truly not cycling and assigned to the no CIDR treatment. These 2 groups of cows are designated by their pretreatment cycling status in Table 4

Effects of treatment of anovulatory cows based on serum concentrations of progesterone are summarized in the top 3 lines of Table 4, and treatment effects on previously cycling cows are summarized in the bottom 3 lines of Table 4. When only previously anovulatory cows were treated or not treated with a CIDR, their pregnancy rates did not differ from those in CL present cows regardless of progesterone concentrations at the time of the $PGF_{2\alpha}$ injection (Table 4). In contrast, regardless of progesterone concentrations at the time of the $PGF_{2\alpha}$ injection, CL absent cows treated with a CIDR and CL cows had greater pregnancy rates than the CL absent cows not treated with a CIDR (Table 4). Overall, cows having prior cycling activity, assessed by blood progesterone before treatment, had 53% greater ($P < 0.001$) pregnancy rates at day 33 than those without prior luteal activity (37.8 vs. 24.7%; Table 4).

Treatment with the CIDR insert in a previous study improved pregnancy rates in cows that were previously noncycling without a functional CL before the $PGF_{2\alpha}$ injection, but not in the present study (Table 4). Further, CIDR treatment in previously cycling cows having low concentrations of progesterone before $PGF_{2\alpha}$ injection (early CL regression) had numerically greater pregnancy rates in the present study as in a previous study. Interpretation of the results in the previous study indicated that the CIDR insert would improve conception rates in cows having no active CL or low progesterone before $PGF_{2\alpha}$ injection regardless of previously cycling or luteal status, whereas in the present study, we could only verify improved fertility for previously cycling cows having low progesterone (early CL regression). Although interpretation of results in the present study relative to luteal status before $PGF_{2\alpha}$ injection should be conservative because luteal status was confounded with CIDR-supplemented progesterone concentrations, nearly 26% of the CL absent cows treated with a CIDR had concentrations of progesterone < 1 ng/mL when the insert was removed. Clearly, these cows had no CL at this time. Concentrations of progesterone in cows bearing a luteal structure are generally not different before and after the insert is removed.

Concentrations of progesterone in blood serum 2 days before the second GnRH injection (day of $PGF_{2\alpha}$ injection of Ovsynch and removal of CIDR inserts), before the second GnRH injection, and 5 and 12 days later are illustrated in Figure 2. At insert removal, cows treated with CIDR inserts had concentrations of progesterone that did not differ from those in cows without inserts, but both treatments had less ($P < 0.05$) progesterone than in CL present cows. This same pattern existed 48 hours later when the second GnRH injection was given (0 h). At 5 days after AI, no differences in progesterone concentration were detected among treatments, but at 12 days after the second GnRH injection or 11 to 12 days after AI, CL absent cows, regardless of CIDR treatment, had greater ($P < 0.05$) concentrations of progesterone than CL present cows. The greater progesterone 12 days later is consistent with an earlier report in which cows treated with CIDR inserts during the Ovsynch protocol had greater progesterone concentrations than controls. It seems that maturing follicles exposed to progesterone during CIDR treatment may, after ovulation, differentiate into CL having greater progesterone secretory capacity.

As expected, pregnancy status had no effect on concentrations of progesterone at 5 days after the second GnRH injection, but at 12 days, pregnant cows had greater ($P < 0.001$) concentrations of progesterone than nonpregnant cows (4.7 ± 0.2; n = 686 vs. 3.6 ± 0.1 ng/mL; n = 381).

In summary, identifying cows with a CL was more accurate after the single ultrasound examination than during the 28 days when heatmount detectors were applied to cows. When lactating dairy cows were found to have no CL (but had evidence of increased concentrations of progesterone in blood serum during 28 days before initiating the Ovsynch protocol) at the onset of the Ovsynch protocol and treated with progesterone (via a CIDR), pregnancy rates were greater than similar CL absent cows not treated with a CIDR. Pregnancy rates of the former did not differ from cows having a CL at the initiation of the TAI protocol. Our study finds no evidence that including progesterone in a TAI protocol for previously noncycling cows is warranted, regardless of luteal status before the $PGF_{2\alpha}$ injection of the Ovsynch protocol. Insemination of cows at 0 vs. 24 hours after the second GnRH injection did not differ significantly, even though a numerical advantage (5.4 percentage points) occurred for CL present cows inseminated at 24 vs. 0 hours.

Table 1. Selected Outcomes of Lactating Dairy Cows Enrolled at Each Location

	Location						
Item	IN	KS	MI	MN	MO	WI	Total
Cows enrolled, no.	80	217	153	194	242	186	1,072
Calving difficulty score[1]	1.5[a]	1.3[a,b]	1.2[c]	1.7[d]	1.4[a]	1.3[b,c]	1.3**
Body condition score post-calving[1]	2.9[a]	2.6[b]	2.7[c]	3.1[d]	2.9[a]	…	2.8**
Body condition score at Presynch[1,2]	…	2.3[a]	2.4[b]	2.8[c]	2.7[d]	2.9[c]	2.6**
Days postpartum at first AI[3]	72[a]	79[b]	82[c]	69[d]	75[e]	72[a]	75**
Pregnancy rate at 33 d[4], %	37.5	42.9	36.0	29.4	33.1	36.3	35.7
Pregnancy rate at 61 d[4], %	37.5[a]	37.3[a]	33.3[a]	22.7[b]	29.8[a,b]	35.2[a]	32.0*
Pregnancy loss (33 to 61 d), %	0.0[a,c]	12.9[b]	7.3[a,c]	22.8[b]	10.0[c]	3.0[c]	10.2*
Luteal activity before treament[5], %	92.5[a]	91.2[a]	89.5[a]	69.1[b]	79.3[c]	87.3[a,c]	83.7**
Regression of CL[6], %	88.4[a,b]	86.7[b,c]	87.4[b]	81.9[b]	94.8[a]	90.6[a,c]	88.5**

**Location effect ($P < 0.01$).

*Location effect ($P < 0.05$).

[a,b,c,d,e]Location values having different superscript letters differ ($P \leq 0.05$).

[1]Range of 1 to 5. Standard deviations ranged from 0.44 to 0.48.

[2]Assessed before first Presynch PGF$_{2\alpha}$ injection.

[3]Standard deviation was 7.0.

[4]Determined by transrectal ultrasonography after the first postpartum AI.

[5]Based on progesterone concentrations measured in a total of 3 blood samples collected before each Presynch PGF$_{2\alpha}$ injection and before ultrasonography at the time of the first GnRH injection of the Ovsynch protocol. Cutoff values for luteal activity were based on progesterone ≥ 1 ng/mL and < 1 ng/mL for no luteal activity.

[6]Cows having elevated concentrations of progesterone 48 to 72 hours before AI in which blood progesterone was < 1 ng/mL at 48 hours after the PGF$_{2\alpha}$ injection of the Ovsynch protocol.

Table 2. Ovarian Characteristics and Fertility in Response to Presence or Absence of a Corpus Luteum (CL) Assessed by Transrectal Ultrasonography and Progesterone Treatment (CIDR insert) at the Onset of the Ovsynch Protocol

	CL absent[1]		
Item	No CIDR	CIDR	CL present[1]
Ovulation after first GnRH[2], % (no.)	76.8[a] (69)	47.1[b] (104)	43.4[c,x] (604)
Presence of CL before $PGF_{2\alpha}$[2], % (no.)	76.8[a] (69)	51.0[b] (104)	95.7[c,x] (610)
Corpus luteum before $PGF_{2\alpha}$[3]	0.9[a] ± 0.1 (55)[4]	0.7[a] ± 0.1(78)	1.4[b] ± 0.1 (450)
Diameter of ovulatory follicle before second GnRH[2], mm	16.7[a] ± 0.4 (65)[4]	16.2[a] ± 0.4 (103)	15.2[b,x] ± 0.1 (588)
Ovulation after second GnRH[2], % (no.)	77.9 (68)	75.0 (104)	80.7 (605)
Pregnancy rate at day 33, % (no.)	24.1[a] (116)	32.3[b] (155)	38.0[b,x] (797)
Pregnancy rate at day 61, % (no.)	20.7[a] (116)	29.0[b] (155)	34.3[b,x] (797)
Pregnancy loss from day 33 to 61, % (no.)	14.3 (28)	10.0 (50)	9.9[y] (303)

[a,b,c]Means having different superscript letters differ ($P \leq 0.05$).

[x]Different ($P \leq 0.05$) from CL absent cows.

[y]Tended ($P \leq 0.10$) to differ from CL absent cows.

[1]Absence or presence of a CL assessed by transrectal ultrasonography at the first GnRH injection of the Ovsynch protocol.

[2]Assessed at only 3 of 6 locations.

[3]Assessed at only 2 of 6 locations.

[4]Mean ± SE and number of observations.

Table 3. Pregnancy Rates at Day 33 After Timed Insemination on the Basis of Time of AI After the Second GnRH Injection and Location

Item	CL absent[1]		CL present[1]	Overall
	No CIDR	CIDR		
AI time, hour after second GnRH	-------------------- % (no.) --------------------			
0	24.1[a] (54)	33.8[a] (74)	35.4[a] (401)	34.0 (530)
24	24.2[a] (62)	30.9[a] (81)	40.8[b,x] (395)	37.4 (538)
Location[2]				
IN	22.2 (9)	18.8 (16)	45.5 (55)	37.5 (80)
KS	21.7 (23)	26.1 (23)	48.0 (171)	42.9 (217)
MI	12.5 (16)	58.3 (12)	36.8 (125)	35.9 (153)
MN	21.6 (37)	32.4 (34)	30.9 (123)	29.4 (194)
MO	35.0 (20)	29.0 (62)	34.4 (160)	33.1 (242)
WI	36.4 (11)	62.5 (8)	35.2 (162)	36.3 (182)

[a,b]Means within AI timing having different superscript letters differ ($P \leq 0.05$).

[x]Different ($P \leq 0.05$) from corpus luteum (CL) absent cows at 24 hours.

[1]Absence or presence of a CL assessed by transrectal ultrasonography at the first GnRH injection of the Ovsynch protocol.

[2]Interaction ($P = 0.08$) of treatment × location.

Table 4. Pregnancy Rates at Day 33 After Timed Insemination According to Luteal Activity Before Treatment and Concentrations of Progesterone at the Time of $PGF_{2\alpha}$ Injection

Pretreatment cycling status[1]	Serum progesterone before $PGF_{2\alpha}$[2]	CL absent[3]		CL present[3]	Pretreatment cycling total
		No CIDR	CIDR		
		---------------------------- % (no.) ----------------------------			
Noncycling	High	29.4 (34)	32.1 (56)	32.0[a,b] (25)	
	Low	17.6 (17)	6.7 (30)	16.7[a,x] (12)	
	Total	25.4[a] (51)	23.3[a] (86)	27.0[a] (37)	24.7 (174)
Cycling	High	25.9 (54)	44.1 (59)	40.2[b] (651)	
	Low	9.1 (11)	40.0 (10)	28.7[a,b] (108)	
	Total	23.1[a] (65)	43.5[b](69)	42.8[b](759)	37.8* (893)

[a,b]Treatment × cycling status interaction ($P = 0.08$).

*Differed ($P < 0.05$) from noncycling cows.

[1] Based on progesterone concentrations measured in 3 blood serum samples collected before each Presynch $PGF_{2\alpha}$ injection and before the first GnRH injection of the Ovsynch protocol.

[2] Low = concentrations of progesterone < 1 ng/mL, and high = ≥ 1 ng/mL.

[3] Absence or presence of a corpus luteum (CL) assessed by transrectal ultrasonography at the first GnRH injection of the Ovsynch protocol. Some CIDR-treated cows may have had elevated progesterone because of the CIDR insert.

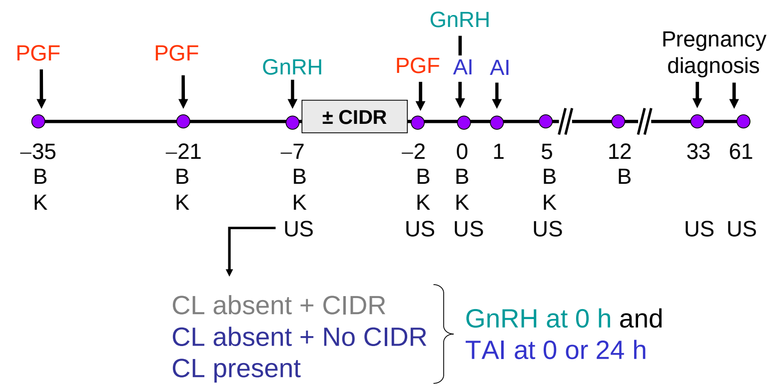

Figure 1. Experimental Protocol Showing Design of Treatments. Heatmount detectors (K) were applied to detect mounted activity. Activated heatmount detectors were replaced at each evaluation. Blood (B) was collected before various injections and twice post AI. Presence of a corpus luteum (CL) was detected by transrectal ultrasonography (US) and, alternatively, cows without a CL received a progesterone-releasing intravaginal controlled internal drug release (CIDR) insert. Cows having a CL present received no CIDR. All cows received GnRH at 48 hours after $PGF_{2\alpha}$ (PGF) and were inseminated before the second GnRH injection (0 hour) or 24 hours later in the 3 treatments. Pregnancy was diagnosed at 33 days after timed AI and reconfirmed at 61 days after timed AI.

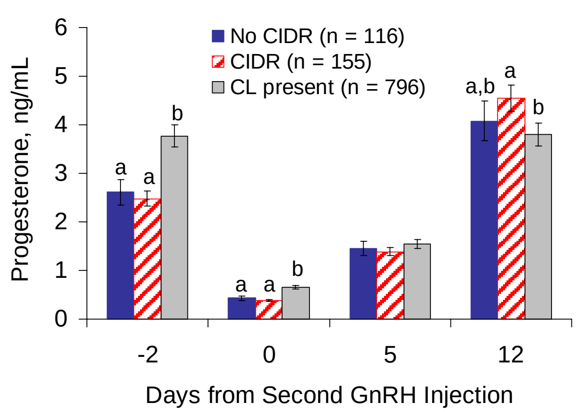

Figure 2. Concentrations of Progesterone in Serum of Lactating Dairy Cows Before Injections of $PGF_{2\alpha}$ [time of progesterone (CIDR) Insert Removal; day –2] and the Second GnRH Injection (day 0), and at 5 and 12 Days After GnRH. [a,b] Means having different superscript letters differ ($P < 0.05$).