

Treatment of Cycling and Noncycling Lactating Dairy Cows with Progesterone During Ovsynch¹

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ABSTRACT

Our objective was to determine whether progesterone (P4) supplementation during an Ovsynch protocol would enhance fertility in lactating dairy cows. Lactating dairy cows ($n = 634$) at 6 locations were assigned randomly within lactation number and stage of lactation to receive the Ovsynch protocol [OVS; synchronization of ovulation by injecting GnRH 7 d before and 48 h after PGF_{2 α} , followed by one fixed-time AI (TAI) 16 to 20 h after the second GnRH injection] or Ovsynch plus a controlled internal drug release (CIDR) P4-releasing insert for 7 d, beginning at the first GnRH injection (OVS + CIDR). Blood was sampled to quantify P4 10 d before the first GnRH injection, immediately before the first GnRH injection, at the time of CIDR removal, before the PGF_{2 α} injection (1 to 2 h after CIDR insert removal), and 48 h after the PGF_{2 α} injection to determine cyclicity status before initiation of treatment, luteal status at the PGF_{2 α} injection, and incidence of luteal regression. Overall, conception rates at 28 (40 vs. 50%) and 56 d (33 vs. 38%) after TAI differed between OVS and OVS + CIDR, respectively; but a treatment \times location interaction was detected. Compared with OVS, pregnancy outcomes were more positive for OVS + CIDR cows at 4 of 6 locations 28 d after TAI and at 3 of 6 locations 56 d after TAI. An interaction of luteal status (high vs. low) before CIDR insert removal and PGF_{2 α} injection with pretreatment cycling status indicated that cows having low P4 at PGF_{2 α} injection benefited most from P4 supplementation (OVS + CIDR = 36% vs. OVS = 18%), regardless of pretreatment cycling

status. Pregnancy loss between 28 and 56 d after TAI was greater for noncycling cows (31%) compared with cycling cows (16%). Pregnancy loss for cows receiving P4 (21%) did not differ from that for cows not receiving P4 (21%). Supplementation of P4, pretreatment cycling status, and luteal status before PGF_{2 α} injection altered follicular diameters at the time of the second GnRH injection, but were unrelated to pregnancy outcomes. Incidence of multiple ovulation was greater in noncycling than in cycling cows. Further, cows having multiple ovulations had improved pregnancy outcomes at 28 and 56 d after TAI. In summary, a CIDR insert during the Ovsynch protocol increased fertility in lactating cows having low serum P4 before PGF_{2 α} injection. Improved pregnancy outcomes were observed at some, but not all locations.

Key words: controlled internal drug release, Ovsynch, dairy cow, fertility

INTRODUCTION

Poor conception rate in lactating dairy cows ranks as one of the most limiting factors to dairy profitability. Conception rates decreased from 66% in 1951, to 50% during 1973 to 1985 (Butler and Smith, 1989), to about 45% for cows inseminated at spontaneous estrus and 35% for cows receiving a timed AI (TAI) in 2000 (Lucy, 2001). This decrease in fertility was concurrent with a dramatic increase in DMI and milk production per cow.

A negative relationship exists between DMI and circulating concentrations of progesterone (P4) in lactating dairy cows (Sangsrivavong et al., 2002); and lactating dairy cows have serum concentrations of P4 less than those in nonlactating nulliparous heifers (Wiltbank et al., 2000; Wolfenson et al., 2004). Progesterone is important to fertility as demonstrated by a positive correlation between serum P4 before AI and subsequent conception rate (Fonseca et al., 1983; Folman et al., 1990).

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Several studies have examined the effect of exogenous progestin supplementation on fertility in lactating dairy cows. Conception rate was greater for cows that received a P4-releasing intravaginal device during 7 d before the second of 2 injections of PGF_{2α} (14 d between injections), compared with controls (Folman et al., 1990). Decreased conception rates, however, were reported in lactating dairy cows and heifers without a corpus luteum (CL) at the end of treatments with P4 or norgestomet, compared with females having a CL at the end of progestin treatment (Smith and Stevenson, 1995).

Combining progestin treatments with protocols similar to Ovsynch (synchronization of ovulation by injecting GnRH 7 d before and 48 h after PGF_{2α} followed by one fixed-time AI 16 h after the second GnRH injection) administered to noncycling suckled beef cows improved conception rates resulting from inseminations after estrus or those resulting from TAI (Lamb et al., 2001; Stevenson et al., 2003). Similar studies in lactating dairy cows reported improved conception rates in first-lactation cows treated with intravaginally placed P4-releasing controlled internal drug release (CIDR) inserts during an Ovsynch protocol (experiment 1; El-Zarkouny et al., 2004; Moreira et al., 2004a) and in all cows treated with CIDR inserts during an Ovsynch protocol in which estrous cycles were presynchronized (**Presynch**; Moreira et al., 2004b). In contrast, no positive effects of the P4 via the CIDR insert were detected in another study in which cows were treated with Presynch + Ovsynch (experiment 2; El-Zarkouny et al., 2004).

The current experiment was designed to test the hypothesis that providing P4 (via a CIDR insert) during Ovsynch before TAI would improve fertility, particularly in noncycling cows and those having no CL at the time of PGF_{2α} injection. Specifically, our objective was to determine whether P4 administration before induced luteal regression might improve overall fertility by either increasing conception rates or reducing pregnancy losses in lactating dairy cows determined retrospectively to be cycling or noncycling before the onset of treatment.

MATERIALS AND METHODS

Experimental Locations

This study was a collaborative project of the North Central Regional Research Project 113 of the Cooperative States Research, Education, and Extension Service (CSREES). Similar treatments were applied to lactating Holstein cows in 6 locations (Illinois, Kansas, Michigan, Missouri, Ohio, and Wisconsin) in which coauthors were located. A total of 634 cows were enrolled between

December 1998 and June 1999. A similar experimental design was used at each location. Cows were organized into breeding clusters in which they were inseminated on the same day in each particular breeding cluster. Details, variations in data collection, and methodologies for each location are listed below.

Illinois. Lactating cows enrolled at this location were part of the University of Illinois research herd composed of 200 Holstein cows, with an annual rolling herd average of 9,500 kg of milk. Cows were housed in stanchions and were milked twice daily. The experiment was conducted in 96 cows organized in 5 breeding clusters in which cows were inseminated between February and May 1998. Although DIM in all cows ranged from 60 to 396 at TAI, 58 of the 96 cows were inseminated before 100 DIM.

Ultrasonography was conducted by using a transrectal 5.0-MHz linear-array transducer (Aloka 500V; Corometrics Medical Systems, Inc., Wallingford, CT). Serum P₄ concentrations were quantified by enzyme immunoassay (Kesler et al., 1990), with intra- and interassay coefficients of variation of 5 to 10%.

Kansas. Lactating cows were enrolled on a commercial dairy farm in northeastern Kansas. The herd consisted of 500 Holstein cows, with an annual rolling herd average of 11,500 kg of milk. Cows were milked thrice daily and fed a TMR consisting of chopped alfalfa, corn silage, whole cottonseed, and a concentrate-mineral mix (offered twice daily) to meet or exceed NRC (1989) recommendations for lactating cows. Cows had access to fresh water ad libitum at 3 locations in each 100-cow pen, which consisted of feed-line self-locking head gates and free stalls bedded with sand. All procedures, including hormone injections, blood collection, TAI, and ovarian ultrasonography, were conducted while cows were locked up at the feed line after the morning milking. The experiment was conducted in 184 cows organized in 8 breeding clusters in which cows were inseminated between January and June 1999. All cows were inseminated at first service between 50 and 77 DIM. Some of these results were reported earlier (El-Zarkouny et al., 2004).

Ultrasonography was conducted by using a transrectal 5.0-MHz linear-array transducer (Aloka 500V; Corometrics Medical Systems, Inc.). Serum P₄ concentrations were quantified by radioimmunoassay (Skaggs et al., 1986) with intra- and interassay coefficients of variation of 6.9 and 6.4%, respectively.

Michigan. Lactating cows were enrolled at a commercial dairy farm composed of 750 Holstein cows, with an annual rolling herd average of 12,720 kg of milk. Cows were housed in freestall barns, fed a TMR balanced for milk production, and milked thrice daily. The experiment was conducted in 94 cows organized in 4

breeding clusters in which cows were inseminated between May and June 1998. Although DIM in all cows ranged from 74 to 264 at TAI, 69 of the 94 cows were inseminated before 100 DIM.

Ultrasonography was conducted by using a transrectal 7.5-MHz linear-array transducer (Aloka 900; Corometrics Medical Systems, Inc.). Serum P4 concentrations were quantified by radioimmunoassay (Progesterone Coat-a-Count kit, Diagnostic Products Cooperation, Los Angeles, CA) with intra- and interassay coefficients of variation of 5.6 and 9.1%, respectively.

Missouri. Lactating cows were enrolled at the University of Missouri experimental herd composed of 225 Holstein cows, with an annual rolling herd average of 9,500 kg of milk. The experiment was conducted in 99 cows organized in 10 breeding clusters in which cows were inseminated between December 1998 and May 1999. Although DIM ranged from 67 to 154 in all cows, 78 of the 99 cows were inseminated before 100 DIM. Cows were fed a TMR consisting of alfalfa hay, alfalfa silage, corn silage, whole cottonseed, soybean hulls, soybean meal, ground corn, and a premix containing minerals and vitamins. Diets were based on NRC (1989) recommendations. Cows were housed in a freestall barn with feed provided twice daily in an alley adjacent to the freestall area. For blood sample collections, hormone injections, TAI, and ultrasonographic examination of ovaries, cows were moved from the freestall area to a laboratory barn with holding facilities for the procedures.

Ultrasonography was conducted by using a transrectal 7.5-MHz linear-array transducer (Aloka 200; Corometrics Medical Systems, Inc.). Serum P₄ concentrations were quantified by radioimmunoassay (Progesterone Coat-a-Count kit, Diagnostic Products Cooperation; Kirby et al., 1997) with intra- and interassay coefficients of variation of 4.2 and 8.2%, respectively.

Ohio. Holstein cows were enrolled at 2 commercial dairy farms consisting of approximately 300 cows each. Cows were milked twice daily and fed a TMR consisting of chopped alfalfa, corn silage, whole cottonseed, and a concentrate-mineral mix (offered twice daily) to meet or exceed NRC (1989) recommendations for lactating cows. All procedures, including hormonal injections, blood collection, TAI, and ovarian ultrasonography, were conducted while cows were locked up at the feed line or when cows were moved through a working alley and cattle chute. The experiment was conducted in 93 cows organized in 6 breeding clusters in which cows were inseminated between May and July 1998. Although DIM ranged from 50 to 292 in all cows, 52 of the 93 cows were inseminated before 100 DIM.

Ultrasonography was conducted by using a transrectal 5.0-MHz linear-array transducer (Aloka 500V; Corometrics Medical Systems, Inc.).

Serum P4 concentrations were quantified by radioimmunoassay (Clapper et al., 1990) with intra- and interassay coefficients of variation of 7.7 and 10.7%, respectively.

Wisconsin. Holstein cows were enrolled at a commercial dairy farm in southern Wisconsin. The herd consisted of 1,100 lactating Holstein cows, having an annual rolling herd average of 11,000 kg of milk. Cows were milked thrice daily and fed a TMR consisting of chopped alfalfa, corn silage, whole cottonseed, and a concentrate-mineral mix (offered twice daily) formulated to meet or exceed NRC (1989) recommendations for lactating cows. Cows had access to fresh water ad libitum at 3 locations in each pen, which consisted of feed-line self-locking head gates and free stalls bedded with sand. All procedures, including hormonal injections, blood collection, TAI, and ovarian ultrasonography, were conducted while cows were locked up at the feed line. All 68 cows were inseminated in a single breeding cluster. Although DIM ranged from 57 to 223 in all cows, 59 of the 68 cows were inseminated before 100 DIM.

Ultrasonography was conducted by using a transrectal 7.5-MHz linear-array transducer (Aloka 500V; Corometrics Medical Systems, Inc.). Serum P4 concentrations were quantified by enzyme-linked immunosorbent assay (Rasmussen et al., 1996) with intra- and interassay coefficients of variation of 4.3 and 15.5%, respectively.

Treatments

Within location, cows were blocked by lactation number, stratified by DIM, and assigned randomly to receive either of 2 treatments. Controls received the Ovsynch protocol (n = 321) consisting of 100- μ g i.m. injections of GnRH (Cystorelin; Merial, Ltd., Duluth, GA) 7 d before and 48 h after a 25-mg i.m. injection of PGF_{2 α} (Lutalyse; Pharmacia Animal Health, Kalamazoo, MI). Treated cows (n = 313) received the Ovsynch protocol (OVS) and received at the time of the first GnRH injection a controlled internal drug release (OVS + CIDR) insert containing 1.9 g of P4 (CIDR-B; InterAg, Hamilton, New Zealand), which was removed 1 to 2 h before the PGF_{2 α} injection. The first injection of GnRH was administered at random stages of the estrous cycle.

Cows in both treatments received one TAI, 16 to 20 h after the second GnRH injection. At each location, herd personnel who were generally unaware of treatments to which cows were assigned performed inseminations. Semen used for AI was chosen by herd managers at each location as part of routine management of their herd. Therefore, semen and inseminators were confounded with location.

Blood Collection

Blood samples were collected from all cows by venipuncture of the median caudal vein or artery 10 d before the first GnRH injection, immediately before the first GnRH injection and CIDR insertion, at the time of removal of the CIDR insert [at only 2 (Missouri and Wisconsin) of the 6 locations were all controls and treated cows sampled at this time], immediately before PGF_{2α} injection (1 to 2 h after CIDR insert removal), and 48 h after the PGF_{2α} injection. Blood sera samples were stored at -20°C until concentrations of P4 were measured as described previously for each location.

Our rationale for waiting 1 to 2 h after CIDR insert removal before collecting blood before the PGF_{2α} injection was based on P4 clearance rates reported for ovariectomized cows (Rathbone et al., 2002). In that study, after a 7-d insertion period, a waiting period of 1 to 2 h was sufficient for concentrations of P4 in 4 ovariectomized cows to decrease from 2.8 ng/mL (upon insert removal) to 0.75 ng/mL by 1 h and 0.5 ng/mL by 2 h after insert removal.

Transrectal Ultrasonography

Ovarian structures (antral follicles ≥ 5 mm and CL) were monitored by using transrectal ultrasonography as described previously (Lamb et al., 2001). Ovulatory response to the second GnRH injection was determined by the presence of a single, or multiple, large antral follicle(s) at the time of the second GnRH injection, and absence of the follicle(s) 48 h later.

Pregnancy status was assessed by using ultrasonography in all cows 28 d after TAI and was reassessed 56 d after TAI for cows diagnosed pregnant at 28 d. A positive pregnancy diagnosis was confirmed by presence of uterine fluid and a large CL or by visualization of a viable (heart beat) of the embryo or fetus. Conception rate was defined as the percentage of inseminated cows that were pregnant at 28 and 56 d after TAI. Pregnancy loss between 28 and 56 d after TAI was calculated as the proportion of cows that were not pregnant 56 d after TAI, expressed as a percentage of cows pregnant at 28 d after TAI.

Definitions of Various Reproductive Statuses

Occurrence of estrous cycles (cycling vs. noncycling) before the onset of treatments was determined by serum concentrations of P4 assessed in samples collected 10 d before and immediately before the first GnRH injection. When both samples of blood serum contained concentrations of P4 < 1 ng/mL (low P4; Low-Low), the cow was classified as noncycling. When either or both of the paired samples contained concentrations of P4

≥ 1 ng/mL (high P4; High-High, Low-High, or High-Low), the cow was classified as cycling.

Induced ovulation was defined as the proportion of cows that were not cycling at the onset of treatments, but in which high P4 was detected 7 d after the first GnRH injection. Consequently, noncycling cows having low P4 7 d after the first GnRH injection (at time of the PGF_{2α} injection) were defined as having no induced CL, whereas those with high P4 were assumed to have an active induced CL. Cycling cows having high P4 7 d after the first GnRH injection (at time of the PGF_{2α} injection) were defined as having an active CL, whereas those having low P4 were assumed to have had early luteolysis (Lamb et al., 2001).

Regression of the CL was defined as the proportion of cows with high P4 in serum at the time of the PGF_{2α} injection, but in which concentrations of P4 decreased to < 1 ng/mL after 48 h (i.e., low P4).

Ovulation was defined as the proportion of cows that ovulated 1 or more follicles (multiple ovulation) by 48 h after the second GnRH injection.

Statistical Analyses

Models used to analyze conception rates at 28 and 56 d, pregnancy loss from 28 to 56 d, incidence of single and multiple ovulation after the second GnRH injection, induced ovulation, incidence of high concentrations of P4 before PGF_{2α}, incidence of low P4 by 48 h after PGF_{2α}, follicle diameters, incidence of ovulation (including multiple ovulation), concentrations of P4 in serum just before CIDR insert removal (2 locations only) and 1 to 2 h later just before PGF_{2α} injection (all locations), and CL regression consisted of the following independent variables: treatment (n = 2), location (n = 6), lactation number (1 vs. 2+), pretreatment cycling status (0 vs. 1), daily milk yield and DIM at TAI as covariates, and 2-way interactions of pretreatment cycling status, location, and lactation number with treatment. Follicle diameters at the time of the second GnRH injection were categorized into 2 size classes (≤ 15 or > 15 mm) to examine relationships with milk yield (< 34 , 35 to 43, and > 43 kg). Analyses of follicle diameters were conducted using ANOVA (procedure GLM), whereas all binominal variables were analyzed by using procedure GENMOD (SAS Institute, Inc., Cary, NC).

Pretreatment cycling status (0 vs. 1), concentrations of P4 (≥ 1 vs. < 1 ng/mL) or luteal status before PGF_{2α}, and their interaction were added to the other independent variables cited previously. Analysis of pretreatment cycling status was conducted by using procedure GENMOD consisting of a model having location, lactation number, location \times lactation number, plus daily milk yields and DIM at TAI as covariates. Differences

Table 1. Characteristics of lactating dairy cows in response to the Ovsynch protocol or the combination of a controlled internal drug-releasing (CIDR) insert plus Ovsynch

Location	Daily milking frequency	Total cows, n	Days in milk, ¹ d	Milk yield, ¹ kg/d	Cycling status, ²	Induced ovulation, ³	High P4 at PGF _{2α} ⁴	Low P4 48 h after PGF _{2α} ⁵	CL regressed ⁶	Ovulated after GnRH ⁷	Multiple ovulation ⁷
							(%)				
IL	2×	96	114 ± 54	30 ± 8	94	83	76	83	82	95	6.3
KS	3×	184	60 ± 6	46 ± 11	41	52	63	95	94	88	14.7
MI	3×	94	107 ± 45	39 ± 11	89	70	80	90	89	85	10.6
MO	2×	99	88 ± 17	39 ± 9	91	56	80	95	94	92	5.1
OH	2×	93	109 ± 54	40 ± 8	63	68	84	97	96	90	6.5
WI	3×	68	83 ± 28	37 ± 7	76	50	66	98	98	87	14.9
Average			89 ± 42	39 ± 11	71	57	73	93	92	89	10.3

¹Average DIM or milk yield (± SD) at the time of AI.

²Proportion of cows that had at least one sample having elevated (≥1 ng/mL) concentration of progesterone (P4) 0 to 10 d before initiating the Ovsynch (OVS) protocol (injections of GnRH 7 d before and 48 h after PGF_{2α}, plus timed AI 16 h after the second GnRH injection). Percentage of cows at each location determined to be cycling and subsequently treated with OVS or OVS + CIDR were: IL (94, 94); KS (39, 43); MI (89, 90); MO (90, 91); OH (69, 57); and WI (61, 89), respectively.

³Proportions of noncycling cows (low P4 in 2 blood samples collected 0 and 10 d before the first GnRH injection) with high P4 at 7 d after the first GnRH injection of Ovsynch.

⁴Proportion of cows having high (≥1 ng/mL) concentrations of P4 in serum at PGF_{2α} injection. Blood samples were collected 1 to 2 h after CIDR insert removal just before the injection of PGF_{2α}.

⁵Proportion of cows having low (<1 ng/mL) P4 at 48 h after PGF_{2α}.

⁶Proportion of cows with high P4 in serum at the time of the PGF_{2α} injection of Ovsynch in which concentrations of P4 decreased to <1 ng/mL after 48 h.

⁷Proportions of cows that ovulated one or more follicles by 48 h after the second GnRH injection of Ovsynch.

among treatments and pretreatment cycling status were made by *F*-tests resulting from ANOVA. Differences between more than 2 means were tested by χ^2 within procedure GENMOD or by the PDIFF option in procedure GLM, when protected by a significant ($P \leq 0.05$) *F*-test.

RESULTS

Cycling Status

Numbers of cows and their DIM at TAI differed ($P < 0.01$) among locations (Table 1). Treatment inseminations were made entirely at first service only at the Kansas location. Although service number varied at all other locations as indicated by the mean and standard deviation for DIM (Table 1), 79% of cows at all locations were inseminated before 100 DIM.

Proportions of cows cycling before onset of treatments ranged from 41 to 94% and differed ($P < 0.001$) among locations (Table 1). Lactation number, DIM, and milk yield, however, did not influence the proportions of cows cycling before onset of treatments. Percentages of cycling (72%) and noncycling (70%) cows subsequently treated with OVS or OVS + CIDR were similar (see footnote 2 in Table 1 for distribution of cows in each location).

Pregnancy Outcomes

The primary objective of this study was to determine whether P4 supplementation from a 1.9-g CIDR insert

before AI would increase conception rates in lactating dairy cows. The design of this study also allowed determination of the effect of pretreatment cycling status with the CIDR insert on fertility in cycling and noncycling cows, as well as its effect on cows having an active CL (high P4) before the PGF_{2α} injection.

As a result of the CIDR insert, pregnancy outcomes at d 28 after TAI were improved ($P < 0.05$) by 10 percentage points (Table 2). Inconsistencies in pregnancy outcomes among locations ($P < 0.05$), however, accounted for an interaction ($P < 0.05$) of treatment × location at d 28 and 56. In response to P4 treatment via the CIDR insert, cows in Illinois, Kansas, Missouri, and Ohio had numerically greater conception rates at d 28, and also at d 56 (except in Missouri); cows in Michigan and Wisconsin had poorer conception rates at d 28 and 56.

Even though 4 locations (Illinois, Kansas, Missouri, and Ohio) had overall numerically greater conception rates in response to the CIDR insert, neither DIM nor cycling status accounted for these similarities (Table 1). At the extremes for DIM at TAI (least in Kansas and most in Illinois) and pretreatment cycling status (least in Kansas and most in Illinois), conception rates were the greatest at both locations (Tables 1 and 2). For cows in Missouri and Ohio, pretreatment cycling status and DIM at TAI were intermediate between the extremes for Illinois and Kansas, but both locations had numerical advantages for conception rates.

In an attempt to explain the treatment × location interaction on pregnancy outcomes, pretreatment cy-

Table 2. Conception rates in lactating dairy cows at 28 and 56 d after timed AI in response to Ovsynch (OVS) or Ovsynch + controlled internal drug release (CIDR) insert (OVS + CIDR), and subsequent pregnancy losses from d 28 to 56

Location	Pregnant at 28 d ¹			Pregnant at 56 d ²			Pregnancy loss ³ 28 to 56 d		
	No. of cows	OVS	OVS + CIDR	No. of cows	OVS	OVS + CIDR	No. of cows	OVS	OVS + CIDR
	%		%		%		%		
IL	96	44	72	96	30	48	58	30	36
KS	184	38	62	184	24	51	86	41	17
MI	94	40	33	94	31	25	36	23	14
MO	99	40	44	99	39	38	42	5	10
OH	93	25	41	93	24	30	32	10	28
WI	68	56	49	68	47	37	35	18	25
Average		40	50		33	38		21	21

¹Treatment ($P < 0.05$), location ($P < 0.01$), and treatment \times location interaction ($P < 0.05$).

²Treatment \times location interaction ($P < 0.05$).

³Location ($P < 0.05$) and treatment \times location interaction ($P = 0.09$).

cling status was examined, in addition to whether serum P4 was high or low before PGF_{2 α} was administered. In other words, cows were categorized by pretreatment cycling status and luteal status before the PGF_{2 α} injection (Table 3). Across treatments, conception rates at 56 d after TAI in 184 noncycling cows (31%) did not differ from those of 450 cycling cows (35%). Of the 184 noncycling cows in which serum P4 was low before treatment, 57% of the cows had high P4 before the PGF_{2 α} injection, whereas 79% of 450 cycling cows had high P4 before the PGF_{2 α} injection (Table 3).

It is clear, however, that cycling cows having an active CL (OVS = 39% vs. OVS + CIDR = 43%) and noncycling cows having an active induced CL (OVS = 39% vs. OVS + CIDR = 34%) did not have improved conception rates at d 56 in response to additional P4 provided by the CIDR insert, respectively (Table 3).

In contrast, the treatment \times luteal status interaction ($P < 0.05$) indicated that regardless of pretreatment cycling status, noncycling cows treated with a CIDR insert without an active induced CL (OVS + CIDR = 33% vs. OVS = 17%) or cycling cows without an active CL (OVS + CIDR = 38 vs. OVS = 19%) before the PGF_{2 α} injection had greater conception rates than OVS cows of similar luteal status.

Pregnancy Losses

Pregnancy loss between 28 and 56 d after TAI did not differ between treatments (Table 2), but effects of location ($P < 0.05$) and a tendency ($P = 0.09$) for a treatment \times location interaction were detected. Although OVS + CIDR cows in Kansas and Michigan seemed to have fewer pregnancy losses than OVS cows, those at

Table 3. Conception rates in lactating dairy cows at 56 d after timed AI based on cycling status before treatment and on serum concentrations of progesterone (P4) at the time of PGF_{2 α} injection of the Ovsynch (OVS) or Ovsynch + controlled internal drug release (CIDR) insert (OVS + CIDR) protocol

Pretreatment cycling status	Serum P4 before PGF _{2α} ¹	Luteal status before PGF _{2α}	Treatment ²		Total ³
			OVS	OVS + CIDR	
			% (no. of cows)		
Noncycling	High	Active induced corpus luteum (CL) ⁴	39 (58)	34 (47)	37 (105)
	Low	No induced CL ⁴	17 (38)	33 (41)	25 (79)
Cycling	High	Active CL	39 (178)	43 (179)	41 (357)
	Low	Early luteolysis	19 (47)	38 (46)	28 (93)
Total	High	Active CL	39 (236)	39 (226)	
	Low	No CL	18 (85)	36 (87)	

¹PGF_{2 α} injection given before timed AI. High = ≥ 1 ng/mL and low = < 1 ng/mL.

²Two-way interaction ($P < 0.05$) of treatment \times luteal status before PGF_{2 α} . No three-way interaction of treatment \times pretreatment cycling status \times luteal status before PGF_{2 α} .

³Conception rate in 184 noncycling cows (31%) did not differ from that of 450 cycling cows (35%).

⁴In response to the first GnRH injection.

Table 4. Pregnancy loss in lactating dairy cows from 28 to 56 d after timed AI, based on cycling status before treatment and on serum concentrations of progesterone (P4) at the time the PGF_{2α} injection of the Ovsynch (OVS) or Ovsynch + controlled internal drug release (CIDR) insert (OVS + CIDR) protocol

Pretreatment cycling status	Serum P4 before PGF _{2α} ¹	Luteal status before PGF _{2α}	Treatment ²		
			OVS	OVS + CIDR	Total ³
			% (no. of cows)		
Noncycling	High	Active induced corpus luteum (CL) ⁴	18 (25)	38 (28)	29 (53)
	Low	No induced CL ⁴	42 (8)	24 (20)	29 (28)
Cycling	High	Active CL	15 (81)	12 (91)	13 (172)
	Low	Early luteolysis	34 (16)	5 (20)	18 (36)
Total	High	Active CL	17 (106)	25 (119)	
	Low	No CL	38 (24)	14 (40)	

¹PGF_{2α} injection given before timed AI. High = ≥1 ng/mL and low = <1 ng/mL.

²Two-way interaction ($P < 0.01$) of treatment × luteal status before PGF_{2α}. No three-way interaction of treatment × pretreatment cycling status × luteal status before PGF_{2α}.

³Pregnancy loss in 81 noncycling cows (31%) differed ($P < 0.05$) from loss in 208 cycling cows (16%).

⁴In response to the first GnRH injection.

other locations had numerically greater losses between 28 and 56 d after TAI in response to P4 supplementation (Table 2).

Accounting for pretreatment cycling status of cows, and whether an active CL was present before PGF_{2α} was administered, did not eliminate the location or treatment × location effects (Table 4). Noncycling cows (31%; $n = 81$), however, had greater ($P < 0.05$) pregnancy loss than that for cycling cows (16%; $n = 208$). Although pregnancy losses were greater for cows classified as noncycling, overall losses were reduced for all cows receiving CIDR inserts when serum P4 was low before PGF_{2α} in noncycling cows without an induced CL (OVS + CIDR = 24% vs. OVS = 42%) and in cycling cows without an active CL (OVS + CIDR = 5% vs. OVS = 34%) before the PGF_{2α} injection (Table 4). In contrast, the interaction ($P < 0.01$) of treatment × serum P4 status before PGF_{2α} injection showed that pregnancy loss was greater in OVS + CIDR than in OVS when serum P4 was high (25 vs. 17%), regardless of pretreatment cycling status.

Concentrations of P4 at CIDR Insert Removal

Supplementing P4 via the CIDR insert did not increase relative serum concentrations of P4 in cows. Concentrations of P4 measured in blood samples collected just before CIDR insert removal at 2 locations did not differ between OVS (2.2 ± 0.3 ng/mL; $n = 84$) and OVS + CIDR cows (2.4 ± 0.3 ng/mL; $n = 80$). Just before CIDR insert removal, concentrations of P4 in 19 noncycling CIDR-treated cows (1.9 ± 0.6 ng/mL) did not differ from those of 16 noncycling OVS (1.8 ± 0.5 ng/mL). Likewise, at CIDR insert removal, concentrations of P4 were similar among 71 cycling OVS cows (2.8 ± 0.2 ng/mL) and

68 cycling cows treated with CIDR inserts (2.7 ± 0.2 ng/mL).

Induced Ovulation in Noncycling Cows

Concentrations of P4 exceeded 1 ng/mL in some noncycling cows because of P4 supplementation provided by the CIDR insert and because 57% of all noncycling cows (54% at the 2 sampled locations) ovulated in response to the first GnRH injection. After the first GnRH injection, ovulation was induced in 52 to 83% of all noncycling cows. Proportions of cows having induced ovulation did not differ among locations (Table 1).

Concentrations of P4 Before PGF_{2α} Injection

Decline in concentrations of P4 for cows treated with a CIDR insert between the time of insert removal and 1 to 2 h later when the PGF_{2α} injection was given averaged only 0.5 ± 0.1 ng/mL, ranged from -0.2 ± 0.1 to 0.8 ± 0.1 ng/mL among locations. These differences in P4 suggested that the P4 contribution of the CIDR insert to peripheral serum concentrations of P4 was small. When P4 was assessed in blood samples collected before PGF_{2α} injection from cows at all locations at least 1 h after CIDR insert removal, OVS + CIDR cows (2.4 ± 0.2 ng/mL; $n = 313$) did not differ from OVS (2.5 ± 0.2 ng/mL; $n = 321$). Cycling cows had greater concentrations of P4 (3.3 ± 0.1 ng/mL; $n = 450$) than noncycling cows (1.6 ± 0.2 ng/mL; $n = 184$). Concentrations of P4 exceeded 1 ng/mL in noncycling cows because cycling status was determined before treatment. Therefore, some cows classified as noncycling had ovulated in response to the first GnRH injection (Table 1) and had elevated serum P4 during and after the time of CIDR insert removal.

Table 5. Diameter of the ovulatory follicle before the second GnRH injection of the Ovsynch (OVS) or Ovsynch + controlled internal drug release (CIDR) insert (OVS + CIDR) protocol, based on cycling status before treatment and on serum concentrations of progesterone (P4) at the time of PGF_{2α} injection

Pretreatment cycling status	Serum P4 before PGF _{2α} ¹	Luteal status before PGF _{2α}	Treatment ²	
			OVS	OVS + CIDR
			— Mean ± SE (no. of cows) —	
Noncycling	High	Active induced corpus luteum (CL) ⁴	15.5 ± 0.4 (47)	16.4 ± 0.4 (39)
	Low	No induced CL ⁴	14.2 ± 0.5 (27)	15.5 ± 0.5 (31)
Cycling	High	Active CL	14.9 ± 0.2 (154)	14.8 ± 0.2 (160)
	Low	Early luteolysis	14.9 ± 0.4 (40)	16.3 ± 0.4 (41)

¹PGF_{2α} injection given before timed AI. High = ≥1 ng/mL and low = <1 ng/mL.

²Three-way interaction ($P < 0.01$) of treatment × pretreatment cycling status before treatment × luteal status before PGF_{2α}.

³Follicle diameter in noncycling cows (15.4 ± 0.3 mm; n = 144) did not differ from that of cycling cows (15.2 ± 0.2 mm; n = 395).

⁴In response to the first GnRH injection.

Percentages of cows in which concentrations of P4 were ≥1 ng/mL before the PGF_{2α} injection ranged from 63 to 84% among locations (Table 1) and differed ($P = 0.05$) among locations. Fewer ($P < 0.001$) noncycling cows (58%; n = 168) than cycling cows (79%; n = 434) had high P4 before PGF_{2α} injection.

Concentrations of P4 after PGF_{2α} Injection

Proportions of all cows in which serum P4 approached basal (proestrus) concentrations by 48 h after PGF_{2α} were affected by treatment differently at various locations (treatment × location interaction; $P < 0.001$; Table 1). Among OVS + CIDR cows, the percentages ranged from 83 to 99%, whereas among OVS, they ranged from 79 to 100%. Although differences were small, at 3 locations, more OVS + CIDR cows than OVS cows had low P4, whereas the reverse was true at 3 other locations. Interactions were unrelated to overall cycling status at each location, but more ($P < 0.05$) noncycling cows (97%; n = 184) than cycling cows (92%; n = 447) had low P4 by 48 h after PGF_{2α} injection. Proportion of cows undergoing CL regression followed the same trends as percentages of cows having low P4 by 48 h after PGF_{2α} injection (Table 1).

Ovulatory Responses

Incidence of ovulation in 602 cows examined by 48 h after the second GnRH injection ranged from 85 to 95%, but did not differ among locations (Table 1). More cycling cows (91%; n = 434) than noncycling cows (84%; n = 168) ovulated at least 1 follicle. Proportion of cows having multiple ovulations was not affected by location (Table 1). Multiple ovulations occurred less ($P < 0.05$) frequently in 434 cycling than 168 noncycling cows (7.9 vs. 15.2%; respectively).

Follicle Size

Of 602 cows examined for follicular diameters and evidence of ovulation, only 2 cows failed to ovulate when at least 1 follicle was identified >5 mm in diameter. The remaining 62 cows failed to have at least 1 follicle in excess of 5 mm in diameter and did not ovulate. Overall, cows treated with P4 had larger ($P < 0.01$) follicles than did cows not treated with P4 [(15.7 ± 0.2 mm, n = 271) vs. (15.1 ± 0.2 mm, n = 268); Table 5], but a 3-way interaction ($P < 0.01$) between treatment, luteal status before PGF_{2α}, and pretreatment cycling status was detected. In 3 of the 4 treatment contrasts in Table 5, CIDR-treated cows had larger follicles, except in cycling cows having an active CL (high P4) before PGF_{2α} injection.

No relationship was detected between follicular size and conception rates. Across treatments, however, pregnancy loss between d 28 and 56 tended ($P = 0.08$) to be greater when follicle diameter was ≤15 mm (27%; n = 134) than when > 15 mm (18%; n = 134).

Multiple Ovulations

Incidence of multiple ovulations assessed in 602 cows averaged 10.3%, ranged from 5.1 to 14.9% across locations, but did not differ among locations (Table 1). Noncycling cows had greater incidences of multiple ovulations (15.2%; n = 168) than cycling cows (5.7%; n = 434). The incidence of multiple ovulations based on luteal status before the PGF_{2α} injection is summarized by treatment in Table 6. No treatment interactions were detected.

Conception rates were greater ($P < 0.01$) at 28 d (68 vs. 42%) and 56 d (53 vs. 34%) after TAI for cows having multiple (n = 62) vs. single (n = 540) ovulation, respectively. Pregnancy loss tended to be greater in OVS cows

Table 6. Incidence of multiple ovulation 48 h after the second GnRH injection of the Ovsynch (OVS) or Ovsynch + controlled internal drug release (CIDR) insert (OVS + CIDR) protocol, based on ovulatory status before treatment and on serum concentrations of progesterone (P4) at the time of PGF_{2α} injection

Pretreatment cycling status	Serum P4 before PGF _{2α} ¹	Luteal status before PGF _{2α}	Treatment ²		
			OVS	OVS + CIDR	Total ³
			% (no. of cows)		
Noncycling	High	Active induced corpus luteum (CL) ⁴	15.4 (51)	14.9 (43)	15.2 (94)
	Low	No induced CL ⁴	16.5 (38)	14.2 (36)	15.3 (74)
Cycling	High	Active CL	7.1 (169)	11.8 (175)	9.5 (344)
	Low	Early luteolysis	0.0 (46)	5.7 (44)	1.9 (90)

¹PGF_{2α} injection given before timed AI. High = ≥1 ng/mL and low = <1 ng/mL.

²No treatment interactions.

³Incidence of multiple ovulation in 168 noncycling cows (15.2%) differed ($P < 0.01$) from incidence in 434 cycling cows (5.7%).

⁴In response to the first GnRH injection.

having multiple (31%; $n = 18$) vs. single (19%; $n = 106$) ovulation, whereas the reverse trend was detected for OVS+CIDR cows (15%, $n = 24$; vs. 26%, $n = 129$), respectively, (treatment \times multiple ovulation; $P = 0.08$).

DISCUSSION

The objective of our study was to determine whether P4 supplementation from a 1.9-g CIDR insert before TAI, as part of the Ovsynch protocol, would improve fertility. Overall, compared with OVS, conception rates were 10 and 5 percentage points greater for cows treated with P4 at 28 and 56 d after TAI, respectively. Conception rates were positive for both cycling and noncycling cows treated with the CIDR insert, but only at 4 of the 6 locations at 28 d and at 3 of 6 locations at 56 d. This inconsistent response is corroborated by other large-scale studies summarized from the literature in Table 7. In one study, conception rates were improved by addition of a CIDR insert (El-Zarkouny et al., 2004) compared with cows inseminated at estrus or after the Ovsynch protocol. In a 5-location study conducted during summer in Mexico, in which cows were treated with Ovsynch alone, conception rates were only improved in first-lactation cows in which a CIDR insert also was included (Moreira et al., 2004a).

In another study conducted in Mexico, in which estrous cycles were presynchronized (Presynch) in all cows before treatment with Ovsynch, addition of a CIDR insert improved conception rates (Moreira et al., 2004b), whereas no positive effect occurred in 2 other studies of similar treatments (El-Zarkouny et al., 2004; Thatcher et al., 2006), unless cows were in late diestrus at the time of CIDR insertion (Thatcher et al., 2006). In another large study, estrous cycles were presynchronized (Presynch) and a CIDR insert was incorporated into a TAI protocol using estradiol cypionate to induce estrus and ovulation (Galvão et al., 2004). Conception

rate in lactating dairy cows was not improved in response to the CIDR insert; however, few noncycling cows received a CIDR insert ($n = 52$).

In the present study, blood P4 concentrations at time of PGF_{2α} injection influenced conception rates (Table 3). Cows having low serum P4 before the PGF_{2α} injection, whether cycling or not, had greater conception rates when treated with a CIDR insert. Noncycling cows in which an active CL was induced and cycling cows having an active CL at the time of PGF_{2α} injection did not have improved conception rates as a result of P4 supplementation. Therefore, only cows without an active CL before PGF_{2α} injection had improved pregnancy outcomes. Our hypothesis was that noncycling cows and cycling cows having low P4 before the PGF_{2α} injection should have improved conception rates when treated with the Ovsynch protocol in combination with the CIDR insert. Not all results to date confirm that hypothesis.

The mechanism by which conception rates were improved in CIDR-treated cows having low P4 before the PGF_{2α} injection may include several possibilities. A lack of synchrony between luteolysis, ovulation, and TAI for cycling OVS cows having early luteolysis may have been remedied in part by the CIDR insert because estrus and ovulation were prevented by the supplemental P4. Our results are consistent with improved conception rates for similarly treated cycling suckled beef cows in which blood P4 was low before PGF_{2α} injection (Lamb et al., 2001).

For noncycling cows in which no CL was induced and serum P4 was low before the PGF_{2α} injection (Table 3), conception rates were increased in OVS+CIDR cows. Our results corroborate at least 3 other studies in beef cattle (Lamb et al., 2001; Stevenson et al., 2003; Larson et al., 2006), in which use of the CIDR insert improved conception rates in noncycling cows without an induced CL. It is possible that the supplemental P4 served a

Table 7. Studies from the literature in which Ovsynch or Presynch + Ovsynch in combination were compared with similar treatments that included an intravaginal progesterone-releasing controlled interval drug release (CIDR) insert in lactating dairy cows

Trait	Ovsynch		Presynch + Ovsynch		Reference
	No CIDR	CIDR	No CIDR	CIDR	
	— % (no. of cows) —		— % (no. of cows) —		
Conception rate at 29 d ²	36.3 (91)	59.3 (91)			
Conception rate at 57 d ²	19.8 (91)	45.1 (91)			El-Zarkouny et al., 2004 (experiment 1)
Conception rate at 29 d ³	42.9 (154)	32.0 (150)	48.4 (153)	45.1 (153)	El-Zarkouny et al., 2004 (experiment 2)
Conception rate at 40 to 45 d					
First lactation ⁴	20.1	38.2			Moreira et al., 2004a
≥Second lactation	27.5	22.3			
Conception rate at 40 to 45 d ⁵			37 (415)	43 (414)	Moreira et al., 2004b
Conception rate at 27 d			38.8 (338)	35.8 (335)	Galvão et al., 2004

¹Greater ($P < 0.05$) conception rates for cows treated with CIDR inserts having no CL before the PGF_{2 α} injection.

²Greater ($P < 0.05$) conception rates for Ovsynch cows treated with a CIDR insert.

³Greater ($P < 0.05$) conception rates for Presynch cows; no effect of CIDR insert.

⁴Greater ($P < 0.05$) conception rates for CIDR-treated first-lactation cows only.

⁵Greater ($P < 0.05$) conception rates for Presynch + Ovsynch cows treated with CIDR inserts.

priming function to facilitate ovulation of a follicle in response to the second GnRH injection. In suckled non-cycling beef cows treated with GnRH + norgestomet (analogous to the first GnRH + CIDR insert in our study), size of the recruited follicle after GnRH was not increased, but greater subsequent concentrations of estradiol enhanced the GnRH-induced LH release at 48 h after PGF_{2 α} and increased the occurrence of normal luteal function after induced ovulation (Thompson et al., 1999). In a recent study, a greater proportion of anovulatory lactating dairy cows treated with a CIDR insert had high P4 by 62 ± 7 DIM compared with controls (Cerri et al., 2005). Factors influencing why the CIDR was beneficial in some, but not all, anestrous cows need to be identified.

Concentrations of P4 in cows with or without an active CL were only slightly increased by the CIDR insert in the present study. Other studies in beef cattle concluded that the CIDR insert provided relatively small increases (0.5 to 1 ng/mL) in serum concentrations of P4 (Stevenson et al., 2003). Endogenous P4 production was decreased by insertion of a P4-releasing intravaginal device in late diestrus (d 10 to 17), whereas no change occurred when treatment was initiated earlier, on d 5 to 10 of the estrous cycle (Robinson et al., 1989). These observations are consistent with the concept that the CL has receptors for P4 (Schams and Berisha, 2002) allowing for some self-autoregulation of P4 biosynthesis, and thus partly influencing peripheral P4 concentrations, in addition to liver-regulated clearance rates of P4 (Sangsritavong et al., 2002).

Further, initiating P4 treatment (P4-releasing intravaginal device) during the luteal phase increased conception rates in cows having reduced P4 concentrations, but decreased conception rates in cows having greater

P4 concentrations (Folman et al., 1990). Therefore, P4 treatment may have improved fertility in cows with low peripheral P4 concentrations during the luteal phase (e.g., starting treatment in late diestrus; Thatcher et al., 2006), or when treatment was started earlier during the luteal phase of the estrous cycle. Our results (Table 3) are consistent with those reported by Folman et al. (1990) in which a P4-releasing intravaginal device was used.

Other studies demonstrated that heifers had greater fertility (Pursley et al., 1997) and greater blood concentrations of P4 (Wiltbank et al., 2000; Wolfenson et al., 2004) during the estrous cycle than did lactating dairy cows. If subnormal P4 concentrations were limiting fertility of lactating dairy cows, it would seem logical that conception rates in cycling cows might increase in response to CIDR inserts. Concentrations of P4, however, at the time of CIDR removal did not differ between treatments. Because blood samples were not collected each day the CIDR was in situ, we cannot determine whether P4 was significantly altered during the treatment period. In suckled beef cows in which ovulation was induced after treatment with GnRH and norgestomet, serum concentrations of P4 were greater after 7 d compared with cows treated with GnRH alone (Thompson et al., 1999), although their peak increase rarely exceeded 1.5 ng/mL in previously noncycling cows. In the previous cases, cows had newly formed, induced CL in addition to an exogenous source of progestin. In 4 ovariectomized cows treated with 1.38-g CIDR inserts, concentrations of P4 exceeded 5 ng/mL by 1 h after its insertion, but decreased linearly to about 2.8 ng/mL after 7 d (Rathbone et al., 2002). Concentrations of P4 decreased to 0.75 and 0.5 ng/mL by 1 and 2 h, respectively, after insert removal. It is not clear

how the CIDR insert affects circulating concentrations of P4 in cycling cows at different stages of the estrous cycle. In the current study, regardless of treatment, no trend was detected for increased conception rate when endogenous P4 was high at the time of insert removal. Perhaps duration of exposure to P4 during the week before PGF_{2α} injection is more important than the absolute magnitude of P4 concentrations in serum.

Diameters of the follicles detected before the second GnRH injection were greater for all cows treated with the CIDR insert, except for cycling cows having an active CL before luteolysis was induced (Table 5). Because concentrations of P4 were less in noncycling cows, regardless of luteal status before the PGF_{2α} injection, and in cycling cows in which early luteolysis occurred, supplemental P4 probably caused more rapid follicular growth while the CIDR insert was in situ. Without a normally functioning CL that produces luteal phase concentrations of P4, one would expect LH pulse frequency to increase and cause more rapid follicular maturation and increased diameters in all cows relative to those in which an active CL is present (Smith and Stevenson, 1995). Our results for follicle diameters in cows treated with a CIDR insert are consistent with that observation.

Pregnancy losses between 28 and 56 d after TAI were greater in noncycling cows and were not affected by treatment, except when cows had low P4 before PGF_{2α} injection. Our results are consistent with a recent report summarizing 6,123 lactating dairy cows from 9 studies on 5 dairy farms (Rutigliano and Santos, 2005). They reported that pregnancy rates were greater for cycling than noncycling cows at 30 d (40 vs. 28%) and 58 d (34 vs. 22%) after TAI, but pregnancy losses were greater in noncycling cows (19 vs. 14%). The CIDR inserts applied in our study, however, reduced losses in cows having low P4 at time of PGF_{2α} (Table 4). For noncycling cows without an active CL at PGF_{2α}, reduced losses may be associated with a greater percentage of normal luteal activity detected after induced ovulations in cows preceded by supplementation of norgestomet (Thompson et al., 1999). Pregnancy losses differed among locations with losses of 31.4% in Kansas and 10% in Missouri. Although Ovsynch has been speculated as a cause of increased embryonic loss in lactating dairy cows, embryonic loss from 31 to 45 (±3) d after AI was similar for cows receiving AI after removed tail chalk and for cows receiving TAI after Ovsynch (10.4 vs. 13.2%; Chebel et al., 2004).

How pregnancy loss can be affected 28 to 56 d after conception in response to events that occur before AI is unclear. Smaller ovulatory follicles may predispose subsequently formed CL to have fewer luteal cells and secrete less P4. Pregnancy losses were twice as great

among noncycling than cycling cows (Table 4). In cases in which the follicle was larger in OVS+CIDR than OVS cows (noncycling cows having no induced CL and cycling cows having early luteolysis), follicle diameters were greater and pregnancy losses were less (Tables 4 and 5). The reverse was true for cows having an active CL at PGF_{2α} injection, even though follicle diameters were increased in cows having an active induced CL by supplemental P4, but not when cycling cows had an active CL before PGF_{2α} injection. Because of the interaction between treatment and luteal status before PGF_{2α} administration, pregnancy losses were likely not associated with follicle diameter, but related to the lack of established estrous cycles before the onset of treatment. Because conception rates seemed to improve in all cows having reduced P4 just before PGF_{2α} injection (Table 3), with cycling cows having less loss than noncycling cows, the effects of P4 are more likely related to a mechanism involving both improved uterine function and earlier establishment of estrous cycles. We detected greater pregnancy loss for cows having follicles ≤15 mm compared with those >15 mm in diameter. Reduced conception rates and increased embryonic mortality in beef cows were only observed when follicles were ≤11 mm in diameter following TAI (Perry et al., 2005). In contrast, when inseminations occurred after detected estrus and spontaneous ovulation in that same study, follicle size was not a factor in reduced fertility.

In summary, fertility of lactating dairy cows is poor, and has decreased more than 50% since 1970 (Butler and Smith, 1989). Improving fertility of lactating dairy cows is economically important to the dairy industry. Understanding the pharmacological impact of P4 supplementation on conception rates may lead to a better understanding of the physiological reasons for reduced fertility of lactating dairy cows. In the current study, the CIDR improved chances for conception and reduced pregnancy losses between 28 and 56 d after TAI in cows at some locations in which no luteal activity was detected before the PGF_{2α} injection, regardless of their pretreatment cycling status.

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