Pregnancy in Dairy Cows After Synchronized Ovulation Regimens With or Without Presynchronization and Progesterone¹

S. Z. El-Zarkouny,² J. A. Cartmill,³ B. A. Hensley, and J. S. Stevenson

Department of Animal Sciences and Industry
Kansas State University, Manhattan 66506-0201

ABSTRACT

Two experiments examined pregnancy after synchronized ovulation (Ovsynch) with or without progesterone (P4) administered via controlled internal drug release (CIDR) intravaginal inserts. In experiment 1, 262 lactating cows in one herd were in 3 treatments: Ovsynch (n = 91), Ovsynch + CIDR (n = 91), and control (n = 80). The Ovsynch protocol included injections of GnRH 7 d before and 48 h after an injection of PGF_{2α}. Timed artificial insemination (TAI; 57 to 77 d postpartum) was 16 to 20 h after the second GnRH injection. Cows in the Ovsynch + CIDR group also received a CIDR (1.9 g of P4) insert for 7 d starting at first GnRH injection. Control cows received A-I when estrus was detected using an electronic estrus detection system. Based on serum P4, 44.1% of cows were cyclic before Ovsynch. Pregnancy rates at 29 d (59.3 vs. 36.3%) and 57 d (45.1 vs. 19.8%) after TAI and embryo survival (75.9 vs. 54.5%) from 29 to 57 d were greater for Ovsynch + CIDR than for Ovsynch alone. In experiment 2, 630 cows in 2 herds received TAI at 59 to 79 d postpartum after 6 treatments. Estrous cycles were either presynchronized (2 injections of PGF_{2α} 14 d apart; n = 318) or not presynchronized (n = 312). Within those groups, Ovsynch was initiated 12 d after second presynchronization PGF_{2α} and used alone (n = 318) or with CIDR inserts for 7 d (1.38 g of P4/insert, n = 124 or 1.9 g of P4/insert, n = 188). Before Ovsynch, 80% of cows were cyclic. Presynchronization increased pregnancy (46.8 vs. 37.5%) at 29 d after TAI, but CIDR inserts had no effect on pregnancy in experiment 2. Overall embryonic survival between 29 and 57 d in experiment 2 was 57.7%. Use of CIDR inserts with Ovsynch improved conception and embryo survival in experiment 1 but not in experiment

2, in part due to differing proportions of cyclic cows at the outset. Presynchronization before Ovsynch enhanced pregnancy rate.

(**Key words:** progesterone, Ovsynch, dairy cow, pregnancy rate)

Abbreviation key: CIDR = controlled internal drug-releasing intravaginal insert containing either 1.38 (CIDR-1.38) or 1.9 g of progesterone (CIDR-1.9), CL = corpus luteum, ECM = energy-corrected milk, **High P4** = concentrations of progesterone in blood serum ≥ 1 ng/mL, **Low P4** = concentrations of progesterone in blood serum < 1 ng/mL, **Ovsynch** = injection of GnRH 7 d before and 48 h after an injection of PGF_{2α} with timed AI at 16 to 20 h after the second GnRH injection, **Ovsynch + CIDR** = same as Ovsynch plus a CIDR insert for 7 d at the time of first GnRH injection, **P4** = progesterone; **Presynch** = presynchronization—2 injections of PGF_{2α} 14 d apart with the second injection given 12 d before initiating Ovsynch, **PRID** = progesterone-releasing intravaginal device; **TAI** = timed AI.

INTRODUCTION

Lactating dairy cows with high genetic merit and outstanding milk production are likely to be more vulnerable to fertility problems, such as lower AI conception rates, weaker expression of estrus, and greater embryonic loss after insemination than lower-producing cows (Lucy, 2001).

When estrus was synchronized and AI was scheduled after detected estrus in earlier studies, fertility was greater than when AI was made at fixed times after synchronization without regard to detected estrus (Dailey et al., 1983; Lucy et al., 1986; Larson and Ball, 1992). Poorer fertility after timed AI (**TAI**) often was attributed to insufficient synchrony of estrus and ovulation to allow appropriate timing of AI relative to ovulation (Fogwell et al., 1986).

A protocol of synchronized ovulation (**Ovsynch**) was developed (Pursley et al., 1997a, 1997b) to synchronize follicular growth and maturation coupled with luteolysis before ovulation. The Ovsynch protocol uses GnRH and PGF_{2α}, and cows are inseminated at fixed times

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Corresponding author: J. S. Stevenson; e-mail: jss@ksu.edu.

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²Present address: Department of Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan.

³Present address: Department of Animal Science, Louisiana State University, Baton Rouge.

after the second of 2 GnRH injections without detection of estrus (Burke et al., 1996, 1997a, 1997b; Stevenson et al., 1999; Xu and Burton, 2000).

Deficiencies in luteal function (Dizerega and Hodgen, 1981), either before or after insemination, are associated with reduced fertility in beef (Odde, 1990) and dairy cattle (Fonseca et al., 1983). In addition, concentrations of progesterone (P4) in blood 34 to 48 h before the preovulatory surge of LH were greater in cows that conceived compared with those that failed to conceive (Erb et al., 1976). Thus, the magnitude of P4 concentrations before estrus may be associated with factors that increase the probability of conception. Blood concentrations of P4 during the luteal phase before insemination are associated positively with conception rate (Folman et al., 1973, 1990; Erb et al., 1976; Holness et al., 1981; Fonseca et al., 1983; Rosenberg et al., 1990a, 1990b).

Increased pregnancy rate was reported after intravaginal inserts containing P4 were applied to cows that were synchronized with PGF_{2α} (Xu et al., 1997). Therefore, reproductive performance of cows receiving the Ovsynch protocol may be improved if P4 is administered during the 7 d between the first GnRH and the only PGF_{2α} injections. Progesterone should prevent premature estrus and ovulation during the period in which spontaneous luteolysis may occur in small percentages of cows whose dominant follicles are not responsive to the first GnRH injection (Twagiramungu et al., 1992; Pursley et al., 1995; Roy and Twagiramungu, 1999; Vasconcelos et al., 1999; Xu and Burton, 2000).

Greater pregnancy rates in dairy cows were reported when the Ovsynch protocol was initiated on d 5 to 12 of the estrous cycle (Vasconcelos et al., 1999; Cartmill et al., 2001; Moreira et al., 2001). Initiation of the Ovsynch protocol during the late luteal phase (i.e., d 13 to 17 of the estrous cycle) often leads to premature regression of the corpus luteum (CL) and estrus before the second injection of GnRH (Moreira et al., 2000). Moreover, initiation of the Ovsynch protocol during metestrus may lead to failure of the first GnRH injection to synchronize a new follicular wave (Vasconcelos et al., 1999; Moreira et al., 2000). Such a failure may cause the subsequent ovulatory follicle to form a subnormal CL that produces less P4 following ovulation and consequently reduces conception (Vasconcelos et al., 1999; Moreira et al., 2000). It is possible to manipulate the estrous cycle of cows such that they are at an ideal stage of the estrous cycle (d 5 to 12) when the Ovsynch protocol is initiated (Cartmill et al., 2001; Moreira et al., 2001).

The objective of the first experiment was to test the hypothesis that providing P4 before TAI to lactating dairy cows would improve fertility at first services. Therefore, P4 was administered via a controlled internal drug release (CIDR) insert during the first 7 d

of the Ovsynch protocol. The objective of the second experiment was to investigate whether presynchronizing the estrous cycles of cows before applying the Ovsynch protocol with or without supplemental P4 administered via a P4-releasing insert would improve pregnancy rates.

MATERIALS AND METHODS

Experiment 1

Herd management. Lactating Holstein cows (n = 262) were housed in a 4-row, free-stall barn at a cooperating dairy in northeast Kansas. The herd consisted of 500 cows with an annual rolling herd average of 11,500 kg of milk. Cows were milked 3 times daily and fed a total mixed diet consisting of chopped alfalfa, corn silage, whole cottonseed, and a concentrate-mineral mix (offered twice daily) to meet or exceed National Research Council (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 head lockups and free stalls bedded with sand. All procedures, including injections, blood collection, TAI, and ovarian ultrasonography, were conducted while cows were locked up at the feed line.

Experimental design. Breeding clusters were formed at 21-d intervals as cows calved and, within each cluster, cows were assigned randomly to either of 3 treatments before AI was carried out between 57 and 77 d postpartum. Inseminations were performed between January and June 1999. The experiment consisted of 8 breeding clusters. Treatment protocols are illustrated in Figure 1. Ovulation was synchronized in 91 cows using the Ovsynch protocol consisting of two 100-μg injections of GnRH (Cystorelin, Merial, Iselin, NJ) 9 d apart with a 25-mg injection of PGF_{2α} (Lutalyse, Pharmacia Animal Health, Kalamazoo, MI) administered 48 h before a second injection of GnRH. The first injection of GnRH was given at random stages of the estrous cycle. In the second treatment (Ovsynch + CIDR), 91 cows received the Ovsynch protocol plus an intravaginal insert containing 1.9 g of P4 (CIDR; InterAg, Hamilton, NZ) at the time of first GnRH injection. The CIDR insert was removed 7 d later at least 1 to 2 h before the PGF_{2α} injection. The control group consisted of 80 cows that were fitted with an electronic estrus detection device (HeatWatch, DDx, Inc., Denver, CO). Cows in the Ovsynch and Ovsynch + CIDR treatments were inseminated between 16 and 20 h after the second GnRH injection. Controls were inseminated after detected estrus according to the a.m.-p.m. rule no earlier than 54 DIM. Artificial inseminations were conducted by either of 2 AI technicians.

Figure 1. Treatment protocols applied to lactating dairy cows before first service in experiment 1. Blood (B) samples were collected from all cows including controls, but ultrasonography (US) of follicle diameter (d -1) and incidence of ovulation (d +1) were only performed in cows treated with the Ovsynch or Ovsynch + CIDR protocols. Injections (i.m.) of GnRH (100 μ g) and PGF_{2 α} (25 mg), and CIDR = controlled internal drug release (intravaginal P4 [1.9 g] insert) were administered as illustrated. Control cows were inseminated between 54 and 113 DIM according to the AM-PM rule after detection of estrus supplemented by an electronic estrus-detection system.

Blood collection. Blood samples were collected via coccygeal venipuncture from all cows (including controls) before hormonal or placebo injections (d -20, -10, -3, and -1; d 0 = TAI) and stored overnight at 5°C until sera were harvested after centrifugation. A blood sample was collected 1 to 2 h after CIDR removal and before PGF_{2 α} was injected on d -3 to detect changes in serum P4. Sera samples were stored at 20°C until assayed for concentrations of P4 by radioimmunoassay (Skaggs et al., 1986). The inter- and intraassay coefficients of variance of P4 assays were 6.9 and 6.4%, respectively.

Pregnancy diagnosis. Pregnancy was confirmed by transrectal ultrasonography at 29 d after TAI in the Ovsynch and Ovsynch + CIDR treatments and confirmed by palpation of the uterus at 40 d by the herd veterinary practitioner. Pregnancy was diagnosed in control cows by palpation of the uterus at 40 to 46 d after AI. Embryo survival was determined in pregnant cows (no controls) by reassessing pregnancy status by ultrasonography at 57 d after TAI.

Cyclic status and luteal function. We assumed that concentrations of P4 were <1 ng/mL on d 0 to 4 (estrus and metestrus) and d 19 to 21 (proestrus) of the estrous cycle, whereas on d 5 to 18 (early diestrus: d 5 to 11; late diestrus: d 12 to 18), concentrations were $\geq$ 1 ng/mL. Cyclic status (cyclic or anestrus) before d -10

was determined by serum concentrations of P4 on d -20 and -10 (Figure 1). When both samples of blood serum contained concentrations of P4 < 1 ng/mL (**low P4**; Low-Low), the cow was classified as anestrus. When either of the paired samples contained concentrations of P4 $\geq$ 1 ng/mL (**high P4**; High-High, Low-High, or High-Low), the cow was classified as cyclic. When the cow was classified as anestrus (Low-Low) and any of the subsequent samples on d -3 or -1 contained high P4, ovulation was assumed to have occurred (induced) in response to the first GnRH injection on d -10. Luteolysis was indicated when serum P4 was high on d -3 and low 48 h later. Cows were defined to be synchronized when they had low or high P4 on d -3, then low P4 48 h later (i.e., High-Low or Low-Low). Note that anestrus cows are synchronized according to this definition.

Pretreatment stages of the estrous cycle at the onset of treatment on d -10 were classified based on those paired concentrations on d -20 and -10: early diestrus (Low [d -20]- High [d -10]); late diestrus (High-High); proestrus, estrus, or metestrus (High-Low); or anestrus (Low-Low). During, and as a result of, treatment, stages of the estrous cycle on d -10 were classified similarly, based on paired sample concentrations on d -10 and -3: early diestrus (High [d -10]-High [d -3]); late diestrus (High-Low); proestrus, estrus, or metestrus (Low-High); or anestrus (Low-Low).

Follicular measures. In the Ovsynch and Ovsynch + CIDR cows, diameters of the largest- and second-largest ovarian follicles were measured (using electronic calipers; average of vertical and horizontal distance across follicle) by transrectal ultrasonography (Aloka 500V, Wallingford, CT) using a 5-MHz transducer, before the second GnRH injection (d -1; Figure 1). Incidence of ovulation of either or both of the 2 largest follicles in response to the second GnRH injection was determined 48 h later on d +1.

Statistical analyses. Various analyses were conducted by ANOVA (procedures CATMOD, GLM, or both, SAS Inst., Cary, NC). Dependent variables included: pregnancy rates measured on d 29, 40 to 46, and 57; concentrations of P4 in serum on various days; percentages of cows with either high or low P4 concentrations on d -3; incidence of luteolysis; synchronization rate; serum P4 patterns that defined stage of the estrous cycle and proportions of cows therein on d -20 and -10, and on d -10 and -3; induced ovulation by d -3 in cows previously classified as anestrus; and incidences of ovulation (single follicle [single-largest follicle or second-largest follicle], two follicles [double], or the combined data from both single and double-ovulating cows) by 48 h after the second GnRH injection. Independent variables in the general model included: treatment

($n = 3$); lactation number (1 vs. 2+); serum P4 pattern ($n = 4$), or cyclic status before treatment in some models, and their interactions. Diameters of the 2 largest follicles, incidences of ovulation of one or two follicles, pregnancy rates via ultrasonography or palpation, and embryo survival were analyzed similar to that described above with the addition of cyclic status, lactation number, and their interactions with treatment. In selected models, P4 pattern was substituted for cyclic status.

Cyclic status in all models included only those cows that had resumed estrous cycles before treatments were initiated (d -10; cyclic vs. noncyclic [anestrus] cows). Cyclic status as an independent variable was analyzed before treatments were applied on d -10 in a model that included lactation number. In addition, BCS (assigned to cows on d -10 were based on a scale of 1 to 5 [1 = thin and 5 = obese]; Wildman et al., 1982) and average energy-corrected milk (ECM) yield for the first 150 DIM were included as covariates in all of the preceding models.

Experiment 2

Lactating Holstein cows were housed at 2 cooperating dairy herds in northeast Kansas. Herd sizes ranged from 400 to 600 cows, with rolling herd averages of 10,000 to 11,500 kg of milk. Cows were milked 3 times daily, housed in either 2- or 4-row barns containing free stalls bedded with sand. Cows were provided water and fed diets similar to those described in experiment 1. One of the 2 herds used in this experiment was the same herd used in experiment 1.

Handling of cows. Cows ($n = 630$) were assigned randomly in 21-d breeding clusters to 2×3 factorial arrangement of 6 treatments before TAI was carried out between d 59 and 79 postpartum. Inseminations were performed between November 1999 and June 2000. The experiment consisted of 16 breeding clusters in each herd. Treatment protocols are illustrated in Figure 2. Ovulation was synchronized according to the Ovsynch protocol as described in experiment 1. Before the Ovsynch protocol, estrous cycles in 318 cows were presynchronized (**Presynch**) using two 25-mg injections of $\text{PGF}_{2\alpha}$ 14 d apart with the second injection given 12 d before initiating the Ovsynch protocol. Remaining cows ($n = 312$) received no $\text{PGF}_{2\alpha}$ injections before the start of the Ovsynch protocol. At the time of first GnRH injection in the Ovsynch protocol, one-half of the cows received a CIDR insert (InterAg, Hamilton, NZ) containing 1.38 g of P4 (CIDR-1.38, $n = 124$), 1.9 g of P4 (CIDR-1.9, $n = 188$), or no P4 ($n = 318$). All sources and doses of GnRH and $\text{PGF}_{2\alpha}$ were as described in experiment 1.

Figure 2. Treatment protocols for presynchronization (Presynch or No Presynch) and CIDR treatments (2×3 factorial arrangement of 6 treatments) applied to lactating dairy cows before first services in experiment 2. All cows received the Ovsynch protocol (with or without a CIDR insert and were time inseminated [TAI] 16 to 20 h after second GnRH injection; B = blood collection). First 8 breeding clusters of cows ($n = 188$) received a CIDR containing 1.9 g of P4, whereas the last 8 clusters of cows ($n = 124$) received a CIDR containing 1.38 g of P4.

Cows were inseminated artificially between 16 and 20 h after the second GnRH injection by either of 2 AI technicians at each dairy. Pregnancy was diagnosed by transrectal ultrasonography at 29 d after TAI and confirmed by palpation of the uterus at 40 to 41 d by the same veterinary practitioner at both herds. Embryo survival was determined in pregnant cows by reassessing pregnancy maintenance by ultrasonography at d 57 after TAI. A BCS was assigned to cows on d -10 as in experiment 1.

Blood collection. Blood samples were scheduled to be collected from all cows via coccygeal venipuncture from all cows just before hormonal injections (d -36, -22, -10, -3, and -1; d 0 = TAI) and stored at 5°C overnight until serum was harvested after centrifugation. Serum samples were stored at -20°C until assayed for P4 concentrations as in experiment 1. The inter- and intraassay coefficients of variance of 14 assays were 7.2 and 5.5%, respectively. Because of missing blood samples, not all cows were included in various classifications in which concentrations of P4 were used as a basis for determining cyclic status, induced ovulation, luteal function, synchronization rate, luteolysis, and various serum P4 patterns in response to treatments.

Cyclic status and luteal function. Cyclic status (cyclic vs. anestrus) before the onset of the Ovsynch protocol (d -10) was based on serum concentrations of P4 on d -36, -22, and -10 (d 0 = TAI). When P4 concentrations in each of those 3 samples were low (i.e., Low-Low-Low), the cows were classified as anestrus. If

the concentration was high in any one of those 3 samples, then the cows were classified as cyclic (any one of the 7 remaining permutations of Low and High). To determine the stage of the estrous cycle at the onset of the Ovsynch protocol (d -10), P4 concentrations were considered to be High-High (early diestrus), High-Low (late diestrus), Low-High (proestrus, estrus, or metestrus), or Low-Low (anestrus) on d -10 and -3, respectively, as in experiment 1. Only these days could be used to estimate stage of cycle because of changes in serum P4 that resulted from injections of PGF_{2α} given on d -22 to all Presynch cows. Definitions for induced ovulation after d -10 in response to GnRH, luteal function on d -3, luteolysis by d -1, and synchronization rates were as described in experiment 1.

Statistical analyses. Percentages of cows cyclic by d -10 were analyzed by ANOVA (procedures CATMOD and GLM; SAS Inst. Inc., Cary, NC). Herd, lactation number (1 vs. 2+), their interaction, and regression variables (BCS and DIM at TAI) were included in the model. Pregnancy rates measured on d 29 and 57 were analyzed with main effects (Presynch vs. No Presynch: CIDR-1.3, CIDR-1.9 vs. no CIDR) consisting of 6 treatments, lactation number, herd, cyclic status or serum P4 patterns (based on P4 patterns on d -10 and -3 as described in experiment 1), and their interactions with treatment in the model.

Luteal function, incidence of luteolysis, synchronization rate, induced ovulation in cows previously classified as anestrus, and serum concentrations of P4 on various days or proportions of cows on various days having high P4 were analyzed in a separate model including treatment, herd, lactation number, regression variables and all interactions with treatment. Further analyses of pregnancy rates were conducted in which both cyclic status (defined above) and (or) serum P4 patterns on d -10 and -3 were considered in a model that included treatments, lactation number, herd, and all interactions with treatment plus BCS. Means were separated using the PDIFF function (Tukey option for adjustment of means) in procedure GLM when a significant *F*-test was detected by ANOVA.

RESULTS

Experiment 1

Cyclic status. Based on low concentrations of P4 in blood sera on d -20 and -10, fewer than half of the cows (44.3%) had resumed estrous cycles. However, a higher percentage (50 vs. 42%; $P < 0.05$) of multi-lactation ($n = 78$) than first-lactation ($n = 184$) cows had resumed estrous cycles before treatments were initiated. After calving, BCS varied among cows and affected the percentage of cows that had resumed cyclicity. For each

unit increase in BCS (ranging from 1.25 to 3.5), cyclic status increased ($P < 0.01$) by $48 \pm 10\%$. Although 150-d ECM had no effect on cyclic status, multiple-lactation cows produced ($P < 0.05$) more ECM than first-lactation cows (49.1 ± 0.8 vs. 44.3 ± 0.8 kg).

Induced ovulation. Induction of ovulation in anestrus cows in response to the first GnRH injection on d -10 in both Ovsynch and Ovsynch + CIDR treatments was 3.9 to 4.6 times ($P < 0.01$) that of the controls, but not different between the 2 treatments (Table 1).

Progesterone patterns. Average concentrations of P4 in Ovsynch + CIDR cows (unknown CL status) decreased from 2.6 ± 0.2 ng/mL upon CIDR removal to 2.0 ± 0.2 ng/mL at least 1 to 2 h after its removal. Average concentrations of P4 were ≥ 1 ng/mL in both treatments at the time of PGF_{2α} injection. Addition of the CIDR (2.0 ± 0.2 ng/mL) did not increase serum P4 on d -3 beyond that of Ovsynch alone (2.0 ± 0.2 ng/mL). Average P4 concentrations in both the Ovsynch and Ovsynch + CIDR cows decreased ($P < 0.01$) to 0.5 ± 0.2 ng/mL on d -1 in response to the PGF_{2α} injection, whereas elevated concentrations of P4 (1.9 ± 0.2 ng/mL) were maintained in control cows.

Luteal function. Percentage of cows that had high concentrations of P4 at the time of PGF_{2α} injection was 1.7 to 1.8 times greater ($P < 0.001$) after treatment with Ovsynch and Ovsynch + CIDR than in controls (Table 1). For each unit increase in BCS (ranging from 1.25 to 3.25), a $20 \pm 10\%$ increase ($P < 0.05$) in the percentage of cows that had elevated P4 at the time of PGF_{2α} injection was detected.

Incidence of luteolysis. Greater percentages ($P < 0.001$) of cows treated with Ovsynch and Ovsynch + CIDR had luteolysis than controls (Table 1). More first-lactation cows treated with Ovsynch tended ($P = 0.07$) to have luteolysis in response to PGF_{2α} injection (72%; $n = 61$) than multiple-lactation cows (47%; $n = 30$). However, both lactation groups had similar rates of luteolysis (55% [$n = 67$] vs. 54% [$n = 24$]; respectively) when they received the Ovsynch + CIDR treatment.

Synchronization rate. More ($P < 0.001$) cows in the Ovsynch and Ovsynch + CIDR treatments were synchronized than controls (Table 1). Based on our definition of synchronization rate, all anestrus cows (Low-Low) were synchronized when P4 was low on d -3 and -1. In fact, of the synchronized cows in Table 1, 50 cows (59%) treated with Ovsynch and 55 cows (63%) treated with Ovsynch + CIDR were anestrus. For each unit increase in BCS, ranging from 1.25 to 3.5, a $23 \pm 7\%$ decrease ($P < 0.05$) in the synchronization rate was detected after the PGF_{2α} injection, favoring thinner, noncyclic cows (BCS = 2.3 ± 0.03) compared with cyclic cows (BCS = 2.5 ± 0.03).

Table 1. Percentages of cows in which ovulation was induced in response to GnRH on d -10, luteal function on d -3, luteolysis after PGF_{2α} on d -1, synchronization rates, pregnancy rates, and subsequent embryo survival (experiment 1).

Item	Treatment ¹		
	Ovsynch	Ovsynch + CIDR	Control
Induced ovulation in anestrus cows ²	56.4 ^x (55)	48.0 ^x (55)	12.2 ^y (41)
Luteal function ³	65.9 ^x (91)	60.4 ^x (91)	36.3 ^y (79)
Luteolysis ⁴	63.7 ^x (91)	54.9 ^x (91)	2.5 ^y (79)
Synchronization rate ⁵	96.7 ^x (91)	93.4 ^x (91)	49.4 ^y (79)
Pregnancy rate		% (no.)	
d 29 ultrasonography	36.3 (91)	59.3* (91)	
d 40–46 palpation	20.9 ^x (91)	45.1 ^y (91)	38.8 ^y (80)
d 57 ultrasonography	19.8 (91)	45.1* (91)	
Embryo survival			
d 29 to d 40–46	57.6 (33)	75.9* (54)	
d 29 to d 57	54.5 (33)	75.9* (54)	
40–46 to d 57	94.7 (33)	100 (54)	

^{x,y}Mean percentages within item with dissimilar superscript letters differ ($P < 0.05$).

*Different ($P < 0.05$) from Ovsynch.

¹Ovsynch = injection of GnRH (d -10) 7 d before and 48 h (d -1) after an injection of PGF_{2α} (d -3), with one timed AI (d 0) 12 to 20 h after GnRH injection on d -1. Ovsynch + CIDR = as Ovsynch plus insertion of a P4-releasing intravaginal insert (CIDR) for 7 d beginning on d -10 and removed on d -3. Control cows were inseminated after detected estrus. See figure 1 for further details.

²Cows with low (< 1 ng/mL) progesterone (P4) on d -20 and -10, but had high (≥ 1 ng/mL) P4 on d -3, 7 d after GnRH injection on d -10.

³Percentage of cows with high P4 on d -3.

⁴Percentage of cows with high P4 on d -3 just before injection of PGF_{2α}, and low P4 48 h later (sample collected at least 1 to 2 h after CIDR inserts were removed).

⁵Percentage of cows with either low or high P4 on d -3, then low P4 48 h later.

Follicular measures. No cow had follicles >24 mm in diameter on d -1. There was an interaction ($P < 0.05$) between treatment and lactation number for diameters of the largest follicle and the eventual ovulatory follicle on the day of the second GnRH injection. For the ovulatory follicle, multiple-lactation cows treated with Ovsynch + CIDR had larger (16.2 ± 0.6 mm; $n = 24$) follicles than those treated with Ovsynch alone (14.1 ± 0.6 mm; $n = 27$), whereas no treatment effect was detected in the first-lactation cows (14.6 ± 0.4 ; $n = 60$ vs. 14.6 ± 0.5 mm; $n = 50$, respectively). Diameter of the ovulatory follicle was not influenced by cyclic status at the time of PGF_{2α} injection, BCS, or 150-d ECM. Increased follicular diameter in multiple-lactation cows treated with Ovsynch + CIDR was independent of P4 because blood concentrations of P4 at the time of follicle assessment did not differ among treatments, nor were they different in first (2.0 ± 0.1 ng/mL; $n = 183$) and multiple lactation (1.9 ± 0.2 ng/mL; $n = 78$) cows.

Incidence of ovulation. Incidences of ovulation after the Ovsynch and Ovsynch + CIDR treatments are illustrated in Figure 3. Use of a CIDR with Ovsynch increased the combined percentage of cows with either single or double ovulations (92.3 vs. 84.6%; $P < 0.05$); increased the percentage of cows with single ovulations (79.1 vs. 67.0%; $P < 0.01$); and decreased the percentage

of cows failing to ovulate (7.7 vs. 15.3%; $P < 0.05$). Neither BCS nor 150-d ECM affected ovulatory responses. Occurrences of double ovulations (15.4%), ovulation of the single largest follicle (79.6%), and ovulation of the second largest follicle (8.8%) were unaffected by treatment or cyclic status. Double ovulation was not affected by 150-d ECM or BCS.

Pregnancy rates. Cows treated with Ovsynch + CIDR had greater ($P < 0.01$) pregnancy rates at d 29 after TAI than those treated with the Ovsynch protocol (Table 1). An interaction ($P < 0.01$) of magnitude between treatment and pretreatment cyclic status was detected. Anestrus cows treated with Ovsynch + CIDR had a greater pregnancy rate (64%; $n = 50$) than anestrus cows (27%; $n = 55$) treated with Ovsynch alone, whereas cyclic cows receiving Ovsynch + CIDR had a pregnancy rate (54%; $n = 41$) similar to that of cyclic cows receiving Ovsynch alone (47%; $n = 36$).

Pregnancy rates assessed in all cows by palpation on d 40 to 46 after AI were greater ($P < 0.01$) after Ovsynch + CIDR than Ovsynch, whereas no difference was detected between Ovsynch + CIDR and controls (Table 1). Control cows received their first services between 54 and 113 DIM compared with between 57 and 77 DIM for cows in both treated groups. Pregnancy rates on d 40 to 46 were greater ($P < 0.05$) in cyclic cows (44%;

Figure 3. Incidence of ovulation (48 h after the second injection of GnRH; d +1 in Figure 1) for follicles identified before the second GnRH injection (d -1 in Figure 1) in 91 cows treated with the Ovsynch protocol (open bars) and in 91 cows treated with Ovsynch + CIDR (shaded bars) in experiment 1. Incidence includes that of either a single or double ovulation (combined), ovulation of a single follicle (single), ovulation of 2 follicles (double), ovulation of the single largest of 2 follicles (largest), ovulation of the second largest follicle (second), and no ovulation (none).

n = 116) than in anestrus cows (27%; n = 146). In addition, older cows had greater ($P < 0.05$) pregnancy rates (42%; n = 78) than first-lactation cows (32%; n = 184). More ($P < 0.01$) cows treated with Ovsynch + CIDR were still pregnant on d 57 after TAI than after Ovsynch (Table 1).

Relationships of serum P4 patterns and pregnancy rates were investigated. Based on pretreatment serum P4 concentrations as either "High" or "Low" on d -20 and -10, cows in the 2 treatments were equally distributed among P4 categories, and most were classified as Low-Low (anestrus; 59%), with few cows having Low-High (early diestrus; 11%) or High-Low (proestrus, estrus, or metestrus; 8.8%) patterns of serum P4, while those having High-High patterns (late diestrus) were intermediate (21.4%; Table 2).

Cows were then reclassified based on serum P4 patterns on d -10 and -3 (Table 2). Seven days after onset of treatment, cows were redistributed to different stages of the estrous cycle based on the serum P4 patterns observed before treatment. Although proportions of cows changed very little in the High-High and High-Low patterns, most of the cows in the Low-Low pattern before treatment were now redistributed into either the Low-High and Low-Low patterns (Table 2). Movement of anestrus cows from the Low-Low pattern into the Low-High pattern was consistent, with more than a 50% incidence of ovulation in the anestrus cows (Table 1).

Averaged across treatments, overall pregnancy rates on d 29 were less ($P < 0.05$) in cows with the High-Low serum P4 pattern (those initiating treatments in late diestrus; Table 2) than those in the Low-High pattern

(initiating treatment in either proestrus, estrus, or metestrus). Treatment with supplemental P4 improved pregnancy rates compared with Ovsynch alone by 9.1 to 35.6 percentage points in all but the High-Low pattern (Table 2). The most improvement was observed for cows initiating the CIDR treatment with low concentrations of P4 on d 10 (Low-High pattern), which likely included anestrus cows and those in proestrus, estrus, or metestrus.

No relationship was detected for the diameter of the ovulatory follicle on d -1 and pregnancy rates measured on d 29 after TAI when the diameter was included as a regression variable. Pregnancy rates were examined in cows in which the diameter of their ovulatory follicle was smaller or larger than the mean diameter (14.8 ± 0.2 mm) of 161 treated cows that ovulated. Cows treated with Ovsynch alone had lower ($P < 0.01$) pregnancy rates than cows receiving Ovsynch + CIDR cows when their ovulatory follicles were smaller (<14.8 mm; 29% [n = 34] vs. 62% [n = 37], respectively), or larger (≥ 14.8 mm) than the mean diameter (49% [n = 43] vs. 64% [n = 47], respectively).

Pregnancy rates tended ($P = 0.16$) to be greater in cows with double ovulation (Ovsynch = 9/16 [56%] vs. Ovsynch + CIDR = 8/12 [67%] or a total of 17/28 [60.7%]) than in cows with single ovulation (Ovsynch = 22/61 [36%] vs. Ovsynch + CIDR = 45/72 [63%] or a total of 67/133 [50.4%]).

Embryo survival. Embryo survival between 29 d and 40 to 46 d and between 29 and 57 d was greater ($P < 0.05$) in cows receiving prebreeding supplemental P4 compared with the Ovsynch protocol (Table 1). No difference in embryo survival between 40 to 46 d and 57 d was detected between treatments, indicating that most embryo loss occurred before d 40 to 46. A tendency ($P = 0.08$) for improved embryo survival occurred between d 29 and 40 to 46 for cows classified as cyclic (80%; n = 40) before treatments compared with those that were anestrus (60%; n = 47). Further, a tendency ($P = 0.05$) for improved embryo survival occurred in cows that had the High-High serum P4 pattern compared with other patterns (Table 2).

Days open. Average intervals between calving and conception were not affected by treatment, but were less ($P < 0.01$) in cyclic (129 ± 9 d; n = 100) than in anestrus (160 ± 9 d; n = 128) cows. First-lactation cows tended ($P = 0.09$) to have greater days open than multiple-lactation cows (156 ± 7 d, n = 164; vs. 133 ± 11 d, n = 64). For each unit increase in BCS (range from 1.25 to 3.25), the number of days open decreased ($P < 0.05$) by 35 ± 20 d.

Experiment 2

Cyclic status. Of 630 cows, 624 were classified as cyclic or anestrus according to their blood serum concen-

Table 2. Proportions of lactating dairy cows, stage of the estrous cycle, serum concentrations of progesterone (P4), and pregnancy rates on d 29 after timed AI relative to serum progesterone patterns before and during treatments (experiment 1).

Item	Serum P4 pattern ¹			
	High-High	High-Low	Low-High	Low-Low
	d -10 (before first GnRH injection) ^{1,3}			
Serum P4	4.2 ± 0.2 ^x	0.5 ± 0.3 ^z	3.3 ± 0.2 ^y	0.2 ± 0.1 ^z
Proportions in each P4 pattern, (no.)	(39)	(16)	(20)	(107)
Ovsynch ²	19.8	7.7	11.0	61.5
Ovsynch+CIDR ²	23.1	9.9	11.0	56.0
	d -3 (before PGF _{2α} injection) ^{1,4}			
Serum P4	3.9 ± 0.2 ^x	0.4 ± 0.3 ^z	2.2 ± 0.2 ^y	0.5 ± 0.2 ^z
Proportions in each P4 pattern, (no.)	(44)	(15)	(71)	(52)
Ovsynch	24.2	6.6	41.8	27.5
Ovsynch+CIDR	24.2	9.9	36.3	29.7
	% (no.)			
Overall pregnancy rates	45.5 ^{x,y} (43)	33.3 ^y (15)	57.7 ^x (71)	38.5 ^{x,y} (52)
Pregnancy rates				
Ovsynch	40.9 (22)	33.3 (6)	42.1 (38)	20.0 (25)
Ovsynch+CIDR	50.0 (21)	33.3 (9)	75.8 (33)	55.6 (27)
Embryo survival, d 29 to 40–46	85.7 (21)	60 (5)	65.9 (41)	60 (20)

^{x,y,z}Mean percentages within item with dissimilar superscript letters differ ($P < 0.05$).

¹Concentrations of P4 were low (< 1 ng/mL) or high (≥ 1 ng/mL) in each of the two paired blood sera samples before treatment (d -20 and -10) and during treatment (d -10 and -3).

²Ovsynch = injection of GnRH (d -10) 7 d before and 48 h (d -1) after an injection of PGF_{2α} (d -3), with one timed AI (d 0) 12 to 20 h after GnRH injection on d -1. Ovsynch + CIDR = as Ovsynch plus insertion of a P4-releasing intravaginal insert (CIDR) for 7 d beginning on d -10 and removed on d -3. See Figure 1 for further details.

³Paired samples collected on d -20 (10 d before onset of treatment) and d -10 (before first GnRH injection).

⁴Paired samples collected on d -10 (before first GnRH injection) and d -3 (1 to 2 h after CIDR removal and just before PGF_{2α} injection).

trations of P4 collected on d -36, -22, and -10 before TAI: 80% (501/624) were cyclic and 20% (123/624) were anestrus. Incidence of anestrus tended ($P = 0.12$) to be greater in first-lactation (22%; $n = 306$) than multiple-lactation cows (18%; $n = 318$). After calving, BCS varied among cows and affected the percentage of cows that resumed estrous cycles. For each unit increase in BCS (ranging from 1.25 to 3.5), cyclic status increased ($P < 0.01$) by $13 \pm 4\%$. In addition, for every 10-d increase in DIM (ranging from 59 to 79 d at TAI), cyclic status increased ($P < 0.05$) by $9 \pm 4\%$.

Redistribution of estrous cycles. Compared with no presynchronization, the two Presynch PGF_{2α} injections (d -36 and -22) increased the proportions of cows with high P4 on d -22 ($P < 0.05$) and -10 ($P < 0.01$) by 8 to 13 percentage points, respectively (Table 3). By d 3, the proportion of cows with elevated P4 did not differ among treatments, but the presence of the CIDR masked the true effects of Presynch vs. no presynchronization. Use of Presynch without subsequent use of a CIDR produced more ($P = 0.07$) cows (90% of 160) with high P4 on d -3 compared with cows receiving no presynchronization or CIDR (82% of 156). At all 3 sampling times (d -22, -10, and -3), for each unit increase in BCS (ranging from 1.25 to 3.5), the proportions of cows

with high P4 increased ($P < 0.01$) by 21 ± 5 , 12 ± 5 , and $8 \pm 3\%$, respectively.

Induced ovulation. Ovulation in anestrous cows in response to the first GnRH injection (d -10) varied from 68 to 73% but was not different among treatments (no interactions of main effects; Table 4). For each unit increase in BCS (ranging from 1.25 to 3.5), induced ovulation increased ($P < 0.01$) by $51 \pm 18\%$.

Concentrations of progesterone. Average P4 concentrations in serum on d -3 were greater ($P < 0.01$) in Presynch cows (4.2 ± 0.1 ng/mL; $n = 316$) than in those not presynchronized (3.4 ± 0.1 ng/mL; $n = 306$). In addition, the CIDR insert used with Ovsynch increased ($P < 0.01$) concentrations of P4 in those cows treated with CIDR-1.38 (3.8 ± 0.2 ng/mL; $n = 122$) and CIDR-1.9 (4.2 ± 0.2 ng/mL; $n = 184$) compared with cows receiving Ovsynch without a CIDR (3.4 ± 0.1 ng/mL; $n = 316$). Concentrations of serum P4 tended ($P = 0.10$) to be higher for cows receiving a CIDR insert containing 1.9 g P4 than for those receiving a CIDR insert with 1.38 g of P4. For each unit increase in BCS (ranging from 1.25 to 3.5), serum concentrations of P4 increased ($P < 0.001$) by 0.8 ± 0.2 ng/mL.

Incidence of luteolysis. Because blood concentrations of P4 on d -3 tended to differ between the two

Table 3. Proportions of cows with high serum concentrations of progesterone (P4) on d -22 and -10 in response to Presynch PGF_{2α} injections (d -36 and -22) and on d -3 in response to GnRH (d -10) and/or CIDR insertion (d -10 to -3), respectively (experiment 2).

Day	Treatment				
	No Presynch	Presynch	No CIDR	CIDR - 1.38	CIDR - 1.9
	% (no.)				
Before second Presynch (d -22) ²	52 (308)	60* (286)	...	...	...
Before first GnRH (d -10) ³	59 (310)	72** (312)	...	...	...
Before CIDR insert removal (d -3) ⁴	87 (306)	91 (316)	87 (316)	90 (122)	92 (184)

*Different ($P < 0.05$) from No Presynch.

**Different ($P < 0.01$) from No Presynch.

¹Main effects in the experiment: Presynch = injections of PGF_{2α} on d -36, -22, and -3; injections of GnRH on d -10 and -1; and timed AI on d 0. No Presynch = same as Presynch without injections of PGF_{2α} on d -36 and -22. CIDR-1.38 = insertion of a P4-releasing intravaginal insert (CIDR) containing 1.38 g of P4 for 7 d beginning on d -10 and removed on d -3. CIDR-1.9 = insert contained 1.9 g of P4. See Figure 2 for further details. Numbers of observations differ because of missing blood samples.

²Effect of first Presynch injection of PGF_{2α} on d -36.

³Effect of second Presynch injections of PGF_{2α} on d -22.

⁴Interaction ($P = 0.07$) of Presynch and CIDR. See text.

⁵Before insertion of CIDR treatment on d -10.

CIDR treatments, occurrence of luteolysis was greater ($P < 0.01$) in the CIDR-1.9 than for the CIDR-1.38 (Table 4). Further, an interaction ($P < 0.05$) between main effects was detected due to a higher incidence of luteolysis for cows receiving Presynch but no CIDR than for cows receiving neither Presynch nor CIDR (75%; $n = 159$ vs. 64%; $n = 150$).

Synchronization rate. Overall, synchronization rate was 84% and was not affected by Presynch (Table 4). In contrast, cows that received the CIDR-1.9 had greater ($P < 0.01$) synchronization rates than those that received the CIDR-1.38. Synchronization rate in the

second herd tended ($P = 0.07$) to be greater than that of the first herd (87%; $n = 307$ vs. 81%; $n = 304$).

Pregnancy rates. Based on serum P4 patterns for cows on d -10 and -3, most (61.5%) of the cows had a High-High pattern compared with 27.5% with a Low-High pattern (Table 5). Less than 11% of the cows had either High-Low or Low-Low patterns of P4. The proportions of cows in early diestrus (High-High) at the onset of the Ovsynch protocol was increased ($P < 0.01$) for those receiving Presynch. Differences in proportions were due to more cows being redistributed from the Low-High (proestrus, estrus, and metestrus) and the

Table 4. Percentages of cows in which ovulation was induced in response to GnRH on d -10, luteolysis after PGF_{2α} on d -1, and synchronization rates (experiment 2).

Item	Treatment ¹				
	No Presynch	Presynch	No CIDR	CIDR-1.38	CIDR-1.9
	% (no.)				
Induced ovulation in anestrus cows ²	73 (64)	68 (56)	70 (66)	74 (27)	70 (27)
Luteolysis ³	72 (299)	78 (309)	70 (309)	67 (121)	91* (178)
Synchronization rate ⁴	85 (299)	83 (312)	83 (309)	75 (121)	92* (181)

*Different ($P < 0.01$) from CIDR-1.38.

¹Main effects in the experiment: Presynch = injections of PGF_{2α} on d -36, -22, and -3; injections of GnRH on d -10 and -1; and timed AI on d 0. No Presynch = same as Presynch without injections of PGF_{2α} on d -36 and -22. CIDR-1.38 = insertion of a P4-releasing intravaginal insert (CIDR) containing 1.38 g of P4 for 7 d beginning on d -10 and removed on d -3. CIDR-1.9 = insert contained 1.9 g of P4. See Figure 2 for further details. Numbers of observations differ because of missing blood samples.

²Cows with low (< 1 ng/mL) progesterone (P4) on d -20 and -10, but had high (≥ 1 ng/mL) P4 on d -3, 7 d after GnRH injection on d -10.

³Percentage of cows with high P4 on d -3 just before the injection of PGF_{2α} and subsequent low serum P4 48 h later. Interaction ($P < 0.05$) of Presynch and CIDR. See text.

⁴Percentage of cows with either low or high P4 on d -3, then low P4 48 h later.

Table 5. Pregnancy rates on d 29 and 57 based on proportions of lactating dairy cows with various patterns and concentrations of serum progesterone (P4) on d -10 and -3 (experiment 2).

Serum P4 pattern ¹	No Presynch, % of 304 cows	Presynch**, % of 310 cows	Serum P4 on d -3, ng/mL	Pregnancy rates	
				d 29	d 57
High-High	52.6	70.3	4.7 ± 0.1 ^x	47.3 ^x	28
High-Low	6.9	1.9	0.6 ± 0.5 ^y	37.0 ^y	14.8
Low-High	34.2	21	2.8 ± 0.2 ^z	33.7 ^y	20.7
Low-Low	6.3	6.7	0.7 ± 0.4 ^y	32.5 ^y	12.5

^{x,y,z}Means within columns with dissimilar superscript letters differ ($P < 0.01$).

**Presynchronized (Presynch) cows differed ($P < 0.01$) from expected proportions of cows not presynchronized (No Presynch).

¹Concentrations of P4 were low (< 1 ng/mL) or high (≥ 1 ng/mL) in each of the 2 paired sera samples on d -10 and -3, making up four permutations (High-High, High-Low, Low-High, and Low-Low; respectively).

High-Low patterns (late diestrus) to the High-High pattern (early diestrus) by the Presynch treatment. Average concentrations of P4 on d -3 were consistent with the change in serum P4 pattern during the 7-d period after the first GnRH injection. Pregnancy rates at d 29, but not at d 57, were greater ($P < 0.05$) for all cows with the High-High serum P4 pattern, compared with other permutations. First-lactation cows had greater ($P < 0.05$) pregnancy rates (47%; $n = 306$) on d 29 than older lactating cows (37%; $n = 318$).

Pregnancy rates on d 29 were greater ($P < 0.01$) for all Presynch cows, compared with those not presynchronized (Table 6). This was true for all but 3 of the 12 permutations of serum P4 patterns and treatment. No interaction of Presynch and use of CIDR inserts was detected. At the lower dose of 1.38 g of P4, use of a CIDR insert with Ovsynch resulted in numerically, but not significantly, lower pregnancy rates, compared with

groups receiving Ovsynch without a CIDR insert whether presynchronized or not.

Cyclic status before the onset of treatment was coupled with whether serum P4 was high or low on d -3 to determine their effects on pregnancy rates on d 29 (Table 7). The main effect of Presynch was significant ($P < 0.05$), and no interaction of Presynch and CIDR was detected. Use of Presynch increased pregnancy rate by 3.7 to 33.9 percentage points, regardless of cyclic status before Ovsynch or luteal status upon CIDR removal and PGF_{2α} injection.

Embryo survival. Neither Presynch nor use of CIDR inserts influenced embryo survival between d 29 and 57 after TAI; overall embryo survival was 150/260 or 57.7%. Embryo survival tended ($P = 0.09$) to increase by 10.6% for every unit increase in BCS (range of 1.25 to 3.5).

Table 6. Pregnancy rates on d 29 in lactating dairy cows according to serum patterns of progesterone (P4) on d -10 and -3 (experiment 2).

Treatment ²	Serum P4 pattern ¹				
	High-High	High-Low	Low-High	Low-Low	Total
No Presynch/No CIDR	48.6 (74)	26.7 (15)	40.4 (52)	38.5 (13)	42.9 (154)
No Presynch/CIDR-1.38	35.5 (31)	0.0 (3)	13.0 (23)	25.0 (4)	24.6 (61)
No Presynch/CIDR-1.9	41.8 (55)	33.3 (3)	31.0 (29)	0.0 (2)	37.1 (89)
No Presynch	43.8 (160)	23.8 (21)	31.7 (104)	31.6 (19)	37.5 (304)
Presynch/No CIDR	51.9 (108)	66.7 (3)	42.9 (35)	27.3 (11)	48.4 (157)
Presynch/CIDR-1.38	38.1 (42)	100 (1)	38.5 (13)	25.0 (4)	38.3 (60)
Presynch/CIDR-1.9	54.4 (68)	100 (2)	23.5 (17)	50.0 (6)	49.5 (93)
Presynch	50.0 (218)	83.3 (6)	36.9 (65)	33.3 (21)	46.8*** (310)
Total	47.4 ^x (378)	37.0 ^{x,y} (27)	33.7 ^y (169)	32.5 ^{x,y} (40)	

**Overall Presynch effect ($P < 0.01$).

^{x,y}Means within row with dissimilar superscript letters differ ($P < 0.01$).

¹Concentrations of P4 were low (< 1 ng/mL) or high (≥ 1 ng/mL) in each of the 2 paired sera samples and d -10 and -3, making up four permutations (High-High, High-Low, Low-High, and Low-Low; respectively).

²Main effects in the experiment (no interactions): Presynch = injections of PGF_{2α} on d -36, -22, and -3; injections of GnRH on d -10 and -1; and timed AI on d 0. No Presynch = same as Presynch without injections of PGF_{2α} on d -36 and -22. CIDR-1.38 = insertion of a P4-releasing intravaginal insert (CIDR) containing 1.38 g of P4 for 7 d beginning and d -10 and removed on d -3. CIDR-1.9 = insert contained 1.9 g of P4. See Figure 2 for further details. Numbers of observations differ because of missing blood samples.

Table 7. Pregnancy percentages (n) on d 29 in lactating dairy cows based on cyclic status before treatment with Ovsynch and CIDR inserts (d -10) and concentrations of serum progesterone (P4) on d -3 (experiment 2).

Cyclic status ²	Serum P4 on d -3 ³	Treatment main effects ¹				
		No Presynch	Presynch*	No CIDR	CIDR - 1.38	CIDR - 1.9
Cyclic	High	40.8 (218)	48.4 (246)	50.0 (224)	32.2 (90)	44.7 (150)
Cyclic	Low	21.7 (23)	55.6 (9)	27.3 (22)	20.0 (5)	60.0 (5)
Anestrus	High	29.8 (47)	42.1 (38)	37.0 (46)	30.0 (20)	36.8 (19)
Anestrus	Low	35.2 (17)	38.9 (18)	40.0 (20)	28.6 (7)	37.5 (8)
Cyclic total		39.0 (241)	48.7 (255)	48.0 (246)	31.6 (95)	45.2 (155)
Anestrus total		31.2 (64)	41.1 (56)	37.9 (66)	29.6 (27)	37.0 (27)

*Presynch effect ($P < 0.05$).

¹Main effects in the experiment: Presynch = injections of PGF_{2α} on d -36, -22, and -3; injections of GnRH on d -10 and -1; and timed AI on d 0. No Presynch = same as Presynch without injections of PGF_{2α} on d -36 and -22. CIDR-1.38 = insertion of a P4-releasing intravaginal insert (CIDR) containing 1.38 g of P4 for 7 d beginning on d -10 and removed on d -3. CIDR-1.9 = insert contained 1.9 g of P4. See Figure 2 for further details. Numbers of observations differ because of missing blood samples.

²If any concentration of P4 on d -36, -22, or -10 was ≥ 1 ng/mL then cows were assumed to be cyclic. Otherwise, when concentrations were < 1 ng/mL on all 3 d, cows were considered to be anestrus.

³Concentrations of P4 in serum were low (< 1 ng/mL) or high (≥ 1 ng/mL) on d -3.

DISCUSSION

Supplementation of P4 for 7 d concurrent with the Ovsynch protocol improved pregnancy rates on d 29 after TAI in experiment 1 (Table 1). This finding is consistent with lower pregnancy rates (43%) in dairy cows having low concentrations of P4 before the injection of PGF_{2α} compared with those receiving supplemental P4 via the P4-releasing intravaginal device ([PRID]; 63%; Folman et al., 1990). Further, beef cows that received a single PRID had lower P4 concentrations before insert removal and consequently lower pregnancy rates (53%) than those cows that received two PRID inserts with higher P4 concentration and greater pregnancy rates (77%; Wehrman et al., 1993). Cows from that study with low P4 concentrations were found to have higher circulating estrogen concentrations associated with reduced conception rates.

Improved fertility in the Ovsynch + CIDR treatment of experiment 1 might be attributed to the fact that, under P4 dominance, the dominant follicle either grows, turns over, or ovulates and a new follicular wave emerges (Adams et al., 1992; Smith and Stevenson, 1995). A further advantage for applying P4 concurrently with the first GnRH injection of the Ovsynch protocol is that 87% of cows had a normal luteal phase after insemination, compared with 71% of cows receiving only GnRH or 43% of cows receiving only a norgestomet implant before PGF_{2α} (Thompson et al., 1999). Further, in experiment 1, and in another larger multilocation study of lactating dairy cows (Pursley et al., 2001), use of the CIDR insert concurrent with the Ovsynch protocol increased pregnancy rates, particularly in cows that were classified as anestrus before applying the CIDR insert concurrent with the Ovsynch protocol.

Supplementing the Cosynch protocol (same as Ovsynch except that TAI occurs at 48 h after PGF_{2α}, coincident with the second GnRH injection) with a CIDR insert increased pregnancy rates of suckled beef cows after TAI (Lamb et al., 2001; Stevenson et al., 2003). Progesterone improved pregnancy rates in suckled beef cows that were cyclic but in the later stages of the estrous cycle at first injection of GnRH and consequently had no luteal structure at the PGF_{2α} injection (Cosynch + CIDR = 79% vs. Cosynch = 43%). Among anestrus cows, pregnancy rates were also greater in the Cosynch + CIDR (59%) than for Cosynch (39%). Addition of P4 improved pregnancy rates in anestrus cows, regardless of whether they were induced to ovulate after the first GnRH injection (Lamb et al., 2001; Stevenson et al., 2003). What is unique about experiment 1 is that P4 supplementation increased pregnancy rates on d 29 after TAI among anestrus cows (Low-Low) as well as those anestrus cows that were induced to ovulate (Low-High) in response to the first GnRH injection (Table 2).

Average incidence of ovulation after TAI was improved in CIDR-treated cows (Figure 3). The incidence observed in experiment 1 (88.5%) was similar to that reported earlier (Vasconcelos et al., 1999) when the Ovsynch protocol was initiated in the latter half (80%; d 12 to 22) or earlier half (91%; d 1 to 12) of the estrous cycle. Further, for cows with low concentrations of P4 on d -3 when PGF_{2α} was injected to initiate luteolysis before TAI, incidence of ovulation (one or two follicles) was improved by the addition of the CIDR in experiment 1.

A tendency for greater pregnancy rates occurred in cows that had double vs. single ovulations, as reported earlier (Fricke and Wiltbank, 1999). Incidence of double

ovulation in experiment 1 (15.4%) was not influenced by P4 treatment and was consistent in magnitude with an earlier report (14.1%; Fricke and Wiltbank, 1999) for cows treated with the Ovsynch protocol.

Stage of the estrous cycle or continued anestrus was assessed by concentrations of P4 on d -10 and -3 in both experiments. Relative proportions of cows in those various categories were quite different for cows in experiment 1 when treatments were initiated at random stages of the cycle (Table 2) and for No Presynch cows in experiment 2 (Table 5). The major differences between experiments were the proportions of Ovsynch cows in early diestrus, late diestrus, and anestrus, because of a greater incidence of anestrus and higher percentage (70%) of first-lactation cows studied in experiment 1 compared with experiment 2.

Greater pregnancy rates on d 29 after TAI generally occurred in cows initiating treatments with the CIDR in experiment 1 during early diestrus (High-High), proestrus, estrus, and metestrus (Low-High), and anestrus (Low-Low). In all categories, except late diestrus (High-Low), the addition of the CIDR insert improved pregnancy rates (Table 2). In experiment 2, greater pregnancy rates on d 29 after TAI occurred because a greater percentage of cows were early diestrus at the onset of the Ovsynch protocol as a result of the two presynchronizing injections of PGF_{2α} (Table 5). As a consequence, the Presynch treatment proportionally reduced the numbers of cows in late diestrus (High-Low), and in proestrus, estrus, and metestrus (Low-High) before the onset of treatments on d -10. Cows in proestrus, estrus, or metestrus (Low-High; but ovulated in response to the first GnRH injection on d -10) at the onset of the Ovsynch protocol may or may not respond to PGF_{2α} injection 7 d later (Vasconcelos et al., 1999; Moreira et al., 2000). Our results are also consistent with other studies (Vasconcelos et al., 1999; Moreira et al., 2001) that demonstrated the greatest pregnancy rates when the Ovsynch protocol was initiated in cows during early diestrus (d 5 to 12).

Improved pregnancy rates observed after presynchronizing the estrous cycles of cows (Presynch treatment) with the first 2 injections of PGF_{2α} in experiment 2 was not only due to its ability to increase the proportion of cows in early diestrus at the onset of the Ovsynch protocol but also because of greater concentrations of P4 on d -3 (Table 5). Increased P4 concentrations at the time of PGF_{2α} injection or before insemination were associated positively with pregnancy rates (Folman et al., 1973; Erb et al., 1976; Holness et al., 1981; Fonseca et al., 1983; Rosenberg et al., 1990a, 1990b). These findings are in agreement with those reported by Moreira et al. (2001) and with the hypothesis that fertility is improved when cows are exposed to higher concentra-

tions of P4 during the luteal phase before AI (Fonseca et al., 1983) or before PGF_{2α} injection (Folman et al., 1984). Increased P4 concentrations in cows whose estrous cycles were presynchronized (experiment 2) also may be the consequence of reduced variation in the stage of the estrous cycle at which the Ovsynch protocol was initiated (Table 5). High incidences of ovulation (96%) occurred in response to first GnRH injection when the Ovsynch protocol was initiated early diestrus (d 5 to 9) compared with 23% when initiated at d 1 to 4 of the estrous cycle (Vasconcelos et al., 1999). Based on those results, it is likely that more presynchronized cows with the High-High P4 pattern on d -10 and -3 would have a higher incidence of ovulation in response to the first GnRH injection, compared with cows whose estrous cycles were not presynchronized (Vasconcelos et al., 1999; Moreira et al., 2001).

Treatment of dairy cows with 2 injections of PGF_{2α} given 14 d apart followed by TAI and GnRH injection 48 h after the second PGF_{2α} injection produced pregnancy rates equal to that produced by the Ovsynch protocol (Cartmill et al., 2001). Further, we (Cartmill et al., 2001) reported that a single injection of PGF_{2α} 12 d preceding the onset of the Ovsynch protocol produced greater pregnancy rates than the Ovsynch protocol in multiple-lactation cows because this treatment grouped a greater proportion of cows into early diestrus before the initiation of the Ovsynch protocol. Therefore, 2 presynchronizing injections of PGF_{2α} given 14 d apart with the second injection given 12 d before the Ovsynch protocol in the present study and elsewhere (Moreira et al., 2001) should be more effective than one presynchronizing PGF_{2α} injection, thus resulting in greater pregnancy rates than what was produced by Ovsynch alone.

Why the CIDR insert did not improve pregnancy rates in experiment 2 as in experiment 1 is bothersome. One of the herds used in experiment 1 was one of the 2 herds studied in experiment 2, and seasons of insemination excluded summer in both experiments. Differences in experiments included proportionally more first-lactation cows and a greater rate of pretreatment anestrus in experiment 1 compared with experiment 2. Synchronization rates were lower in experiment 2, but incidences of luteolysis and percentage of cows with high P4 on d -3 were greater in experiment 2. If the CIDR is more effective in anestrus cows, as observed in experiment 1, it was not consistent in experiment 2 among anestrus cows, although fewer anestrus cows were treated in experiment 2. These same inconsistencies were observed in a multiple-location experiment conducted in 6 Midwestern states (Pursley et al., 2001). In that experiment, numerically greater pregnancy rates after the Ovsynch + CIDR than after Ovsynch protocol were detected in 3 herds (cyclic status before

treatment = 41, 63, and 94%), no effects in one herd (cyclic status = 91%), and slightly negative effects in 2 herds (cyclic status = 77 and 92%). No obvious correlation between herd cyclicity and pregnancy rate was evident, but pregnancy rates were improved in all anestrus cows despite herd location. That observation is consistent with the results of experiment 1 but not with experiment 2.

Endogenous P4 production was decreased by insertion of a PRID 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). Further, initiating P4 treatment (PRID) during the luteal phase increased conception rates in those cows with lower P4 concentrations, but decreased conception rates on cows with higher P4 concentrations (Folman et al., 1990). These results may partly explain why using the CIDR insert in Presynch cows seemed to reduce pregnancy rates because of their higher concentrations of P4 during the luteal phase as a result of the CIDR insert and less endogenous P4 production by the CL. Therefore, P4 treatment best improved fertility in those cows with low peripheral P4 concentrations during their luteal phase or when treatment started earlier during the luteal phase of the estrous cycle. Factors influencing why the CIDR insert was beneficial in some, but not all, anestrus cows need to be identified.

Losses of embryos between 29 and 57 d after TAI varied from about 14 to 45% for various subgroups in the 2 experiments. Such losses are costly for producers. Improved embryo survival was observed for cows in experiment 1 that were supplemented with P4 via the CIDR insert before TAI but was not confirmed by results of experiment 2. Nearly all of the losses in experiment 1 occurred after d 29 but before d 40 to 46 (Table 1). Progesterone synthesis and secretion by the CL plays a major role in regulating the secretory pattern of maternal endometrium. Bovine embryos recovered from P4-treated cows on d 14 after inseminations were advanced in development compared with those from controls (Garrett et al., 1988). Progesterone was found to increase the synthesis and release of polypeptides from endometrial explant cultures assessed on d 5 after insemination. Therefore, it was concluded that bovine conceptus development is regulated, at least in part, by endometrial secretions, which are influenced by the time and quantity of P4 secreted or administered (Garrett et al., 1988). When P4 was administered early in the estrous cycle, it effectively advanced the uterine receptivity for the transfer of older bovine embryos (Geisert et al., 1991). Treatment with P4 during the first 4 d of the estrous cycle of the ewe hastened the development of the diestrus uterus so that on d 6 it was able to provide an acceptable environment for 10-

d old embryos (Lawson and Cahill, 1983). Therefore, improved rates of embryo survival in experiment 1 may be due, in part, to the carryover effect of P4 treatment on the bovine uterus during the 7 d of P4 exposure before insemination so the uterus was able to provide a more acceptable environment for establishing pregnancy. Further, these results also are consistent with higher posttreatment incidences of ovulation (perhaps more synchronized) in cows treated with the CIDR insert before insemination in experiment 1.

Body condition influenced the outcomes of many important reproductive traits assessed in both experiments. Cyclic status and concentrations of P4 or percentage of cows with high P4 at the onset of the Ovsynch protocol and/or CIDR treatment, and embryo survival were consistently improved in cows with greater BCS in both experiments. These effects indicate that more optimal body condition is highly related to reproductive outcomes. Further, they confirm the importance of adequate DMI to promote early ovulation that was associated with reduced BW loss (higher BCS) and higher milk production of lactating dairy cows (Staples et al., 1990), as well as subsequently better fertility as demonstrated in our studies.

In summary, supplementation of P4 during the Ovsynch protocol improved pregnancy rates and embryo survival after TAI in experiment 1. Combining the Presynch treatment with the Ovsynch protocol increased the percentage of cows with elevated P4 concentrations at the time of PGF_{2α} just before TAI, increased the proportion of cows in early diestrus at the start of the Ovsynch protocol, and consequently increased pregnancy rates on d 29 after TAI. The CIDR treatment for 7 d concurrent with Ovsynch protocol did not improve pregnancy rates on d 29 after TAI in experiment 2 as in experiment 1 where more cows were in their first lactation and more pretreatment anestrus was detected.

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