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RESYNCHRONIZATION OF ESTRUS IN CATTLE OF UNKNOWN PREGNANCY STATUS USING ESTROGEN, PROGESTERONE, OR BOTH

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Abstract

Our objectives were to develop a treatment applied to cattle of unknown pregnancy status that would synchronize the repeat estrus of nonpregnant females. In Exp. 1, previously inseminated dairy and beef heifers were assigned randomly to three treatments at 13 d after AI: 1) no further treatment (controls; n = 44); 2) 0.5 mg of estradiol cypionate (ECP) was given i.m. on d 13 and 20 at the time of insertion and removal of a used intravaginal P4-releasing insert (CIDR-B; P4+ECP; n=44); and 3) same as P4+ECP without the injections of ECP (P4; n = 42). The P4+ECP (>90%) and P4 (>75%) protocols effectively synchronized repeat periods of estrus to 2 d and did not harm established pregnancies, but tended to reduce conception rates of heifers inseminated after CIDR removal. In Exp. 2, treatments were similar to those in Exp. 1 were applied to previously inseminated beef heifers in which feeding 0.5 mg of melengestrol acetate (MGA) replaced the CIDR as a source of progestin and it was fed on d 13 through 19 after AI. Reinsemination rates exceeded 86% in all treatments but conception at the resynchronized estrus was reduced ($P<0.05$) to 61 to 71% of controls. In Exp. 3, previously inseminated lactating beef cows at four locations were assigned within herd to three threatments according to days postpartum, parity, breed, (primiparous vs. multiparous), and previous synchronization treatment used before their initial AI: 1) no further treatment (control; n = 307); 2) as in Exp. 1, but P4 + 1 mg of estradiol benzoate on d 13 and 20 (P4+EB; n = 54); and 3) as in Exp. 1, P4+ECP (n=148). At two locations, insertion of the used CIDRs and first estradiol (EB or ECP) injections occurred on d 11 after timed AI (TAI) and CIDR removal and second estradiol injections on d 18.
Treatments with P4 +ECP did not reduce pregnancy rates in pregnant cows at any location, but increased ($P<0.05$) the 20- to 23-d return rate from 29% in controls, to 86% in P4+EB, and 65% in P4+ECP cows. Conception rates were not reduced when treatments occurred between d 13 and 20. Overall 23-d pregnancy rates after two inseminations and embryo survival between d 29-33 and 54-61 of pregnancy did not differ among treatments. In Exp. 4, lactating beef cows were assigned as in Exp. 3 to three treatments: 1) no further treatment(controls; n = 51); 2) P4 +ECP (n = 47); and 3) a single injection of ECP on d 13 (n = 48). Although established pregnancies were not harmed, return rates of nonpregnant cows were not different among treatments and the single injection of ECP caused more than 30% of the returns to occur after 8 d and more than 60% on 11 to 12 d after ECP injection. In both heifers and lactating beef cows, the resynchronization treatments increased synchronized return rates in cases where estrus-detection rates were poor, seemed to have no negative effects on established pregnancies, but seemed to reduce conception rates in heifers but not cows at the resynchronized estrus.

Introduction

Pregnancy outcome after synchronization of estrus and ovulation is unknown until cows return to estrus (19 to 24 d after AI) or after diagnosis of pregnancy. In such cases, the full extent of the advantages for synchronizing estrous cycles is not realized. Because the cycles of most nonpregnant cows are closely synchronized, resynchronization for a subsequent AI is possible. From an economic standpoint, if the second estrus can be resynchronized with little additional cost because of the existing synchrony from the first synchronization, costs associated with the first estrus can be spread out over a second breeding. New and previously used progestin-releasing inserts or implants have been used successfully to reduce the variation in returns to service after AI in previously synchronized cows and heifers (Stevenson and Mee, 1991; Favero et al., 1993, 1995; Van Cleeff et al., 1996; Purvis and Whittier, 1997). The variability in fertility results from these studies may imply that the synchrony of follicular growth could be improved. Reinsemination of nonpregnant females at the first eligible estrus can be facilitated by resynchronization of estrus (Van Cleeff et al., 1996) and may increase conception at the resynchronized estrus (Stevenson and Mee, 1991). Reinsertion of a progestin insert or implant between 13 and 20 d after AI in addition to an injection of estradiol benzoate (EB) given at insertion and removal of the progestin treatment increased the probability of identifying nonpregnant cows within 2 d after the second EB injection (Macmillan et al., 1997). Moreover, treatment of EB 13 d after the first insemination was effective in turning over the dominant follicle without affecting CL function or pregnancies established at a previous AI (Macmillan et al., 1997; Burke et al., 2000).

The only estrogen product available in the U.S. for use in the bovine is estradiol cypionate (ECP). It has multiple label indications including one "to correct anestrus [absence of heat period] in the absence of follicular cysts" at doses of 3 to 5 mg. Esterification of estradiol-17β to produce EB or ECP increases the half-life of the estrogen compared with metabolism of the parent estradiol product following hydrolysis (Vynckier et al., 1990). Studies using 10 mg of ECP produced peak concentrations of plasma estradiol at 20 h that remained elevated for up to 5 d (Vynckier et al., 1990). Administration of 2 mg of ECP was sufficient to cause ovariectomized heifers to display estrus (Lefebvre and Block, 1992). It has been demonstrated that 1 or 0.5 mg of ECP induces an LH surge in lactating dairy cows (Pancarci et al., 2002; Stevenson et al., 2002) and dairy heifers (Lopes et al., 2000) when given 24 h after a luteolytic dose of $PGF_{2\alpha}$.

The present study was comprised of four experiments to determine if resynchronization of estrus was feasible in beef cattle. The objectives were to determine whether treatments would: 1) reduce pregnancy rates in previously inseminated cattle whose pregnancy status was unknown at the onset of treatments; 2) increase AI resubmission rates with subsequent normal fertility; and 3) increase cumulative pregnancy rates after two inseminations. The first experiment utilized progesterone (P4; via a used CIDR-B insert) and P4 + ECP as tools to resynchronize estrus in dairy and beef heifers compared to controls. The second experiment tested MGA as the progestin plus or minus ECP with controls in beef heifers. The third experiment tested P4 + either EB or ECP with controls in suckled beef cattle. The fourth experiment tested P4 + ECP and ECP alone with controls in suckled beef cattle.

Materials and Methods

Experiment 1

Previously inseminated Holstein heifers (n = 68) were studied between September 2000 and August 2001 in six replications and purebred Angus, Simmental, and Hereford heifers (n = 62) during the spring 2001 breeding season. Heifers were assigned randomly within breed to three treatments on d 13 (range: 11 to 15 d) after first insemination (Figure 1): 1) no further treatment (controls; n = 44); 2) 0.5 mg of ECP (Pharmacia Animal Health, Kalamazoo, MI) was given i.m. on d 13 and 20 at the time of insertion and removal of a used intravaginal progesterone-releasing insert (CIDR-B; P4+ECP; n = 44); and 3) same as P4+ECP without the injections of ECP (P4; n = 42).

Beef heifers were observed visually for signs of estrus at multiple times in daylight hours during periods of expected returns to estrus (d 18 to 26). Dairy heifers were fitted with electronic estrus-detection devices (HeatWatch, DDx Inc., Denver, CO) for detection of standing estrus. Once estrus was detected, inseminations were performed 8 to 14 h later by one technician for dairy heifers and three technicians for beef heifers. Pregnancy was diagnosed once between 27 and 34 d after AI by transrectal ultrasonography to visualize fluid, embryo, or both.

Blood samples were collected from all heifers via puncture of a coccygeal vessel on d 13 and 20 after the initial AI corresponding to when the CIDR was inserted and removed. Progesterone was measured in blood sera using a specific validated radio-immunoassay (Skaggs et al., 1986). The inter- and intra-assay coefficients of variation were 11.3% and 8.6%, respectively, for two assays.

Characteristics of estrus for dairy heifers were calculated from HeatWatch data (duration of estrus, number of standing events, duration of all standing events, and duration of individual standing events) for the first post-insemination estrus (18 to 26 d). Conception rates at the initial insemination and those at the resynchronized estrus, concentrations of progesterone on d 20 when CIDR inserts were removed, percentages of heifers with low (<1 ng/mL) or high ($1 ng/mL) concentrations of progesterone on d 20, 26-d pregnancy rate (proportion pregnant after two inseminations), interval between inseminations, and percentage of nonpregnant heifers detected in estrus (AI resubmission rate for second service after resynchronization) were analyzed using procedure GLM (SAS, 2001). The model consisted of treatment, location (dairy vs. beef), and its interaction. Because AI technicians and sires were unique to each location, those effects were confounded with location. Means were separated by orthogonal contrasts (control vs. both P4 treatments and P4 vs. P4 + ECP) or by LSD tests when associated with a protected F-test (P #0.05) in the GLM procedure. Levene's test for heterogeneity of variance (Milliken and Johnson, 1984) was used to analyze the variability of return-to-estrus patterns after resynchronization treatments.

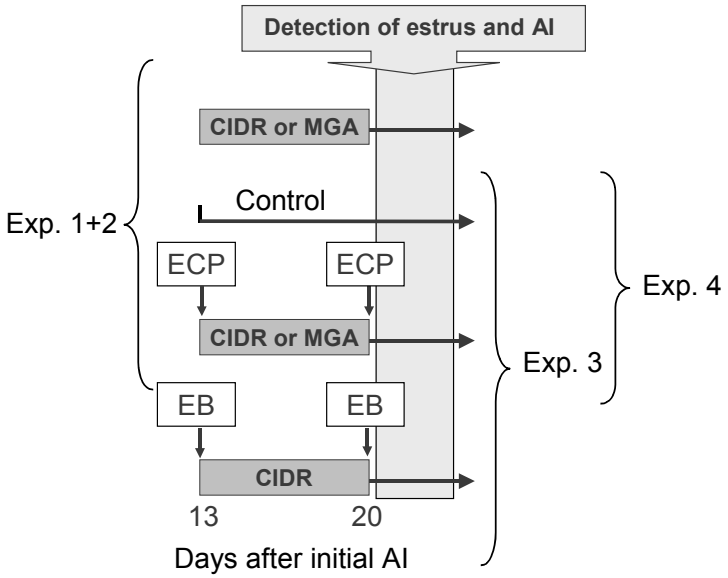

Figure 1. Experimental protocols employed in each of four experiments. Blood samples were collected on d 13 and 20 after first insemination when CIDR inserts were placed intravaginally and removed, respectively. Experiment 1 consisted of three treatments: CIDR (progesterone), progesterone + 0.5 mg of ECP (estradiol cypionate), and control. Experiment 2 consisted of three treatments: MGA (melengestrol acetate was fed on d 13 through 19), MGA + 0.5 mg of ECP (estradiol cypionate), and control. Experiment 3 consisted of three treatments: CIDR, CIDR + 1 mg of EB (estradiol benzoate), and CIDR + 0.5 mg of ECP. Experiment 4 consisted of three treatments: CIDR + 0.5 mg of ECP, single injection of 0.5 mg of ECP on d 13 (not shown), and control. Females were observed for estrus and inseminated during MGA feeding (Exp. 2) through d 33 or on or after CIDR inserts removal (d 20). CIDR = controlled internal drug release insert that is placed intravaginally through which progesterone is released.

Experiment 2

This experiment was conducted at Losey Land and Cattle, Agra, KS with yearling Angus and Hereford and Angus crossbred heifers. Heifers used in this study were previously synchronized with a standard MGA + $PGF_{2\alpha}$ protocol; 0.5 mg of MGA fed per head per day for 14 d and 25 mg of $PGF_{2\alpha}$ (Lutalyse, Pharmacia Animal Health, Kalamazoo, MI) 19 d after the last feeding of MGA. Heifers were inseminated based on the AM-PM rule until 72 h after $PGF_{2\alpha}$, at which time all heifers that had not shown estrus were inseminated. The average day of insemination was considered to be d 0 of the current study. A majority (431 of 439) were inseminated from 1.5 d before to 1.5 d after d 0, and the remaining heifers had been time inseminated but then were observed in estrus 2 to 2.5 d later and were reinseminated Immediately following the initial AI, heifers were returned to new pens based on the time they were inseminated. Five days before resynchronization treatments were begun, each pen of heifers was gate cut to divide the heifers into three treatments. Heifers that returned to estrus before sorting (d 3 to d 8) into resynchronization treatments were excluded from the experiment. Treatments for this experiment (Figure 1) were: 1) no further treatment (control; n = 87); 2) MGA (n = 176) fed at 0.5 mg$\text{Chd}^{-1}\text{Cd}^{-1}$ from d 13 (d 0 = mean day of previous insemination) through d 19; and 3) MGA + ECP (n = 176), which was the same as the previous treatment plus 0.5 mg (i.m.) of ECP administered on d 13 and 20.

Heifers were observed for estrus at least twice daily from d 0 to d 33 and were reinseminated according to the AM-PM rule.

Pregnancy was determined on d 33 (check of initial insemination) and d 59 (check of resynchronized insemination) by transrectal ultrasonography to visualize embryo, fluid or both. Data were analyzed with the mixed models procedure (SAS, 2001). Treatment and type of first AI (estrus AI or TAI) were considered fixed effects and sire and technician were considered random effects.

Experiment 3

This experiment was conducted at four locations: University of Minnesota Research and Outreach Center, Grand Rapids (81 Angus cows); DarLynn Ranch, Pierz, MN (149 Angus, Hereford, and South Devon cows); Kansas State University Purebred Beef Unit, Manhattan (161 Angus, Hereford, and Simmental cows); and Thielen Ranch, Dorrance, KS (218 cows consisting of a three-way rotational cross of Angus, Hereford, and Simmental). Cows were inseminated previously in another experiment (Stevenson et al., 2002) and assigned within herd to three treatments according to days postpartum, parity (primiparous vs. multiparous), and previous synchronization treatment used before their initial TAI (Figure 1): 1) no further treatment (control; n = 307); 2) a previously used CIDR insert was reinserted 13 d after TAI for a period of 7 d and 1 mg of estradiol benzoate (EB; Sigma Chemical, St. Louis, MO) diluted in sesame oil was administered i.m. on d 13 when the CIDR was inserted and on d 20 when it was removed (P4 + EB; n = 154); and 3) as in the previous treatment but 0.5 mg of ECP was injected at insertion and removal of the used CIDR (P4 + ECP; n = 148). At the two Minnesota locations, insertion of the used CIDR's and first estradiol (EB or ECP) injections occurred on d 11 after TAI and CIDR removal and second estradiol injections on d 18. Blood samples were collected at insertion and removal of the CIDR insert as in Exp. 1 and assayed for progesterone (Skaggs et al, 1986) in six assays with intra- and interassay CV's of 6.4 and 7.8%, respectively.

Cows were observed for estrus two or three times daily from d 19 to 23 d after TAI at the Kansas locations and from d 17 to 21 at the Minnesota locations. Cows in estrus were inseminated 8 to 14 h after first detected estrus. Sires and inseminators were distributed equally among treatments and were confounded within herd. Pregnancy was diagnosed 29 to 33 d after the initial TAI and again 54 to 61 d after TAI (or 35 to 42 d after the second AI that followed resynchronization treatments) by transrectal ultrasonography to visualize fluid, embryo, or both.

All variables (described in Exp. 1) were analyzed using procedure GLM (SAS, 2001) using a model that consisted of resynchronization treatment, location (MN vs. KS), cycling status at the time of the first AI (based on previous blood collection [Stevenson et al.,2002]), parity (primiparous vs. multiparous), all two-way interactions with treatment, with days postpartum and body condition score (BCS; 1 = thin and 9 = fat; Whitman, 1975) as regression variables. Least-square means or unadjusted mean percentages were separated using LSD tests (SAS, 2001) when associated with a protected F-test (P #0.05) in procedure GLM or by orthogonal contrasts (control vs. both CIDR treatments; and P4 +ECP vs. P4 + EB).

Experiment 4

This experiment was conducted at the Kansas State University Purebred Unit with 146 previously inseminated purebred Angus, Hereford, and Simmental lactating cows used in a previous experiment (Stevenson et al., 2002). After the initial timed insemination, cows were assigned randomly to three resynchronization treatments based on breed, parity (primiparous vs. multiparous), days postpartum, and previous synchronization treatment used before TAI (Figure 1): 1) no further treatment (control; n = 51); 2) a previously used CIDR insert was reinserted on d 13 d after TAI for a period of 7 d and 0.5 mg of ECP was injected at its insertion and removal (P4 + ECP; n = 47); and 3) a single 0.5 mg injection of ECP on d 13 (ECP; n = 48). Blood samples were collected as in Exp. 1 and assayed for progesterone (Skaggs et al, 1986) in two assays with inter- and intraassay CV's of 7.5 and 6.8%, respectively.

Cows were observed twice daily for estrus after the initial TAI and were reinseminated as in Exp. 3. Pregnancy was diagnosed as described above at 35 to 36 d after the TAI and after second AI that followed resynchronization treatments. All variables (described for Exp. 1 and 3) were analyzed in procedure GLM (SAS, 2001) in a model including resynchronization treatments, parity (primiparous vs. multiparous), breed, and cycling status at the initial TAI (based on previous blood collection [Stevenson et al., 2002]). All two-way interactions with treatment were included with days postpartum and BCS as regression variables. Least-square means or adjusted mean percentages were separated using LSD tests (SAS, 2001) when associated with a protected F-test (*P*#0.05) in procedure GLM or by orthogonal contrasts (control vs. P4+ECP and ECP; and P4+ECP vs. ECP).

These studies were conducted while availability of new CIDR inserts was limited so used CIDR inserts were employed. The used CIDR's contained either 1.38 or 1.9 g of progesterone when new and had been used once or twice previously in lactating cows. We have conducted previous studies with new CIDR inserts under the authorization of the U.S. Food and Drug Administration Investigational New Animal Drug 6450. The intent of the current treatments was to test the efficacy of supplying progesterone to prevent premature occurrence of repeat estrus during the resynchronization treatment period rather than testing the used CIDR insert itself, which could not be done without the concurrent administration of new CIDR inserts as controls. Application of the previously used CIDR inserts in no way implies that we endorse their reuse. Although precautions were taken to clean and sanitize the used CIDR inserts prior to their reuse, no guarantee of their purity, potency, or sterility can be made.

Results and Discussion

Experiment 1

Distribution of estrus after CIDR removal for the nonpregnant dairy (visual observations plus electronic estrus-detection system) and beef heifers (visual observation) is illustrated in Figure 2. Most of the heifers in the P4 + ECP (64%) treatment were in estrus the day after CIDR removal. The majority of the controls came into estrus before (25%), on the day of CIDR removal (25%), or 4 or more d after CIDR removal (19%). On the day following the second ECP injection (d 1), more ($P < 0.05$) P4+ECP heifers (64%) were in estrus than in either of the other treatments (P4 = 33%; control = 13%). In fact, the P4+ECP treatment produced nearly as many heifers in estrus on d 1 (64%) as the P4 treatment did for

2 d (d 1 and 2; 76%). The variability of the return pattern was less (P<0.01) in both P4 treatments than in that of the control.

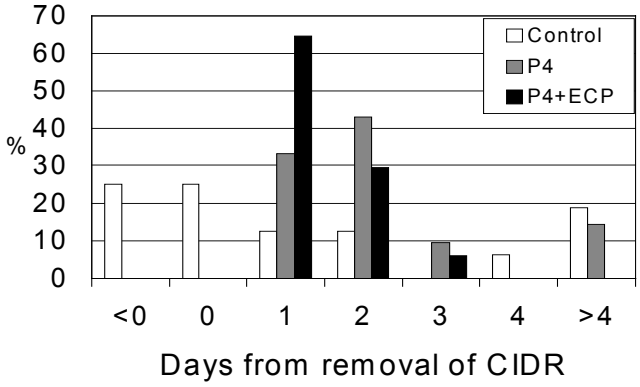

Figure 2. Distribution of repeat estrus in previously inseminated heifers relative to CIDR insert removal for those treated with CIDR inserts for 7 d beginning on d 13 after AI; CIDR inserts + estradiol cypionate (ECP); or controls. The pattern of return was less (P<0.01) variable in both resynchronization treatments that employed the CIDR compared to controls (Exp. 1).

Table 1 summarizes the reproductive characteristics of all heifers to which the resynchronization treatments were applied. Based on elevated ($1 ng/mL) concentrations of progesterone on d 20 (CIDR removal), more (P < 0.05) P4-treated heifers had high progesterone than controls. However, concentrations of progesterone for the P4 treatment tended (P = 0.09) to be greater than those of the P4+ECP treatment, suggesting that the prior ECP injection on d 13 may have reduced progesterone secretion. Serum concentrations of progesterone were less (P<0.05) in beef than in dairy heifers on d 13 (4.1 " 0.5 vs. 5.5 " 0.2 ng/mL) and 20 (3.4 " 0.4 vs. 4.5 " 0.4 ng/mL). Although the proportion pregnant varied from 47 to 60%, the resynchronization treatments seemed to have no adverse effect on the proportion of heifers that conceived to the previous insemination before their application. Of those heifers that failed to conceive to the first insemination, only numerically fewer controls were detected in estrus following resynchronization treatments than after the P4 or P4+ECP treatments. But 84 to 90% of our nonpregnant heifers were detected in estrus after resynchronization compared to 75% of the heifers that were resynchronized with a CIDR alone for 5 d (d 17 to 22; Van Cleeff et al., 1996). Although limited numbers of heifers were reinseminated, the P4 and P4+ECP treatments tended (P = 0.13) to suppress conception at the repeat service compared to controls. The 26-d pregnancy rate, those conceiving after either the first or second insemination, did not differ among treatments. Further, the interval between first and second AI did not differ among treatments, confirming earlier observations (Stevenson and Mee, 1991; Van Cleeff et al., 1996).

Table 1. Reproductive characteristics of dairy and beef heifers (Exp. 1)

	Resynchronization treatments[a]		
Item	Control	P4	P4 + ECP
High P4 on d 20 after first AI, %	68.2[c] (30/44)	88.1(37/42)	81.8 (36/44)
Concentration of P4 on d 20, ng/mL	3.6 " 0.4 (44)	4.7 " 0.5[d] (42)	3.6 " 0.4 (44)
Pregnancy rate after first AI, %	53.2 (25/47)	46.7 (21/45)	60.4 (29/48)
Returned to estrus 18-26 d after initial AI, %	72.7 (16/22)	84.0[e] (21/25)	89.5 (17/19)
Conception of repeat AI, %	60.0[f] (9/15)	33.3 (7/21)	35.2 (6/17)
26-d pregnancy rate[b], %	72.3 (34/47)	60.0 (27/45)	72.9 (35/48)
Interval between 1st and 2nd AI, d	22.4 " 0.7 (16)	22.6 " 0.6 (21)	21.8 " 0.6 (17)

[a]See Figure 1. Progesterone (P4) was administered via a CIDR insert for 7 d, beginning on d 13 after the first insemination, or 0.5 mg of estradiol cypionate (ECP) was given on d 13 and 20 when the CIDR insert was inserted and removed.
[b]Heifers conceiving after two inseminations.
[c]Different ($P < 0.05$) from both P4 treatments.
[d]Different ($P \# 0.09$) from P4 + ECP and control.
[e]One pregnant heifer was detected in estrus after CIDR removal.
[f]Different ($P = 0.13$) from both P4 treatments.

Sexual behavioral characteristics of dairy heifers whose estrus was resynchronized were summarized in Table 2. No significant differences among treatments were detected for the duration of standing estrus, total number and duration of all standing events, or duration of individual standing events (Table 2). However, the P4+ECP treatment had the greatest number of standing events and the duration of total standing time. Variability of these traits were not different among treatments.

All nonpregnant heifers in the P4+ECP treatment were observed visually in estrus compared to less than 64% of heifers in the P4 and control heifers. The ECP injection may have enhanced the proportion of heifers visually detected in estrus because of a tendency for more estrual activity induced by ECP (Lefebvre and Block, 1992). Further, because no significant differences were detected, one could conclude that the 0.5 mg dose of ECP used is apparently producing a normal physiological estrus. Estradiol-17β (2 mg) administered 24 h after removal of the progestin caused 98 to 100% of cows to show estrus during a 48-h period (Wiltbank et al., 1971). Similar findings were observed after 0.5 mg of ECP in dairy heifers (Lopes et al., 2000). In the latter study, 100% of heifers treated with ECP were synchronized, whereas 89% of the estrous periods of controls were synchronized. Administration of EB 24 h after CIDR removal seemed to increase the number of heifers exhibiting estrus (Hanlon et al., 1996).

Table 2. Sexual behavioral characteristics of dairy heifers whose estrus was resynchronized based on the HeatWatch system (Exp. 1)

	Resynchronization treatments[a]		
Item	Control	P4	P4 + ECP
No. of heifers	7	11	10
Duration of standing estrus, h	14.0 " 1.9	11.2 " 1.5	11.1 " 1.6
Total number of standing events	28.6 " 9.5	28.4 " 7.6	40.6 " 7.9
Total duration of standing events, s	59.2 " 22.5	70.1 " 18.1	94.9 " 19.1
Duration of individual standing events, s	2.1 " 0.4	2.9" 0.3	2.3 " 0.3
Observed visually, %	57.1	63.6	100

[a]See Figure 1. Progesterone (P4) was administered via a CIDR insert for 7 d, beginning on d 13 after the first insemination, or 0.5 mg of estradiol cypionate (ECP) was given on d 13 and 20 when the CIDR insert was inserted and removed.

Experiment 2

Resynchronization treatments had no negative effect on the pregnancy rates resulting from the initial insemination; 58/87 (67%), 132/176 (75%) and 112/176 (64%) for control, MGA, and MGA + ECP, respectively. Mean hours from the second injection of ECP to estrus tended ($P<0.07$) to be less for control (54 " 9) than for MGA (72 " 6) or MGA+ECP (74 " 5). Further, the variance of the interval to estrus was greater ($P<0.05$) for control than MGA or MGA+ECP (1160, 819 and 912, respectively). Greater ($P<0.05$) proportions of control than MGA or MGA+ECP treated heifers were in estrus before and during MGA feeding (Table 3). Feeding MGA seemed to delay estrus in the MGA and MGA+ ECP treated heifers because 14% of heifers in each treatment were in estrus more than 25 d after the first insemination, whereas none of the controls were in estrus during this period (Figure 3). Distribution of estrus after the second ECP injection shows no clear peak for the MGA or MGA+ECP treatments. Conception rates were greater in control than MGA ($P<0.05$) or MGA + ECP ($P<0.09$) treated heifers (Table 3). These differences seem to be due to lower conception rates during the targeted resynchronization period (d 20 to 25) for MGA and MGA + ECP heifers than control heifers (Table 3). Total pregnancy rates resulting from the first and second AI did not differ among treatments. If only a 5-d period of detected estrus had been employed for the second insemination, the 25-d pregnancy rate would not show an advantage for the resynchronization treatments.

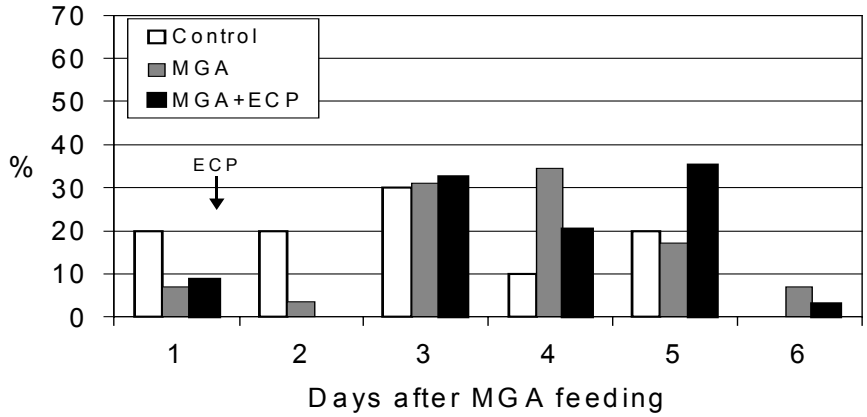

Figure 3. Distribution of repeat estrus in previously inseminated heifers after receiving MGA for 7 d; MGA + estradiol cypionate (ECP); or controls (Exp. 2).

That resynchronization had no effect on pregnancy rates to the first insemination agrees with previous studies (Purvis and Whittier, 1997). Despite this, more MGA-treated heifers were in estrus beyond d 25 suggesting that embryonic loss occurred in these heifers. The total percentage of heifers reinseminated was similar among treatments, so perhaps MGA "spared" embryos for a short period of time, but were eventually lost.

Conception rate of beef heifers to a second insemination following resynchronization with MGA was not different from controls, but was numerically lower in MGA-treated heifers (Purvis and Whittier, 1997). Lower conception rates in the MGA and MGA + ECP heifers indicated that some persistent follicles might have developed in heifers assigned to these treatments (Chenault et al., 1990). Based on previous results with estradiol benzoate (Burke et al., 1999) and ECP (El-Zarkouny and Stevenson; 2002), it was expected that the ECP injection on d 13 would initiate a new wave of follicular growth, if the majority of heifers had two follicular waves. A greater variation in cycle duration in yearling heifers may make attempts more difficult to resynchronize estrus, partially because a greater percentage of heifers may have three follicular waves and the existing dominant follicle on d 13 after AI may not be LH-dependent and thus respond to the loss of LH pulses (turn over and lose its dominance) after an estrogen injection (Burke et al., 1999).

Table 3. Reproductive traits of heifers after resynchronization with MGA or MGA plus ECP[a] (Exp 2)

	Treatment		
	Control	MGA	MGA+ECP
Number	87	176	176
No. pregnant to first insemination	58	132	112
Pregnancy rate (%)	67	75	64
No. not pregnant	29	44	59
No. reinseminated (%)			
Before MGA (< d 13)	3 (10.3)	1 (2.2)	6 (10.2)
During MGA (d 13 to 19)	13 (31.0)	3 (6.8)	3 (5.1)
After MGA (d 20 to 25)	10 (34.5)	29 (65.9)	34 (57.6)
After MGA (>d 25)	0	6 (13.6)	8 (13.6)
Total	26 (89.7)	39 (88.6)	51 (86.4)
Conception rate (%)			
Before MGA (<d 13)	3/3	1/1	4/ 6 (66.7)
During MGA (d 13 to 19)	9/13 (69.2)	2/ 3 (66.7)	2/ 3 (66.7)
After MGA (d 20 to 25)	7/ 9 (77.8)	10/29 (34.5)	16/34 (47.1)
After MGA (>d 25)	0	5/6 (83.3)	6/ 8 (75)
Total	19/25 (76)	18/39 (46)[b]	28/52 (55)[c]

[a]See Figure 1. MGA was fed for 7 d, beginning on d 13 after the first insemination, or 0.5 mg of estradiol cypionate (ECP) was given on d 13 and 20 when the CIDR insert was inserted and removed. Day 0 = mean day of first insemination.
[b]Mean differs (P<0.05) from Control.
[c]Mean differs (P<0.09) from Control.

Experiment 3

Timing of administration of resynchronization treatments affected the distribution of returns to estrus (Figure 4) between the Kansas and Minnesota herds. Only one pregnant cow was detected in estrus as a consequence of the P4+EB treatment and she was eliminated from further analyses. In the Minnesota herds, no incidence of return to estrus was observed before CIDR removal in either estrogen treatment (Figure 4; lower panel). In contrast, some Kansas controls returned to estrus on d ! 1 and 0 (d 19 and 20 after TAI; Figure 4; upper panel). The P4+EB treatment concentrated estrus on d 1 and 2, with the majority on d 1. In contrast, the P4+ECP treatment concentrated estrus on d 2 and 3, but mainly on d 2.

In the Minnesota herds, the second injection of estrogen may not have been as effective in inducing estrus and the LH surge because subsequent estrus was distributed across more than 3 d. In both cases, the second injections of EB and ECP were given on d 18 after TAI when endogenous concentrations of progesterone may yet have been elevated sufficiently to block the LH surge. In either P4+estrogen combination, return to estrus was uniformly distributed over a 3-d period. Similar distribution patterns of estrus on d 1 (43%) and d 2 (42%) after CIDR removal were reported in dairy cattle when a single injection of EB was administered on d 13 (CIDR insertion) and on d 20 at CIDR removal (Macmillan et al., 1997).

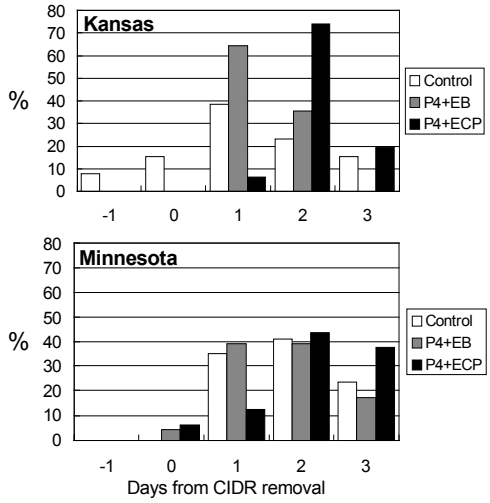

Figure 4. Distribution of repeat estrus in previously inseminated lactating beef cows after CIDR insert removal for those treated with CIDR inserts for 7 d beginning on d 13 after AI (d11 in MN) and removed on d 20 (d 18 in MN) with either estradiol benzoate (EB) or estradiol cypionate (ECP) injections given on d 13 and d 20 (d 11 and d 18 in MN herds); or controls (Exp 3).

That estrus activity in the P4+EB treatment was concentrated and occurred earlier than that of the P4+ECP treatment is consistent with the half-lives of the two forms of estradiol as well as their rates of absorption and conversion to estradiol-17β. Plasma estradiol-17β reached supraphysiologic concentrations 1 to 23 h after EB treatment and remained elevated for 20 to 30 h (Vynckier et al., 1990; Lammoglia et al., 1998). A pronounced increase in the peak of plasma estradiol-17β does not occur after ECP injection. Rather, maximum concentrations are observed 13 to 31 h after treatment and remain elevated for 170 h before decreasing steadily (Vynckier et al., 1990). Therefore, differences in the pattern of estrus distribution may occur either because EB produces adequate concentrations of estradiol-17β to induce follicular atresia earlier than ECP (d 13 injection) or because of prolonged concentration of estradiol-17β after the ECP injection (d 20 injection). Asynchronous emergence or delay of a new follicular wave may occur because of prolonged elevated concentrations of estradiol-17β (Bo et al., 2000). The difference in dosage between EB (1 mg) and ECP (0.5 mg) also may have influenced emergence of the new follicular wave.

More ($P<0.05$) cows treated with P4+estrogen than controls had elevated concentrations of progesterone on d 20 after TAI when the CIDR insert was removed (Table 4). However, average concentrations of progesterone were only greater ($P<0.05$) in the P4+ECP treatment compared to controls, with the P4+EB treatment being intermediate. As expected, fewer ($P<0.001$) cows (53%; n = 62) that were anestrus just prior to the TAI had elevated serum progesterone on d 20 than cycling cows (87%; n = 526), which is reflected in average concentrations of progesterone (anestrus = 2.0 " 2.0 ng/mL; n = 62 vs. cycling = 3.9 " 2.5 ng/mL; n = 526). For each unit increase in BCS, concentrations of progesterone on d 20 were increased by 0.7 " 0.2 ng/mL.

No harm occurred to pregnancies established in cows when resynchronization treatments were applied (Table 4). Pregnancy rates varied from 44 to 52% across treatments, with fewer ($P<0.05$) anestrous cows conceiving (33%; n = 66) than cycling cows (52%; n = 543).

Table 4. Reproductive characteristics of suckled cows exposed to resynchronization treatments (Exp.3)

	Resynchronization treatments[a]		
Item	Control	P4 + EB	P4 + ECP
High P4 on d 20 after initial AI, %	78.1[c] (228/292)	86.1[d] (130/151)	90.3[d] (131/145)
Concentration of P4 on d 20, ng/mL	3.1 " 0.3[c] (292)	3.4 " 0.4[c,d] (151)	4.1 " 0.4[d] (145)
Pregnancy rates after initial AI, %	51.8 (159/307)	44.4 (70/153)	51.7 (77/149)
Returned to estrus 20-23 d after initial AI, %	29.1[c] (43/148)	83.5[d] (71/85)	65.3[d] (47/72)
23-d pregnancy rate[b], %	54.7 (168/307)	60.1 (92/153)	68.5 (102/149)
Embryo survival, %	86.1 (99/115)	90.0 (45/50)	94.8 (55/58)

[a]See Figure 1. Progesterone (P4) was administered via a CIDR insert for 7 d, beginning on d 13 after the first insemination, plus either 1 mg of estradiol benzoate (EB) or 0.5 mg of estradiol cypionate (ECP) was given on d 13 and 20 when the CIDR insert was inserted and removed.
[b]Cows conceiving after two inseminations.
[c,d]Means with uncommon superscript letters differ (P<0.05).

The total proportion of nonpregnant cows returning to estrus between d ! 1 and 3 after CIDR removal (d 0) was 2.2 to 2.9 times greater (P<0.05) after both P4+estrogen treatments than in controls (Table 4). No difference was detected between estrogen treatments, but proportions of primiparous cows (41%; n = 59) that returned to estrus were less (P<0.05) than those of multiparous cows (56%; n = 246).

Intervals between TAI and the repeat estrus were influenced by a treatment H location interaction (P<0.05; Table 5). Because of the earlier administration of the resynchronization treatments in the Minnesota herds, concentrations of serum progesterone on d 18 may have blocked the ability of the second injection of estradiol to induce estrus and the LH surge. Intervals to returned estrus among treatments were not different. In contrast, in the Kansas herds in which resynchronization treatments were administered between d 13 and 20, the P4+ECP treatment prolonged (P < 0.05) the average interval to estrus compared to controls and P4+EB.

Table 5. Interval to estrus and conception rate after resynchronization of estrus at Kansas and Minnesota locations (Exp.3)

		Resynchronization treatments[a]		
Item	Location	Control	P4 + EB	P4 + ECP
Interval between 1st and 2nd AI, d	KS	20.8 " 0.3[b] (26)	21.2 " 0.2[b] (48)	22.4 " 0.2[c] (31)
	MS	21.6 " 0.3 (17)	21.6 " 0.2 (23)	22.3 " 0.3 (16)
Conception rate of repeat AI, %	KS	65.4 (26)	52.1 (48)	64.5 (31)
	MN	52.9[d] (17)	17.4[e] (23)	50.0[d,e] (16)

[a]See Figure 1. Progesterone (P4) was administered via a CIDR insert for 7 d, beginning on d 13 after the first insemination, plus either 1 mg of estradiol benzoate (EB) or 0.5 mg of estradiol cypionate (ECP) was given on d 13 and 20 when the CIDR insert was inserted and removed.
[b,c]Treatment H location interaction (P<0.05)
[d,e]Treatment H location interaction (P=0.07).
Interval to estrus: DPP per 10 d = 7.9 " 3.5% (P<0.05). CR: BCS per unit = 17.1 " 7.2% (P<0.05).

As a consequence, conception rates also were affected differently between locations ($P < 0.05$). No difference among treatments were observed for the Kansas herds, but for the Minnesota herds, the P4+EB treatment tended (P=0.07) to reduce conception rates compared to controls.

The 23-d pregnancy rates tended ($P = 0.15$) to be greater after both P4+estrogen treatments, with the biggest difference between the P4+ECP and controls (Table 4). The 23-d pregnancy rate was greater in cycling cows (62%; n = 543) than in anestrous cows (41%; n = 66). This relationship is substantiated by the fact that for each 10-d increase in days since calving at the time of first AI, a 2.8 " 1.1% increase (P<0.01) in 23-d pregnancy rates was detected. Pregnancy rate is a product of the rates of detected estrus or AI submission rate and conception rates. Therefore, because both P4+estrogen treatments increased return rates and did not affect conception rates, it is not surprising that pregnancy rates tended to improve relative to controls. In another study, the combination of EB+CIDR effectively synchronized returns to service of nonpregnant cows, and as a consequence, overall pregnancy rates increased (Macmillan et al., 1997). Our results further indicated that the P4+ECP treatment was as effective as EB+CIDR in synchronizing returns to estrus and subsequently increasing total 23-d pregnancy rates. In fact, nearly 69% of the cows treated with P4+ECP were pregnant after two inseminations. Based on the fact that the only estrogen available in the U.S. market is ECP, this study opens the possibility for its use in mature or lactating beef cattle for resynchronization of estrus.

Embryo survival in pregnant cows after the initial pregnancy diagnosis between d 29 and 33 after the TAI was affected (P< 0.05) by herd. Although survival was numerically greater in the P4+ECP cows, it did not differ from the other two treatments. Most embryo deaths seem to occur between d 8 and 16 or 18 (Roche, 1981). Pregnant cows have elevated concentrations of progesterone when compared to nonpregnant and cycling cows from d 6 to d 14 or 18 after estrus, which ensures a quiescent uterus during early stages of embryo development (Robinson et al., 1989). Luteal insufficiency is considered to be one cause of embryonic death. Interestingly, the proportion of cows in each treatment that had elevated progesterone on d 20 (Table 5) paralleled the percentages of embryo survival (Table 4). Supplementing exogenous progesterone may prevent low concentrations of progesterone from occurring in the maternal circulation and prevent embryo losses (Stevenson and Mee, 1991; Van Cleeff et al., 1996). In dairy cows, providing supplemental progesterone with a progesterone-releasing intravaginal device (PRID) increased pregnancy rates when treatments were initiated no earlier than d 3 after AI (Robinson et al., 1989; Van Cleeff et al., 1991), but not consistently (Stevenson and Mee, 1991). Supplemental progestin during the luteal phase tended to increase conception rates (Wilmut et al., 1986) or calving rates of beef heifers (Favero et al., 1993). In Exp. 3, the CIDR was inserted between d 13 and 20 (Kansas herds) or d 11 and 18 (Minnesota herds) after TAI, thus covering that time interval when embryo loss is reported to be greatest. It can be assumed that supplemental progesterone from the CIDR might prevent early embryo death when combined with the appropriate form of estradiol, but further observations are warranted.

Experiment 4

Distribution of estrus after a single injection of ECP on d 13 or the 7-d CIDR plus injections of ECP on d 13 and 20 is illustrated in Figure 5. Nearly 67% of the P4+ECP cows were in estrus on d 2 after removal of the CIDR, with the pattern of distribution of estrus after the single ECP injection skewed to the right of the figure. More than 62% of the ECP-treated cows were in estrus on or after d 4 ($d 24 or $11 d after ECP injection). Our results don't dispute previous observations that estrus occurred 9 to 10 d after a single injection of EB was administered on d 13 of the estrous cycle (Burke et al., 2000). However, the apparent bimodal distribution of return to estrus among cows treated with only ECP on d 13 suggests the possibility that perhaps cows may respond differently depending on whether or not they have a dominant follicle that is responsive to estrogen (i.e., larger second-wave follicle or a smaller third wave follicle).

Results of Exp. 4 are summarized in Table 6. Treatments did not affect the proportion of cows with elevated progesterone or the concentration of progesterone on d 20. Pregnancy rates of cows exposed to these resynchronization treatments were not different, but as in Exp. 3, anestrous cows at the time of first AI had lower ($P<0.05$) pregnancy rates (20%; n = 10) than cycling cows (46%; n = 136). Rates of return to estrus between 0 and 5 d after CIDR insert removal (20 to 25 d after TAI) were unaffected by treatment. Intervals between inseminations tended ($P=0.09$) to be longer in P4+ECP and ECP-treated cows compared to controls. Conception rates and 25-d pregnancy rates after resynchronization did not seem to be affected by treatments, although an insufficient number of cows were tested in this experiment to detect differences.

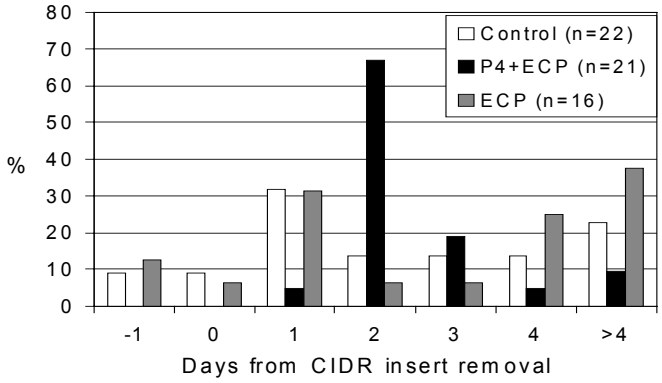

Figure 5. Distribution of repeat estrus in previously inseminated lactating beef cows relative to CIDR insert removal for those treated with CIDR inserts for 7 d beginning on d 13 after AI + estradiol cypionate (ECP) on d 13 and 20; ECP on d 13; or controls (Exp. 4).

Table 6. Reproductive characteristics of suckled cows exposed to resynchronization treatments (Exp.4)

	Resynchronization treatments[a]		
Item	Control	P4 + ECP	ECP
High P4 on d 20 after initial AI, %	60.8 (31/51)	61.7 (29/47)	62.5 (30/48)
Concentration of P4 on d 20, ng/mL	2.0 ± 1.0	2.4 ± 0.7	2.1 ± 0.7
Pregnancy rates after initial AI, %	41.2 (21/51)	42.6 (20/47)	47.9 (23/48)
Returned to estrus 20-25 d after initial AI, %	73.0 (22/30)	75.0[c] (21/28)	61.5[c] (16/26)
Interval between 1st and 2nd AI, d	20.1 ± 1.8[d] (22)	22.3 ± 1.7 (21)	25.1 ± 1.3 (16)
Conception rate of repeat AI, %	81.0 (17/21)	65.0 (13/20)	53.3 (8/15)
25-d pregnancy rate[b], %	74.5 (38/51)	70.2 (33/47)	62.5 (30/48)

[a]See Figure 1. Progesterone (P4) was administered via a CIDR insert for 7 d, beginning on d 13 after the first insemination, plus 0.5 mg of estradiol cypionate (ECP) was given on d 13 and 20 when the CIDR insert was inserted and removed or a single injection of ECP was given on d 13.
[b]Cows conceiving after two inseminations.
[c]One cow in each treatment showed estrus and was reinseminated when pregnant.
[d]Different (P=0.09) from P4 + ECP and ECP. Breed × treatment interaction (P<0.01).

General Discussion

Recently, it was suggested that the combination of estradiol and progesterone may be useful to manipulate ovarian follicular development and may have important implications for use of AI (Bo et al., 1995). Our results confirm those conclusions and suggest that either P4+EB or P4+ECP are effective in synchronizing returns to estrus in lactating cattle. Perhaps because of high rates of return and possible reduced conception rates in Exp. 1 and 2, resynchronization treatments may not be needed or cost effective for heifer. One purpose of these studies was to determine if the first eligible estrus after insemination could be resynchronized without harming established pregnancies. It seems clear from all four experiments that administering either estrogen alone or in combination with a 7-d treatment with progesterone via the CIDE insert or MGA that established pregnancies were unaffected. This finding was true for heifers and lactating cows. Established pregnancies likewise were unaffected. This finding was true for heifers and lactating cows. Established pregnancies likewise were unaffected when injections of EB were administered on d 12, 13 or 14 after AI (Macmillan et al, 1997). In addition, injections of 1 mg of EB or 0.5 mg of ECP administered at insertion and at removal of a used CIDR did not compromise the ability of the CL to maintain pregnancies already established in our studies. Some caution is warranted in interpreting the lack of harm of resynchronization protocols attempted. In none of the individual experiments were there sufficient observations to avoid making a type II error. However, collectively, the data suggest that no harm was caused to ongoing pregnancies.

Our results show that administering progesterone via the CIDR was also effective in preventing occurrence of spontaneous estrus before its removal. The combination of estradiol and progesterone were effective in inducing atresia of dominant follicles regardless of their age or diameter (Burke et al., 1999). Further, they suggested that duration of progesterone treatment must be sufficient to prevent occurrence of estrus until complete spontaneous

luteolysis has occurred. Occurrence of estrus in control cows in the Kansas herds in Exp. 3 on d !1 and 0 was consistent with occurrence of luteolysis before d 19 of the estrous cycle. In contrast, because detection of estrus in the Minnesota herds started on d 17, and no estrus was observed in control cows until d 19 (d 1 after CIDR removal), then the duration of progesterone treatment must have been sufficient. Control heifers in Exp. 2 came into estrus consistently during the resynchronization treatments and even a few MGA treated heifers were in estrus during MGA feeding. Ensuring adequate consumption of MGA in a resynchronization treatment or synchronization protocol is a limitation to its use.

Concentrating the distribution of estrus into a short, predictable time frame provides advantages for an AI program. Detection of estrus is both time consuming and labor intensive, especially for repeat estrus periods after a failed AI because the interval to estrus is more variable than in noninseminated females (Van Cleeff et al., 1996). Our results with either EB or ECP plus P4 confirm previous observations that injections of estrogen on d 13 and 20 of the estrous cycle corresponding to insertion and removal, of a used CIDR respectively, synchronized returns to estrus of nonpregnant cows (Macmillan et al., 1997).

The purpose of the first estrogen injection is to initiate a new follicular wave so that the timing of the new dominant follicle corresponds to withdrawal of progesterone when the CIDR is removed. The progesterone released via the CIDR likewise can turnover dominant follicles and initiate a new follicular wave (Kang et al., 1999). Exogenous estradiol is normally luteolytic when administered early in the estrous cycle (Wilbank and Kasson, 1968). Although estrogen is an integral component of the natural luteolytic mechanism, the effect of exogenous estrogen is variable and it should not be considered equipotent to the putative luteolysin, $PGF_{2\alpha}$ (Burke et al., 1999).

The purpose of the second injection of estrogen was to induce a LH surge and subsequently reduce the period necessary to detect estrus. Further, the second injection of EB reduced the time to the next eligible estrus so that more nonpregnant cows returned to estrus sooner (Macmillan et al., 1997). Thus, in the present experiments, in both P4 + estrogen treatments, most of the returns to estrus occurred earlier than expected, probably due to the second injection of estrogen. In cattle, an increased titer of endogenous estradiol normally promotes the preovulatory LH surge by stimulating the number of GnRH receptors in the anterior pituitary while concentrations of progesterone are basal (Hansel and Convey, 1983). Therefore, administration of exogenous estrogen after luteolysis may induce a LH surge (Fike et al., 1997; Lammoglia et al., 1998). A dose of 1 mg of EB has been shown to be sufficient to elicit behavioral signs of estrus in anestrous cows (Fike et al., 1997). It has been demonstrated that 1 or 0.5 mg of ECP induces an LH surge in lactating dairy cows (Pancarci et al., 2002; Stevenson et al., 2002) and dairy heifers (Lopes et al., 2000) when given 24 h after a luteolytic dose of $PGF_{2\alpha}$. Administration of EB at CIDR removal on d 20 of the cycle has been used to reduce variability in timing of the LH surge (Hanlon et al., 1996). When administering EB 24 h after CIDR removal, an LH surge occurred approximately 24 h later, with females exhibiting estrus and ovulating earlier than those with no EB injection after insert removal (Hanlon et al., 1996). Based on our results, a greater percentage of returns to estrus occurred 8 d after EB injection on d 13, which was one day earlier than predicted based on the latter report. We suggest that the initial injection of ECP probably induced emergence of a new wave 1 d later than EB, consistent with the delayed estrus in ECP vs. EB-treated cows in Exp. 3. Therefore the expected day of estrus occurred 10 to 11 d after ECP administration in Exp. 3 and 4

(following a single injection of ECP on d 13). However, peak estrus occurred on d 9 after ECP or 2 d after CIDR removal when ECP injection was combined with the CIDR insert.

One report indicated that the EB + CIDR treatments increased fertility of dairy cows as a consequence of promoting three follicular waves (Macmillan et al., 1997). This was evident when conception rates were less in cows in which the fertilized oocyte was derived from the second (58%) compared to the third (95%) follicular wave of the estrous cycle in beef (Ahmad et al., 1997) and dairy cows (30 vs. 68%; Townson et al., 2002). Even though our findings are not consistent with the previous report where fertility was enhanced, the fact that neither estrogen treatment in lactating cows, compromised conception at the resynchronized estrus may have important implications for increasing use of AI in the beef cattle industry. However, given the current data, resynchronization of estrus may not be productive for heifers.

The final and most important practical goal of estrus-synchronization programs is to facilitate and increase the usage of AI. Unfortunately, less than 6% of beef cows in the U.S. are inseminated annually (NAHMS, 1997). According to that survey of cattle producers, the most common reasons for not utilizing estrus-synchronization programs and AI include lack of time and labor (37%), too complicated (20%), other (20%), cost (13%), lack of facilities (8%) and do not work (2%). Resynchronization treatments based on estrogen and the used CIDR tended to increase the pregnancy rates after two inseminations in lactating cows during the early breeding season, and also were inexpensive to apply. This may represent a strategic tool to increase the utilization of AI and increase profits on cow-calf operations.

Implications

Resynchronization of estrus beginning 13 d following insemination by feeding MGA, inserting a CIDR, or CIDR + estrogen injection increased the synchrony of estrus and visual detection of estrus (when ECP was added) of nonpregnant heifers. In lactating beef cows, incorporation of either estradiol benzoate or estradiol cypionate and a CIDR insert to resynchronize repeat estrus increased AI resubmission rate, tended to increase 25-d pregnancy rates, and reduced the period needed for detection of estrus. In both heifers and lactating beef cows, the resynchronization treatments had no negative effects on established pregnancies, but tended to reduce conception rates after resynchronization in heifers. Resynchronization provides another tool for cow-calf producers enterprises to facilitate the use of AI and make genetic progress.

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