Ovarian Traits After Gonadotropin-Releasing Hormone-Induced Ovulation and Subsequent Delay of Induced Luteolysis in an Ovsynch Protocol¹

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ABSTRACT

Our objective was to determine whether delaying the PGF_{2α} injection by 24 or 48 h after the first GnRH injection in an Ovsynch protocol (from a standard 7 d) altered ovarian characteristics in lactating dairy cows. Beginning 9 d after removal of a progesterone-releasing controlled internal drug release (CIDR) insert and injection of PGF_{2α} (d 6.4 of the estrous cycle), 36 Holsteins (average body weight = 707 ± 12 kg and body condition score = 2.3 ± 0.1) were administered 100 µg of GnRH (81 ± 2 d in milk) and assigned randomly to receive a treatment injection of PGF_{2α} 7, 8, or 9 d later. Timed artificial insemination was performed at 48 h after PGF_{2α} at which time a second injection of GnRH was administered. Ovarian structures were mapped by ultrasonography on d 0 (first GnRH injection); on d 2 to determine responses to the first GnRH injection; at PGF_{2α} injection; and daily thereafter through 72 h after PGF_{2α} to monitor ovulation of preovulatory follicles. Blood was collected on d 0, 2, at PGF_{2α} injection, and at 24 and 48 h after PGF_{2α} to monitor serum changes in estradiol-17β (E2-17β) and progesterone (P4). Based on serum P4 and ovarian exams, 2 cows were eliminated because of anestrus and their failure to ovulate a follicle in response to the first GnRH injection. Two other cows in which luteolysis failed to occur after PGF_{2α} treatment also were eliminated. Final numbers of cows per treatment were: 7 d (n = 13), 8 d (n = 9), and 9 d (n = 10). Twenty-nine of 32 cows ovulated (90.6%) in response to the first GnRH injection. Of those cows not ovulating in response to the first GnRH injection, 2 had 1 original corpus luteum and 1 had 2 original corpora lutea. Despite a 24- or 48-h delay between first GnRH and PGF_{2α} injections, the diameter (mm) and volume (mm³) of the ovulatory follicle did not differ among treatments: 14.3 ± 0.6 and 1,526 ± 62 at 7 d; 14.1 ± 0.8 and 1,479 ± 97 at 8 d; and 15.3 ± 0.9 and

1,490 ± 69 at 9 d. In all 32 cows, at least 1 follicle ovulated after treatment, but ovulation rates did not differ: 1.2 ± 0.1, 1.1 ± 0.1, and 1.3 ± 0.2, respectively, for the 7-, 8-, and 9-d treatments. Serum concentrations of E2-17β did not differ among treatments. Four cows in the 7-d treatment were inseminated 24 h late and were excluded before assessing conception rates, which were 5/9 (55.6%), 5/9 (55.6%), and 1/10 (10%), respectively. We conclude that delaying PGF_{2α} injection by 24 h had no effect on outcomes.

Key words: Ovsynch, ovulation, follicle, timing of luteolysis

INTRODUCTION

Before the advent of the Ovsynch protocol (injection of GnRH 7 d before and 48 h after an injection of PGF_{2α}, with 1 timed AI at 12 to 16 h after the second GnRH injection; Pursley et al., 1997), 33% of all dairy operations were using some type of systematic PGF_{2α} program to synchronize estrus (National Animal Health Monitoring System, 1996), with rates of use greater for operations with 200 or more cows (50.2%) than for those with 100 to 199 cows (45%) or less than 100 cows (31.1%). Since then, timed AI (TAI) protocols likely have become even more popular among dairy producers and are used in nearly 10% of all US dairy herds (Lucy, 2001).

Development of synchronized ovulation was based on earlier reports in which a new follicular wave was initiated in response to an injection of GnRH 6 to 7 d before PGF_{2α}-induced luteolysis (Thatcher et al., 1989; Twagiramungu et al., 1995; Pursley et al., 1997). Emergence of a new follicular wave in response to GnRH led to greater homogeneity of ovarian follicular inventories among cows at the time of induced luteolysis (Twagiramungu et al., 1995). Improved synchrony of estrus resulting from coordinated follicular maturation and luteal regression (after administering GnRH 7 d before PGF_{2α}) was first demonstrated in dairy heifers (Thatcher et al., 1989), and later in lactating dairy (Stevenson et al., 1999) and beef cattle (Twagiramungu et al., 1992a,b). In consequence, GnRH treatment of cows 6 to 7 d before induced luteolysis resulted in 70 to 83% of heifers or

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cows in estrus during a 4-d period (Thatcher et al., 1989; Twagiramungu et al., 1992a,b). Furthermore, using a GnRH agonist 6 d before PGF_{2α}-induced luteal regression improved precision of estrus that occurred without any detrimental effect on fertility of beef cows (Twagiramungu et al., 1995).

Administration of GnRH reduces occurrence of estrus during 6 to 7 d after GnRH injection (Thatcher et al., 1989; Twagiramungu et al., 1992a,b). Reduced estrus during the post-GnRH period occurs because of ovulation of the dominant follicle and formation of a new or ancillary corpus luteum (CL) during the luteal phase (Twagiramungu et al., 1994a,b; Pursley et al., 1995), resulting in decreased peripheral concentrations of serum estradiol-17β (E2-17β; Twagiramungu et al., 1994b). In lactating dairy cows, the average incidence of ovulation is about 64% when cows are injected across various stages of the estrous cycle (Vasconcelos et al., 1999).

Once a new dominant follicle is selected, concentrations of E2-17β increase, LH pulses increase, and the selected dominant follicle becomes the preovulatory follicle. Maximum concentrations of E2-17β in serum preceding ovulation, however, are 30% less in lactating dairy cows than in nulliparous heifers, even though ovulatory follicles are 13% greater in diameter (Sartori et al., 2004). Further, maximum concentrations of progesterone (P4) are 30% less in cows than in heifers, despite cows having 53% more luteal tissue (Sartori et al., 2004). Discrepancies between sizes of ovarian structures and serum steroid concentrations may result from greater rates of steroid metabolism in lactating dairy cows than in heifers (Sangsratavong et al., 2002) and in cows of various milk-producing abilities (Lopez et al., 2004).

Reduced serum steroid concentrations may have numerous potential physiologic consequences that compromise fertility in lactating cows. We hypothesized that greater concentrations of serum E2-17β in lactating cows may occur when using the Ovsynch protocol if PGF_{2α}-induced luteolysis is delayed after the first GnRH injection. Our objective was to determine whether lengthening the interval between the first GnRH injection and PGF_{2α} from 7 d to 8 or 9 d might result in greater serum concentrations of E2-17β and larger follicles, possibly leading to increased fertility after a TAI.

MATERIALS AND METHODS

Herd Management

The experiment was conducted at the Kansas State University Dairy Teaching and Research Center, with 36 lactating Holstein cows that calved between August and September 2004 and had an average BCS of 2.3 ± 0.1. Daily test-day milk yield of these cows nearest to the day of first AI averaged 49.6 ± 1.7 kg (3.5% fat and

Figure 1. Experimental design for presynchronizing estrous cycles in lactating dairy cows before applying treatments. All cows received a controlled internal drug release (CIDR) insert for 7 d, with an injection of PGF_{2α} given 24 h before removal of the CIDR insert. Nine days after CIDR insert removal, cows were allocated randomly to 1 of 3 treatments: PGF_{2α} at 7, 8, or 9 d after the first GnRH injection (d 0). Inseminations were administered at 48 h after PGF_{2α}, at which time the second GnRH injection was administered. Pregnancy was diagnosed 32 to 34 d after the timed AI (TAI).

3.0% protein) and 305-d mature-equivalent yield averaged 15,356 ± 429 kg. Cows were housed in covered freestalls bedded with sand, and were fed thrice daily a TMR that met or exceeded NRC (2001) requirements for lactating cows. The TMR consisted of 30% chopped alfalfa hay, 19% wet corn gluten meal, 15% corn silage, 9.3% whole cottonseed, 4.4% solvent-extracted soybean meal, 3.3% expeller soybean meal, 13% corn grain, 1.3% menhaden fish meal, 1% sugar cane wet molasses, and 3.7% mineral-vitamin premix. Cows had ad libitum access to fresh water. Pens were covered with shade cloth, and water was applied by sprinklers 6 times per hour for 1 min during May to October.

Experimental Design

Beginning at 65 ± 2 DIM, estrous cycles were synchronized in lactating dairy cows (BW = 707 ± 12 kg) by applying a P4-releasing, intravaginally placed, controlled internal drug release (CIDR) insert (Eazi-Breed CIDR, Pfizer Animal Health, New York, NY) for 7 d, plus 25 mg of PGF_{2α} (Lutalyse, Pfizer Animal Health, New York, NY) given 24 h before removal of the CIDR insert (Figure 1). Nine days after removal of the CIDR insert (approximately d 6 of the estrous cycle), cows received 100 μg of GnRH (Cystorelin, Merial Ltd., Iselin, NJ) and then were allocated randomly to 1 of 3 treatments in which they received 25 mg of PGF_{2α} at d 7, 8, or 9 after the first GnRH injection (d 0; 81 ± 2 DIM).

Inseminations were administered at 48 h after PGF_{2α} (91 ± 2 DIM), at which time the second 100-μg injection of GnRH was administered. Pregnancy was diagnosed

32 to 34 d after TAI by using transrectal ultrasonography (5.0-MHz linear-array transducer, Aloka 500V; Corometrics Medical Systems, Inc., Wallingford, CT). A positive diagnosis included confirmation of a CL, uterine fluid, and an embryonic heart beat.

Ultrasonography was conducted on d 0, d 2, and daily thereafter, beginning with PGF_{2α} treatment through 72 h after PGF_{2α}, to monitor ovarian structures. Ovarian follicles were mapped and sized on d 0 and 2 to determine responses to the first GnRH injection. In subsequent scans, all follicles were mapped and sized to monitor the dominant ovarian follicle that developed after the first GnRH injection, and that later became the preovulatory follicle. Follicles were assumed to be spherical. Diameter of structures was determined by averaging their largest cross-sectional width and height measured by ultrasound electronic calipers. Volume of follicles and CL was calculated as follows:

Radius = (W/2 + H/2)/2, where W = largest width
and H = largest height of structure;

Volume = $4/3 \times R^3 \times \pi$, where R = radius and $\pi = 3.14159$.

Blood samples were collected from a coccygeal vessel on d 0, d 2, and then daily beginning with PGF_{2α} treatment through 72 h after PGF_{2α}, d 14, and d 21. The samples were stored on ice, and serum concentrations of P4 were later quantified by RIA (Skaggs et al., 1986). Intra- and interassay coefficients of variation for the P4 RIA were 7.7 and 11.4%, respectively, for a sample that averaged 3.2 ± 0.1 ng/mL. In blood samples collected on d 0, d 2, and daily beginning with PGF_{2α} treatment through 48 h after PGF_{2α}, serum concentrations of E2-17β were quantified in a single RIA (Perry et al., 1991). To increase assay sensitivity, modifications included incubating dried serum extracts with E2-17β antiserum for 24 h at 20°C before adding ¹²⁵I-labeled E2-17β and incubating assay tubes for an additional 6 h at 20°C. After that 6-h incubation, bound E2-17β was separated from free E2-17β by adding cold (5°C) dextran-coated charcoal. The intraassay coefficient of variation was 7.8% for a sample that averaged 18.9 ± 0.6 pg/mL. Sensitivity of the assay was <0.05 pg/tube.

Statistical Analyses

All analyses were performed by using SAS software (SAS Inst. Inc., Cary, NC). All pretreatment variables were analyzed by ANOVA (GLM procedure) by applying a model that consisted of only lactation number (1, 2, and 3+). Posttreatment continuous variables were analyzed similarly with a model that included treatment (7-, 8-, or 9-d interval between the first GnRH injection and

the treatment PGF_{2α} injection), lactation number, and treatment by lactation number interaction. Binomial data (percentages and proportions) were analyzed by logistic regression (GENMOD procedure) using the same model.

Changes in follicular diameters, follicular volumes, and serum concentrations of P4 and E2-17β, across days of the experiment or standardized to the day of PGF_{2α} treatment, were analyzed by using a mixed model procedure (MIXED procedure) to account for repeated measures. Because lactation number had no effects on treatment outcomes, the model consisted of treatment, cow within treatment (random), day, and treatment by day interaction. The covariance structure varied with model. The final model chosen for each variable produced the smallest Akaike's information criteria (AIC). Most covariance structures were heterogeneous autoregressive, but a few were heterogeneous compound symmetry. A priori contrasts were constructed to compare treatment on d 7 (control) with each of the other 2 treatments.

RESULTS

Pretreatment Responses

Estrus was expressed by 22 of 36 cows after removal of the CIDR insert, as detected by electronic, pressure-sensitive HeatWatch transmitters (CowChips LLC, Denver, CO). Interval to estrus after CIDR insert removal was 52.1 ± 4.6 h (ranged from 24 to 114 h). Average number of standing events was 3.8 ± 0.7 , with a total duration of standing time of 9 ± 2 s. Duration of estrus was 6.3 ± 1.1 h. Of those cows that expressed estrus, days from estrus to injection of GnRH was $d 6.4 \pm 0.2$ (range of d 3.7 to 7.4).

Ovarian responses and characteristics of cows before treatments were imposed are summarized in Table 1. Of 36 cows treated, 4 cows were found anestrous by using information from ovarian scans, lack of expressed estrus after CIDR insert removal, and serum concentrations of P4. Two of those 4 cows failed to ovulate in response to GnRH. The CL failed to regress in response to PGF_{2α} treatment in 2 additional cows. Therefore, 4 cows (2 anestrous cows and 2 cows having no luteolysis) were eliminated from the study, leaving a total of 32 cows in the 3 treatments.

In the 32 cows studied, pretreatment responses summarized in Table 2 include numbers of original, induced, and total CL; total numbers of follicles ≥10 mm in diameter before GnRH; and diameter and volume of the largest follicle and resulting diameter and volume of the CL that formed after ovulation (assessed 7 d after the first injection of GnRH). In addition, in 29 of 32 (90.6%) cows, at least 1 follicle ovulated in response to GnRH. Of the

Table 1. Ovarian responses of 36 cows assigned to treatment assessed at 7, 8, or 9 d after the first GnRH injection of a modified Ovsynch protocol

Item	Interval between first GnRH injection and PGF _{2α} , d		
	7	8	9
Cows assigned, no.	13	11	12
Expressed estrus after CIDR insert removal, no.	7	7	8
Cows having original CL, no.	11	9	11
Anestrous cows, ¹ no.	1	2	1
Anestrous cows that ovulated, no.	1	1	0
Cows having induced CL, no.	11	7	11
Cows having no induced CL, no.	2	4	1
Cows having no luteolysis, ² no.	0	1	1
Total cows studied per treatment, no.	13	9	10

¹Two anestrous cows that did not ovulate in response to the first GnRH injection were eliminated from all analyses (1 cow in the 8- and 1 cow in the 9-d treatments).

²Two cows not having luteolysis were eliminated from all analyses.

3 cows not ovulating in response to the first GnRH injection, 1 had 2 original CL and 2 had 1 original CL.

Distribution of CL is shown in Figure 2 based on the number of CL per cow assessed before and 7 d after GnRH. Two anestrous cows had no CL before GnRH, whereas 1 cow had 3 CL before GnRH was administered. One new CL was induced in 23 cows and 2 new CL in 4 cows, so the number of total CL ranged from 1 CL in 4 cows, 2 CL in 17 cows, and 3 or more CL in 11 cows.

Treatment Responses

Changes in average diameter of the largest (putative dominant) follicle, monitored after the first GnRH injection, that subsequently ovulated are illustrated in Figure 3. Small differences were detected among treatments in the diameter of the largest follicle from d 2 to 10. Cows in the 9-d treatment had slightly smaller ($P < 0.05$) follicle diameters on d 2 to 9. Average diameter or average volume of the resulting preovulatory follicles assessed at 72 h after PGF_{2α}, did not differ among treatments (Table 3). When diameters (Figure 4) and volumes (not shown) of the preovulatory follicles were standardized to the day when PGF_{2α} treatment was administered, no

differences were detected among treatments. At least 1 follicle ovulated per cow (range of 1 to 2), and ovulation rates did not differ among treatments (Table 3).

Serum concentrations of E2-17β differed ($P \leq 0.05$) among treatments when plotted by days since the first GnRH injection (Figure 5; upper panel). On d 8, and because of earlier treatment-induced luteolysis, cows in the 7-d treatment (24 h after PGF_{2α}) already had greater concentrations of E2-17β than did cows in the 8- and 9-d treatments; this trend continued to d 9. Once concentrations of E2-17β were expressed relative to days since PGF_{2α} treatment, differences were no longer evident (Figure 5; bottom panel).

A treatment by day interaction ($P < 0.001$) was detected for serum concentrations of P4 for d 0 to 9 (Figure 6; upper panel). Concentrations of P4 were greater ($P < 0.05$) on d 0 to 9 for cows in the 8- and 9-d treatments than for those in the 7-d treatment. When concentrations of P4 were reanalyzed as a percentage of concentrations on d 0, similar patterns of P4 emerged and yielded a significant interaction that was likely caused by differences in designed timing of PGF_{2α}-induced luteolysis. Further, patterns of luteal regression, as assessed by decreasing concentrations of P4 at 24 and 48 h after

Table 2. Pretreatment follicular and luteal characteristics, before and in response to, the first GnRH injection

Item	Average	SD
Original corpus luteum (CL) per cow, no.	1.38 (32)	0.68
New CL per cow, no.	0.97 (32)	0.54
Total CL per cow, no.	2.25 (32)	0.76
Cows that ovulated at least 1 follicle, %	90.6 (32)	
Total follicles ≥ 10 mm before first GnRH injection, no.	1.88 (32)	0.94
Diameter of largest ovulating follicle, mm	12.6 (28) ¹	2.4
Volume of largest ovulating follicle, mm ³	1,163 (28) ¹	755
Diameter of resulting CL, mm	20.7 (29)	3.9
Volume of resulting CL, mm ³	4,687 (29)	2,340

¹In one cow, the follicle was missed on d 0, but a new CL was identified at 48 h and later.

Figure 2. Distribution of original corpus luteum (CL) and those induced by the first GnRH injection, grouped by subsequent treatments of PGF_{2α} administered 7, 8, or 9 d after GnRH.

PGF_{2α}, were not different when expressed relative to day of PGF_{2α} treatment injection (Figure 6; bottom panel). All 32 cows subsequently ovulated and formed new CL (Table 3) after treatment as is further evidenced by increased concentrations of P4 in serum at 14 and 21 d after the first GnRH injection (Figure 6; top panel).

Four cows in the 7-d treatment were inseminated 24 h late (i.e., not according to protocol). Of the remaining cows, 5 of 9 cows (55.6%) conceived in the 7-d treatment, and 5 of 9 cows (55.6%) conceived in 8-d treatment, compared with only 1 of 10 cows (10%) in the 9-d treatment. Conception rates of individual 7- and 8-d treatments

tended ($P = 0.07$) to differ from that of the 9-d treatment. Two of the 4 cows (50%) that failed to ovulate after the first GnRH injection conceived, whereas 9 of 24 cows (37.5%) conceived that ovulated at least 1 follicle after the first GnRH injection.

DISCUSSION

Our model allowed us to test the hypothesis that delaying luteolysis up to 48 h after a normal 7-d interval between GnRH and PGF_{2α} in an Ovsynch protocol would increase follicle size and E2-17 β secretion. The uniform group of cows tested included 22 cows that were known to be between d 3.7 and 7.4 of the estrous cycle, and 29 of 32 cows had at least 1 follicle ovulate in response to the first GnRH injection. The large incidence (90.6%) of ovulation in response to the first GnRH injection is consistent with earlier reports for cows at similar stages of the estrous cycle (Vasconcelos et al., 1999).

We hypothesized that greater concentrations of serum E2-17 β in lactating cows might occur when using the Ovsynch protocol if PGF_{2α}-induced luteolysis was delayed after the first GnRH injection. Our treatments, however, failed to produce larger follicles and did not result in greater concentrations of serum E2-17 β . Neither diameter or volume of the preovulatory follicle nor concentrations of E2-17 β were increased by lengthening the period of dominance for the newly recruited dominant follicle.

In heifers, restricting duration of dominance of the preovulatory follicle to ≤ 4 d at the onset of estrus led to

Figure 3. Changes in average diameter of the dominant follicle (DF) that eventually ovulated after each of 3 treatments in which PGF_{2α} was administered either 7 d ($n = 13$), 8 d ($n = 9$), or 9 d ($n = 10$) after the first GnRH injection (d 0).

Table 3. Largest diameter and volume of preovulatory follicles that ovulated after treatment in response to the second GnRH injection and resulting ovulation rate

Measure ¹	Interval between first GnRH injection and PGF _{2α} , d		
	7	8	9
Diameter of largest follicle, mm	14.3 ± 0.6	14.1 ± 0.8	15.3 ± 0.9
Volume of largest follicle, mm ³	1,526 ± 62	1,479 ± 97	1,490 ± 69
Ovulation rate	1.2 ± 0.1	1.1 ± 0.1	1.3 ± 0.2

¹Assessed 72 h after PGF_{2α} in each treatment.

more precise estrus and a higher conception rate (Austin et al., 1999). This duration of dominance would be most consistent with cows in our 7- or 8-d treatments. In a recent review (Tenhagen, 2005), it was concluded that intervals of 7 d between GnRH and PGF_{2α} were most effective relative to fertility when the second GnRH followed PGF_{2α} by 48 h and insemination occurred 16 to 20 h later.

Clear differences seem to exist between lactating and nonlactating cows with regard to size of ovarian structures and their steroid-secreting capacity, indicating that lactation per se, age, or both, creates these differences. Lactating cows have a much greater incidence of ovulation failure after luteolysis, more multiple ovulations, lower serum E2-17β concentrations, and larger follicles and CL than do nulliparous heifers (Sartori et al., 2004). Moreover, expression of estrus (Dransfield et al., 1998) and estrus-detection rates (Stevenson, 2001) are reduced in lactating dairy cows probably as a result of less estrogen exposure during the peri-estrous period. Among lactating cows, cows producing more milk have shorter durations of estrus, fewer numbers of standing events, and less total standing time than do lower-producing cows (Lopez et al., 2004). It seems clear that

lactating cows require a larger follicle and greater E2-17β production to achieve adequate circulating concentrations of E2-17β necessary to produce an LH surge and ovulation compared with nonlactating cows (Sartori et al., 2002). In our study, concentrations of E2-17β were not increased by prolonged growth and dominance of the newly recruited follicles after the first GnRH injection.

Discrepancies between sizes of ovarian structures and serum steroid concentrations may be due to greater rates of steroid metabolism in lactating dairy cows than in

Figure 4. Average diameter of the preovulatory (dominant) follicle (DF) in cows treated with PGF_{2α} either 7 (n = 13), 8 (n = 9), or 9 (n = 10) d after the first injection of GnRH. Diameters were standardized to the day of PGF_{2α} treatment. **Figure 5.** Serum concentrations of estradiol-17β (E2-17β) in cows after the first GnRH injection, and in response to PGF_{2α} treatment administered 7 (n = 13), 8 (n = 9), or 9 (n = 10) d after the first injection of GnRH (top panel). Serum E2-17β is standardized to the day of PGF_{2α} injection (bottom panel).

Figure 6. Serum concentrations of progesterone (P4) in cows from the first GnRH injection, and in response to PGF_{2α} treatment administered 7 (n = 13), 8 (n = 9), or 9 (n = 10) d after the first injection of GnRH (top panel). Serum P4 is standardized to the day of PGF_{2α} injection (bottom panel); *different ($P \leq 0.05$) from d 7 (control).

heifers (Sangsrivong et al., 2002). Further, it is clear that these differences cannot be explained by differences in patterns of follicular waves (Sartori et al., 2004). Based on those studies (Sangsrivong et al., 2002; Sartori et al., 2004), it seems that the lactating cow metabolizes E2-17 β more quickly, resulting in inadequate peripheral concentrations of E2-17 β to support events associated with normal fertility. It is not known, however, whether E2-17 β biosynthesis by antral follicles may be inferior because of lactation. Further, high-producing cows had lower E2-17 β concentrations than low-producing cows, despite having larger preovulatory follicles (Lopez et al., 2004). Interestingly, E2-17 β concentrations were not correlated with diameter of the preovulatory follicle, but milk production was correlated with both E2-17 β concentrations and diameter of the preovulatory follicle. It is clear that prolonging the life span of the dominant follicle (preovulatory follicle) in our study failed to increase either follicle size or peripheral concentrations of serum E2-17 β .

Other attempts to increase concentrations of estrogen during the peri-estrous period have been made. Studies

in which 0.5 to 1 mg of estradiol cypionate (ECP) was injected in combination with ovulation synchronization indicated that injecting ECP 24 h after PGF_{2α} may not increase conception rates in lactating dairy cows (Pancarci et al., 2002; Stevenson and Tiffany, 2004; Stevenson et al., 2004). Further, when 0.25 mg of ECP was administered at 48 h after PGF_{2α}, concomitant with the second GnRH injection of Ovsynch, concentrations of serum E2-17 β were greater at 6 and 12 h after injection than in controls, which only received GnRH, but conception rates did not differ between treatments (Sellars et al., 2006).

Lactating dairy cows were administered 1 mg of E2-17 β (shorter acting and shorter half-life estrogen than ECP; Vynckier et al., 1990) 8 h before the second GnRH injection and compared with cows treated with an Ovsynch-like protocol, in which the second GnRH injection was delayed to 54 h after PGF_{2α}, and TAI occurred 16 h later (Souza et al., 2005). Treatment with E2-17 β tended to improve conception rate in cows having lower BCS (≤ 2.5), cows in their first lactation, and cows ovulating medium-size (15 to 19 mm) follicles compared with cows treated with Ovsynch alone.

Injection of estrogen or increased exposure of cows to endogenous estrogen increased the incidence of estrus in previous studies (Pancarci et al., 2002; Stevenson et al., 2004), which may benefit fertility in some instances (Souza et al., 2005). Furthermore, other positive benefits of estrogen include induction of normal estrual characteristics, such as mucus secretion, uterine tone, and resulting sexual behavior. These traits are positive indicators of estrus for inseminators because they validate the likelihood of the cow being in estrus (Pancarci et al., 2002).

Although too few cows were inseminated to test differences in conception rates, only 1 of 10 cows in the 9-d treatment conceived when inseminated at 48 h after PGF_{2α} compared with 5 of 9 cows conceiving in each of the 7- or 8-d treatments. In cows whose estrous cycles are presynchronized before Ovsynch, reproductive outcomes may not be reduced when PGF_{2α} injection is delayed by 24 h from the standard 7-d period between GnRH and PGF_{2α} injections, but 48 h may be too long to prevent a potentially reduced conception rate.

In conclusion, prolonging the life span of a newly recruited dominant follicle in the presence of a functional CL by 24 to 48 h, from the standard 7-d interval between the first GnRH injection of the Ovsynch protocol and the injection of PGF_{2α}, failed to increase serum concentrations of E2-17 β or the diameter of that dominant follicle. Further study is warranted to verify whether delaying PGF_{2α} injections by 24 h has no effects on fertility and whether PGF_{2α} injections may be given late (d 8), when not given at d 7 as planned.

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