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## Delaying Injection of prostaglandin F2 $\alpha$ in an ovsynch protocol

### Abstract

Our objective was to determine whether delaying the PGF2 $\alpha$  injection by 24 or 48 hr after the first GnRH injection in an Ovsynch protocol (from a standard 7 days) altered ovarian characteristics in lactating dairy cows. Estrous cycles were synchronized in 36 Holsteins after removal of a progesterone-releasing controlled internal drug release (CIDR) insert and injection of PGF2 $\alpha$ . On day 6 of the estrous cycle, cows were administered 100  $\mu$ g of GnRH (81  $\pm$  2 days postpartum) and assigned randomly to receive a treatment injection of PGF2 $\alpha$  7, 8, or 9 days later. Timed artificial insemination (TAI) was performed at 48 hr after PGF2 $\alpha$ , at which time a second injection of GnRH was administered. Ovarian structures were mapped by ultrasonography on day 0 (first GnRH injection), on day 2 to determine responses to the first GnRH injection, at PGF2 $\alpha$  injection, and daily thereafter through 72 hr after PGF2 $\alpha$  to monitor ovulation of preovulatory follicles. Blood was collected on day 0, day 2, at PGF2 $\alpha$  injection, and at 24 and 48 hr after PGF2 $\alpha$ , to monitor serum changes estradiol-17 $\beta$  and progesterone. On the basis of serum progesterone 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 PGF2 $\alpha$  treatment also were eliminated. Final numbers of cows per treatment were: 7 days (n = 13), 8 days (n = 9), and 9 days (n = 10). Twenty-nine of 32 cows ovulated (90.6%) in response to the first GnRH injection. Despite a 24- or 48-hr delay between first GnRH and PGF2 $\alpha$  injections, the diameter (mm) and volume (mm<sup>3</sup>) of the ovulatory follicle did not differ among treatments. In all 32 cows, at least 1 follicle ovulated after treatment, but ovulation rates did not differ. Serum concentrations of estradiol-17 $\beta$  did not differ among treatments. Two cows in the 7-day treatment and 2 cows in the 8-day treatment were inseminated 24 hr late and were excluded before assessing conception rates: 5/9 (55.6%), 5/9 (55.6%), and 1/10 (10%), respectively. We concluded that delaying PGF2 $\alpha$  injection by 24 hr had little effect on outcomes.; Dairy Day, 2006, Kansas State University, Manhattan, KS, 2006;

### Keywords

Diary Day, 2006; Kansas Agricultural Experiment Station contribution; no. 07-118-S; Report of progress (Kansas State University. Agricultural Experiment Station and Cooperative Extension Service); 965; Dairy; Follicle; Ovsynch; Ovulation; Pregnancy rate

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## **DELAYING INJECTION OF PROSTAGLANDIN F<sub>2α</sub> IN AN OVSYNCH PROTOCOL**

*J. S. Stevenson, M. A. Portaluppi, and D. E. Tenhouse*

### **Summary**

Our objective was to determine whether delaying the PGF<sub>2α</sub> injection by 24 or 48 hr after the first GnRH injection in an Ovsynch protocol (from a standard 7 days) altered ovarian characteristics in lactating dairy cows. Estrous cycles were synchronized in 36 Holsteins after removal of a progesterone-releasing controlled internal drug release (CIDR) insert and injection of PGF<sub>2α</sub>. On day 6 of the estrous cycle, cows were administered 100 µg of GnRH (81 ± 2 days postpartum) and assigned randomly to receive a treatment injection of PGF<sub>2α</sub> 7, 8, or 9 days later. Timed artificial insemination (TAI) was performed at 48 hr after PGF<sub>2α</sub>, at which time a second injection of GnRH was administered. Ovarian structures were mapped by ultrasonography on day 0 (first GnRH injection), on day 2 to determine responses to the first GnRH injection, at PGF<sub>2α</sub> injection, and daily thereafter through 72 hr after PGF<sub>2α</sub> to monitor ovulation of preovulatory follicles. Blood was collected on day 0, day 2, at PGF<sub>2α</sub> injection, and at 24 and 48 hr after PGF<sub>2α</sub>, to monitor serum changes estradiol-17β and progesterone. On the basis of serum progesterone and ovarian exams, 2 cows were eliminated because of anestrus and their failure ovulate a follicle in response to the first GnRH injection. Two other cows in which luteolysis failed to occur after PGF<sub>2α</sub> treatment also were eliminated. Final numbers of cows per treatment were: 7 days (n = 13), 8 days (n = 9), and 9 days (n = 10). Twenty-nine of 32 cows ovulated (90.6%) in response to the first GnRH injection.

Despite a 24- or 48-hr delay between first GnRH and PGF<sub>2α</sub> injections, the diameter (mm) and volume (mm<sup>3</sup>) of the ovulatory follicle did not differ among treatments. In all 32 cows, at least 1 follicle ovulated after treatment, but ovulation rates did not differ. Serum concentrations of estradiol-17β did not differ among treatments. Two cows in the 7-day treatment and 2 cows in the 8-day treatment were inseminated 24 hr late and were excluded before assessing conception rates: 5/9 (55.6%), 5/9 (55.6%), and 1/10 (10%), respectively. We concluded that delaying PGF<sub>2α</sub> injection by 24 hr had little effect on outcomes.

(Key Words: Follicle, Ovsynch, Ovulation, Pregnancy Rate.)

### **Introduction**

Before advent of the Ovsynch protocol [injection of GnRH 7 days before and 48 hr after an injection of PGF<sub>2α</sub>, with one timed AI (TAI) at 12 to 16 hr after the second GnRH injection], 33% of all dairy operations were using some type of programmed breeding system that used prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>) to synchronize estrus, 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 U.S. dairy herds.

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 days before PGF<sub>2α</sub> injection. 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. Improved synchrony of estrus resulting from coordinated follicular maturation and luteal regression (after administering GnRH 7 days before PGF<sub>2α</sub>) was first demonstrated in dairy heifers, and later in lactating dairy cattle.

Once a new dominant follicle is selected, concentrations of estradiol-17β increase, LH pulses increase, and the selected dominant follicle becomes the preovulatory follicle. Maximum concentrations of estradiol-17β in serum preceding ovulation, however, are 30% less in lactating dairy cows than in heifers, even though ovulatory follicles are 13% larger in diameter. Further, maximum concentrations of progesterone are 30% less in cows than in heifers, despite cows having 53% more luteal tissue. 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 and among cows of various milk-producing abilities.

Reduced serum steroid concentrations may have numerous potential physiologic consequences that compromise fertility in lactating cows. We hypothesized that greater concentration of serum estradiol-17β in lactating cows may occur when using the Ovsynch protocol if PGF<sub>2α</sub>-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<sub>2α</sub> from 7 to 8 or 9 days might result in greater serum concentrations of estradiol-17β and larger follicles, possibly leading to increased fertility after AI.

## Procedures

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 body condition score =  $2.3 \pm 0.1$ . Daily test-day milk yield of these cows nearest to the day of first AI averaged  $109 \pm 4$  lb (3.5% fat and 3.0% protein) and 305-d mature equivalent yield averaged  $33,854 \pm 946$  lb. Cows were housed in covered free stalls bedded with sand, and were fed a TMR 3 times daily.

Beginning at  $65 \pm 2$  days postpartum, estrous cycles were synchronized in lactating dairy cows (average body weight =  $1,559 \pm 26$  lb) by applying a progesterone-releasing controlled internal drug release (CIDR) insert (Eazi-Breed CIDR, Pfizer Animal Health, New York, NY) for 7 days, plus 25 mg of PGF<sub>2α</sub> (Lutalyse, Pfizer Animal Health, New York, NY) given 24 hr before CIDR insert removal. Nine days after CIDR insert removal (approximately day 6 of the estrous cycle), cows received 100 μg of GnRH (Cystorelin, Merial Limited, Iselin, NJ) and then were allocated randomly to 1 of 3 treatments in which they received 25 mg of PGF<sub>2α</sub> at days 7, 8, or 9 after the first GnRH injection (day 0 was  $81 \pm 2$  days postpartum).

Inseminations were administered at 48 hr after PGF<sub>2α</sub> ( $91 \pm 2$  postpartum), at which time the second 100-μg injection of GnRH was administered. Pregnancy was diagnosed, 32 to 34 days after TAI, by using transrectal ultrasonography. A positive diagnosis included confirmation of a CL, uterine fluid, and an embryonic heart beat.

Ultrasonography was conducted on days 0 and 2, and daily thereafter, beginning with

PGF<sub>2α</sub> treatment and continuing through 72 hr after PGF<sub>2α</sub>, to monitor ovarian structures. Ovarian follicles were mapped and sized on days 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.

Blood samples were collected from a coccygeal vessel on days 0 and 2; daily thereafter, beginning with PGF<sub>2α</sub> treatment and continuing through 72 hr after PGF<sub>2α</sub>; on day 14; and on day 21. The samples were stored on ice, and serum concentrations of progesterone and estradiol-17β were later quantified by radioimmunoassay.

## Results and Discussion

Changes in average diameter of the largest (putative dominant) follicle that subsequently ovulated, monitored after the first GnRH injection, are illustrated in Figure 1. Small differences were detected among treatments in the diameter of the largest follicle from days 2 to 10. Cows in the 9-day treatment had slightly smaller ( $P < 0.05$ ) follicle diameters on days 2 to 9. Average diameter or average volume of the resulting preovulatory follicles, however, assessed at 72 hr after PGF<sub>2α</sub>, did not differ among treatments (Table 1). When diameters and volumes (not shown) of the preovulatory follicles were standardized to the day when PGF<sub>2α</sub> 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 1).

When serum concentrations of estradiol-17β (not shown) were expressed relative to days since PGF<sub>2α</sub> treatment, no differences were evident among treatments. Patterns of luteal regression, as assessed by decreasing

concentrations of progesterone at 24 and 48 hr after PGF<sub>2α</sub> (not shown), were not different when expressed relative to day of PGF<sub>2α</sub> treatment injection. All 32 cows subsequently ovulated and formed new CL (Table 1) after treatment, as is further evidenced by increased concentrations of progesterone in serum at 14 and 21 days after the first GnRH injection (not shown).

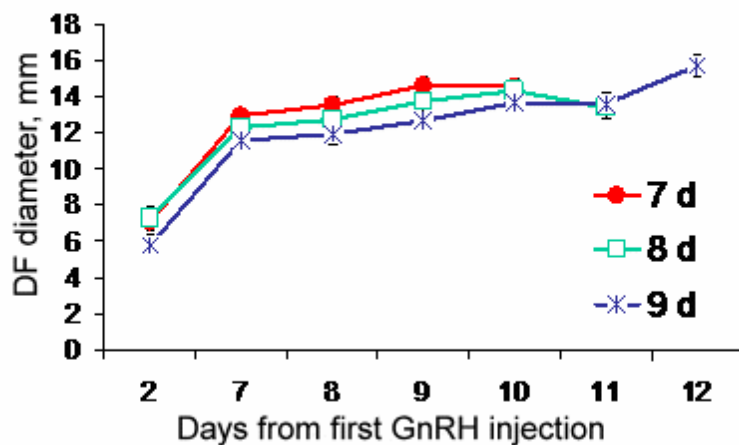
Two cows each in the 7- and 8-day treatments were inseminated 24 hr late (not according to protocol). Of the remaining cows in each of these 2 treatments, 5 of 9 cows (55.6%) conceived in the 7-day treatment, and 5 of 9 cows (55.6%) conceived in the 8-day treatment, compared with only 1 of 10 cows (10%) in the 9-day treatment. Conception rates of individual 7- and 8-day treatments tended ( $P = 0.07$ ) to differ from that of the 9-day 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. Although too few cows were inseminated to test differences in conception rates, reproductive outcomes may not be reduced when PGF<sub>2α</sub> injection is delayed by 24 hr from the standard 7-day period between GnRH and PGF<sub>2α</sub> injections, but 48 hr may be too long to prevent a potentially reduced conception rate.

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

**Table 1. Largest Diameter and Volume of Preovulatory Follicles that Ovulated After Treatment in Response to the Second GnRH Injection, and Resulting Ovulation Rate**

Item <sup>1</sup>	Interval Between First GnRH Injection and PGF <sub>2α</sub> , days		
	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 <sup>3</sup>	1526 ± 62	1479 ± 97	1490 ± 69
Ovulation rate	1.2 ± 0.1	1.1 ± 0.1	1.3 ± 0.2

<sup>1</sup>Assessed 72 hr after PGF<sub>2α</sub> in each treatment.



**Figure 1. Changes in Average Diameter of the Dominant Follicle that Eventually Ovulated After Each of 3 Treatments in Which PGF<sub>2α</sub> was Administered Either 7 Days (n = 13), 8 Days (n = 9), or 9 Days (n = 10) After the First GnRH Injection (d 0).**