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M.A. Portaluppi

D.E. Tenhouse

Jeffrey S. Stevenson

DELAYING INJECTION OF PROSTAGLANDIN $F_{2\alpha}$ IN AN OVSYNCH PROTOCOL

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

Summary

Our objective was to determine whether delaying the $PGF_{2\alpha}$ 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 $PGF_{2\alpha}$. 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 $PGF_{2\alpha}$ 7, 8, or 9 days later. Timed artificial insemination (TAI) was performed at 48 hr after $PGF_{2\alpha}$, 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 $PGF_{2\alpha}$ injection, and daily thereafter through 72 hr after $PGF_{2\alpha}$ to monitor ovulation of preovulatory follicles. Blood was collected on day 0, day 2, at $PGF_{2\alpha}$ injection, and at 24 and 48 hr after $PGF_{2\alpha}$, to monitor serum changes estradiol-17ß 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 $PGF_{2\alpha}$ 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 $PGF_{2\alpha}$ injections, the diameter (mm) and volume (mm^3) 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-17ß 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 $PGF_{2\alpha}$ 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 $PGF_{2\alpha}$, 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 $F_{2\alpha}$ ($PGF_{2\alpha}$) 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 $PGF_{2\alpha}$ 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 $PGF_{2\alpha}$) was first demonstrated in dairy heifers, and later in lactating dairy cattle.

Once a new dominant follicle is selected, concentrations of estradiol-17ß increase, LH pulses increase, and the selected dominant follicle becomes the preovulatory follicle. Maximum concentrations of estradiol-17ß 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-17ß in lactating cows may occur when using the Ovsynch protocol if $PGF_{2\alpha}$-induced luteolysis is delayed after the first GnRH injection. Our objective was to determine whether lengthening the interval between the first GnRH injection and $PGF_{2\alpha}$ from 7 to 8 or 9 days might result in greater serum concentrations of estradiol-17ß 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 ± 0.1. Daily test-day milk yield of these cows nearest to the day of first AI averaged 109 ± 4 lb (3.5% fat and 3.0% protein) and 305-d mature equivalent yield averaged 33,854 ± 946 lb. Cows were housed in covered free stalls bedded with sand, and were fed a TMR 3 times daily.

Beginning at 65 ± 2 days postpartum, estrous cycles were synchronized in lactating dairy cows (average body weight = 1,559 ± 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 $PGF_{2\alpha}$ (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 $PGF_{2\alpha}$ at days 7, 8, or 9 after the first GnRH injection (day 0 was 81 ± 2 days postpartum).

Inseminations were administered at 48 hr after $PGF_{2\alpha}$ (91 ± 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

$PGF_{2\alpha}$ treatment and continuing through 72 hr after $PGF_{2\alpha}$, 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 $PGF_{2\alpha}$ treatment and continuing through 72 hr after $PGF_{2\alpha;}$ on day 14; and on day 21. The samples were stored on ice, and serum concentrations of progesterone and estradiol-17ß 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 $PGF_{2\alpha}$, did not differ among treatments (Table 1). When diameters and volumes (not shown) of the preovulatory follicles were standardized to the day when $PGF_{2\alpha}$ 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-17ß (not shown) were expressed relative to days since $PGF_{2\alpha}$ treatment, no differences were evident among treatments. Patterns of luteal regression, as assessed by decreasing concentrations of progesterone at 24 and 48 hr after $PGF_{2\alpha}$ (not shown), were not different when expressed relative to day of $PGF_{2\alpha}$ 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 $PGF_{2\alpha}$ injection is delayed by 24 hr from the standard 7-day period between GnRH and $PGF_{2\alpha}$ 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 $PGF_{2\alpha}$, failed to increase serum concentrations of estradiol-17ß or diameter of that dominant follicle. Further study is warranted to verify whether delaying $PGF_{2\alpha}$ injections by 24 h has no effects on fertility and whether $PGF_{2\alpha}$ 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

	Interval Between First GnRH Injection and $PGF_{2\alpha}$, days		
Item[1]	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	1526 ± 62	1479 ± 97	1490 ± 69
Ovulation rate	1.2 ± 0.1	1.1 ± 0.1	1.3 ± 0.2

[1]Assessed 72 hr after $PGF_{2\alpha}$ in each treatment.

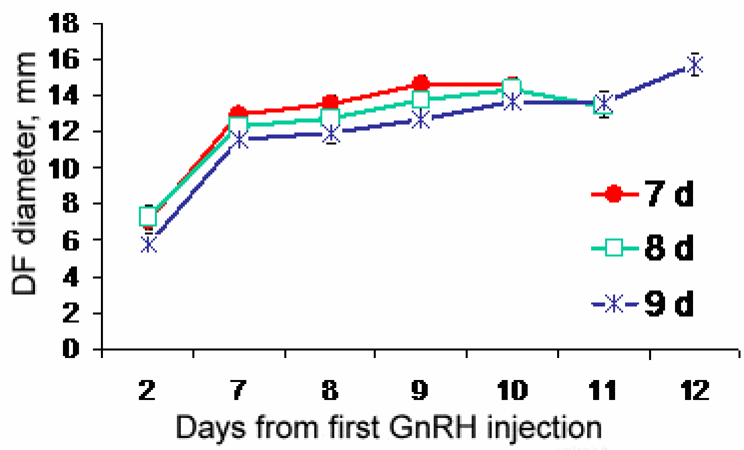

Figure 1. Changes in Average Diameter of the Dominant Follicle that Eventually Ovulated After Each of 3 Treatments in Which $PGF_{2\alpha}$ was Administered Either 7 Days (n = 13), 8 Days (n = 9), or 9 Days (n = 10) After the First GnRH Injection (d 0).