J. Dairy Sci. 90:1281–1288

Ovarian Traits After Gonadotropin-Releasing Hormone-Induced Ovulation and Subsequent Delay of Induced Luteolysis in an Ovsynch Protocol[1]

J. S. Stevenson,[2] M. A. Portaluppi, and D. E. Tenhouse
Department of Animal Sciences and Industry, Kansas State University, Manhattan 66506-0201

ABSTRACT

Our objective was to determine whether delaying the $PGF_{2\alpha}$ 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 $PGF_{2\alpha}$ (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 $PGF_{2\alpha}$ 7, 8, or 9 d later. Timed artificial insemination was performed at 48 h after $PGF_{2\alpha}$ 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 $PGF_{2\alpha}$ injection; and daily thereafter through 72 h after $PGF_{2\alpha}$ to monitor ovulation of preovulatory follicles. Blood was collected on d 0, 2, at $PGF_{2\alpha}$ injection, and at 24 and 48 h after $PGF_{2\alpha}$ 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 $PGF_{2\alpha}$ 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 $PGF_{2\alpha}$ injections, the diameter (mm) and volume (mm^3) 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 $PGF_{2\alpha}$ 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 $PGF_{2\alpha}$, 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 $PGF_{2\alpha}$ 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 $PGF_{2\alpha}$-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 $PGF_{2\alpha}$) 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

Received July 26, 2006.
Accepted October 11, 2006.
[1]Contribution no. 07-004-J, Kansas Agricultural Experiment Station, Manhattan.
[2]Corresponding author: jss@k-state.edu

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 $PGF_{2\alpha}$-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 (Sangsritavong 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 $PGF_{2\alpha}$-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 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 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.

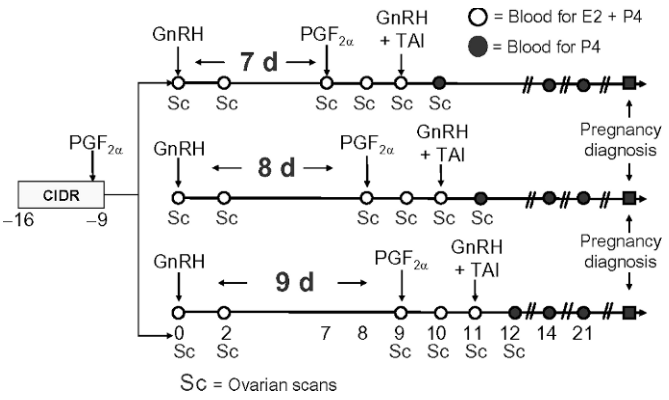

Figure 1. Experimental design for presynchronizing estrous cycles in lactating dairy cows before applying treatments. All cows received a controlled internal drug release (CIDR) insert for 7 d, with an injection of $PGF_{2\alpha}$ given 24 h before removal of the CIDR insert. Nine days after CIDR insert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

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 $PGF_{2\alpha}$ (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 $PGF_{2\alpha}$ at d 7, 8, or 9 after the first GnRH injection (d 0; 81 ± 2 DIM).

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