# Alternative programs to presynchronize estrous cycles in dairy cattle before a timed artificial insemination program<sup>1</sup>

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#### **ABSTRACT**

The objective was to test potential presynchronization programs applied to cows before a timed artificial insemination (TAI) program to increase the percentage of cows ovulating in response to both GnRH injections of a TAI program and having a functional corpus luteum before the first GnRH injection of the TAI program. At calving, cows were blocked by lactation (1 vs. 2+) and assigned randomly to receive 1 of 5 presynchronization treatments. Two variants of the standard Presynch program were tested in which 2 injections of  $PGF_{2\alpha}$  were administered 14 d apart with either 14 d (Pre14; n = 122), 12 d (Pre12; n = 123), or 10 d (Pre10; n = 151) intervening before a TAI program was initiated. Two other presynchronization programs consisted of administering a progesterone-releasing controlled internal drug release (CIDR) insert for 7 d plus PGF<sub>20</sub> administration at insert removal. Insert removal occurred either 10 d (CIDR10; n = 157) or 3 d (CIDR3; n = 117) before a TAI program was initiated. The TAI program was a standard Cosynch program [injection of GnRH 7 d before (GnRH-1) and 72 h after (GnRH-2)  $PGF_{2\alpha}$  with TAI administered 72 h after  $PGF_{2\alpha}$ ). Cosynch served as the control (n = 157), and cows were assumed to be starting this program at random stages of the estrous cycle. From a subset of cows per treatment (ranging from 49 to 51 cows each), blood samples were collected from coccygeal vessels by using evacuated tubes at d-28, -14, 0 (onset of TAI program), 7, 9, 14, and 21. Ovarian scans were conducted on d 0, 7, 9, 14, and 21 by transrectal ultrasonography. Diameters of follicles and corpus luteum were measured at each exam, and ovulation was determined on d 7 (response to GnRH-1 on d 0) and d 14 (response to GnRH-2 on d 10). Ovulatory incidence after GnRH-1 (47.1 to

Received April 26, 2010.

Accepted September 20, 2010.

67.3%) and GnRH-2 (78 to 90.2%) varied but did not differ among treatments. Before GnRH-1, progesterone concentrations were less in the CIDR3 treatment than in all other treatments. Before GnRH-2, progesterone was greater in the CIDR3 treatment than in all other treatments. Luteal regression and synchronization rate (successful luteolysis and ovulation after GnRH-2) did not differ among treatments. Pregnancy rate per AI at 32 and 60 d post TAI was less in CIDR3 cows than in cows in all other treatments. None of the Presynch treatments improved key responses (ovulation, luteolysis, and synchronization rate) known to improve fertility compared with a standard Cosynch program without presynchronization.

Key words: luteolysis, ovulation, timed AI

### INTRODUCTION

When timed artificial insemination (TAI) programs were first attempted using Ovsynch [injection of GnRH 7 d before (GnRH-1) and 48 h (GnRH-2) after PGF<sub>2 $\alpha$ </sub> with TAI administered 16 to 20 h after GnRH-2], lactating dairy cows treated with this program between d 5 and 12 of the estrous cycle had a pregnancy rate per AI (PR/AI) advantage relative to cows treated at other stages of the cycle (Vasconcelos et al., 1999). On the basis of the hypothesis that fertility after a TAI program was related to the stage of the estrous cycle, presynchronization of estrous cycles was attempted before the Ovsynch program by using 2 injections of PGF<sub>20</sub> administered 14 d apart (Presynch). The second Presynch injection given 12 d before the onset of the TAI program (Moreira et al., 2001) resulted in a larger proportion of cows between d 5 and 12 of the estrous cycle at the onset of the TAI program. These cows had a significant improvement in PR/AI compared with cows at random stages of the estrous cycle (Moreira et al., 2001) and cows in subsequent experiments in which estrous cycles were presynchronized after administration of 1 (Cartmill et al., 2001) or 2 presynchronizing injections of  $PGF_{2\alpha}$  (El-Zarkouny et al., 2004; Navanukraw

 $<sup>^{1}\</sup>mathrm{Contribution}$  number 10-316-J from the Kansas Agricultural Experiment Station, Manhattan 66506.

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et al., 2004; Galvão et al., 2007). In the latter 2 studies, the interval from the second Presynch injection to onset of the TAI program was 14 d rather than 12 d. In other experiments, however, single injections of  $PGF_{2\alpha}$  given 3 d (Meyer et al., 2007), 10 d (LeBlanc and Leslie, 2003), or 12 d (Cartmill et al., 2001) before initiating a TAI program failed to improve PR/AI.

In subsequent experiments initiated after the present study, presynchronization of estrous cycles accomplished by injecting  $PGF_{2\alpha}$  and GnRH (Bello et al., 2006) or by using an Ovsynch program before the TAI Ovsynch program (Souza et al., 2008) resulted in improved PR/AI. Applying an intravaginal progesterone insert (Chebel et al., 2006; Bicalho et al., 2007; Rutigliano et al., 2008) at various intervals before initiating a TAI program to appropriately stage the estrous cycle failed to improve PR/AI compared with a standard Presynch program but may have benefitted some anovulatory cows (Chebel et al., 2006) by inducing estrous cycles without affecting fertility (Cerri et al., 2009).

Improved PR/AI in TAI programs is associated with (1) greater ovulation response to the first GnRH injection of Ovsynch (Vasconcelos et al., 1999; Bello et al., 2006), (2) more cows having a functional corpus luteum (CL) at the first GnRH injection (Galvão and Santos, 2010), (3) greater ovulatory response to the first GnRH injection increasing ovulatory response to the second GnRH injection (Rutigliano et al., 2008), and (4) greater ovulation response after TAI (Galvão and Santos, 2010). Researchers concluded that presynchronization with 2 injections of  $PGF_{2\alpha}$  (Presynch) and initiation of the Ovsynch 14 d later decreased ovulatory responses to the first and second GnRH injections. Therefore, a 14-d interval between presynchronization and the first GnRH injection might not be adequate to optimize fertility in dairy cows (Galvão and Santos, 2010).

The objective was to test potential presynchronization programs administered before a TAI program that might improve the percentage of cows having a functional CL, having elevated progesterone concentrations, and successfully ovulating in response to both GnRH injections of a TAI Ovsynch-like program. The hypothesis was that reducing the interval from the second Presynch  $PGF_{2\alpha}$  injection from 14 to 10 d would increase the proportion of cows ovulating in response to GnRH-1. Further, applying progesterone via a controlled internal drug release (CIDR) insert for 7 d and removing the CIDR 10 d before GnRH-1 might have favorable effects on anovulatory or anestrous cows and enhance responses to a TAI program. In contrast, applying a CIDR for 7 d and removing it 3 d before initiation of a TAI program would produce unfavorable effects on responses to ovulation synchronization, including fertility.

#### **MATERIALS AND METHODS**

#### **Experimental Cows**

The current studies were approved by the Kansas State University Institutional Animal Care and Use Committee. Lactating Holstein cows were enrolled between October 2004 and January 2009 at the Kansas State University Dairy Teaching and Research Center. Cows were housed in covered freestalls and fed twice or thrice (summer) daily a TMR calculated to meet nutrient requirements for lactating dairy cows producing 50 kg of 3.5% milk (NRC, 2001). The diet consisted of alfalfa hay, corn silage, soybean meal, whole cotton seed, corn or milo grain, sweet bran, vitamins, and minerals. Cows were milked twice daily and treated with recombinant bST according to label directions.

#### Experimental Design

At calving, cows were blocked by lactation number (1 vs. 2+) and assigned randomly to receive 1 of 5 presynchronization treatments (Figure 1). Two permutations of the standard Presynch program were tested in which 2 injections of  $PGF_{2\alpha}$  (5 mL of Lutalyse Sterile Solution; Pfizer Animal Health, New York, NY) were administered 14 d apart with either 14 d (Pre14; n = 122), 12 d (**Pre12**; n = 123), or 10 d (**Pre10**; n = 151) intervening before a TAI program was initiated. The rationale for decreasing the number of intervening days between the second  $PGF_{2\alpha}$  injection and the onset of the TAI program was to place more cows at a time in their cycle when the first-wave dominant follicle would be likely to ovulate in response to GnRH-induced LH release (Vasconcelos et al., 1999; Moreira et al., 2000). Figure 1 shows the predicted days of the estrous cycle for these treatments. Two other presynchronization programs consisted of administering a progesteronereleasing Eazi-Breed CIDR cattle insert (Pfizer Animal Health) containing 1.38 g of progesterone for 7 d plus PGF<sub>20</sub> administration at insert removal. Insert removal occurred either 10 d (CIDR10; n = 157) or 3 d (CIDR3; n = 117) before a TAI program was initiated. The TAI program was a standard Cosynch program [100 µg of GnRH 7 d before (GnRH-1) and 72 h after (GnRH-2) 25 mg of  $PGF_{2\alpha}$  with TAI administered 72 h after  $PGF_{2\alpha}$ ]. Cows administered Cosynch served as the control (n = 157), and cows were assumed to be starting this program at random stages of the estrous cycle. The CIDR3 treatment was a negative control; the hypothesis was that these cows would be at a disadvantage by starting the TAI program near estrus in a low-progesterone environment. Body condition scores were assigned on median d -28. The GnRH products

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