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Concentrations of luteinizing hormone and ovulatory responses in dairy cows before timed artificial insemination[1]

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ABSTRACT

The objective was to determine the incidence of LH surges and ovulatory responses in lactating dairy cows enrolled in a timed artificial insemination (TAI) program. Cows were assigned randomly to 2 presynchronization treatments: (1) Pre10 (n = 37): 2 injections of $PGF_{2\alpha}$ (PG; PG-1 and PG-2) 14 d apart (Presynch); or (2) PG-3-G (n = 33): one 25-mg injection of PG (Pre-PG) administered 3 d before a 100-μg GnRH injection (Pre-GnRH). Ten days after PG-2 or Pre-PG, all cows were enrolled in a 7-d Ovsynch TAI program [injection of GnRH (GnRH-1) 7 d before PG (PG-3) and GnRH (GnRH-2) administered at either 56 or 72 h after PG-3; TAI at 72 h]. Blood was collected to determine LH at (1) Pre-GnRH: 48 to 80 h after PG-2 and hourly from 72 to 78 h (Pre-GnRH at 72 h); (2) GnRH-1: 0 to 6 h after GnRH-1; and (3) GnRH-2: 48 to 80 h after PG-3 and hourly from 56 to 62 h or 72 to 78 h for cows injected with GnRH-2 at 56 or 72 h after PG-3, respectively. Ovaries were scanned and pregnancy per TAI (P/AI) was diagnosed 31 and 61 d post-TAI by transrectal ultrasonography. The Pre-GnRH injection increased the incidences of LH surges (100 vs. 43%) and ovulation (91 vs. 60%) and subsequent concentrations of progesterone in PG-3-G cows compared with Pre10 cows, respectively. Seven days later, incidence of ovulation (48 to 62%) and occurrence of LH surges (100%) did not differ between treatments after GnRH-1. In contrast, LH concentrations and area under the LH curve of Pre10 cows were greater than that of PG-3-G cows because progesterone was greater in PG-3-G than in Pre10 cows (4.6 ± 0.4 vs. 2.8 ± 0.4 ng/mL), respectively. Concentrations of LH did not differ after GnRH-2 at either 56 or 72 h; however, 1 cow receiving GnRH-2 at 56 h and 3 cows at 72 h had early spontaneous LH surges before GnRH-2. Ovulation was suppressed overall in 210 blood collection windows in cows with elevated progesterone concentrations. When progesterone was <1 ng/mL after either PG-2 or PG-3 injections, GnRH-induced LH surges occurred in more than 90% of cows, and incidence of ovulation exceeded 80%. Pregnancy per AI tended to differ for PG-3-G (56.7%) compared with Pre10 (37.8%) and for 56 h (54.5%) compared with 72 h (38.2%), with the Pre10–72 h treatment combination producing less than half (22.2%) the pregnancies compared with all other treatment combinations. Furthermore, in these same cows, post-TAI luteal tissue volume tended to be compromised. We conclude that incidences of GnRH-induced LH surges and ovulation are suppressed in cows with elevated progesterone, possibly contributing to some loss in P/AI in TAI programs.
Key words: luteal function, luteinizing hormone, ovulation, presynchronization, progesterone

INTRODUCTION

Synchronization of ovulation to allow for timed artificial insemination (**TAI**) has become one of the most adopted reproductive technologies by dairy producers (Caraviello et al., 2006; Moeller et al., 2010). The most commonly used TAI programs in the dairy industry are variants of the original Ovsynch protocol [Pursley et al., 1998; injection of GnRH 7 d before and 48 h after $PGF_{2\alpha}$ (**PG**) with TAI 16 h following the last GnRH injection]. Presynchronizing the estrous cycles of cows improves pregnancy per AI (**P/AI**) compared with cows starting Ovsynch at random stages of the estrous cycle (Moreira et al., 2001; El-Zarkouny et al., 2004; Navanukraw et al., 2004). Improved P/AI resulting from presynchronization programs before Ovsynch has been associated with synchronizing the majority of estrous cycles to d 5 through 12, which improved ovulation incidence to the first GnRH injection of Ovsynch and resulting P/AI compared with cows treated at random stages of the estrous cycle (Vasconcelos et al., 1999).

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A standard PG-presynchronization protocol involves 2 PG injections given 14 d apart, with initiation of the Ovsynch protocol 12 d later (14 × 12; Moreira et al., 2001). Other intervals between the second PG Presynch injection and the onset of Ovsynch, 14 d (14 × 14; Navanukraw et al., 2004), 11 d (14 × 11; Galvão et al., 2007), and 10 d (14 × 10; Stevenson et al., 2012), have been investigated. The 14 × 11 Presynch program increased P/AI compared with Presynch 14 × 14 (Galvão et al., 2007).

Other presynchronization programs including PG and GnRH (PG-3-G: Peters and Pursley, 2002; Stevenson et al., 2012; Stevenson and Pulley, 2012; or G-6-G: Bello et al., 2006; Ribeiro et al., 2011) and application of a nonbreeding Ovsynch-like treatment before the TAI Ovsynch program (Double Ovsynch; (Souza et al., 2008; Ribeiro et al., 2011; Herlihy et al., 2012) has generally increased P/AI compared with Ovsynch alone or with other Presynch-PG programs. Presynchronization programs that include GnRH improved ovulatory responses before Ovsynch (Stevenson et al., 2012), increased the proportion of cows having progesterone concentrations ≥1 ng/mL at GnRH-1 (Herlihy et al., 2012; Stevenson et al., 2012; Ayres et al., 2013), increased the number of CL in anovular cows at GnRH-1 (Herlihy et al., 2012; Stevenson et al., 2012), and increased P/AI during summer (Stevenson and Pulley, 2012) compared with current Presynch-PG programs.

Despite wide application of TAI programs, relatively little is known about the characteristics of LH concentrations or incidences of LH surges in association with the PG-induced luteolysis and GnRH injections associated with these TAI programs. Concentrations of LH in response to either 50 or 100 μg of different GnRH products have been reported (Souza et al., 2009). One product tended to release less LH after both doses and induce fewer ovulations than the other 3 products in diestrous lactating dairy cows treated 7 d after TAI (Souza et al., 2009). Furthermore, this same GnRH product stimulated fewer ovulations and less LH release in beef heifers compared with another GnRH product (Martinez et al., 2003). In virgin heifers and lactating dairy cows treated with 2 injections of PG 11 d apart with GnRH administered 72 h after the last PG injection, LH surges were detected in all females, with 39% being induced by GnRH and 61% occurring spontaneously (Lucy and Stevenson, 1986). It is not known what proportion of LH surges occur before or after GnRH injections in various TAI programs. In addition, the relationship of the characteristics of LH concentrations associated with each GnRH injection and the steroid milieu had not been determined when this study was initiated during autumn 2011.

Thus, this study was designed to determine the incidence of spontaneous and predictable GnRH-induced LH surges (peak LH magnitude, area under the LH secretion curve, and time to peak LH concentration) and subsequent ovulation in lactating dairy cows enrolled in a TAI program preceded by presynchronization of estrous cycles. Ancillary measures of progesterone, estradiol, and ovarian structures were also made to confirm our previous findings in response to the same 2 treatments (Stevenson et al., 2012) as well as improved P/AI in a previous large 4-herd study (Stevenson and Pulley, 2012).

MATERIALS AND METHODS

Cows, Housing, and Diets

The current studies were approved by the Kansas State University Institutional Animal Care and Use Committee. Lactating Holstein cows were enrolled at calving from September 2011 through March 2012 at the Kansas State University Dairy Teaching and Research Center. Cows were considered to be structurally sound and were housed individually in a tiestall barn equipped with feed bunks, automatic waterers, and stall mats covered with wood shavings. Cows were moved to a double-6 herringbone parlor and milked thrice daily. Cows were fed individually ad libitum twice daily at 0630 and 1600 h. A TMR calculated to meet nutrient requirements for lactating dairy cows producing 50 kg of 3.5% milk (NRC, 2001) consisted of alfalfa hay, corn silage, soybean meal, whole cottonseed, corn or milo grain, corn gluten feed, vitamins, and minerals. Cows were evaluated daily for health status by trained farm personnel.

Experimental Design and Treatments

A total of 70 cows were enrolled in 10 weekly clusters according to calving date and were assigned randomly within lactation number (1 vs. ≥2) to receive 1 of 2 presynchronization treatments (Figure 1). The first presynchronization treatment (**PG-3-G**; n = 33; Stevenson and Pulley, 2012; Stevenson et al., 2012) consisted of a 25-mg injection (i.m.) of $PGF_{2\alpha}$ (**Pre-PG**; 5 mL Lutalyse Sterile Solution, Zoetis, Florham Park, NJ) 3 d before a 100-μg (i.m.) injection of GnRH (**Pre-GnRH**; 2 mL Factrel, Zoetis). The second treatment (**Pre10**; n = 37) was timed so that the second of 2 (administered 14 d apart) 25-mg injections (i.m.) of PG (**PG-2**; 5 mL Lutalyse Sterile Solution, Zoetis) was administered on the same day as the Pre-PG injection of the PG-3-G treatment (Figure 1). Beginning