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Pregnancy per artificial insemination in lactating dairy cows subjected to 2 different intervals from presynchronization to initiation of Ovsynch protocol

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ABSTRACT

A protocol for presynchronization of ovarian status with 2 injections of $PGF_{2\alpha}$ given 14 d apart, with the last $PGF_{2\alpha}$ injection given 12 or 14 d before Ovsynch increases pregnancy per artificial insemination (P/AI) in dairy cows. We determined the efficacy of reducing the interval from the last $PGF_{2\alpha}$ injection (500 μg of cloprostenol) of presynchronization to initiation of Ovsynch on response to treatment and P/AI. Lactating dairy cows were assigned to an Ovsynch protocol, with the initial injection of GnRH given either 9 (PRE-9; n = 135) or 12 d (PRE-12; n = 135) after the second $PGF_{2\alpha}$ injection of presynchronization. The Ovsynch protocol consisted of 2 injections of 100 μg of GnRH given 9 d apart and 1 injection of $PGF_{2\alpha}$ given 7 d after the initial GnRH injection, and cows were subjected to timed artificial insemination (TAI; 70 ± 3.5 DIM) approximately 16 h after the second GnRH injection. Body condition score (1–5 scale) was recorded at TAI. Blood samples were taken for progesterone determination at the $PGF_{2\alpha}$ injection of Ovsynch, at TAI, and at 11 d after TAI. Ultrasonographic examinations were done in all cows at the second $PGF_{2\alpha}$ injection of presynchronization, initial GnRH injection, $PGF_{2\alpha}$ injection of Ovsynch, at TAI, and 24 h after TAI for cyclicity status and ovarian responses to treatments, and at 32 and 60 d after TAI for confirmation of pregnancy. Overall, 29 cows (10.7%) were determined acyclic or cystic and excluded from the study. The percentage of cows responding to initial GnRH injection (62.2 vs. 61.5%) did not differ between PRE-9 and PRE-12 but more cows in the PRE-9 group failed to respond to $PGF_{2\alpha}$ treatment of Ovsynch compared with PRE-12 (22.7 vs. 10.7%). Body condition score at TAI (2.9 ± 0.02) and mean ovulatory follicle diameter (16.4 ± 0.2 mm) were not different between treatments. Overall P/AI at 32 d was reduced in PRE-9 (33.6%) compared with PRE-12 (44.3%) but pregnancy losses (5.0 vs. 3.7%) did not differ between treatments. Primiparous cows in the PRE-12 group had higher mean progesterone concentration 11 d after TAI and greater P/AI 32 after TAI than primiparous cows in the PRE-9 group (6.4 ± 0.5 vs. 4.6 ± 0.5 ng/mL and 55.8 vs. 30.0%, respectively). In conclusion, reducing the interval from the last $PGF_{2\alpha}$ injection of the presynchronization treatment to initiation of Ovsynch (from 12 to 9 d) did not affect ovulatory response to initial GnRH injection but reduced response to $PGF_{2\alpha}$ injection of Ovsynch and P/AI at 32 and 60 d after TAI. The reduction in P/AI was particularly evident in primiparous cows of the PRE-9 group.

Key words: presynchronization, Ovsynch, dairy cow, pregnancy per artificial insemination

INTRODUCTION

Protocols that synchronize ovulation (e.g., Ovsynch) to facilitate timed AI (**TAI**) are available management tools to improve reproductive performance in lactating dairy cows, as they increase the insemination risk (Pursley et al., 1997). However, Ovsynch does not improve conception rate mainly because the percentage of cows with a regressed corpus luteum (**CL**) and ovulating within 24 after TAI in Ovsynch-treated cows is only about 70% (Colazo et al., 2009, 2013). The stage of the estrous cycle at which Ovsynch protocol is initiated affects the synchronization rate (Vasconcelos et al., 1999; Moreira et al., 2000). To initiate the Ovsynch protocol in the most favorable stage of the estrous cycle, a presynchronization strategy that involves 2 injections of $PGF_{2\alpha}$, each given 12 d apart, has been developed (Moreira et al., 2001). Indeed, pregnancy per AI (**P/AI**) was increased when Ovsynch was initiated 12 (Moreira et al., 2001; El-Zarkouny et al., 2004) or 14 d (Navanukraw et al., 2004) after the last $PGF_{2\alpha}$ injection of the presynchronization protocol compared with Ovsynch, followed by TAI in randomly cycling lactating dairy cows.

It seems that synchronization and, eventually, pregnancy outcome to Ovsynch is influenced by the ovulatory response to initial GnRH injection (Vasconcelos et al., 1999; Bello et al., 2006; Galvão et al., 2007). Sev-

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eral factors may determine whether ovulation occurs in response to GnRH injection. The most important is perhaps the day of the estrous cycle (Martínez et al., 1999; Vasconcelos et al., 1999; Moreira et al., 2000; Bello et al., 2006). High progesterone concentrations suppressed pituitary release of LH and reduced the ovulatory response following the administration of GnRH, indicating that circulating progesterone concentration can influence ovulation response to initial GnRH injection of Ovsynch, regardless of the stage of the estrous cycle (Colazo et al., 2008).

Vasconcelos et al. (1999) has shown that dairy cows at mid cycle (d 5 to 9 of the estrous cycle) have a greater probability of ovulating to GnRH injection than cows early (d 1 to 4) or late (d 10 to 16) in the cycle. Similarly, Galvão et al. (2007) has shown that initiation of a TAI protocol (i.e., Heatsynch) on approximately d 5 to 8 of the estrous cycle compared with d 8 to 11 increased the proportion of lactating dairy cows ovulating to initial GnRH injection (61.4 vs. 40.5%). Moreover, Bello et al. (2006) reported that ovulation rate to initial GnRH injection in dairy cows treated 3, 4, or 5 d after induced ovulation were 56, 67, and 85%, respectively. In 2 other studies with heifers, the ovulatory response to GnRH was optimized 3 to 5 d after ovulation (Martínez et al., 1999; Moreira et al., 2000). Therefore, controlling the interval from the second $PGF_{2\alpha}$ injection of presynchronization to initial GnRH injection, to have most cows between d 3 and 6 of the estrous cycle at initiation of Ovsynch, might increase ovulatory response to the first GnRH injection. We hypothesized that reducing the interval between the last $PGF_{2\alpha}$ injection of presynchronization and initiation of Ovsynch treatment from 12 to 9 d would further increase ovulatory response to initial GnRH and subsequent P/AI in lactating dairy cows. Consequently, we compared ovarian responses, P/AI, and pregnancy loss in lactating dairy cows subjected to Ovsynch protocol initiated either 9 or 12 d after the second $PGF_{2\alpha}$ injection of the presynchronization protocol.

MATERIALS AND METHODS

All animal procedures were approved by the Animal Care and Use Committee for Livestock (University of Alberta, Edmonton, AB, Canada), and conducted in accordance with the guidelines of the Canadian Council on Animal Care (1993).

Animals and Experimental Design

This study was conducted with lactating Holstein cows from the Dairy Research Unit, University of Alberta, with an annual rolling herd average of 10,370 kg of milk. Cows were housed in tie-stalls and had unrestricted access to water. They were fed individual rations once daily at approximately 0900 h, allowed 2 h of exercise during the day, and milked twice daily between 0400 and 0600 h and 1530 and 1730 h. Cows received a TMR formulated for lactating dairy cows according to NRC (2001) guidelines. Main ingredients were silage (barley and alfalfa), grain (barley or corn), hay (alfalfa or grass), and mineral supplements. Body condition score was determined before TAI by 1 individual using a scale of 1 (emaciated) to 5 (overconditioned; Edmonson et al., 1989).

Cows (n = 270) were blocked by parity and date of calving, and randomly assigned, every week, to 1 of 2 treatments. All cows received their first and second $PGF_{2\alpha}$ (500 μg of cloprostenol, Estrumate; Schering-Plough Animal Health, Pointe-Claire, QC, Canada) injections of the presynchronization protocol 14 d apart, with the first $PGF_{2\alpha}$ injection given approximately 5 wk after calving (Table 1). Cows were then subjected to an Ovsynch protocol (Pursley et al., 1997), initiated either 9 (**PRE-9**; n = 135) or 12 d (**PRE-12**; n = 135) after the last $PGF_{2\alpha}$ injection of presynchronization. Cows assigned to the PRE-9 group received their first $PGF_{2\alpha}$ injection of the presynchronization protocol 3 d after the first $PGF_{2\alpha}$ injection in cows assigned to the PRE-12 group. This staggered pattern of starting the presynchronization protocol was deliberately planned to ensure that TAI following the Ovsynch protocol occurred at the same DIM in both groups. The Ovsynch protocol consisted of 100 μg of GnRH (gonadorelin acetate; Fertiline, Vétoquinol NA Inc., Lavaltrie, QC, Canada) on d 0, followed 7 d later by a $PGF_{2\alpha}$ injection and 48 h later by a second GnRH injection, with TAI performed approximately 16 h after the second GnRH injection. All treatments were given i.m. and a trained technician performed all inseminations with frozen-thawed commercial semen. Treatment protocols and activities during this study are illustrated in Figure 1.

Ultrasonographic Examinations

Transrectal ultrasonography (Aloka-500V scanner equipped with a 7.5-MHz linear transducer; Aloka Co., Tokyo, Japan) was performed at second $PGF_{2\alpha}$ injection of presynchronization and during the Ovsynch protocol (e.g., at initial GnRH injection, at $PGF_{2\alpha}$ injection, at TAI, and 24 h later). The diameter and location of follicles and CL were recorded as previously described by Pierson and Ginther (1984). Cows with detectable luteal tissue at the second $PGF_{2\alpha}$ injection of presynchronization or first GnRH injection of Ovsynch, or both, were considered cyclic (Colazo et al., 2010). The