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Effect of timing of initiation of resynchronization and presynchronization with gonadotropin-releasing hormone on fertility of resynchronized inseminations in lactating dairy cows

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ABSTRACT

Lactating Holstein cows (n = 1,456) were randomized in a 2 × 2 factorial design to compare the main effects of day of initiation of resynchronization after artificial insemination (AI; 32 vs. 39 d) and presynchronization with GnRH 7 d before initiation of resynchronization on fertility to timed AI (TAI). This design resulted in the following 4 resynchronization treatments: (1) resynchronization (GnRH treatment, $PGF_{2\alpha}$ treatment 7 d later, GnRH treatment 56 h later, and TAI 16 h later), initiated 32 ± 3 d after AI; (2) presynchronization with 100 μg of GnRH 25 ± 3 d after AI and resynchronization initiated 32 ± 3 d after AI at nonpregnancy diagnosis; (3) resynchronization initiated 39 ± 3 d after AI (GPG39); and (4) presynchronization with 100 μg of GnRH 32 ± 3 d after AI at nonpregnancy diagnosis and resynchronization initiated 39 ± 3 d after AI. Overall, 344 cows were inseminated at estrus between enrollment (25 ± 3 d after AI) and TAI of the resynchronization treatments, and 1,112 cows received TAI. Progesterone (P4) was analyzed in blood samples collected from all cows at the first GnRH injection of the resynchronization protocols (G1), and ovarian structures were evaluated and blood samples were collected at G1, at the $PGF_{2\alpha}$ injection, and at the TAI of the resynchronization protocols in a subgroup of cows (n = 417). When analyzed as main effects, cows presynchronized with GnRH had more pregnancies per AI (P/AI) than nonpresynchronized cows (38.9 vs. 33.8%), whereas timing of initiation of resynchronization did not affect P/AI. Although cows with high P4 at G1 had greater P/AI than cows with low P4 (38.7 vs. 31.8%), presynchronization with GnRH did not increase the proportion of cows with high P4 (>1.0 ng/mL) at G1 but moved cows from a low-P4 environment to an intermediate-P4 level. Presynchronization with GnRH also decreased the percentage of cows with low P4 at the $PGF_{2\alpha}$ injection, thereby increasing synchrony to the protocol. Cows with high P4 at G1 had a decreased ovulatory response to G1 compared with cows with low P4 (40.9 vs. 69.1%), and cows that ovulated to G1 had decreased luteal regression after $PGF_{2\alpha}$ compared with cows that did not ovulate (78.5 vs. 87.3%). We conclude that presynchronization with GnRH 7 d before initiation of resynchronization increased fertility in dairy cows, whereas timing of initiation of resynchronization did not.
Key words: presynchronization, resynchronization, gonadotropin-releasing hormone, dairy cow

INTRODUCTION

Many confinement-based dairy systems in the United States have adopted systematic synchronization protocols that allow for timed AI (**TAI**) for first AI service, thereby reducing the impact of poor detection of estrus (Pursley et al., 1997; Caraviello et al., 2006). The strategy for resynchronization of ovulation on most dairy farms often relies on the timing of initiation of the Ovsynch protocol or variations of the Ovsynch protocol after insemination (Fricke et al., 2003; Bartolome et al., 2005; Sterry et al., 2006a). In most experiments, there are usually fewer pregnancies per AI (**P/AI**) to TAI after resynchronization compared with TAI at first service (Galvão et al., 2007; Silva et al., 2009; Thompson et al., 2010), indicating that fertility to TAI after resynchronization is not optimized.

Poor fertility of resynchronized cows is related to progesterone (**P4**) concentration at initiation of the resynchronization protocol. Between 15 and 26% of cows lack a corpus luteum (**CL**) or have low P4 at initiation of resynchronization (Fricke et al., 2003; Sterry et al., 2006b; Silva et al., 2009), resulting in development of the preovulatory follicular wave in a low-P4 environment. Development of follicles in a low-P4 environment is associated with reduced embryo quality (Rivera et al., 2011) and fertility (Folman et al., 1990; Wiltbank et al., 2012) in lactating dairy cows. Therefore, we hypothesized that presynchronization with GnRH 7 d before initiation of the resynchronization protocol would increase fertility by inducing ovulation in some

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cows, thereby increasing P4 7 d later at the first GnRH injection of the resynchronization protocol.

Many dairies start the resynchronization protocol after a negative pregnancy diagnosis 39 ± 3 d after AI or 32 ± 3 d after AI (Pursley et al., 1997; Stevenson et al., 2003); however, no data exist directly comparing fertility of cows that initiate the resynchronization protocol 32 ± 3 versus 39 ± 3 d after a previous AI. Based on a 23-d interovulatory interval in lactating cows (Sartori et al., 2004) and data supporting that the best time to start the Ovsynch protocol is during the early luteal phase, around 5 to 9 d after ovulation (Vasconcelos et al., 1999), we hypothesized that the ideal time to initiate resynchronization would occur between 28 to 32 d after a previous AI.

In the present study, we compared the main effects of (1) presynchronization with GnRH 7 d before initiating the resynchronization protocol and (2) initiating the protocol either 32 ± 3 or 39 ± 3 d after a previous insemination. Our hypotheses were (1) that P/AI would be greater for cows presynchronized with GnRH 7 d before initiation of resynchronization compared with cows not presynchronized and (2) that P/AI would be greater for cows initiating the resynchronization protocol at 32 ± 3 d compared with 39 ± 3 d after a previous insemination.

MATERIALS AND METHODS

Cows and Reproductive Management

This experiment was conducted in collaboration with a dairy farm located near Pickett (Wisconsin) milking approximately 8,000 cows. Cows were housed in cross-ventilated, freestall barns and were fed a TMR once daily to meet or exceed the dietary requirements for lactating Holstein cows weighing 680 kg and producing 45 kg of 3.5% FCM (NRC, 2001). Cows were milked 3 times daily at approximately 8-h intervals and received bovine somatotropin (bST; Posilac, 500 mg; Elanco Animal Health, Indianapolis, IN) starting at approximately 63 d postpartum and continuing every 14 d until cows were dried off.

Cows were submitted to a Presynch-Ovsynch protocol for first service AI as described by Navanukraw et al. (2004). Briefly, cows received 2 intramuscular injections of $PGF_{2\alpha}$ (25 mg i.m. of Dinoprost tromethamine, Lutalyse; Pfizer Animal Health, New York, NY) 14 d apart at 39 ± 3 and 53 ± 3 DIM. Cows detected in estrus based on tail chalk removal conducted daily after the second $PGF_{2\alpha}$ injection of the presynchronization protocol were inseminated by professional AI technicians, whereas cows not detected in estrus within 14 d of the second $PGF_{2\alpha}$ injection initiated an Ovsynch-56 protocol as described by Brusveen et al. (2008). Cows enrolled in the Ovsynch-56 protocol received the first GnRH injection (100 μg i.m. of Gonadorelin diacetate tetrahydrate, Fertagyl; Intervet Animal Health, Millsboro, DE), an injection of $PGF_{2\alpha}$ 7 d later (25 mg i.m. of Dinoprost tromethamine), and the second GnRH injection (100 μg i.m. of gonadorelin diacetate tetrahydrate) 56 h after the $PGF_{2\alpha}$ injection. Cows received a TAI approximately 16 to 20 h after the second GnRH injection.

Experimental Treatments

After first service and until cows were enrolled in the resynchronization of ovulation treatments, cows were detected in estrus based on removed tail chalk and were inseminated as described previously. Each week, a cohort of cows at various DIM and having at least 1 AI service were blocked by parity and assigned to a 2 × 2 factorial design 25 ± 3 d after AI (if not detected in estrus and inseminated) to test the main effects of day of initiation of the resynchronization protocol after AI (32 ± 3 vs. 39 ± 3 d) and the effect of presynchronization with GnRH 7 d before initiation of resynchronization. Throughout the experiment, cows detected in estrus either before or after enrollment were inseminated. The number of cows enrolled into the 4 resynchronization treatments and the number of cows receiving TAI for each treatment differed because the interval from AI to initiation of resynchronization differed among treatments. As the interval from AI to initiation of resynchronization increased, the number of cows inseminated at a detected estrus increased and the number of cows completing the protocols and receiving TAI decreased (Table 1).

Four experimental treatments resulted from the 2 × 2 factorial design (Figure 1) as follows: (1) **GGPG32** cows (n = 384) were presynchronized with GnRH (100 μg) at 25 ± 3 d after AI, nonpregnancy diagnosis was performed 32 ± 3 d after AI, and nonpregnant cows were submitted for resynchronization of ovulation with the Ovsynch-56 protocol (GnRH treatment, $PGF_{2\alpha}$ treatment 7 d later, and GnRH treatment 56 h later) and received TAI 16 to 20 h later (n = 335); (2) **GPG32** cows (n = 378) were diagnosed for pregnancy 32 ± 3 d after AI and nonpregnant cows were submitted to the Ovsynch-56 protocol to receive their next TAI (n = 289); (3) **GGPG39** cows (n = 356) were submitted for nonpregnancy diagnosis 32 ± 3 d after AI and nonpregnant cows were presynchronized with GnRH (100 μg) and 7 d later were submitted to the Ovsynch-56 protocol to receive their next TAI (n = 269); and (4) **GPG39** cows (n = 338) were diagnosed for pregnancy 32 ± 3 d after AI and nonpregnant cows