



Resynchronized Ovulation in Lactating Dairy Cattle of Unknown Pregnancy: Occurrence and Timing of Gonadotropin-Releasing Hormone¹

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ABSTRACT

The objectives were to determine if the first gonadotropin-releasing hormone (GnRH) injection of a resynchronization protocol is essential to achieve acceptable fertility and if the timing of the standard second GnRH injection would improve pregnancy rates per AI (P/AI) when administered at 56 versus 72 h after prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Lactating dairy cows of unknown pregnancy status ($n = 763$) were treated with either GnRH or saline to initiate ovulation resynchronization, which was completed 7 d later when cows were diagnosed as nonpregnant.

Within treatments, nonpregnant cows were administered $PGF_{2\alpha}$ and then were either injected with GnRH 56 h later and inseminated 16 h later or injected and inseminated concurrently at 72 h. Injection of GnRH at 56 h produced more ($P < 0.001$) P/AI than injection at 72 h

when AI was administered at 72 h (29.9 vs. 14%, respectively). Further, beginning the resynchronization with GnRH improved the resulting P/AI but was time dependent. When the nonpregnant status was determined between d 29 and 35 after previous AI, the GnRH injection (7 d before a nonpregnant diagnosis) did not increase the resulting P/AI compared with saline (27.4 vs. 24.8%, respectively). In contrast, when a nonpregnant diagnosis occurred after d 35 and GnRH was given 7 d earlier, P/AI were greater ($P = 0.036$) with GnRH than with saline (32.4 vs. 15.6%, respectively). Injection of GnRH 7 d before a pregnancy diagnosis in pregnant cows had no effect on subsequent pregnancy loss. Administering the second GnRH injection at 56 h rather than 72 h after $PGF_{2\alpha}$, with AI at 72 h, improved the pregnancy outcome. The first GnRH injection of the ovulation resynchronization program did not improve the pregnancy outcome when the nonpregnant diagnosis was determined before d 35, but after d 35, the first GnRH injection improved the resulting fixed-timed P/AI compared with saline.

Key words: gonadotropin-releasing hormone, Ovsynch, pregnancy rate, resynchronization, timing

INTRODUCTION

In a recent survey, 77% of large dairy herd owners reported resynchronizing ovulation in cows diagnosed as not pregnant (Caraviello et al., 2006). Most herds initiate the Ovsynch program [injection of gonadotropin-releasing hormone (**GnRH**) 7 d before and 48 h after prostaglandin $F_{2\alpha}$ (**PGF_{2α}**) with a fixed-time AI administered between 16 and 24 h after the second GnRH injection] 7 d before pregnancy status is diagnosed. Resynchronization can then be completed with reinsemination 72 h after a nonpregnancy status is confirmed.

Many factors determine the success of such resynchronizations, including precise follicular maturation and its synchronization with the demise (luteolysis) of the corpus luteum or corpora lutea (**CL**; Stevenson et al., 2007). Follicular synchronization may occur

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when the resynchronization protocol is initiated with GnRH 7 d before a nonpregnant diagnosis, but its success is related to the stage of follicular dominance (Vasconcelos et al., 1999). To maximize fertility at the resynchronized AI, timing of a pregnancy diagnosis relative to the stage of the estrous cycle in nonpregnant cows may be critical to the subsequent reinsemination outcome. Depending on the timing of the nonpregnancy diagnosis, the first (up-front) GnRH injection of the Ovsynch protocol may not be necessary for cows that are metestrus or early diestrus at its initiation (El-Zarkouny et al., 2004).

Ovulation resynchronization initiated 7 d before (d 26) a nonpregnancy diagnosis on d 33 after a previous AI resulted in fewer pregnancies per AI (P/AI) than one initiated on d 33 (Sterry et al., 2006). The contribution of the first GnRH injection to subsequent P/AI is unknown, particularly when nonpregnant cows on d 26 after a previous AI are expected to be very early in the estrous cycle when a follicle may not ovulate in response to GnRH-induced luteinizing hormone release (Vasconcelos et al., 1999).

Further, giving the second GnRH injection at 56 h after PGF_{2α} in the Ovsynch protocol, followed in 16 h by timed AI has produced more P/AI in first- and repeat-service dairy cows (Brusveen et al., 2008; Nebel et al., 2008) than concurrent insemination

and GnRH injection at 72 h. These results contrast with those of another study (Portaluppi and Stevenson, 2005) in which the first-service timed P/AI were greater when the second GnRH injection was given concurrently with AI at 72 h after PGF_{2α}.

These reports on the effect of timing of the second GnRH injection relative to PGF_{2α} and AI are inconsistent. Therefore, the present study had 3 objectives: 1) to determine if GnRH is necessary to achieve acceptable P/AI when the nonpregnant diagnosis occurs earlier (d 29 to 35) postinsemination rather than later (d 35 to 42); 2) to determine if initiating resynchronization with a GnRH injection in pregnant cows would exacerbate pregnancy loss during the next 6 wk compared with injecting saline; and 3) to determine if the timing of the standard second GnRH injection would improve P/AI when administered at 56 versus 72 h after PGF_{2α}.

MATERIALS AND METHODS

Experimental Design

The current studies were approved by the Kansas State University Institutional Animal Care and Use Committee. The experiment was conducted between October 2006 and January 2009 at the Kansas State University Dairy Teaching and Research Center. Lactating Holstein cows (n =

763), milked twice daily, treated with recombinant bovine somatotropin according to label, previously inseminated, and of unknown pregnancy status, were assigned randomly but unequally to a 2 × 2 factorial experiment consisting of 4 treatments (Figure 1) 7 d before pregnancy status was determined by transrectal ultrasonography (5.0-MHz linear-array transducer, Aloka 500V, Corometrics Medical Systems Inc., Wallingford, CT). The main effects were 1) first injection of GnRH (100 μg i.m.; 2 mL of Fertagyl, Intervet Inc., Millsboro, DE) to initiate a resynchronization program or saline 7 d before a nonpregnant diagnosis, and 2) timing of the second GnRH injection (56 vs. 72 h) after injecting PGF_{2α} i.m. (2 mL of Estrumate, Schering Plough Animal Health, Union, NJ) of the Ovsynch or Cosynch (same as Ovsynch, but with AI occurring concurrently with the second GnRH injection) program. Therefore, the 4 treatments were 1) no GnRH injection (saline) to initiate Ovsynch-56 h; 2) no GnRH injection (saline) to initiate Cosynch-72 h; 3) Ovsynch-56 h; and 4) Cosynch-72 h. The treatments represented either a standard Ovsynch or Cosynch program with one timed AI administered at 72 h after PGF_{2α}, with the exception of replacing the standard up-front GnRH injection with saline.

Our rationale for unequal random assignment of cows to the 4 treatments was economically based. Because we anticipated that using saline to initiate a resynchronization protocol would produce poorer fertility (Buttrey et al., 2007; Brusveen et al., 2008), the number of cows treated with saline was limited (Buttrey et al., 2007) as was the number of cows inseminated and treated with GnRH at 72 h after PGF_{2α} (Brusveen et al., 2008).

One AI technician performed 90.3% of all inseminations, and multiple sires were used. Pregnancy status (presence of fluid and a CL or viable embryo) was determined between 29 and 42 d after the last AI (mean ± SE = 35.1 ± 1.4 d; median = 33 d). More than 80% of the cows were at either d 31

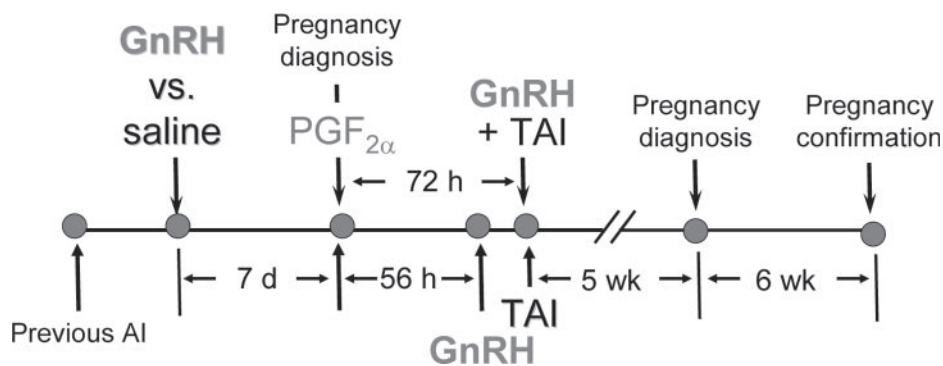


Figure 1. A 2 × 2 factorial design of 4 treatments was applied to previously inseminated dairy cattle of unknown pregnancy status. The ovulation resynchronization program was initiated with either saline or gonadotropin-releasing hormone (GnRH). Seven days after pregnancy diagnosis (29 to 42 d post-AI), nonpregnant cows were administered prostaglandin F_{2α} (PGF_{2α}) and received GnRH at 56 or 72 h later. All cows were inseminated at 72 h (timed AI; TAI). Cows pregnant at the initial pregnancy diagnosis were reconfirmed 6 wk later to determine pregnancy loss.

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