Kansas Agricultural Experiment Station Research Reports

Volume 0
Issue 2 *Dairy Research (1984-2014)*

Article 208

2008

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Abstract

Lactating dairy cows and replacement virgin heifers of unknown pregnancy status were treated with either gonadotropin-releasing hormone (GnRH) or saline to initiate a resynchronization program that was continued 7 days later when a not-pregnant diagnosis was determined. Nonpregnant cattle were administered prostaglandin F2αand then either injected with GnRH 56 hours later and artificially inseminated (AI) by appointment at 72 hours or injected and inseminated concurrently at 72 hours. Injection of GnRH at 56 hours produced more pregnancies than injection of GnRH at 72 hours when AI was administered at 72 hours in both treatments (30.9 vs. 15.2%). Further, starting the resynchronization with GnRH was beneficial to resulting pregnancy rates but was timing dependent. When a not-pregnant status was determined between day 30 and 36 after AI, upfront GnRH injection (7 days before pregnancy diagnosis) may not be necessary because stage of cycle is 1 to 7 days (days 3 to 4 in 71% of cattle) and resulting pregnancy rates after GnRH or saline did not differ (27.5 vs. 26.6 %, respectively). In contrast, when pregnancy status was determined after day 36 (days 37 to 43; cycle days 10 to 11 in 71% of cattle), upfront GnRH as part of the resynchronization protocol nearly doubled the number of pregnancies compared with saline (31.0 vs. 15.1%).; Dairy Day, 2008, Kansas State University, Manhattan, KS, 2008; Dairy Research, 2008 is known as Dairy Day, 2008

Keywords

Dairy Day, 2008; Kansas Agricultural Experiment Station contribution; no. 09-134-S; Report of progress (Kansas State University. Agricultural Experiment Station and Cooperative Extension Service); 1002; Dairy; Resynchronized pregnancy rate; Gonadotropin-releasing hormone; Artifical insemination

Resynchronized Pregnancy Rates in Dairy Cattle: Timing of Gonadotropin-Releasing Hormone Injection before Timed Artificial Insemination

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Summary

Lactating dairy cows and replacement virgin heifers of unknown pregnancy status were treated with either gonadotropin-releasing hormone (GnRH) or saline to initiate a resynchronization program that was continued 7 days later when a not-pregnant diagnosis was determined. Nonpregnant cattle were administered prostaglandin $F_{2\alpha}$ and then either injected with GnRH 56 hours later and artificially inseminated (AI) by appointment at 72 hours or injected and inseminated concurrently at 72 hours. Injection of GnRH at 56 hours produced more pregnancies than injection of GnRH at 72 hours when AI was administered at 72 hours in both treatments (30.9 vs. 15.2%). Further, starting the resynchronization with GnRH was beneficial to resulting pregnancy rates but was timing dependent. When a not-pregnant status was determined between day 30 and 36 after AI, upfront GnRH injection (7 days before pregnancy diagnosis) may not be necessary because stage of cycle is 1 to 7 days (days 3 to 4 in 71% of cattle) and resulting pregnancy rates after GnRH or saline did not differ (27.5 vs. 26.6 %, respectively). In contrast, when pregnancy status was determined after day 36 (days 37 to 43; cycle days 10 to 11 in 71% of cattle), upfront GnRH as part of the resynchronization protocol nearly doubled the number of pregnancies compared with saline (31.0 vs. 15.1%).

Introduction

Achieving acceptable pregnancy rates in previously inseminated dairy cows after a not-pregnant diagnosis is a challenge. Most dairy producers find that first-service timed artificial insemination (**TAI**) pregnancy rates are much greater than those achieved in open cows reinseminated after a not-pregnant diagnosis. A number of factors determine the success of such reinseminations including precise follicular maturation and its synchronization with the demise (luteolysis) of the corpus luteum or corpora lutea (**CL**). Good follicular synchronization usually occurs when the resynchronization protocol is initiated with GnRH 7 days before prostaglandin $F_{2\alpha}$ (**PGF$_{2\alpha}$**) is administered to the open female at a not-pregnant diagnosis. Administering GnRH causes ovulation in more than 60% of dairy cows and reinitiates new follicular growth and a new dominant follicle after 4 to 5 days. Timing of pregnancy diagnosis relative to the stage of the estrous cycle in nonpregnant females may not require the upfront GnRH injection for those that are early in the estrous cycle at initiation of the resynchronization protocol.

Our first objective was to determine whether gonadotropin-releasing hormone (**GnRH**) is necessary to achieve acceptable pregnancy rates when the not-pregnant diagnosis occurs earlier (days 30 to 36) post-insemination rather than later (days 37 to 43). The earlier diagnosis corresponds to when transrectal ultrasonography is generally used, whereas the later diagnosis corresponds to when transrectal palpation is applied for diagnosing pregnancy in dairy cows and heifers.

Our second objective was to determine whether timing of the standard second GnRH injection would improve pregnancy rates if administered at 56 vs. 72 hours after $PGF_{2\alpha}$. The earlier timing at 56 hours would more closely align with the standard Ovsynch protocol (injection of GnRH at 7 days and 48 hours after $PGF_{2\alpha}$ with TAI occurring 16 hours after the second GnRH injection) but requires another cow handling event before the TAI.

Experimental Procedures

The experiment was conducted between October 2006 and July 2008 at the Kansas State University Dairy Teaching and Research Center (Manhattan, KS). Lactating dairy cows (n = 704) and 125 replacement heifers (12 to 16 months of age) previously inseminated and of unknown pregnancy status were assigned randomly but unequally to a 2 × 2 factorial experiment consisting of 4 treatments 7 days before pregnancy status was determined by transrectal ultrasonography (5.0 MHz linear-array transducer, Aloka 500V; Corometrics Medical Systems, Inc., Wallingford, CT). Pregnancy status was determined every 2 weeks.

Main effects were upfront injection of GnRH (100 µg; 2 mL Fertagyl, Intervet, Millsboro, NJ) or saline 7 days before a not-pregnant status (30 to 43 days after last AI) and timing of GnRH injection (56 vs. 72 hours) after $PGF_{2\alpha}$. Therefore, the 4 treatments were (1) saline + Ovsynch-56, (2) saline + Cosynch-72, (3) GnRH + Ovsynch-56, and (4) GnRH + Cosynch-72 (Figure 1). The treatments represented either a standard Ovsynch or Cosynch program with 1 TAI administered 72 hours after $PGF_{2\alpha}$, with the exception of replacing the standard upfront GnRH injection with saline. One AI technician performed 90.3% of all inseminations, and multiple sires were used. Pregnancy status was determined 32 to 39 days after TAI.

Results were analyzed by logistic regression (procedures LOGISITIC and GLM, SAS Institute, Inc., Cary, NC). The model to determine pregnancy rate included upfront injection (GnRH vs. saline), time of GnRH injection (56 vs. 72 hours), interaction of GnRH and time, season, and lactation number (0 1, 2, and 3+).

Results and Discussion

Pregnancy rates resulting from treatments are summarized in Table 1. Initiating the resynchronization program with GnRH increased ($P < 0.01$) TAI pregnancy rates from 21.1 to 30.2%. Initiating a resynchronized ovulation program by injecting GnRH to cause ovulation of the dominant follicle, however, was timing dependent. When GnRH or saline was administered between 23 and 30 days after the last AI and pregnancy diagnosis then occurred 7 days later (days 30 to 36), the resulting TAI pregnancy rates did not differ from one another (Table 2; GnRH = 27.5% and saline = 26.6%). In contrast, when the program was initiated between days 30 and 37 and pregnancy diagnosis occurred 7 days later (days 37 to 43), pregnancy rates were doubled ($P = 0.044$) when GnRH (31%) was used rather than saline (15.2%).

Because TAI is used rather extensively in our herd, more than 70% of inseminations are closely synchronized. For cows diagnosed not pregnant at the earlier interval (days 30 to 36), 71.2% of the diagnoses were made on days 32 or 33 since last AI. If we assume that the estrous cycle averages 22 days in duration, these cattle were likely on days 3 or 4 of the estrous cycle when GnRH or saline was injected. We would not expect a large proportion of cows to ovulate in response to GnRH at this stage of follicular growth, early in the estrous cycle. In fact, the proportion of cattle having 2 or more CL at the not-pregnant diagnosis for this earlier interval was similar (saline = 23.9%, n = 46 vs. 22.6%, n =115). Thus, no benefit in resulting TAI pregnancy rates was accrued from administering the GnRH injection in these cattle receiving GnRH before the earlier not-pregnant diagnosis.

In contrast, for cattle diagnosed at the later interval (days 37 to 43), 71.8% of the diagnoses were made on days 39 or 40 since last AI. These cattle were likely on days 10 or 11 of the estrous cycle when GnRH or saline was injected. The GnRH injection was beneficial to the resulting pregnancy rates because of greater GnRH-induced ovulation to initiate the resynchronized ovulation program. The proportion of cattle having 2 or more CL at the not-pregnant diagnosis for this later interval tended ($P = 0.12$) to favor the GnRH treatment (34.5%, n = 58) compared with saline (15.8%, n = 19). Thus, the improvement in pregnancy rates likely occurred because of greater follicular synchrony in those cattle receiving GnRH to initiate the resynchronization program at the later post-AI interval. This trend for a difference in CL proportions among treatments is validated by differences in concentrations of progesterone in blood serum of cattle having 1 or 2+ CL at the not-pregnant diagnosis (7 days post-treatment; Figure 2). Serum progesterone did not differ among GnRH- and saline-treated cattle having only 1 CL, but among those having 2+ CL, GnRH treatment increased ($P < 0.05$) concentrations of progesterone.

As expected, the resynchronized TAI pregnancy rates tended ($P = 0.058$) to be greater in replacement heifers (44.8%, n = 29) than in the lactating cows: first lactation (26.7%, n = 359), second lactation (24.7%, n = 162), or third and greater lactation numbers (24.6%, n = 126).

When ultrasound is used to diagnose pregnancies at earlier post-AI intervals (days 30 to 36), reinitiating a resynchronized ovulation program with a GnRH injection in cows of unknown pregnancy status 7 days before a not-pregnant diagnosis is contraindicated because resulting pregnancy rates were not improved. In contrast, for herds in which pregnancy diagnosis is made at a later post-AI interval (days 37 to 43), either by transrectal ultrasound or palpation, initiating the resynchronization program requires GnRH to improve resulting TAI pregnancy rates. Further work to improve resynchronization treatments and resulting TAI pregnancy rates is warranted.

Table 1. Pregnancy rates in dairy cattle in response to resynchronized ovulation initiated with either saline or gonadotropin-releasing hormone (GnRH) and subsequent timing of GnRH before timed artificial insemination (TAI)

	Time of GnRH before TAI, hours		
Item	56	72	Total
Upfront treatment		——— % (no./no.) ———	
GnRH	34.1 (107/314)	17.5 (17/97)	30.2^x (124/411)
Saline	25.3 (45/178)	12.6 (11/87)	21.1^y (56/265)
Total	30.9^a (152/492)	15.2^b (28/184)	26.6 (180/676)

$^{a-b}$ Means having different superscript letters differ ($P < 0.05$).
$^{x-y}$ Means having different superscript letters differ ($P < 0.001$).

Table 2. Pregnancy rates in dairy cattle are affected by the timing of resynchronization program whether initiated with either saline or gonadotropin-releasing hormone (GnRH)

	Timing of upfront treatment, days since last AI	
Item	23 to 30	30 to 37
Upfront treatment	——— % (no./no.) ———	
GnRH	27.5[a,b] (57/207)	31.0[a] (54/174)
Saline	26.6[a,b] (33/124)	15.1[b] (16/106)
Total	27.2 (90/331)	25.0 (70/280)

[a-b] Interaction of timing of upfront treatment and type of upfront treatment ($P = 0.044$).

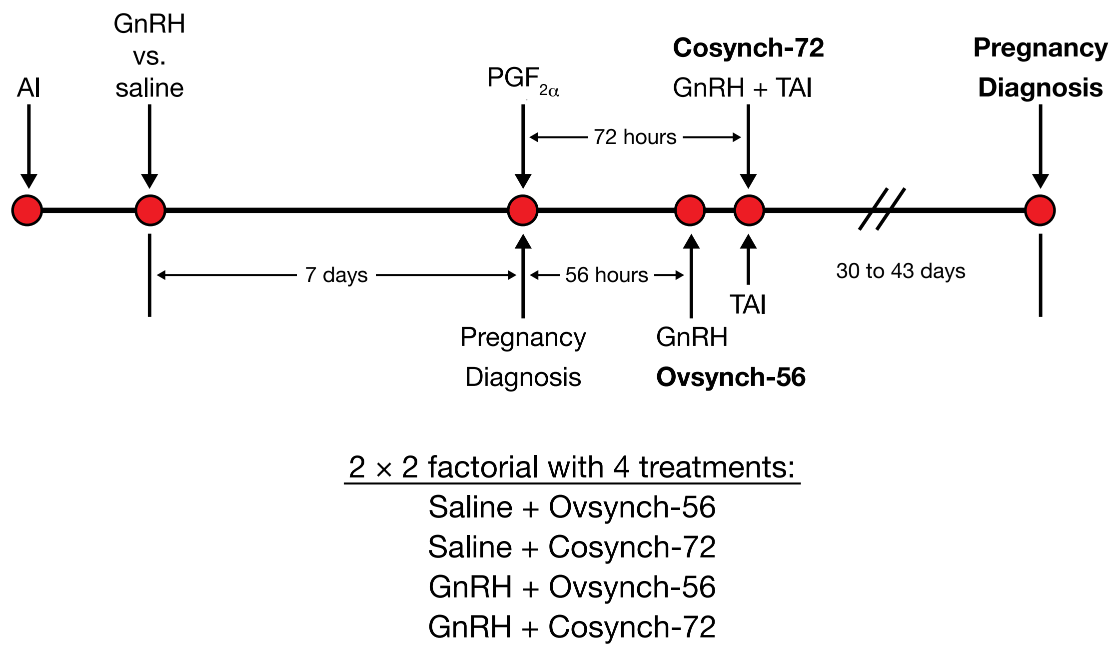

Figure 1. Experimental design of treatments for previously inseminated dairy cattle of unknown pregnancy status treated 7 days before a not-pregnant diagnosis conducted at 30 to 43 days post-insemination. AI = artificial insemination; TAI = timed artificial insemination; GnRH = gonadotropin-releasing hormone; $PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$.

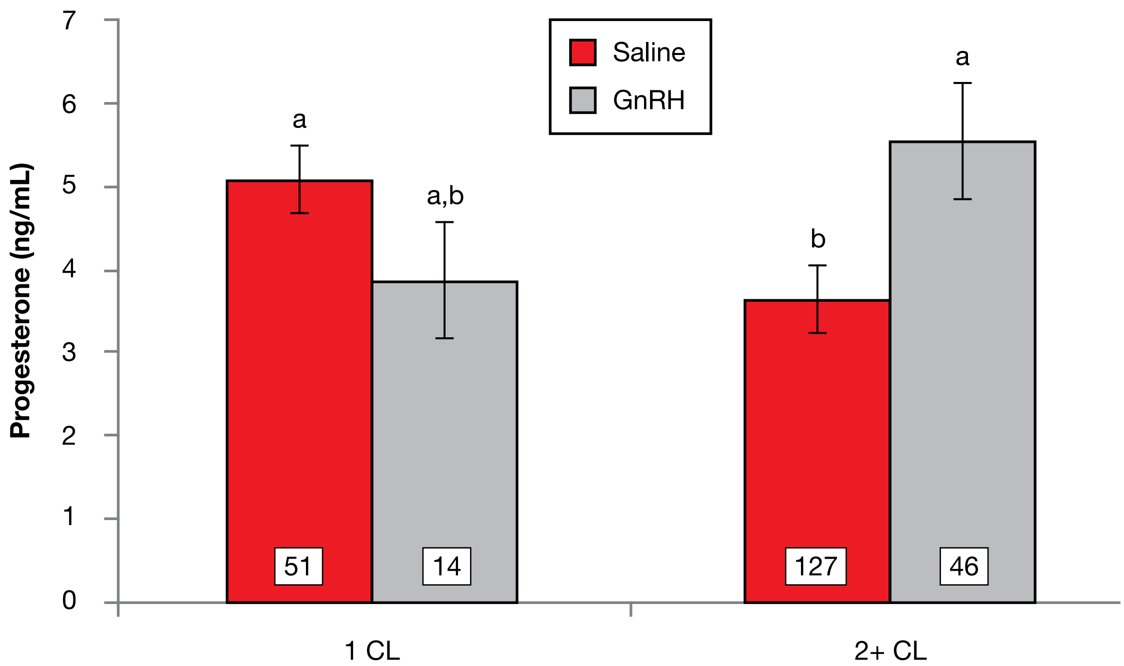

Figure 2. Concentrations of progesterone in blood serum at the time of pregnancy diagnosis (7 days post-treatment) and number of corpora lutea (CL) identified at not-pregnant diagnosis. Numbers of observations are shown for each bar. [a-b] Means with different letters differ ($P < 0.05$).