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Factors Influencing Upfront Single- and Multiple-Ovulation Incidence, Progesterone, and Luteolysis Before a Timed Insemination Resynchronization Protocol[1]

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ABSTRACT

Our objectives were to determine relationships among factors influencing responses to the first GnRH injection in a timed artificial insemination (TAI) protocol and subsequent fertility after altering timing of the second GnRH injection and AI relative to $PGF_{2\alpha}$ injection. Replacement heifers (n = 86) and 613 lactating cows previously inseminated were diagnosed not pregnant to form 77 breeding clusters spanning 36 mo. At not-pregnant diagnosis (d 0), females received 100 μg of GnRH, and then 7 d later, they received 25 mg of $PGF_{2\alpha}$. Females in 2 treatments received GnRH 48 h (G48) after $PGF_{2\alpha}$ injection and TAI at the time of the second GnRH injection (G48 + TAI48) or 24 h later (G48 + TAI72). Females in the third treatment received GnRH 72 h after $PGF_{2\alpha}$ when inseminated (G72 + TAI72). Neither timing of GnRH nor time of AI altered TAI pregnancy rates (average of 20.4%). Ovaries of females in 65 clusters were scanned on d 0 (first GnRH injection) and 7 d later ($PGF_{2\alpha}$ injection). Ovarian structures were mapped and ovulation in response to the first GnRH injection was evaluated on d 7. When estrus was detected before scheduled TAI, females were inseminated; otherwise, TAI conception of remaining females was based on timing of GnRH and AI in 3 treatments. On d 7, 1 or more new corpora lutea (CL) were detected in 43% of females and their pregnancy rate was subsequently greater (28 vs. 18%) than those not ovulating. Follicle diameters on d 0 did not differ between females that did (11.9 ± 0.3 mm) and did not (11.8 ± 0.4 mm) subsequently ovulate in response to GnRH. Follicle diameter and number of follicles ≥5 mm increased with increasing lactation number, but decreased with increasing number of CL. Diameter of follicles in which more than 1 follicle ovulated decreased linearly from that in which only 1 follicle ovulated. Incidence of ovulation increased with increasing lactation number and total number of follicles ≥5 mm, but decreased with increasing number of CL. Incidence of multiple ovulations (15%) was greater in females having more follicles ≥5 mm and in those in early diestrus. Multiple ovulation did not occur in heifers, but was decreased in cows having more than 1 CL. In cows having more than 1 CL, luteal regression was reduced by 5.6 percentage units compared with those having 1 CL. In a TAI protocol, pregnancy rate was greater for females in early diestrus compared with females in other stages of the cycle, in those that ovulated after the first GnRH injection, in those having luteolysis, and in those inseminated during nonsummer months.
Key words: gonadotropin-releasing hormone, Ovsynch, ovulation, pregnancy rate

INTRODUCTION

Several factors are known to influence fertility of a timed AI (**TAI**) protocol in dairy cattle when a follicular wave is synchronized in an Ovsynch-like protocol (GnRH injection given 7 d before and 48 h after luteolysis is induced by $PGF_{2\alpha}$). Day of the estrous cycle at the onset of such protocols influenced incidence of ovulation and follicle diameter after the second ovulatory GnRH injection that followed $PGF_{2\alpha}$-induced luteolysis (Vasconcelos et al., 1999). In that study, cows treated between d 1 and 4 of the estrous cycle had the smallest incidence of ovulation (23%), followed by those between d 10 and 16 (54%), d 17 to 21 (77%), and d 5 to 9 (96%). No information is available about diameters of follicles in cows that ovulated.

Cows in early (d 1 to 4) or late (d 17 to 21) portions of the estrous cycle at the first GnRH injection, however, had larger diameter ovulatory follicles 7 d later than those on d 5 to 13, whereas pregnancy rates were greatest for cows in which the Ovsynch protocol was initiated between d 5 and 14 (42%) and less for those on d 1 to 4 and 14 to 21 (32%; Vasconcelos et al., 1999). Because pregnancy rate is greater among cows that ovulated in

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response to the first GnRH injection (Vasconcelos et al., 1999), determining those variables that influence ovulation is essential to understanding the Ovsynch protocol. Little is known, however, about factors associated with ovulatory responses to the first GnRH injection that may include, in addition to the stage of the estrous cycle, ovarian follicular inventory, number of luteal structures, season, and lactation status.

Timing of the GnRH injection and AI influence TAI pregnancy rates. When GnRH was administered at 48 h after the $PGF_{2\alpha}$ injection of the Ovsynch protocol and cows were inseminated at 48, 56, 64, 72, or 80 h after $PGF_{2\alpha}$, pregnancy rates at first service were maximal at 64 h or 16 h after GnRH (Pursley et al., 1997). In lactating dairy cows inseminated after 2 presynchronizing injections of $PGF_{2\alpha}$ given 14 d apart (Presynch) in which the Ovsynch protocol was initiated 12 d after the second Presynch injection, females were treated with GnRH and various administration times of GnRH and TAI were tested. Those females treated with GnRH at 48 h after the $PGF_{2\alpha}$ injection of Ovsynch and inseminated at that time (48 h after $PGF_{2\alpha}$) or 24 h later (Cosynch48) had lower pregnancy rates than cows injected and inseminated at 72 h after $PGF_{2\alpha}$ (Cosynch72; Portaluppi and Stevenson, 2006). The obvious advantage of such treatments is the convenience of carrying out all hormonal injections and TAI at the same time of the day when cows are conveniently restrained by feed-line lockups.

Recently, similar treatments initiated 11 d after Presynch (Cosynch48 and Cosynch72), produced lesser pregnancy rates in dairy cows compared with administering GnRH at 56 h after $PGF_{2\alpha}$ and inseminating cows 16 h later (72 h after $PGF_{2\alpha}$; Brusveen et al., 2006). These results also were consistent in that study for cows in which these variations of the Ovsynch protocol were applied after a not-pregnant diagnosis.

The objective of our study was to examine various factors that influence the leading first GnRH-induced ovulatory response and resulting pregnancy rates in conjunction with altered timing of the second GnRH injection and TAI.

MATERIALS AND METHODS

Herd Management

The experiment was conducted at the Kansas State University Dairy Teaching and Research Center (Manhattan) using 68 replacement Holstein heifers and 249 lactating cows (total of 86 inseminations in heifers and 613 in cows during 36 mo). Previously inseminated females in 77 biweekly (every other week) breeding clusters were diagnosed not pregnant between October 2002 and October 2005. Cows were housed in covered free-stalls bedded with sand, and were fed a TMR at least twice daily that met or exceeded NRC (2001) requirements for lactating cows. The TMR consisted of 30% chopped alfalfa hay, 19% wet corn gluten meal, 15% corn silage, 9.3% whole cottonseed, 4.4% solvent-extracted soybean meal, 3.3% expeller soybean meal, 13% corn grain, 1.3% menhaden fish meal, 1% sugar cane wet molasses, and 3.7% mineral-vitamin premix. Cows had ad libitum access to fresh water. Pens housing lactating cows also had shade cloth covering part of the pens over the feed bunk and water applied by sprinklers 6 times per hour for 1 min along the feed line during May to October.

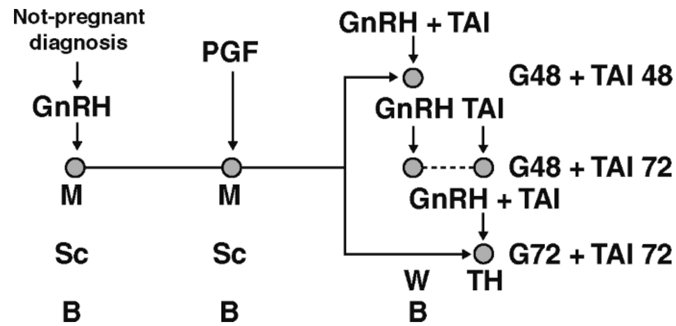

Figure 1. Experimental design of treatments. Nonpregnant females were injected with the first GnRH injection upon not-pregnant diagnosis and then 7 d later were injected with $PGF_{2\alpha}$. Females were injected with a second GnRH injection at 48 h after $PGF_{2\alpha}$ (G48) and inseminated at 48 (TAI48) or 72 h (TAI72) after $PGF_{2\alpha}$ or injected with GnRH at 72 h (G72) concurrent with timed AI (TAI72). M = Monday, W = Wednesday, TH = Thursday; B = blood sample to determine concentration of progesterone; Sc = ovarian scans by transrectal ultrasonography; GnRH = gonadotropin-releasing hormone; PGF = $PGF_{2\alpha}$; and TAI = timed AI.

Replacement heifers were maintained in dirt lots with covered free-stalls and a concrete feed apron. They were fed a TMR consisting of chopped prairie or alfalfa hay, corn or milo grain, soybean meal, and minerals and vitamins to exceed NRC (2001) guidelines for growing heifers by 10 to 15% for all nutrients.

Experimental Design

Biweekly pregnancy diagnosis was conducted when females were between 30 and 43 d since last AI. Number of previous inseminations averaged 2.4 ± 1.2 (mean ± SD; range 1 to 4). Lactating females ranged from 78 to 537 DIM, and averaged 190 d. Replacement heifers ranged from 12 to 16 mo of age. Not-pregnant females were assigned randomly to 3 treatments (Figure 1). All females received 100 μg of GnRH (Factrel, Fort Dodge Laboratories, Fort Dodge, IA, or Cystorelin, Merial Ltd., Iselin, NJ) followed in 7 d by a 25-mg injection of $PGF_{2\alpha}$ (Lutalyse, Pharmacia Animal Health, Kalamazoo, MI).