

## Altering the Time of the Second Gonadotropin-Releasing Hormone Injection and Artificial Insemination (AI) During Ovsynch Affects Pregnancies per AI in Lactating Dairy Cows

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### ABSTRACT

Based on previous research, we hypothesized that Cosynch at 72 h [GnRH-7 d-PGF<sub>2α</sub>-72 h-GnRH + artificial insemination (AI)] would result in a greater number of pregnancies per AI (P/AI) than Cosynch at 48 h. Further, we hypothesized that P/AI would be improved to a greater extent when GnRH was administered at 56 h after PGF<sub>2α</sub> before AI at 72 h due to a more optimal interval between the LH surge and AI. Nine hundred twenty-seven lactating dairy cows ( $n = 1,507$  AI) were blocked by pen, and pens rotated through treatments. All cows received GnRH followed 7 d later by PGF<sub>2α</sub> and then received one of the following: 1) GnRH + timed AI 48 h after PGF<sub>2α</sub> (Cosynch-48); 2) GnRH 56 h after PGF<sub>2α</sub> + timed AI 72 h after PGF<sub>2α</sub> (Ovsynch-56); or 3) GnRH + timed AI 72 h after PGF<sub>2α</sub> (Cosynch-72). Pregnancy diagnoses were performed by ultrasound at 31 to 33 d post-AI and again at 52 to 54 d post-AI. Overall P/AI were similar for the Cosynch-48 (29.2%) and Cosynch-72 (25.4%) groups. The Ovsynch-56 group had a greater P/AI (38.6%) than Cosynch-48 or Cosynch-72. Presynchronized first-service animals had greater P/AI than cows at later services in Cosynch-48 (36.2 vs. 23.0%) and Ovsynch-56 (44.8 vs. 32.7%) but not in Cosynch-72 (24.6 vs. 26.2%). Similarly, primiparous cows had greater P/AI than multiparous cows in Cosynch-48 (34.1 vs. 22.9%) and Ovsynch-56 (41.3 vs. 32.6%), but not Cosynch-72 (29.8 vs. 25.3%). In conclusion, we found no advantage to Cosynch at 72 h vs. 48 h. In contrast, we found a clear advantage to treating with GnRH at 56 h, 16 h before a 72-h AI, probably because of more-optimal timing of AI before ovulation.

**Key words:** Ovsynch, Cosynch

### INTRODUCTION

Timed AI (TAI) protocols such as Ovsynch (GnRH-7 d-PGF<sub>2α</sub>-48-72 h-GnRH), have been developed to improve pregnancy rates by increasing AI submission rates. Ovsynch utilizes a final treatment with GnRH that synchronizes the time of ovulation within an 8-h period (Pursley et al., 1995). This precise timing of ovulation should allow optimization of the time of AI in relation to the time of ovulation. A number of studies have provided information relating to a potential optimal time of AI.

One important study on the optimal time of AI utilized the original Ovsynch protocol and then had AI performed at 0, 8, 16, 24, or 32 h after the final GnRH treatment (Pursley et al., 1998). This study found a quadratic effect of time of AI on number of pregnancies per AI (P/AI) with P/AI increasing from 0 to 16 h with a subsequent decrease at 24 h and a further decrease when cows received AI after the expected time of ovulation (32 h). Nevertheless, only AI after the expected time of ovulation (32 h after final GnRH) produced a significant decrease in both percentage calving per AI and P/AI determined at the first pregnancy diagnosis (Pursley et al., 1998). Analysis of calving data suggested that AI at any time between 0 and 24 h after the final GnRH resulted in similar rates of calving. Thus, this study suggested that there may be an optimal time of AI (~16 h after final GnRH) but also suggested that there may be substantial flexibility in the time of AI in relation to ovulation, provided that AI was done before the time of ovulation.

Insemination near or after the time of ovulation may provide insufficient time for optimal sperm capacitation and transport and result in an aged oocyte before the sperm is capable of fertilization (Hunter and Wilmut, 1983; Wilmut and Hunter, 1984; Hawk, 1987). Alternatively, excessive time from insemination to ovulation (>24 h) also appears to reduce fertility. Indeed, results from the earliest (Trimberger and Davis, 1943; Trimberger, 1944) up to the most recent studies (Dransfield et al., 1998; Pursley et al., 1998; Saacke

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et al., 2000; Dalton et al., 2001) on time of AI have generally shown a decline in P/AI in cows inseminated at the onset of estrus or at the time of the LH surge (induced by GnRH treatment; Pursley et al., 1998) compared with later times. Decreases in fertilization rate have been reported when cows were inseminated at the onset of estrus compared with breeding 12 or 24 h later (Dalton et al., 2001). Therefore, a loss of sperm viability is likely responsible for the declines in fertilization rate and P/AI that have been observed in many (Trimberger and Davis, 1943; Trimberger, 1944; Pursley et al., 1998; Dalton et al., 2001) but not all (Portaluppi and Stevenson, 2005) studies when there were long intervals between AI and ovulation.

Despite these results, many dairies utilize a protocol in which AI is done concurrently with the final GnRH of the Ovsynch protocol, a protocol known as Cosynch (Geary and Whittier, 1998). This procedure requires one less time to lock up, identify, and handle each cow, thereby potentially decreasing labor costs, heat stress, and other cow-handling problems associated with utilizing a TAI protocol. Some studies have compared Cosynch to an Ovsynch protocol in which cows were inseminated 24 h after the GnRH treatment. Vasconcelos et al. (1997) found that inseminating at 24 h after GnRH improved pregnancy success compared with Cosynch at 48 h (**Cosynch-48**). In contrast, 2 recent studies utilizing only presynchronized first-service animals found no difference in P/AI for animals receiving Cosynch at 48 h compared with Ovsynch with a 24-h interval between GnRH and AI (Portaluppi and Stevenson, 2005; Cornwell et al., 2006). Portaluppi and Stevenson (2005) not only compared these 2 protocols in their study with presynchronized first-service animals, but also included a treatment group that received Cosynch at 72 h (**Cosynch-72**). The latter cows had better P/AI than when the results from the other 2 groups were combined, suggesting that delaying the time of final GnRH as well as the time of AI may improve Ovsynch results, at least during the first AI after Presynch. Nevertheless, the authors cautioned that this protocol had not been evaluated for synchronizing cows at second and later services (Resynch). Thus, although Cosynch has become quite popular in the dairy industry, there are conflicting results about whether this protocol produces P/AI that are similar to protocols that have AI at a time closer to the time of ovulation.

Problems with detection of estrus have led to the widespread use of TAI in the dairy industry (Caraviello et al., 2006), making optimization of TAI protocols of economic importance. In addition, the substantial variation in reproductive efficiency reported after altering the timing of the final GnRH treatment and

time of AI in some of the previous studies suggests that substantial gain might occur from optimizing these intervals. In this study we again compared the Cosynch-48 protocol to the Cosynch-72 protocol at first AI (after Presynch) but also at later AI (Resynch). We hypothesized that Cosynch-72 would result in better P/AI than Cosynch-48 at first AI but not at later AI based on the previous results from Portaluppi and Stevenson (2005). In addition, a group was included that had the same time of AI as Cosynch-72 (72 h after PGF<sub>2α</sub>) but with a more optimal time of induced ovulation in relation to AI. This was done by administering the GnRH at 56 h after the PGF<sub>2α</sub> treatment and then performing AI 16 h later (**Ovsynch-56**). We hypothesized that optimizing the time of AI in relation to the final GnRH of Ovsynch would increase P/AI compared with Cosynch-72 or Cosynch-48 based on previous results of Pursley et al. (1998) suggesting that 16 h after GnRH was the optimal time of AI during Ovsynch. Nevertheless, the interval from PGF until GnRH can also impact fertility (Peters and Pursley, 2003) and this cannot be excluded in the present experimental design.

## MATERIALS AND METHODS

### Animals

Nine hundred twenty-seven lactating Holstein cows, receiving a total of 1,507 AI, were housed in free-stall barns at a commercial dairy farm near Juneau, Wisconsin. Cows were milked thrice daily and fed a TMR twice daily that consisted of corn silage and alfalfa silage as forage and a corn/soybean meal-based concentrate. The TMR was formulated to meet or exceed minimum nutritional requirements for lactating dairy cows (NRC, 2001). Cows in the study started to receive bST (500 mg/dose; Posilac, Monsanto, St. Louis, MO) at about 60 d postpartum. Commercial frozen-thawed semen from multiple sires was used across all treatments (10 to 150 units from each sire used during the study). All injections and inseminations were performed by 1 of 5 researchers from our laboratory. The University of Wisconsin-Madison, College of Agriculture and Life Sciences Animal Care Committee approved all animal procedures.

### Experimental Design

This study was conducted from August to December 2005. Cows were blocked by pen (n = 8 pens) where all cows in one pen received the same treatment during 1 wk, and each pen rotated through the 3 treatments during a 3-wk period. Primiparous and multiparous cows were present and intermingled in each of the

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