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# Ovarian characteristics and timed artificial insemination pregnancy risk after presynchronization with gonadotropin-releasing hormone 7 days before $PGF_{2\alpha}$ in dairy cows

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#### ABSTRACT

The objective was to determine the benefit of including GnRH and PGF<sub>2</sub>(PG) as a part of a presynchronization option before enrolling cows in a timed artificial insemination (AI) program. Holstein cows in one herd were assigned weekly at calving from January 2012 to August 2014 to a completely randomized design consisting of two presynchronization treatments. Cows in the Presynch-11 (n = 290) control were administered two PGF<sub>2a</sub> injections (Presynch PG-1 and Presynch PG-2) 14 days apart starting at 39  $\pm$  4 days postpartum (study Days 0 and 14). Cows receiving the experimental presynchronization treatment (Gsynch-11, n = 287) were treated with GnRH (pre-GnRH) on study Day 7 and PG (pre-PG) on study Day 14. On study Day 25, all cows were enrolled in the Ovsynch-56 timed AI program: GnRH-1 on study Day 25, PG on study Day 32, GnRH-2 on study Day 34, 56 hours after PG, and timed AI on study Day 35, 16 hours after GnRH-2. In a subsample of 255 cows, ovarian structures were monitored for size and ovulation, and blood samples were collected on study Days 7, 14, 25, 32, 34, and 41 to measure progesterone. Concentrations of progesterone were greater (P < 0.05) in Gsynch-11 than Presynch-11 cows before pre-GnRH was administered (3.3  $\pm$  0.3 vs. 2.1  $\pm$  0.3 ng/mL), respectively, and ovulatory response to the pre-GnRH treatment also was greater (P = 0.008) in Gsynch-11 than Presynch-11 cows (53.2 vs. 35.0%), respectively. One week later, the dominant follicle was larger (P = 0.045) in Presynch-11 than Gsynch-11 cows. Eleven days after completing the presynchronization treatments, ovulatory response to the Ovsynch GnRH-1 treatment was greater (P = 0.016) in Presynch-11 than Gsynch-11 cows (62.2% vs. 45.6%), respectively. At the time of the Ovsynch-PG treatment, more (P = 0.019) Presynch-11 than Gsynch-11 cows had at least one CL. Subsequent luteal regression (>96%), ovulation to GnRH-2 (>90%), and synchronization risk (>88%) did not differ between treatments, but incidence of multiple ovulation after GnRH-2 was larger (P = 0.036) in Presynch-11 than Gsynch-11 cows (28.4% vs. 15.9%), respectively. Pregnancy per AI at 32 days (36.4% vs. 35.1%) and 60 days (30.0% vs. 29.0%) after AI did not differ between Gsynch-11 and Presynch-11 cows, respectively, but was suppressed during summer months in both treatments to less than 70% of the pregnancy per AI of nonsummer months. Because more than 90% of the cows were ovular as treatments were applied, the GnRH treatment of Gsynch-11 could not be assessed for its benefit in anovular cows. The Gsynch-11 presynchronization treatment performed comparably with the standard Presynch-11 program and provides a viable presynchronization option for use before first AI in dairy herds.

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#### 1. Introduction

Preliminary studies formed the foundation for developing bovine timed AI programs when GnRH administered to control follicle waves was followed in 6 days [1] or 7 days







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[2] by PGF<sub>2 $\alpha$ </sub> (PG) to regress functional luteal tissue in a coordinated fashion [3]. Lactating dairy cows treated with the Ovsynch program (GnRH-1–7 days–PG–56 hours–GnRH-2–16 hours–timed artificial insemination) beginning on Day 5 through 12 of the estrous cycle had greater incidences of ovulation and pregnancy per AI (P/AI) than cows treated at other stages of the estrous cycle [4].

Based on the hypothesis that fertility after a timed AI program was related to the stage of the estrous cycle (i.e., stage of the first follicular wave), presynchronization of estrous cycles was attempted before the Ovsynch program by using two injections of PG administered 14 days apart (Presynch). The second Presynch injection given 12 days before the onset of the timed AI program [5] resulted in a larger proportion of cows in diestrus at the onset of the timed AI program and greater P/AI than cows initiating the timed AI programs at random stages of the estrous cycle [5–7]. Overall, lactating dairy cows exposed to PG-presynch programs have 42% greater odds of pregnancy compared with cows receiving only the timed AI program [8].

Presynchronization programs before first postpartum AI that include combinations of GnRH and PG (i.e., GnRH-PG presynch options): PG-3-G [9,10]; G-6-G [11]; or Double Ovsynch [12,13] before a timed AI program have become alternatives to standard PG-presynch programs because they often further improved P/AI. The mechanism of advantage of these programs includes inducing ovulation in anovulatory cows [9], decreasing the percentage of cows with low circulating progesterone concentrations (<0.50 ng/mL) at GnRH-1, increasing the percentage of cows with medium progesterone concentrations (0.50 < progesterone < 3.0 ng/mL) at GnRH-1, and increasing the proportion of cows with a CL at GnRH-1 [9,14]. In addition, these GnRH-PG presynch programs also increased the percentage of cows with high progesterone (>3.0 ng/mL) at the Ovsynch PG and tended to increase average circulating progesterone at PG [9,14].

Thus, GnRH-PG presynch options induced ovulation in anovular cows and seemed to improve most aspects of synchronization before or during the Ovsynch protocol. Cows that lack a CL at GnRH-1, which includes anovular cows, and ovular cows treated during proestrus, estrus, and metestrus, had reduced concentrations of progesterone compared with cows with a CL. Anovular cows may represent approximately 5 to 41% of cows subjected to a fixed-time AI, and are those that have the poorest pregnancy outcomes to timed AI [8].

Administering PG 2 or 3 days before GnRH is consistent with the GnRH-PG presynch options [9–11,15]. In earlier studies [1,2], a treatment of GnRH was administered 7 days before PG treatment. The proportions of cows [1] or heifers [2] detected in estrus during 6 to 7 days after GnRH were decreased, whereas proportions of cows or heifers were increased after PG was administered 6 or 7 days after GnRH compared with a single PG injection. Anecdotally, observations on dairy farms, in which PG-presynch treatments were given 14 days apart before enrolling cows in a timed Al program 10 to 14 days later, indicate that occurrence of estrus averaged approximately 25% after each PG treatment. In contrast, when GnRH was administered 7 days before PG, expression of estrus increased to more than 60%.

We hypothesized that using a GnRH-PG presynch approach (GnRH 7 days before PG) would be as effective as a standard PG-presynch option in lactating dairy cows. In this experiment, we chose to not inseminate cows detected in estrus between or after the GnRH-PG presynch option to determine its value as a standard presynch alternative in which cows were inseminated only at the appointed fixed time. Our objective was to compare this GnRH-PG presynch option with a standard PG presynch by examining resulting ovarian characteristics, serum progesterone, and subsequent pregnancy risk.

#### 2. Materials and methods

#### 2.1. Experimental cows

Lactating Holstein cows (n = 577) in the Kansas State University Dairy Teaching and Research Center were enrolled in this study beginning at calving in January 2012 through August 2014. Cows were housed in covered free stalls and fed twice or thrice (summer) daily a total mixed diet calculated to meet nutrient requirements for lactating dairy cows producing 45 kg of 3.5% milk per day [16]. The diet consisted of alfalfa hay, corn silage, soybean meal, whole cottonseed, corn or milo grain, corn gluten feed, vitamins, and minerals.

#### 2.2. Experimental design

Weekly clusters of cows were stratified at calving according to parity (primiparous vs. multiparous), calving difficulty score, and calving body condition score, and then cows were assigned to a completely randomized design consisting of two presynchronization treatments (Fig. 1). Body condition scores were assigned to cows during the first week after calving and on study Day 7 [17]. Cows (n = 290) in the Presynch-11 control were administered two PGF<sub>2a</sub> injections (Presynch PG-1 and Presynch PG-2) 14 days apart starting at 39  $\pm$  4 days postpartum (study Days 0 and 14). Cows receiving the experimental presynchronization treatment (Gsynch-11, n = 287) were treated with GnRH (pre-GnRH) on study Day 7 and PG (pre-PG) on study Day 14. On study Day 25, all cows were enrolled in the Ovsynch-56 timed AI program: GnRH-1 on study Day 25, PG on study Day 32, GnRH-2 on study Day 34,



**Fig. 1.** Illustration of experimental presynchronization treatments (Presynch-11 and Gsynch-11) administered before the timed artificial insemination (TAI) Ovsynch-56 program. PG =  $PGF_{2z}$ ; GnRH = gonadotropin-releasing hormone; DIM = days in milk; Day = day of the week; and S = blood sampling.

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