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# Supplemental progesterone and timing of resynchronization on pregnancy outcomes in lactating dairy cows

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## ABSTRACT

The objective was to determine the effect of exogenous progesterone ($P_4$) in a timed artificial insemination (TAI) protocol initiated at 2 different times post-AI on pregnancies per AI (P/AI) in lactating dairy cows. Cows (n = 1,982) in 5 dairy herds were assigned randomly at a nonpregnancy diagnosis 32 ± 3 d post-AI to 1 of 4 resynchronization (RES) treatments arranged in a 2 × 2 factorial design using the Ovsynch-56 (GnRH, 7 d later $PGF_{2\alpha}$, 56 h later GnRH, 16 h later TAI) protocol. Treatments were as follows: cows initiating RES 32 ± 3 d after AI with no supplemental $P_4$ (d 32 RES-CON; n = 516); same as d 32 RES-CON plus a controlled internal drug release (CIDR) insert containing $P_4$ at the onset of Ovsynch-56 (d 32 RES-CIDR; n = 503); cows initiating RES 39 ± 3 d after AI (d 39 RES-CON; n = 494); and same as d 39 RES-CON plus a CIDR (d 39 RES-CIDR; n = 491). Cows were inseminated if observed in estrus before TAI. The P/AI was determined 32 and 60 d after TAI. In a subgroup of cows (n = 1,152), blood samples were collected and ovarian structures examined by ultrasonography on the days of the first GnRH (G1) and $PGF_{2\alpha}$ of Ovsynch-56. Percentage of cows with a corpus luteum (CL) at G1 was unaffected by timing of treatments, but percentage of cows with a CL at $PGF_{2\alpha}$ was greater for d 32 than for d 39 cows (87.9 vs. 79.4%). In addition, percentage of cows with $P_4 \geq 1$ ng/mL at G1 was unaffected by timing of treatments, but was increased for d 32 compared with d 39 RES cows on the day of the $PGF_{2\alpha}$ of the RES protocols (86.5 vs. 74.3%). Treatment did not affect ovulation to G1 or P/AI 32 d after RES TAI (d 32 RES-CON = 30.1%, d 32 RES-CIDR = 28.8%, d 39 RES-CON = 27.5%, d 39 RES-CIDR = 30.5%). A greater percentage of d 39 RES cows underwent premature luteolysis during the RES protocol compared with d 32 RES cows. An interaction was detected between day of RES initiation and CIDR treatment, in which the CIDR increased P/AI 60 d after TAI for d 39 (CON = 23.7% vs. CIDR = 28.0%), but not for d 32 (CON = 26.9% and CIDR = 24.2%) cows. Pregnancy loss was unaffected by treatment. In addition, cows had improved P/AI 60 d after TAI when they received a CIDR and did not have a CL (CON-CL = 28.2%, CON-No CL = 19.2%, CIDR-CL = 27.0%, and CIDR-No CL = 26.5%) or had $P_4$ <1 ng/mL (CON-High $P_4$ = 27.8%, CON-Low $P_4$ = 15.0%, CIDR-High $P_4$ = 25.0%, and CIDR-Low $P_4$ = 29.4%) at G1, but not if a CL was present or $P_4$ was ≥1 ng/mL at G1. In conclusion, addition of a CIDR insert to supplement $P_4$ during the RES protocol increased P/AI for cows initiating RES 39 ± 3 d after AI but not 32 ± 3 d after AI.
**Key words:** controlled internal drug release insert, dairy cow, resynchronization

## INTRODUCTION

Dairy herd managers continue to be challenged by inadequate detection or expression of estrus in lactating dairy cows previously inseminated, and resynchronization (**RES**) protocols before timed AI (**TAI**) are often recommended for re-insemination of nonpregnant lactating dairy cows to increase the AI service rate. The limitation of RES protocols has traditionally been poor pregnancies per AI (**P/AI**) compared with TAI after first service (Bruno et al., 2013). Poor P/AI seems to result from the inability to properly synchronize the estrous cycle before or after nonpregnancy diagnosis (**NPD**) when cows start the RES protocol at random stages of the estrous cycle.

A controlled internal drug release (**CIDR**) insert containing 1.38 g of progesterone (**$P_4$**) induces cyclicity in anovular cows and heifers (Rhodes et al., 2003), concentrates the return to estrus in nonpregnant cows (Chenault et al., 2003), and improves synchronization

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of the estrous cycle when used during a TAI protocol (Lima et al., 2009). Some studies have shown variable results when including a CIDR insert as part of synchronization protocol for first AI (Stevenson et al., 2006; Chebel et al., 2010). These studies, however, did not evaluate the value of a CIDR in a RES protocol for subsequent AI. Recently, a 7-percentage-point increase in P/AI was observed when a CIDR was incorporated in an Ovsynch protocol for RES beginning at 39 ± 3 d after AI (Dewey et al., 2010).

Resynchronization programs are frequently used as a reproductive management strategy to reduce the interval between AI and increase the overall rate at which cows become pregnant. The interval between AI and the start of RES protocols depends on the interval from AI to NPD. The day of the estrous cycle on which the RES protocol is initiated is critical in determining the probability of ovulation to the first GnRH injection of the Ovsynch protocol, timing of luteolysis during the protocol, duration of dominance of the ovulatory follicle, and ultimately synchrony of the estrous cycle (Moreira et al., 2001; Fricke et al., 2003). Vasconcelos et al. (1999) observed that initiating the Ovsynch protocol between d 5 and 9 of the estrous cycle increased the percentage of cows that ovulated after the first GnRH (>90%) injection. In addition, a new follicular wave is recruited 24 to 48 h after the GnRH injection and a corpus luteum (**CL**) is present at the time of the prostaglandin (**PG**) $F_{2\alpha}$ injection administered on d 7 of the protocol. These orchestrated responses result in a properly timed luteolysis when synchronized ovulation is induced with the second GnRH administered 48 to 56 h after $PGF_{2\alpha}$ (Vasconcelos et al., 1999). Considering that duration of the estrous cycle of lactating dairy cows is approximately 23 d (Sartori et al., 2004) and the optimal time to begin a synchronization protocol is from d 5 to 9 of the estrous cycle (Vasconcelos et al., 1999), it may be advantageous to initiate RES protocols between 28 and 32 d after AI. Initiation of a RES protocol 19 to 21 d after AI resulted in reduced P/AI (Fricke et al., 2003), whereas P/AI was similar when RES was initiated 25 to 28 d after AI compared with starting the RES 32 to 33 d after AI (Fricke et al., 2003; Chebel et al., 2003; Sterry et al., 2006).

The hypothesis of the current experiment was that cows resynchronized with an Ovsynch protocol including a CIDR insert would have increased P/AI when RES began at either 32 or 39 ± 3 d post-AI. We also hypothesized that initiating the RES protocol 32 ± 3 d post-AI would improve P/AI because most cows would begin the RES protocol between d 5 and 12 of their estrous cycle, assuming that an undetected estrus occurred 20 to 27 d after the previous AI. Therefore, the objectives of the current experiment were to determine the effects of $P_4$ supplementation via a CIDR insert during the Ovsynch protocol on P/AI of dairy cows initiating the RES program at different intervals after AI. A second objective was to determine if timing of initiation of RES after AI (d 32 vs. d 39) influences P/AI in lactating dairy cows.

## MATERIALS AND METHODS

### *Cows and Facilities*

The experiment was conducted from October 2009 through February 2010 at 5 dairy farms located in 5 states (Minnesota, Kansas, Wisconsin, Texas, and Florida). Lactating Jersey (Minnesota; n = 417), Holstein × Jersey (Texas; n = 418), and Holstein (Kansas, Wisconsin, and Florida; n = 1,169) cows previously inseminated were enrolled in the experiment 32 ± 3 d after last AI. Cows in Kansas and Florida were housed in naturally ventilated freestall barns, Texas cows were in an open dry-lot, and Minnesota and Wisconsin cows were housed in cross-ventilated freestall barns.

### *Treatments*

Within each site, all cows were enrolled in the experiment 32 ± 3 d after AI and assigned randomly to 1 of 4 treatments arranged in a 2 × 2 factorial design (Figure 1). Treatments were initiation of the RES protocols either 32 or 39 ± 3 d post-AI, with or without inclusion of a CIDR insert (1.38 g of $P_4$; EaziBreed CIDR, Zoetis, Madison, NJ) on the day of the first GnRH injection of the RES protocol. Nonpregnancy was diagnosed by ultrasonography at 32 ± 3 d after AI for all cows regardless of when RES treatments were initiated. At NPD, cows were submitted to an Ovsynch-56 protocol [GnRH, followed 7 d later by $PGF_{2\alpha}$ (25 mg of dinoprost tromethamine; Lutalyse, Zoetis), 56 h later a second GnRH injection was administered, and TAI occurred 12 to 16 h later] initiated 32 d after AI or initiated 1 wk later for d 39 cows. The CIDR was inserted concurrent with the first GnRH (100 μg of gonadorelin diacetate tertahydrate; Fertagyl, Merck Animal Health, De Soto, KS) injection of the Ovsynch-56 protocol and was removed 7 d later at the $PGF_{2\alpha}$ injection. Therefore, the 4 treatments (Figure 1) were (1) cows examined for NPD 32 ± 3 d after AI and initiating the Ovsynch-56 protocol at NPD (**d 32 RES-CON**; n = 516); (2) cows examined for NPD 32 ± 3 d after AI and initiating the Ovsynch-56 protocol with a CIDR at NPD (**d 32 RES-CIDR**; n = 503); (3) cows examined for NPD 39 ± 3 d after AI and initiating the Ovsynch-56 protocol at NPD (**d 39 RES-CON**; n = 494); and (4) cows examined for NPD 39 ± 3 d after AI and initiating