

# Presynchronization with Double-Ovsynch improves fertility at first postpartum artificial insemination in lactating dairy cows

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#### **ABSTRACT**

The objective of this study was to compare circulating progesterone (P4) profiles and pregnancies per AI (P/AI) in lactating dairy cows bred by timed artificial insemination (TAI) following Ovsynch-56 after 2 different presynchronization protocols: Double-Ovsynch (DO) or Presynch-Ovsynch (PS). Our main hypothesis was that DO would increase fertility in primiparous cows, but not in multiparous cows. Within each herd (n = 3), lactating dairy cows (n = 1,687; 778) primiparous, 909 multiparous) were randomly assigned to DO  $[n = 837; GnRH-7d-PGF_{2\alpha}-3d-GnRH-7d-Ovsynch-56]$  $(GnRH-7d-PGF_{2\alpha}-56h-GnRH-16hTAI)$  or PS (n = 850;  $PGF_{2\alpha}$ -14d- $PGF_{2\alpha}$ -12d-Ovsynch-56). In 1 herd, concentrations of P4 were determined at the first GnRH (GnRH1) of Ovsynch-56 and at d 11 after TAI (n =739). In all herds, pregnancy was diagnosed by palpation per rectum at 39 d. In 1 herd, the incidence of late embryo loss was determined at 74 d, and data were available on P/AI at the subsequent second service. Presynchronization with DO reduced the percentage of animals with low P4 concentrations (<0.50 ng/mL) at GnRH1 of Ovsvnch-56 (5.4 vs. 25.3\%, DO vs. PS). A lesser percentage of both primiparous and multiparous cows treated with DO had low P4 concentrations at GnRH1 of Ovsynch-56 (3.3 vs. 19.7%, DO vs. PS primiparous; and 8.8 vs. 31.9%, DO vs. PS multiparous). Presynchronization with DO improved P/AI at the first postpartum service (46.3 vs. 38.2%, DO vs. PS). Statistically, a fertility improvement could be detected for primiparous cows treated with DO (52.5 vs. 42.3%, DO vs. PS, primiparous), but only a tendency could be detected in multiparous cows (40.3 vs. 34.3\%, DO vs. PS, multiparous), consistent with our original hypothesis. Presynchronization treatment had no effect on the incidence of late embryo loss after first service (8.5 vs. 5.5%, DO vs. PS). A lower body condition score increased the percentage of cows with low P4 at GnRH1 of Ovsynch-56 and reduced fertility to the TAI. In addition, P4 concentration at d 11 after TAI was reduced by DO. The method of presynchronization at first service had no effect on P/AI at the subsequent second service (34.7 vs. 36.5%, DO vs. PS). Thus, presynchronization with DO induced cyclicity in most anovular cows and improved fertility compared with PS, suggesting that DO could be a useful reproductive management protocol for synchronizing first service in commercial dairy herds.

**Key words:** presynchronization, Ovsynch, dairy cow, pregnancy rate

#### INTRODUCTION

Reproductive efficiency in lactating dairy cows is not optimal, encouraging the development of several different reproductive management strategies focused on improving the use of AI and pregnancy rates in dairy herds (Thatcher et al., 2006). One of the most common strategies is Ovsynch (Pursley et al., 1995), in which GnRH and PGF<sub>2 $\alpha$ </sub> are combined to synchronize the time of ovulation, allowing timed AI (TAI). The Ovsynch-56 protocol specifically includes an injection of GnRH 7 d before and 56 h after an injection of  $PGF_{2\alpha}$  (Brusveen et al., 2008), resulting in ovulation 24 to 32 h after GnRH (Pursley et al., 1995), with the highest fertility (pregnancies per AI; P/AI) achieved when TAI is completed 16 h after the second GnRH treatment (Pursley et al., 1998). It is interesting that when Ovsynch was initiated on d 1 to 4 of the estrous cycle, ovulation rates as low as 23% were observed because of the lower ovulatory capacity of follicles early in the estrous cycle.

Received December 13, 2011. Accepted June 16, 2012. <sup>1</sup>Corresponding author: wiltbank@wisc.edu 7004 HERLIHY ET AL.

Conversely, when Ovsynch was initiated on d 5 to 9 of the estrous cycle, GnRH-induced ovulatory responses were increased to 85 to 96% (Vasconcelos et al., 1999; Bello et al., 2006). Greater ovulatory responses to the first GnRH (**GnRH1**) of Ovsynch have been shown to positively affect synchronization and P/AI in lactating dairy cows (Vasconcelos et al., 1999; Galvão and Santos, 2010). Thus, presynchronization of ovarian function before Ovsynch could improve fertility to the TAI.

Based on this idea, protocols have been developed to presynchronize cows before Ovsynch that traditionally use 2 injections of  $PGF_{2\alpha}$  administered 14 d apart with initiation of the TAI protocol 11 d (Galvão et al., 2007), 12 d (Moreira et al., 2001), or 14 d (Navanukraw et al., 2004) later. These protocols have been termed Presynch-Ovsynch (PS) and have been found to increase P/AI compared with Ovsynch in cyclic cows (Moreira et al., 2001) or in all cows (Navanukraw et al., 2004; Galvão et al., 2007). Earlier studies that used 2 injections of  $PGF_{2\alpha}$  to presynchronize estrous cycles recognized that this approach was not effective for anovular cows (Moreira et al., 2001). Gümen et al. (2003) reported that a high percentage of anovular cows ovulated to GnRH1 of Ovsynch albeit dependent on the stage of the follicular wave at the time of this GnRH injection. However, anovular cows still had greatly reduced fertility to the TAI protocol. Thus, strategies that induce production of a corpus luteum (CL) before initiation of the Ovsynch protocol are likely to improve fertility in anovular cows.

One strategy that has been reported to induce cyclicity in anovular dairy cows is the use of an Ovsynch protocol to presynchronize and induce cyclicity in cows before the use of a breeding Ovsynch protocol, termed Double-Ovsynch (DO; Souza et al., 2008; Wiltbank et al., 2011b). A field study (Souza et al., 2008) comparing the standard PGF<sub>20</sub>-based PS with DO showed improved P/AI in cows receiving the DO strategy. However, the experiment was not large (n = 337), and unexpectedly, improvements were limited to primiparous cows and were not observed in multiparous cows. Although these results suggested that DO could be a good alternative for presynchronization of lactating dairy cows, further trials using larger numbers of experimental units were required to confirm these preliminary findings. Thus, our primary hypothesis for this study was that DO would improve fertility compared with the PS protocol for first-service TAI, particularly in primiparous cows. A second hypothesis was that DO would increase the percentage of cows that were cycling at the initiation of Ovsynch. In addition, the effect of presynchronization strategy on circulating concentrations of P4 on d 11 after TAI, and fertility at the subsequent AI were tested.

#### MATERIALS AND METHODS

#### Farms and Animals

This experiment was performed on 3 commercial farms in south-central Wisconsin from March 2007 to April 2010. Lactating dairy cows (n = 1,687; 778 primiparous, 909 multiparous) were housed in free-stall facilities bedded with sand and equipped with feedline head lockup gates, and had ad libitum access to fresh feed and water. Diets were typical for Wisconsin, using corn silage as the major forage and balanced by a professional nutritional consultant for protein, vitamins, and minerals. A similar experimental protocol was used on all 3 farms. Fertility evaluations were performed on all 3 farms, and additional ultrasound evaluations of the ovaries and measurement of circulating progesterone (P4) were completed on 1 farm. On the farm with physiological measurements, 739 lactating Holstein cows (398 multiparous, 341 primiparous) were enrolled in the study. All cows received bST (Posilac, 500 mg/ dose; Monsanto Co., St. Louis, MO) starting at 57 to 68 d postpartum and continuing every 11 d throughout the study. Cows were milked 3 times daily and fed a TMR twice daily that consisted of corn silage and alfalfa silage as forage with a corn- and soybean meal-based concentrate. The TMR was balanced to meet or exceed minimum nutritional requirements for dairy cows (NRC, 2001). Lists for scheduled injections (completed by AI personnel) and pregnancy examination (completed by the herd veterinarian) for individual cows were generated weekly using a commercial on-farm software program (Dairy Comp 305; Valley Agricultural Software, Tulare, CA). This program was also used to track and record reproductive outcomes, individual cow events, and milk production records for each cow enrolled in the experiment. All animal procedures were approved by the Animal Care Committee of the College of Agriculture and Life Sciences, University of Wisconsin, and were conducted while cows were restrained in the feedline lockup gates.

#### Presynchronization Treatments and Al

Cohorts of cows on a weekly basis were randomly assigned to 1 of 2 hormonal protocols to facilitate first postpartum TAI, either PS or DO. Double-Ovsynch uses an Ovsynch protocol for presynchronization, followed by the Ovsynch-56 protocol 7 d later to facilitate TAI (Brusveen et al., 2008). Presynch-Ovsynch uses 2 injections of  $PGF_{2\alpha}$  administered 14 d apart for presynchronization, followed by the Ovsynch-56 protocol 12 d later to facilitate TAI. Animals were assigned

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