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# A new presynchronization system (Double-Ovsynch) increases fertility at first postpartum timed AI in lactating dairy cows

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**Abstract**

This study evaluated a novel presynchronization method, using Ovsynch prior to the Ovsynch-timed AI protocol (Double-Ovsynch) compared to Presynch-Ovsynch. Lactating Holstein ($n = 337$) cows, were assigned to two treatment groups: (1) Presynch ($n = 180$), two injections of PGF 14 d apart, followed by the Ovsynch-timed AI protocol 12 d later; (2) Double-Ovsynch ($n = 157$), received GnRH, PGF 7 d later, and GnRH 3 d later, followed by the Ovsynch-timed AI protocol 7 d later. All cows received the same Ovsynch-timed AI protocol: GnRH (G1) at 68 ± 3 DIM (mean ± SEM), PGF 7 d later, GnRH (G2) 56 h after PGF, and AI 16 to 20 h later. Pregnancy was diagnosed 39–45 d after timed AI. Double-Ovsynch increased the pregnancies per AI (P/AI) compared to Presynch-Ovsynch (49.7% vs 41.7%, $P = 0.03$). Surprisingly, Double-Ovsynch increased P/AI only in primiparous (65.2% vs 45.2%; $P = 0.02$) and not multiparous (37.5% vs 39.3%) cows. In a subset of 87 cows, ovarian ultrasonography and progesterone (P4) measurements were performed at G1 and 7 d later. Double-Ovsynch decreased the percentage of cows with low P4 (<1 ng/mL) at G1 (9.4% vs 33.3%) and increased the percentage of cows with high P4 (≥3 ng/mL) at PGF (78.1% vs 52.3%). Thus, presynchronization of cows with Double-Ovsynch increased fertility in primiparous cows compared to a standard Presynch protocol, perhaps due to induction of ovulation in non-cycling cows and improved synchronization of cycling cows. Future studies are needed, with a larger number of cows, to further test the hypothesis of higher fertility with Double-Ovsynch, and to elucidate the physiological mechanisms that underlie apparent changes in fertility with this protocol.


*Keywords:* Ovsynch; Presynch; Timed AI; Dairy cow; Fertility

## 1. Introduction

Reproductive efficiency in high-producing lactating dairy cows is low due to reductions in fertility (pregnancies per AI; P/AI), expression of estrus, and detection of estrus [1–3]. Therefore, protocols that allow for timed AI (TAI), such as Ovsynch [4], have been developed. The Ovsynch protocol combines treatments with GnRH and prostaglandin F2α to synchronize the time of ovulation (GnRH–7 d–PGF–2 d-GnRH–16 h–AI). The P/AI following Ovsynch-like protocols has been directly compared to P/AI after detected estrus with reported rates that are similar [5], higher [6], or lower [7]. Previous experiments using lactating dairy cows [8] and dairy heifers [9] found that the ideal phase to initiate the Ovsynch protocol is from Days 5 to 12 of the estrous cycle. Based on this idea, researchers have developed pre-synchronization systems that attempt to increase the proportion of cows in the ideal part of the estrous cycle on the day of the first

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GnRH of Ovsynch. For instance, Moreira and coworkers [10] reported that two PGF treatments 14 d apart increased the percentage of cows in the early to mid-luteal phase and improved fertility in cycling cows when Ovsynch was initiated 12 d later. However, anovular cows did not benefit from this pre-synchronization protocol [10]. Other studies using similar Presynch protocols with two PGF treatments reported an improvement [11,12] in fertility following an Ovsynch protocol; however, a single treatment with PGF prior to Ovsynch was not effective [13].

Anovular cows have been found to be well synchronized by the Ovsynch protocol [14], but have greatly reduced fertility to the TAI protocol [14–16]. This reduced fertility may be due to the increased percentage of short cycles in anovular cows following Ovsynch [14]. There are a substantial percentage of cows that are anovular (20–30%) at the time of the first GnRH of Ovsynch [10,14,17], highlighting the importance of targeting presynchronization protocols to stimulate cyclicity in anovular cows. Chebel et al. [16] and Bicalho et al. [18] used a progesterone-releasing device (CIDR) during the Presynch protocol to reduce the percentage of anovular cows starting Ovsynch. Although an increased percentage of previously anovular cows were cycling at the start of Ovsynch, there was no increase in fertility to TAI. Novel presynchronization protocols should continue these attempts to increase cyclicity of anovular cows prior to beginning Ovsynch.

Another limitation of the standard PGF-based Presynch protocol is that follicular and luteal stages are not precisely synchronized, due to the variability in time to estrus/ovulation following PGF treatments. A recent study [19] used a PGF-GnRH protocol (PGF–2d–GnRH–6d-Ovsynch) to increase ovulation rates to the first GnRH of Ovsynch. Ovulation to the first GnRH of Ovsynch increased circulating progesterone (P4) at the time of PGF, reduced variation in the size of the ovulatory follicle, and increased synchronization rates during Ovsynch [19]. Although a large fertility trial was not attempted in this physiological study, it demonstrated the concept that increased synchronization of follicular waves/luteal function may improve outcomes during Ovsynch.

Thus, the main objective of this trial was to compare conception rates following Ovsynch in postpartum dairy cows previously treated with different presynchronization systems. One of the presynchronization protocols was the standard Presynch protocol using two treatments with PGF, 14 d apart, followed by the Ovsynch protocol initiated at 12 d after the final PGF. We decided to use a complete Ovsynch protocol for a comparison presynchronization procedure, as this protocol is known to induce cyclicity in a high percentage of anovular cows [14] and would provide tight synchrony of follicular and luteal function at the start of the Ovsynch-TAI protocol. Our hypothesis was that Double-Ovsynch would increase fertility to the Ovsynch-TAI protocol by increasing ovulation rate to the first GnRH and by increasing the percentage of cows with a CL at the beginning of Ovsynch.

## 2. Materials and methods

### 2.1. Cows, housing, and feeding

Cows were housed in free stall facilities on two commercial dairy farms located in south-central Wisconsin during the months of February through June 2007. Lactating Holstein cows ($n = 337$; 142 primiparous and 195 multiparous) were used in the present study. Cows were milked three times daily at approximately 8 h intervals and fed twice daily a total mixed ration that consisted of corn and alfalfa silage as forage with a corn and soybean meal-based concentrate. On both farms, the ration was balanced to meet or exceed minimum nutritional requirements for dairy cattle [20]. The pens on both farms had feedline head lockups, access to fresh water ad libitum, and free stalls bedded with mattress/saw dust. All procedures, including injections, blood collection, TAI, and ovarian ultrasonography, were approved by the Animal Care Committee of the College of Agriculture and Life Sciences, University of Wisconsin-Madison and conducted while cows were locked up at the feedline. All cows in the study received bovine somatotropin (500 mg/dose; Posilac, Monsanto Co., St. Louis, MO, USA) every 14 d, starting at approximately 60 d postpartum. On the day of the second GnRH treatment of the breeding Ovsynch protocol, cows had their body condition and locomotion scored using 5-point systems: 1 = thin to 5 = fat (BCS; [21]); and 1 = normal to 5 = severely lame (LS; [22]), respectively.

### 2.2. Treatments and AI

Weekly, a cohort of 30–50 cows at 42 $\pm$ 3 DIM were stratified by parity and DIM, and randomly assigned to one of two treatments: Presynch or Double-Ovsynch. Presynch cows ($n = 180$) received two injections of PGF (Prostamate®, 25 mg dinoprost tromethamine, IVX Animal Health, Inc., St. Joseph, MO, USA) at 42 $\pm$ 3 and 56 $\pm$ 3 DIM, then began the Ovsynch-TAI protocol