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

A.H. Souza [a,b,1], H. Ayres [a,b,1], R.M. Ferreira [a,b], M.C. Wiltbank [b,*]

[a] Department of Animal Reproduction, University of São Paulo, São Paulo 05508, Brazil
[b] Department of Dairy Science, University of Wisconsin, 1675 Observatory Drive, Madison, WI 53706, USA

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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 $68 \pm 3$ DIM (mean $\pm$ 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 ($\geq$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

* Corresponding author. Tel.: +1 608 263 9413; fax: +1 608 263 9412.
*E-mail address:* wiltbank@wisc.edu (M.C. Wiltbank).
[1] Co-first authors.

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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

12 d later. Double-Ovsynch cows ($n = 157$), received GnRH (Ovacyst®, 100 μg of gonadorelin diacetate tetrahydrate, IVX Animal Health, Inc.) at 51 ± 3 DIM, followed by an injection of PGF 7 d later and GnRH 72 h after PGF, then began the Ovsynch-TAI protocol 7 d later. All cows received the Ovsynch protocol: GnRH (G1) at 68 ± 3 DIM, PGF 7 d later, GnRH (G2) 56 h after PGF, and AI 16 to 20 h later (Fig. 1).

### 2.3. Ovarian ultrasonography, ovulatory responses, and pregnancy diagnosis

Ultrasonographic evaluations of the ovaries were performed using a 7.5-MHz linear transducer (Easi-Scan®/portable ultrasound, BCF Technology Ltd., Livingston, UK) in a subset of 87 cows (on the same farms) at 68 ± 3 and 75 ± 3 DIM, coinciding with G1 and PGF injections of the Ovsynch-TAI protocol. Data from ultrasonography were used to determine presence of CL at G1 and at PGF of the Ovsynch-TAI protocol and to evaluate ovulation to G1. Ovulation was characterized by appearance of a new CL in either ovary at the time of the PGF treatment of Ovsynch (Fig. 1). Pregnancy was diagnosed by transrectal palpation 39–45 d after TAI.

### 2.4. Blood sampling and radioimmunoassay

Blood samples were collected by coccygeal venipuncture just before the administration of each injection (G1 and PGF). Refrigerated samples were centrifuged ($3000 \times g$ for 20 min) within 1 h after collection and stored at −20 °C until assayed for progesterone concentrations. Circulating progesterone was evaluated from unextracted sera using an antibody-coated-tube RIA kit (Diagnostic Products Corporation, Los Angeles, CA). Inter-assay coefficient of variation was 3.0%.

### 2.5. Statistical analyses

Binomially-distributed data (i.e. presence of CL at G1 or at PGF, ovulation after G1, and pregnancies per AI), concentration of P4 at G1, and concentration of P4 at PGF, were analyzed by logistic regression, using the GLIMMIX procedure of SAS [23]. Explanatory variables considered for inclusion in the models were treatment, farm, month, parity (primiparous vs multiparous), days in milk, BCS (categorized as <2.75 or ≥2.75), LS (categorized as 1, 2, 3 or greater), and interactions. The final logistic regression model removed variables by a backward elimination based on the Wald statistics criterion when $P > 0.20$. The variables that were included in the final model for analysis of fertility were: treatment, parity (primiparous vs multiparous), BCS (categorized as <2.75 or ≥2.75), and interaction between treatment and parity. The final statistical models for analysis of concentrations of progesterone (continuous variable and distribution of cows within P4 categories) and percentage of cows that ovulated to GnRH included only the effect of parity. A univariable analysis with Proc GLIMMIX was used for analyses of treatment effects on days in milk, lactation number, BCS, and LS (Table 1).

## 3. Results

### 3.1. General results

A total of 450 cows (218 on Double-Ovsynch and 232 on Presynch) were enrolled in the present study. Some cows were excluded from the analysis (Double Ovsynch, $n = 61$; Presynch, $n = 52$) because they were culled before the end of the study or had the wrong breeding date (failure to comply with the research protocol). Therefore, 337 cows were available for analysis: 251 from farm A and 86 from farm B.

Average DIM ($P = 0.57$), number of lactations ($P = 0.64$), BCS ($P = 0.54$), and LS ($P = 0.34$) did not differ among the treatment groups. Also, similar percentages of pregnant cows after TAI were achieved on both farms [46.6% (117/251) and 41.9% (36/86), $P = 0.83$], and during all months [February – 44.2% (19/43), March – 44.9% (53/118), April – 54.1% (33/61), and May – 41.8% (48/115), $P = 0.37$]. The following interactions were not significant during analysis of P/AI: treatment × farm ($P = 0.51$), treatment × month ($P = 0.30$), and treatment × BCS class ($P = 0.67$). These variables were removed from the final model. An interaction between treatment and parity was kept in the model, because a $P$-value of 0.15 was observed.

### 3.2. Effect of presynchronization protocol on pregnancies per AI

Double-Ovsynch had greater P/AI ($P = 0.03$) than Presynch (Table 1; 49.7% vs 41.7%, respectively). Parity (primiparous vs multiparous) had an effect ($P = 0.05$) on pregnancies per AI (Table 2) with primiparous (54.9%) having greater P/AI than multiparous (38.5%) cows. Unexpectedly, Double-Ovsynch increased P/AI only in primiparous cows (65.2% vs 45.2%; $P = 0.02$), with no effect of treatment observed in multiparous cows (37.5% vs 39.3%; $P = 0.58$).

There was also an effect ($P = 0.04$) of BCS on P/AI (Table 3; high = 48.3% vs low = 28.3%). This negative impact of low BCS on P/AI was numerically similar in Double-Ovsynch (20.1% decrease) and Presynch (19.5% decrease) cows. In addition, there were numerically similar effects of Double-Ovsynch on P/AI in cows with both high (Double-Ovsynch 12.5% greater than Presynch; $P = 0.06$) and low (Double-Ovsynch 11.9% greater than Presynch; $P = 0.25$) BCS; although, statistical differences were clearly not detected in the small number of cows with low BCS in this study (only 46 cows in this classification).

### *3.3. Ovarian morphology and progesterone concentration*

#### *3.3.1. First GnRH of breeding Ovsynch*

At G1, there was no significant difference in P4 concentration ($P = 0.97$) between Double-Ovsynch vs Presynch (Table 4). However, analysis of the distribution of cows by categories of P4 concentrations at G1 was informative (Fig. 2A). Presynchronization with Double-Ovsynch decreased ($P = 0.03$) the percentage of cows with low (<1 ng/mL) P4 concentrations at G1. Conversely, Double-Ovsynch dramatically increased the percentage of cows with medium (1.0 to <3 ng/mL) P4 concentrations (Double-Ovsynch = 75.0% vs Presynch = 35.7%; $P < 0.01$) at G1. Nevertheless, there was no effect ($P = 0.24$) of Double-Ovsynch on the percentage of cows with a CL detected by ultrasound at G1 (Table 1). There was also no effect of Double-Ovsynch vs Presynch ($P = 0.53$) on the percentage of cows that ovulated to the first GnRH treatment of Ovsynch (Table 4).

#### *3.3.2. PGF treatment of Ovsynch*

The P4 concentration at the time of the PGF treatment (Table 4) was greater ($P = 0.03$) in Double-Ovsynch than Presynch cows ($4.19 \pm 0.38$ ng/mL vs $3.16 \pm 0.31$ ng/mL; mean ± SEM). Nevertheless, the percentage of cows with low P4 (<1 ng/mL) was not different between the two groups ($P = 0.73$). The difference in P4 concentrations between the two groups was due to an altered distribution of cows within the medium (1.0 to <3.0 ng/mL) and high (≥3.0 ng/mL) P4 categories (Fig. 2B). Double-Ovsynch decreased the percentage of cows with medium P4 ($P = 0.03$). Conversely, Double-Ovsynch increased the percentage of cows with high P4 ($P = 0.03$).

## 4. Discussion

This study evaluated a more complex presynchronization protocol than most previous studies in an attempt to achieve a more synchronous stage of the cycle at the initiation of the Ovsynch protocol. The use of a presynchronization Ovsynch before beginning the breeding Ovsynch may be regarded as extreme, but our overall objective is to develop protocols that optimize fertility in lactating dairy cows by optimizing follicular size and hormonal environment. This research should allow better understanding of factors that limit fertility in dairy cows and may allow development of practical methods to increase reproductive performance on commercial dairy farms. Accordingly, this research analyzed the Double-Ovsynch protocol on two commercial dairy farms compared to the Presynch-Ovsynch protocol.

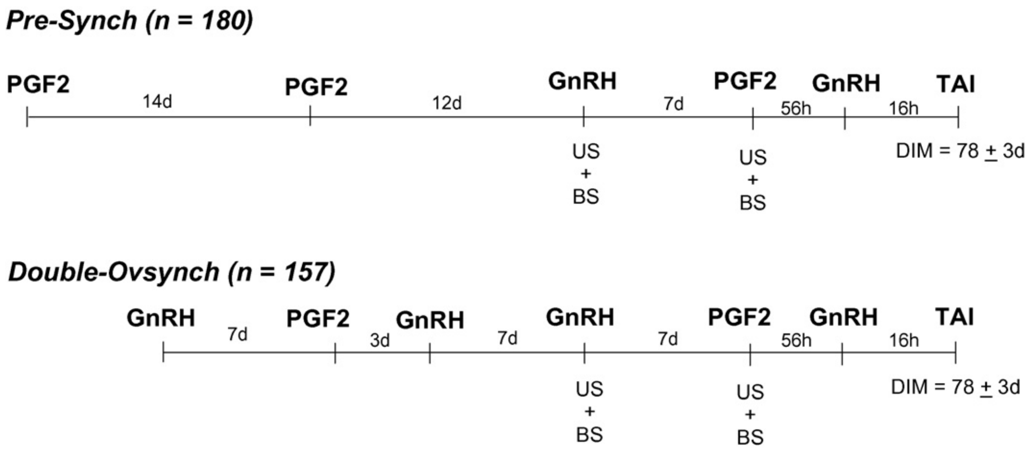


Fig. 1. Schematic diagram of hormonal treatments, ultrasonographic exams (US), and blood samples (BS) in lactating dairy cows (mean ± range of DIM).

Table 1
Mean (±SEM) effects of synchronization protocol on pregnancies per AI (P/AI), days in milk, number of lactations, body condition score (BCS) and locomotion score in lactating cows

| | Double-Ovsynch | Presynch | *P*-value |
|---|---|---|---|
| Pregnancies per AI (P/AI)[#] | 49.7% (78/157) | 41.7% (75/180) | 0.03 |
| Days in milk (range) | 77.0 ± 0.16 (74–81) | 77.2 ± 0.15 (74–81) | 0.57 |
| Lactation number (range) | 2.2 ± 0.12 (1–11) | 2.4 ± 0.13 (1–10) | 0.64 |
| BCS (range) | 2.91 ± 0.03 (2.25–4.00) | 2.89 ± 0.02 (2.25–4.25) | 0.54 |
| Locomotion score (range) | 1.45 ± 0.08 (1–5) | 1.48 ± 0.07 (1–4) | 0.34 |

# This analysis was done with GLIMMIX and accounted for parity and BCS in the model.

The Presynch-Ovsynch protocol used in this study had two PGF injections given 14 d apart, with Ovsynch initiated 12 d after the final PGF. This protocol has produced significantly greater fertility at first postpartum AI, at least in cycling cows, compared to the Ovsynch protocol alone [10], and is currently the U.S. industry standard for first TAI. Recent research [24] suggests that a shorter Presynch protocol (11 d from final PGF of Presynch to first GnRH of protocol) produces greater fertility (40.5% vs 33.5%; $P = 0.06$) than a longer protocol (14 d from final PGF to first GnRH); however, the Heatsynch protocol was utilized in that study (this protocol uses estradiol cypionate in lieu of the final GnRH for synchronizing ovulation). The ovulation rate to the first GnRH after Presynch (12 d between PGF and Ovsynch) in our study (66.7%) appeared to be similar to what was reported after the 11 d Presynch protocol (61.4% [24]) and higher than what has been reported for the 14 d protocol (44.7% [24]; 35.8% [12]). These data were consistent with the selected Presynch-Ovsynch protocol being a good control protocol for comparison to the Double-Ovsynch protocol. This is further reinforced by the high fertility to the TAI (41.7%) in the cows treated with Presynch-Ovsynch in our study. In spite of the high fertility to Presynch-Ovsynch, cows presynchronized with Double-Ovsynch had even greater fertility than Presynch-Ovsynch cows ($P = 0.03$), with P/AI reaching close to 50% for Double-Ovsynch (49.7%). While this result reached statistical significance using a statistical model in GLIMMIX that accounted for parity and BCS effects, it should be viewed with some caution because a simple Chi-square analysis of the data did not yield statistical significance ($P = 0.14$). Future studies, with greater statistical power, are needed to provide more information on the practical value of this novel protocol. As discussed below, the underlying physiology producing improved fertility with Double-Ovsynch in primiparous cows appears to be improved treatment of anovular cows and increased synchronization of stage of the cycle at initiation of Ovsynch.

In response to the high frequency of anovulation in dairy cows (~20%) [10,14,17], various protocols have been tested for treating this condition. The earliest studies with Presynch-Ovsynch recognized that this protocol was not effective in anovular cows [10] because only PGF was used during the presynchronization protocol. Subsequent studies used a CIDR during Presynch [16,18]. In these studies, a CIDR was inserted 7 d after the first PGF of Presynch, and removed at the time of the second PGF. In both studies the percentage of cows with low P4 at the first GnRH of Ovsynch decreased from 30.6% in control cows to 17.4% in CIDR-treated cows [18] or from 29.6% in control cows

Table 2
Effects of number of lactations on pregnancies per AI (P/AI) following presynchronization of dairy cows with a standard Presynch protocol or Double-Ovsynch

| | Primiparous | Multiparous | *P*-value*,# |
|---|---|---|---|
| Double-Ovsynch | 65.2% (45/69) | 37.5% (33/88) | 0.02 |
| Presynch | 45.2% (33/73) | 39.3% (42/107) | 0.71 |
| *P*-value**,# | 0.02 | 0.58 | – |
| Overall | 54.9% (78/142) | 38.5% (75/195) | 0.05 |

* *P*-values for comparisons of primiparous vs multiparous.
** *P*-values for comparisons of Double-Ovsynch vs Presynch.
\# These analyses were done with GLIMMIX and accounted for BCS in the model.

Table 3
Effects of body condition score (BCS) on pregnancies per AI (P/AI) in dairy cows following presynchronization with a standard Presynch protocol or Double-Ovsynch

| | High (≥2.75) | Low (<2.75) | *P*-value*,# |
|---|---|---|---|
| Double-Ovsynch | 55.1% (59/107) | 35.0% (7/20) | 0.26 |
| Presynch | 42.6% (55/129) | 23.1% (6/26) | 0.08 |
| *P*-value**,# | 0.06 | 0.25 | – |
| Overall | 48.3% (112/236) | 28.3% (13/46) | 0.04 |

* *P*-values for comparisons of high vs low BCS cows.
** *P*-values for comparisons of Double-Ovsynch vs Presynch.
\# These analyses were done with GLIMMIX and accounted for parity in the model.

Table 4
Mean (±SEM) effects of synchronization protocol various reproductive end points in lactating dairy cows

| | Double-Ovsynch | Presynch | *P*-value |
|---|---|---|---|
| Presence of CL at 1st GnRH | 84.6% (33/39) | 75.0% (36/48) | 0.24 |
| Concentration of P4 at 1st GnRH (ng/mL) | 1.94 ± 0.18 (0.00–4.45) | 1.91 ± 0.27 (0.00–9.63) | 0.97 |
| Percentage of cows with [P4] < 1.0 ng/mL at 1st GnRH | 9.4% (3/32) | 33.3% (14/42) | 0.03 |
| Ovulation to 1st GnRH | 71.8% (28/39) | 66.7% (32/48) | 0.53 |
| Concentration of P4 at PGF (ng/mL) | 4.19 ± 0.38 (0.00–9.02) | 3.16 ± 0.31 (0.03–6.95) | 0.03 |
| Percentage of cows with [P4] ≥ 1.0 ng/mL at PGF | 84.4% (27/32) | 81.0% (34/42) | 0.73 |

to 20.9% in CIDR-treated cows ([16]. In the present study, there was a decrease in percentage of cows with low P4 at the first GnRH of Ovsynch from 33.3% in Presynch-treated cows to 9.4% in Double-Ovsynch-treated cows. This result is consistent with the idea that Double-Ovsynch is effective in inducing cyclicity in anovular cows; however, further studies are needed to confirm these results. Previous studies using GnRH in the presynchronization protocol also indicated an induction of cyclicity. For example in the previous study comparing the interval from Presynch to the first GnRH [24], there was a third group of cows that were treated with GnRH 4 d after the final PGF of Presynch (7 d before initiation of Heatsynch); only 11.8% of these cows lacked a CL at time of the first GnRH of Ovsynch, compared to 25.8% in cows treated only with Presynch. In a previous study from our laboratory [25], cows were treated with GnRH during the Presynch protocol (7 d before the last PGF) and this induced ovulation in anovular cows (80% with GnRH vs 31% spontaneous ovulation in control anovular cows). Nevertheless, none of these previous studies found an overall improvement in fertility or an improvement in fertility in anovular cows after treatment with CIDR or GnRH during the presynchronization protocol. In the present study, we did not identify the cows that were anovular prior to beginning the protocol and therefore no conclusions can be made about whether Double-Ovsynch specifically improved fertility in anovular cows. Nevertheless, the reduction in cows with low progesterone at the time of initiation of the breeding Ovsynch was consistent with the first Ovsynch inducing cyclicity in anovular cows prior to beginning the breeding Ovsynch.

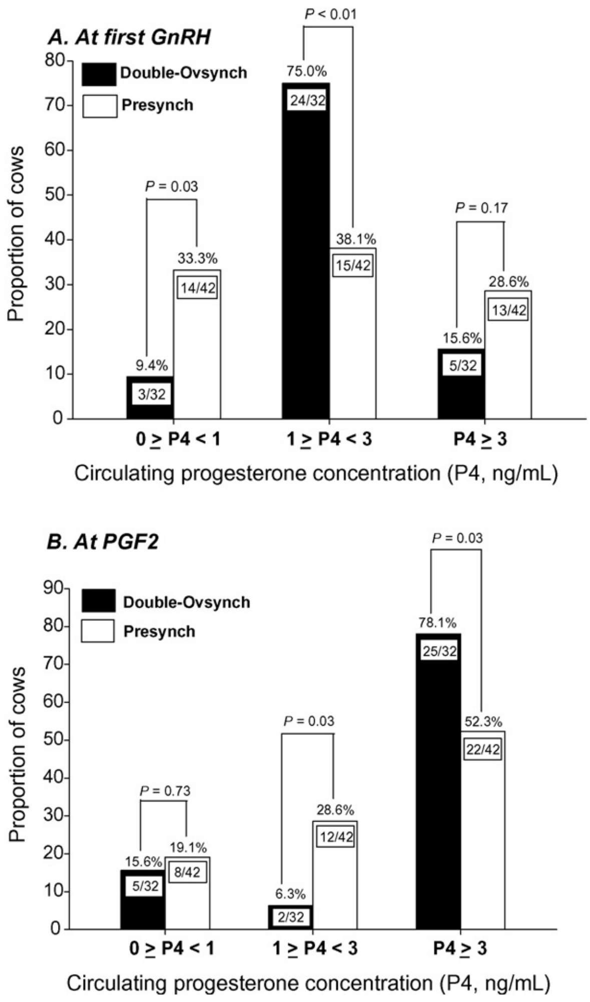


Fig. 2. Effects of synchronization protocol on proportion of lactating dairy cows distributed in three progesterone (P4) classes at 1st GnRH (A) and at PGF (B).

In addition to treatment of anovular cows, it seems likely that Double-Ovsynch more tightly synchronized the stage of the estrous cycle at initiation of Ovsynch compared to Presynch. Ovulation to the first GnRH of the Ovsynch protocol and fertility during the Ovsynch protocol was dependent on stage of the estrous cycle at Ovsynch initiation [8,26]. In addition, cows ovulating to the first GnRH of the Ovsynch have greater fertility compared with cows not ovulating to this first GnRH [16,19]. Unexpectedly, we did not detect a difference in ovulation rate to the first GnRH of Ovsynch in this trial. In designing the Double-Ovsynch protocol it seemed likely that cows on Day 7 of the estrous cycle, the stage selected for initiation of the breeding Ovsynch, would be very likely to ovulate in response to the first GnRH of

Ovsynch [8]. However, further trials with larger numbers of cows are needed to determine whether a higher ovulation rate does not occur or whether a Type II error is responsible for our negative results. The greater circulating P4 (Table 4) and the greater percentage of cows with high P4 ($\geq$3 ng/mL; Fig. 2) at the time of PGF were consistent with a greater synchrony of cows at Ovsynch initiation and/or a greater percentage ovulating to the first GnRH of Ovsynch. Increased ovulation to G1 may produce a dominant follicle at G2 that is less variable and closer to the ideal size [19,27]. In addition, greater synchrony during Ovsynch may decrease the percentage of cows that ovulate at inopportune times during Ovsynch [8], increase circulating P4, and increase fertility [19]. For example, Chebel et al. [16] found higher fertility ($\sim$40% vs $\sim$10%) in cows having a CL on the day of PGF during Ovsynch, compared with cows without a CL. These results were in agreement with a reported linear increase in probability of pregnancy as circulating concentrations of P4 at the time of PGF of Ovsynch increased [19]. Thus, improved fertility during Double-Ovsynch may be at least partially related to the hormonal milieu during follicular development. Greater circulating P4 during follicular development could decrease LH pulsatility possibly improving the competency of the dominant follicle and/or the quality of the ovulated oocyte [28,29].

Perhaps the two PGF treatments of Presynch-Ovsynch had some positive effects on uterine health that were not observed with Double-Ovsynch, but these effects were offset by more dramatic positive effects of Double-Ovsynch on fertility in other areas (i.e. anovular cows or another subgroup). These potentially divergent effects of the two presynchronization protocols in different subsets of cows could not be differentiated in our experiment, but require future research to determine if specific subsets of cows are better treated with specific presynchronization protocols.

Surprisingly, the improvement in fertility during Double-Ovsynch seemed to be mostly due to increases in fertility in primiparous rather than multiparous cows. It is generally accepted that primiparous cows are more likely to be anovular compared with multiparous cows [11,16,30]. Thus, the parity differences in effects of Double-Ovsynch might be related to the percentage of anovular cows in each lactation group. For instance, Cartmill et al. [31] used a single PGF 12 d before initiation of Ovsynch and reported greater conception rates in multiparous but not primiparous cows. Similarly, DeJarnette and Marshall [32] found that two PGF treatments can be advantageous for multiparous but not primiparous cows. Thus, a high percentage of anovular cows could result in reduced effectiveness of Presynch-Ovsynch in primiparous cows. In contrast, Double-Ovsynch may effectively treat anovular cows and the increased percentage of primiparous cows that are anovular may make this protocol more useful for primiparous cows. Further studies are needed to confirm and extend these findings.

Thus, based on the results of this study and other studies, fertility in Ovsynch-like protocols in lactating dairy cows is likely to improve by priming with P4 before synchronizing ovulation for TAI, increasing percentage of cows in early diestrus at Ovsynch initiation, increasing percentage of cows ovulating after G1, and increasing circulating P4 during the Ovsynch protocol. The Double-Ovsynch procedure offers one method to potentially improve these variables during Ovsynch and increase fertility to the TAI.

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