Theriogenology 79 (2013) 159–164

Contents lists available at SciVerse ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Double-Ovsynch in high-producing dairy cows: Effects on progesterone concentrations and ovulation to GnRH treatments

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

[a] Department of Animal Reproduction, University of São Paulo, São Paulo, Brazil
[b] Department of Dairy Science, University of Wisconsin, Madison, Wisconsin, USA

ARTICLE INFO

Article history:
Received 5 April 2012
Received in revised form 1 October 2012
Accepted 1 October 2012

Keywords:
Ovsynch
Estrus synchronization
Timed AI
Dairy cow
Reproduction

ABSTRACT

Previous studies reported increased fertility using Ovsynch for presynchronization before Ovsynch (Double-Ovsynch), as compared with presynchronization with two prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) treatments before Ovsynch (Presynch-Ovsynch). This study compared ovarian follicular dynamics and hormone concentrations during Double-Ovsynch versus Presynch-Ovsynch. Lactating Holstein cows (N = 193) were assigned to one of two treatment groups: (1) Presynch (N = 93), two injections of $PGF_{2\alpha}$ 14 days apart, followed by the Ovsynch-timed AI protocol 12 days later; and (2) Double-Ovsynch (N = 100), one injection of GnRH, $PGF_{2\alpha}$ 7 days later, and GnRH 3 days later, followed by the Ovsynch-timed AI protocol 7 days later. All cows received the same Ovsynch-timed AI protocol: GnRH (G1) at 68 ± 3 days in milk (mean ± SEM), $PGF_{2\alpha}$ 7 days later, and GnRH (G2) 56 hours after $PGF_{2\alpha}$. Ultrasonographic evaluations of the ovaries and blood sampling were performed at G1, $PGF_{2\alpha}$, G2, and 6 days after the G2 injection of the Ovsynch-timed AI protocol. Double-Ovsynch decreased the percentage of cows with low circulating progesterone (P4) concentrations (<0.50 ng/mL) at G1 (12.0% vs. 30.1%; P = 0.003) and increased the percentage of cows with medium P4 concentrations (0.50 > P4 ≤ 3.0 ng/mL) at G1 (80.0% vs. 57.0%; P < 0.01), and with CL at G1 (94.0% vs. 67.8%; P < 0.01). Double-Ovsynch also increased the percentage of cows with high P4 (>3.0 ng/mL) at $PGF_{2\alpha}$ (88.0% vs. 76.3%; P = 0.04) and tended to increase average circulating P4 at $PGF_{2\alpha}$ (3.52 ± 0.17 ng/mL vs. 3.09 ± 0.21 ng/mL; P = 0.11). Double-Ovsynch also tended to increase percentage of cows ovulating to G1 (80.0% vs. 69.9%; P = 0.11) and G2 (98.0% vs. 93.5%; P = 0.08). Thus, presynchronization of cows with Double-Ovsynch induced ovulation in noncycling cows and appeared to increase most aspects of synchronization during the Ovsynch protocol.

1. Introduction

Reproductive efficiency in high-producing lactating dairy cows has been decreasing because of reductions in fertility (pregnancies per AI; P/AI), expression of estrus, and detection of estrus [1,2]. Therefore, protocols that include timed AI, such as Ovsynch [3], have been developed. The Ovsynch protocol combines treatments with GnRH and $PGF_{2\alpha}$ to synchronize the time of ovulation (GnRH-7 days-$PGF_{2\alpha}$-2 days-GnRH-16 hours-AI). The P/AI after Ovsynch-like protocols has been directly compared with P/AI after detected estrus in a number of studies and, although fairly variable among studies, on average, the risks of pregnancy were similar for the two methods [4,5]. In previous experiments using lactating dairy cows [6] and dairy heifers [7], initiation of the Ovsynch protocol on Days 5 to 12 of the estrous cycle produced better results than initiation of Ovsynch either earlier or later in the cycle. Based on this idea, presynchronization systems were developed in an attempt to increase the proportion of cows in the ideal

* Corresponding author. Tel.: +1 608 263 9413; fax: +1 608 263 9412.
E-mail address: wiltbank@wisc.edu (M.C. Wiltbank).
[1] H. Ayres and R.M. Ferreira are co-first authors.

0093-691X/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2012.10.001

part of the estrous cycle on the day of the first GnRH of Ovsynch. For instance, Moreira and coworkers [8] reported that two $PGF_{2\alpha}$ treatments 14 days apart increased the percentage of cows in the early to midluteal phase and improved fertility in cycling cows when Ovsynch was initiated 12 days later. However, anovular cows did not benefit from this presynchronization protocol [8]. Other studies using similar Presynch protocols with two $PGF_{2\alpha}$ treatments reported improved [9,10] fertility following an Ovsynch-timed AI protocol; however, a single treatment with $PGF_{2\alpha}$ before Ovsynch was not effective [11].

Anovular cows were well synchronized by the Ovsynch protocol [12], but had greatly reduced fertility to the timed AI protocol [12–14]. This reduced fertility might be because of the increased percentage of short cycles in anovular cows after Ovsynch [12]. There is a substantial percentage of lactating dairy cows that are anovular or have low circulating progesterone (P4) (20%–30%) at the time of the first GnRH of Ovsynch [1,8,12,15,16], highlighting the importance of using presynchronization protocols that not only regulate the timing of Ovsynch initiation in cycling cows, but also stimulate cyclicity of anovular cows. Thus, there are limitations to the standard $PGF_{2\alpha}$-based Presynch protocol, including lack of induction of cyclicity in anovular cows and lack of precise synchronization of follicular and luteal stages, because of the variability in time to estrus and ovulation after $PGF_{2\alpha}$ treatments.

In two recent studies [17,18], the Double-Ovsynch protocol increased fertility to the Ovsynch-timed AI (TAI) protocol compared with a Presynch-12-Ovsynch protocol. In both studies, Double Ovsynch increased the percentage of cows with elevated P4 at the time of first GnRH treatment. Furthermore, one of the studies reported an improvement in P4 concentrations at the time of $PGF_{2\alpha}$ treatment. Thus, the authors speculated that use of Double-Ovsynch might effectively treat anovular cows and might improve synchronization during the final Ovsynch-TAI protocol.

Thus, the main objective of this trial was to compare the responses to each of the treatments of the Ovsynch protocol in postpartum dairy cows previously treated with two presynchronization systems. Our hypothesis was that Double-Ovsynch would increase the percentage of cows with CL at initiation of Ovsynch, increase the percentage of cows with medium P4 at first GnRH, and increase the percentage of cows that ovulate to the first GnRH.

2. Materials and methods

2.1. Animals, housing, and feeding

Animals were housed in free stall facilities on a commercial dairy farm located in south central Wisconsin during the months of June through November 2007. Lactating Holstein cows (N = 193, including 87 primiparous and 106 multiparous) were used in the present study. Animals were milked three times daily at approximately 8-hour intervals and fed twice daily a total mixed reaction that consisted of corn and alfalfa silage as forage with a corn and soybean meal-based concentrate. The total mixed ration was balanced to meet or exceed minimum nutritional requirements for dairy cattle [19]. The farm had feed-line 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 feed-line. All animals in the study received bovine somatotropin (500 mg per dose; Posilac, Monsanto Co., St. Louis, MO, USA) every 14 days, starting at approximately 60 days postpartum. On the day of the second GnRH treatment of the breeding Ovsynch protocol, cows had their body condition and locomotion scored using five-point systems: 1 = thin, to 5 = fat (body condition score [20]) and 1 = normal, to 5 = severely lame (locomotion score [21]).

2.2. Treatments and AI

Weekly, a cohort of 30 to 50 cows at 42 ± 3 days in milk (DIM) were stratified by parity and DIM, and randomly assigned to one of two treatments: Presynch-Ovsynch or Double-Ovsynch. Presynch-Ovsynch cows (N = 100) received two injections of $PGF_{2\alpha}$ (Prostamate, 25 mg dinoprost tromethamine; IVX Animal Health, Inc., St. Joseph, MO, USA) at 42 ± 3 and 56 ± 3 DIM, then began the Ovsynch-timed AI protocol 12 days later. Double-Ovsynch cows (N = 93), received GnRH (Ovacyst, 100 μg of gonadorelin diacetate tetrahydrate; IVX Animal Health, Inc.) at 51 ± 3 DIM, followed by an injection of $PGF_{2\alpha}$ 7 days later and GnRH 72 hours after $PGF_{2\alpha}$, then began the Ovsynch-timed AI protocol 7 days later. All cows received the same Ovsynch protocol: GnRH (G1) at 68 ± 3 DIM, $PGF_{2\alpha}$ 7 days later, and GnRH (G2) 56 hours after $PGF_{2\alpha}$.

2.3. Ovarian ultrasonography, and ovulatory responses

Ultrasonographic evaluations of the ovaries were performed using a 7.5-MHz linear transducer (Easi-Scan portable ultrasound; BCF Technology Ltd., Livingston, UK) at G1 (68 ± 3 DIM), $PGF_{2\alpha}$ (75 ± 3 DIM), G2 (77 ± 3 DIM), and 6 days after G2 (83 ± 3 DIM) injections of the Ovsynch-timed AI protocol. Data from ultrasonography were used to determine presence of CL at G1 and at $PGF_{2\alpha}$, size of the dominant follicle, and endometrial thickness at G2 of the Ovsynch-timed AI protocol, and to evaluate ovulation to G1 and G2. Sizes of follicles and endometrial thickness were estimated by use of the grid on the Easi-Scan screen. Ovulation was characterized by disappearance of a dominant follicle and appearance of a new CL in either ovary at the time of examination.

2.4. Blood sampling and radioimmunoassay

Blood samples were collected by venipuncture of the median caudal vein or artery using evacuated tubes (Becton Dickinson, Franklin Lakes, NJ, USA), just before administration of each injection (G1, $PGF_{2\alpha}$, and G2), in both treatment groups. Refrigerated samples were centrifuged (3000 × *g* for 20 minutes) within 1 hour after collection. Serum was harvested and stored at approximately 20 °C

Table 1
Effects of two presynchronization treatments before the Ovsynch-timed AI on various aspects of ovarian function in lactating dairy cows.

	Double-Ovsynch (N = 100)	Presynch-Ovsynch (N = 93)	P
Presence of CL at first GnRH, % (N/N)	94.0 (94/100)	67.8 (63/93)	0.0003
Presence of CL at $PGF_{2\alpha}$, % (N/N)	91.0 (91/100)	86.0 (80/93)	0.53
Ovulation to 1st GnRH, % (N/N)	80.0 (80/100)	69.9 (65/93)	0.11
Percentage of animals with P4 ≤ 0.50 ng/mL at 1st GnRH, % (N/N)	12.0 (12/100)	30.1 (28/93)	0.003
Percentage of animals with P4 ≥ 1.00 ng/mL at $PGF_{2\alpha}$, % (N/N)	88.0 (88/100)	76.3 (71/93)	0.04
Luteolysis, % (N/N)	95.0 (95/100)	93.5 (87/93)	0.67

Abbreviation: P4, progesterone.

until assayed to determine concentrations of P4 and estradiol (E2).

Serum P4 concentrations were evaluated from unextracted sera using a solid-phase RIA kit for P4 (Coat-a-Count Progesterone, Diagnostic Products Corp., Los Angeles, CA, USA). The interassay coefficient of variation for the quality control samples was 3.0%.

Serum E2 concentrations were evaluated using a commercial RIA kit (Double Antibody Estradiol; Diagnostic Products Corp.). The procedure in our laboratory has been described for cattle [22], except with the following modifications. Standards (0.78 to 100 pg/mL) were prepared in steroid-free serum (charcoal-treated bovine plasma). The standards and plasma samples (500 μL in duplicate) were extracted with 3 mL of diethyl ether, frozen in a dry ice/methanol bath, decanted into assay tubes, and dried overnight under the hood. The dried samples and standards were resuspended with 100 μL of assay buffer (0.1% gelatin in PBS). The intra-assay and interassay CVs and sensitivity were 6.2%, 7.7%, and 0.08 pg/mL, respectively.

2.5. Statistical analyses

Binomially-distributed data (i.e., presence of CL at G1, ovulation percentage at G1, percentage of animals with P4 ≤ 0.50 ng/mL at G1, presence of CL at $PGF_{2\alpha}$, percentage of animals with P4 ≥ 1.00 ng/mL at $PGF_{2\alpha}$, luteolysis percentage, and ovulation percentage at G2) and normally-distributed data (i.e., concentrations of P4, concentrations of E2, size of the dominant follicle, and endometrial thickness) were analyzed using the GLIMMIX procedure of SAS software version 9.1 [23]. Statistical differences were considered if $P < 0.05$ and a tendency if $P < 0.15$.

3. Results

3.1. General results

A total of 200 cows (100 Double-Ovsynch and 100 Presynch) were enrolled in the present study. Seven cows from the Presynch group were excluded from the analysis because they were culled before the end of the study or had the wrong breeding date (failure to comply with the research protocol). Therefore, 193 cows were available for analysis. Average DIM (77.7 ± 0.22 vs. 77.4 ± 0.21; P = 0.27), number of lactations (2.0 ± 0.12 vs. 2.1 ± 0.13; P = 0.84), body condition score (2.91 ± 0.04 vs. 2.85 ± 0.04; P = 0.29), and locomotion score (1.48 ± 0.07 vs. 1.43 ± 0.07; P = 0.55) did not differ among the treatment groups (Double-Ovsynch vs. Presynch-Ovsynch, respectively).

3.2. Concentrations of P4 and ovulation to first GnRH

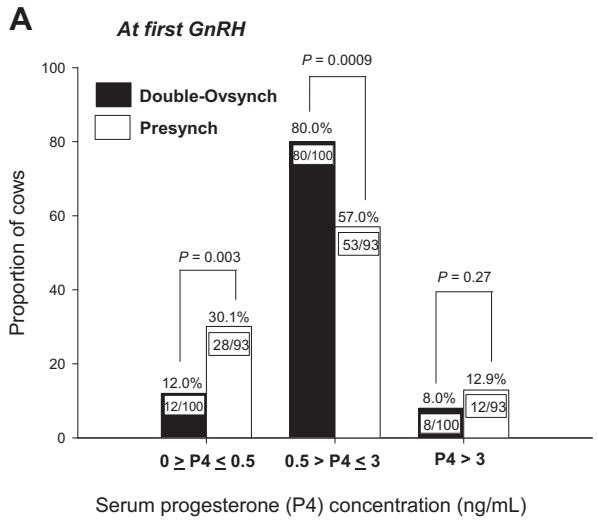
At G1, there was an effect (P = 0.0003) of Double-Ovsynch on the percentage of cows with CL detected ultrasonographically at G1 (Table 1). However, there was no difference in mean P4 concentrations (P = 0.36) between Double-Ovsynch versus Presynch for all cows at G1 (Table 2). Further analysis of the distribution of cows by categories of P4 concentrations at G1 was informative (Fig. 1A). Presynchronization with Double-Ovsynch decreased the percentage of cows with low (≤0.50 ng/mL) P4 at G1 (Double-Ovsynch = 12.0% vs. Presynch = 30.1%; P = 0.003). Conversely, Double-Ovsynch substantially increased the percentage of cows with medium (0.50 to <3 ng/mL) P4 concentrations at G1 (Double-Ovsynch = 80.0% vs. Presynch = 57.0%; P = 0.0009), based on P4 concentrations at G1 for cows with a CL, compared with cows without a CL, there was no treatment effect on P4 concentrations in cows without a CL but there were dramatically higher P4 concentrations in cows with a CL in the Presynch-Ovsynch cows compared with the Double-Ovsynch cows (Table 2). However, as shown (Table 1), there was a much larger percentage of cows with low P4 at G1 in the Presynch-Ovsynch group (30.1%) than in the Double-Ovsynch group (12.0%), leading to the similar mean P4 concentrations at G1. Analysis by parity of the P4 concentrations at G1 showed no difference between treatment groups (Table 2).

Overall, there was a tendency for an effect of Double-Ovsynch versus Presynch (P = 0.11) on the percentage of

Table 2
Effects (mean ± SEM) of two presynchronization treatments before the Ovsynch-timed AI protocol on concentration of P4 at 1st GnRH and at $PGF_{2\alpha}$.

	Double-Ovsynch (N = 100)	Presynch-Ovsynch (N = 93)	P
[P4] at 1st GnRH (ng/mL)			
All cows	1.57 ± 0.09	1.74 ± 0.16	0.36
Presence of CL at 1st GnRH			
No	0.23 ± 0.19	0.06 ± 0.02	0.77
Yes	1.67 ± 0.09	2.67 ± 0.23	<0.01
By lactation			
Primiparous	1.37 ± 0.11	1.52 ± 0.20	0.95
Multiparous	1.73 ± 0.14	1.91 ± 0.25	0.89
[P4] at PGF 2α (ng/mL)			
All cows	3.52 ± 0.17	3.09 ± 0.21	0.11
Ovulation at 1st GnRH			
No	2.92 ± 0.40	2.88 ± 0.42	0.95
Yes	3.68 ± 0.19	3.17 ± 0.25	0.11
By lactation			
Primiparous	3.31 ± 0.25	3.09 ± 0.32	0.95
Multiparous	3.71 ± 0.24	3.08 ± 0.29	0.33

Abbreviation: P4, progesterone.



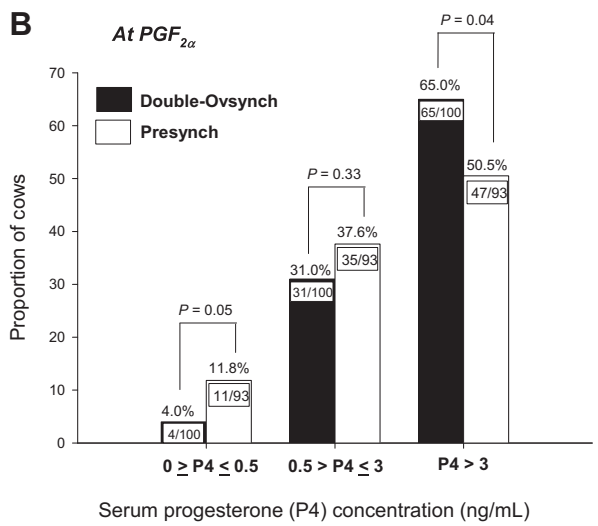


Fig. 1. Effects of two presynchronization treatments before the Ovsynch-timed AI protocol on proportion of lactating dairy cows distributed into three progesterone (P4) classes at first GnRH (A) and at $PGF_{2\alpha}$ (B).

cows that ovulated to the first GnRH treatment of Ovsynch (Table 1). Analysis of ovulation to G1 treatment by the circulating P4 at G1 by logistic regression (Fig. 2) indicated that there was decreasing ovulation to G1 with increasing P4 concentration. Nevertheless, there were no differences between treatment groups at any P4 concentration.

3.3. Concentrations of P4 and luteolysis to $PGF_{2\alpha}$ of Ovsynch

Circulating P4 concentrations at $PGF_{2\alpha}$ treatment tended to be greater (P = 0.11) in Double-Ovsynch than Presynch cows (3.52 ± 0.17 ng/mL vs. 3.09 ± 0.21 ng/mL; Table 2). Analysis of circulating P4 at $PGF_{2\alpha}$ for cows that ovulated or did not ovulate to the first GnRH also indicated no treatment group differences, although there was a tendency (P = 0.11) for greater P4 in Double-Ovsynch than Presynch for cows that ovulated to the first GnRH (Table 2). There were

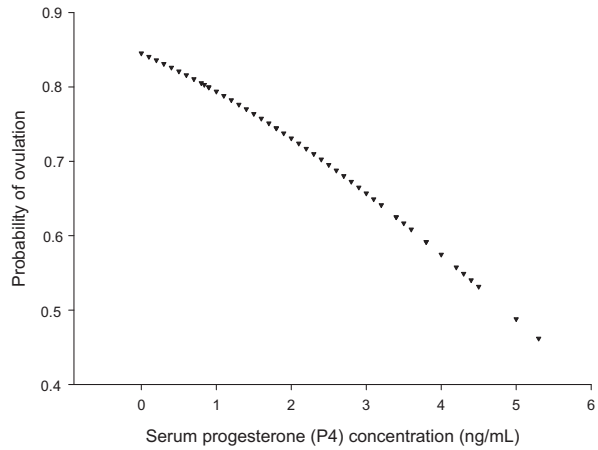


Fig. 2. Probability of ovulation for cows with different circulating progesterone (P4) concentrations at first GnRH. Cows with higher circulating P4 concentrations had lower probability of ovulation (P = 0.05).

no treatment group differences if cows were divided by parity (Table 2). In addition, the presence of a CL at $PGF_{2\alpha}$ was not different between the two groups (Double-Ovsynch = 91.0% vs. Presynch = 86.0%; P = 0.53; Table 1). In contrast, the percentage of cows with very low P4 concentration (≤0.50 ng/mL; Double-Ovsynch = 4.0% vs. Presynch = 11.8%; P = 0.05) or low P4 concentrations (≤1.0 ng/mL; Double-Ovsynch = 12.0% vs. Presynch = 23.7%; P = 0.04) at the time of $PGF_{2\alpha}$ was different between groups, indicating that more cows with an identified CL were undergoing CL regression in the Presynch than the Double-Ovsynch group. In addition, the percentage of cows with high P4 concentrations (>3 ng/mL) was greater (P = 0.04) in Double-Ovsynch (65.0%) than Presynch (50.5%) treatment group (Fig. 1B). However, Double-Ovsynch did not change the percentage of cows with medium P4 concentration (Double-Ovsynch = 31.0% vs. Presynch = 37.6%; P = 0.33; Fig. 1B) or the percentage of cows with complete luteolysis (Double-Ovsynch = 95.0% vs. Presynch = 93.5%; P = 0.67; Table 1).

3.4. Second GnRH of breeding Ovsynch

At G2, there was no difference in circulating P4 concentrations (0.18 ± 0.03 ng/mL vs. 0.19 ± 0.03 ng/mL; P = 0.82), circulating E2 concentrations (3.50 ± 0.22 pg/mL vs. 3.12 ± 0.17 pg/mL; P = 0.16), or endometrial thickness (10.4 ± 0.12 mm vs. 10.4 ± 0.12 mm; P = 0.99) between cows submitted to Double-Ovsynch versus Presynch. Nevertheless, the size of the dominant follicle tended (P = 0.13) to be larger in Double-Ovsynch (15.95 ± 0.31 mm) than Presynch (15.26 ± 0.32 mm) cows. The percentage of cows that ovulated to the second GnRH treatment also tended (P = 0.08) to be greater in cows treated with Double-Ovsynch (98.0%) than Presynch (93.5%).

4. Discussion

This study evaluated more intensively a presynchronization protocol, Double-Ovsynch, first described by Souza

et al. in 2008 [17]. This protocol attempts to induce cyclicity in anovular cows and to achieve a more synchronous stage of the cycle at the initiation of the Ovsynch protocol (Day 7). Previous studies reported improved fertility when using Double-Ovsynch compared with Presynch-12-Ovsynch for first timed AI in lactating dairy cows [17,18]. In addition, a recent study demonstrated improved P/AI using Double-Ovsynch during a resynchronization program [24]. The present study provided clear evidence that Double-Ovsynch not only increased the percentage of cows with a CL at the time of Ovsynch initiation (reduced percentage of anovular cows), but also tended to improve specific hormonal and ovarian measures during the Ovsynch protocol. These changes in hormonal and ovarian profiles might underlie the improvement in fertility that has been observed in cows treated with Double-Ovsynch.

Previous studies have shown that stage of the estrous cycle at initiation of Ovsynch affects physiological response to the protocol and fertility to timed AI [6,7,25]. Based on this idea, the Presynch-Ovsynch protocol was developed in which two $PGF_{2\alpha}$ injections were given 14 days apart, with Ovsynch initiated 11 to 14 days after the final $PGF_{2\alpha}$ treatment. The Presynch-Ovsynch protocol increased fertility, as compared with the Ovsynch protocol alone [8–10,26], and is currently the industry standard in the United States for the first timed AI. The percentage of cows that ovulated to the first GnRH after Presynch (12 days between $PGF_{2\alpha}$ and Ovsynch) in our study (69.9%) was similar to that reported after the 11-day Presynch protocol (61.4% [27]) and the 12-day protocol (66.7% [17]), but higher than that reported for the 14-day protocol (44.7% [9,27]).

Nevertheless, the Presynch-Ovsynch protocol does not appear to improve fertility in anovular dairy cows [8]. Anovulation occurs at a surprisingly high frequency in high-producing dairy cows [8,12,15,17]. In a recent summary [16] of 13 previous studies of large commercial dairy herds in the United States, the prevalence of anovulation was 23.3% in 5818 cows that that were examined approximately 50 to 65 days postpartum using multiple P4 and/or ultrasonography. These previous values correspond to the 32.2% prevalence of anovulation in our study (based on ultrasonographic examination for a CL) or 30.1% (based on P4 $<$0.5 ng/mL). Treatment with Double-Ovsynch decreased the percentage of anovular cows to 6% (based on CL) or 12% (based on P4). This was similar to the decrease from 33.3% (Presynch) to 9.4% (Double-Ovsynch) observed by Souza et al. [17], or the decrease from 24.7% (Presynch) to 6.3% (Double-Ovsynch) observed by Herlihy et al. [18]. Thus, all of these studies are consistent with the idea that use of Double-Ovsynch is very effective in inducing ovulation in anovular cows before initiation of the Ovsynch protocol.

In addition, Double-Ovsynch is designed to more completely optimize the stage of the estrous cycle at Ovsynch initiation than can be achieved with $PGF_{2\alpha}$ treatments alone. The optimal stage of the estrous cycle for Ovsynch initiation is likely to be Days 6 or 7 [6,25] when P4 would be increasing and a dominant follicle of the first follicular wave would have ovulatory capacity. This would be expected to increase ovulation to the first GnRH treatment of Ovsynch potentially optimizing P4 concentrations and follicle size and age at the time of $PGF_{2\alpha}$ and the second GnRH treatment of Ovsynch [28,29]. Previous studies have shown that cows ovulating to the first GnRH of Ovsynch have greater fertility compared with cows not ovulating to this first GnRH [14,25,26,30]. Increased ovulation to G1 might produce a dominant follicle at G2 that is less variable and closer to the ideal size and will increase circulating P4 concentrations [17,25,29].

In our study, a very high percentage of cows ovulated to the first GnRH treatment during Double-Ovsynch (80%); this tended ($P = 0.11$) to be greater than found in Presynch (69.9%) cows. Cows with low and medium P4 had a high ovulation response to the first GnRH treatment in either Double-Ovsynch or Presynch cows, although ovulation response tended to decrease as circulating P4 increased. This was likely because of a decrease in the GnRH-induced LH surge when P4 concentrations are increased [24].

The greater synchrony produced by Double-Ovsynch was most apparent in the distribution of cows by P4 concentrations at the time of $PGF_{2\alpha}$ in our study (Fig. 1). The reduction in percentage of cows with low P4 ($<$0.50 ng/mL) and the greater percentage of cows with high P4 ($>$3 ng/mL) at the time of $PGF_{2\alpha}$ were consistent with a greater synchrony of cows at Ovsynch initiation and/or a greater percentage ovulating to the first GnRH of Ovsynch. Cows with low P4 at the time of $PGF_{2\alpha}$ are likely to have undergone premature luteolysis and can have an LH surge and ovulation prior to the final GnRH treatment [6]. Alternatively, cows with high P4 at the time of the $PGF_{2\alpha}$ treatment are likely to ovulate a more optimally sized follicle and have better fertility [24,29]. In this study, the size of the dominant follicle at the final GnRH treatment tended to increase in Double-Ovsynch compared with Presynch-Ovsych. A size of approximately 16 mm, as ovulated by cows in Double-Ovsynch, is similar to the size observed in heifers [31,32] and might be close to the optimal size for fertility [33]. In addition, greater synchrony during Ovsynch might decrease the percentage of cows that ovulate at inopportune times during Ovsynch [6], increase circulating P4, and increase fertility [25]. Thus, the improved fertility during Double-Ovsynch [17,18,24] might 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, the quality of the ovulated oocyte, and the quality of the uterine environment [34–36].

4.1. Conclusions

Based on the results of this study and other studies, use of an Ovsynch protocol before initiation of a breeding Ovsynch with timed AI can induce ovulation in anovular cows and thus increase percentage of cows with medium circulating P4 at initiation of Ovsynch. Previous studies have clearly shown that initiation of Ovsynch in a low P4 environment decreases fertility to timed AI [29,37,38]. In addition, it appears that many other aspects of synchrony during the Ovsynch protocol might be improved by use of Double-Ovsych as compared with Presynch-Ovsynch including P4 concentration at $PGF_{2\alpha}$, size of the dominant

follicle, and ovulation to second GnRH. Further studies are needed to determine which of these changes are most critical for increasing fertility during the Double-Ovsynch protocol.

Acknowledgments

The authors thank Wagner Dairy, and his staff for all the help and the use of his herd to conduct this on-farm trial. IVX Animal Health, Inc., now TEVA Animal Health, Inc., donated hormones used for this research. This research was supported by the Wisconsin State Experiment Station, USDA-IFAFS grant 2001-52101-11252, AFRI Competitive Grant No. 2010-85122-20612 from the US Department of Agriculture-NIFA, and a fellowship from FAPESP of Brazil to Henderson Ayres (05/57425-7).

References

[1] Wiltbank M, Lopez H, Sartori R, Sangsritavong S, Gumen A. Changes in reproductive physiology of lactating dairy cows due to elevated steroid metabolism. Theriogenology 2006;65:17–29.
[2] Lopez H, Satter LD, Wiltbank MC. Relationship between level of milk production and estrous behavior of lactating dairy cows. Anim Reprod Sci 2004;81:209–23.
[3] Pursley JR, Mee MO, Wiltbank MC. Synchronization of ovulation in dairy cows using PGF2alpha and GnRH. Theriogenology 1995;44: 915–23.
[4] Pursley JR, Kosorok MR, Wiltbank MC. Reproductive management of lactating dairy cows using synchronization of ovulation. J Dairy Sci 1997;80:301–6.
[5] Rabiee AR, Lean IJ, Stevenson MA. Efficacy of Ovsynch program on reproductive performance in dairy cattle: a meta-analysis. J Dairy Sci 2005;88:2754–70.
[6] Vasconcelos JL, Silcox RW, Rosa GJ, Pursley JR, Wiltbank MC. Synchronization rate, size of the ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the estrous cycle in lactating dairy cows. Theriogenology 1999; 52:1067–78.
[7] Moreira F, de la Sota RL, Diaz T, Thatcher WW. Effect of day of the estrous cycle at the initiation of a timed artificial insemination protocol on reproductive responses in dairy heifers. J Anim Sci 2000; 78:1568–76.
[8] Moreira F, Orlandi C, Risco CA, Mattos R, Lopes F, Thatcher WW. Effects of presynchronization and bovine somatotropin on pregnancy rates to a timed artificial insemination protocol in lactating dairy cows. J Dairy Sci 2001;84:1646–59.
[9] Navanukraw C, Redmer DA, Reynolds LP, Kirsch JD, Grazul-Bilska AT, Fricke PM. A modified presynchronization protocol improves fertility to timed artificial insemination in lactating dairy cows. J Dairy Sci 2004;87:1551–7.
[10] El-Zarkouny SZ, Cartmill JA, Hensley BA, Stevenson JS. Pregnancy in dairy cows after synchronized ovulation regimens with or without presynchronization and progesterone. J Dairy Sci 2004; 87:1024–37.
[11] LeBlanc SJ, Leslie KE. Short communication: presynchronization using a single injection of PGF2alpha before synchronized ovulation and first timed artificial insemination in dairy cows. J Dairy Sci 2003;86:3215–7.
[12] Gumen A, Guenther JN, Wiltbank MC. Follicular size and response to Ovsynch versus detection of estrus in anovular and ovular lactating dairy cows. J Dairy Sci 2003;86:3184–94.
[13] Galvao KN, Santos JE, Juchem SO, Cerri RL, Coscioni AC, Villasenor M. Effect of addition of a progesterone intravaginal insert to a timed insemination protocol using estradiol cypionate on ovulation rate, pregnancy rate, and late embryonic loss in lactating dairy cows. J Anim Sci 2004;82:3508–17.
[14] Chebel RC, Santos JE, Cerri RL, Rutigliano HM, Bruno RG. Reproduction in dairy cows following progesterone insert presynchronization and resynchronization protocols. J Dairy Sci 2006;89:4205–19.
[15] Lopez H, Caraviello DZ, Satter LD, Fricke PM, Wiltbank MC. Relationship between level of milk production and multiple ovulations in lactating dairy cows. J Dairy Sci 2005;88:2783–93.
[16] Bamber RL, Shook GE, Wiltbank MC, Santos JE, Fricke PM. Genetic parameters for anovulation and pregnancy loss in dairy cattle. J Dairy Sci 2009;92:5739–53.
[17] Souza AH, Ayres H, Ferreira RM, Wiltbank MC. A new presynchronization system (Double-Ovsynch) increases fertility at first postpartum timed AI in lactating dairy cows. Theriogenology 2008;70: 208–15.
[18] Herlihy MM, Giordano JO, Souza AH, Ayres H, Ferreira RM, Keskin A, et al. Presynchronization with Double-Ovsynch improves fertility at first postpartum artificial insemination in lactating dairy cows. J Dairy Sci 2012. pii: S0022-0302(12)00704-7. http://dx.doi.org/10.3168/jds.2011-5260.
[19] NRC. Nutrient requirements of dairy cattle. Seventh Revised Edition. Washington, DC: National Academy of Sciences; 2001.
[20] Ferguson JD, Galligan DT, Thomsen N. Principal descriptors of body condition score in Holstein cows. J Dairy Sci 1994;77:2695–703.
[21] Sprecher DJ, Hostetler DE, Kaneene JB. A lameness scoring system that uses posture and gait to predict dairy cattle reproductive performance. Theriogenology 1997;47:1179–87.
[22] Kulick LJ, Kot K, Wiltbank MC, Ginther OJ. Follicular and hormonal dynamics during the first follicular wave in heifers. Theriogenology 1999;52:913–21.
[23] SAS Institute. SAS user's guide: statistics, 9.1 for Windows edition. Cary, NC: SAS Institute, 2002-2003.
[24] Giordano JO, Wiltbank MC, Guenther JN, Pawlisch R, Bas S, Cunha AP, et al. Increased fertility in lactating dairy cows resynchronized with Double-Ovsynch compared with Ovsynch initiated 32 d after timed artificial insemination. J Dairy Sci 2012;95: 639–53.
[25] Bello NM, Steibel JP, Pursley JR. Optimizing ovulation to first GnRH improved outcomes to each hormonal injection of ovsynch in lactating dairy cows. J Dairy Sci 2006;89:3413–24.
[26] Galvao KN, Santos JE. Factors affecting synchronization and conception rate after the Ovsynch protocol in lactating Holstein cows. Reprod Domest Anim 2010;45:439–46.
[27] Galvao KN, Sa Filho MF, Santos JE. Reducing the interval from presynchronization to initiation of timed artificial insemination improves fertility in dairy cows. J Dairy Sci 2007;90:4212–8.
[28] Wiltbank MC, Sartori R, Herlihy MM, Vasconcelos JL, Nascimento AB, Souza AH, et al. Managing the dominant follicle in lactating dairy cows. Theriogenology 2011;76:1568–82.
[29] Wiltbank MC, Souza AH, Carvalho PD, Bender RW, Nascimento AB. Improving fertility to timed artificial insemination by manipulation of circulating progesterone concentrations in lactating dairy cattle. Reprod Fertil Dev 2012;24:238–43.
[30] Vasconcelos JL, Sartori R, Oliveira HN, Guenther JG, Wiltbank MC. Reduction in size of the ovulatory follicle reduces subsequent luteal size and pregnancy rate. Theriogenology 2001;56:307–14.
[31] Sartori R, Haughian JM, Shaver RD, Rosa GJ, Wiltbank MC. Comparison of ovarian function and circulating steroids in estrous cycles of Holstein heifers and lactating cows. J Dairy Sci 2004;87:905–20.
[32] Wolfenson D, Inbar G, Roth Z, Kaim M, Bloch A, Braw-Tal R. Follicular dynamics and concentrations of steroids and gonadotropins in lactating cows and nulliparous heifers. Theriogenology 2004;62: 1042–55.
[33] Souza AH, Gumen A, Silva EP, Cunha AP, Guenther JN, Peto CM, et al. Supplementation with estradiol-17beta before the last gonadotropin-releasing hormone injection of the Ovsynch protocol in lactating dairy cows. J Dairy Sci 2007;90:4623–34.
[34] Mihm M, Baguisi A, Boland MP, Roche JF. Association between the duration of dominance of the ovulatory follicle and pregnancy rate in beef heifers. J Reprod Fertil 1994;102:123–30.
[35] Revah I, Butler WR. Prolonged dominance of follicles and reduced viability of bovine oocytes. J Reprod Fertil 1996;106:39–47.
[36] Pursley JR, Martins JP. Impact of circulating concentrations of progesterone and antral age of the ovulatory follicle on fertility of high-producing lactating dairy cows. Reprod Fertil Dev 2012;24: 267–71.
[37] Bisinotto RS, Chebel RC, Santos JE. Follicular wave of the ovulatory follicle and not cyclic status influences fertility of dairy cows. J Dairy Sci 2010;93:3578–87.
[38] Bisinotto RS, Santos JE. The use of endocrine treatments to improve pregnancy rates in cattle. Reprod Fertil Dev 2012;24:258–66.