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# Effects of presynchronization with gonadotropin-releasing hormone-prostaglandin $F_{2\alpha}$ or progesterone before Ovsynch in noncyclic dairy cows

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## ABSTRACT

The aim of this study was to evaluate the efficiency of presynchronization with GnRH and $PGF_{2\alpha}$ or with progesterone on overall Ovsynch (OVS) outcomes in noncyclic dairy cows. Cows were scanned 7 d apart with ultrasonography to determine cyclicity. Noncyclic cows (n = 281; no corpus luteum on ovaries at both examinations) were randomly divided into 3 groups. In the GP group (n = 108), the cows received GnRH and $PGF_{2\alpha}$ (PGF) administrations 7 d apart, and OVS was started 11 d after PGF (GnRH-7 d-PGF-11 d-OVS). In the P4 group (n = 90), the cows were treated for 7 d with an intravaginal progesterone (P4) implant (PRID), and then OVS was started 11 d after removal of the implant (7 d PRID-11 d-OVS). The control group (CON, n = 83) did not receive any presynchronization, and OVS was started at the same time as in the other groups (18 d-OVS). The percentage of cows that became cyclic at the beginning of OVS was lower in the CON group (38.6%; 32/83) than in the presynchronization groups (66.7%, 72/108 in GP; 71.1%, 64/90 in P4). The response to the first GnRH of OVS did not differ among groups (63.9%, 53/83 in CON; 67.6%, 73/108 in GP; 63.3%; 57/90 in P4), and synchronization rates were similar among the groups (74–82%). The cows that responded to presynchronization treatments (GP or P4) had higher pregnancy per artificial insemination (P/AI) than did nonresponding cows. Pregnancy per AI at 31 d did not differ between groups (30.1%, 25/83 in CON; 43.5%, 47/108 in GP; and 35.6%, 32/90 in P4). However, CON cows (24.1%, 20/83) had lower P/AI at 62 d than GP cows (41.7%, 45/108). Embryonic loss was higher in CON (20%, 5/25) compared with the P4 group (3%, 1/32). The administration of GnRH followed by PGF or exogenous progesterone (PRID) similarly increased the percentage of cows that became cyclic before Ovsynch in noncyclic cows, but fertility did not improve. However, the cows that responded to presynchronization had higher fertility rates than the nonresponding cows.

**Key words:** noncyclic cow, Ovsynch, presynchronization, progesterone

## INTRODUCTION

One of the most important factors contributing to interrupted reproductive efficiency is the noncyclic condition of a dairy herd (Wiltbank et al., 2002). Noncyclic cows lead to remarkable economic loss in the herd due to longer intervals to conception, prolonged calving intervals, and so on (McDougall, 2010). In addition, the reported percentage of noncyclic cows is high, 20 to 30%, in lactating dairy cows (Moreira et al., 2001; Gümen et al., 2003; Lopez et al., 2005). Especially for high-yielding dairy cows, reducing the percentage of cows with an extended interval from calving to conception provides important economic benefits to the herd (Bicalho et al., 2007).

Stimulation of ovulation can be achieved successfully in noncyclic cows using GnRH analogs in cows with a functional dominant follicle (Rhodes et al., 2003). Moreover, previous studies (Gümen et al., 2003; Galvão and Santos, 2010) have indicated that noncyclic cows are more responsive than cyclic cows to GnRH treatment. However, ovulation after GnRH administration is usually followed by a short luteal phase (Rhodes et al., 2003), or a return to the noncyclic condition follows the treatment (McDougall et al., 1995).

The administration of progesterone is generally used to induce cyclicity in noncyclic cows (Rhodes et al., 2003). Progesterone treatments reset the hypothalamus, which becomes nonresponsive to estradiol due to extended progesterone deprivation (Gümen and Wiltbank, 2005), resulting in a normal length of luteal phase of the estrous cycle (Rhodes et al., 2003). However, the response to progesterone administration in noncyclic cows is variable, and using progesterone alone

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does not include synchronization of ovulation (Rhodes et al., 2003).

The Ovsynch protocol is an effective breeding program used worldwide that allows for timed AI (**TAI**) synchronization of a new follicular wave, luteal regression, and time of ovulation in lactating dairy cows. The protocol involves administration of GnRH on d 0, followed by $PGF_{2\alpha}$ (**PGF**) on d 7, and an additional administration of GnRH 56 h later (Pursley et al., 1995). Although the Ovsynch program effectively synchronizes ovulation in both cyclic and noncyclic cows (Gümen et al., 2003), the pregnancy rate is lower in noncyclic compared with cyclic cows (Gümen et al., 2003; Gãlvao et al., 2004; Chebel et al., 2006). This impotency after Ovsynch in noncyclic cows, which is associated with decreased pregnancy rates and increased early embryonic losses, may have resulted from the lack of effect of progesterone for some time before the Ovsynch protocol. Thus, to achieve the well-known success of the Ovsynch program for cyclic cows, a presynchronization program needs to be developed for noncyclic cows in which the ovaries of noncyclic cows undergo at least 7 d of progesterone exposure (Gümen and Wiltbank, 2005) before Ovsynch, which would, therefore, regulate the ovaries and prevent shortened duration of the luteal phase.. This type of presynchronization protocol, which is performed 11 d before Ovsynch, may increase the percentage of cyclic cows at the beginning of Ovsynch and provide an opportunity to begin Ovsynch in these newly cyclic cows in the optimal phase of the cycle (d 5 to 12; Vasconcelos et al., 1999) to obtain the best results from Ovsynch.

The objectives of our study were to determine the effects of presynchronization with GnRH administration followed by PGF 7 d later (endogenous progesterone) or presynchronization with progesterone administration for 7 d (exogenous progesterone), given 11 d before the Ovsynch protocol, on increasing cyclicity before Ovsynch. Additionally, this study aimed to identify the advantages and disadvantages of these 2 presynchronization protocols on overall outcomes of the Ovsynch protocol.

## MATERIALS AND METHODS

### *Animals*

Noncyclic lactating dairy cows (n = 281) from a commercial dairy farm located in the South Marmara region of Turkey were enrolled in the present study. Cows were housed in freestall barns with self-catching headlocks, and all barns were equipped with fans and sprinklers that were activated during the hotter months of the year. All cows were grouped according to their milk production and were milked 3 times daily at approximately 8-h intervals. The mean milk production of the herd was 9,880 ± 69.7 kg (305 d) per cow. Cows had free access to water and were fed complete mixed rations according to the National Research Council recommendations (NRC, 2001). The daily milk yield, reproductive health, and management records for each cow were collected from Alpro 2000 (DeLaval, Sweden). The average milk production for each cow was recorded for 7 d before and after AI. Body condition scores were determined for all cows at the beginning of the study using a 5-point (1 = thin to 5 = fat) scoring system (Ferguson et al., 1994). All protocols involving cows used in this research were approved by the Lalahan Livestock Central Research Institute Animal Care Committee.

### *Experimental Design*

Noncyclic cows at various DIM (n = 281) were randomly assigned to 3 groups after ultrasonographic examinations of ovaries 7 d apart (Figure 1). In the first group (n = 108), PGF (cloprostenol, 500 μg i.m., Juramate, EGE-VET, Turkey) was administered 7 d after a GnRH treatment (buserelin acetate, 10 μg i.m., Receptal, Intervet, Turkey), and the Ovsynch protocol (**OVS**) was started 11 d after PGF administration (**GP**; GnRH-7 d-PGF-11 d-OVS). In the second group (n = 90), the cows received 1.55 g of progesterone (Progesterone Releasing Intravaginal Device, without estradiol capsule, PRID, Ceva-DIF, Istanbul, Turkey) for 7 d, and OVS was started 11 d after device removal (**P4**; 7 d PRID-11 d-OVS). The control group (n = 83) received only OVS at the same time as the other 2 groups, which was 18 d after the beginning of the study (**CON**; 18 d-OVS). The OVS was initiated with a GnRH treatment (buserelin acetate, 10 μg i.m., Receptal) followed by PGF (cloprostenol, 500 μg i.m., Juramate) 7 d later. A second GnRH was administered 56 h after the PGF, and all cows were inseminated at a fixed time (TAI; 16 to 18 h) after the second GnRH.

### *Ultrasonographic Evaluations*

The transrectal ultrasonographic evaluations were performed with a Honda HS 2000 ultrasonogram equipped with a 7.5-MHz transducer (Honda, Tokyo, Japan). The cows were examined 7 d apart (−7 and 0 d) to determine cyclicity [no corpus luteum (**CL**) at each examination], and their maximum follicle size was measured. Ultrasonographic examinations were performed on d 7 (to determine the response to the GnRH

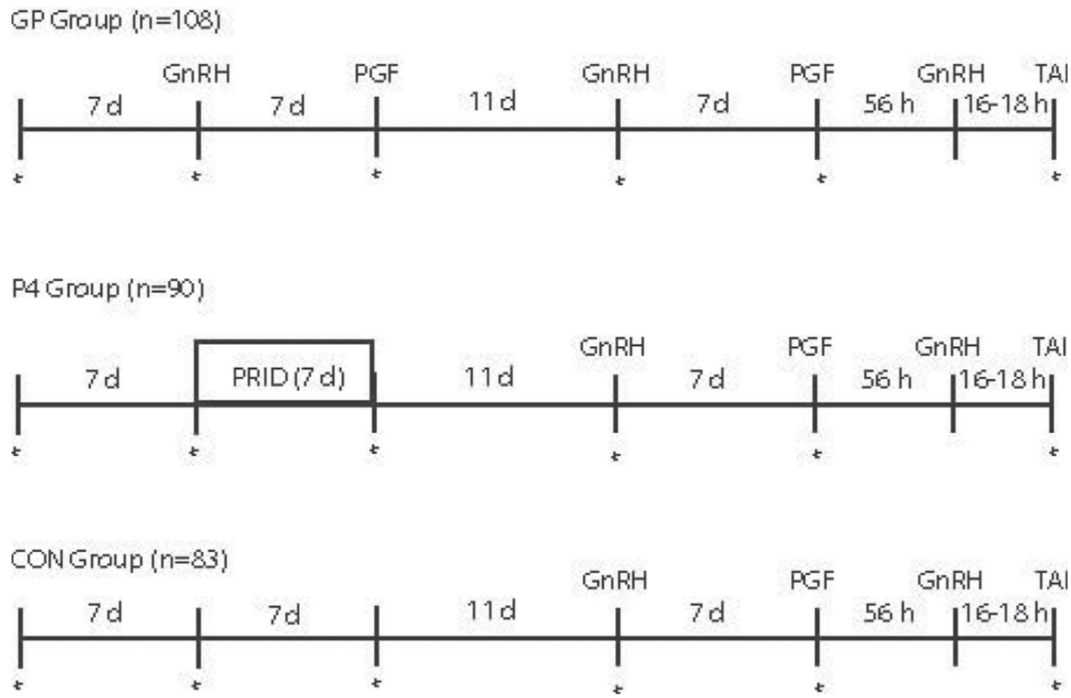


**Figure 1.** Experimental design of the study. Cows were examined 7 d apart to determine cyclicity (no corpus luteum at each examination). Noncyclic cows (n = 281) were randomly assigned to 1 of 3 groups. In the P4 group (n = 90), the cows received a progesterone implant (PRID, Ceva-DIF, Istanbul, Turkey) for 7 d, and the Ovsynch protocol (OVS) was started 11 d after device removal (7 d-PRID-11 d-OVS). In the GP group (n = 108), $PGF_{2\alpha}$ (PGF) was administered 7 d after a GnRH treatment, and OVS was started 11 d after PGF (GnRH-7 d-PGF-11 d-OVS). The CON group (n = 83) received only OVS at the same time as the other 2 groups, which was 18 d after the beginning of the study (18 d-OVS). Asterisks indicate ultrasound evaluation days. TAI = timed AI.

of Presynch), d 18 (to determine the percentage of the cows that became cyclic before the protocol), d 25 (to determine ovulation in response to the first GnRH of Ovsynch and presence or absence of a CL), d 28 (to determine size of the follicles), and d 35 (to determine ovulation in response to the second GnRH of Ovsynch). Ovulation in response to the GnRH treatments was characterized by disappearance of the responsive follicle and the appearance of a new CL. Cows with early ovulation (no follicle at the time of TAI), no ovulation (no CL 7 d after TAI), and no CL at the time of the PGF of Ovsynch were defined as nonsynchronized cows. Recovery rate was defined as the percentage of the cows that became cyclic before the protocol in the control (spontaneous) or Presynch groups (induced by either GnRH or progesterone). Pregnancy diagnoses were performed by ultrasonography on 31 and 62 d after TAI.

### Statistical Analysis

Statistical analyses were conducted by using SAS (version 9.2; SAS Institute, 2009). Data were evaluated using PROC LOGISTIC, PROC GLM and PROC FREQ in SAS. The statistical model included the effects of treatments, parity, DIM, BCS, service number, milk production, responses to first and second GnRH treatments of the Ovsynch, recovery rate, maximum follicle size at the beginning of the study, and follicle size at the time of TAI. The GLM procedure was used to compare the differences among groups for milk production, DIM, BCS, maximum follicle size at the beginning of study and follicle size at the time of TAI among groups. Chi-squared results, obtained using the PROC FREQ procedure, were used to test for response to GnRH administrations, recovery rate, pregnancy per AI (**P/AI**, 31 and 62 d), and embryonic loss among groups. The LOGISTIC procedure was performed to determine the effect of covariant factors, such as DIM, BCS, and milk production, on responses to GnRH administrations, recovery rate, and P/AI (31 and 62 d).

## RESULTS

### General Results and Nonsynchronization Rates

Groups did not differ with regard to BCS, DIM, milk production, service number, lactation number, or maximum follicle size before the protocol. The maximum follicle sizes at the time of TAI were similar among

**Table 1.** Mean BCS, milk production, DIM, service number, lactation number, and maximum follicle sizes at the beginning of the study and at the time of timed AI (TAI) among the groups

| Item | Group[1] | | | $P$-value |
|---|---|---|---|---|
| | CON | GP | P4 | |
| BCS | 2.63 ± 0.04 | 2.63 ± 0.03 | 2.56 ± 0.04 | 0.50 |
| Milk production (kg/d) | 34.7 ± 0.89 | 34.9 ± 0.79 | 34.6 ± 0.86 | 0.96 |
| DIM | 141.2 ± 8.9[a] | 114.8 ± 8.1[b] | 134.3 ± 8.7[ab] | 0.07 |
| Service number | 0.83 ± 0.11 | 0.62 ± 0.10 | 0.76 ± 0.11 | 0.36 |
| Lactation number | 1.92 ± 0.12 | 1.81 ± 0.10 | 1.93 ± 0.11 | 0.68 |
| Maximum follicle size at start, mm | 19.80 ± 0.67 | 19.84 ± 0.59 | 19.13 ± 0.64 | 0.55 |
| Maximum follicle size at TAI, mm | 16.01 ± 0.29[a] | 15.29 ± 0.24[ab] | 15.09 ± 0.28[b] | 0.06 |

[a,b]Means within a row with different superscript letters differ at $P < 0.05$.

[1]Groups: CON = control (Ovsynch protocol only); GP = presynchronization with GnRH and PGF followed by Ovsynch protocol; P4 = presynchronization with progesterone followed by Ovsynch protocol (Figure 1).

groups (Table 1). The distribution of maximum follicle size was as follows: 28% of cows had follicles 9 to 15 mm in diameter, 53% of cows had follicles 16 to 24 mm, and 19% of cows had follicles ≥25 mm (Figure 2). Fifty-six (n = 21 in CON, n = 19 in GP, n = 16 in P4) of 281 cows were not synchronized. Nonsynchronization rates due to early ovulation, no CL at the time of PGF, or no ovulation in response to the second GnRH of Ovsynch did not differ among the groups (Table 2).

### Effect of Presynchronization Protocols on Recovery Rate, Synchronization Rate, and P/AI

Although ovulation in response to the GnRH treatment of presynchronization in the GP group was found in 86.1% (93/108), approximately 20% of these cows (66.7%, 72/108) became noncyclic (no CL) at the beginning of Ovsynch. Recovery rates were higher ($P$ = 0.0001) in groups GP (66.7%; 72/108) and P4 (71.1%; 64/90) than in the CON group (39.8%; 33/83). Responses to the first (63.3–67.8%) and second GnRH (74.7–82.4%) were similar among groups, as shown in Table 3. Although P/AI at 31 d was similar among groups (30.1%, 25/83 in CON; 43.5%, 47/108 in GP; 35.7%, 32/90 in P4), P/AI at 62 d was lower ($P$ = 0.008) in the CON group (24.1%, 20/83) compared with that in the GP group (41.7%, 45/108). Additionally, pregnancy loss between 31 and 62 d was higher ($P$ = 0.05) in the CON group (20.0%, 5/25) than in the other groups (4.2%, 2/47 in GP; 3.1%, 1/32 in P4).

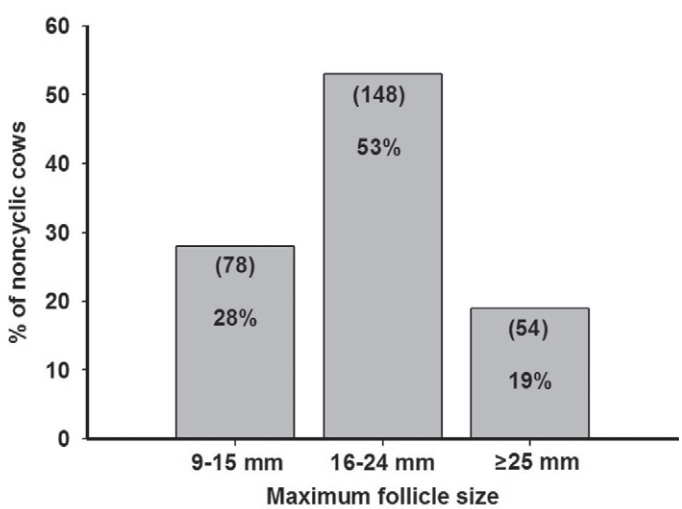


**Figure 2.** Distribution of maximum follicle sizes in noncyclic cows. The numbers of cows are shown in parentheses in each follicle size group.

### Effect of Response to First GnRH of Ovsynch on Ovsynch Outcomes

When all cows were evaluated, regardless of treatment group, the cows responsive to the first GnRH treatment of Ovsynch (90.7%, 166/183) had a greater ($P$ = 0.0001) synchronization rate than the nonresponsive cows (60.2%, 59/98), and P/AI at 31 d was higher ($P$ = 0.02) in responding cows (42.1%, 77/183) compared with cows that did not respond (27.6%, 27/98). In addition, P/AI at 62 d was greater ($P$ = 0.03) in cows that responded to the first GnRH of Ovsynch (38.8%; 71/183) compared with the nonresponding cows (25.5%; 25/98).

The synchronization rates were not different among the treatment groups in cows either responsive (84.9–94.5%) or nonresponsive (56.7–66.7%) to the first GnRH of Ovsynch. However, synchronization rates did differ between the responsive and nonresponsive cows within each treatment group, as shown in Table 4.

When P/AI was evaluated among the treatment groups in terms of response to first GnRH, we observed no difference between responsive and nonresponsive cows to the GnRH of Ovsynch in the GP and P4 groups.

**Table 2.** Proportion (%; number/total in parentheses) of nonsynchronized cows [early ovulation, no ovulation in response to second GnRH of Ovsynch, or no corpus luteum (CL) at the time of $PGF_{2\alpha}$ of Ovsynch] among the groups

| Item | Group[1] CON | GP | P4 | *P*-value |
|---|---|---|---|---|
| Early ovulation | 6.0 (5/83) | 4.6 (5/108) | 3.3 (3/90) | 0.70 |
| No ovulation to second GnRH of Ovsynch | 6.0 (5/83) | 2.8 (3/108) | 4.4 (4/90) | 0.54 |
| No CL at $PGF_{2\alpha}$ of the Ovsynch | 13.3 (11/83) | 10.2 (11/108) | 10.0 (9/90) | 0.75 |

[1]Groups: CON = control (Ovsynch protocol only); GP = presynchronization with GnRH and PGF followed by Ovsynch protocol; P4 = presynchronization with progesterone followed by Ovsynch protocol (Figure 1).

However, in the CON group, P/AI was higher ($P = 0.03$) in the cows that responded (37.7%, 20/53) than in the cows that did not respond to the first GnRH (16.7%, 5/30; Table 4).

### *Effect of Covariant Factors*

When we evaluated covariant factors that may have had an effect on response to the first GnRH, lactation number, treatment, recovery after treatment, and maximum follicle size before the protocol had no effect on response to the first GnRH of Ovsynch. However, spontaneous recovery ($P = 0.04$) did have an effect on response to the first GnRH.

Milk production, DIM, BCS, service number, synchronization rate, maximum follicle size before the protocol, and maximum follicle size at the time of TAI had no effect on P/AI at 31 or 62 d. Recovery rate ($P = 0.0007$) and ovulatory response to first GnRH of Ovsynch ($P = 0.05$) had a significant effect on P/AI at 31 and 62 d.

When the cows were evaluated according to recovery rates after presynchronization or spontaneous recovery, responses to the first GnRH of Ovsynch did not differ among the groups (Table 5). However, synchronization rates and P/AI at 31 and 62 d were higher in the cows that became cyclic than in the cows that did not become cyclic before Ovsynch within the presynchronization groups (Table 5).

## DISCUSSION

In the present study, we aimed to increase cyclicity in noncyclic dairy cows before the Ovsynch protocol by performing presynchronization with either GnRH and PGF administrations 7 d apart or progesterone administration for 7 d, which were given 11 d before the Ovsynch protocol. In addition, the study aimed to evaluate the effect of these presynchronizations on Ovsynch outcome and fertility. The Ovsynch program has effectively synchronized ovulation in both cyclic and noncyclic cows (Gümen et al., 2003), but it has not improved the reproductive performance of noncyclic cows (Gümen et al., 2003; Galvão et al., 2004; Chebel et al., 2006). Therefore, optimal fertility would be achieved if noncyclic cows became cyclic before being subjected to the Ovsynch protocol. In our study, the percentages of cows that became cyclic after presynchronization in both the P4 and GP groups were higher than the percentage of cows that spontaneously became cyclic in the CON group. Recent studies (Chebel et al., 2006; Bicalho et al., 2007) have reported that presynchroni-

**Table 3.** Responses (%; number/total in parentheses) to GnRH treatments, pregnancy per AI (P/AI) at 31 and 62 d, and embryonic loss among the groups

| Item | Group[1] CON | GP | P4 | *P*-value |
|---|---|---|---|---|
| Recovery rate | 39.8[a] (33/83) | 66.7[b] (72/108) | 71.1[b] (64/90) | 0.0001 |
| Ovulation to first GnRH of Ovsynch | 63.9 (53/83) | 67.6 (73/108) | 63.3 (57/90) | 0.79 |
| Synchronization rate | 74.7 (62/83) | 82.4 (89/108) | 82.2 (74/90) | 0.34 |
| P/AI at 31 d | 30.1 (25/83) | 43.5 (47/108) | 35.6 (32/90) | 0.15 |
| P/AI at 62 d | 24.1[c] (20/83) | 41.7[d] (45/108) | 34.4[cd] (31/90) | 0.04 |
| Embryonic loss | 20.0[e] (5/25) | 4.2[ef] (2/47) | 3.1[f] (1/32) | 0.05 |

[a–f]Means within a row with different superscript letters differ at [a,b]$P < 0.0002$, [c,d]$P = 0.008$, or [e,f]$P = 0.05$.

[1]Groups: CON = control (Ovsynch protocol only); GP = presynchronization with GnRH and PGF followed by Ovsynch protocol; P4 = presynchronization with progesterone followed by Ovsynch protocol (Figure 1).

**Table 4.** Results (%; number/total in parentheses) of pregnancy per AI (P/AI) at 31 and 62 d of pregnancy in cows according to their response (positive or negative) to the first GnRH treatment of Ovsynch (GnRH-1) among groups

| | Synchronization rate | | | 31 d of pregnancy | | | 62 d of pregnancy | | |
|---|---|---|---|---|---|---|---|---|---|
| Group[1] | GnRH-1 (+) | GnRH-1 (−) | *P*-value | GnRH-1 (+) | GnRH-1 (−) | *P*-value | GnRH-1 (+) | GnRH-1 (−) | *P*-value |
| CON | 84.9 (45/53) | 56.7 (17/30) | 0.006 | 37.7 (20/53) | 16.7 (5/30) | 0.03 | 30.2 (16/53) | 13.3 (4/30) | 0.06 |
| GP | 94.5 (69/73) | 57.1 (20/35) | 0.0001 | 47.9 (35/73) | 34.3 (12/35) | 0.17 | 45.2 (33/73) | 34.3 (12/35) | 0.27 |
| P4 | 91.2 (52/57) | 66.7 (22/33) | 0.006 | 38.6 (22/57) | 30.3 (10/33) | 0.42 | 38.6 (22/57) | 27.3 (9/33) | 0.26 |
| *P*-value | 0.18 | 0.65 | | 0.42 | 0.26 | | 0.23 | 0.15 | |

[1]Groups: CON = control (Ovsynch protocol only); GP = presynchronization with GnRH and PGF followed by Ovsynch protocol; P4 = presynchronization with progesterone followed by Ovsynch protocol (Figure 1).

zation with exogenous progesterone (CIDR, controlled internal drug-releasing device) before Ovsynch resulted in a higher percentage of newly cyclic cows, which is similar to our finding that 71.1% of the cows became cyclic after exogenous progesterone (PRID) administration. Additionally, previous reports that used GnRH administration in presynchronization, either integrated into the Presynch protocol (7 d before last PGF; Gümen et al., 2005) or into the Ovsynch performed before Ovsynch-TAI (Souza et al., 2008), reported that GnRH administration had a beneficial effect on increasing cyclicity before Ovsynch in noncyclic cows. Gümen et al. (2005) reported a 58% increase in the percentage of cyclic cows after GnRH administration (89% of cows received GnRH, and 31% of cows did not receive GnRH during Presynch). Similarly, in our study, GnRH administration followed by PGF in the GP group also increased the incidence of cyclicity (86.1% of GP cows at the beginning of Ovsynch versus 39.8% of control cows). However, in the present study, approximately 20% of cows in the GP group that ovulated after the GnRH treatment during presynchronization became noncyclic (no CL) at the beginning of Ovsynch (66.7%), which is consistent with the data from McDougall et al. (1995). Thus, presynchronization of noncyclic cows by exogenous progesterone (PRID) or by endogenous progesterone (GnRH administration followed by PGF) had equal effectiveness in increasing cyclicity before the Ovsynch protocol.

During the Ovsynch protocol, early or absent ovulation from the dominant follicle or absence of the CL at the time of PGF can occur and result in nonsynchronization of the cows. In this study, nonsynchronized cows were evaluated, and the presynchronization methods tested had no effect on the percentage of these nonsynchronized cows. The study of Galvão and Santos (2010) intended to obtain responses to every hormone administration of the Ovsynch protocol, in which 2.2% of the cows had ovulated before the second GnRH of the Ovsynch; however, the authors did not evaluate the cows that had ovulated between the second GnRH of Ovsynch and TAI. In our study, cows without an ovulatory follicle at the time of TAI were defined as early ovulatory cows. Thus, the percentage of early ovulatory cows in our study was higher (6.0%) than that reported by Galvão and Santos (2010). This difference between the studies may be due to the inclusion of cows that ovulated between the second GnRH of Ovsynch and TAI in our study. Additionally, the percentage of cows that did not ovulate after the second GnRH administration of Ovsynch in our study averaged 4.3%. In our study, ultrasonography confirmed the presence or absence of the CL at the time of PGF of Ovsynch, and the results showed that, on average, 11.2% of the cows had no CL at the time of PGF. In this measure, groups did not differ, and the percentage is similar to that (9%) reported by Galvão and Santos (2010). Thus, in the present study, up to 25% of the cows in the CON group had early or absent ovulation or no CL at the time of PGF (i.e., no possibility of pregnancy).

In this study, we also evaluated the maximum follicle sizes in noncyclic cows at the beginning of the study before presynchronization. The distribution of maximum follicular size was similar to that in previous reports (Gümen et al., 2003). In both studies, only ~20% of noncyclic cows had cystic size follicles (≥25 mm), and most of the noncyclic cows (53–58%) had follicle sizes between 15 and 24 mm. Because large (≥25 mm) and medium-sized (16–24 mm) follicles in noncyclic cows share the same etiology (lack of LH surge), the size in the definition of follicular cysts may be reconsidered.

In recent studies, the ovulatory responses to the first GnRH administration of Ovsynch were between 45 and 75% in dairy cows (Bello et al., 2006; Galvão and Santos, 2010; Keskin et al., 2010). Previous studies have reported that noncyclic cows have a higher ovulation response to the first GnRH of Ovsynch (Gümen et al., 2003; Galvão and Santos, 2010). In our study, the responses to the first GnRH of Ovsynch were between 63 and 68% and did not differ among the groups.

In most studies (Vasconcelos et al., 1999; Moreira et al., 2001; Bello et al., 2006; Galvão and Santos, 2010;

**Table 5.** Evaluation of the response (%; number/total in parentheses) to the first GnRH of Ovsynch (GnRH-1), synchronization rate, and pregnancy per AI (P/AI) at 31 and 62 d of pregnancy according to recovery (Rec) after Presynch (in GP and P4 groups) or spontaneous recovery (in CON group) before Ovsynch among the groups

| | Ovulation to GnRH-1 | | | Synchronization rate | | | P/AI at 31 d | | | P/AI at 62 d | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Group[1] | Rec (+) | Rec (−) | *P*-value | Rec (+) | Rec (−) | *P*-value | Rec (+) | Rec (−) | *P*-value | Rec (+) | Rec (−) | *P*-value |
| CON | 54.5 (18/33) | 70.0 (35/50) | 0.15 | 87.9 (29/33) | 66.0 (33/50) | 0.02 | 39.4 (13/33) | 24.0 (12/50) | 0.13 | 30.3 (10/33) | 20.0 (10/50) | 0.28 |
| GP | 65.3 (47/72) | 72.2 (26/36) | 0.47 | 91.7 (66/72) | 63.9 (23/36) | 0.0004 | 55.6 (40/72) | 19.4 (7/36) | 0.0004 | 54.2 (39/72) | 16.7 (6/36) | 0.0001 |
| P4 | 64.1 (41/64) | 61.5 (16/26) | 0.82 | 92.2 (59/64) | 57.7 (15/26) | 0.0001 | 46.9 (30/64) | 7.7 (2/26) | 0.0004 | 45.3 (29/64) | 7.7 (2/26) | 0.0007 |
| *P*-value | 0.55 | 0.65 | | 0.76 | 0.77 | | 0.28 | 0.22 | | 0.07 | 0.38 | |

[1]CON = control (Ovsynch protocol only); GP = presynchronization with GnRH and PGF followed by Ovsynch protocol; P4 = presynchronization with progesterone followed by Ovsynch protocol (Figure 1).

Keskin et al., 2010), higher ovulation rates after the first GnRH of Ovsynch increased synchronization rates (response to second GnRH of Ovsynch) and pregnancy rates after the Ovsynch protocol in lactating dairy cows. The synchronization rates in previous studies were between 75 and 95% (Bello et al., 2006; Galvão and Santos, 2010; Keskin et al., 2010). In our study, synchronization rates ranged from 75 to 82% and did not differ among the groups, which seems reasonable because the responses to the first GnRH also did not differ among groups. However, similar to previous studies (Vasconcelos et al., 1999; Moreira et al., 2001; Bello et al., 2006), the cows in our study that responded to the first GnRH, regardless of treatment, had higher synchronization rates compared with cows that did not respond. In addition, when the cows were evaluated according to recovery after presynchronization or spontaneous recovery, the synchronization rates in the cows that become cyclic before Ovsynch (91.7, 92.2, and 87.9%) were higher ($P \leq 0.006$) than those in the cows that did not become cyclic (63.9, 57.7, and 66.0%) in all 3 groups (GP, P4, and CON, respectively). Thus, our results show that cyclicity before the Ovsynch protocol improves the synchronization rate in noncyclic cows, which is similar to the results reported by some previous studies (Cartmill et al., 2001; Galvão and Santos, 2010), but in contrast to the data reported by Gümen et al. (2003).

In our study, fertility after Ovsynch was similar among the cows presynchronized with GnRH followed by PGF, cows presynchronized with exogenous progesterone (PRID), and control cows that were not presynchronized, possibly because of the similar ovulation responses to the first GnRH of Ovsynch and synchronization rates. The fertility after Ovsynch reported in the previous studies ranged between 7 and 30% in noncyclic cows (Cordoba and Fricke, 2001; Moreira et al., 2001; Gümen et al., 2003; Maiero et al., 2006). Similar to Cordoba and Fricke (2001), in our study, P/AI at 31 d in cows that did not receive any presynchronization treatment (the CON group) was 30%. However, it should be emphasized that, in our study, 40% of the cows became cyclic during the period before Ovsynch. In our study, fertility did not improve, even though ~70% of the cows that received presynchronization treatments became cyclic before Ovsynch (44% in GP and 36% in P4). Similar to previous studies, our study found that the presynchronization protocols with exogenous progesterone (Chebel et al., 2006; Bicalho et al., 2007) or GnRH (Gümen et al., 2005; Souza et al., 2008) increased the number of the cows that became cyclic before Ovsynch, but the cows did not gain fertility.

Cows that respond to the first GnRH of Ovsynch have higher synchronization rates and, therefore, pregnancy rates (Keskin et al., 2010; Galvão and Santos, 2010). In our study, similar to previous results, we found that the CON cows that responded to the first GnRH of Ovsynch (37.7%) had higher fertility than the nonresponding CON cows (16.7%). However, the response to the first GnRH of Ovsynch had an effect on fertility only in CON cows and not in the presynchronized cows, most likely depending on whether the number of CON noncyclic cows at the beginning of Ovsynch was greater than the number of Presynch group cows that became cyclic at the beginning of Ovsynch. When cows were evaluated according to recovery rate (becoming cyclic after presynchronization or spontaneously), pregnancy rates were higher in the cows that became cyclic before Ovsynch than in the cows that did not become cyclic; this difference was found in presynchronized cows but not in CON cows. This difference might be due to the cows that did not respond to presynchronization treatments also not responding to the Ovsynch protocol; these cows also failed to achieve fertility, similarly to the cows that responded to presynchronization treatments.

In our relatively small number of cows to evaluate P/AI, we observed no increase in fertility, yet embryonic loss was greater in CON cows than in presynchronized cows, which is similar to reports that embryonic loss was higher (Galvão et al., 2004) or tended to be higher (El-Zarkouny et al., 2004) in noncyclic cows compared with cyclic cows. The factors that contribute to late embryonic loss are complicated and not adequately explained (Santos et al., 2004). Galvão et al. (2004) reported that embryonic loss in noncyclic cows might be due to the mechanism that causes a short cycle concomitant with suboptimal priming of progesterone in the previous cycle.

## CONCLUSIONS

Although presynchronization, either by GnRH administration followed by PGF or by exogenous progesterone (PRID), increased the number of cows that became cyclic before Ovsynch, the treatments did not improve fertility in noncyclic cows.

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