J. Dairy Sci. 96:3817–3824
http://dx.doi.org/10.3168/jds.2012-6496


# Reducing the duration between gonadotropin-releasing hormone (GnRH) and prostaglandin $F_{2\alpha}$ treatment in the Ovsynch protocol to 6 days improved ovulation to second GnRH treatment, but inclined to reduce fertility

**G. Yilmazbas-Mecitoglu,* E. Karakaya,* A. Keskin,* A. Alkan,† and A. Gumen*[1]**
*Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, University of Uludag, Gorukle 16059, Bursa, Turkey
†Tarfas Company, Karacabey 16700, Bursa, Turkey

## ABSTRACT

The aim of this study was to test Ovsynch (OVS) versus modified OVS (decreasing the interval between first GnRH and $PGF_{2\alpha}$ to 6 d) protocols on pregnancy per artificial insemination (AI) and OVS outcomes in cyclic dairy cows. Cyclic cows (n = 920) were assigned to 1 of 2 groups: the OVS7 group (n = 459) received the OVS protocol [GnRH treatment, $PGF_{2\alpha}$ treatment 7 d later, a second GnRH (GnRH2) treatment 56 h later, and timed AI (TAI) 16 to 18 h after the GnRH2 treatment], and the OVS6 group (n = 461) received a modified OVS protocol, in which the interval between the first GnRH and $PGF_{2\alpha}$ was decreased to 6 d (GnRH treatment, $PGF_{2\alpha}$ treatment 6 d later, GnRH2 treatment 56 h later, and TAI 16 to 18 h after the GnRH2 treatment). The response to the first GnRH of OVS was similar between OVS7 (54.5%, 250/459) and OVS6 (54.2%, 250/461) groups. The ovulatory response to GnRH2 of OVS was higher in OVS6 (91.3%, 421/461) than OVS7 (84.5%, 388/459). The follicle size (mean ± standard error of the mean) at the time of TAI was smaller in OVS6 (15.23 ± 0.11 mm) than OVS7 (16.04 ± 0.11 mm). When all cows were evaluated, the pregnancy per AI at 31 d tended to be lower in OVS6 (38.0%, 175/461) than in OVS7 (43.8%, 201/459). Moreover, the pregnancy per AI at 31 d was lower in OVS6 (40.9%, 172/421) compared with OVS7 (50.3%, 195/388) in synchronized cows. In conclusion, although the modified OVS protocol decreased the follicle size at the time of AI and increased the ovulatory response to GnRH2 of OVS, it unexpectedly reduced fertility in cyclic lactating dairy cows.
**Key words:** cyclic cow, gonadotropin-releasing hormone, Ovsynch, prostaglandin $F_{2\alpha}$

## INTRODUCTION

Milk yield and fertility are important factors that affect the productivity and profitability of dairy herds. A high milk yield results in decreasing the duration of estrus, ovulation without signs of estrus, and anovulatory conditions that are directly related to fertility (Wiltbank et al., 2006, 2008). Thus, the basic reproductive management tool, estrus detection followed by AI, has lost its effectiveness in many dairies, and synchronization protocols, including the use of hormones that regulate and control estrous cycles and ovulation, have become common in practice (Pursley et al., 1995; Rabiee et al., 2005; Souza et al., 2008). The Ovsynch (**OVS**) protocol is an effective worldwide timed AI (**TAI**) program that includes administrations of GnRH 7 d before and 48 h after $PGF_{2\alpha}$ and TAI 16 to 18 h after the second administration of GnRH (Pursley et al., 1995). After the development and common usage of the OVS protocol, many studies have focused on improving the pregnancy rates achieved with OVS, with modifications intending to optimize outcomes of the OVS protocol. These attempts include many different modifications of the OVS protocol, such as performing TAI at the time of second GnRH (Cosynch; Small et al., 2001), using double $PGF_{2\alpha}$ administrations before the protocol (Presynch; Moreira et al., 2001), replacing the second GnRH administration with estradiol (Heatsynch; Pancarci et al., 2002; Stevenson et al., 2004), supplementing an exogenous progesterone source between the first GnRH and $PGF_{2\alpha}$ treatments (El-Zarkouny et al., 2004; Stevenson et al., 2006), and replacing the first GnRH treatment of OVS with human chorionic gonadotropin (Keskin et al., 2010).

The OVS protocol is a TAI program that is able to synchronize new follicular wave emergence in cows that respond to the first GnRH of the protocol and ovulation of the dominant follicle from the new wave. The cycle of development of a dominant follicle in the course of a follicular wave goes through a selection, dominance,

Received December 18, 2012.
Accepted February 25, 2013.
[1]Corresponding author: agumen@uludag.edu.tr

and atresia or ovulation phase (Matton et al., 1981). The duration between selection and ovulation or atresia phases is defined as the dominance period of the dominant follicle. Previous studies reported that pregnancy (Mihm et al., 1994; Bleach et al., 2004) and embryo quality (Cerri et al., 2009) were inversely related to the length of follicle dominance. For example, the prolonged dominance of the ovulatory follicle is associated with decreased fertility, which is reduced as the duration of dominance increases from 4 to 8 d (Mihm et al., 1994). Similarly, Bleach et al. (2004) demonstrated that cows that had an interval from follicular emergence to estrus that was 1 d shorter were more likely to become pregnant. Because previous studies (Mihm et al., 1994; Bleach et al., 2004; Cerri et al., 2009) indicated that the extended period of dominance of the ovulatory follicle might have a negative effect on pregnancy, reducing the interval from follicular emergence to TAI in an OVS protocol might be considered as one of the modifications that need to be evaluated. Likewise, Santos et al. (2010) evaluated reducing the interval between GnRH and $PGF_{2\alpha}$ to 5 d in a Cosynch protocol (5-d TAI protocol) in dairy cows, and achieved improved fertility with a shorter duration of follicle dominance. However, the 5-d TAI protocol requires a double dose of $PGF_{2\alpha}$ (at least 7 h apart; Kasimanickam et al., 2009; Santos et al., 2010) because the new corpus luteum (**CL**) induced by the first GnRH of the protocol is not responsive to $PGF_{2\alpha}$ during the first 5 d after ovulation (Tsai and Wiltbank, 1998; Miyamoto et al., 2005). A recent study (Ribeiro et al., 2012) that aimed to overcome this impractical issue of the 5-d TAI program reported that using a double dose of $PGF_{2\alpha}$ as a single administration (1 mg per cow) on d 5 resulted in inadequate CL regression, whereas dividing the same dose into 2 administrations 24 h apart (0.5 mg/cow at each) resulted in success.

Thus, the current study aimed to evaluate a potentially more practical modification of the OVS protocol by reducing the interval between the first GnRH and $PGF_{2\alpha}$ to 6 d (6-d TAI program), in which only 1 dose of $PGF_{2\alpha}$ administration should be sufficient, and compare all OVS outcomes (preovulatory follicle size, responses to $PGF_{2\alpha}$, and GnRH administrations and pregnancy rates) in OVS with a modified OVS protocol in cyclic lactating dairy cows.

## MATERIALS AND METHODS

### *Animals*

Cyclic lactating dairy cows (n = 920) from a commercial dairy farm located in the South Marmara region (Bursa, Turkey) were enrolled in the present study. The cows were housed in freestall barns with self-catching headlocks, and all barns had fans and sprinklers that were activated during the hotter months of the year. All cows were grouped according to their milk production and milked 3 times daily at approximately 8-h intervals. The mean milk production of the herd was 9,880 ± 69.7 kg (305 d) and 32.4 kg/d per cow. The cows had free access to water and were fed complete mixed rations according to the NRC (2001) recommendations. The daily milk yield, reproductive health, and management records for each cow were collected from Alpro 2000 software (DeLaval, Tumba, Sweden). The average milk production for each cow was recorded from the 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 (Lalahan, Ankara, Turkey).

### *Treatment Groups*

After determination of the cyclicity of the cows by ultrasonographic examination, cyclic cows (n = 920) were randomly assigned to 1 of 2 groups. In the **OVS7** group (n = 459), the cows received the OVS protocol: a GnRH treatment (**GnRH1**; buserelin acetate, 10 μg, i.m.; Receptal; Intervet, Istanbul, Turkey) followed by $PGF_{2\alpha}$ (cloprostenol, 500 μg, i.m.; Estrumate; Ceva-DIF, Istanbul, Turkey) 7 d later. A second GnRH treatment (**GnRH2**) was administered 56 h after $PGF_{2\alpha}$, and all cows were inseminated at a fixed time (TAI) 16 to 18 h after GnRH2 treatment. In the **OVS6** group (n = 461), the cows received a modified OVS protocol (6-d TAI program) in which $PGF_{2\alpha}$ was administered 6 d after GnRH1. The GnRH2 administration was performed 56 h after $PGF_{2\alpha}$, and all cows were inseminated at TAI 16 to 18 h after GnRH2, similar to the OVS7 group.

### *Ultrasonographic Examinations*

All transrectal ultrasonographic evaluations in this study were performed with a Honda HS 2000 ultrasound scanner equipped with a 7.5-MHz transducer (Honda, Tokyo, Japan). Ultrasonographic examinations were performed at the time of GnRH1 administration on d 0 for OVS7 and d 1 for OVS6 (to determine the size of the largest follicle and presence of the CL in the ovaries), at the time of $PGF_{2\alpha}$ administration on d 7 (to determine ovulation in response to GnRH1), at the time of TAI on d 10 (to determine the presence and size of the ovulatory follicle), and 7 d after TAI on d 17 (to determine ovulation in response to GnRH2).

Ovulations in response to the GnRH treatments were characterized by disappearance of the responsive follicle and appearance of a new CL. The cows that had early ovulation (no follicle at the time of TAI) or had no ovulation (no CL 7 d after TAI) were defined as nonsynchronized cows. The cows that showed signs of estrus after $PGF_{2\alpha}$ administration were inseminated according to the a.m./p.m. rule, and they were also included as early ovulatory cows. Pregnancy diagnoses were performed by ultrasonography by imaging of the embryonic vesicle and embryo on 31 d and by imaging of fetal fluids and fetus on 62 d after TAI.

### Blood Sampling and Progesterone Analysis

Blood samples were collected from 77 cows (n = 46 in OVS7; n = 31 in OVS6) as a subgroup by puncture of the coccygeal vein or artery into evacuated tubes at the time of $PGF_{2\alpha}$ administration, at different times after $PGF_{2\alpha}$ administration (12 h, 24 h, and 48 h), at the time of TAI (72–74 h), and 7 d after TAI to evaluate serum progesterone concentrations. Samples were stored at 4°C overnight, and on the next day, they were centrifuged at 3,000 × g for 15 min at 4°C to harvest serum. Samples were frozen at −20°C until the analysis of serum concentrations of progesterone by RIA with a commercial kit (Coat-A-Count, RIA progesterone PITKPG-9; Siemens Healthcare Diagnostics GmbH, Eschborn, Germany). The lower measurement range was 0.02 ng/mL. The intra- and interassay coefficients of variation were 3.5 and 3.9%, respectively.

### Statistical Analysis

Statistical analyses were conducted using SAS (SAS Institute, 2009). The 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, average milk production, response to GnRH1 and GnRH2 of the OVS protocol, and preovulatory follicle size at the time of TAI. The PROC GLM was used to compare the differences between groups in milk production, DIM, BCS, preovulatory follicle size at the time of TAI, and serum progesterone concentrations. Chi-squared results were obtained using PROC FREQ for independent tests between the response to GnRH administrations and pregnancy/AI (**P/AI**; 31 and 62 d) between groups. The PROC LOGISTIC was performed to determine the effect of covariant factors (such as milk production, DIM, BCS, parity, ovulatory follicle size at the time of TAI, and response to GnRH1) on P/AI (31 and 62 d).

## RESULTS

Although milk production (mean ± SEM: 38.0 ± 0.45 and 39.4 ± 0.45 kg/d) was different ($P = 0.03$) between the groups (OVS7 and OVS6, respectively), BCS (mean ± SEM: 2.82 ± 0.02 and 2.79 ± 0.02), DIM (mean ± SEM: 150.1 ± 4.3 and 139.0 ± 4.3), service number (mean ± SEM: 1.29 ± 0.07 and 1.15 ± 0.07), and lactation number (mean ± SEM: 1.97 ± 0.04 and 2.04 ± 0.04) were not different between OVS7 and OVS6 groups.

In total, 12% (111/920) of the cows (n = 71 in OVS7; n = 40 in OVS6) were not synchronized. The no-ovulation rate in response to GnRH2 of the OVS protocol was not different between the groups (approximately 2%); however, early ovulation was higher ($P = 0.0004$) in OVS7 (13.3%, 61/459) than in OVS6 (6.3%, 29/461), as shown in Table 1. Only 24.1% (7/29) of the OVS6 and 44.3% (27/61) of the OVS7 cows were in estrus in early ovulatory cows. The percentages of cows that showed signs of estrus and were inseminated before TAI (early AI) were also higher ($P = 0.0005$) in the OVS7 group (5.9%, 27/459) than the OVS6 group (1.5%, 7/461).

The rate of ovulations to GnRH1 of OVS (~54%) were similar between groups (Table 1); however, the ovulatory response to GnRH2 of OVS was higher ($P = 0.002$) in OVS6 (91.3%, 421/461) than in the OVS7 group (84.5%, 388/459). As expected, the maximum follicle size (mean ± SEM) at the time of TAI, whether in cows responsive to GnRH1 or in all cows, was smaller ($P < 0.0004$) in OVS6 (15.04 ± 0.13 and 15.23 ± 0.11 mm, respectively) than in OVS7 (15.72 ± 0.14 and 16.04 ± 0.11 mm, respectively) cows, as shown in Table 1.

The serum progesterone concentrations at the time of $PGF_{2\alpha}$ administration; at 12, 24, and 48 h after $PGF_{2\alpha}$ administration; and 7 d after TAI were not different (Figure 1). In both groups, serum progesterone concentrations were lowest at the time of TAI, and progesterone levels were similar and above 2 ng/mL 7 d after TAI.

Pregnancy per AI at 31 d was not different ($P = 0.10$) between OVS7 (42.5%, 195/459) and OVS6 (37.3%, 172/461) groups. Pregnancy per AI at 62 d was also not different between groups (37.7%, 173/459 in OVS7 and 33.8%, 156/461 in OVS6). Pregnancy loss between 31 and 62 d was similar between the groups (11.3%, 22/195 in OVS7 and 9.3%, 16/172 in OVS6). In contrast, when only synchronized cows were evaluated, the P/AI at 31 d was higher ($P = 0.008$) in the OVS7 group (50.3%, 195/388) than the OVS6 group (40.9%, 172/421). In synchronized cows, P/AI at 62 d was also

**Table 1.** Effects of reducing the interval between GnRH and $PGF_{2\alpha}$ treatments to 6 d in Ovsynch (OVS) on synchronization parameters and pregnancy rates

| Item | Treatment[1] OVS7 | OVS6 | *P*-value |
|---|---|---|---|
| Early ovulation[2] [% (no./total no.)] | 13.3 (61/459) | 6.3 (29/461) | 0.0004 |
| Early AI[3] [% (no./total no.)] | 5.9 (27/459) | 1.5 (7/461) | 0.0005 |
| No ovulation to second GnRH [% (no./total no.)] | 2.2 (10/459) | 2.4 (11/461) | 0.83 |
| Diameter of follicle at TAI (mm) | | | |
| All cows[4] | 16.04 ± 0.11 | 15.23 ± 0.11 | 0.0001 |
| Cows responsive to first GnRH[4] | 15.72 ± 0.14 | 15.04 ± 0.13 | 0.0004 |
| Ovulation to first GnRH [% (no./total no.)] | 54.5 (250/459) | 54.2 (250/461) | 0.94 |
| Ovulation to second GnRH [% (no./total no.)] | 84.5 (388/459) | 91.3 (421/461) | 0.002 |
| Pregnancy/AI [% (no./total no.)] | | | |
| All cows | | | |
| At 31 d | 42.5 (195/459) | 37.3 (172/461) | 0.10 |
| At 62 d | 37.7 (173/459) | 33.8 (156/461) | 0.22 |
| Pregnancy loss | 11.3 (22/195) | 9.3 (16/172) | 0.53 |
| Synchronized cows | | | |
| At 31 d | 50.3 (195/388) | 40.9 (172/421) | 0.008 |
| At 62 d | 44.6 (173/388) | 37.1 (156/421) | 0.03 |
| Pregnancy loss | 11.3 (22/195) | 8.1 (14/172) | 0.31 |

[1]OVS7 = OVS protocol; OVS6 = modified OVS protocol (reducing the interval between first GnRH and $PGF_{2\alpha}$ treatments to 6 d).

[2]Early ovulatory cows [included cows that had no ovulatory follicle at the time of timed AI (TAI) and cows that showed signs of estrus and were inseminated before TAI].

[3]Only cows that showed signs of estrus and were inseminated before TAI.

[4]Results are reported as mean ± SEM.

higher ($P = 0.03$) in the OVS7 group compared with the OVS6 group (Table 1). Pregnancy loss was similar between synchronized cows in both groups (Table 1).

The early ovulation rate in the OVS7 group was higher ($P = 0.001$) compared with the OVS6 group in cows that were not responsive to GnRH1 and tended to be higher ($P = 0.09$) in cows that were responsive to GnRH1 (Table 2). The no-ovulation rate (no response to GnRH2) was similar within and between groups with respect to cows that were responsive or not responsive to GnRH1 (Table 2).

The ovulatory response to GnRH2 of OVS was not different between the cows that were responsive or not responsive to GnRH1 within the groups (Table 2). However, cows that were not responsive to GnRH1 in the OVS6 group had a higher ($P = 0.002$) ovulatory response to GnRH2 (92.4%, 195/211) compared with cows that were not responsive to GnRH1 in the OVS7 group (82.8%, 173/209), whereas no difference was observed between the cows that were responsive to GnRH1 in both groups.

Pregnancy per AI was not different between cows that were responsive or not responsive to GnRH1 within and between two groups. However, in the OVS6 group, P/AI at 31 d tended to be higher ($P = 0.09$) in the cows that were responsive to GnRH1 (40.8%, 102/250) compared with the cows that were not responsive (33.2%, 70/211). When the synchronized cows were evaluated, in cows that were not responsive to GnRH1, P/AI at 31 d was lower ($P = 0.01$) in the OVS6 group (35.9%, 70/195) compared with the OVS7 group (49.1%, 85/173). Additionally, in cows that were not responsive to GnRH1, in the OVS6 group, P/AI was lower ($P = 0.05$) at 31 d and tended to be lower ($P = 0.09$) at 62 d compared with cows that were responsive to GnRH1 (Table 3). In synchronized cows, pregnancy loss was only different between cows that were responsive (7.3%, 8/110) or not responsive to GnRH1 (16.5%, 14/85) in the OVS7 group.

Regardless of the treatment, when evaluating the effect of response to GnRH1 on P/AI in all cows, cows that were responsive to GnRH1 tended to have higher ($P = 0.09$) P/AI at 31 d and significantly higher ($P \leq 0.04$) P/AI at 62 d compared with cows that were not responsive to GnRH1 (Table 4). Also, regardless of the treatment, when evaluating the effect of parity on P/AI in all cows, primiparous cows tended to have higher ($P = 0.07$) P/AI at 31 d and significantly higher ($P = 0.04$) P/AI at 62 d compared with multiparous cows (Table 4).

## DISCUSSION

In the present study, it was hypothesized that reducing the interval between GnRH1 and $PGF_{2\alpha}$ treatments in the OVS protocol to 6 d would improve pregnancy rates compared with the OVS protocol due to the ovulatory follicle coming from a new follicular wave

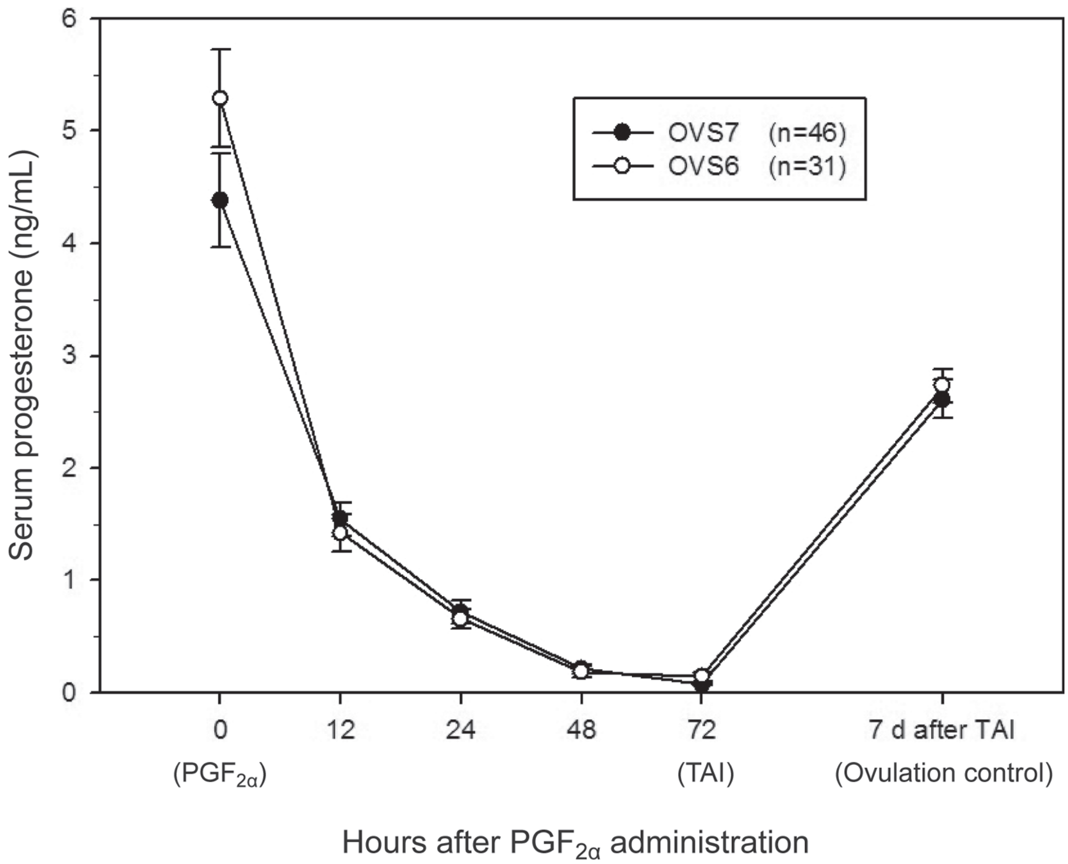


**Figure 1.** Serum progesterone concentrations (ng/mL) in a modified Ovsynch (OVS) protocol: OVS6 [GnRH treatment, $PGF_{2\alpha}$ treatment 6 d later, a second GnRH (GnRH2) treatment 56 h later, and timed AI (TAI) 16 to 18 h after the GnRH2 treatment] and in OVS7 (GnRH treatment, $PGF_{2\alpha}$ injection 7 d later, GnRH2 treatment 56 h later, and TAI 16 to 18 h after the GnRH2 treatment) after $PGF_{2\alpha}$ administration of the OVS protocol in cyclic dairy cows.

induced by GnRH1 having 1 less day of dominance. Likewise, Bleach et al. (2004) demonstrated that cows that had an interval from follicular emergence to estrus that was 1 d shorter were more likely to become pregnant. In addition, a recent study (Santos et al., 2010) investigating the effect of follicle dominance on P/AI in cows subjected to the TAI protocol (5-d TAI program) reported higher fertility in cows that had a shorter interval between the GnRH1 and $PGF_{2\alpha}$ compared with the cows that had a 7-d interval. Unexpectedly, in the present study, modifying the OVS protocol by reducing the interval between GnRH1 and $PGF_{2\alpha}$ to 6 d did not improve fertility; moreover, it tended to decrease pregnancy in cyclic lactating dairy cows. Also, in synchronized cows, the fertility of the OVS6 group was significantly lower compared with the OVS7 group.

Some previous studies (Vasconcelos et al., 2001; Wiltbank et al., 2011) indicated that the ovulation of smaller follicles resulted in lower fertility. In our study, the maximum diameter of the ovulatory follicle in the OVS6 group was smaller than that of the OVS7 group. Vasconcelos et al. (2001) attributed the lower fertility of the smaller follicles to decreased concentrations of progesterone (at 6 d after TAI) produced by a smaller CL following the ovulation of smaller follicles, which is not valid for our study because the progesterone concentrations at 7 d after TAI were similar between the groups (Figure 1). Wiltbank et al. (2011) also reported other possible reasons for decreased fertility in cows ovulating small follicles, including reduced estradiol concentrations before AI and ovulation of a less mature oocyte. In the present study, the OVS7 group had a higher percentage of early ovulatory cows (13.3%, 61/459), and a higher percentage of cows showed signs of estrus before TAI (5.9%, 27/456) compared with the OVS6 group (6.3%, 29/461 and 2.4%, 11/461, respectively). These clinical observations might be due to different estradiol concentrations between the two groups. Thus, the hypothesis of decreased fertility due to reduced estradiol concentrations before AI might explain the declined fertility in synchronized cows of the OVS6 group in the present study.

**Table 2.** Ovulation to the second GnRH treatment (GnRH2), early ovulation, and no ovulation to GnRH2 in cows responsive (+) or not responsive (−) to the first GnRH treatment (GnRH1) between the groups

| | Ovulation to GnRH2 | | | Early ovulation[1] | | | No ovulation to GnRH2 | | |
|---|---|---|---|---|---|---|---|---|---|
| Item | GnRH1(+) | GnRH1(−) | *P*-value | GnRH1(+) | GnRH1(−) | *P*-value | GnRH1(+) | GnRH1(−) | *P*-value |
| OVS7 [% (no./total no.)] | 86.0 (215/250) | 82.8 (173/209) | 0.34 | 11.2 (28/250) | 15.8 (33/209) | 0.15 | 2.8 (7/250) | 1.4 (3/209) | 0.30 |
| OVS6 [% (no./total no.)] | 90.4 (226/250) | 92.4 (195/211) | 0.44 | 6.8 (17/250) | 5.7 (12/211) | 0.62 | 2.8 (7/250) | 1.9 (4/211) | 0.51 |
| *P*-value | 0.13 | 0.002 | | 0.09 | 0.001 | | 1.00 | 0.71 | |

[1]Early ovulatory cows (included cows that had no ovulatory follicle at the time of timed AI (TIA) and cows that showed signs of estrus and were inseminated before TAI).

**Table 3.** Pregnancy per AI (P/AI) and pregnancy loss in synchronized cows according to response to the first GnRH treatment (GnRH1)[1]

| | P/AI at 31 d | | | P/AI at 62 d | | | Pregnancy loss | | |
|---|---|---|---|---|---|---|---|---|---|
| Item[2] | GnRH1(+) | GnRH1(−) | *P*-value | GnRH1(+) | GnRH1(−) | *P*-value | GnRH1(+) | GnRH1(−) | *P*-value |
| OVS7 [% (no./total no.)] | 51.2 (110/215) | 49.1 (85/173) | 0.69 | 47.4 (102/215) | 41.0 (71/173) | 0.20 | 7.3 (8/110) | 16.5 (14/85) | 0.04 |
| OVS6 [% (no./total no.)] | 45.1 (102/226) | 35.9 (70/195) | 0.05 | 40.7 (92/226) | 32.8 (64/195) | 0.09 | 9.8 (10/102) | 8.6 (6/70) | 0.78 |
| *P*-value | 0.20 | 0.01 | | 0.15 | 0.10 | | 0.51 | 0.14 | |

[1]+ = cows were responsive to GnRH1; − = cows were not responsive to GnRH1.

[2]OVS7 = Ovsynch (OVS) protocol: GnRH treatment, $PGF_{2\alpha}$ treatment 7 d later, a second GnRH (GnRH2) treatment 56 h later, and timed AI (TAI) 16 to 18 h after the GnRH2 treatment; OV6 = modified OVS protocol: GnRH treatment, $PGF_{2\alpha}$ injection 6 d later, GnRH2 treatment 56 h later, and TAI 16 to 18 h after the GnRH2 treatment.

**Table 4.** Regardless of the treatments, ovulatory response to the second GNRH treatment (GnRH2) and pregnancy rates according to the parity or responses to the first GNRH treatment (GnRH1) of cows

| | Parity | | | Response to GnRH1 | | |
|---|---|---|---|---|---|---|
| Item | Primiparous | Multiparous | *P*-value | Responsive | Not responsive | *P*-value |
| Ovulation to GnRH2 [% (no./total no.)] | 88.9 (240/270) | 87.5 (569/650) | 0.57 | 88.2 (441/500) | 87.6 (368/420) | 0.78 |
| Pregnancy/AI [% (no./total no.)] | | | | | | |
| All cows | | | | | | |
| At 31 d | 44.4 (120/270) | 38.0 (247/650) | 0.07 | 42.4 (212/500) | 38.9 (155/420) | 0.09 |
| At 62 d | 40.7 (110/270) | 33.7 (219/650) | 0.04 | 38.8 (194/500) | 32.1 (135/420) | 0.04 |
| Pregnancy loss | 8.3 (10/120) | 11.3 (28/247) | 0.35 | 8.5 (18/212) | 12.9 (20/155) | 0.18 |

Although fertility did not increase in our study, the ovulatory response to GnRH2 of OVS was markedly improved in cows that had a 1-d shorter duration of follicular development (91.3% in OVS6) compared with cows that had the conventional duration of follicular development (84.5% in OVS7). Santos et al. (2010) achieved increased pregnancy rates by reducing the duration of follicular development (Cosynch vs. 5-d Cosynch) without having an increased response to either the first or second GnRH treatment. However, most studies (Vasconcelos et al., 1999; Moreira et al., 2001; Galvão and Santos, 2010; Keskin et al., 2010) demonstrated that a higher response to GnRH1 increased the ovulatory response to GnRH2 and, therefore, the pregnancy rate. Because no difference existed in the ovulation response to GnRH1 (~54%) between groups, the improvement in the ovulatory response to GnRH2 in the OVS6 group was interpreted as the effect of reducing the interval between GnRH and $PGF_{2\alpha}$. This improvement in the ovulatory response to GnRH2 might originate from cows that were not responsive to GnRH1 due to the low incidence of early ovulation in those cows in the modified group.

One of the important limitations of reproductive synchronization protocols is the ineffectiveness of exogenous $PGF_{2\alpha}$ during the first few days of the estrous cycle. Exogenous $PGF_{2\alpha}$ causes regression of the bovine CL only between d 5 and 16 after estrus. In the OVS protocol, the cows have progesterone concentrations above the basal levels at the time of TAI considered as the cows have incomplete luteal regression. Incomplete luteal regression after $PGF_{2\alpha}$ administration in the OVS protocol has been reported and associated with lower pregnancy rates by many researchers (Moreira et al., 2000; Kim et al., 2003; Brusveen et al., 2009). Santos et al. (2010) reported that using only 1 dose of $PGF_{2\alpha}$ was insufficient and determined the requirement of a double dose of $PGF_{2\alpha}$ 12 h apart in a 5-d TAI program. In the present study, the regression of the CL after $PGF_{2\alpha}$ administration was evaluated through the progesterone concentrations in serum samples, which were collected at the time of $PGF_{2\alpha}$ administration, during the subsequent hours, and at the time of TAI. The progesterone concentrations were not different at any sampling time between treatments (Figure 1). Additionally, Brusveen et al. (2009) showed that progesterone levels above 0.4 ng/mL were associated with decreased pregnancy. In the current study, in both OVS6 and OVS7 cows, serum progesterone concentrations at the time of TAI were below this critical level (0.4 ng/mL). Thus, even though blood samples were not taken from all the cows in the current study, the authors think that it is possible to achieve a complete regression of CL in the OVS6 protocol.

## CONCLUSIONS

Modifying the OVS protocol by reducing the interval between the GnRH1 and $PGF_{2\alpha}$ administration to 6 d decreased the ovulatory follicle size at the time of TAI as expected. It also increased the ovulatory response to GnRH2 due to a reduced percentage of cows with early ovulation. However, this shortened protocol did not improve pregnancy rates in cyclic lactating dairy cows and also unexpectedly decreased fertility in synchronized cows.

## ACKNOWLEDGMENTS

The authors thank Tarfas Co. (Bursa, Turkey) for the use of their herd and facilities. Our appreciation is extended to Selim Aslan (University of Ankara, Ankara, Turkey) and Heinrich Bollwein (University of Zurich, Zurich, Switzerland) for their help in the analysis of progesterone samples. Support was provided by the Scientific and Technological Research Council of Turkey (TUBITAK) grant no. TOVAG 107O227. G. Yilmazbas-Mecitoglu was supported by the TUBITAK–Scientist Support Department (BIDEB) International Postdoctoral Research Fellowship Programme.

## REFERENCES

Bleach, E. C. L., R. G. Glencross, and P. G. Knight. 2004. Association between ovarian follicle development and pregnancy rates in

dairy cows undergoing spontaneous oestrous cycles. Reproduction 127:621–629.
Brusveen, D. J., A. H. Souza, and M. C. Wiltbank. 2009. Effects of additional prostaglandin $F_{2\alpha}$ and estradiol-17β during Ovsynch in lactating dairy cows. J. Dairy Sci. 92:1412–1422.
Cerri, R. L., H. M. Rutigliano, R. C. Chebel, and J. E. P. Santos. 2009. Period of dominance of the ovulatory follicle influences embryo quality in lactating dairy cows. Reproduction 137:813–823.
El-Zarkouny, S. Z., J. A. Cartmill, B. A. Hensley, and J. S. Stevenson. 2004. Pregnancy in dairy cows after synchronized ovulation regimens with or without presynchronization and progesterone. J. Dairy Sci. 87:1024–1037.
Ferguson, J. D., D. T. Galligan, and N. Thomsen. 1994. Principal descriptors of body condition score in Holstein cows. J. Dairy Sci. 77:2695–2703.
Galvão, K. N., and J. E. P. Santos. 2010. Factors affecting synchronization and conception rate after the Ovsynch protocol in lactating Holstein dairy cows. Reprod. Domest. Anim. 45:439–446.
Kasimanickam, R., M. L. Day, J. S. Rudolph, J. B. Hal, and W. D. Whittier. 2009. Two doses of prostaglandin improve pregnancy rates to timed-AI in a 5-day progesterone-based synchronization protocol in beef cows. Theriogenology 71:762–767.
Keskin, A., G. Yilmazbas-Mecitoglu, A. Gumen, E. Karakaya, R. Darici, and H. Okut. 2010. Effect of hCG vs. GnRH at the beginning of the Ovsynch on first ovulation and conception rates in cyclic lactating dairy cows. Theriogenology 74:602–607.
Kim, I.-H., G.-H. Suh, and D.-S. Son. 2003. A progesterone-based timed AI protocol more effectively prevents premature estrus and incomplete luteal regression than an Ovsynch protocol in lactating dairy cows. Theriogenology 60:809–817.
Matton, P., V. Adelakoun, Y. Couture, and J. J. Dufour. 1981. Growth and replacement of the bovine ovarian follicles during the estrous cycle. J. Anim. Sci. 52:813–820.
Mihm, M., A. Baguisi, M. P. Poland, and J. F. Roche. 1994. Association between the duration of dominance of the ovulatory follicle and pregnancy rate in beef heifers. J. Reprod. Fertil. 102:123–130.
Miyamoto, A., K. Shirasuna, M. P. B. Wijayagunawardane, S. Watanabe, M. Hayashi, D. Yamamoto, M. Matsui, and T. J. Acosta. 2005. Blood flow: A key regulatory component of corpus luteum function. Domest. Anim. Endocrinol. 29:329–339.
Moreira, F., C. Orlandi, C. A. Risco, R. Mattos, F. Lopes, and W. W. Thatcher. 2001. Effects of presynchronization and bovine somatotropin on pregnancy rates to timed artificial insemination protocol in lactating dairy cows. J. Dairy Sci. 84:1646–1659.
Moreira, F., C. A. Risco, M. F. A. Pires, J. D. Ambrose, M. Drost, and W. W. Thatcher. 2000. Use of bovine somatotropin in lactating dairy cows receiving timed artificial insemination. J. Dairy Sci. 83:1237–1247.
NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington, DC.
Pancarci, S. M., E. R. Jordan, C. A. Risco, M. J. Schouten, F. L. Lopes, F. Moreira, and W. W. Thatcher. 2002. Use of estradiol cypionate in a presynchronized timed artificial insemination program for lactating dairy cattle. J. Dairy Sci. 85:122–131.
Pursley, J. R., M. O. Mee, and M. C. Wiltbank. 1995. Synchronization of ovulation in dairy cows using $PGF_{2\alpha}$ and GnRH. Theriogenology 44:915–923.
Rabiee, A. R., I. J. Lean, and M. A. Stevenson. 2005. Efficacy of Ovsynch program on reproductive performance in dairy cattle: A meta-analysis. J. Dairy Sci. 88:2754–2770.
Ribeiro, E. S., R. S. Bisinotto, M. G. Favoreto, L. T. Martins, R. L. A. Cerri, F. T. Silvestre, L. F. Greco, W. W. Thatcher, and J. E. P. Santos. 2012. Fertility in dairy cows following presynchronization and administering twice the luteolytic dose of prostaglandin $F_{2\alpha}$ as one or two injections in the 5-day timed artificial insemination protocol. Theriogenology 78:273–284.
Santos, J. E. P., C. D. Narciso, F. Rivera, W. W. Thatcher, and R. C. Chebel. 2010. Effect of reducing the period of follicle dominance in a timed artificial insemination protocol on reproduction of dairy cows. J. Dairy Sci. 93:2976–2988.
SAS Institute. 2009. SAS/STAT User's Guide. Version 9.2. SAS Institute Inc., Cary, NC.
Small, J. A., J. D. Ambrose, W. P. McCaughey, D. R. Ward, W. D. Sutherland, N. D. Glover, and R. Rajamahendran. 2001. The effects of gonadotropin releasing hormone in prostaglandin $F_{2\alpha}$-based timed insemination programs for beef cattle. Can. J. Anim. Sci. 81:335–343.
Souza, A. H., H. Ayres, R. M. Ferreira, and M. C. Wiltbank. 2008. A new presynchronization system (Double-Ovsynch) increases fertility at first postpartum timed AI in lactating dairy cows. Theriogenology 70:208–215.
Stevenson, J. S., J. R. Pursley, H. A. Garverick, P. M. Fricke, D. J. Kesler, J. S. Ottobre, and M. C. Wiltbank. 2006. Treatment of cycling and noncycling lactating dairy cows with progesterone during Ovsynch. J. Dairy Sci. 89:2567–2578.
Stevenson, J. S., S. M. Tiffany, and M. C. Lucy. 2004. Use of estradiol cypionate as a substitute for GnRH in protocols for synchronizing ovulation in dairy cattle. J. Dairy Sci. 87:3298–3305.
Tsai, S.-J., and M. C. Wiltbank. 1998. Prostaglandin $F_{2\alpha}$ regulates distinct physiological changes in early and mid-cycle bovine corpora lutea. Biol. Reprod. 58:346–352.
Vasconcelos, J. L. M., R. Sartori, H. N. Oliveira, J. G. Guenther, and M. C. Wiltbank. 2001. Reduction in size of the ovulatory follicle reduces subsequent luteal size and pregnancy rate. Theriogenology 56:307–314.
Vasconcelos, J. L. M., R. W. Silcox, G. J. M. Rosa, J. R. Pursley, and M. C. Wiltbank. 1999. 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 52:1067–1078.
Wiltbank, M. C., A. Gumen, H. Lopez, and R. Sartori. 2008. Management and treatment of dairy cows that are not cycling or have follicular cysts. Cattle Pract. 16:14–19.
Wiltbank, M., H. Lopez, R. Sartori, S. Sangsritavong, and A. Gümen. 2006. Changes in reproductive physiology of lactating dairy cows due to elevated steroid metabolism. Theriogenology 65:17–29.
Wiltbank, M. C., R. Sartori, M. M. Herlihy, J. L. M. Vasconcelos, A. B. Nascimento, A. H. Souza, H. Ayres, A. P. Cunha, A. Keskin, J. N. Guenther, and A. Gumen. 2011. Managing the dominant follicle in lactating dairy cows. Theriogenology 76:1568–1582.