Comparing the Effect of the Presynch and Ovsynch Protocols Combination as a Presynchronization Protocol with the Current Presynch Protocol Before Performing Ovsynch + FTAI Program on the Conception Rate of Milking Holstein Cows

Hesam Kohsari*,1, Ali Alavi Tabatabaee2, Parviz Tajik 3

1 Department of Veterinary Medicine, Kermanshah Branch, Islamic Azad University, Kermanshah, Iran
2 Department of Specialized Veterinary Medicine, Tehran Branch, Islamic Azad University, Tehran, Iran, a_alavit@yahoo.com.
3 Department of Clinical Sciences, College of Veterinary Medicine, University of Tehran, Tehran, Iran, ptajik@ut.ac.ir.

ABSTRACT
To determine if the current Presynch-Ovsynch (PO) protocol can be replaced by Modified Double-Ovsynch (MDO) protocol to improve the conception rate of the milking Holstein cows. One hundred and twenty-nine milking Holstein cows, on average 90 ± 5 days post-partum, were assigned to one of the two following protocols randomly. Protocols were PO (n = 62) and MDO (n = 67). Timing of the hormonal injections for PO and MDO protocols were the followings respectively: PGF2α-14d-PGF2α-12d-GnRH-7d-PGF2α-2d-GnRH-18h–FTAI and PGF2α-7d-GnRH-7d-PGF2α-3d-GnRH-7d-PGF2α-2d-GnRH-18h-FTAI. For all the cows ovarian ultrasonographic examination and blood samples collection were done at the times of the 1st GnRH and PGF2α injections in the Ovsynch + Fixed-time artificial insemination (OVS + FTAI) program. Pregnancy diagnosis examinations of the cows were done 30-35 days after AIs by ultrasonography. Average conception rate of the cows that were assigned to PO and MDO protocols were 29% (18/62) and 29.8% (20/67) respectively. On the average, the cows that were allocated in PO protocol had lower sera progesterone concentrations at both times of the 1st GnRH and PGF2α injections in the OVS + FTAI program in comparison to the cows that were allocated in MDO protocol (P > 0.05). MDO protocol cannot increase the average conception rate of the milking Holstein cows in comparison to the current PO protocol.

Keywords: Presynch-Ovsynch; Modified Double-Ovsynch; Holstien; Conception Rate.

INTRODUCTION
Calving intervals should not be more than 365 days to obtain optimum benefit in dairy industry (1). To obtain a calf from each cattle per year calving to ovarian rebound interval should not be more than 60 days (2). Otherwise, calving rate will be reduced during the economic age of the cattle; consequently, milk production will be reduced because milk production is directly dependent on pregnancy and parturition (3). It is estimated that, increasing calving interval per day for each cow costs 4.7 dollars (4). One of the most important aspects of the researches to improve the cattle’s reproductive performance, specially in the recent years, is hormonal manipulations in the period of the parturition to the next pregnancy. Hormonal manipulations in this period is based on our knowledge of follicular growth, ovulation and corpus luteum regression mechanisms. Goals of the hormonal manipulations in this period are ovarian rebound acceleration after parturition, ovarian cycle synchronization and improving uterine health of the cows. Several synch and FTAI programs have been developed for dairy cows and have been improved over time to reach better results. The most important need for any synch program is the proper and predictable ovulatory response through a 12-24 h period that in pursuit high rate of conception occurs after performing FTAI program. PO is one of the most important hormonal programs that is used widely in industrial dairy farms to cover the triplex goals. Timing of the hormonal injections for PO protocol is the following: PGF2α-14d-PGF2α-12d-GnRH-7d-PGF2α-2d-GnRH-18h-FTAI. This program cannot cover the triplex goals properly and there is a sense, that the program need to be modified. DO protocol is recently used in the dairy farms. Timing of the hormonal injections for DO protocol is the following: GnRH-7d-PGF2α-3d-GnRH-7d-GnRH-7d-PGF2α-2d-GnRH-18h-FTAI. Cordoba and Fricke (5) showed in the Ovsynch protocol that if PGF2α injection cannot decrease circulatory pro-
gesterone concentration because of the failure to corpus luteum regression, 55.6% of the treated cows will have immature and early estrus that is diagnosed as a heat 0-17 days post-insemination, otherwise only 8.9% of the treated cows will have immature and early estrus. In the Ovsynch protocol, results from another study showed that if the treated cows did ovulate at the time of the 1st GnRH injection, they would have smaller ovulatory follicles on their ovaries at the time of the insemination in comparison to the cows that did not ovulate at this time (15.9 ± 0.12 mm vs. 16.4 ± 0.16 mm respectively, P = 0.02) (6). Eight millimetre is the minimum size of the ovulatory follicle of the cows when the LH receptors are sufficiently present to respond to LH secretion. There are many reports that confirm the improvement of reproductive performance of the cows after performing OVS + FTAI program in comparison to heat detection of the cows and AI at the proper time (5-7). For the dairy cows, when the Ovsynch protocol was started at proestrus, metestrus and late diestrus in comparison to early diestrus, ovulation rate at the time of the 1st GnRH injection and conception rate decreased (8, 9). It was demonstrated in a study when the cows having higher levels of the circulatory progesterone concentration at the time of the PGF2α injection in the OVS + FTAI program underwent luteolysis with higher probability and had higher conception rates than the cows with lower levels of the circulatory progesterone concentration at the same time (10).

It has been reported that conception rate for cyclic cows is higher (49.4%) than non-cyclic cows (19.4%) after performing Ovsynch protocol (11). To perform Ovsynch protocol, the best situation is when the cows be cyclic and have a corpus luteum on their ovaries that remains functional 7 days in the period of the 1st GnRH to PGF2α injections. Furthermore, the cows should have a dominant follicle on their ovaries that ovulates after the 1st GnRH injection (8, 9). Ovulatory response to the 1st GnRH injection in the Ovsynch protocol differs between the cows in accordance to the age of the corpus luteum or size or age of the dominant follicle. During the first four days of the ovarian cycle of the cows, follicular recruitment occurs in absence of a dominant follicle. At this time, presence of the small follicles (less than 8 mm diameter) on the ovaries is responsible for low ovulatory response to the 1st GnRH injection in the Ovsynch protocol. After the 4th day of the ovarian cycle, follicular selection occurs in which one (sometimes two) dominant follicle grows more than the others. Between days 5-10 of the ovarian cycle, there is usually a dominant follicle on the ovaries with more than 10 mm diameters, and of course there is also a functional corpus luteum on the ovaries which explains the high rate of the ovulatory response to the 1st GnRH injection in the Ovsynch protocol at this time. This period is the best time to begin the Ovsynch protocol. On days 11-16 of the ovarian cycle, ovulatory response to the 1st GnRH injection in the Ovsynch protocol is different. It is because of the dominant follicle which is yielded from the first or the second follicular wave, if the atresia process is underway, it may ovulate or not. This is the period that yields the minimums result in the aspect of the ovulatory response to the 1st GnRH injection in the Ovsynch protocol. Since the majority of the dominant follicles become atretic. There is a dominant follicle on the ovaries again after the 16th day of the ovarian cycle that responds to the 1st GnRH injection in the Ovsynch protocol however the ovulatory response is higher when GnRH is injected at days 5-10 of the ovarian cycle. It may be because of the low circulatory progesterone concentration at the latter time. If the age of the corpus luteum is 1-4 days at the time of the 1st GnRH injection in the Ovsynch protocol, ovulation probability of the dominant follicle will be 23%. If the age of the corpus luteum is 5-10 days, ovulation probability of the dominant follicle will be 96% at this time. If the age of the corpus luteum is 11-16 and 17-21 days, ovulation probability of the dominant follicle is 5% and 77% respectively at the mentioned times (8).

Two dose PGF2α injections 14 days apart and then performing Ovsynch protocol 11-14 days later assures that more than 70% of the treated cows are on days 5-12 of the estrus cycle at the beginning of the Ovsynch protocol. PO protocol is more effective in cyclic cows, hence a relatively high percent of the treated cows (20-30%) will not benefit from the protocol at the early stages of the milk production, because they are not cyclic (9).

Since conception rate of the non-cyclic dairy cows after performing PO protocol was low another synth program was introduced recently called DO. DO protocol is based on the followings:

1) At the time of the 1st GnRH injection in the OVS + FTAI program more cows will ovulate. 2) At the mentioned time, more cows will have a functional corpus luteum on their ovaries. These situations will increase the average conception rate of the cows that receive DO protocol than the cows that receive the Ovsynch protocol only. In a study which cows were treated with DO protocol, percentage of the cows with low levels of the circulatory progesterone concentration at the time of the 1st GnRH injection in the OVS + FTAI program was reduced to 9.4% in comparison to 33.3% of the cows that were treated with the current PO protocol (12). It is in agreement with the idea that DO protocol can induce cyclicity in non-cyclic cows.

Increasing the conception rate of the cows after performing DO protocol at least partly is related to the hormonal environment in which, ovulatory follicle is developed. High circulatory progesterone concentration at the time of the follicular development reduces LH secretion and this phenomenon will enhance dominant follicle sufficiency or oocyte quality (13, 14). It seems that DO protocol is more effective in primipa-
rous than multiparous cows because post-partum long-term inactive ovaries incidence is higher in primipara cows (15, 16). It was demonstrated in a study that DO protocol increases the conception rate of the milking dairy cows because of the reducing the synchrony rate of the cows in the OVS + FTAI program (17). It has been shown that synth programs such as Ovsynch create lower than optimum synchrony rates in the treated cows because of the reduction of the ovulation rate of the cows subsequent to the 1st GnRH injection or inadequate luteolysis after PGF2α injection in the OVS + FTAI program (18).

Herlihy et al (19) demonstrates that a few cows that receive DO protocol had lower circulatory progesterone concentration levels (< 0.5 ng/mL) at the time of the 1st GnRH injection in the OVS + FTAI program in comparison to the cows that receive PO protocol (5.4% vs. 25.3% respectively). Cows that receive DO protocol had higher conception rates than the cows that receive PO protocol in the first AI post-partum (46.3% vs. 38.2% respectively) and this increase is mostly related to the primipara cows (52.5% vs. 42.3% respectively) than the multipara cows (40.3% vs. 34.3% respectively).

Performing the 1st Ovsynch program in DO protocol can treat follicular and luteal ovarian cysts and enhance conception rate of the cows at this aspect too. It seems the proposed program that combines Presynch and Ovsynch programs before performing OVS + FTAI protocol eliminates PO program defects and improves reproductive efficiency. The reason is that the treated cows benefit from either Presynch and Ovsynch presynchronization programs before performing the OVS + FTAI protocol.

**MATERIALS AND METHODS**

**COWS, HOUSING AND FEEDING**

The research was conducted in accordance with Local Bioethics Committee of Veterinary Faculty of Tehran University. This study was performed in an industrial dairy farm near Tehran during February 2013 to November 2014 on 129 milking Holstein cows. The cows were milking three times and feed two times daily in T.M.R form. Fresh water was always available for the cows. They housed in semi-open areas. All the treatment procedures, including hormone injections, collecting blood samples, AIs and ovarians and uterine ultrasonographic examinations were done when the cows were restricted.

**TREATMENTS AND AI**

During the study, the cows were categorized based on parity and days in milk and assigned to one of two treatment groups randomly.

Treatment groups were: PO and MDO.

Timing of the hormonal injections and FTAI for the cows that were assigned to PO and MDO protocols are shown in Figure 1.

**Figure 1.** Schematic schemes of the hormonal injections, ovarian ultrasonographic examinations (US) and collecting blood samples for progesterone concentration assay (BS) in milking Holstein cows (DIM: Range of the days in milk of the cows at the time of the initiation of the OVS+FTAI program)

Gonadrolone acetate (gonabreed, parnell, Australia) was the GnRH analogue that was applied with the dose of 100 μg per cow at each injection time.
Cloprostenol sodium (estroplan, parnell, Australia) was the \(\text{PGF}_{2\alpha}\) analogue that was applied with the dose of 500 μg per cow at each injection time.

Initiation of the OVS + FTAI program for the cows that were assigned to PO and MDO protocols was 91 ± 5 and 89 ± 5 days post-partum respectively. Out of 62 cows that were assigned to PO protocol, 20 cows were in the first parity, 25 cows in the second parity and 17 cows in the third or more parity. Out of 67 cows that were assigned to MDO protocol, 19 cows were in the first parity, 26 cows in the second parity and 22 cows in the third or more parity.

**OVARIAN ULTRASONOGRAPHIC EXAMINATIONS, OVULATION RESPONSES AND PREGNANCY DIAGNOSIS**

Ovarian ultrasonographic examinations were done for all the cows with 7.5 MHz linear transducer (Ibex/portable ultrasound, Colorado USA) at the times of the 1st GnRH and \(\text{PGF}_{2\alpha}\) injections in the OVS + FTAI program. At the time of the 1st GnRH injection in the OVS + FTAI program, presence or absence of a corpus luteum on the ovaries of the cows was assessed. At the time of the \(\text{PGF}_{2\alpha}\) injection in the OVS + FTAI program, presence or absence of a follicle with ovulation competence on the ovaries and ovulatory response to the 1st GnRH injection in the OVS + FTAI program were assessed. Ovulatory response to the 1st GnRH injection in the OVS + FTAI program was marked as a new corpus luteum development on the ovaries of the cows at the time of the \(\text{PGF}_{2\alpha}\) injection in the OVS + FTAI program. Pregnancy diagnosis examinations of the cows were done 30-35 days after AIs by ultrasonography. Cows that were detected in heat between AI to pregnancy diagnosis examination period were assigned non-pregnant and were inseminated at the proper time.

**BLOOD SAMPLE COLLECTION AND PROGESTERONE CONCENTRATION ASSAY**

Blood sample collections were done before the 1st GnRH and \(\text{PGF}_{2\alpha}\) injections in the OVS + FTAI program via coccygeal vein. Blood samples were placed in a refrigerator and centrifuged (3000 g for 20 m) within 1h after the collection to attain sera samples. Then sera samples were placed at -20° C till progesterone assay. For progesterone concentration assessment in the sera samples Monobind Inc (lake forest, CA 92630, USA) ELISA kits were used.

**STATISTICAL ANALYSIS**

To run data statistical analysis, SPSS21 statistical software and chi-square test were used. When variance analysis showed meaningful difference at the level of the, results were analyzed with multiple range Duncan test.

**RESULTS**

Table 1. Comparison of Some Reproductive Parameters between the Cows that Were Assigned to PO and MDO Protocols

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PO</th>
<th>MDO</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conception rate (average)</td>
<td>29% (18/62)</td>
<td>29.8% (20/67)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Days in milk at the time of the beginning of the OVS + FTAI program (in range)</td>
<td>91 ± 5 (86-96)</td>
<td>89 ± 5 (84-94)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Parity (in range)</td>
<td>2.22 (1-8)</td>
<td>2.29 (1-7)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Average diameter of the ovulatory follicle at the time of the 1st GnRH injection in the OVS + FTAI program (cm)</td>
<td>1.74</td>
<td>1.55</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Average diameter of the follicle with ovulation competence at the time of the (\text{PGF}_{2\alpha}) injection in the OVS + FTAI program (cm)</td>
<td>1.58</td>
<td>1.66</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Average concentration of the sera progesterone at the time of the 1st GnRH injection in the OVS + FTAI program (ng/mL)</td>
<td>2.48</td>
<td>3.01</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Average concentration of the sera progesterone at the time of the (\text{PGF}_{2\alpha}) injection in the OVS + FTAI program (ng/mL)</td>
<td>2.87</td>
<td>3.30</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>
Table 2. Comparison of the Parity Effect on the Conception Rate of the Cows that Were Assigned to PO and MDO Protocols

<table>
<thead>
<tr>
<th>Parity sub-groups</th>
<th>PO</th>
<th>2</th>
<th>3 ≤</th>
<th>P-value a</th>
</tr>
</thead>
<tbody>
<tr>
<td>PO</td>
<td>30% (6/20)</td>
<td>20% (5/25)</td>
<td>41.1% (7/17)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>MDO</td>
<td>36.8% (7/19)</td>
<td>19.2% (5/26)</td>
<td>36.3% (8/22)</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>P-value b</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>---</td>
</tr>
<tr>
<td>Total</td>
<td>33.3% (13/39)</td>
<td>19.6% (10/51)</td>
<td>38.4% (13/39)</td>
<td>---</td>
</tr>
</tbody>
</table>

a Comparison of the conception rate between the cows that were assigned to the different parity sub-groups for each protocol.

b Comparison of the conception rate between the cows that were assigned to the same parity sub-groups between PO and MDO protocols.

Different superscripts in the total row show meaningful statistical difference (P < 0.05).

Table 3. Comparison of the Treatment Effects on the Ovulation Presumption and Existence Presumption of a Functional Corpus Luteum on the Ovaries of the Cows at the Time of the 1st GnRH Injection in the OVS + FTAI Program in PO and MDO Protocols

<table>
<thead>
<tr>
<th>Sub-groups</th>
<th>PO</th>
<th>MDO</th>
<th>P-value c</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Ovu a + CL b</td>
<td>14.5% (9/62) d</td>
<td>29.8% (20/67) d</td>
<td>&lt; 0.05</td>
<td>22.4% (29/129) d</td>
</tr>
<tr>
<td>2. CL</td>
<td>48.3% (30/62) e</td>
<td>56.7% (38/67) e</td>
<td>&gt; 0.05</td>
<td>52.7% (68/129) e</td>
</tr>
<tr>
<td>3. Ovu</td>
<td>16.1% (10/62) ef</td>
<td>10.4% (7/67) ef</td>
<td>&gt; 0.05</td>
<td>13.1% (17/129) ef</td>
</tr>
<tr>
<td>4. —</td>
<td>20.9% (13/62) ef</td>
<td>2.9% (2/67) ef</td>
<td>&lt; 0.01</td>
<td>11.6% (15/129) ef</td>
</tr>
</tbody>
</table>

a Ovulation

b Functional corpus luteum

c Comparison of the same sub-groups between PO and MDO protocols.

Different superscripts in the same column show meaningful statistical differences. For PO column: 1 vs 2 and 2 vs 3 sub-groups (P < 0.001). 2 vs 4 sub-groups (P < 0.01). For MDO column: 1 vs 2 and 1 vs 3 sub-groups (P < 0.01). 1 vs 4, 2 vs 3 and 2 vs 4 sub-groups (P < 0.01).

Table 4. Comparison of the Treatment Effects on the Existence Presumption of a Large Follicle With Ovulation Competence and a Functional Corpus Luteum on the Ovaries of the Cows at the Time of the PGF2α Injection in the OVS + FTAI Program in PO and MDO Protocols

<table>
<thead>
<tr>
<th>Sub-groups</th>
<th>PO</th>
<th>MDO</th>
<th>P-value c</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. LF a + CL b</td>
<td>75.8% (47/62) d</td>
<td>74.6% (50/67) d</td>
<td>&gt; 0.05</td>
<td>75.1% (97/129) d</td>
</tr>
<tr>
<td>2. CL</td>
<td>6.4% (4/62) ef</td>
<td>10.4% (7/67) ef</td>
<td>&gt; 0.05</td>
<td>8.5% (11/129) ef</td>
</tr>
<tr>
<td>3. LF</td>
<td>14.5% (9/62) ef</td>
<td>14.9% (10/67) f</td>
<td>&gt; 0.05</td>
<td>14.7% (19/129) f</td>
</tr>
<tr>
<td>4. —</td>
<td>3.2% (2/62) ef</td>
<td>0% (0/67) ef</td>
<td>&gt; 0.05</td>
<td>1.5% (2/129) ef</td>
</tr>
</tbody>
</table>

a Large follicle with ovulation competence.

b Functional corpus luteum.

c Comparison of the same sub-groups between PO and MDO protocols.

Different superscripts in each column show meaningful statistical differences. For PO column: 1 vs 2, 1 vs 3 and 1 vs 4 sub-groups (P < 0.001). 3 vs 4 sub-groups (P < 0.05). For MDO column: 1 vs 2, 1 vs 3 and 1 vs 4 sub-groups (P < 0.001). 2 vs 4 and 3 vs 4 sub-groups (P < 0.01). For total column: 1 vs 2, 1 vs 3, 1 vs 4 and 3 vs 4 sub-groups (P < 0.01). 2 vs 4 sub-groups (P < 0.05).
Table 5. Comparison of the Conception Rate between the Cows that Were Assigned to PO and MDO Protocols Based on the Ovulatory Response and Presence or Absence of a Functional Corpus Luteum on their Ovaries at the Time of the 1st GnRH Injection in the OVS + FTAI Program

<table>
<thead>
<tr>
<th>Sub-groups</th>
<th>PO</th>
<th>MDO</th>
<th>P-value c</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Ovu a + CL b</td>
<td>22.2% (2/9) d</td>
<td>20% (4/20)</td>
<td>&lt; 0.05</td>
<td>20.6% (6/290)</td>
</tr>
<tr>
<td>2. CL</td>
<td>46.6% (14/30) a</td>
<td>34.2% (13/38)</td>
<td>&lt; 0.05</td>
<td>39.7% (27/68)</td>
</tr>
<tr>
<td>3. Ovu</td>
<td>10% (1/10) e</td>
<td>28.5% (2/7)</td>
<td>&lt; 0.05</td>
<td>17.6% (3/17)</td>
</tr>
<tr>
<td>4. —</td>
<td>7.6% (1/13) f</td>
<td>50% (1/2)</td>
<td>&lt; 0.05</td>
<td>13.3% (2/15)</td>
</tr>
</tbody>
</table>

c: Comparison of the conception rate of the cows in the same sub-groups between PO and MDO protocols.
Different superscripts in total column show meaningful statistical differences (P < 0.05).

Table 6. Comparison of the Conception Rate between the Cows that Were Assigned to PO and MDO Protocols Based on the Existence Presumption of a Large Follicle with Ovulation Competence and Presence or Absence of a Functional Corpus Luteum on their Ovaries at the Time of the PGF2α Injection in the OVS + FTAI Program

<table>
<thead>
<tr>
<th>Sub-groups</th>
<th>PO</th>
<th>MDO</th>
<th>P-value c</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. LF a + CL b</td>
<td>34% (16/47)</td>
<td>34% (17/50)</td>
<td>&gt; 0.05</td>
<td>34% (33/97) d</td>
</tr>
<tr>
<td>2. CL</td>
<td>0% (0/4)</td>
<td>0% (0/7)</td>
<td>&gt; 0.05</td>
<td>0% (0/11) a</td>
</tr>
<tr>
<td>3. LF</td>
<td>22.2% (2/9)</td>
<td>30% (3/10)</td>
<td>&gt; 0.05</td>
<td>26.3% (5/19) e</td>
</tr>
<tr>
<td>4. —</td>
<td>0% (0/2)</td>
<td>0% (0/0)</td>
<td>&gt; 0.05</td>
<td>0% (0/2) e</td>
</tr>
</tbody>
</table>

a: Large follicle with ovulation competence.
b: Functional corpus luteum.
c: Comparison of the conception rate of the cows in the same sub-groups between PO and MDO protocols.
Different superscripts in total column show meaningful statistical differences (P < 0.05)

**DISCUSSION**

In the present study, average conception rates for the cows assigned to PO and MDO protocols were 29% (18/62) and 29.8% (20/67) respectively (P < 0.05). Here the control group consisting of cows which were inseminated at the proper time after standing heat was not used because as mentioned before several studies showed beneficial effects of different synch hormonal programs to improve reproductive efficiency in dairy cows. At present, PO protocol is widely used in dairy farms. It has recently been reported that DO has more beneficial effects on the reproductive efficiency of the dairy cows than PO protocol. In this study before performing OVS + FTAI program, Presynch presynchronization program was combined with Ovsynch so that the treated cows receive the benefits of both programs. Moreover, several reproductive parameters were compared among the cows assigned to current PO and MDO protocols.

For PO protocol follicular wave development that guaranties ovulation at the beginning of the Ovsynch program, is created naturally while in DO protocol it is artificially created by GnRH injection. On the other hand, for ovarian cysts treatment without ovarian cyst type detection, Ovsynch is more successful than Pre-synch as a presynchronization program. This is one of the advantages for DO over PO protocol to increase the conception rate in milking dairy cows. Conception rate increase of the cows after performing DO protocol, partly depends on the hormonal environment in which ovulatory follicle is developed. Higher circulatory progesterone concentration levels at the time of the ovulatory follicle development decreases LH secretion and this phenomenon increases dominant follicle efficiency or oocyte quality (13, 14).

It seems that the most beneficial effects of MDO over PO protocol are in the cows with inactive ovaries at the beginning of the treatment, and cows with high incidence of follicular cysts and uterine post-partum infections. It seems that none of the mentioned cases had high frequency in the cows assigned to the present study otherwise the average conception rate of the cows in MDO protocol was dramatically higher than PO. Generally, MDO in comparison to PO protocol makes the uterus of the treated cows to be under the effect of the high progesterone concentration levels before performing OVS + FTAI program for a shorter period of time.

Souza et al. (12) compared the conception rate of the cows after performing DO with the current PO protocol. The beginning of the OVS + FTAI program for the cows was 68 ± 3 days post-partum. They showed that DO in comparison to PO protocol increased the conception rate only in primipara cows (P = 0.02). They stated that the probable causes are ovulation induction in non-cyclic cows and increasing synchrony rate of the ovarian events in the primipara cows assigned to DO protocol. However; similar result was not achieved in the present study. In the present study on the average, initiation of the OVS + FTAI program was on the cows.
was more effective than PO, because this increased the percentage of the cows that ovulated and had a functional corpus luteum on their ovaries at the time of the 1st GnRH injection in the OVS + FTAI program (P < 0.05). Bello et al. (23) demonstrated that the cows that ovulated at the time of the 1st GnRH injection in the OVS + FTAI program had higher circulatory progesterone concentrations at the time of the PGF₂α injection. Ovulatory follicle size changes reduced in these cows and they had higher synchrony rate. Performing the first Ovsynch program in DO protocol can induce high synchrony rate in the treated cows at the beginning of the OVS + FTAI program. MDO in comparison to PO protocol decreased the percentage of the cows that did not ovulate and did not have a functional corpus luteum on their ovaries at the time of the 1st GnRH injection in the OVS + FTAI program (P < 0.01). More cows in MDO in comparison to PO protocol did not ovulate but had a functional corpus luteum on their ovaries at the time of the 1st GnRH injection in the OVS + FTAI program. More cows in PO in comparison to MDO protocol ovulated but did not have a functional corpus luteum on their ovaries at the time of the 1st GnRH injection in the OVS + FTAI program. Furthermore, these results showed that regarding the presence of the functional structures on the ovaries at the time of the 1st GnRH injection in the OVS + FTAI program and their effects on the conception rate of the cows MDO protocol is more effective than PO. In this study, most cows assigned to PO and MDO protocols did not ovulate but had a functional corpus luteum on their ovaries at the time of the 1st GnRH injection in the OVS + FTAI program, and a few cows at this time did not ovulate and did not have a functional corpus luteum on their ovaries (P < 0.05). These results are in agreement with Ayres et al. (24) which showed more cows ovulated in DO than PO protocol at the time of the 1st GnRH injection in the OVS + FTAI program (P < 0.05). They showed that more cows would have a functional corpus luteum on their ovaries at this time if they received DO protocol (P < 0.01). It was concluded in Ayres et al (24) study that presynchronization of the cows with Ovsynch program induced ovulation in non-cyclic cows and improved the reproductive efficiency of the cows in the OVS + FTAI program. In the present study, regardless of 50% conception rate which was achieved in the cows without ovulation and without a functional corpus luteum on their ovaries at the time of the 1st GnRH injection in the OVS + FTAI program, these conditions were as follows:

When cows ovulated and had a functional corpus luteum on their ovaries, average conception rate was 42.2%. When cows did not ovulate but had a functional corpus luteum on their ovaries, average conception rate was 37.7%. When cows ovulated but did not have a functional corpus luteum on their ovaries, average conception rate was 27.6% and finally, when cows did not ovulate and did not have a functional corpus luteum on their ovaries too, average conception rate was 15.4% (22). Thus, in the present study MDO protocol
ovulated and did not have a functional corpus luteum on their ovaries at the mentioned time. This result is in agreement with Galvao et al. (22). Comparison of the total conception rates of the cows in the same subgroups for PO and MDO protocols showed that the highest conception rate was achieved in the cows that had a functional corpus luteum on their ovaries at the time of the 1st GnRH injection in the OVS + FTAI program and the least conception rate was achieved in the cows that did not ovulate and did not have a functional corpus luteum on their ovaries at this time. These differences were not statistically significant. In conclusion, these results confirmed the positive and important roles of high concentrations of circulatory progesterone on the ovulatory follicle or oocyte efficiency which is in agreement with other studies (14, 25). PO and MDO protocols caused more cows to have a functional corpus luteum and a large follicle with ovulation competence on their ovaries at the time of the PGF2α injection in the OVS + FTAI program. The highest conception rate of the cows was in the mentioned subgroup for both PO and MDO protocols. Comparison of the conception rates of the cows in both PO and MDO protocols based on the probable presence of a large follicle with ovulation competence and a functional corpus luteum on their ovaries at the time of the PGF2α injection in the OVS + FTAI program showed no significant statistical difference. It may be because of the low number of the samples. All in all, PO and MDO protocols caused most cows to have a large follicle with ovulation competence and a functional corpus luteum on their ovaries at the time of the PGF2α injection in the OVS + FTAI program and a few cows did not have a large follicle with ovulation competence and did not have a functional corpus luteum on their ovaries at this time (P < 0.0001). Total conception rates of the cows in the same subgroups for PO and MDO protocols based on the presence or absence of a large follicle with ovulation competence and a functional corpus luteum on the ovaries showed that the highest conception rate belonged to the cows having a large follicle with ovulation competence and a functional corpus luteum on their ovaries at the time of the PGF2α injection in the OVS + FTAI program and the least conception rate belonged to the cows that did not have a large follicle with ovulation competence on their ovaries at this time (P < 0.05). These results also confirm the positive effects of the high circulatory progesterone concentrations on the ovulatory follicle or oocyte quality.

The present study showed that PO and MDO protocols improve the reproductive efficiency in milking Holstein cows. Performing MDO protocol costs more than PO because it needs two more GnRH doses. If this extra charge is recommended in comparison to lower parturition to AI interval, MDO protocol can be used; otherwise, it is not justifiable. Moreover, on the average, initiation time of the OVS + FTAI program was on the cows with 90 ± 5 days post-partum. The most positive effects of MDO protocol to improve the reproductive efficiency are probably on the cows that receive AI on earlier periods after parturition. Further studies can test that how MDO protocol affect the reproductive efficiency of the cows that receive AI on the shorter periods after parturition.

ACKNOWLEDGMENTS: This work was funded by research and technology assistance of Islamic Azad University of Iran, Kermanshah branch. We are very thankful to Dr. Zahra Nikousefat for technical consulting.

CONFLICT OF INTEREST: The research was conducted in accordance with Local Bioethics Committee of Veterinary Faculty of Tehran University.

Dr. Hesam Kohsari has no conflict of interest.

Dr. Ali Alavi Tabatabaee has no conflict of interest.

Dr. Parviz Tajik has no conflict of interest.

REFERENCES


