ADMINISTRATION OF GnRH TREATMENT PRIOR TO OVSYNCH PROTOCOL TO STIMULATE OVARIAN CYCLE IN COWS WITH FUNCTIONAL ANOESTRUS

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Abstract

The study aimed to stimulate ovarian cycle and increase conception rates with administration of GnRH prior to the synchronisation of ovulation; by the use of Ovsynch protocol in cows (n=58) with anoestrus. For group I, GnRH was administered one week prior to Ovsynch protocol. For group II, only Ovsynch protocol was applied. The synchronisation rate was statistically higher (P<0.01) in group II (95.2%) than that in group I (71.0%). The conception rate was higher (P<0.01) in group I (40.0%) than that in group II (20.8%). At the beginning of the Ovsynch protocol, the percentage of cows with progesterone levels higher than 1 ng/ml was 83.3% in group I, and 29.2% in group II, and cycling rate, based on progesterone levels above 1ng/ml, was higher in group I (50.0%) than that in group II (20.8%) among cows with body condition score (BCS) ≥ 2.5. In conclusion, additional GnRH treatment one week prior to Ovsynch protocol, positively affected the conception rate and ovarian cycling, and this programme could be used for the treatment of functional anoestrus in cows.

Key words: cow, anoestrus, GnRH, Ovsynch, body condition score.

The dysfunction of ovaries due to endogenous and exogenous reasons is termed as true anoestrus (6). The reason of ovary dysfunction is due to, either insufficient secretion of the gonadotropin releasing hormone leading to the absence of folliculogenesis, or no response of the ovaries, in spite of normal secretion, to the gonadotropin releasing hormone (7). Nutritional deficiency during pre- and postpartum and lactation, could result in anoestrus in cows (3). Postpartum inactivity of the ovaries is attributed to low secretion of LH. Despite stress factors suppressing LH secretion, insufficient and unbalanced nutrition causes interruption in ovary functions (4). In animals identified with true anoestrus, ovaries were found to be small, hard, and dysfunctional (7) and plasma progesterone levels were below 1 ng/ml (6). True anoestrus is a significant cause of infertility in cows (9). Combination of gonadotropin releasing hormone and PGF2α was used to facilitate oestrous detection and timed artificial insemination (8). Among these combinations, Pursley et al. (13) developed Ovsynch protocol for the synchronisation of ovulation leading to timed artificial insemination. Several researchers carried out studies on improving pregnancy rates in cows with anoestrus using the Ovsynch protocol (1, 2, 10, 15). Bartolome et al. (2) reported higher pregnancy rates with additional GnRH injection prior to Ovsynch protocol in anestrous cows. Klindworth et al. (8) observed that the pregnancy rates were increased by 25% in anoestrous cows, following the Ovsynch protocol. Body condition score (BCS) higher or lower than 3 negatively, affects follicular development leading to a lower pregnancy rate in cows (8, 9, 14, 18). The relationship between BCS and fertility has been presented by many researchers (8, 17, 18).

The aim of this study was to stimulate the ovarian cycle, and increase conception rates in anoestrus cows by synchronising ovulation, using the Ovsynch protocol one week after the stimulation of follicle development with GnRH1.

Material and Methods

Cows (n=58) of various breeds, being 60-420 d postpartum, were used. The study was performed in the winter and spring of 2005. Dystocia occurred in two of the animals. Mean milk yield was 6.5 L/d. BCSs of the cows were determined using the method of Edmonson et
al. (5) on the scale of 1-5 with intervals of 0.25. The cows were classified as having optimal BCS (≥ 2.5) or poor BCS (< 2.5). The animals were fed with hay and straw. Rectal palpation was done at 10-d intervals to establish whether the cows were in anoestrus. Cows with small and hard ovaries with dysfunctional structure, and those with a small and soft uterus were eliminated.

The study was carried out in 2 groups. Treatment was applied in 33 cows in group I and 25 cows in group II. The Ovsynch protocol was carried out according to the method described by Pursley et al. (13). For group I, GnRH (Lesirelin acetate, 25 µg, Dalmarlin®, Vetas, Turkey,) was injected (day 0). Seven days after GnRH injection, the Ovsynch protocol started. On day 7, PGF$_{2α}$ (Cloprostenol sodium, 263 mcg, 2 mL, Estrumate®, DIF, Turkey) was injected i.m. On day 9, GnRH was administered once again and artificial insemination was performed 18 h later. For group II, only the Ovsynch protocol was applied. For the inseminations, (all done by the same experienced person) there thawed straws were used according to the correct techniques, and containing 30-50x10$^6$/ml of motile spermatozoa in a single insemination dose with at least 55% motility and 85% vitality. Prior to any procedure, blood was collected from the jugular vein and then centrifuged for 20 min at 3 000 rpm. The sera obtained were stored at -20°C. The progesterone levels were determined using ELISA, according to the method described by Prakash et al. (12). Progesterone concentrations higher than 1 ng/ml before commencing the Ovsynch protocol were marked as H, and those lower than 1 ng/ml were marked as L. Afterwards, the cows were classified to four groups, based on progesterone concentrations at the first GnRH and PGF$_{2α}$ injections as follows: L-H, H-L, H-H and L-L. Pregnancy rates were compared among these subgroups based on progesterone concentrations. Pregnancy diagnosis was done via rectal palpation on day 60, following artificial insemination.

**Statistical analysis.** Statistical analysis was done with the SPSS statistics programme, using the t-test, standard deviation, Chi-square and Anova test.

**Results**

Due to the inconsistency between results of rectal palpation and very high serum progesterone levels, 4 cows (3 cows in group I and 1 in group II) were not included in the assessment.

Pregnancy rates following the first insemination were found to be 40.0% (12/30) in group I and 20.8% (5/24) in group II. A statistically significant difference (P<0.01) between the groups was noted with respect to pregnancy rate. While a success rate of 71.4% (20/28) for synchronisation was reached in group I, a synchronisation success rate of 95.2% (20/21) was achieved in group II. A statistically significant difference (P<0.01) was found between the groups in relation to synchronisation rate. Progesterone levels were observed to remain below 1 ng/ml throughout the treatment in 5 cows (2 cows in group I (0.07%) and 3 cows in group II (0.13%), and these cows were unresponsive to the treatment. No statistically significant difference (P>0.05) was found between the groups, regarding anoestrus rates. The progesterone levels were higher than 1 ng/ml at the first GnRH administration, and PGF$_{2α}$ injection in 23 of 30 cows (76.7%) in group I and in 6 of 24 cows (25.0%) in group II. The pregnancy rate of these cows was found to be higher in group I compared to group II (Table 1).

**Table 1**

<table>
<thead>
<tr>
<th>Group I (n=30)</th>
<th>n</th>
<th>Pregnancy rate (%)</th>
<th>Group II (n=24)</th>
<th>n</th>
<th>Pregnancy rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GnRH</td>
<td>PGF$_{2α}$</td>
<td></td>
<td>GnRH</td>
<td>PGF$_{2α}$</td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>H</td>
<td>3</td>
<td>33.3$^a$</td>
<td>(1/3)</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>L</td>
<td>2</td>
<td>50.0$^c$</td>
<td>(1/2)</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>H</td>
<td>23</td>
<td>43.5$^e$</td>
<td>(10/23)</td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>L</td>
<td>2</td>
<td>0$^g$</td>
<td>(0/2)</td>
<td></td>
</tr>
<tr>
<td>Total pregnancy rate (%)</td>
<td>40.0</td>
<td>(12/30)$^i$</td>
<td>Total pregnancy rate (%)</td>
<td>20.8</td>
<td>(5/24)$^j$</td>
</tr>
</tbody>
</table>

L: low progesterone (1 ng/ml<)   H: high progesterone (1 ng/ml>)

b:a= P<0.001, c:d= P<0.05, e:f= P<0.01, g:h= P>0.05, = P<0.01 (different superscripts differ within the row)
The percentage of cows with plasma progesterone levels higher than 1 ng/ml at the first GnRH administration in the Ovsynch protocol was 83.3% (25/30) in group I, and pregnancy rates in these animals was 44.0%. For group II, 29.2% (7/24) of the cows displayed plasma progesterone levels higher than 1 ng/ml and 28.6% of these animals were pregnant. A statistically significant difference (P<0.01) was observed between cows of both groups with progesterone levels higher than 1 ng/ml at the first GnRH administration in the Ovsynch protocol (Fig. 1).

The percentage of cows with progesterone levels higher than 1 ng/ml at PGF_{2α} administration was found to be 71.4% in group I and 95.2% in group II. Pregnancy rates of these cows were found to be 30.0% in group I and 20.0% in group II. No statistically significant difference between the groups was noted in this period with respect to pregnancy rates (P>0.05).

BCS of cows in group I, ranged from 2 to 4, and of cows in group II from 2 to 3.5. The number of cows with BCS ≥ 2.5 was 19 in group I and 18 in group II. Based on the results, pregnancy rates in cows with a BCS above 2.5 and those with a BCS below 2.5 were 37.8% and 23.5%, respectively, and this difference was statistically significant (P<0.01). Pregnancy rate in cows with a BCS ≥ 2.5 was found to be higher in group I (42.1%) than that in group II (36.3%); however, no statistically significant difference (P>0.05) was determined. For cows with a BCS < 2.5, pregnancy rates were 33.3% in group I and 0% in group II. There was a statistically significant difference (P<0.05) in relation to pregnancy rates between both groups of cows with a BCS < 2.5. A statistically significant difference (P<0.01) was also found between cows with a BCS ≥ 2.5 and those below 2.5 in group II (Table 2).

At the initiation of the Ovsynch protocol, 50.0% (15/30) of cycling cows and 60.0% (9/15) pregnancy rates were recorded in group I in cows with progesterone levels above 1 ng/ml and BCS ≥2.5. In group II, rate of cycling cows was found to be 20.8% and pregnancy rate was 40.0% (2/5). A statistical difference (P<0.05) was found between the groups in relation to rate of cycling cows. At the first GnRH administration, 33.3% of cycling cows and 20.0% pregnancy rates were achieved in group I in cows with a BCS < 2.5 and progesterone levels above 1 ng/ml. Eight per cent of cycling cows was noted in group II; however, no pregnancy was detected. A significant difference (P<0.05) was found between the groups among cows with BCS < 2.5 and progesterone levels above 1 ng/ml (Table 3).

![Fig. 1](image_url) Percentages of cows with plasma progesterone concentrations above or below 1 ng/ml at the first GnRH injection according to Ovsynch protocol (a vs b= P<0.01).

<table>
<thead>
<tr>
<th>BCS &lt;2.5</th>
<th>BCS ≥2.5</th>
</tr>
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<tbody>
<tr>
<td>Pregnancy rate (%):</td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>42.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group II</td>
<td>33.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group I+Group II</td>
<td>37.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

a:b=P<0.01, c:d=P<0.05, e:f=P<0.01
on ovarian cycle in cows with BCS<2.5. GnRH administration one week before the Ovsynch protocol was shown to have a positive effect upon pregnancy rates. A statistically significant difference (P<0.01) was demonstrated in pregnancy rates between the groups. It has been reported that the first postpartum progesterone rise in cows is observed approximately 15-30 d after parturition. True anoestrus is seen in 10-30% of cows not presenting oestrus 2 months after calving (11). In this study, the cows were in the 60-420 d postpartum and no outward sign of oestrus was observed. In cows with anoestrus, Klindworth et al. (8) found the progesterone levels to be below 1 ng/ml prior to the study. The researchers applied the Ovsynch programme to these cows, and following the first GnRH injection, found progesterone levels to be higher than 1 ng/ml in 81.8% of the cows. In the present study, the progesterone levels were seen to be higher than 1 ng/ml in 83.3% of the cows in group I at the time of GnRH administration, and 44.0% of these cows became pregnant. For group II, the progesterone levels were higher than 1 ng/ml in 29.2% of the cows at the time of GnRH administration, and 28.6% became pregnant. A statistically significant difference (P<0.001) was detected between the groups following assessment of progesterone levels. The purpose of this study was to prevent infertility and improve pregnancy rates in cows detected to be anoestrous, by administering GnRH one week before the Ovsynch protocol (13). The effect of body condition score on pregnancy rates and progesterone levels were also investigated.

Klindworth et al. (8) achieved the highest pregnancy rate, after the first insemination in cows with BCS of 3.0. These researchers demonstrated that the pregnancy rates were lower in cows with a BCS above and below this value. Several authors detected a significant relationship between BCS and fertility (16, 18). Stevenson et al. (17) found that the pregnancy rate to be 16.7% in cows with a BCS>3. A significant relationship between BCS and fertility was also found in this study. While pregnancy rate was 37.8% in cows with BCS≥2.5, it was 23.5% in cows with BCS<2.5. A significant difference (P<0.01) in relation to pregnancy rates was found between the groups. A significant difference (P<0.005) with respect to pregnancy rates was observed between groups I and II, where the cows had a BCS<2.5. GnRH administration one week before the Ovsynch protocol was shown to have a positive effect on ovarian cycle in cows with 2.5< BCS<2.5. The rate of cycling cows was also found to have a positive effect upon pregnancy rates.

In the study carried out on cows with anoestrus, Ahuja et al. (1) achieved a 28.0% pregnancy rate. Bartolome et al. (2) determined the pregnancy rates using GnRH+Ovsynch and Ovsynch protocols at the dioestrus, metaoestrus and prooestrus stages in cows with anoestrus. The values were 30.3%, 26.6%, and 22.9% in the GnRH+Ovsynch group, and 20.2%, 18.5%, and 14.7% in the Ovsynch group, respectively. Shephard (15) achieved a 48.4% pregnancy rate, following the Ovsynch programme in non-cycling cows. Klindworth et al. (8) reached a pregnancy rate of 25.0% using the Ovsynch method in cows with anoestrus. In this study, a 40.0% pregnancy rate was achieved in the GnRH+Ovsynch group and 20.8% in the Ovsynch group. The pregnancy rates achieved in this study are parallel to the findings of other researchers. A statistically significant difference (P<0.01) was demonstrated in pregnancy rates between the groups.

In conclusion, higher synchronisation and pregnancy rates could be achieved, and infertility could be prevented by using the GnRH+Ovsynch programme.

### Table 3

<table>
<thead>
<tr>
<th>BCS &lt;2.5</th>
<th>BCS ≥2.5</th>
<th>BCS &lt;2.5</th>
<th>BCS ≥2.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>3/18</td>
<td>2/12</td>
<td>13/18</td>
</tr>
<tr>
<td>H</td>
<td>15/18</td>
<td>10/12</td>
<td>5/18</td>
</tr>
</tbody>
</table>

L: low progesterone (1 ng/ml <),  H: high progesterone (1 ng/ml >)

L: low progesterone (1 ng/ml <),  H: high progesterone (1 ng/ml >)

a:b=P<0.05  c:d=P<0.05
in non-cycling cows. In addition, oestrus cycle and pregnancy rates could be higher in anestrous cows with an optimal BCS.

References


