Abstract

The objective of this study was to compare the efficacy of human chorionic gonadotropin (hCG) for the stimulation of ovulation within 48 h after treatment in mares, which had a ≥35 mm follicle. A total of 41 oestrus cycles of 21 mares (9 Thoroughbred, 6 Dutch Warmblood, and 6 Friesian), between 7 and 12 years of age were used for this study. All the mares were intramuscularly treated with Hcg, at the dose of 3 000 IU when a follicle ≥35 mm was detected. They were examined daily by transrectal ultrasonography, until the ovulation was confirmed. It was found that the diameter of follicle before ovulation was more significant, and oestrus duration was longer in Friesian mares than those of Thoroughbred and Dutch Warmblood ones. Moreover, the percentage of mares ovulating within 48 h after hCG treatment was significantly lower in Friesian mares, when compared to that of the other breed mares. There was no significant difference in these parameters between Thoroughbred and Dutch Warmblood mares. In conclusion, the efficacy of hCG may be different among mares of different breeds. The percentage of ovulation within 48 h after hCG treatment was lower in Friesian mares when compared to the other breeds. Therefore, various strategies are needed for the stimulation of ovulation in Friesian mares.

Key words: mare, human chorionic gonadotropin, breed, ovulation.

Mares are seasonally polyoestrous animals. This means that a non-pregnant mares display recurring oestrous cycles during certain times of year. The duration of an average oestrous cycle is 21-22 d; however, the length of oestrus is rather variable (ranging from 2 to 12 d or more), and the ovulation generally occurs 24 to 48 h prior to the end of oestrus (8). Therefore, mating or insemination must be made close to the time of ovulation, in order to increase conception rates (3). In many breeding programmes, mares are inseminated once every 48 h after the onset of oestrus, until ovulation is detected by rectal palpation or ultrasonographic examination (11). However, the increase in the number of insemination or natural mating during oestrus period; causes the waste of valuable semen (6) and raises the risk of contamination of the mare’s reproductive tract (16).

Dutch Warmblood is a modern sporting horse breed, which derives from the selective breeding of German, French, and English horses crossed with the native Dutch breeds. They are powerful horses moreover, especially; their legs are strong with a long forearm. Therefore, they are used for dressage and jumping competitions. The Friesians are cold-blooded horses, although they are considered warm-blooded due to their temperament. The Friesian horse is truly an ancient breed; and it has provided the bloodline for many European breeds such as the Shire and Gelderlander. They are strong and durable horses. Therefore, they are suitable for use as workhorses.

Human chorionic gonadotropin (hCG), due to its luteinizing hormone (LH)-like activity, has been extensively used to induce ovulation in mares for many years. The administration of hCG generally causes ovulation within 48 h in mares having a follicle >35 mm (3, 6). In this way, a single timed insemination after hCG treatment may result in a high pregnancy rate (5). However, the interval from hCG treatment to ovulation can be quite variable, depending on individual sensitivity or breed differences. The delay of ovulation after treatment, may lead to the insemination of the mare at an incorrect time. There not much research about the comparison of the efficacy of hCG on the stimulation of ovulation among the mares of different breeds.

The objective of this study was to compare the efficacy of hCG on the stimulation of ovulation within 48 h after treatment in Thoroughbred, Dutch
Warmblood, and Friesian mares which had a ≥35 mm follicle.

**Material and Methods**

**Animals.** This experiment was carried out between April 1 and June 30. This time is accepted as the breeding season for mares in Turkey. A total of 41 oestrus cycles of 21 mares (9 Thoroughbred, 6 Dutch Warmblood, and 6 Friesian), between 7 and 12 years of age were used. The animals were kept in an outdoor paddock during the season. They were fed grain and alfalfa hay, and drinking water was provided *ad libitum*.

**Oestrus detection and treatment.** Oestrus detection was mainly based on observations of the mare's behaviour towards a stallion. When they first showed typical signs of oestrus, such as tail raise, frequent urination, winking of the vulva, and the position of pelvis region, the onset of oestrus was recorded. The follicular growth was monitored daily by transrectal ultrasonography (Pie Medical Scanner 200 Vet). All the mares were intramuscularly injected with hCG (Pregnyl®, Organon), at a dose of 3 000 IU when a follicle ≥35 mm was detected. They were examined in the morning everyday by transrectal ultrasonography, until the ovulation was confirmed, and the interval from the treatment to the ovulation, and the percentage of the ovulations within 48 h was determined. In addition, all the mares were observed until they ceased signs of oestrus. Then the oestrus duration was recorded.

**Statistical analysis.** All values are presented as mean ± standard error of means (SEM). The differences of the interval from the treatment to the ovulation, and oestrus duration among groups were considered to be significant at P<0.05. The statistical analyses were performed using analysis of variance (One-way ANOVA) and the post-hoc Tukey test using the SPSS/PC (version 10.0) software programme. The average percentage of the ovulations within 48 h among group was analysed by the Fisher’s exact Chi-square test.

**Results**

The hCG was administered to 21 mares during 41 oestrus cycles. The mean reproductive parameters for the mares in all groups are presented in Table 1. There was no significant difference (P>0.05) in follicle size at the time of treatment among groups. The percentage of mares ovulating within 48 h after treatment was significantly (P<0.05) lower in Friesian mares, when compared to that of Thoroughbred and Dutch Warmblood ones. However, there was no significant difference (P>0.05) in these parameters between Thoroughbred and Dutch Warmblood mares.

The diameter of the follicle before ovulation and the number of ovulating mares of three different breeds in time after hCG treatment are presented in Figs 1 and 2, respectively. It was found that the diameter of follicle before ovulation was larger (P<0.05) in Friesian mares, than those of Thoroughbred and Dutch Warmblood mares. In addition, the interval from the treatment to the ovulation and oestrus duration were longer (P<0.05) in Friesian mares than those of Thoroughbred and Dutch Warmblood mares.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Thoroughbred</th>
<th>Dutch Warmblood</th>
<th>Friesian</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of mares</td>
<td>9</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Number of oestrous cycles</td>
<td>14</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>Follicle size at the time of treatment (mm)</td>
<td>36.8±0.18</td>
<td>37.1±0.24</td>
<td>36.9±0.24</td>
</tr>
<tr>
<td>The average interval from treatment to ovulation (h)</td>
<td>42.9±6.3a</td>
<td>44.6±5.5a</td>
<td>94.2±7.9b</td>
</tr>
<tr>
<td>The percentage of ovulations within 48 h (%)</td>
<td>85.7 (12/14)a</td>
<td>78.6 (11/14)a</td>
<td>15.4 (2/13)b</td>
</tr>
<tr>
<td>Oestrus duration (d)</td>
<td>5.71±0.30a</td>
<td>5.79±0.33a</td>
<td>7.93±0.42b</td>
</tr>
</tbody>
</table>

a,b: Different superscripts within the same line designate significant differences between groups (P<0.05).

±S.E.M.
Fig. 1. Number of ovulated mares of three different breeds in time (h) after hCG treatment.

Fig. 2. Diameter of the largest follicle before ovulation (mm) in mares of three different breeds.
Discussion

Hormonal induction of ovulation with hCG, has an important role as therapeutic tool for optimisation of reproductive performance in veterinary management of broodmares (13). It has been demonstrated that a single administration of hCG at the dose of 3 000 IU, is effective for the hastening of ovulation in mares (19). However, the percentage of ovulations within 48 h after hCG treatment is an accurate criterion for evaluating its effectiveness in mares with a follicle ≥35 mm diameter (12).

In this study, it was found that the ovulation rate within 48 h was 85.3% (12/14) in hCG treated Thoroughbred mares. This result was similar to those reported in several previous studies (1, 4, 7). In addition, the percentage of ovulations within 48 h after hCG treatment was recorded as 78.6% (11/14) in Dutch Warmblood mares, and there was no significant difference in ovulation response within 48 h after treatment between Thoroughbred and Dutch Warmblood mares. However, the ovulation can sometimes be delayed, and it does not occur within 48 h after hCG treatment in some mares. In the present study, the ovulation occurred between 48 and 72 h after treatment in some Thoroughbred (n=3) and Dutch Warmblood (n=2) mares. This situation might be due to the variation of individual sensitivity to hCG among mares. Pierson and Ginther (14) reported that diameter of follicle, and thickness of the follicular wall may change during the preovulatory period, and the increase in the thickness of the follicular wall can delay the ovulation. This may cause individual differences in ovulation time after hCG treatment.

There is controversy, whether or not the repeated use of hCG within a breeding season causes a decrease in its efficacy on the induction of ovulation. Sullivan et al. (18) reported that efficiency of hCG on the stimulation of the ovulation was reduced, if it was administered more than twice during the same breeding season. The use of repeated administration of hCG has been associated with hCG antibody formation. hCG antibodies do not interfere with normal ovulation, but the efficacy of hCG may be reduced (7). However, it was reported that there was no decrease in the response rates when hCG was administered, during up to four different oestrous cycles within the same breeding season (4). Repeated administration of hCG, stimulates the generation of antibodies, but the hCG antibodies were not associated with a reduction in its effectiveness. Ovulation spontaneously occurred in mares with high antibody titres, because the antibodies did not react with LH (15).

On the other hand, Barbacini et al. (2) reported that the percentage of mares, which did not ovulate within 48 h after hCG administration, was the highest in barren and older (>16 years of age) mares. Similarly, McCue et al. (10) concluded that the decrease in efficacy of hCG on the stimulation of the ovulation, may be related to the age of mares, and it declines with the advancement of age. In this study, it was observed that there was no relationship between the repeated administration of hCG during the same breeding season, relating to the age of a mare, and ovulation time. The percentage of mares ovulating within 48 h after treatment, was significantly lower in the present study in Friesian mares when compared to that of Thoroughbred and Dutch Warmblood mares. Furthermore, the oestrus duration was longer in Friesian mares than that of other breeds.

In mares, the ovulation generally occurs when the diameter of mature follicle reaches 40-45 mm. However, the follicle diameter may dramatically differ before ovulation among the mares of different breeds. It was observed in this study that ovulations occurred after the diameter of dominant follicle approached ≥45 mm in Friesian mares, and the follicle size was larger before ovulation in Friesian mares than those of Thoroughbred and Dutch Warmblood mares.

These results are in agreement with the views of Stout (17), who reported that a mare with a follicle diameter of 35 mm is ready for hormonal stimulation of ovulation. However, this may be insufficient in Friesian mares, which have a longer oestrous period, due to the fact that their follicle maturation is slower, and their dominant follicle can develop up to size of 60 mm before ovulation. Harrison et al. (9) also reported that the reason for delay or failure of ovulation in some mares is the inadequate concentration of follicle stimulating hormone (FSH) to stimulate follicular growth. FSH is necessary for the maturation of follicle and regulation of LH receptors in ovaries.

In conclusion, the efficacy of hCG may be different among mares of different breeds. The percentage of the ovulations within 48 h after hCG treatment was lower in Friesian mares when compared to the other breeds. Therefore, various strategies are needed for the stimulation of ovulation in Friesian mares.

References