The effectiveness of supplemental administration of progesterone with GnRH, hCG and PGF$_{2\alpha}$ on the fertility of Tuj sheep during the non-breeding season

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**A B S T R A C T**

This study investigated the effects on fertility parameters of combined administration of flugestone acetate, GnRH, hCG and PGF$_{2\alpha}$ to Tuj ewes during the non-breeding season. In the study, 60 Tuj ewes were separated into four groups. Seven days before administering an intravaginal gestagen (20 mg Flugestone acetate, sponge) to the ewes in Group I (GI, n = 15), they were given an injection of GnRH (8.4 $\mu$g Buserelin acetate). Seven days after the sponge was administered, the ewes were given a second intramuscular injection of GnRH. The sponge was left in the vagina for 14 days. Immediately after removing the sponge, an intramuscular injection of PGF$_{2\alpha}$ (15 mg) was administered. The ewes in Group II (GII, n = 15) were administered hCG injections at a dose of 1000 IU/ewe instead of the GnRH injection that was given to the first group. The ewes in Group III (GIII, n = 15) were only administered intravaginal sponge. The ewes in Group IV (n = 15) were kept as a control group and were not given any treatment. Immediately after the sponges were removed, rams were introduced in all groups, ensuring that ewes exhibiting estrus were mated. The percentage of ewes exhibiting estrus was found to be significantly higher in all the study groups (GI, GII and GIII) than in the control group (GIV) ($P$ < 0.05). It was determined that the pregnancy ratio for ewe mating at the first estrus was higher in group GII than in all the other groups (46.6%) ($P$ < 0.05). The overall pregnancy rate and lambing rate was found to be significantly higher in GII (100%) than the other study groups (GI: 66.6% and 66.6%; GIII: 66.6% and 46.6%, respectively, $P$ < 0.05). On day 7 after the sponge was administered, the mean plasma progesterone level (3.64 ng/ml) was found to be higher in the ewes in GII, and this difference was found to be statistically significant ($P$ < 0.01). We concluded in this study that administering hCG and vaginal sponges to Tuj ewes during the non-breeding season had a positive effect on pregnancy and lambing rates.

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1. Introduction

Lambs are the most important source of revenue when raising sheep. However, due to a rather long anestrus period after lambing, it is usually only possible for an ewe to give birth once per year. For this reason, various hormones are administered in order to increase the fertility of ewes and to obtain lambs twice in one year or three times in two years (Ainsworth et al., 1982; Bazer et al., 2007; Gordon, 1997; Goulet and Castonguay, 2002).
The hypothalamus is quite sensitive to the negative feedback effect of estradiol in ewes during the seasonal anestrous period. During this period, LH is secreted less frequently, resulting in a lack of estrus and ovulation. For this reason, gonadotropin is needed for small and medium-sized follicles to continue developing (Leyva et al., 1998).

Administration of GnRH can stimulate ovulation in anestrous ewes, but premature luteal regression can occur in these animals. In order to prevent this, progesterone is administered prior to ovulation to inhibit oxytocin receptors in the endometrium, thus reducing the uterine response to oxytocin for the secretion of PGF2α (Leyva et al., 1998).

Intravaginal sponges are usually preferred for the purpose of estrus synchronization in animals that are cyclic and in seasonal anestrous (Wildeus, 2000). It has been determined that progesterone treatment followed by GnRH injections in ewes during the anestrous period results in follicular development, ovulation and luteal function that are similar to ewes in the breeding season (Mcleod et al., 1982; Wright et al., 1983, 1984). Administration of hCG in ewes during the anestrous period is usually performed after mating or insemination. This aids CL development and helps to increase the secretion of progesterone. This effect prevents early embryonic losses during the anestrous period (Fukui et al., 2001). Administering a PGF2α injection one day before the vaginal sponges are removed or on the day the sponges are removed prevents the occurrence of luteolysis at different times and prevents the delay of the estrus (Ataman et al., 2006; Dogan and Nur, 2006; Wildeus, 2000).

The goal of this study was to research how fertility parameters are affected by the combined use of GnRH, hCG and PGF2α administered together with a vaginal sponge for the purpose of estrus synchronization during the non-breeding season in the Tuj breed of ewes raised in the province of Kars and the surrounding area.

2. Materials and methods

2.1. Location

The study was performed between the months of May and July of 2010 on Tuj ewes at the Kafkas University Veterinary Faculty Research and Application Farm in Kars province, which is located at 40.5° north latitude and 43° east longitude.

2.2. Animals

Sixty multiparous Tuj ewes aged three to six years old with a body condition score (Thompson and Meyer, 1994) of between 2 and 3 and 6 Tuj rams aged four to five years old were used in the study. The animals were grazed freely in a pasture during the day. The animals were given approximately 300 gr of concentrate feed per day and water ad libitum. The groups were selected such that the condition scores and age distribution were even. The ewes in each group were marked with different colors.

This study was approved by Kafkas University, Animal Local Ethics Committee (KAU-HADYEK; 2010/12).

2.3. Experimental groups

2.3.1. Group I (n = 15)

Seven days before the animals in this group were administered a vaginal sponge (20 mg Flugestone acetate, Chronogest® CR, MSD, Turkey) (day 7), they were given intramuscular (IM) an injection of GnRH (0.0084 mg Buserelin acetate, Receptal®, MSD, Turkey). The sponge was administered intravaginally (on day 0) and left in the vagina for 14 days. Seven days after the sponge was administered, the ewes were given a second injection of GnRH. The sponge was removed on the 14th day and immediately afterward an IM injection of PGF2α (7.5 mg/per animal, Dinoprostone, Dinolytic®, Pfizer, Turkey) was administered. On the same day, rams were joined to this group, enabling them to mate with ewes in estrus.

2.3.2. Group II (n = 15)

Seven days before a vaginal sponge was administered to the animals in this group, they were given an IM injection of 1000 IU hCG (Chorulon®, MSD, Turkey). Seven days after the injection, a sponge containing FGA 7 (7.5 mg/per animal, Dinoprostone, Dinolytic®, Pfizer, Turkey) was placed in the vagina. Seven days after the sponge was administered, a second IM injection of hCG was given. Other applications were same as in GI.

2.3.3. Group III (n = 15)

The ewes in this group were administered an intravaginal sponge for a period of 14 days. On day 14, the sponge was removed and the other applications were same as in the other treatment groups.

2.3.4. Group IV (n = 15)

The ewes in this group were set aside as a control group and no hormones were administered to them. On the same days as the other three groups (21 days after the first hormone treatment), teaser rams were joined to ensure that the ewes in estrus mated. Study design in groups is shown in Fig. 1.

In order to determine progesterone levels, blood was taken from the jugular vein of all animals using EDTA 10 ml tubes on days −7, 0, 7 and 14 and during the first, second and third estrus periods.

2.4. Diagnosis of pregnancy

To determine pregnancy status of ewes that mated with fertile rams, transrectal ultrasonography was performed using a Pie Medikal 100 Falko

Fig. 1. Study design in experimental groups. B: blood samples.
Vet. Equipment with a linear (6.0–8.0 MHz) probe on days 29–31 after mating.

### 2.5. Progesterone analysis

Progesterone in the blood samples was tested with the radioimmunoassay (RIA) method using commercial kits (Immunotech®, France). The intra-assay and inter-assay coefficients for the kits were 6.5% and 7.2% respectively.

### 2.6. Evaluation of fertility parameters

The ewes were observed for symptoms of estrus for the 7 days after joining the rams. The ear number and the date and time that estrus was observed were recorded for each ewe. Ewes that did not exhibit symptoms of estrus were monitored for two cycles. The fertility parameters, calculated following treatment, were: estrus occurrence rate (number of ewes exhibiting estrus/total number of ewes in the group × 100), first estrus pregnancy rate (number of pregnant ewes/number of ewes mated in each group × 100), total pregnancy rate (pregnancy ratios were calculated separately for three cycles and then the total pregnancy ratio was determined for each group), lambing rate (number of ewes that lambed/number of pregnant ewes × 100) and lamb weight (the lamb was weighed on the day it was born – within the first 24 h) (Karaca et al., 2009; Zarkawi et al., 1999, 1999b; Zonturlu et al., 2011).

### 2.7. Statistical analysis

Statistical analysis of the data obtained in the study was performed using the SPSS 16.0 statistical program. Differences between the groups were calculated with the ANOVA test. Pregnancy and lambing rates in the groups were statistically evaluated using the chi-squared test.

### 3. Results

Estrus symptoms following ram joining began the earliest in the control group. Ewes in this group were found to begin exhibiting estrus symptoms in an average of 16.7 ± 3.2 h. The average estrus start time was found to be 54.8, 50.3 and 59.6 h in treatment Group I, Group II and Group III, respectively. The statistical evaluation revealed a significant difference between Group III and Group IV with regard to the start time of estrus symptoms (P < 0.05). No statistically significant difference was identified between Group I, Group II and Group IV (P > 0.05).

The estrus occurrence rates, pregnancy rates and lambing rates for the study and control groups have been shown in Table 1. No statistically significant difference was identified between the study groups (Groups I, II and III) in an evaluation of the estrus rates, but all of the study groups were found to be statistically higher than the control group (P < 0.05). We determined that the pregnancy rate at first estrus was higher in the group that was administered hCG (Group II) than in the other three groups (P < 0.05).

The total pregnancy rates were found to be statistically higher in II than in all the other groups (P < 0.01; P < 0.05). A statistically significant difference was also found between the other study groups and the control group with regard to total pregnancy rates (P < 0.01). Similarly, when all the ewes were evaluated, the lambing rate was found to be statistically higher in II than both the other study groups and the control group (P < 0.01) Table 1.

Progesterone levels increased in the study groups until day 7. Furthermore, it was determined that the progesterone levels in the ewes in Group II were significantly higher than the control group (P < 0.01) (Fig. 2). Lamb weights were determined by weighing the lambs in the first 24 h following birth. The results revealed that there was no statistically significant difference between the male and female lambs with regard to weight (P > 0.05; Table 2). There was also no significant difference found between the groups with regard to average lamb weights (P > 0.05).

### Table 1

<table>
<thead>
<tr>
<th>Parameters</th>
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<tbody>
<tr>
<td></td>
<td>Group I</td>
</tr>
<tr>
<td>Number of ewes (n)</td>
<td>15</td>
</tr>
<tr>
<td>First estrus response (%)</td>
<td>66.6 a (10/15)</td>
</tr>
<tr>
<td>Pregnancy rate at first estrus (%)</td>
<td>6.6 a (1/15)</td>
</tr>
<tr>
<td>Ram joining-start of estrus (h)</td>
<td>54.8 a,b</td>
</tr>
<tr>
<td>Total pregnancy rate (%)</td>
<td>66.6 a (10/15)</td>
</tr>
<tr>
<td>Lambing rate (%)</td>
<td>66.6 a (10/15)</td>
</tr>
<tr>
<td>Percentage of twins</td>
<td>0 a</td>
</tr>
</tbody>
</table>

Values with different superscripts (a, b) in the same row are significantly significant. First estrus response: a,b P < 0.05; Pregnancy rate at first estrus: a,b P < 0.05; Total pregnancy rate: a,b P < 0.05; Lambing rate: a,b P < 0.05; a,a,b,c P < 0.01.

### Table 2

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<td>Average lamb weight</td>
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<tr>
<td></td>
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</tr>
<tr>
<td>Number of ewes (n)</td>
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<tr>
<td>First estrus response (%)</td>
<td>3.9 ± 0.5</td>
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<tr>
<td>Pregnancy rate at first estrus (%)</td>
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<tr>
<td>Rams joining-start of estrus (h)</td>
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Fig. 2. The mean concentration of plasma progesterone (P4) in the groups.
4. Discussion

It has been reported that the pregnancy rate in ewes administered progestagen treatment during the anestrous period is 22–70% (Karagiannidis et al., 2001). This difference depends on breed, diet, whether the ewe is lactating, which period of the anestru the animal is in when the treatment starts, the treatment protocol and the fertility of the ram (Echternkamp et al., 1976; Hulet and Stormshak, 1972; Karagiannidis et al., 2001; Moenei et al., 2007).

In a study carried out by Bekyurek (1993), on Tuj ewes in the anestrous period, vaginal sponges containing MAP were administered to one group. Vaginal sponges were administered to the first and second groups for 14 days. On the day the sponges were removed, an intramuscular injection of 500 IU PMSG was administered in the second group only. No treatment was given to the ewes in the third group, which was kept as a control group. The estrus occurrence rates were 60% in the first group and 80% in the second group, but no sexual activity was identified in the control group (0%). The duration of estrus was determined to be 61 ± 19 and 42 ± 13 h in Group I and Group II. The study identified pregnancy rates of 30% and 70%, respectively, in the groups that received treatment, but no pregnancies were observed in the control group.

The results obtained in this study were similar to those of the aforementioned study. In our study, first estrus occurrence rates varied from 60% to 73.3% in the study groups, while pregnancy rates varied from 66.6% to 100% after combination therapies. These results indicate that estrus occurrence and pregnancy rates will vary depending on the method that is used.

Similarly, it was observed that the estrus start time after treatment varied depending on the treatment that was performed for estrus synchronization during the anestrous period. It has been reported that for Tuj ewes during the non-breeding season, the time from the conclusion of treatment in ewes which are given CIDR alone or CIDR and PMSG on the day the CIDR is removed until the time that estrus symptoms begin varies from 34.5 h to 74 h (Gungor et al., 2007, 2009; Kacar et al., 2008). In our study, we determined that the time until the onset of estrus symptoms varied from 50.3 h to 59 h in the different study groups. In the present study exhibited signs of estrus at 54.8 (G1), 50.3 (GII) and 59.6 (GIII) h, while it was observed in the control group at 16.7 h. We must note that the fact that the first estrus occurrence rate in the control group was 20%, in other words that we could only evaluate the results for 3 out of 15 ewes, is what caused this result.

In our study, although the estrus rate in Tuj ewes that received GnRH was close to the rate of the group that received hCG, the pregnancy rate after first estrus obtained in ewes in this group was 6.6% less than in the group that was administered hCG (46.6%) (P < 0.01). It has been reported that administration of hCG and GnRH has different effects on the vitality of the embryo, and that better results are obtained with hCG (Cam and Kuran, 2004).

Because secretion of gonadotropin is insufficient during the periovulatory phase during the non-breeding season, fully functional CL does not occur and progesterone secretion is insufficient (Spencer et al., 2004). For this reason, alternative treatment methods have been developed in which treatment with hCG is administered in order to increase the pregnancy rate (Gomez-Brunet et al., 2007). Human chorionic gonadotropin injection has a similar effect to the luteinizing hormone, helps the oocyte to mature and stimulates ovulation (Schmitt et al., 1996), causes the conceptus to grow and leads to significant increases in placentation and the number of lambs born (Khan et al., 2003). In our study, the administration of hCG and sponges in Tuj ewes increased the total pregnancy and lambing rate to 100%, which supports the aforementioned findings. The result was determined to be statistically different from both sponge combined with GnRH and the control group (P < 0.01 and P < 0.05). We think that the administration of hCG supported the luteal structure, thus causing an increase in the pregnancy rate. We base this on reports that the increase in pregnancy rates is related to the fact that hCG enhances progesterone production and increases uterine secretions that are embryotrophic (Nephew et al., 1994; Cam and Kuran, 2004). This view is supported by the fact that the P4 levels increased significantly more on day 7 in Group II than in the other study groups, even though the increase was not statistically significant, and that the levels were significantly higher in Group II than in the control group (P < 0.01).

Other studies have shown that progesterone levels are significantly higher than the control group in groups that are administered hCG on days 11, 12 and 13 after mating subsequent to administer progesterone (Kittok et al., 1983). These results lead to the conclusion that human chorionic gonadotropin stimulates ovulation in this process, thus causing an increase in progesterone levels.

We identified fetal deaths in three of pregnant ewes in Group III and one ewe in the control group. It has been found that GnRH has a positive effect on the vitality of the embryo by increasing and regulating LH stimulation (Cam et al., 2002) and that in a similar fashion, administration of hCG causes the same effect by increasing progesterone levels because it causes the accessory corpus lutea to form (Beck et al., 1998; Nephew et al., 1994). Furthermore, studies performed with color Doppler have found that administering hCG increases the blood flow in the corpus luteum significantly more than GnRH (Aslan et al., 2011), which is an indication of the reason that the group receiving hCG had much higher rates of continued pregnancy and lambing than all the other ewes.

It is thought that hCG treatment results in the development of a stronger conceptus, more regular and higher secretion of IFN-tau, and suppression of the luteolytic mechanism, which increases the lambing rate because the lamb develops better (Nephew et al., 1994; Spencer et al., 1996). The increase in the number of placentomes forming after hCG treatment proves this fact (Khan et al., 2003).

It was determined that total pregnancy rates after administering gonadotropin releasing hormone together with vaginal sponges were the same as in the group that was administered only a vaginal sponge. No statistically significant difference was found with regard to lambing rates either (Fukui et al., 1985, 1991; Kridli et al., 2003). Certain publications have reported no increase in P4 serum/plasma levels after administering GnRH and even
that drops have been observed (Lucy and Stevenson, 1986; Ryan et al., 1991; Naohisa et al., 1999). In this study, we did not observe any increase in P4 serum levels due to administering GnRH either.

Similar to what was found by Saharra et al. (1998), our study also shows that the GnRH has a lower effect on fertility in ewes than hCG. It has been reported that hCG helps the blastocyst to develop and down regulates estradiol and oxytocin, thus strongly suppressing the secretion of PGF$_{2\alpha}$ and causing the blastocyst to secrete more IFN-$\tau$ (Nephew et al., 1994). This may explain why the administration of a vaginal sponge and hCG provided better fertility results than other groups during the non-breeding season.

Lashari and Tasawar (2010), gave PGF$_{2\alpha}$ injections to ewes during the breeding season at 11 day intervals and then mated the ewes that showed signs of estrus; on the day they were mated, half of these ewes were given an injection of GnRH in an attempt to identify the effect on lamb weight. It was determined that the birth weights of lambs in the group that were given GnRH injections were higher than the control group. In contrast, Cam et al. (2002), reported that the GnRH injection (4 µg) gave 12 days after ewes were mated during the breeding season did not affect the weight of the newborn lambs. In our study, the fact that no difference was identified between the groups with regard to birth weights supports the view that the treatments that were administered had no effect on the lamb weights.

5. Conclusions

We concluded in this study that administering hCG together with vaginal sponges to Tuj ewes during the non-breeding season increased the pregnancy and lambing rates.

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References


