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Effects of Melatonin Implants on Hormone Profile and Mating Behaviour in Rams

ABSTRACT

Objective: This study investigated the effects of melatonin implants on the mating behaviours and hormone concentrations of melatonin (MEL), GnRH and testosterone (TES) before the breeding season were investigated in Tahirova rams.

Materials and Methods: Six rams were used for hormone analysis. For the sexual behaviour test, six rams were mated with 69 ewes. The rams' sexual behaviour was tested for 10 minutes. Half of the rams and ewes were implanted with 18 mg melatonin, three pellets in rams and one pellet in females. MEL, GnRH, and TES hormone analyses were performed by the ELISA method from the blood serum of the rams before implanting (Day 0) and 21, 42, 63, 94 and 129 days after implant. **Results:** Results showed that melatonin implantation significantly reduced MEL hormone concentration in Tahirova rams (P≤0.05). MEL concentration decreased to its lowest concentration on Day 63 in the treatment (T) group and differed significantly from the control (C) group (P≤0.05). While GnRH and TES hormone concentrations were similar between the groups, TES hormone concentrations in the C group differed according to the observation days (P≤0.05). Ano-genital sniffing, foreleg kicking, and tongue flicks behaviours were observed at a higher frequency in the T group than the C group (P≤0.05). While rams in group T had 64.8% mating efficiency, rams in group C had 86.9% mating efficiency (P≤0.05).

Conclusion: The administration of subcutaneous melatonin implants before the breeding season in Tahirova rams, which induced a significant decrease in the MEL hormone concentration, is important in increasing sexual behaviours.

Keywords: Tahirova sheep, GnRH, testosterone, negative response, sexual behaviour, mating efficiency

Koçlarda Melatonin Implantının Hormon Profili ve Asım Davranışlarına Etkisi

ÖZ

Amaç: Bu çalışmada Tahirova koçlarında melatonin implantlarının çiftleşme davranışları üzerindeki etkileri ve üreme sezonu öncesi melatonin (MEL), GnRH ve testosteron (TES) hormon konsantrasyonları araştırılmıştır.

Materyal ve Methot: Hormon analizi için 6 baş koç kullanılmıştır. Seksüel davranış testi için 6 koç 69 koyunla çiftleştirilmiştir. Koçların seksüel davranışları 10 dk. boyunca test edilmiştir. Koç ve koyunların yarısına 18 mg melatonin implantı olmak üzere, koçlara üç pellet, dişilere ise bir pellet olarak yerleştirilmiştir. Koçların implantasyon öncesi (0. gün) ve implantasyon sonrası 21., 42., 63., 94. ve 129. günlerde kan serumlarından ELISA yöntemiyle MEL, GnRH ve TES hormon analizleri yapılmıştır.

Bulgular: Bulgular melatonin implantasyonunun Tahirova koçlarında MEL hormon konsantrasyonunu önemli ölçüde azalttığını göstermiştir (P≤0.05). MEL konsantrasyonu, uygulama (T) grubunda 63. günde en düşük konsantrasyonuna düşmüş ve kontrol (C) grubundan önemli ölçüde farklılaşmıştır (P≤0.05). Gruplar arasında GnRH ve TES hormon konsantrasyonları benzer iken, C grubunda TES hormon konsantrasyonları gözlem günlerine göre farklılık göstermiştir (P≤0.05). Ano-genital koklama, ön ayak vurma ve dil çıkarma davranışları T grubunda C grubuna göre daha yüksek sıklıkta gözlenmiştir (P≤0.05). Aşım etkinliği T grubundaki koçlarda %64,8 iken, C grubundaki koçlarda %86,9 olmuştur (P≤0,05).

Sonuç: Tahirova ırkı koçlarda aşım sezonu öncesinde deri altı melatonin implantı uygulaması, MEL hormonu konsantrasyonunda önemli bir azalmaya neden olurken, seksüel davranışları da teşvik etmiştir.

Anahtar Kelimeler: Tahirova koyunu, GnRH, testosteron, negatif yanıt, seksüel davranış, aşım etkinliği

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INTRODUCTION

The "ram effect" is one of the most important factors in stimulating the extra seasonal oestrus cycle in sheep exhibiting seasonal oestrus. The effectiveness of the ram effect depends on the sexual activity or behaviour of male animals, their smell, sight, and voice (Rosa and Bryant, 2002; Ungerfeld et al. 2020). The ram effect is effective in the occurrence of oestrus in females the increase of libido in males, and inter-sex interactions (Rosa and Bryant, 2002). It is reported that strong and intense sexual behaviour in rams stimulates the oestrus cycle and increases fertility in sheep (Perkins and Fitzgerald, 1994; Stellflug et al. 2006; Kleemann et al. 2014; Calderon-Leyva et al. 2018).

Rams to be used in oestrus cycle synchronized sheep should be primed for breeding by increasing their libido. One of the most important methods practiced in males is the light management method (Chemineau et al. 1988; Abecia et al. 2017). The basic goal of light management is to increase melatonin concentrations by increasing the dark period during the diurnal cycle. It is well known that the release of melatonin increases in the dark. The use of the exogenous melatonin application is based on imitating melatonin release during short days. The stimulation of GnRH in males via melatonin and the resultant release of testosterone, FSH and LH, promotes sperm production and increased libido (Lincoln and Clarke 1997; Casao et al. 2008).

The 18 mg melatonin-containing implants used to induce oestrus in off-season sheep are reported to secrete the appropriate hormone for between 40 and 70 days, and this effect can last up to 120 days (Forcada et al. 2002; Abecia et al. 2007). It is shown that three pellets of melatonin implants applied in rams begin to show their effect after 36-45 days. While examining the effect of melatonin implants on the reproductive hormones in male animals, studies have mainly focused on testosterone concentrations and changes (Kaya et al. 2000; Faigl et al. 2009; Abecia et al. 2017). There are also studies examining the changes in melatonin concentrations (Rosa et al. 2000; Casao et al. 2013; Egerszegi et al. 2014). The melatonin implants have been reported to increase melatonin and testosterone concentrations in male animals (Casao et al. 2013).

The milk yield and lamb yield of Tahirova sheep breed, which is obtained as a result of crossing the Turkish native sheep breed Kıvırcık and East Friesian dairy sheep, is good performance levels (Tölü and Yazgan, 2022; Tölü et al. 2022). There is not enough information about the breeding season and off-season oestrus synchronization and performance of Tahirova sheep. While melatonin application is related to females in Turkey, there is no regulation in the breeding season or non-breeding season with rams. While ewes can be sexually stimulated by exogenous hormone applications, flushing and ram effect, rams to mate with females in the herd may be insufficient in terms of sexual hormones and sexual activity. Administration of subcutaneous melatonin implants has already been determined to increase the frequency of sexual behaviours in males and females (Abecia et al. 2018; Calderon-Leyva et al. 2018; Kleemann et al. 2021). However, there reports indicate no effects of the melatonin implants on sexual behaviours (Rosa et al. 2000). This study investigated the effects of pre-breeding season melatonin implantation on mating behaviours, and the melatonin, GnRH and testosterone concentrations in rams.

MATERIAL and METHODS

Animals and management

The study was carried out in the Animal Production Research Unit (40° 07’ N, 26° 26’ E) of the Faculty of Agriculture at Çanakkale Onsekiz Mart University, Turkey. The Tahirova breed used in the study was developed by crossing East Friesian sheep with native Kıvırcık sheep. Ewes and rams were housed separately in semi-open barns throughout the year and during summer were housed outside in shaded areas. Hand-mating was carried out in the flocks at the end of August and September.

In the study, ewes only participated in mating and no findings were made regarding ewes. The experiment was set up with the treatment group (T) having melatonin implantation and the control group (C) being non-treated. On June 26, half of the rams and ewes were implanted with melatonin (Regulin ®). Three pellets in males and one pellet in females were applied (Kleemann et al., 2014). Thirty-six days after the melatonin implantation, an aproned (a teaser) ram was placed in the flock of ewes to detect the oestrus cycle. The ewes found to be in oestrus were mated with their designated group, and mating was carried out for 8 to 12 hours.

Experimental design

In the study, ewes only participated in mating and no findings were made regarding ewes. The experiment was set up with the treatment group (T) having melatonin implantation and the control group (C) being non-treated. On June 26, half of the rams and ewes were implanted with melatonin (Regulin ®). Three pellets in males and one pellet in females were applied (Kleemann et al., 2014). Thirty-six days after the melatonin implantation, an aproned ram was placed in the flock of ewes to detect the oestrus cycle. The ewes found to be in oestrus were mated with their designated group, and mating was carried out for 8 to 12 hours.

Tests and recordings

Recordings of the mating of each ram were made for 10 min. in a 2.5 x 4.0-m pen. The mating test of each sheep was done once. The frequency and duration of the courtship and mating behaviours were recorded using direct observation and video recordings. Ano-genital sniffing, foreleg kicks, tongue flicks, vocalizations, lateral approaches, mount frequency (without ejaculation), and mating frequency (with ejaculation) and their duration were recorded as indices of sexual behaviour. The total number of the mentioned behaviours (ano-genital sniffings + foreleg kicks + tongue flicks + vocalizations + lateral approaches), and the total number of mounts (mounts + matings) and the ratio of the mating/total number of mounts (mating efficiency) were calculated (Ungerfeld et al. 2020).

Hormone measurements

Blood was collected (08:00-12:00 am in the morning) by jugular venepuncture before the melatonin implantation (Day 0) and 21, 42, 63, 94, and 129 days after the implantation. The blood samples were centrifuged at 3500 rpm for 10 minutes. Blood serum was transferred to sterile storage tubes and kept in a deep freezer at -20 ºC until hormone analysis. Melatonin (MEL), GnRH, and testosterone (TES) analyses were performed by the enzyme-linked immunosorbent assay (ELISA) method. A Thermo Scientific Multiskan FC Microplate Photometer was used for analysis. Hormone analyses of MEL, GnRH and TES were performed with sheep specific commercial hormone kits. The kit (Shanghai Sunred Biological Technology Co.) uses a double-antibody sandwich enzyme-linked immunosorbent assay. The sensitivity of the melatonin, GnRH and testosterone hormone was 2.631 ng/L, 0.036 ng/ml and 0.512 nmol/L, respectively.

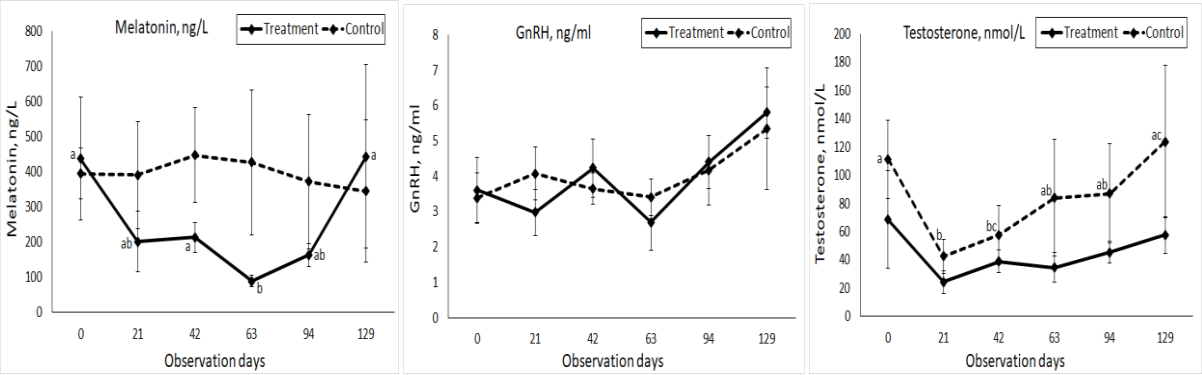
Statistical Analysis

Repeated measurements variance analysis was performed after applying logarithmic transformation (LOG10(y+10)) to the mating behaviours. The model included treatment group (T, C) and female age (1,…4) as the animal's fixed effects and random effects. Melatonin, GnRH and testosterone data were analysed by Kruskal–Wallis test. While each hormone analysis was carried out on a group (T, C) basis, within-group analyses between observation days were also made. All analyses were made in the SAS (1999) statistical software package.

RESULTS

Hormone analysis

Melatonin implantation before the breeding season caused a significant decrease in MEL in the Tahirova rams (Fig. 1). MEL concentration showed a different trend in the T group than in the C group and decreased until 63 days after implantation. MEL concentrations between the T and C groups on Day 63 differed significantly ($P \leq 0.05$). After the 63rd day, MEL in the T group increased and reached its initial value on the 129th day. MEL concentrations in group C were similar from the first day to Day 129.



a-c: Difference between hormone means on observation days within group is significant, P≤0.05.

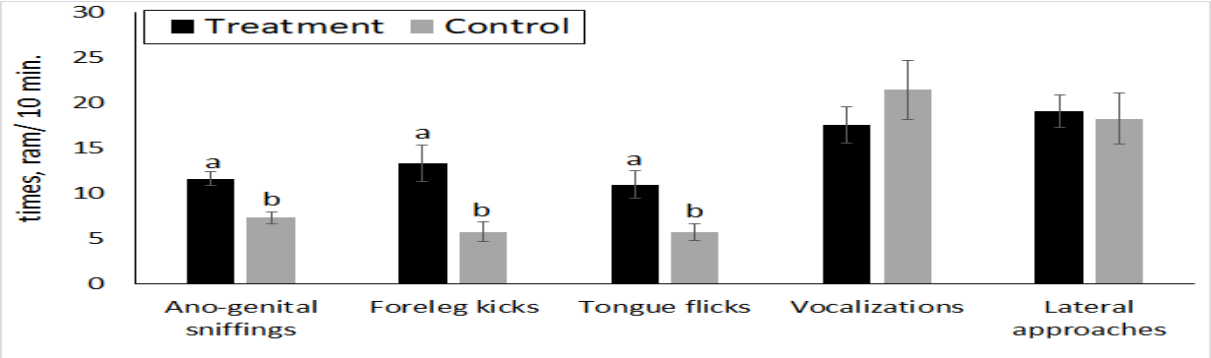
Figure 1. Means and standard errors of MEL, GnRH and TES hormone concentrations according to observation days by treatment and control groups in Tahirova rams

Şekil 1. Tahirova koçlarında uygulama ve kontrol gruplarında gözlem günlerine göre MEL, GnRH ve TES hormon konsantrasyonlarının ortalamaları ve standart hataları

GnRH concentrations were similar in both groups (Fig. 1; P>0.05). While the TES concentrations were similar in both groups, the changes in TES values between the observation days of the C group were significant (P≤0.05). TES decreased significantly on the 21st post-implantation day in group C and increased significantly afterwards. In the T group, the trend of TES was similar with a slight increase until Day 63, after a decrease on Day 21, increasing slightly more on days 94 and 129 (P>0.05).

Courtship and mating behaviours

The frequency of the mating behaviour traits of the T-group rams was higher (except vocalizations) than the C-group rams (Fig. 2). The frequency of ano-genital sniffing behaviour, foreleg kicks and tongue licking behaviours differed significantly between groups (P≤0.05). Only in tongue licking behaviour was the ram x ewe groups interaction significant (P=0.0481; data not shown).

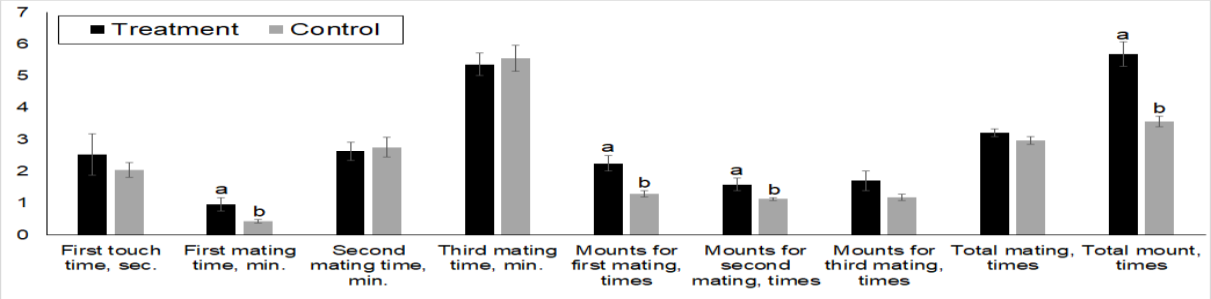


a-b: Difference between group means in each behaviour is significant, P≤0.05.

Figure 2. Means and standard errors of frequency of mating behaviours (times, ram/10 min.) by group in Tahirova rams

Şekil 2. Tahirova koçlarında gruplara göre aşım davranışlarının sıklığına ilişkin ortalamalar ve standart hatalar

Rams were observed to contact the ewes immediately after release into the test pen (Fig. 3). Group C rams performed the first mating in a significantly shorter time than group T rams (P≤0.05). While the second and third matings were similar in both groups, the T group performed more frequent mounting behaviour for the first and second mating than the C group (P≤0.05). Also, total mounting behaviour frequency of T group was higher than C group (P≤0.05). Although the total mounting behaviour frequency was higher in the T group, the overall mating frequency of the two groups was similar (P>0.05).

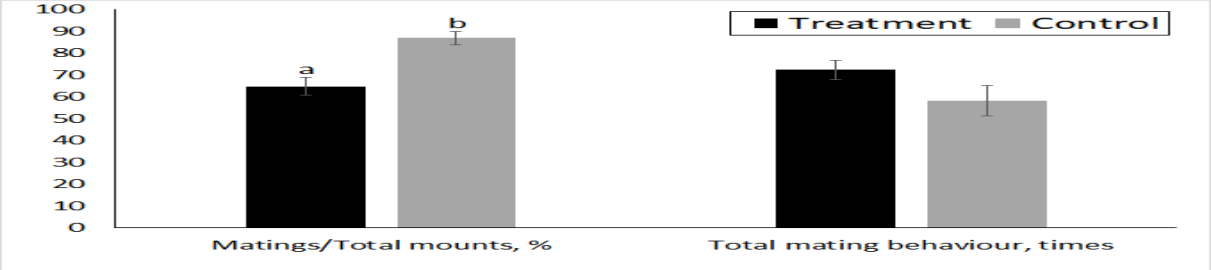


a-b: Difference between group means in each behaviour is significant, P≤0.05.

Figure 3. Means and standard errors of mating frequency and duration by group in Tahirova rams

Şekil 3. Tahirova koçlarında gruplara göre aşım sıklığı ve aşım süresine ilişkin ortalamalar ve standart hatalar

Rams in group T exhibited slightly higher total mating behaviour than rams in group C (Fig. 4; P>0.05). However, the mating efficiency of rams in group C was higher than rams in group T (P≤0.05). A mating efficiency of 64.8% for rams in the T group and 86.9% in the C group was determined.



a-b: Difference between group means in each behaviour is significant, P≤0.05.

Figure 4. Means and standard errors of mating efficiency (total mating / total mounts x 100) and total mating behaviour frequency by group in Tahirova rams

Şekil 4. Tahirova koçlarında aşım etkinliğine (toplam çiftleşme / toplam biniş x 100) ve gruplara göre toplam aşım davranışı sıklığına ilişkin ortalamalar ve standart hatalar

DISCUSSION

Melatonin implantation before the breeding season significantly reduced the MEL concentration in Tahirova rams. These findings in the present study differ from previous studies. It is known that the melatonin implant in rams significantly increases the MEL concentration (Rosa et al. 2000; Casao et al. 2013; Rekik et al. 2015; Pool et al. 2020). It can be said that the 54 mg subcutaneous melatonin implants have a negative feedback effect on the serum melatonin hormone concentration in the treatment ram group. In the pre-study period, the rams were housed in a closed shelter with 1/20 window area. When the trial began, rams were housed in the shade of trees in the garden of the sheepfold. Rams were housed in the same area throughout the trial period. Therefore, the decrease in melatonin hormone level may result from the different interaction of the natural dark period and the melatonin implant. However, the reduction in melatonin concentrations in the present study was unexpected and this issue awaits further exploration. Melatonin hormone concentrations remained significantly stable in the dark period of the day in sheep treated with melatonin implants compared to the control group (Kennaway et al. 1983). It is seen that the prolonged secretion of melatonin implant in the present study maintains its negative effect for a long time in the rams in the treatment ram group. The lowest MEL value in the T group was determined on Day 63, when the secretion from the implants reached its expected highest concentration (Forcada et al. 2002; Abecia et al. 2007).

Therefore, it can be said that the rams used in the study had satisfactory concentrations of melatonin hormone before the breeding season. Moreover, it may be stated that the melatonin hormone implant performed in Tahirova rams on these dates may have an adverse effect on the MEL concentration. In addition, the value of 88.48 ng/L of MEL measured on the 63rd day in the rams in the study was lower than in similar studies (Rosa et al. 2000; Egerszegi et al. 2014). However, it is known that melatonin stimulates the release of GnRH (Lincoln and Clarke 1997; Casao et al. 2008), but it should be noted that the decrease in MEL in the T group did not affect the GnRH concentration (Fig. 1).

TES concentrations decreased after the initial measurements in both groups. TES values on Day 0 in group C were lower than on days 21 and 42 (Fig. 1). One of the reasons for the decrease in TES of T group may be the decrease in MEL. However, the decrease in group C cannot be explained with this justification. The rise in TES concentration after the 21st day may have been due to the rams' contact with ewes 36 days after post-implantation. It has been determined that the concentration of TES increases in rams with melatonin implants (Casao et al. 2013; Pool et al. 2020). TES concentrations in the T and C groups were similar in the present study. In Konya Merino rams, while the TES concentrations on days 0-70 were similar in the groups during the breeding season, higher TES was detected out of the breeding season in the melatonin group, than in the control group the 70th day (Kaya et al. 2000). TES concentration in Poll Dorset rams was significantly higher in the melatonin administration group than in the control group at 6 and 30 weeks (Pool et al. 2020). TES concentrations of melatonin treated group differed from the control group and increased at 75 and 90 days after implantation in Rasa Aragonesa rams (Abecia et al. 2018).

In the present study, rams in the T group exhibited mating behaviours at a higher frequency (except for vocalizations) than throse in the C group (Fig. 2). While the mating behaviour of Border Leicester rams with melatonin implants was similar to those in Lucindale, the latency to the nose, nosing, courting and mounting behaviours of rams in the Turretfield region were significantly higher in the treatment group than in the control group (Kleemann et al. 2021). In Rasa Aragonesa rams, melatonin implants significantly increased the frequency of nudging, ano-genital sniffing and self-urination (Abecia et al. 2018). Also, lateral approaches, mount attempts, and total activity times were significantly higher in the melatonin implanted group in Barbarine rams (Rekik et al. 2015).

It can be said that melatonin and other exogenous hormone administrations positively effect on the frequency of sexual behaviours and sexual activity in rams. Likewise, it was determined that ano-genital sniffing, total inspective behaviour, and the number of matings and mating efficiency were higher in the treatment group than in the control group in Saint Croix rams (Ungerfeld et al. 2020). Similarly, in our study, the rams in T group performed more mating behaviours and a higher frequency of mounting behaviours. However, the total number of ejaculations in the groups was similar. On the other hand, rams in the T group performed more mounting behaviours overall. For this reason, the mating efficiency was higher in C group than T group. This may be due to the individual mating abilities of the rams in the treatment group. And the lack of energy and skills required for overcoming may have occurred due to the excessive frequency of overdoing behaviour. However, it should be noted that the mating efficiency or a number of matings in our study was at a sufficient concentration (Abecia et al. 2017; Ungerfeld et al. 2020).

The sexual behaviour of the male animal can, of course, also be affected by the behaviour of females in the display of mating behaviour. In our study, only tongue licking behaviour was significant compared to the ram x ewe subgroups. Also, the rams in the T group showed higher mating behaviour frequencies when they were matched with the ewes from both groups. The lowest tongue licking behaviour was observed in group C with treated ewes (4.22 times ram / 10 min. test; data not shown); this differed significantly from the treated ram x treated ewe groups (13.77 times ram / 10 min. test; data not shown). Therefore, it can be said that the frequency of mating behaviour was higher in the T group (except the treated ram x control ewe group), and the melatonin implant had a reinforcing effect on the frequency of sexual behaviours in Tahirova rams.

CONCLUSIONS

Melatonin implantation before the breeding season induced a significant decrease in the melatonin hormone concentration in Tahirova rams. The fact that the lowest concentration of melatonin hormone was at 63 days when the hormone release of the implant was expected to be at the highest concentration suggests that the implant had an adverse effect on the melatonin release. While there was no significant change in GnRH concentrations, the testosterone hormone increased significantly, especially in group C on Day 21, and probably increased after contact with females. As a result of all these, it may be recommended to repeat the study with more rams of the same breed in the same region, especially in terms of hormone results.

Melatonin implants significantly increased the frequency of mating behaviours during the 10-minute mating test in Tahirova rams. However, rams in group T with increased frequency of mating behaviours and

mounts performed their first mating later than the rams in group C. Also, although the rams in the T group had more mounts, the amount of mating was similar between the groups.

In conclusions, while implant administration induced a significant decrease in the melatonin hormone concentration, it promoted courtship behaviours.

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Data availability: Data will be made available upon reasonable request.

Author contributions: All authors contributed equally to the preparation of the article.

Competing interests.: There is no conflict of interest between the authors in this study

Ethical statement: All animal handling and experimental procedures were performed in accordance with the Committee on Animal Research and Ethics of Çanakkale Onsekiz Mart University (Turkey) on animal use (no. 2018/12-13).

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