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Research Article

The Effect of Subcutaneously Administered Melatonin Crystal's Oily Solution on Estrus and Conception Rates in Ewes

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ABSTRACT

The study aims to evaluate the effects of subcutaneously (SC) injected oily solution of melatonin crystal at different doses in ewes during the anoestrus period on serum melatonin and prolactin levels, mating, conception, and pregnancy rates. A total of 350 ewes were used in the study. Ewes were divided into 5 different groups as MEL10 (n=70, 10 mg melatonin), MEL20 (n=70, 20 mg melatonin), MEL40 (n=70, 40 mg melatonin), placebo (n=70), and control (n=70) groups. The melatonin groups received SC injections of oily melatonin solution 11 days before ram introduce. Only oily solution was used in the placebo group. No treatment was applied to the control group. Blood samples were collected from all groups before and 11 days after melatonin injection. It was found that melatonin levels in blood samples collected before and after melatonin injection increased in MEL10 ($P<0.05$) and MEL20 ($P>0.05$) groups. In addition, prolactin levels were lower in the second blood sample collected from all groups except MEL40 group ($P>0.05$). In terms of reproductive parameters, estrus rates in MEL10, MEL20, MEL40, placebo and control groups were 75.71%, 58.57%, 54.28%, 51.42% and 44.28%, respectively. Mating rates were 61.42%, 57.14%, 52.85%, 48.57% and 47.14% and pregnancy rates were 60%, 52.85%, 42.85%, 41.42% and 38.57%, respectively. Conception rates were 97.67%, 92.50%, 81.08%, 85.29% and 81.81% in MEL10, MEL20, MEL40, placebo and control groups, respectively. In conclusion, SC melatonin injection at appropriate doses in ewes may indirectly imitate the breeding season and stimulate the heat. SC injection of melatonin, especially at doses of 10-20 mg, is thought to be a potential alternative to melatonin implants or different synchronization methods in the future.

Keywords: *Conception, estrus, melatonin, pregnancy, sheep.*

Merinos Irkı Koyunlarda Subkutan Uygulanan Melatonin Kristalinin Yağlı Çözeltisinin Östrüs ve Konsepsiyon Oranları Üzerine Etkisi

ÖZET

Bu çalışma, anöstrus dönemindeki koyunlarda farklı dozlarda deri altı (SC) enjekte edilen yağlı melatonin kristali çözeltisinin serum melatonin ve prolaktin düzeyleri, çiftleşme, konsepsiyon ve gebelik oranları üzerindeki etkilerini değerlendirmeyi amaçlamaktadır. Çalışmada toplam 350 koyun kullanıldı. Koyunlar MEL10 (n=70, 10 mg melatonin), MEL20 (n=70, 20 mg melatonin), MEL40 (n=70, 40 mg melatonin), plasebo (n=70) ve kontrol (n=70) grupları olmak üzere 5 farklı gruba ayrıldı. Melatonin gruplarına koç katımından 11 gün önce yağlı melatonin solüsyonunun SC enjeksiyonları yapıldı. Plasebo grubunda sadece yağlı solüsyon kullanıldı. Kontrol grubuna herhangi bir tedavi uygulanmadı. Melatonin enjeksiyonundan önce ve enjeksiyondan sonraki 11. günde tüm gruplardan kan örnekleri toplandı. Melatonin enjeksiyonundan önce ve sonra toplanan kan örneklerindeki melatonin seviyelerinin MEL10 ($P<0,05$) ve MEL20 ($P>0,05$) gruplarında arttığı tespit edildi. Ayrıca, tüm gruplardan alınan ikinci kan örneğinde MEL40 grubu haricinde prolaktin seviyesi daha düşüktü ($P>0,05$). Üreme parametreleri açısından MEL10, MEL20, MEL40, plasebo ve kontrol gruplarında östrüs oranı sırasıyla %75,71, %58,57, %54,28, %51,42 ve %44,28 olarak tespit edildi. Çiftleşme oranı aynı gruplarda sırasıyla; %61,42, %57,14, %52,85, %48,57 ve %47,14 ve gebelik oranı %60, %52,85, %42,85, %41,42 ve %38,57 olarak belirlendi. Konsepsiyon oranı MEL10, MEL20, MEL40, plasebo ve kontrol gruplarında sırasıyla %97,67, %92,50 %81,08 %85,29 ve %81,81 olarak bulundu. Sonuç olarak, koyunlara uygun dozlarda SC melatonin enjeksiyonu, üreme mevsimini dolaylı olarak taklit edebilir, östrüsü uyarabilir. Melatoninin SC enjeksiyonunun, özellikle 10-20 mg dozlarda kullanılmasının, gelecekte melatonin implantlarına veya farklı senkronizasyon yöntemlerine potansiyel bir alternatif olacağı düşünülmektedir.

Anahtar kelimeler: *Konsepsiyon, östrüs, melatonin, gebelik, koyun.*

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Introduction

The breeding season for small ruminants in the northern hemisphere occurs during late summer, autumn, and early winter, coinciding with the shortening of daylight hours. Photoperiodic rhythm is regulated by the hormone melatonin secreted from the pineal gland (Kalkan and Horoz, 2010). The reproductive process is controlled by the hypothalamus, anterior pituitary, and ovarian axis, where the pineal gland and melatonin play an essential initiating role (Abecia et al., 2012). Melatonin stimulates the hypothalamus and initiates the secretion of gonadotropin-releasing hormone (GnRH) from the pituitary gland. The gonadotropin cells in the anterior pituitary gland produce follicle-stimulating hormone (FSH), which induces the development of ovarian follicles, and GnRH, which triggers the secretion of luteinizing hormone (LH) from mature Graafian follicles, leading to ovulation (Bartlewski et al., 2011).

Melatonin (5-methoxy-N-acetyltryptamine) is an indoleamine with a molecular weight of 232, secreted from pinealocytes in the pineal gland during the dark phase. It serves as the primary hormone released by the pineal gland (Liu et al., 2004). Generally, melatonin is synthesized from circulating tryptophan and converted into serotonin (Klein and Moore, 1979). The mRNAs encoding enzymes involved in melatonin synthesis are expressed in the pineal gland, regulating the day/night rhythm (Bernard et al., 1999). Due to its role in initiating changes in melatonin concentration, photoperiod length significantly impacts the seasonal and diurnal variations in prolactin (PRL) concentration (Morgan, 2000). An oscillator within the suprachiasmatic nucleus of the hypothalamus responds to the daily changes in PRL concentration (Misztal et al., 1997; Misztal et al., 2001).

Various methods such as the ram effect (Rosa and Bryant, 2002), flushing effect (Özbey and Tatlı, 2002), and artificial lighting (Du Preez et al., 2001), as well as medical treatment involving hormones such as melatonin (Abecia et al., 2007), progestagens, estrogens, PGF2α, equine chronionic gonadotropin (eCG), GnRH, and human chorionic gonadotropin (hCG) are used alone or in the combination for estrus synchronization in small ruminants (Liu et al., 2004; Salt et al., 2017; Rosasco et al., 2019; Skliarov et al., 2021). In veterinary medicine, melatonin is commonly administered as an implant. For sheep and goats, the implant is subcutaneously placed in the ear area. Dermatonin® (Melatek LLC, USA), Melovine® (Ceva, Canada), Ferretonin® (Melatek LLC, USA), and Regulin® (Ceva, Türkiye) are among the most frequently used in clinical practice (Saat et al., 2015).

Subcutaneous single-dose injection of melatonin at different doses has been studied in recent years. Depending on the dose, varying results can be obtained. Kumar and Purohit (2009) randomly administered subcutaneous (SC) melatonin injections to goats in January and May. These injections contained 20 mg and 40 mg of melatonin per animal. The percentage of goats that responded to melatonin treatments was 80% and 90% in the 20 mg and 40 mg treatments, respectively. However, the oestrus induction interval was significantly longer in the 20 mg treatment group compared to the 40 mg treatment group. No significant differences were observed in pregnancy rates. In another study, the effect of a single subcutaneous administration of melatonin on reproductive performance in Singharey goats during the non-breeding period was investigated. Different groups received a single SC injection containing 10 mg, 20 mg, and 40 mg melatonin doses per animal. As a result, it was concluded that a single subcutaneous injection of 10 mg of melatonin can initiate reproductive activity in Singharey goats (Singh et al., 2020). Abdelnaby et al. (2020) injected melatonin at doses of 25 and 50 mg intramuscularly in sheep and observed different rates of oestrus and pregnancy rates.

The study aimed to evaluate the effects of melatonin crystal's oily solution injected subcutaneously at different doses on serum melatonin and prolactin levels, mating, conception, and pregnancy rates during anoestrus in Merino ewes.

Materials and Methods

Ethical Approval

This study received ethical approval from Burdur Mehmet Akif Ersoy University Animal Experiments Local Ethics Committee under reference number 21-95/826.

Animal Material and Management

The animal material used in this study was obtained from a sheep farm located in the province of Burdur where intensive breeding of sheep (a total of 600 sheep) was conducted. The study groups were composed of 350 multiparous Merino ewes, with a body condition score between 2.5 and 2.75, in the 3rd month of lactation. Clinically healthy ewes with no abnormal uterine discharge were included in the study. The study was conducted between May and July.

Study Groups

The study groups consisted of melatonin (MEL), control and placebo groups. The MEL10, MEL20, and MEL40 groups were received 10 mg, 20 mg, and 40 mg of melatonin solution, respectively. In contrast, melatonin solution was not administered to the control group. The placebo group received a treatment that did not contain the active drug (melatonin). The groups were categorized as follows: MEL10 (n=70), MEL20 (n=70), MEL40 (n=70), placebo (n=70), and control (n=70), based on the respective doses of melatonin administration.

Preparation of Melatonin Injectable Solution

For the preparation of the melatonin solution, 98% pure melatonin crystal (Sigma-Aldrich, USA, catalog no: M5250) was utilized. Following the injection of the solution into the animals, a commercial preparation Ademin® (Ceva, Türkiye) containing vitamins A (500.000 IU), D_3

Table 1. Melatonin levels according to groups (pg/ml)

	First sample (Before treatment)	Second sample (11^{th} day after treatment)	P value
MEL10 (n:70)	33.77±8.16	39.59±7.58	0.045
MEL20 (n:70)	29.02±5.75	30.39±2.08	NS
MEL40 (n:70)	31.95±3.85	30.67±3.37	NS
Placebo (n:70)	33.28±3.82	30.58±3.40	NS
Control (n:70)	35.03±7.15	32.09±5.47	NS
P value	NS	NS	

The statistical evaluation was performed both within groups (columns) and between groups (rows). NS: Non significant Data are presented as mean±standard error.

Table 2. Prolactin levels according to groups (ng/ml)

Groups	First sample (Before treatment)	Second sample (11^{th} day after treatment)	P value
MEL10 (n:70)	78.47±3.16	72.88±3.20	NS
MEL20 (n:70)	68.05±4.88	63.88±5.30	NS
MEL40 (n:70)	76.04±2.82	78.96±2.88	NS
Placebo (n:70)	81.95±5.81	73.80±3.40	NS
Control (n:70)	79.58±6.79	76.52±9.31	NS
P value	NS	NS	

The statistical evaluation was performed both within groups (columns) and between groups (rows). NS: Non significant Data are presented as mean±standard error.

(75.000 IU) and E (50 mg), in the form of an oily solution, was used to facilitate slow absorption (Kumar and Purohit, 2009). A total of 2 ml volume of the melatonin solution at doses of 10 mg/2ml, 20 mg/2ml, and 40 mg/2ml was administered (SC) to the animals in the MEL10, MEL20, and MEL40 groups, respectively.

Blood Sampling and Melatonin Administration

These samples were taken from the jugular vein of all animals between 18.00 and 20.00 hours. The first blood samples were taken 11 days before ram introduce, immediately before melatonin injections. Following the first blood sampling, melatonin injections were administered. A second blood samples were collected 11 days after melatonin injection (Singh et al., 2020). The collected blood samples were centrifuged at 3000 rpm for 15 minutes, and the resulting serums were separated. The serum samples were stored at a temperature of -20°C for laboratory analysis. The levels of species-specific melatonin (BT-Lab®, China, catalog no: E0108Sh) and prolactin (BT-Lab®, China, catalog no: E0022Sh) in the collected blood sera were assessed using ELISA kits. The data from the microplate reader (Bio-Tek ELx800) were used to process the results.

Rams Protocol and Estrus Detection

The rams were housed in a designated area within the facility. An intensive feeding program was implemented for the rams. Three implants (Regulin®, Ceva, Türkiye) containing 18 mg of melatonin were applied to the back of the ear (ear root) 30 days before introducing the rams to the herd. Mating activities were conducted twice a day, between 06.00-08.00 and 19.00-21.00, and were recorded accordingly. The criteria for determining estrus rates in ewes included behavioral changes, interest in males or males' interest in females, and mating status. Observations were made daily to record the heat data of the animals within specific time frames: 1-15 days (early period), 15-30 days (mid-term), and 30-60 days (late period) after ram introduction. The rams were kept in the herd throughout the study.

Ultrasonographic Examination and Determination of Pregnancy

The ultrasound examination (Hasvet 838, Türkiye) for pregnancy determination was conducted using transrectal and transabdominal methods (5-7 MHz, Linear probe). To identify animals that became pregnant within 0-30 days following the ram introduce, a pregnancy examination was conducted on the 60^{th} day. Similarly, on the 90^{th} day, a pregnancy examination was performed on animals that became pregnant during the 30-60 day period.

Statistical Analysis

All statistical analyses were performed using the SPSS 25.0 program (Inc., Chicago, IL, USA). The Shapiro-Wilk test was applied to assess the normality of the data. For analyzing hormone levels between groups, ANOVA analysis was conducted for normal distribution, and the

Kruskal-Wallis test was used for cases with non-normal distribution. For analyzing hormone levels within the group between different days, the paired T-test was employed for normally distributed cases, and the Wilcoxon test was utilized for cases with non-normal distribution. The data were presented as mean ± standard error. Also, the Chi-square test was used to compare estrus, mating, pregnancy, and conception rates between the groups. These data were presented as percent or f (%). P<0,05 was considered significant.

Results

In terms of serum melatonin levels, there was no statistically significant difference between the groups on the 11th day and the first blood samples (P>0,05). However, except for the MEL10 and MEL20 groups, melatonin levels were found to be lower in all groups than those measured in the first samples. The increase in melatonin in the MEL10 group was statistically significant (P<0.05). Melatonin levels according to groups are present in Table 1. Similar to the melatonin levels in the first blood samples, there was no statistically significant difference (P>0,05) in serum prolactin levels according to groups (Table 2). However, except for the MEL40 group, prolactin levels were found to be lower in all groups compared to the first samples.

The statistical evaluation was performed both within groups (columns) and between groups (rows). NS: Non significant Data are presented as mean±standard error.

The evaluation of estrus data was based on the number and percentage of animals showing estrus within specific day intervals. Estrus rates according to day intervals are shown in Table 3. The number of heats experienced by the same animal in consecutive days was not added to the next day's interval. It was observed that the animals in the MEL10 group exhibited more estrus symptoms compared to the MEL20 and control groups between days 1-15 (P<0.05). Additionally, a statistically significant difference (P<0.05) was found between the MEL10 group and placebo group in the 15-30 day range, as well as between the MEL10 group and control group in the 30-60 day range. Total estrus data revealed that the MEL10 group had a higher estrus rate than all other groups (P<0.05). In some cases, although animals were in heat, mating did not occur. Mating rates according to day intervals are shown in Table 4. When evaluating pregnancy data, the highest pregnancy rate in all periods and the total pregnancy rate were observed in the MEL10 group. Pregnancy rates are shown in Table 5. Furthermore, when considering the total pregnancy data, the MEL10 groups had significantly higher rates than the MEL40, placebo, and control groups (P<0.05). In terms of conception rates, a statistically significant difference was observed between the groups (P<0.05). The data obtained from ultrasound examinations were used to determine the conception rate in the 0-30 day interval following the 60th-day pregnancy examination. Similarly, the 30-60 day interval following the 90th-day pregnancy examination was used for conception rates. Conception rates according to day intervals are shown in Table 6. In terms of total conception rates, MEL10 and MEL 20

Table 3. Estrus rates according to day interval [f (%)]

Groups	1-15 day	15-30 day	30-60 day	Total
MEL10 (n:70)	12 (17.14)^{a}	19 (27.14)^{a}	22 (31.42)^{a}	53 (75.71)^{a}
MEL20 (n:70)	4 (5.71)^{b}	18 (25.71)^{ab}	19 (27.14)^{ab}	41 (58.57)^{b}
MEL40 (n:70)	7 (10)^{ab}	11 (15.71)^{ab}	20 (28.57)^{ab}	38 (54.28)^{b}
Placebo (n:70)	6 (8.57)^{ab}	9 (12.85)^{b}	21 (30)^{ab}	36 (51.42)^{b}
Control (n:70)	4 (5.71)^{b}	14 (25.71)^{ab}	13 (21.42)^{b}	31 (44.28)^{b}

The statistical evaluation was conducted within groups (columns). Different letters (a, b, c, ...) between columns indicate a statistically significant difference. A significance level of P<0.05 was considered statistically significant.

Table 4. Mating rates according to day interval [f (%)]

Groups	0-30 day	30-60 day	Total
MEL10 (n:70)	26 (37.14)^{a}	17 (24.28)	43 (61.42)
MEL20 (n:70)	22 (31.42)^{ab}	18 (25.71)	40 (57.14)
MEL40 (n:70)	17 (24.28)^{ab}	20 (28.57)	37 (52.85)
Placebo (n:70)	15 (21.42)^{b}	19 (27.14)	34 (48.57)
Control (n:70)	20 (28.57)^{ab}	13 (18.57)	33 (47.14)

The statistical evaluation was conducted within groups (columns). Different letters (a, b, c, ...) between columns indicate a statistically significant difference. A significance level of P<0.05 was considered statistically significant.

Table 5. Pregnancy rates [f (%)]

Groups	60th day	90th day	Total
MEL10 (n:70)	25 (35.71)[a]	17 (37.77)	42 (60)[a]
MEL20 (n:70)	21 (30)[ab]	16 (32.65)	37 (52.85)[ab]
MEL40 (n:70)	14 (20)[b]	16 (28.57)	30 (42.85)[b]
Placebo (n:70)	13 (18.57)[b]	16 (28.07)	29 (41.42)[b]
Control (n:70)	16 (22.85)[ab]	11 (20.37)	27 (38.57)[b]

The statistical evaluation was conducted within groups (columns). Different letters (a, b, c, ...) between columns indicate a statistically significant difference. A significance level of P<0.05 was considered statistically significant.

Table 6. Conception rates according to day interval (%)

Groups	0-30 day	30-60 day	Total
MEL10 (n:70)	96.15[a]	100[a]	97.67[a]
MEL20 (n:70)	95.45[a]	88.88[b]	92.50[b]
MEL40 (n:70)	82.35[b]	80[b]	81.08[c]
Placebo (n:70)	86.66[b]	84.21[b]	85.29[c]
Control (n:70)	80[b]	84.61[b]	81.81[c]

The statistical evaluation was conducted within groups (columns). Different letters (a, b, c, ...) between columns indicate a statistically significant difference. A significance level of P<0.05 was considered statistically significant.

groups had statistically higher rates than MEL40, placebo and control groups (P<0.05).

Discussion

There are limited studies investigating blood levels after melatonin administration. In these studies, melatonin was administered to animals by different routes such as oral administration, injection, and implant, and showed efficacy at different durations. Singh et al. (2020) reported that melatonin values peaked on the 12th day after different doses of melatonin injection treatment in goats within a sub-tropical climate during the anoestrus season between April and September. In the same study, 10 mg, 20 mg, and 40 mg melatonin were administered to goats, resulting in melatonin levels increasing 2, 3, and 5 times, respectively. No significant change was observed in the control group. In the present study, no statistically significant difference was observed in the second sampling, except for the MEL10 group. Additionally, an increase was observed in the MEL20 group, but it was not statistically significant. The reason for the differences between the present study and Singh et al. (2020) study could be attributed to the different study designs and different species. Moreover, variations in melatonin and prolactin levels have been observed in studies conducted with sheep under similar designs. For instance, Kennaway et al. (1984) observed that the serum prolactin level was significantly lower in sheep administered oral melatonin compared to the control group, additionally, the serum prolactin level remained low. In another study by Elhadi et al. (2022), it was noted that the plasma melatonin level significantly increased by the subcutaneous implant of melatonin (18 mg/sheep) in sheep (mean, 111%), and a greater increase was evident between breeds (Manchega ewes compared to Lacaune ewes, 150%-63%, respectively). It was also reported that the plasma prolactin level decreased (an average of 35%) in ewes treated with melatonin, and this decrease was significant only in Manchega sheep (54%) (Elhadi et al., 2022). Szczepkowska et al. (2019) found that ewes implanted with melatonin showed a significantly higher plasma concentration of melatonin and a significant decrease in plasma prolactin concentration as compared to the control group. Above mentioned studies (Kennaway et al., 1984; Szczepkowska et al., 2019; Elhadi et al., 2022) displayed that the melatonin levels increased, and prolactin levels decreased due to the oral or implant formation of melatonin. However, in the present study, an increase in melatonin levels was observed only in the MEL10 and MEL20 groups, whereas melatonin levels decreased in the other groups. These discrepancies depending on the melatonin application may be attributed to seasonality (long day period) which is more dominant during this period. When comparing prolactin levels, a decrease was observed in all groups except the MEL40 group, which was in line with previous studies. The increase in the MEL40 group could be due to individual differences or the high dose of melatonin itself. Considering the mentioned studies, there were significant differences in the percentile. However, when the different responses have been evaluated in terms of increase or decrease, it can be said that only the MEL10 and MEL20 groups are consistent with previous studies.

Uyar and Alan (2008) have reported that the application of melatonin implants (18 mg melatonin) as compared to the nontreated Akkaraman sheep in May revealed a higher pregnancy rate (82.14% vs 0) and shorter time from melatonin treatment to the onset of the first estrus (60.8±0.42 days vs 138.0±1.56 days). In another study, Emrelli et al. (2003) stated that the first estrus was observed within 5 days after the ram introduce (35 days after the melatonin treatment) following the application of implant containing 18 mg of melatonin, the estrus rate was 90%, and the pregnancy rate of those showed estrous was 100%. Zarazaga et al. (2009) reported that the goats implanted with melatonin in March showed 85.7% estrus activity, and of 57.1% showed first estrus within 33 days after the implant placement. In comparison to the other reports, in the present study, it was observed that the injection produced a faster estrus response compared to the above-mentioned implant treatments. Although the data in the MEL10 group was relatively high in the present study, it did not match the data from the implant studies. However, it was noticed that the time from the treatment to the first estrus was longer in the present study, and the mating interval was earlier than the implant treatments. On the other hand, other studies mentioned that until the breeding season, estrus was not observed in the control groups during the off-season. In the present study, higher estrus and pregnancy rates were found in the placebo and control groups. This might be the fact that animals in all groups were exposed to the uniform flashing and the ram effect. Also, the weak seasonal characteristics of merino sheep are an important factor in the lack of similarity of the findings with those obtained from previous studies.

In comparison to implant applications, subcutaneous injection is believed to result in faster absorption of melatonin. Singh et al. (2020) reported that the peak level of melatonin in the blood was observed on the 12th day after melatonin injection. This suggests that the subcutaneous injection allows for a quicker release and absorption of melatonin compared to the slow-release effect of implants. An oil-based depot injection typically contains the active pharmaceutical ingredient along with an oil and potentially a co-solvent. Various vegetable and synthetic oils, such as castor oil, soybean oil, sesame oil, cottonseed oil, and coconut oil, have been utilized to achieve a long-lasting effect. A single dose of injection can generally produce active release for 2-8 weeks. The exact release time for the prepared oily solution used in the study was not specified (Wilkinson et al., 2022). However, based on the conception and estrus data, it is believed that absorption and effectiveness might begin around 2 weeks and extend to an average of 8 weeks.

Kumar and Purohit (2009) evaluated the effect of injecting a single subcutaneous dose of 20 mg and 40 mg melatonin dissolved in an oily solution containing vitamins A, D, and E in goats during January. The results showed that the goats treated with 20 mg melatonin exhibited 70% estrus in 20 days, while those treated with 40 mg melatonin showed 85% estrus in 15 days, with pregnancy rates of 100% and 82.35%, respectively. Only 10% of the goats in the control group showed estrus, and no pregnancies were observed in this group. In another study conducted in May, melatonin doses of 25 mg/ml and 50 mg/ml displayed that 80% and 90% of the sheep exhibited estrus, while only 10% of the sheep in the control group showed estrus. The time interval to estrus following melatonin treatment was earlier in the melatonin groups compared to the control group (Abdelnaby, 2020). Singh et al. (2020) reported that single subcutaneous injection of 10 mg, 20 mg, and 40 mg of melatonin to the goats showed 80%, 100%, and 30% estrus rate, respectively, whereas the estrus rate was 20% in the control group. The time interval to estrus following melatonin treatment was shorter in the 10 mg and 20 mg melatonin groups compared to the control and 40 mg melatonin groups. Pregnancy rates were 20% in the control group, 80% in the 10 mg melatonin group, 100% in the 20 mg melatonin group, and 30% in the 40 mg melatonin group. When the results of the present study have been compared with the previous studies (Kumar and Purohit, 2009; Abdelnaby, 2020; Singh et al., 2020), it is evident that there are variations in estrus and pregnancy rates depending on the melatonin dose and timing of administration. The present study is consistent with Singh et al. (2020) in terms of the lower estrus and pregnancy rates observed with the dose of 40 mg melatonin. It is possible that different environmental factors, melatonin administered time and species-specific responses may influence the effectiveness of melatonin treatment in different studies. The present study supports the notion that the dose of melatonin may play a role in determining its effects on estrus and pregnancy rates in sheep.

Conclusion

The interval of the first estrus varies according to different synchronization methods. Melatonin application is intended to shorten the interval of the first estrus rather than serving as a synchronization method. In this regard, although high rates of pregnancy are not achieved compared to other methods, subcutaneous melatonin application has been observed to be effective in the first estrus response and particularly in conception rates. When the estrus rates, mating, conception, and pregnancy rates in the MEL10 groups are considered, the subcutaneous administration of melatonin crystal's oily solution has of positive effect on fertility compared to the other doses during non-breeding season. In the MEL10 ($P<0.05$) and MEL20 groups, melatonin levels were increased compared to the first sample. This is partially supported in terms of fertility rates in the same groups. One of the limitations of the study is the lack of longer and more frequent melatonin and prolactin measurements. The other is that the number of lambs per pregnancy is not monitored.

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Conflict of interest

The authors declare that they have no conflict of interest.

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