EFFECT OF MELATONIN AND SHORTENED DAYLIGHT ON THE REPRODUCTIVE PERFORMANCE OF JORDANIAN GOATS DURING THE OUT-BREEDING SEASON

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ABSTRACT

This study investigated the impact of melatonin (MEL) and a short photoperiod on the reproductive performance of local Jordanian goats during the out-breeding season. Forty-five nursing does and five bucks were divided into five groups: MEL with sponge (MS), MEL without sponge (M), darkness with sponge (DS), darkness without sponge (D), and a control group (C). The darkness groups were subjected to a 63-day controlled darkness program to simulate decreasing daylight hours. MEL was administered via subcutaneous implants, and the MS and DS groups received progesterone (P4)-impregnated sponges. The results showed that MEL levels significantly increased (P=0.007) in all experimental groups (MS, M, DS, and D) compared to the control group. The MS group exhibited the highest P4 levels (P=0.001). While there were no significant differences in estrus or conception rates among the groups, the MS group had the highest pregnancy rate at days 21 and 28. Fertilization rates were similar across all groups. Fecundity was comparable, but prolificacy was highest in the MS group. Hormonal treatments and reduced daylight influenced the bucks' scrotal circumference, with the MS group displaying the largest ($p<0.05$) scrotal circumference and the highest testosterone concentration. In conclusion, the study suggests that administering MEL to goats, particularly in combination with P4 sponges, may enhance their reproductive capacity during the out-breeding season.

Keywords: hormonal treatment; photoperiod; conception; reproductive seasonality; goats.

مجلة العلوم الزراعية العراقية- 2025 :56 (4):1362-1374 الصمادي وآخرون

تأثير الميلاتونين وفترة الإضاءة القصيرة مع استخدام الاسفنجات على الأداء التناسلي للماعز المحلي الأردني خارج موسم التناسل

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المستخلص

أجريت هذه الدراسة لبيان تأثير هرمون الميلاتونين (MEL) وفترة الإضاءة القصيرة في الأداء التناسلي للماعز الأردني المحلي خلال موسم التناسل الخارجي. قُسِّمَت خمس وأربعون ماعز مُرضعة وخمسة ذكور إلى خمس مجموعات: مجموعة الميلاتونين مع إسفنجة بروجستيرون (MS)، ومجموعة الميلاتونين بدون إسفنجة(M) ، ومجموعة الظلام مع إسفنجة (DS)، ومجموعة الظلام بدون إسفنجة(D)، ومجموعة سيطرة .(C) خضعت مجموعات الظلام لبرنامج ظلام مُحكم لمدة 63 يومًا لمحاكاة تناقص ساعات النهار. أُعطي الميلاتونين عن طريق غرسات تحت الجلد، وتلقت مجموعتا MS و DSإسفنجات البروجستيرون(P4) . أظهرت النتائج أن مستويات الميلاتونين زادت بشكل ملحوظ (P=0.007) في جميع المجموعات التجريبية(MS، M، DS، وD) مقارنة بمجموعة التحكم. أظهرت مجموعة MS أعلى مستويا P4 (P=0.001) . على الرغم من عدم وجود اختلافات كبيرة في معدلات الشبق أو الحمل بين المجموعات، إلا أن مجموعة MS سجلت أعلى معدل حمل في اليومين 21 و 28. كانت معدلات الإخصاب متشابهة بين جميع المجموعات. كانت الخصوبة متقاربة، لكن الإنتاجية كانت الأعلى في مجموعةMS . أثر الميلاتونين وتقليل ضوء النهار في محيط كيس الصفن للذكور، اذ أظهرت مجموعة MS أكبر محيط ($p<0.05$) وأعلى تركيز لهرمون التستوستيرون. يمكن الاستنتاج أن إعطاء الماعز الميلاتونين، خاصةً مترافقة مع إسفنجات البروجستيرون، قد حسن قدرتها على التناسل خارج الموسم.

الكلمات المفتاحية: معاملة هرمونية، فترة الإضاءة، إخصاب، موسمية التناسل، ماعز.

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INTRODUCTION

Goats have been associated with humans for at least 10,000 years (28) and can be found worldwide due to their adaptability to different environmental and climatic conditions (6). This versatile farm animal provides meat, milk, fiber, hide, and organic fertilizer, making it highly beneficial to humans globally (24). The livestock sector, including goat farming, contributes around 40% of the total global value of agricultural products, highlighting its significance worldwide (33). Goats are considered seasonally polyestrous animals under temperate climatic conditions (17). Research in recent decades has aimed to enhance goat reproductive efficiency by eliminating seasonal breeding patterns, primarily through photoperiod manipulation and hormone administration (2). Photoperiod, the length of daylight, is a critical factor influencing seasonal changes and reproductive activity in seasonal breeders like goats (12,14,26,39) particularly in temperate latitudes between 24° and 40° (14) Melatonin (MEL), secreted by the pineal gland in response to darkness, plays a crucial role in regulating the sexual activity and reproductive cycles of short-day breeders like goats (37) by mimicking short-day photoperiods and stimulating the release of gonadotropin-releasing hormone (GnRH), thereby promoting luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion, leading to resumed ovarian activity and estrus behaviour (12,45). Various simple and cost-effective estrous synchronization protocols involving the administration of hormones are used to regulate goat reproductive cycles (2). Subcutaneous MEL implants can control the estrous cycle (37). Additionally, melatonin's antioxidant properties may enhance ovarian follicle quality and embryo viability (29). However, goat reproduction in Jordan has been studied, with a focus on estrus synchronization and male fertility, limited research exists on the reproductive efficiency and seasonality of local goats (5,40). In Jordan, farmers typically breed their does during the warm summer months (June/July) until early autumn to have kids during the winter and spring grazing seasons (21,41). However, there is a significant knowledge gap regarding the seasonality of reproduction in goats in Jordan. This study aims to investigate the effect of MEL hormone administration or application of short-day length using sponges on the reproductive performance of local goats during the out-breeding season under common rearing conditions in Jordan.

MATERIALS AND METHODS

Ethical Institutional Review Board (IRB) approval: The Scientific Research Council at the University of Jordan and the Institutional Animal Care and Use Committee approved the experimental protocols (approval number 182/2023).

Location and weather: This experiment was conducted from March to October 2023, encompassing the non-breeding season (March-June) and extending through the kidding season. The study was carried out at a local commercial goat farm with suitable handling and housing facilities, located in Kherbet Alsouq village, Ajloun province, northern Jordan (latitude 32°33'N), at an altitude of 738 meters above sea level. The region has a Mediterranean climate characterized by moderate and dry summers, as well as cold and rainy winters. This climate is classified as Csa according to the Köppen-Geiger climate classification. The official national station in the Anjarah region, which is 2 km from the farm, provided meteorological data, including the highest and lowest temperatures. During the March–October experimental period, the lowest temperature varied between 5°C and 19°C, with an average of 12.9°C, and the highest temperature ranged between 29°C and 43°C, with an average of 35.1°C.

Animals and feeding: Forty-five local Jordanian lactating multiparous does, and five local bucks were included in the experiment. All animals were vaccinated and dewormed according to the Jordanian Ministry of Agriculture's livestock vaccination program and were confirmed to be clinically healthy. Before the experiment, does were selected during milking. The owner confirmed they showed no estrus signs, indicated by P4 concentrations, confirming anestrus. An ultrasound also confirmed that all does were not pregnant (Mindray Animal Care, Model: DP-50Vet). All goats were provided with a

common feed concentrate available in the local market, consisting of 60% barley, 10% corn, 10% soybean, 19.5% wheat bran, and 0.5% salt, minerals, and vitamins. This concentrate contains 12.4% crude protein and 2612 Kcal/kg of metabolizable energy. Each goat received approximately 500 grams of this concentrate twice daily. The average weight for all the does in the experiment was 46.6 kg. In addition, they had ad libitum access to common vetch (Vicia sativa) hay, which contains 5.7% crude protein. Daily, the does were taken to graze on the surrounding green pasture, which includes various plants such as *Trifolium repens*, *Avena sativa*, *Quercus coccifera*, *Styrax officinalis*, and *Cistus incanus*. All animals were provided with the necessary nutrients based on their body weight, as per (31) NRC guidelines (2007), and had access to water at all times.

Experimental Design: Beginning on day 40 to 50 following kidding, all lactating local hybrid does (mountain black × Shami) were chosen for the experiment. According to (13) and (25), uterine involution is finished by days 19 to 28, which permits does to exhibit their first postpartum estrus 5–10 weeks after kidding (20). Forty-five does were randomly assigned to five experimental groups, stratified by age, body weight (BW), and body condition score (BCS), as shown in Table (1). One doe from the MEL with sponge (MS) group died due to complications unrelated to the hormonal treatment and was excluded from the study, resulting in a group size of n=8 for that treatment. The groups were categorized as follows: MEL with sponge (MS; n = 8), MEL without sponge (M; n = 9), darkness with sponge (DS; n = 9), darkness without sponge (D; n = 9), and a control group without any treatment (C; n = 9). Each group included one buck. All bucks were kept separately from the does in designated pens on the same farm to prevent mating before the scheduled introduction. The experiment lasted for 123 days, beginning on day zero (0) when the darkness groups (DS and D) were placed in light-controlled housing to ensure more than 13 hours of darkness per day. MEL was administered subcutaneously near the ear base in the MEL groups (MS and M) using 18 mg MEL implants (Melovin® from Ceva Santé Animale, La Ballastière, Libourne, France). Bucks received three implants five days' prior the does, and each doe in the MEL groups received one implant, according to the manufacturer's protocol. Progesterone (P4) sponges (Syncropat® 30 mg, from Ceva Santé Animale, La Ballastière, Libourne, France) were inserted on day 42 for the groups treated with sponges (MS and DS). On day 61, 19 days after the sponge insertion, all sponges were removed. Following sponge removal, does were injected intramuscularly with 500 IU of Pregnant Mare Serum Gonadotropin (PMSG) to stimulate ovulation, as described by (16). On day 63, bucks were introduced to their respective doe groups for pen mating, which occurred nightly for two months. Every morning, bucks were separated and returned to their designated pens. On day 108, 45 days post-mating, pregnancy detection was conducted using an ultrasonography device (Mindray Animal Care, Model: DP-50 Vet). The experimental timeline and procedures for each treatment group are illustrated in Figure (1).

Table 1. Age, body weight, and body condition score of Jordanian does at the beginning of the experiment during the out-breeding season (Mean ± SEM)

Parameters \ Treatments[1]	MS (n=8)[2]	M (n=9)	DS (n=9)	D (n=9)	C (n=9)	P value
Age (years)	2.88 ±0.25	3.33 ±0.23	3.33 ±0.23	2.78 ±0.23	2.89±0.23	0.339
Body Weight(kg)	46.00 ±1.29	47.44±1.22	47.44±1.22	47.11 ±1.29	46.33 ±1.22	0.883
BCS (1-5)	2.75±0.11	2.94±0.10	2.94±0.10	2.92±0.10	2.97±0.10	0.502

[1] MS= MEL with sponge, M= MEL without sponge, DS= Darkness with sponge, D= Darkness without sponge, and C= Control. [2] Number of does.

Darkness program: In this study, both bucks and does in the darkness treatment groups (DS and D) were subjected to a controlled darkness program for 63 days, from March 1 (day 0) to May 2 (day 63). This program was designed to simulate decreasing daylight hours, aligning with local sunrise and sunset times and the natural progression of spring. Initially, the goats were housed in light-proof rooms with controlled ventilation for 13 hours and 4 minutes of darkness. Each week, the animals were placed in the dark rooms 14 minutes

earlier, for a total of nine weeks. By May 2 (day 63), the duration of darkness reached 13 hours and 55 minutes. At the start of the program, there was a 33-minute difference in darkness exposure between the darkness groups (DS and D) and the other experimental groups (MS, M, and C). By day 63, this difference had increased to 3 hours and 22 minutes, reflecting the natural increase in daylight during the season. To effectively implement the dimming program, the goats were housed in specially designed dark rooms that blocked all natural light while still providing adequate ventilation. This approach was intended to simulate the effects of decreasing daylight hours and increasing darkness, thereby stimulating the goats' natural secretion of MEL, which is essential for regulating their reproductive cycles.

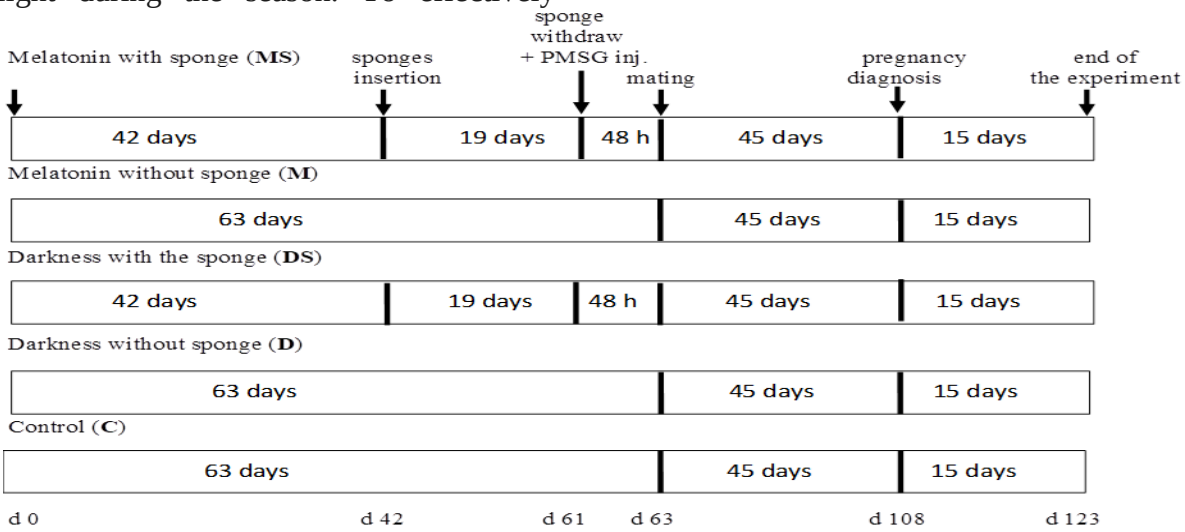

Figure 1. Experimental design to evaluate the effect of MEL or darkness with P4 sponges on the reproductive performance of local goats in the out breeding season.

Blood sampling: Blood samples from does were collected to determine plasma progesterone (P4) and melatonin concentrations (MEL) from the jugular vein into 4-ml vacuum tubes containing lithium heparin on days 0, 14, 28, and 42, prior to sponge insertion, and then on day 49. Additional samples were taken on the day of sponge removal (day 61), the day of mating (day 63, considered day zero post-mating), and then weekly until day 98 (day 35 post-mating). Samples were collected between 6:00 and 8:00 AM from the same six designated does and all bucks in each treatment group. Immediately after collection, the tubes were centrifuged at 3000 rpm for 15 minutes, and the plasma was transferred to 1.5 ml Eppendorf tubes and stored at −20°C until measured for P4, MEL, and testosterone (T) concentrations using an ELISA kit.

Hormonal assay: Plasma MEL, P4, and T were measured using ELISA kits, following the manufacturers' instructions The concentration of MEL was determined in all experimental animals across each treatment group using the MEL ELISA Kit from ELK Biotechnology (Catalog Number: ELK7862), with inter-assay and intra-assay coefficients of variation for MEL were 8.2% and 7.1%, respectively. The concentration of P4 in plasma samples was determined using the P4 ELISA Kit from DiaMetra (Catalog Number: DCM006-10, Ed. 03/2022) with inter-assay and intra-assay coefficients of variation 10.5% and 3.28%, respectively. Additionally, the concentration of testosterone (T) in plasma samples from bucks in all treatment groups was assessed using the Testosterone ELISA (Document Number: MYI002-9, Ed. 09/2018) with inter-assay and intra-assay coefficients of variation 11.1% and 2.24%, respectively.

Reproductive performance: The following parameters and reproductive traits were estimated and compared across the five groups

1-Estrus rate: Estrus was monitored for 72 hours following sponge removal on day 61. The farmer, an experienced observer, reported estrus in all does, based on behavioral signs including bleating, tail wagging, reddened vulva, and occasional mounting by other does. Additionally, P4 concentrations were measured below 1 ng/ml on day 63 from six

does in each treatment group, which supported the behavioural observations. The estrus rate was calculated as Number of does detected in estrus / Number of does treated × 100%. Progesterone concentration is a reliable biomarker for estrus detection, as P4 levels are low during estrus when goats are sexually receptive. This is consistent with previous research (38) that showed goats with progesterone levels below 1ng/ml were in estrus.

2-Conception rate: Conception rates were assessed using two methods: progesterone (P4) concentration and ultrasound. Early conception rates were calculated on days 21, 28, and 35 after mating using P4 concentrations. Pregnancy was assumed when P4 concentration was ≥ 2.5 ng/ml. The calculation was: (number of pregnant does on the specified day/number of does exposed to bucks) × 100% (9,18,22). Overall conception rate for all does was determined by ultrasound and calculated as: the number of pregnant does detected by ultrasound / number of does exposed to bucks) × 100%. Fertilization rate representing the establishment of pregnancy to term, was calculated as (number of kidding does + number of abortion does after 135 days gestation)/number of exposed does to bucks) × 100%

3-Prolificacy and fecundity: Prolificacy in goats = number of kids/number pregnant does × 100%. While fecundity = number of kids / numbers of exposed does × 100% (16).

Scrotal circumference measurement

Scrotal circumference measurements were taken every two weeks throughout the experiment to monitor buck reproductive status. The testicles were gently pulled down by the lower part of the scrotum, and a flexible tape was used to measure the scrotal circumference (in centimetres) at its widest point. No adjustments were made for scrotal skin thickness, as this was consistent across all bucks.

Statistical Analysis: Hormonal concentrations (Plasma P4, MEL, and testosterone) were analyzed using general linear models (GLM) procedures (SAS Institute, 2016). The fixed effects of treatment (MS, M, DS, D, and C) and pregnancy (pregnant or not) were included in the model. Pregnancy for blood samples was determined based on sustained P4 concentrations of ≥ 2.5 ng/ml on days 21, 28, and 35 from six does in each treatment group, meaning that the P4 concentration had to be above 2.5 ng/ml on all three sample days. The sampling day and their interactions were also considered, with repeated measures over time. Where factors were significantly different, the least square means for different groups were compared using the t-test. The effects of treatment groups on estrous and conception rates were analyzed using the chi-square test ($P < 0.05$).

RESULTS AND DISCUSSION

To the best of our knowledge, this is the first study to examine the effects of MEL administration and progesterone (P4) combined with shortened daylight on out-of-breeding season reproductive performance in local Jordanian goats under typical rearing conditions. To ensure a balanced comparison, does were randomly assigned to five treatment groups, with no significant differences in age, live weight, or body condition score observed at the study's commencement (Table 1)

Effect of hormonal treatment and shortening daylight on MEL levels

Figure 2 shows the impact of hormonal treatments and shortened daylight on plasma melatonin (MEL) levels. MEL implants and shortened daylight elevated MEL levels ($P = 0.007$) in the experimental groups (MS, M, DS, D) compared to the control (Table 2). Variations in MEL levels were also observed across sampling days ($P = 0.001$) and in the interaction between treatments and days ($P = 0.001$), as detailed in Figure (2). These results demonstrate the dynamic influence of treatments on plasma MEL concentrations in local goats during the out-of-breeding season.

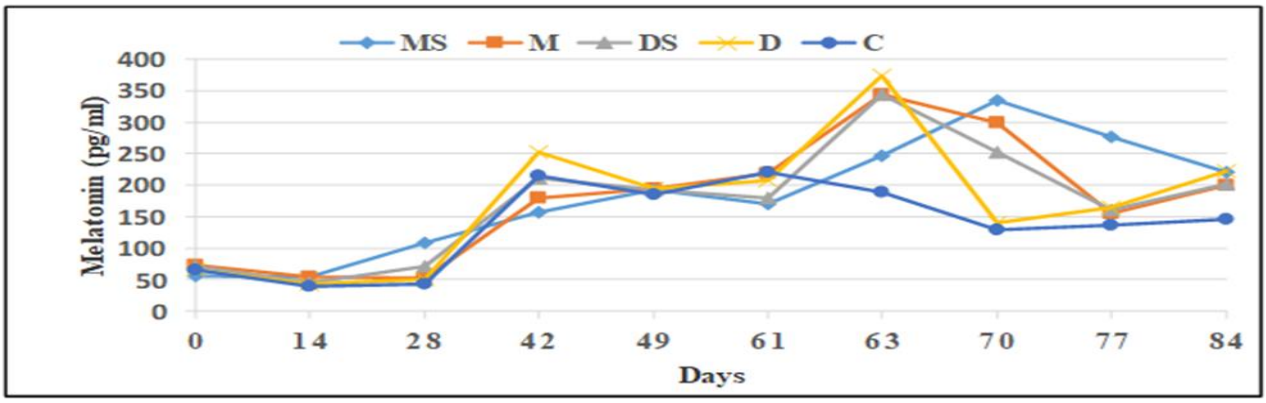

Figure 2. Effect of hormonal treatment and shortening daylight on plasma MEL levels for Jordanian does in various treatment groups during the out-breeding season.

MS= MEL with sponge, M= MEL without sponge, DS= Darkness with sponge, D= Darkness without sponge, and C= Control.

At the experiment's onset (day 0), plasma MEL levels were low in all groups. However, two weeks after implementing treatments in the experimental groups (MS, M, DS, D), MEL levels began a gradual ascent, reaching a peak around day 63 before declining towards day 84. This sustained increase during the experimental period demonstrates the efficacy of MEL implants and shortened daylight in enhancing MEL levels. In contrast, the control group (C) consistently showed low MEL levels throughout the study. These findings corroborate previous research showing that MEL implants (18 mg) elevate MEL levels without suppressing endogenous production (46) and that subcutaneous MEL injections (10 mg) improve hormonal profiles during the non-breeding season (37). Taken together, these studies suggest that continuous, slow-release MEL implants effectively mimic a short-day photoperiod, thereby stimulating breeding activity in seasonally anestrous animals by maintaining elevated MEL levels without inhibiting the body's natural MEL secretion (10,15,47). The present study employed a 63-day artificial dimming program for goats in the darkened treatment groups (DS and D), providing 13 hours and 55 minutes of darkness, in contrast to the 10 hours and 33 minutes of natural darkness experienced by the other groups (MS, M, and C).

Table 2. Effect of treatments on hormone levels (Mean ± SEM) of MEL, P4, and testosterone in local Jordanian goats out breeding season.

Parameters \ Treatments[1]	MS	M	DS	D	C	SEM	P- value Day	P- value Treatment
Does (n=6)[2]								
MEL (pg/ml)	161.5^{a}	155.9^{a}	159.2^{a}	154.8^{a}	130.9^{b}	6.46	0.001	0.007
P4 (ng/ml)	3.81^{a}	1.96^{b}	1.71^{bc}	1.5^{bc}	0.99^{bc}	0.83	0.001	0.001
Bucks (n=1)[3]								
MEL (pg/ml)	215.3^{a}	199.7^{a}	189.9^{a}	160.8^{b}	141.0^{bc}	18.3	0.054	0.050
Testosterone (ng/ml)	7.24^{a}	5.37^{a}	4.74^{b}	2.93^{b}	1.67^{bc}	0.84	0.001	0.002
SC (centimetres)[4]	37.8^{a}	32.5^{bc}	31.5^{c}	34.4^{b}	29.7^{c}	1.48	0.0001	0.0001

[1]MS= MEL with sponge, M= MEL without sponge, DS= Darkness with sponge, D= Darkness without sponge, and C= Control. [2] Number of does. [3]Number of bucks ///[4]Scrotal circumference /^{a,b,c} P <0.05

The DS and D groups showed MEL levels comparable to the MS and M groups, both of which exhibited significantly higher MEL levels than the control group. This observation is consistent with research demonstrating that short photoperiods, such as 8 hours of light and 16 hours of darkness, lead to elevated MEL levels in serum on days 30 and 60 of the experiment in goats (27), noting that short photoperiod treatment increases serum MEL levels to as much as 90 pg in cashmere goats (23). Furthermore, studies suggest that the endogenous rhythm of MEL secretion varies throughout the year in goats, independent of light-adjusted rhythms (4). However, the interaction between MEL implants and breed underscores individual responses to MEL treatment, reflecting genetic variation in MEL secretion (36). This highlights the need to account for genetic factors when evaluating MEL treatment efficacy, given that nocturnal plasma MEL levels are predominantly under genetic control (48).

Effect of hormonal treatment and shortening daylight on progesterone levels : Figure 3 shows the impact of hormonal

treatments and shortened daylight on plasma P4 levels. Compared to the control group, MEL implants and shortened daylight significantly increased P4 levels (P = 0.0001) in the treated groups (MS, M, DS, D). Significant variations in P4 levels were observed across sampling days (P = 0.0001), but there was no significant interaction between treatments and sampling days (P = 0.154), as shown in Table 2. Plasma P4 levels were initially low until day 42, when they began to increase following sponge insertion. The D group showed slightly higher P4 levels on days 49 and 61. Following mating, all treated groups showed a significant increase in P4, indicating successful ovulation, but the MS group exhibited a pronounced increase in P4 levels after day 70, suggesting improved reproductive synchronization. The darkened groups (DS and D) showed moderate P4 levels, with sponge treatment (DS) enhancing the P4 response. The M group, treated with melatonin, showed a noticeable P4 increase, though lower than that of the MS group, likely due to the lack of P4 sponges. Consistently low P4 levels were observed in the control group (C). These findings indicate that MEL-implanted does had higher plasma P4 concentrations compared to non-implanted does, supporting the luteotrophic effects of MEL implants reported by (16). Research has shown that MEL not only increases progesterone but also decreases estradiol, thereby optimizing reproductive function in goats (16).

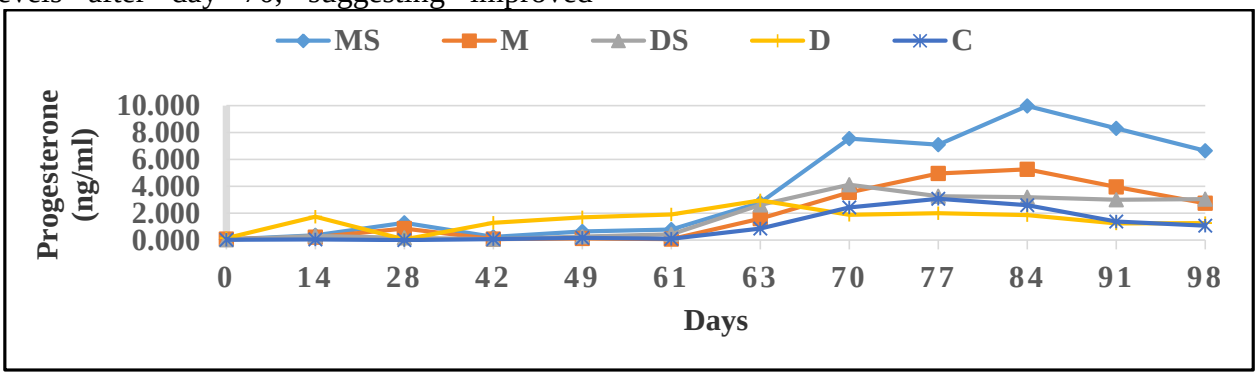

Figure 3. Effect of hormonal treatment and shortening daylight on plasma P4 levels for Jordanian does in various treatment groups during the out-breeding season.

MS= MEL with sponge, M= MEL without sponge, DS= Darkness with sponge, D= Darkness without sponge, and C= Control.

Similar results have been observed following subcutaneous MEL injections during the non-breeding season (37). Melatonin appears to influence hypothalamic regions, augmenting GnRH pulse release through modulation of During days 21, 28, and 35 of the current study, there were some numerical differences in conception rates among the treatment groups (Table 3). Therefore, we cannot conclude with certainty from this data that any of the tested treatments (darkness with or without sponge, MEL with or without sponge) significantly affected the conception rate in comparison to the control group. This may return to small-sized animals per each group (n=6). This finding aligns with the statement that although the administration of exogenous melatonin implant reduced the prolactin levels, it did not markedly increase pregnancy rates (19). estradiol's negative feedback (32). Moreover, MEL melatonin directly stimulates P4 production by the corpus luteum (3).

Effect of hormonal treatment and shortened daylight on reproductive performance

Table 3. Effect of treatments on pregnancy rates based on progesterone concentration in local Jordanian goats out breeding season.

Parameter \ Treatments[1]	MS (n=6)	M (n=6)	DS (n=6)	D (n=6)	C (n=6)	P- value
Conception rate[2]						
d 21	100.00 (6/6)	100.00 (6/6)	66.67 (4/6)	83.33 (5/6)	50.00 (3/6)	0.132
d 28	100.00 (6/6)	100.00 (6/6)	66.67 (4/6)	83.33 (5/6)	50.00 (3/6)	0.132
d 35	83.33 (5/6)	83.33 (5/6)	66.67 (4/6)	66.67 (4/6)	33.33 (3/6)	0.691

[1] MS= MEL with sponge, M= MEL without sponge, DS = Darkness with sponge, D= Darkness without sponge, and C=Control. /[2]Conception rate = no. of does conceiving days 21, 28, and 35 (based on P4 concentration of ≥ 2.3 ng/ml) / (number of does exposed does) ×100%

According to (16), melatonin implants and less daylight did not cause lactating does to exhibit signs of estrus during the first 42 days in any of the study's groups. When compared to untreated controls, (45) discovered that melatonin (MEL) implants given to female Mediterranean goats during the non-breeding season can postpone the onset of estrus by roughly 30 to 45 days. In contrast, the MS group successfully induced estrus in 87.5% of the goats in our study during the non-breeding season two days after the sponge was removed (Table 4). In our study, no statistically significant differences in estrus induction were observed across all treatment groups, despite the MS group's apparent success (P value = 0.843, Table 4). The small sample size we used or the fact that two does had high progesterone (P4) levels for the three weeks before estrus could be the cause of this lack of significant variation. Moreover, the mating system may have obscured possible treatment effects on estrus synchronization. Bucks were introduced for pen mating on day 63 and continued nightly for two months in all groups. All groups' less than 100% estrus rates should be taken into account. Our results are somewhat in line with those of (11), who found that Kilis dairy goats treated with CIDR and melatonin implants had a greater estrus response than a control group. In a similar vein, (46) discovered that, in comparison to controls, this short-day breeding goat breed experienced earlier and more synchronized estrus when exposed to brief daylight and MEL implants. It's interesting to note that (43) found that both the melatonin (M) and control (C) groups had 100% estrus rates. (37) discovered that subcutaneous melatonin injections enhanced the rates of estrus induction in Singharey goats during the non-breeding season when progesterone supplementation was not present. Iraqi local goats, without a melatonin implant, were synchronized with sponges for 14 days and injected with eCG (73.3%) or hCG (90%), resulting in a higher estrus response than the control group (7,8). These inconsistent findings imply that breed (short-day vs. long-day breeders), progesterone treatments (like CIDR), melatonin administration method (implant vs. injection), and environmental cues (daily exposure) can all affect how well melatonin induces estrus.

Table 4. Effect of treatments on reproductive performance of local Jordanian goats out of the breeding season.

Parameter \ Treatments[1]	MS (n=6)	M (n=6)	DS (n=6)	D (n=6)	C (n=6)	P- value
Estrus rate[2]	87.50 (7/8)	77.78 (7/9)	77.78 (7/9)	66.67 (6/9)	66.67 (6/9)	0.843
CR[3]	62.50 (5/8)	44.44 (4/9)	33.33 (3/9)	44.44 (4/9)	33.33 (3/9)	0.747
Fertilization rate[4]	50.00 (4/8)	44.44 (4/9)	22.22 (2/9)	22.22 (2/9)	22.22 (2/9)	0.5534
Prolificacy[5]	1.25 (5/4)	1.00 (4/4)	1.50 (3/2)	1.00 (2/2)	1.00 (2/2)	0.3834
Fecundity[6]	62.50 (5/8)	44.44 (4/9)	33.33 (3/9)	22.22 (2/9)	22.22 (2/9)	0.3789

[1] MS= MEL with sponge, M= MEL without sponge, DS = Darkness with sponge, D= Darkness without sponge, and C= Control. /[2]Estrus rate = (Number of does detected in estrus / Number of does treated) × 100%
[3] Conception rate based on ultrasonography on day 45 /[4]Fertilization rate = (number of kidding does + number of abortion does after 135 days pregnancy)/number of exposed does) × 100% /[5]Prolificacy = (number of kids/number pregnant does) × 100%. /[6]Fecundity = (number of kids/numbers of exposed does) × 100%.

The MS group in our study had a 62.5% conception rate, a 1.25 prolificacy rate, and a 62.5% fecundity rate (Table 4). Both (16) and (43) reported comparable fertilization rates among their treatment groups, which is in line with the similar rates we found across treatment groups in our study. This implies that the various treatments used may not have a major impact on the initial success of fertilization. In support of this, (11) showed that fertility in Kilis goats was unaffected by the combination of melatonin and CIDR treatment. Nevertheless, their results also showed that this combined strategy did not result in appreciable increases in fecundity, prolificacy, or twinning rates, which is similar to the lack of discernible variations in these metrics that we found among our treatment groups (as was previously mentioned with regard to conception rates). This suggests that while fertility (the capacity to conceive) may not be impaired fecundity and prolificacy—two measures that improve overall reproductive output—may necessitate additional or different interventions. In contrast, melatonin implants increased the productivity of does when compared to females who were not treated, according to (10). Additionally, melatonin-

treated Iraqi local goats had noticeably higher pregnancy rates than control groups, according to (1). Although it was not statistically significant in the current study, these studies point to a possible beneficial effect of melatonin on reproductive outcomes in particular breeds or under particular experimental conditions. (43), who recently discovered that melatonin implants produced better reproductive results in Tahirova sheep than in Turkish Saanen goats, further emphasizes the fluctuation in melatonin's efficacy. The significance of taking the animal model into account when assessing how melatonin affects reproductive performance is highlighted by this breed-specific reaction. Our findings, which show numerical but non-significant differences, could be the result of a breed-specific response in the current animals. Figure 4 illustrates the plasma progesterone (P4) profiles in both pregnant (Panel A) and non-pregnant (Panel B) does. In contrast to the other groups, group D showed noticeably higher P4 levels during the first forty-nine days of pregnancy (days 42–49). P4 concentrations were similar for all groups around the time of buck introduction and the start of the estrous cycle (day 63). P4 levels in the MS group were significantly higher ($P < 0.05$) than in the other groups by day 7 of the estrous cycle, which corresponds to day 70, after mating. With P4 levels in the MS group generally higher ($P < 0.10$) by day 14 (day 77) than in the DS or D groups, and either significantly higher ($P < 0.05$) or tending to be higher by day 21 (day 84) than in the DS, D, and M groups, this trend persisted. P4 levels in the MS group were significantly higher ($P < 0.05$) than in all other groups on day 28 (day 91). P4 levels in the MS group were, at last, either significantly higher ($P < 0.05$) or tended to be higher than those in the M, D, C, or DS groups by day 35 (day 98). The initial pregnancy rate was 80% (24 out of 30 does) based on P4 levels on days 21 and 28. By day 35, though, this rate had dropped to 70% (21 out of 30 does). All groups showed a decrease in the rate of conception, which was further supported by ultrasonography on day 45. Table 4 shows that the fertilization rate was statistically comparable for each group. This disparity between the first indication of pregnancy and the subsequent confirmation points to the loss of embryos. Partial or complete embryonic and fetal loss (EFL) in goats between days 20–23 and 26–29 was linked to significant decreases in progesterone (P4) levels (85.06%), according to (34). This suggests endocrine disruption in the corpus luteum (CL). However, total EFL from days 26–29 and 33–36 showed a smaller P4 decrease (24.90%), suggesting a possible effect rather than a direct cause. Furthermore, high levels of estradiol (E2) at day 56 of pregnancy may be a factor in embryonic loss, according to (16). Another possible contributing factor was heat stress, with ambient temperatures during the study ranging from 29 to 43°C. (30) added that a number of variables affect goat conception rates, such as breed, age, nutrition, and season. To prevent premature mating, all bucks and does were housed in separate pens on the same farm. Mean melatonin concentrations differed significantly between treatment groups ($P = 0.050$). Melatonin levels were numerically higher in the males from the MS (215.3 pg/ml), M (199.7 pg/ml), and DS (189.9 pg/ml) groups compared to those from the D (160.8 pg/ml) and C (141.0 pg/ml) groups. Notably, the MS male had significantly higher melatonin levels than the C male ($P < 0.05$). Significant differences were also observed in mean testosterone concentrations between treatments ($P = 0.002$). The male in the MS group exhibited the highest testosterone level (7.24 ng/ml), followed by the males in the M (5.37 ng/ml), DS (4.74 ng/ml), D (2.93 ng/ml), and Control groups (1.67 ng/ml). Regardless of sponge use, buck treated with melatonin exhibited significantly higher testosterone levels compared to the other groups. Within the melatonin-treated bucks, MS was also significantly higher than DS ($P < 0.05$). The administration of melatonin was shown to increase testosterone (T) concentrations in male Shiba goats (35), with a statistically significant difference observed between the melatonin and control groups ($P < 0.05$). The knowledge that melatonin administration affects circulating hormone concentrations is supported by these findings, which are consistent with those of (42), who found that Turkish Saanen goat bucks treated with melatonin implants had higher levels of both melatonin and testosterone. There were

significant differences ($P < 0.0001$) in scrotal circumference, with the MS buck having the largest mean (37.8 cm), followed by D (34.4 cm), M (32.5 cm), DS (31.5 cm), and the Control bucks having the smallest (29.7 cm). The interventions had a significant effect on the bucks' reproductive physiology, as evidenced by the significant P-values for treatment effects on melatonin, testosterone, and scrotal circumference. The higher melatonin levels in the MS, M, and DS males imply that circulating melatonin was raised by exogenous melatonin administration (in MS and possibly M) and decreased daylight exposure (in DS). The MS male had the highest testosterone levels, suggesting that melatonin may have a beneficial effect on testosterone production. The Control male lower testosterone level highlights how the treatments affect hormonal profiles. In addition, compared to the untreated control, the treated groups' larger scrotal circumference, especially that of MS and D indicates that melatonin and/or darkness have a positive impact on testicular development.

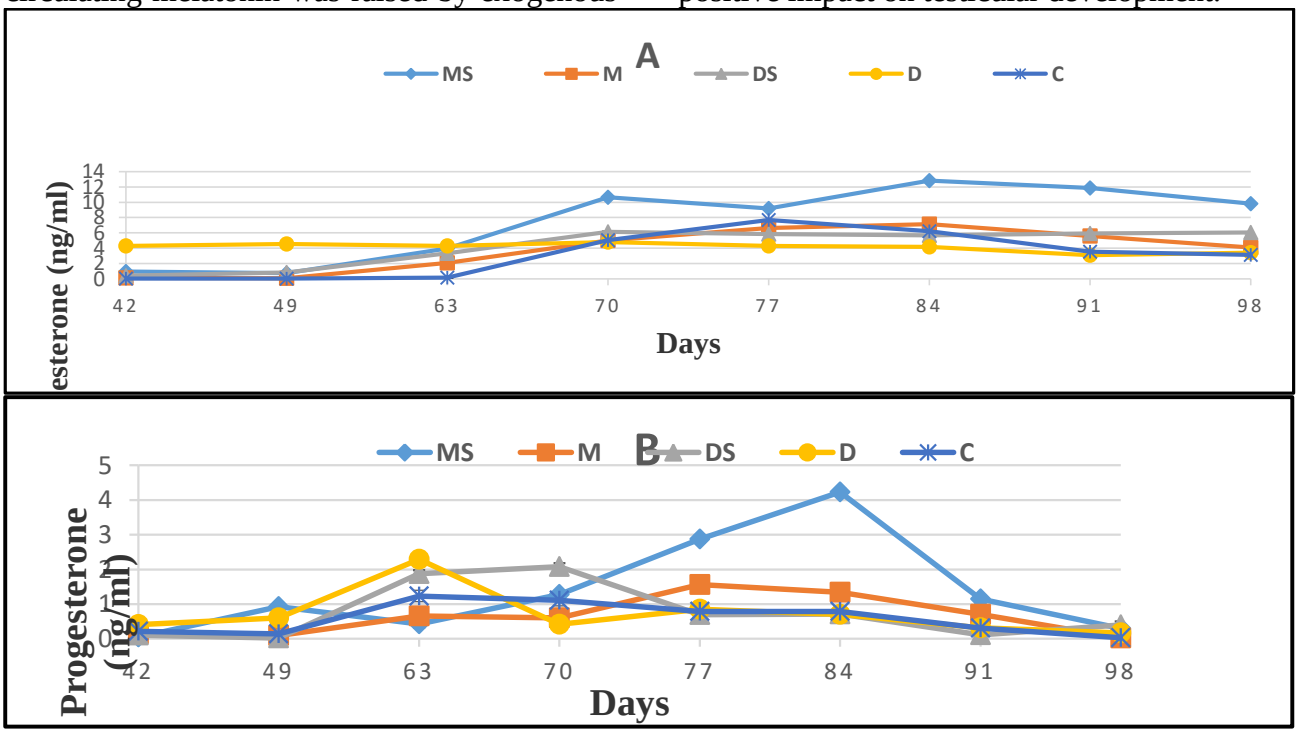

Figure 4. Least square means (±SEM) of plasma P4 concentration post sponge treatment for: (A) Pregnant Jordanian does (♦) MS, n= 4; (■) M, n = 4; (▲) DS, n = 3; (●) D, n=2; (×) C, n = 2; and (B) none pregnant does (♦) MS, n= 2; (■) M, n = 2; (▲) DS, n = 3; (●) D, n = 4; (×) C, n = 4.

This study demonstrates that melatonin, particularly when combined with progesterone sponges, can be a valuable tool for enhancing reproductive performance in local Jordanian goats outside their typical breeding season. Notably, the finding that controlled darkness (DS, D) can stimulate natural melatonin production to levels similar to implantation offers a practical, non-invasive approach. Future research with larger cohorts will strengthen these findings.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

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