



Effect of Melatonin on Ovarian Activity, Hormonal Level, and Reproductive Performance in Local Iraqi Ewes

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ABSTRACT

This study aimed to investigate the effect of dietary supplementation with Melatonin on the hormonal evaluation and estrus performance in local Iraqi ewes. This study was conducted at Safwan/ Al-Basrah Governorate during the period 1/9/2025 until 1/3/2026. Ten multiparous ewes with two breeding rams with average age 2-3 years and the weight of the animals average (30±6) kg, which isolated for 15 days before the start of the experiment and examined by trans-abdominal ultrasound device to ensure that they were empty. The animals were divided equally into two groups randomly (control group G1 n=5) without treatment and (experimental group G2 n=5) were treated by a single dose/day of melatonin 20 mg/kg/day orally by capsule in evening for 30 days. The results showed no significant difference in the level of Follicle-Stimulating Hormone G1, G2 at (0) day. While the results illustrated the level of Follicle-Stimulating Hormone increased significantly ($P<0.05$) values at (15, 30 and 45) days in G2 compared with G1. The Estrogen level also showed no significant difference at day 0 in both groups. On the other hand, the Estrogen level increased significantly values ($P<0.05$) at (15, 30 and 45) days in G2 compare with G1. The Luteinizing Hormone level showed no significant difference at day 0 in both groups, however the results showed increased significantly values ($P<0.05$) of Luteinizing Hormone at (15, 30 and 45) days in G2 group compare with G1. Furthermore, the results showed that the estrus rate was in G2 group (100%) compared with G1 (80%). Also, the results showed significantly values ($P<0.05$) at different time of mating compare between G2 and G1. Moreover, the result showed a significant difference ($p < 0.05$) in the prolificacy in number of kids compared between G2 and G1. In conclusion the study demonstrated that melatonin supplementation led to a significant elevation in hormonal levels compared to the control group. Furthermore, the melatonin-treated group exhibited higher pregnancy and live birth rates, alongside improved reproductive parameters and timing. These findings suggest that melatonin plays a beneficial role in enhancing reproductive outcomes.

INTRODUCTION

Sheep production is a core component of Iraq's livestock sector, supporting rural livelihoods through meat, milk, wool, and manure, and functioning as a risk-

buffer asset under variable climatic and market conditions. In sheep enterprises, reproductive performance (fertility, lambing rate, lamb survival, and weaning rate) is the main driver of flock productivity and profitability; therefore, improvement strategies must integrate genetics, nutrition, health, and reproductive management (Hafez & Hafez, 2000; Noakes *et al.*, 2018).

Iraq's sheep population is largely composed of fat-tailed breeds typical of West Asia. The Awassi (and related local strains/ecotypes) is among the most widely recognized breeds across the Levant and Mesopotamia and is valued for hardiness, milk potential under low-to-medium input systems, and adaptability to heat and limited forage (Galal *et al.*, 2008; Gootwine, 2011).

Most sheep breeds exhibit some degree of seasonal breeding, with reproductive activity influenced by photoperiod and modulated by nutrition and environmental stressors (Hafez & Hafez, 2016). In hot climates, heat stress can impair ovarian function, reduce estrus expression, compromise oocyte/embryo quality, and decrease pregnancy rates — effects that may be magnified when combined with undernutrition and disease challenges (Sejian *et al.*, 2012; Abbas *et al.*, 2024). Nutritional status is a primary determinant of reproductive success. “Flushing” (short-term improvement in energy intake prior to and during mating) can increase ovulation rate and conception in many flocks, while chronic deficits increase anestrus and reduce lamb viability (Robinson *et al.*, 2006; Kenyon *et al.*, 2014).

Many researchers have agreed that ewes are among the seasonal polyestrous animals (Arsoy and Sağmanligil, 2018), and they are named on the basis of multiple cycles of estrus during breeding season. The number of estrus cycles per breeding season ranges between 2-3 estrus cycles (Hafez & Hafez, 2016),

While seasonality is a definitive trait in many temperate sheep breeds, other researchers have found that estrus cycles in ewes can continue throughout the months of the year in some countries as the seasonal phenomenon disappears. (Kridli *et al.*, 2007).

Melatonin (N-acetyl-5-methoxytryptamine) is a highly conserved, naturally occurring indoleamine hormone produced by almost all living organisms. In mammals, it acts as the primary mediator of biological rhythms (circadian and circannual). Beyond its role as a “biological clock,” melatonin is recognized as a potent endogenous antioxidant, a cytoprotective agent, and a modulator of the immune system (Reiter *et al.*, 2016; Habeeb *et al.*, 2026).

Melatonin exerts both direct and indirect influences on ovarian function. Folliculogenesis: Locally, melatonin in the follicular fluid enhances follicular development, increases the ovulation rate, and promotes the health of granulosa cells (Zhang *et al.*, 2022). The goal of this study was to evaluate the effect of melatonin on hormonal levels (FSH, LH, and estrogen) and estrus performance (estrus rate and estrus fertility).

MATERIAL AND METHODS

Ethical approval

This study was approved by the institutional Review (University of Basra/ College of veterinary medicine). the animal care and experimental procedures complied with the relevant institutional and national guidelines for the use of experimental animals. Protocol No. 130/37/2026.

Experimental Animals and Management.

Ten multiparous ewes with two breeding rams that average age 2-3 years and weight average (30±6) kg. Animals were isolated for 15 days before the start of the experiment and examined by trans-abdominal ultrasound (TAU) device to ensure that they were empty. All necessary supplies were available in the field, including sheds and tools to conduct the experiment. The animals were housed in good management conditions and barley was given. A therapeutic and preventive program and a vaccination program were installed. The experiment was conducted at Safwan/ Al-Basrah Governorate during the period 1/9/2025 until 1/3/2026.

Experimental design

Fifteen days post-treatment with vaccines and anthelmintics, the animals were divided equally into two groups randomly (control group G1 n=5) without treatment and (treated group G2 n=5), G2 group were treated by a single dose/day of melatonin 20 mg/kg/day orally by capsule in evening for 30 days (Hafez and Hafez, 2000). After 30 days Estrus synchronization was achieved by applying intravaginal sponges for 14 days then rams were introduced for heat detection, mating and evaluation estrus performance (No. Estrus ewes and Estrus fertility rate). Ewes underwent ultrasonic examination at days (35, 45 and 60) post-mating by trans-abdominal ultrasound, to evaluate reproductive performance (No. Pregnant ewes, No. of lambs born, lambing rate) after 150 days were estimated.

Hormonal Analysis

Blood samples were drawn 10 ml from the jugular vein using (18G) needles anticoagulant tubes from all groups at day (0) before treatment and after treatment (15, 30 and 45 days) to evaluate hormonal FSH, LH and Estrogen. The samples were immediately carried to a laboratory to be separated by centrifugation of the samples (4000 RPM for 15 min.) and stored at -20°C until assay. for this purpose, we utilize a commercial ELISA

kit (Monobind Inc., USA) which operates on the principle of quantitative competitive enzyme immunoassay. This solid-phase technique facilitates precise measurement through the interaction between native antigens and specific antibodies both immobilized and enzyme-conjugated to ensure accurate quantification of the hormone (Bancroft & Gamble, 2008; Ibrahim *et al.*, 2025).

Ultrasonography/ B-mode Ultrasonography

The examination was performed using two-dimensional (B-mode) ultrasound diagnostic technology. The chosen examination technique depends on the stage of pregnancy. Starting from the fifth week of pregnancy, a transabdominal convex transducer was used by placing a probe on the inguinal region and/or abdomen at frequencies of 3.5 MHz to detect pregnancy, including the gestational sac and embryo. The ewe was gently restrained in a sitting position.

STATISTICAL ANALYSIS

All statistical analyses were performed using GraphPad Prism software (San Diego, CA). Each experimental group in the study consisted of five. All experimental testing was carried out three times. A two-way ANOVA were t student test utilized and for each experiment to determine statistical differences. The threshold for statistical significance was set at $P\text{-value} < 0.05$. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$) (Alrafas *et al.*, 2023).

RESULT

The results show that the level of Follicle-Stimulating Hormone at day 0 was not significantly different between groups, but the level of FSH increased significantly at (15) day in G2 compare with G1. Furthermore, at (30) days also increased significantly in G2 compare with G1 group. On the other hand, the study shows the means of FSH increased higher significantly in G2 group compare with G1 group at (45) days in supplement as show in (figure 1). The Estrogen level of our study also showed non-significantly at day (0) in both groups. Moreover, at day (15) the Estrogen level increased significantly in G2 compared with G1. Also, at day (30) the level of Estrogen hormone estimated increased significantly in G2 compare with G1. As well as the study appear increased significantly of Estrogen hormone in G2 at day (45) compare with G1 as show in (figure 1). The Luteinizing Hormone level showed no significant difference at day 0 in both groups, however the study showed increased significantly at day (15) in

G2 compare with G1, as well as the level of LH also significantly at (30 and 45) days in G2 compare with G1, as show in (figure 1).

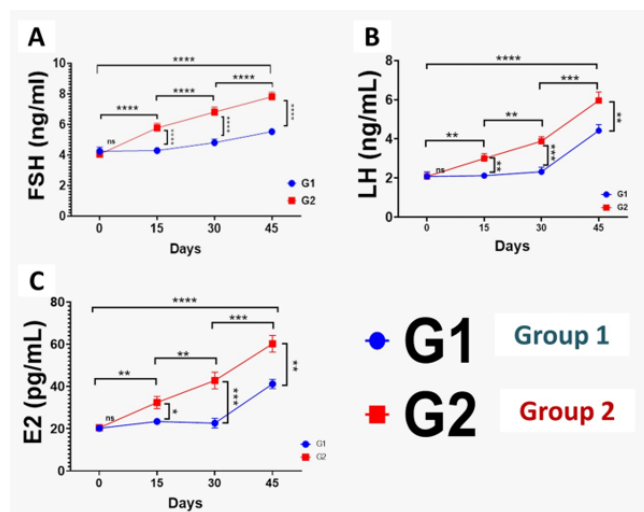


Figure 1: Reproductive parameters hormone in experimental ewes. Indicates a statistically significant difference ($p < 0.05$, $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$; ns = not significant).

Estrus performance and Time of mating analysis:

The results showed that the estrus rate was in G2 group (100%) compared with G1 group (80%), Furthermore, the significantly different at different times of mating in G2 group compare with G1 group as show in (Figure 2).

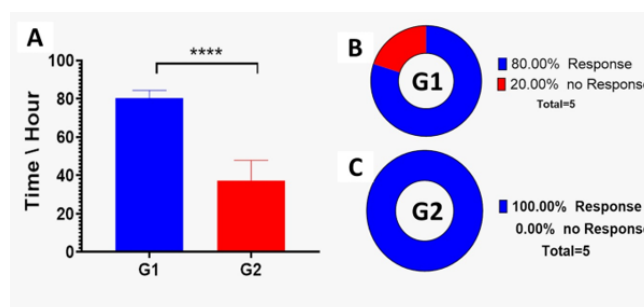


Figure 2: Effect of melatonin on Estrus Expression and Time of mating. **** Indicates a statistically significant difference (**** $p < 0.0001$).

Reproductive performance (No. of fertility ewes' rate%) and No. of lambs born analysis

The result of present study showed that the pregnant ewes were equal in both groups G1, G2 and while No. of lambs born revealed a significant difference in the prolificacy of G2 in number of kids compare with G1 as shown in (Figure 3).

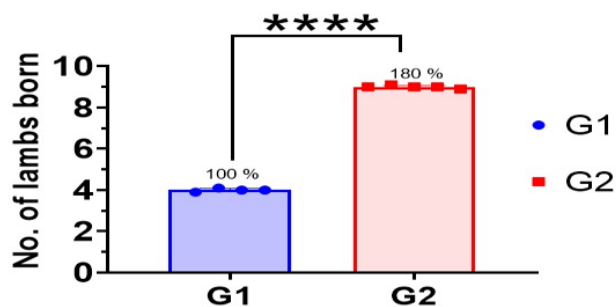


Figure 3: Effect of melatonin on Reproductive performance (No. of fertility rate (%)) and No. of lambs born **** Indicates a statistically significant difference (**** $p < 0.0001$).

Pregnancy detection by ultrasonography

The results showed by using ultrasonography in both experimental groups at 35 days observing the expansion of the uterine lumen and the presence of uterine fluids, both of which are definitive indicators of pregnancy, as shown in (figure 4).

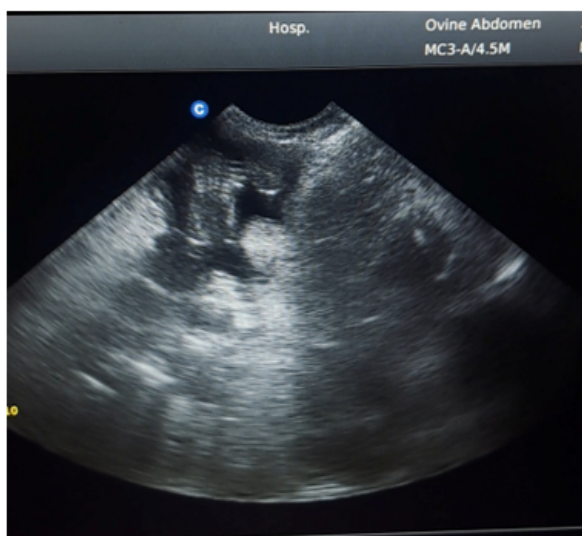


Figure 4: Expansion of the uterine lumen and the presence of uterine fluids

After 45 days post-mating the pregnancy reaches 100% accuracy. This is achieved through the detection of uterine placentomes, fetal heartbeats, and amniotic vesicles (figure 5), and allowed for the differentiation between single-fetus and twin pregnancies as shown in (figure 6).



Figure 5: Uterine placentomes



Figure 6: Twin pregnancies

DISCUSSION

The results of the current study show a significant improvement in hormone levels in ewes treated with melatonin. The gradual increase in follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels, particularly from day 15 to its peak on day 45, indicates that melatonin exerts a strong stimulatory effect on the hypothalamic-pituitary-gonadal (HPG) axis, these findings are consistent with those of Barrell et al. (2000), who demonstrated that melatonin regulates the secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus, which in turn stimulates the anterior pituitary gland to secrete FSH and LH.

The significant increase in FSH levels in group G2 compared to group G1 at day (45) is crucial for ovarian follicle recruitment and selection, this increase is directly related to the marked rise in estrogen levels in group (G2) and according to the “two cells, two gonadotropins” hypothesis, FSH stimulates aromatase activity within granulosa cells, converting androgens to estrogens (Fortune, 1994). The increased concentration of estrogen in the melatonin-treated group indicates improved follicular growth and steroid activity, two key indicators of improved ovarian function, this finding is consistent with (Chemineau *et al.*, 1992).

Furthermore, the significant increase in LH levels in group G2 at day (45) indicates that melatonin treatment contributes to reaching the LH threshold necessary for final follicular maturation and ovulation and these results are consistent with Abecia *et al.* (2002) and Malpoux *et al.* (1997) who suggests that melatonin treatment in small ruminants can mimic the effects of short day photo periods, thereby increasing the frequency of LH secretion and improving reproductive performance during transitional or anestrus seasons.

The time-dependent response observed in this study, where no statistically significant differences appeared until 15 days of treatment, suggests that melatonin requires a cumulative period to normalize hormonal feedback loops in ewes. Stabilizing these hormone levels at higher concentrations contributes to improved oocyte quality and increased ovulation rates, both essential components of reproductive performance these findings are consistent with those of Zuniga *et al.*, (2002).

The results of the current study showed that melatonin treatment (G 2) led to a significant increase in the estrus rate (100%) compared to the control group (80%). More importantly, the melatonin significantly reduced the “coitus time” (time until estrus) from 80 hours in G 1 to 37 hours in G 2. This accelerated onset of estrus is directly attributed to the previously observed elevated levels of FSH and LH. These findings are consistent with the study by Abecia *et al.*, (2002), who found that melatonin affects the tubular region of the pituitary gland, thereby stimulating faster pre-ovulatory follicle growth, which in turn secretes the estrogen necessary to initiate the behavioral estrous cycle. Also, the results showed the reduced mating duration suggests that melatonin acts as a potent regulator of the reproductive cycle in ewes, a finding of significant importance for the management of artificial insemination programs.

While fertility rates reached 100% in both groups (compared to ewes exhibiting signs of estrus), the biological significance lies in the efficiency of the repro-

ductive process. This finding aligns with Forcada *et al.* (1995), who established that melatonin enhances fertility by promoting corpus luteum quality and increasing progesterone secretion, which is crucial for maternal recognition and embryo survival. Moreover, these results confirm that melatonin provides a stable hormonal environment conducive to conception, even under varying environmental conditions.

The most significant finding of the study regarding reproductive performance was the substantial increase in the number of lambs born in the G 2 (9 lambs from 5 ewes) compared to the G1 (4 lambs from 4 ewes). This indicates a higher fertility rate or twinning rate in the melatonin-treated group. This increase in the lambing rate is primarily attributed to the increased concentration of FSH and LH hormones, which stimulate polyovulation (the ovulation of more than one follicle). These findings are consistent with those of Casao *et al.*, (2010), who found that melatonin reduces oxidative stress in the follicular fluid, thus protecting the ova and supporting the development of multiple dominant follicles. On other hand, according to Rosa & Bryant (2003), melatonin treatment increases the ovulation rate by approximately 20–30% in several sheep breeds, a phenomenon clearly demonstrated by the 1.8 lambings per ewe in the second group (G2) compared to 1.0 in the first group (G1).

Thirty-five days after mating, the observation of uterine cavity dilation and accumulation of non-echoic uterine fluid in both groups (Figure 4) is the first conclusive physiological evidence of a successful pregnancy. During this stage, the embryonic vesicle becomes large enough to be observed as a dark (non-echoic) fluid-filled area within the uterine horn. The results, agreement with (Karen *et al.*, 2014) show that detecting amniotic fluid at this stage is a highly reliable indicator, as the volume of amniotic fluid increases rapidly to support fetal growth. Furthermore, this fluid not only serves as a diagnostic marker but also forms a protective environment that facilitates nutrient exchange, reflecting the success of melatonin treatment in maintaining early pregnancy.

Furthermore, in day 45, the diagnostic accuracy reached 100%, as evidenced by the appearance of the placentome, fetal heartbeat, and amniotic sacs (Figure 5). The appearance of the placentome is a crucial indicator of proper placental development and efficient nutrient transfer between the ewe and the fetus. Romano & Christians (2008), indicated that the clarity of these structures between days 40 and 50 of gestation is crucial for predicting pregnancy outcomes, which aligns with our findings. Furthermore, the presence of a well-

defined placenta in the melatonin-treated group (G2) is associated with elevated estrogen and progesterone levels resulting from melatonin regulation of the corpus luteum.

One of the most important findings of this study was the ability to differentiate between singleton and twin pregnancies at day 45 (Figure 6). The identification of multiple amniotic sacs and embryos in the second group (G2) using ultrasound imaging is consistent with previous statistical data showing a significant increase in the number of lambs born (9 lambs in the second group versus 4 in the first group). The phenomenon of twin pregnancies is biologically linked to elevated levels of follicle-stimulating hormone (FSH), as observed in hormonal analysis. Furthermore, Vazquez *et al.*, (2010) and Mosa and Faraj (2021) confirmed that high levels of this hormone during the follicular phase led to the polarization of multiple dominant follicles, resulting in multiple ovulations. Consequently, ultrasound imaging on day 45 provides visual confirmation of melatonin's ability to enhance fertility rates in ewes.

CONCLUSION

The study suggest that melatonin supplementation led to a significant elevation in hormonal levels compared to the control group. Furthermore, the melatonin-treated group exhibited higher pregnancy and live birth rates, alongside improved reproductive parameters and timing. These findings suggest that melatonin plays a beneficial role in enhancing reproductive outcomes. The practical implication for sheep reproductive management in Iraq is that oral administration of melatonin can replace or complement implant-based protocols within the local production system; therefore, we recommend adopting this treatment for field application.

NOVELTY STATEMENT

The novelty of our study lies in understanding the effects of melatonin on hormonal assessment (FSH, LH and estrogen) and estrous performance (estrous rate presentation and estrous fertility) in local Iraqi ewes.

AUTHOR'S CONTRIBUTION

Each of these authors contributed equally

Statement regarding generative artificial intelligence or AI-assisted technology

The authors declare that no generative artificial intelligence was used in the preparation of this manuscript.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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