

# The Effect of the Melatonin hormone on the Performance of the Ovaries in Naimi Sheep when Exposed to Different Periods of Light

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**Abstract:** *The study of the hormone melatonin and hormone progesterone in the serum of the blood of Naimi ewes sheep is carried sheep by exposing groups of ewes to different periods of industrial light between May and the end of August just before the beginning of the normal breeding season. The results showed that melatonin concentration when the group exposed to short photoperiods (8L: 16D), was the highest and reached  $(60.55 \pm 1.55)$  pg / ml compared to the control group prone to natural lighting where melatonin hormone concentration scored  $(39.77 \pm 3.57)$  pg / ml. The differences of concentrations of the hormone melatonin are significant when compared to other groups in the last two weeks while reaching the average concentration of melatonin values in the group, which is subjected to a long period of light (16L: 8D) to the lows  $(36.66 \pm 4.86)$  pg / ml and similarly with the concentrations of the hormone progesterone which it was at its lowest point in a long group  $(0.29 \pm 0.39)$  ng / ml while it was at the top level in the short group  $(0.66 \pm 0.76)$  ng / ml. The results obtained showed a positive correlation between the internal origin melatonin concentration hormone and the length of the optical period and increase reproductive efficiency when Naimi sheep exposed to short photoperiods compared to control groups exposed to longer photoperiod.*

**Keywords:** photoperiods – Melatonin – Neami Ewes

## 1. Introduction

Naimi sheep constitutes one of the main strains that are raised in the Arabian Peninsula, the Levant and Iraq besides the strains of Hari and Nagdi (5). The Naimi sheep are regarded as low in reproductive efficiency compared to many other sheep, as the number of births is estimated to be among 115 to 117 births per a hundred ewes (4), while the average births exceeds 200 when Romanov and Alkiosand Marino German meat reaches 400 births per a hundred sheep in Finn sheep (1). Epstein believes that the decline in reproductive efficiency is related to the attention of breeders in the past for the size qualities and milk production and neglect reproductive efficiency (8) in addition to the preference of single births under nutrition conditions in poor pasture (2). The fertility of sheep constitutes one of the main factors affecting the success of sheep breeding since it is the main determin factor for the number of births and thus the amount of the produced meat (9). The importance of reproductive efficiency of sheep is shown through that you cannot get milk until after the birth process and you cannot get an abundance of meat except after multiple births (14).

Naimi sheep are characterized by its seasonal breeding and its the pattern of reproduction periods includes sexual activities, followed by a seasonal breeding out-offs. The sheep, before and after the breeding season become stable or sexually idle for its sexual efficiency appears to be more evident during the summer months (June - September) (3) when the estrus cycle begins and continues, in generally, for (14-19) days in the late summer and early autumn (4) and extends up to the early winter, when the separation of reproduction becomes more distinctive and shorter in latitude areas away from the equator, while reproductively

continues throughout the majority of the rest of the season at the equator where seasonal variations are less obvious and less distinctive (4).

The photoperiod is regarded as the most important environmental factor affecting the living organisms and ensures its continuation (6) since the photoperiod plays a temporary role in the natural environment of this internal rhythm of the neuroendocrine sexual activity (7). Previous research studies showed that sheep adapt its reproductive cycle to photoperiod changes (4) where they become active sexually at times at short daylight and long night: Short-day breeders, while the temperature of the environment, and nutritional status and exposure to the rams may be modified (10) the ratio between the length of the night and day length is a determining factor for reproduction regulation (11) (16). Therefore several researches are conducted to control breeding season of ewes in Mirino and Suffolk sheep. The most important use of which is the different lighting programs (12) or injecting using Awassi hormone melatonin (13). Reproduction can be activated depending on the previous history of exposure of different periods of light (15) (17). The use of external melatonin hormone improves the breeding capability in non estrus ewes and start reproduction season and works to promote ovulation rate and the appearance of estrus cycles (18) and warns its impact of the toxic impact and its remaining side effects (19), i.e., it has the ability to ovarian stimulation, but was not able to synchronize the timing of ovulation and estrus in the ewes (20). A previous study showed that estrus is linked to the melatonin hormone. There is also synergistic effect between the hormone melatonin is secreted by the pineal gland and hormone progesterone in ovarian stimulation in sexual inactivity season and repeating cycles of heat in the ewes and improves the breeding season (21-22) it is noticed that the

importance of stimulating internal hormone Origin impact different light periods is to reduce the costs and to avoid negative side effects as much as possible and specially the side effect of the externally oriented melatonin hormone which had very long toxic periods, difficult to be avoided or eliminated, when injecting it.

#### **The objectives and the importance of research:**

The scarcity of hormonal studies, especially melatonin on sheep Naimi in terms of changes in the concentration of this hormone with lighting changes and their impact on reproductive efficiency. This also applies to the progesterone hormone which is regarded to be the indicator of the occurrence the oestrus and its cycles.

The research aims to study the effect of photoperiod changes on the concentration of the melatonin hormone and endogenous progesterone in the blood of Naimi ewes serum and its impact on the reproductive efficiency and the frequency of heat outside the traditional breeding season.

## **2. Materials and Research Methods**

### **1. Experimental Animals**

This research was carried out on the Naimi ewes reared in Saudi Arabia, and which come second to Al hari and Najdi sheep in terms of demand for breeding (for its weak reproductivity efficacy) and palatability of its inedible meat (a high proportion of fat in the carcass), two-year old ewes are selected and already had birth, i.e. fertile. The nearly the same weights (54-60) kg before the normal breeding season. They were isolated and divided into six groups, of four in and left in a constant temperature of about  $25 \pm 2^\circ \text{C}$ . The ewes were exposed to artificial lighting as strong as LX 300 for 60 days before estrus period. Three rams were also selected randomly and left with fertile ewes in the pollination season after the end of exposure to artificial lighting.

#### **Lighting programs for each group**

- A- A witness SH group: offered natural lighting.
- B - A group offered lighting neutral (12L: 12D) M. (Light: Dark)
- C - A group offered mutual lighting SL1 been changed then the light has been changed in the middle of the experiment (short (8L: 16D) -to a long-lighting (16L: 8D)).
- D-A group offered mutual lighting LS2 then abruptly changed in the middle of the experiment from long lighting (16 L: 8D) -to a short lighting (8L: 16D)).
- H -A group offered short lighting (8L: 16D) S. Short day.
- (F) A long group offered long lighting (16L: 8D) L. Long Day.

Blood samples were taken weekly from the jugular vein. A nutrition free Ad libitum contained hay and straw and ready-made forage center from, the National Company, which consists of undigested protein 14.90%, equivalent starch 60.7%, calcium 0.73, phosphorus 0.76%, vitamin (A)  $15,000 \text{ IU / kg}$ , vitamin (D3)  $3000 \text{ IU / kg}$ , vitamin unit (E)  $3 \text{ IU / kg}$ .

### **2. Blood samples**

Blood was taken in test pips, and then transferred pot to the laboratory, in a cooling container to precipitate blood then preserved serum at  $-20^\circ \text{C}$  for a time to estimate the concentrations of the melatonin hormone in the serum using enzymatic immunoassays, ELISA (ELISA), technology. The serum of melatonin hormone and progesterone hormone had been estimated and analyzed five weeks before the beginning of the experiment.

### **3. Melatonin Analysis**

The serum analyzed simultaneously using enzymatic immunoassays, (ELISA) using the method Micro plate ELISA and use Automatic Immunodiagnostic Analyzer Model of Personal LABBrioche Immunossystems Italian company. The concentrations of the melatonin hormone are estimated in the blood serum of the ewes experiment using the method: Enzymatic immunoassays, (ELISA) by the way of Micro plate ELISA. Kate using the melatonin hormone IBL HAMBURG DRG Diagnostic EIA-1431.

### **4. Progesterone Analysis**

The concentrations of the progesterone hormone in the blood serum of ewes are estimated using progesterone Kate hormone Novatec Immuno diagnostic GmbH (DNOV006) (German).

The experiments were designed according to Randomized Block Design. Data were analyzed using ANOVA variance, F test and comparing the averages using less significant difference test LSD 5% and Duncan test at the level of 5%, 1%, 0.1%, using the statistical program SAS System for Mixed Models (1996).

## **3. Results and Discussion**

### **1. Study the total concentrations of the melatonin hormone for groups:**

The total average of melatonin  $\pm \text{SE}$  to the control group ( $39.77 \pm 3.57$ ) pg. / ml and reached the highest value at the prone group of minimum illumination ( $60.55 \pm 1.55$ ) pg. / ml, while the lowest value for long lighting reached ( $36.66 \pm 4.86$ ) pg / ml. The concentrations were close to each other in (LS, M,) groups (Table 1). It was noticed that in the photovoltaic short period the melatonin was at the highest levels and it is well established that the secretion of melatonin in the blood responds with changes in the length of the light period and density as it was stimulated by darkness and discouraged by light in all species (23). This shows that there are seasonal changes in the secretion of the melatonin hormone (high level of the melatonin hormone in the winter) and there is a positive daily relationship with the dark period.

The results indicate the presence of significant differences between the groups with each other as well as the matter of weeks of experience as those moral differences were observed between the groups overlap and their relationship to time.

**Table 1:** Overall focus of the averages of melatonin by (the Group)

SE±Mean	The total mean of Melatonin pg/ml	Group
(39.77±3.57)	39.774	SH witness
49.72±3.03	49.72	M neutral
59.62±4.72	59.619	SLshort - long
46.14±4.25	46.14	LSshort - long
(1.55±60.55)	60.558	S short
(36.66±4.86)	36.666	Llong

#### Statistical study of the melatonin hormone:

The results indicated the presence of significant differences between the groups with each other as well as the matter of weeks experience as those moral differences were observed between the groups overlap and their relationship to time (Table 2)

Table 2: analysis of variance of melatonin

Average Squares	degrees of freedom	Sources of variation
**768.00	7	Time in weeks
**3662.46	5	Groups
**492.25	35	Overlap between time and treatment
20.62	240	Random errors

\*\* Very significant - differences \* significant difference - NS not significant

Highly moral impact is noticed for both groups and the time and the overlap between them at the level of melatonin. And show a significant decrease in the proportion of melatonin progress is shown throughout the experiment as it ranged between 35.048 and 53.647 in the fifth week of the experiment and at the beginning of the experiment, respectively. The short sets and short-long are clearly shown at the highest level of melatonin which amounted to 60.558 and 50.619 respectively, while the lowest level of melatonin the long group reached (36.666). By conducting Duncan test, the presence of significant differences is shown between (Time X Group) at the level of melatonin, reaching the highest (98.120) in the short-long group of seventh week and the lowest (16.803) in the group long-short in the fifth week of the experiment (Table 3.0).

**Table 3:** Shows the influencing factors: time and group and overlap in the level of melatonin

Experimental Error	Overlap	Group	Time (A Week)	Affecting Factors
132	35	5	7	Degrees of freedom
20.624	**492.247	**2956.48	**768.004	Average squares
	6.861	2.320	2.801	lsd Value(0.05)

## 2. Study the total concentrations of the Progesterone hormone of the groups

The average for the witness to progesterone limits reached (0.46 ± 0.58) ng / ml and the highest value at the short group reached (0.66 ± 0.76) ng / ml while the lowest value at long (0.29 ± 0.39) ng / ml and the concentrations were greater than 0.5 ng / ml in groups (LS, M, SH). Significant differences between the groups with each other are noticed as well as the matter of weeks of experience those

differences approached the morals by overlapping between the groups and their relationship of time (Table 4).

**Table 4:** The average concentration of progesterone by the transaction

SE±Mean	Total average ng/ml	Treatment
0.46±0.58	0.4623 AB	SH witness
0.52±0.40	0.5167 AB	Mneutral
0.30±0.36	0.3006 B	SLshort - long
0.48±0.54	0.5319 AB	LSshort - long
0.66±0.76	0.6623 A	Sshort
0.29±0.39	0.2885 B	Llong

Ranking: (short) - (Alsarahh / neutral / long -short) - (long-short / long).

#### Statistical Study of progesterone hormone

The results indicate the presence of significant differences between the groups with each other as well as the weeks the of experiment (Table 5)

**Table 5:** Analysis of Variance for progesterone

Sources of variation	Degrees of Freedom	Average squares
Times in weeks	7	**3.62
Transactions	5	*1.00
Overlap between time and treatment	35	NS 0.43
Random errors	240	0.45

\*\* Very significant - differences \* significant difference - NS not significant

The results obtained showed the presence of highly significant P <0.05 for a time in the concentration of progesterone as it ranged between 0.031 and 0.806 at the beginning of the experiment and in the last week, respectively. As it turns out the existence of a significant effect at the level of P <0.05 for the group at the level of progesterone, as characterized by a short set at the top level of progesterone, which amounted to 0.662. Despite the absence of significant effect of interference in the level of progesterone, it is shown through Dunkin test, that there for clear and significant differences between the (time X group) at the level of progesterone, reaching the highest (1.768) in a short set for the week X and the lowest (0.012) in the two groups Tall- short at the beginning of the experiment and long in the first week of experiment (table 6).

**Table 6:** Shows the affecting factors: time and group and overlap in the level of melatonin

Affecting Factors	overlap	Group	The time (a week)	Experimental error
Degrees of freedom	35	5	7	240
Average squares	0.4344	* 0.9994	** 3.6247	0.4454
Value lsd(0.05)	0.759	0.268	0.310	

## 3. Study the concentrations changes of the melatonin hormone and Progesterone at SH Group (the witness):

The average melatonin hormone concentration amounted the witness (50.70 ± 0.73) pg / ml before the beginning of the experiment W0 (week zero) and no significant differences with the rest of the group arrived in the tenth week under the



influence of natural lighting period to  $(32.68 \pm 2.78)$  pg / mL, when the day began decreasing gradually after 22/6 (in the fifth week of the experiment) and natural lighting hours were around  $(14.5L: 9.5D)$  in the experiment zone and reached the melatonin hormone reached on the summer solstice (the longest day) the lowest  $(29.85 \pm 9.74)$  pg/ ml. While the average hormone progesterone concentration reached at the witness  $(0.025 \pm 0.03)$  ng / ml before the start of the experiment W0 (week zero) where there were no estrus cycles at the end of spring. And it reached in the ninth week, its highest level and the differences of individual large under the influence of photoperiod natural to  $(0.933 \pm 1.05)$  ng/ ml and arrived in the second week to the concentration of  $<0.5$  ng / mL  $(0.738 \pm 1.14)$  ng / mL indicating a rut sessions were values  $<0.5$  in the fifth and sixth week  $(0.805 \pm 0.86)$  ng / mL,  $(0.575 \pm 0.64)$  ng / mL, respectively.

#### **4. Study the concentrations changes of the melatonin hormone and progesterone hormone at M Group**

The average of melatonin hormone concentration at Group M reached  $(53.22 \pm 1.59)$  pg / ml before the start of W0 experience and without significant differences with the rest of the groups in the fifth week reached  $(51.56 \pm 3.66)$  pg / mL, and stable its levels stabilized in the following weeks under the influence of neutral light ( $12L: 12D$ ). It resembles the equator areas where the length of the day and night are equal with, and of breeding season of ewes is not clear and undistinguished as it lasts all year long without significant changes in the secretion of the melatonin hormone. While the average of progesterone hormone concentration at Group M reached  $(0.022 \pm 0.01)$  ng / ml before the start of W0 experience and at in the fifth week reached the highest level  $(1.125 \pm 0.29)$  ng / ml. Previous studies show that progesterone concentration that reaches the highest level 1 ng / ml in a single sample determines the beginning of the breeding season, according to Sweeney and O'callaghan. The values reached in the sixth week reached to more than 0.5 ng / ml, and then decreased as a witness of the end of the estrus cycle, but it soon rose in the tenth week to  $(0.780 \pm 0.61)$  ng / ml. It seems that estrus cycles were regular as it is observed a rise greater than 0.5 ng / ml in two weeks: the fifth and sixth and the ninth and tenth weeks and so on. This concurs with Kařatová J & Co., results who pointed out that the ewes in the neutral lighting period were active reproductively and melatonin has a role in the extension of the period of sexual activity, but the timing was not stated for the estrus cycles.

#### **5. Study the concentrations changes of the melatonin hormone and Progesterone with SL1 group**

The average melatonin hormone concentration with SL1 group reached the average of  $(55.67 \pm 0.93)$  pg / ml before the start of W0 experience and without significant differences with the other groups and increased hormone levels when the group was under the influence of a short light period and then got a sharp drop in the melatonin hormone levels in the fifth week when changing highlighting abruptly from short to long and the concentration reached was the focus  $(22.80 \pm 5.50)$  pg / ml, then increased greatly in the following weeks and then the levels go down at the end of the experiment to a level approaching the level of the beginning of the experiment

(W0) as it reached the focus of the week X  $(58.12 \pm 3.16)$  pg / ml.

While the average concentration of the progesterone hormone reached with LS1 group reached  $(0.033 \pm 0.03)$  ng / ml before the beginning of the experiment W0. The total progesterone concentration when the group was under the influence of lighting short period in the second week to the highest level  $(0.743 \pm 0.82)$  ng / ml occurrence of estrus cycles according to Donovan, et al.(1994). The levels began to rise in the tenth week. But it did not reach the levels above 1 ng / ml.

#### **6. Study the concentrations changes of the melatonin hormone and progesterone with mutual LS2 Group**

The average melatonin hormone concentration with mutual 2 Group LS  $(53.70 \pm 2.17)$  pg / ml before the beginning of the experiment W0 and without significant differences with the rest of the groups and decreased hormone levels when the group was under the influence of long light period in the second week  $(32.20 \pm 5.11)$  pg/ ml. Then it got a sharp drop in the melatonin hormone levels in the fifth week when changing lighting abruptly from long to short and reached focus  $(16.80 \pm 4.31)$  pg / ml, then increased in the following weeks to reach  $(62.24 \pm 5.96)$  pg/ ml in a week. While the average concentration of the progesterone hormone reached with the group LS2  $(0.012 \pm 0.01)$  ng / ml before the beginning of the experiment W0. The total progesterone concentration when the group was under the lighting long period impact of less than 0.5 ng / ml and this refers to the lack of estrus cycles while estrus cycle and increase the concentration of progesterone began when changing lighting period abruptly in the fifth and sixth weeks  $(0.833 \pm 0.60)$  ng / ml,  $(0.542 \pm 0.26)$  ng / ml, respectively, according to Donovan, et al.1994 [29] then it go back and go up in the ninth and tenth weeks  $(1.408 \pm 1.73)$  ng / mL,  $(0.883 \pm 0.75)$  ng / mL, respectively. It is noticeable that the levels were higher than 1 ng / ml in the ninth week, which determines the breeding season, according to Sweeney and O'callaghan.

#### **7. Study concentrations changes of the melatonin hormone and progesterone with Group S**

The average hormone melatonin concentration in Group S reached  $(55.61 \pm 2.12)$  pg / ml before the start of W0 experience and without significant differences with other groups and lasted concentrations to rise to the highest levels until the end of the experiment in the tenth week  $(71.14 \pm 1.83)$  pg / mL, and in a manner consistent with the dark period experienced by members of this group. While the average progesterone hormone concentration reached, with the Group S,  $(0.080 \pm 0.01)$  ng / ml before the start of W0 experience and reached in the second week and earlier for the rest of the group to the highest level  $(1.010 \pm 1.62)$  ng / ml, t.e, that is, it the beginning of the breeding season could be determined by the second week according to Sweeney and O'callaghan. The values in the fifth week, reached more than 0.5 ng / mL  $(0.833 \pm 0.60)$  ng / ml, and then decreased after that as an evidence of the end of the estrus cycle, but it soon rose in the tenth week reach its highest peak compared with the rest of the group  $(1.768 \pm 1.67)$  ng / mL. The results show that estrus cycles were regular and more frequent than the rest of the groups.

#### 8. Study the concentrations changes of the melatonin and progesterone hormones with Group L

The average hormone melatonin concentration in Group L reached ( $53.00 \pm 1.36$ ) pg/ ml before the start of W0 experience and without significant differences with the other groups. The concentration continued to drop to the lowest levels until the end of the experiment in the tenth week ( $18.32 \pm 6.91$ ) pg / mL, and in a manner consistent with the long light period which the members of this group were exposed to.

While the average of progesterone concentration reached with Group L reached ( $0.015 \pm 0.01$ ) ng / ml before the start of W0 experience and reached in the ninth and tenth weeks and is too late for other groups to ( $0.538 \pm 0.96$ ) ng / mL, ( $0.693 \pm 0.97$ ) ng/ ml respectively. The results show that estrus cycle was very late compared with other groups.

#### 4. Conclusions

The researcher reached the following conclusion:

The concentration of melatonin hormone decrease during the long period of lighting. The concentration of the melatonin hormone increase during the short period lighting. The animal retains as lighting memory that affect the concentration of melatonin when changing lighting periods.

The timing of the estrus cycles and the early breeding season are controlled by lighting changes.

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