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Effect of melatonin and melatonin plus progestagen protocols on some reproductive performance and blood parameters at the beginning of the breeding season in Hasmer ewes

Efecto de los protocolos de melatonina y melatonina más progestágeno sobre algunos parámetros de rendimiento reproductivo y sanguíneos al inicio de la temporada de cría en ovejas Hasmer

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ABSTRACT

The aim of this study was to investigate the effect of exogenous melatonin administered by different methods at the beginning of the breeding season on estrus onset, fertility, plasma progesterone and malondialdehyde (MDA) concentrations in Hasmer ewes. To this end, the ewes were divided into three groups: Group I (MEL): This group received subcutaneously 18 mg melatonin implant (Regulin™, Ceva–Turkiye) Group II (MELPRO): This group received subcutaneously 18 mg of melatonin implant (Regulin™, Ceva–Turkiye), followed by the intravaginal administration of a sponge containing 20 mg of flugestone acetate (Choronogest CR™–France) for a period of 9 day. This was followed by the intramuscular injection of 250 µg of cloprostenol (Senkrodin™–Vetas–Turkiye) as soon as the sponge was removed. Group III designated as the control group (CON), received 1 mL of saline was administered subcutaneously for placebo effect. Estrus was detected using teaser rams starting 24 h after the melatonin (Group MEL), $PGF_2\alpha$ (Group MELPRO) and physiological saline (Group CON) applications twice a day (06:00 and 17:00). Ewes in oestrus were moved to separate compartments and mated with fertile rams. Blood samples were collected from the vena jugularis on the day of treatment initiation, on the day of mating, and on day 17 of gestation. Pregnancy examinations were performed between 25 and 30 days after mating, and heartbeats were monitored using a 5–8 MHz linear trans–rectal probe in the groups (DP 50 VET™, Mindray Ltd. China). There was no statistical difference in the onset of first oestrus between the groups (P>0.05). A noteworthy finding was the increase in progesterone concentration on the 17th day of pregnancy in the group treated with melatonin alone, compared to other groups, at the beginning of the breeding season (P<0.05).

Key words: Onset of oestrus; malondialdehyde; melatonin; progesterone; reproductive parameters

RESUMEN

En este estudio se investigaron los efectos de la melatonina exógena administrada por diferentes métodos al inicio de la temporada de cría sobre el inicio del celo, la fertilidad, los niveles plasmáticos de progesterona y la concentración de malondialdehído (MDA) en ovejas Hasmer. Para este propósito, las ovejas se asignaron a tres grupos: Grupo I (MEL): Administración subcutánea de 18 mg de melatonina (Regulin®, Ceva–Turquía). Grupo II (MELPRO): Administración subcutánea de 18 mg de melatonina (Regulin®, Ceva–Turquía) + esponja intravaginal que contiene 20 mg de acetato de flugestone durante 9 días (Chronogest CR®, Francia) + al retirar la esponja, $PGF_2\alpha$ intramuscular (250 µg cloprostenol, Senkrodin®–Vetaş–Turquía). Grupo III (CON): Administración subcutánea de 1 mL de solución salina fisiológica para el efecto placebo. El estro se detectó utilizando carneros marcadores comenzando 24 horas después de las aplicaciones de melatonina (Grupo MEL), $PGF_2\alpha$ (Grupo MELPRO) y solución salina fisiológica al 0,09 % (Grupo CON) dos veces al día (06:00 y 17:00). Las ovejas en estro fueron trasladadas a compartimentos separados y apareadas con carneros fértiles. Se tomaron muestras de sangre de la vena yugular el día de inicio del tratamiento, el día del apareamiento y el día 17 de la gestación. Los exámenes de gestación se realizaron entre los días 25 y 30 posteriores al apareamiento, y los como signo de viabilidad embrionaria /primordio cardiaco se monitorearon mediante una sonda lineal transrectal de 5–8 MHz en los grupos. No hubo diferencia estadística en el inicio del primer estro entre los grupos (P>0,05). La melatonina aumenta la concentración de progesterona el día 17 de la gestación en la temporada de cría natural (P<0.05).

Palabras clave: Inicio del estro; malondialdehído; melatonina; progesterona; parámetros reproductivos

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INTRODUCTION

Melatonin is a pivotal regulator of the seasonal modulation of reproductive activity in numerous mammalian species [1]. Melatonin adversely affects animals that reproduce on seasonally longer days (d), while it has a affirmative effect on animals that reproduce on shorter d [2]. This hormone, a naturally occurring hormone that is synthesized in the pineal gland, has been shown to affect the hypothalamus, resulting in the secretion of gonadotropins during the transitional phase in small ruminats [3]. Melatonin has been demonstrated to regulate prolactin synthesis in lactotrophic cells and follicle–stimulating hormone (FSH) and luteinizing hormone (LH) synthesis in gonadotropic cells. [4, 5]. Melatonin has also been demonstrated to have a positive effect on follicular development and ovulation in sheep (*Ovis aries*) [6, 7].

Melatonin effectively reduces oxidative stress induced by various toxins by penetrating all intracellular structures to protect the cell membrane, organelles and nucleus [2]. Reactive oxygen species (ROS) are generated in follicles during the ovulation process, and thus oxidative stress can lead to poor oocyte quality. Melatonin reduces oxidative damage by increasing intrafollicular melatonin concentrations, improves oocyte quality and increases pregnancy rates [8]. The luteotropic effect of exogenous melatonin has a beneficial effect on the viability of the embryo [9]. Moreover, it was established that the number of blastocysts/cells, which is pivotal in the development of the embryo, exhibited an increase in melatonin–containing maturation environments. This increase in the number of blastocysts/cells has been shown to result in enhanced mitosis and improved embryo implantation [10].

The administration of physiological doses of melatonin has been demonstrated to induce the suppression of cortisol formation via the ACTH axis [11]. Cortisol elevates estrogen levels while reducing progesterone levels. Additionally, it increases oxytocin, which enhances myometrial contractions [12, 13], potentially impairing embryo implantation. In women, high cortisol levels can cause failed embryo implantation in *in vitro* fertilisation (IVF) and embryo transfer (ET) [14]. It has been documented that the administration of melatonin during d 7–12 of the oestrus cycle results in a substantial elevation of blood progesterone concentration in ewes [15, 16]. It is imperative to note that low cortisol and high progesterone concentrations are of significant importance for the successful implantation and viability of the embryo. Melatonin applications in ewes (*Ovis aries*) are generally carried out during the anoestrus and transition periods [17]. This study hypothesises that exogenous melatonin administration may improve embryo quality due to its antioxidant effect and protect pregnancy by increasing progesterone levels at the beginning of the breeding season. To investigate this, the effects of exogenous melatonin administered with different protocols at the beginning of the breeding season on onset of oestrus, fertility, plasma progesterone and malondialdehyde (MDA) concentrations were studied in ewes (*Ovis aries*).

MATERIALS AND METHODS

Animals

This study was carried out on 2–4 years old healthy Hasmer ewes (n=75) in Konya Bahri Dağdaş International Agricultural Research Institute. This area is located 37°51'26"N | 32°33'17"E and 1007 m higher from the sea level. The rations were arranged in accordance with the NRC (National Research Council) 2007 criteria, and alfalfa (200 g) and vetch straw (300 g), corn silage (1000 g), wheat and barley stalks (200 g) were added to concentrate feeds (500 g).

Protocols

The research was carried out during the beginning of the breeding season and 75 ewes were divided equally into three groups; Group I (MEL): This group received subcutaneously 18 mg melatonin implant (Regulin™, Ceva–Turkiye) Group II (MELPRO): This group received subcutaneously 18 mg of melatonin implant (Regulin™, Ceva–Turkiye), followed by the intravaginal administration of a sponge containing 20 mg of flugestone acetate (Choronogest CR™–France) for a period of 9 d. This was followed by the intramuscular injection of 250 µg of cloprostenol (Senkrodin™–Vetas–Turkiye) as soon as the sponge was removed. Group III designated as the control group (CON), received 1 mL of isosmotic saline was administered subcutaneously for placebo effect.

Estrus detection

Estrus was detected using teaser rams starting 24 h after the melatonin (Group MEL), $PGF_{2}\alpha$ (Group MELPRO) and physiological saline (Group CON) applications twice a d (06:00 and 17:00). Ewes in oestrus were moved to separate compartments and mated with fertile rams.

Blood samples

Blood samples were collected from the vena jugularis on the first d of treatment, the d of mating, and the 17th d of pregnancy from ewes in each group for the purpose of progesterone and malondialdehyde (MDA) analysis. The samples were then subjected to centrifugation at 2000 g for 10 min (EBA 20, Andreas Hettich ™ GmbH & Co. KG, Germany). The plasmas were stored (Bosch Deep Freeze, Germany) at -20°C until the d of analysis. The determination of plasma progesterone concentrations was conducted in accordance with the CLIA (chemiluminescent enzyme immunoassay) method, employing commercial kits (Artitect i1000 SR Abbott ™ U.S.) [18]. Progesterone concentrations of only pregnant ewes were evaluated. The intra – and inter–assay coefficients of variation (CV) for progesterone were 3.0% and 4.5%, respectively. The assay sensitivity was determined to be 0.05 ng·mL^{-1}.

Buege and Aust [19] established a spectrophotometric method to quantify MDA levels in serum. This method (Shimadzu UV 1700 ™, Japan) is based on the principle that MDA, a byproduct of lipid peroxidation, reacts with thiobarbituric acid (TBA) in an acid medium to form a pink–colored complex. The absorbance of the complex is subsequently measured spectrophotometrically at a wavelength of 536 nm, allowing for the assessment of lipid peroxidation levels. To prepare the stock solution, a 1:1:1 mixture of TBA (0.375% m/v), hydrochloric acid (0.25 N), and trichloroacetic acid (15% w/v) was used. Centrifuge tubes containing whole serum samples were then supplemented with 0.5 mL of this stock solution (totaling 1000 µL) and 10 µL of butylated hydroxytoluene. All tubes were thoroughly mixed using a vortex (M 16, Elektromag™, Turkiye). Subsequently, the tubes were incubated in a hot water bath at 95°C for 25 min. Following incubation, the tubes were rapidly cooled in an ice bath(M

48, Elektromag, Turkiye). They were then centrifuged (M 4800MR, Elektromag, Turkiye) at 7000 g for 5 min at 4°C. The supernatant obtained after centrifugation was carefully collected into separate test tubes for further analysis. The absorbance of the samples at 536 nm was measured using a spectrophotometer (Shimadzu UV 1700™, Japan). The extinction coefficient of the TBA–MDA complex at this wavelength ($\varepsilon = 1.56 \times 10^5$ $cm^{-1} \cdot M^{-1}$) was utilized to calculate the serum MDA concentration, which was expressed in $mol \cdot L^{-1}$.

Pregnancy diagnosis

Pregnancy examinations were conducted between 25 and 30 d post–mating, and fetal heartbeats were monitored using a 5–8 MHz linear (Mindray Ltd., China) trans–rectal probe. Ultrasound imaging was performed using the DP-50 VET device (Mindray Ltd., China).

Reproductive parameters

As reproductive parameters, time of first oestrus (sum of the d of oestrus in ewes/ the number of ewes showing estrus), estrous rate (the number of ewes showing estrus/the total number of ewes × 100), pregnancy rate (the number of pregnant ewes/the number of ewes mated × 100), single lambing rates (the number of ewes lambing singles/ the total number of ewes lambing × 100) multiple lambing rates (the number of ewes lambing twins or triplets/the total number of ewes lambing × 100), litter size (the total number of lambs/the total number of ewes lambing) were calculated [20].

Statistical analysis

The differences between groups in the average time to first estrus were analysed using the Kaplan–Meier test. Chi–square analysis was applied to compare groups in terms of estrous rate, pregnancy rate, single and multiple lambing rates, and litter size. Variations in progesterone and malondialdehyde concentrations were assessed using a one–way ANOVA test. The statistical analyses were performed using SPSS version 23 [21].

RESULTS AND DISCUSSION

The oestrous response, average time to first estrus, pregnancy rate, single lambing rate, multiple lambing rate, litter size, are given in TABLE I.

TABLE I
Reproductive parameters of ewes in melatonin only (MEL), melatonin + intavaginal progesterone + $PGF_{2\alpha}$ (MELPRO) and control (CON) groups

Variable	Groups		
	MEL	MELPRO	CON
n	25	25	25
Oestrous response (%)	96 (24/25)	92 (23/25)	96 (24/25)
Time to first oestrus (d)	15.21 ± 1.41	8.95 ± 2.16	13.87 ± 1.49
Pregnancy rate (%)	96 (24/25)	92 (23/25)	88 (22/25)
Single lambing rate (%)	62.5 (15/24)	52 (12/23)	72 (16/22)
Multiple lambing rate (%)	37.5 (9/24)	48 (11/23)	28 (6/22)
Litter size	1.38 (33/24)	1.48 (34/23)	1.27 (28/22)

When reproductive parameters were evaluated, the differences between the groups were not significant (*P*>0.05)

Despite the absence of statistically significant differences between the initial d of oestrus in the groups (*P*>0.05), it was observed that ewes in the MELPRO group exhibited oestrus in a shorter time frame. In this group, intravaginal sponges containing a combination of progesterone and melatonin were utilised, with $PGF_{2\alpha}$ being injected for luteolytic effects on the d the sponges were removed. Approximately 60% of the ewes in this group exhibited oestrus at the conclusion of the second d. In the control group (CON), oestrus manifested one d after the commencement of the study and concluded on the 36th d. In the MEL group, the initial estrus was observed 12 d after the administration and the estrus terminated on the 46th d (FIG. 1).

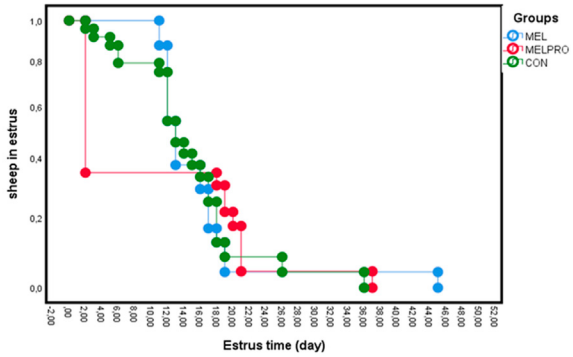

FIGURE 1. Days of oestrus of ewes in melatonin only (MEL), melatonin + intavaginal progesterone + $PGF_{2\alpha}$ (MELPRO) and control (CON) groups

Zarazaga *et al.* [22] reported that the administration of exogenous melatonin via subcutaneous injection resulted in a temporary delay in estrous activity in goats during the natural breeding season. In their study, Kusakari and Osaha [23] reported that the physiological condition of ewes in the early postpartum period caused a delay in reproductive response to melatonin administration in Suffok ewes. However, the suppression of gonadal function by lactation could be overcome by melatonin treatment. In this study, the observation that melatonin administration alone induced a delay in estrous activity is hypothesized to be attributable to melatonin's modulation of ovarian activity [22].

There was no difference between the groups in terms of the rate of pregnancy, multiple lambing and litter size. (TABLE I). Melatonin administration has been reported to increase both implantation and litter size ratios in some studies in mice and ewes [24, 25, 26]. Leyva–Corona *et al.* [27] reported that exogenous melatonin administration increased pregnancy rates in ewes in the anestrous season in northwest Mexico. Abd–Allah and Daghash [28] reported that exogenous melatonin is a useful application for improving reproductive performance in Ossimi ewes. The absence of statistical significance in reproductive parameters observed in the present study may be attributable to the timing of the ewes inclusion during the early stages of the breeding season.

In this study, progesterone concentrations at the beginning of treatment, the d of mating, and d 17 of pregnancy are presented in TABLE II.

TABLE II
Plasma progesterone and malondialdehyde (MDA) concentration of ewes in melatonin only (MEL), melatonin + intavaginal progesterone + $PGF_{2\alpha}$ (MELPRO) and control (CON) groups

	Groups	Progesterone (ng·mL^{-1}) ± SD	*P*	MDA (µmol·L^{-1}) ± SD	*P*
Start day of treatment	MEL	0.45 ± 0.26^{a}		1.32 ± 0.34^{a}	
	MELPRO	0.47 ± 0.33^{a}	NS	1.28 ± 0.10^{a}	NS
	CON	0.44 ± 0.27^{a}		1.33 ± 0.21^{a}	
Mating day	MEL	0.13 ± 0.06^{a}		1.19 ± 0.26^{a}	
	MELPRO	0.19 ± 0.10^{a}	NS	1.37 ± 0.23^{a}	NS
	CON	0.17 ± 0.12^{a}		1.53 ± 0.70^{a}	
17th day of pregnancy	MEL	4.67 ± 1.06^{a}		1.28 ± 0.23^{a}	
	MELPRO	4.00 ± 1.00^{ab}	0.03*	1.35 ± 0.28^{a}	NS
	CON	3.51 ± 0.40^{b}		1.37 ± 0.29^{b}	

*: Means with different subscripts within a column differ at $P<0.05$ level. NS: not significant, SD: standard deviation

Although progesterone concentrations were observed to be higher in the melatonin–treated groups, the difference between the progesterone concentrations of the MEL and CON groups was statistically significant on d 17 of pregnancy ($P<0.05$) (FIG. 2).

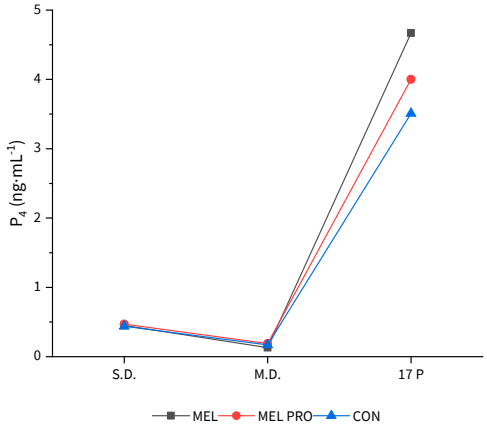

FIGURE 2. Plasma progesterone concentrations in melatonin only (MEL), melatonin + intavaginal progesterone + $PGF_{2\alpha}$ (MELPRO) and control (CON) groups. S.D.: Start day of treatment, M.D.: Mating day, 17 P: 17th day of pregnancy

There are many studies in humans and animals showing that melatonin causes an increase in progesterone concentrations [16, 29]. Taketani *et al.* [30] found that progesterone production was inhibited by hydrogen peroxide (H_2O_2) and melatonin smooth down the inhibitory effect of H_2O_2. Ma *et al.* [31] reported that melatonin stimulated progesterone secretion from theca cells in vitro in ewes. Some studies have reported that melatonin causes an increase in progesterone with its luteotropic effect and a positive effect on the preservation of pregnancy [15, 32]. In this study, higher progesterone concentrations in the MEL group had a numerical effect on the litter size but no statistical difference was observed.

MDA values obtained in this study are given in TABLE II. Although the differences in MDA values between the groups were statistically insignificant ($P>0.05$), MDA values in the melatonin–treated groups were found to be lower than in the control group (FIG. 3).

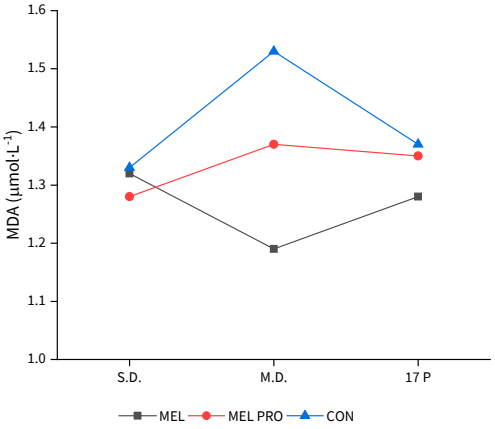

FIGURE 3. Plasma MDA values in melatonin only (MEL), melatonin + intavaginal progesterone + $PGF_{2\alpha}$ (MELPRO) and control (CON) groups. S.D.: Start day of treatment, M.D.: Mating day, 17 P: 17th day of pregnancy

Melatonin is a indirect antioxidant, and it detoxifies a large number of free radicals such as peroxynitrous acid [33] and nitric oxide [34]. Thus melatonin may reduce MDA concentrations by detoxifying these radicals [34]. El–Sawi *et al.* [35] found that melatonin did not cause a significant change in brain, kidney and liver MDA levels in male rats. Pertsov *et al.* [36] reported that melatonin caused a significant decrease in blood MDA levels in rats under stress. During the ovulation process, reactive oxygen species (ROS) are produced in the follicle [37]. Oxidative stimulation plays a critical role in oocyte maturation and follicle wall rupture; however, excessive production of reactive oxygen species (ROS) can compromise oocyte quality. The administration of exogenous melatonin enhances intrafollicular melatonin levels, mitigates oxidative damage, and potentially improves fertilization and pregnancy rates [8].

CONCLUSION

The administration of melatonin with or without progesterone did not statistically affect the time to estrus, pregnancy rate, multiple lambing rate, and litter size. Although progesterone concentration increased on the d of 17 pregnancy in the MEL group compared to the control group, there was no statistical difference in pregnancy rates and litter size, only a numerical increase was found.

Ethics approval

Ethics committee approval was obtained from the Bahri Dağdaş International Agricultural. Research Institute Animal Experiments Local Ethics Committee for this study (05.09.2022/142).

Conflict of interest

The authors declared no potential conflicts of interest with respect to this article.

BIBLIOGRAPHIC REFERENCES

[1] Tamura H, Nakamura Y, Terron MP, Flores LJ, Manchester LC, Tan D, Sugino S, Reíter RJ. Melatonin and pregnancy in the human. Reprod. Toxicol. [Internet]. 2008; 25(3):291–303. doi: https://doi.org/c6dcxj

[2] Atasoy ÖB, Erbaş O. Physiological effects of the melatonin hormone. Ist. Bilim Üniv. Florence Nightingale Tip. Derg. [Internet]. 2017 [cited 12 Nov. 2024]; 3(1):52–62. Available in: https://goo.su/JncWxc

[3] Tsutsui K, Ubuka T. Discovery of gonadotropin–inhibitory hormone (GnIH), progress in GnIH research on reproductive physiology and behavior and perspective of GnIH research on neuroendocrine regulation of reproduction. Mol. Cell. Endocrinol. [Internet]. 2020; 514:110914. doi: https://doi.org/pb6m

[4] Balík A, Kretschmannova K, Mazna P, Svobodova I, Zemkova H. Melatonin action in neonatal gonadotrophs. Physiol. Res. [Internet]. 2004; 53(Suppl. 1):S153–S166. doi: https://doi.org/pb6n

[5] Ferlazzo N, Andolina G, Cannata A, Costanzo MG, Rizzo V, Currò M, Lentile R, Caccamo D. Is melatonin the cornucopia of the 21st century?. Antioxidants [Internet]. 2020; 9(11):1088. doi: https://doi.org/gmgbbz

[6] Vlckova R, Posivak J, Valocký I, Sopkov, D, Andrejcakova, Z, Kostecka Z, Kozioł K, Seidavi A. Effects of short–term melatonin or progestogen with gonadotropic treatments on reproductive performance, hormonal levels and ovarian activity of ewes. Int. J. Vet. Sci. [Internet]. 2022; 11(4):493–497. doi: https://doi.org/pb6p

[7] Xiao L, Hu J, Song L, Zhang Y, Dong W, Jiang Y, Zhang Q, Yuan L, Zhao X. Profile of melatonin and its receptors and synthesizing enzymes in cumulus–oocyte complexes of the developing sheep antral follicle—a potential estradiol–mediated mechanism. Reprod. Biol. Endocrinol. [Internet]. 2019; 17:1. doi: https://doi.org/pb6s

[8] Tamura H, Takasaki A, Miwa I, Taniguchi K, Maekawa R, Asada H, Taketani T, Matsuoka A, Yamagata Y, Shimamura K, Morioka H, Ishikawa H, Reiter RJ, Sugino N. Oxidative stress impairs oocyte quality and melatonin protects oocytes from free radical damage and improves fertilization rate. J. Pineal Res. [Internet]. 2008; 44(3):280–287. doi: https://doi.org/d6bgp7

[9] Vazquez MI, Forcada F, Sosa C, Casao A, Sartore I, Fernández–Foren A, Meikle A, Abecia JA. Effect of exogenous melatonin on embryo viability and uterine environment in undernourished ewes. Anim. Reprod. Sci. [Internet]. 2013; 141(1–2):52–61. doi: https://doi.org/f49w4t

[10] Tian X, Wang F, He C, Zhang L, Tan D, Reiter RJ. Xu J, Ji P, Liu G. Beneficial effects of melatonin on bovine oocytes maturation: a mechanistic approach. J. Pineal Res. [Internet]. 2014; 57(3):239–247. doi: https://doi.org/f6hhr6

[11] Şener G. Karanlığın hormonu: Melatonin [Hormone of darkness: Melatonin]. Marmara Pharm. J. [Internet] 2010 [cited 22 Oct. 2024]; 14:112–120. Turkish. Avaliable in: https://goo.su/AEsSh

[12] Nagel C, Aurich C, Aurich J. Stress effects on the regulation of parturition in different domestic animal species. Anim. Reprod. Sci. [Internet]. 2019; (207):153–161. doi: https://doi.org/pb6x

[13] Akbulut NK, Kal Y. Effects of tocolytic drugs (isoxsuprine hydrochloride) during the implantation period in pregnant sheep. Pol. J. Vet. Sci. [Internet]. 2022; 25(4):557–560. doi: https://doi.org/pb6w

[14] Magarelli PC, Cridennda DK, Cohen M. Changes in serum cortisol and prolactin associated with acupuncture during controlled ovarian hyperstimulation in women undergoing in vitro fertilization–embryo transfer treatment. Fertil. Steril. [Internet]. 2009; 92(6):1870–1879. doi: https://doi.org/db6qnf

[15] Wallace JM, Robinson JJ, Wigzell S, Aitken RP. Effect of melatonin on the peripheral concentrations of LH and progesterone after oestrus, and on conception rate in ewes. The J. Endocrinol. [Internet]. 1988; 119(3):523–530. doi: https://doi.org/bcmnxk

[16] Abecia J, Forcada F, Zuniga O. The effect of melatonin on the secretion of progesterone in sheep and on the development of ovine embryos *in vitro.* Vet. Res. Commun. [Internet]. 2002; (26):151–158. doi: https://doi.org/fws46h

[17] Özyurtlu N, Bademkıran S. Koyunlarda östrus senkronizasyonu ve östrusu uyarma yöntemleri [Estrus synchronization and ınduction of estrus methods in sheep]. Dicle Univ. Vet. Fak. Derg. [Internet]. 2010 [cited 1 Dec. 2024]; 1(1):17–22. Turkish. Available in: https://goo.su/50VLL

[18] Fernandez J, Bruno–Galarraga MM, Sot AT, de la Sota RL, Cueto MI, Lacau IM, Gibbons E. Hormonal therapeutic strategy on the induction of accessory corpora lutea in relation to follicle size and on the increase of progesterone in sheep. Theriogenology [Internet]. 2018; 105:184–188. doi: https://doi.org/pb8v

[19] Buege JA, Aust SD. Microsomal lipid peroxidation. In: Fleischer S, Packer L, editors. Methods in Enzimology [Internet]. Cambridge (MA, USA): Academic Press; 1978. p. 302–310. (Vol. 52). doi: https://doi.org/cgmt6k

[20] Kutlu M, Doğan H, Durmuş, M. The relationship of anogenital distance with fertility and anti–müllerian hormone in Awassi and Cukurova meat ewes. Small Rumin. Res. [Internet]. 2024; 241:107390. doi: https://doi.org/pb8w

[21] International Business Machines Corporation (IBM). Released 2015. IBM SPSS Statistics for Windows, Version 23.0 [Internet]. Armonk (NY, USA): IBM Corp; 2015 [cited 1 Dec. 2024]. Available in: https://goo.su/RTzscym

[22] Zarazaga LA, Gatica M, Celi I, Guzman J, Malpaux B. Artificial long days in addition to exogenous melatonin and daily contact with bucks stimulate the ovarian and oestrous activity in Mediterranean goat females. Animal [Internet]. 2011; 5(9):1414–1419. doi: https://doi.org/fkdznz

[23] Kusakari N, Ohara M. Effect of melatonin feeding on early onset of reproductive activity in postpartum Suffolk ewes lactating during anestrous season. J. Reprod. Develop. [Internet]. 1997; 43(1):97-100. doi: https://doi.org/fwqmrq

[24] Guan S, Xie L, Ma T, Lv D, Jing W, Tian X, Song Y, Liu Z, Xiao X, Liu G. Effects of Melatonin on Early Pregnancy in Mouse: Involving the Regulation of *StAR*, *Cyp11a1*, and *Ihh* Expression. Int. J. Mol. Sci.. [Internet]. 2017; 18:1637. doi: https://doi.org/gbxzbz

[25] Zhang L, Zhang Z, Wang F, Tian X, Ji P, Liu G. Effects of melatonin administration on embryo implantation and offspring growth in mice under different schedules of photoperiodic exposure. Reprod. Biol. Endocrinol. [Internet]. 2017; 15:78. doi: https://doi.org/gb2q4z

[26] Haresign W. The effect of implantation of lowland ewes with melatonin on the time of mating and reproductive performance. Anim. Sci. [Internet]. 1992; 54(1):31-39. doi: https://doi.org/fj8j4d

[27] Leyva–Corona JC, Angulo–Valenzuela NI, Laborin–Escalante BM, Gastelum–Delgado MA, Silva–Avila NJ, Luna–Nevárez P, Aragón–López CE, Sánchez–Castro MA, Morales–Pablos MI. Reproductive performance of hair ewes and rams implanted with Melatonin previous to the anestrus season in Northwest Mexico. Trop. Anim. Health Product. [Internet]. 2023; 55(3):174. doi: https://doi.org/pb84

[28] Abd–Allah M, Daghash MWH. The role of exogenous melatonin and photoperiod on productive and reproductive performance of Ossimi sheep. Egypt. J. Sheep Goats Sci. [Internet]. 2019[cied Dec 13 2024]; 14(3):49-58. Available in: https://goo.su/royIn

[29] Fang L, Li Y, Wang S, Yu Y, Li Y, Guo Y, Yan Y, Sun YP. Melatonin induces progesterone production in human granulosa–lutein cells through upregulation of StAR expression. Aging [Internet]. 2019; 11(20):9013-9024. doi: https://doi.org/pb89

[30] Taketani T, Tamura H, Takasaki A, Lee L, Kizuka F, Tamura I, Taniguchi K, Maekawa R, Asada H, Shimamura K, Reiter RJ, Sugino N. Protective role of melatonin in progesterone production by human luteal cells. J. Pineal Res. [Internet]. 2011; 51(2):207-213. doi: https://doi.org/c7h8kb

[31] Ma J, Wang J, Hu S, Li Y, Zhang Y, Yang Y, Yang C, Huo S, Yang Y, Zhaxi Y, Luo W. Effects of melatonin on development and hormone secretion of sheep theca cells *in vitro.* Theriogenology [Internet]. 2023; 198:172-182. doi: https://doi.org/pb9b

[32] Horoz H, Kaşıkçı G, Ak K, Alkan S, Sönmez C. Controlling the breeding season using melatonin and progestagen in Kıvırcık ewes. Turk. J. Vet. Anim. Sci. [Internet]. 2003 [cited 19 Nov. 2024]; 27(2):301-305. Available in: https://goo.su/qV2S1

[33] Fan W, He Y, Guan X, Gu W, Wu Z, Zhu X, Huang F, He H. Involvement of the nitric oxide in melatonin–mediated protection against injury. Life Sci. [Internet]. 2018; 200:142-147. doi: https://doi.org/gdd6c8

[34] Morvaridzadeh M, Sadeghi E, Agah S, Nachvak SM, Fazelian S, Moradi F, Persad E, Heshmati J. Effect of melatonin supplementation on oxidative stress parameters: a systematic review and meta–analysis. Pharmacol. Res. [Internet]. 2020; 161:105210. doi: https://doi.org/gr5szn

[35] El–Sawi MR, Badawy ME, El–Gharieb NM. Role of melatonin in modulation of oxidative stress induced by delta–aminolevulinic acid in adult male albino rats. Egypt. J. Hosp. Med. [Internet]. 2007; 28(1):233-272. doi: https://doi.org/pb9c

[36] Pertsov SS, Koplik EV, Kalinichenko LS, Alekseeva IV. Effects of melatonin on lipid peroxidation in blood in rats with different behavioral characteristics in acute emotional stress. Neurosci. Behav. Physiol. [Internet]. 2016; 46:133-137. doi: https://doi.org/pb9d

[37] Pasqualotto EB, Agarwal A, Sharma RK, Izzo VM, Pinotti JA, Joshi NJ, Rose B. Effect of oxidative stress in follicular fluid on the outcome of assisted reproductive procedures. Fertil Steril. [Internet]. 2004; 81(4):973-976. doi: https://doi.org/dj3dpv