

The Effect of Melatonin Administration on Colostrum IgG Levels and Oxidative Stress in Advanced Pregnant Awassi Sheep

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ABSTRACT

The aim of the present study was to investigate the effects on oxidative stress markers and colostrum quality levels of subcutaneous implantation of melatonin in the 4th month of pregnancy in Awassi sheep by considering the sex of offspring born. The animals consisted of 60 healthy Awassi female sheep. Progesterone-based oestrus synchronization was applied to the sheep during the breeding season and pregnancy examinations were performed by the transabdominal route. The pregnant sheep were divided into 2 groups: the melatonin (n=30) and control (n=30) groups. In the 4th month of pregnancy, melatonin was implanted subcutaneously behind the ear of each animal in the melatonin group, and 1 ml of saline was applied subcutaneously to the control group. Colostrum samples from all the sheep and blood samples from both sheep and lambs were taken within the first hour after birth, before the lambs were suckled. Colostrum IgG levels were seen to be higher in the melatonin group compared to the control group ($p<0.001$); higher in the group with no female offspring compared to the group with female offspring ($p<0.001$) and higher in twins compared to singletons ($p<0.001$). The colostrum IgG levels were higher in ewes with singleton birth in both the melatonin and control groups compared to those with no female offspring at birth ($p<0.001$). Serum total antioxidant capacity (TAC) levels in sheep was higher in the melatonin group than in the control group ($p<0.001$). Serum total oxidant capacity (TOC) and oxidative stress index (OSI) levels were higher in the control group than in the melatonin group ($p<0.001$). The serum TAC level in lambs was higher in the melatonin group than in the control group ($p<0.001$), and was higher in singleton lambs than in twins ($p<0.001$). The serum TOC and OSI levels were higher in the control group than in the melatonin group ($p<0.001$) and higher in singleton lambs than in twins ($p<0.001$). In conclusion, the use of prepartum melatonin may be considered in sheep breeding enterprises to improve the antioxidant defence system of the body and improve the quality of colostrum for newborn lambs to be able to gain sufficient immunity.

Keywords: Melatonin; Colostrum; IgG; Oxidative Stress; Sheep.

INTRODUCTION

Melatonin is an indolamine, which is synthesized and secreted by the pineal gland in the brain. This molecule, which is abundant in almost all living organisms in nature is a chemical compound with a molecular weight of 232 g/mol with both hydrophilic and hydrophobic properties (1). Oxidative stress can be defined as an imbalance between the amount of free radicals and reactive products or oxidants and

the amount of antioxidants that protect the body from these metabolites. This situation affects the organism as a whole and causes significant damage to the molecules and cellular structures in the body (2). Melatonin removes reactive oxygen species (ROS) and reactive nitrogen species (RNS) from the body and has some important functions halting the formation of free radicals. By decreasing the effect of oxidative stress, it functions as an antioxidant, increasing antioxidant defences,

and thereby protecting cells and tissues from damage. It has both hydrophilic and hydrophobic properties and cleanses hydroxyl radicals (OH) and reduces oxidative stress in both water and lipid parts of the body (3).

Colostrum is a liquid that is synthesized in the last stages of pregnancy. It accumulates in the mammary gland and ceases to be produced immediately after birth. It differs from normal milk in terms of content, taste, color and consistency, as it contains immune complements, growth factors and various nutrients necessary for the defence systems, nutrition and development of newborns after birth (4).

Colostrum is a source of antibodies in passive immunity in newborns as it contains 80 times more antibodies than normal milk (5). There is a significant concentration of immunoglobulin (Ig) (6), and the most important factor affecting the quality of colostrum is the amount of gammaglobulin (IgG) in immunoglobulins. The IgG contained in colostrum accounts for 75% of immunoglobulins. The most commonly used method for calculating the quality of colostrum is the determination of the IgG level (7).

In ruminants, including sheep, the placenta does not allow the transmission of immune substances to the offspring through the maternal bloodstream during pregnancy and therefore lambs are born as agammaglobulinemic. However, the transfer of immune substances in the colostrum provides immunity until the offspring acquire the ability to produce their own antibodies, thus temporary passive immunity is obtained and they are protected from diseases (8).

As the only source for the initiation of postpartum immunity, colostrum is important for the supply of immunoglobulin G, leukocytes, cytokines, growth factors and various nutrients necessary for the immune system in the neonatal period (4).

Lambs and kids need to have sufficient passive immunity to be protected against environmental pathogens in the postpartum period. There is a positive relationship between vitality and passive immunity level in newborns. The neonatal mortality rate has been reported to be high in lambs and kids with passive transfer failure (4, 9).

The use of antioxidants in the prenatal period has been shown to improve the quality of colostrum (10), and it has been reported that antioxidants play a role in the synthesis of IgG and its migration through mammary-specific receptors (11). It has been stated that the dry period serum antioxidant level affects the quality of colostrum (12), while antioxidants

in the colostrum content protect immunoglobulina in the colostrum against oxidative stress damage (13).

The aim of this study was to investigate the effect of subcutaneous implantation of melatonin in the 4th month of pregnancy in Awassi sheep on oxidative stress markers (blood serum TAC and TOC levels) and colostrum quality (colostrum IgG levels) by considering the sex of offspring. The data obtained were examined in terms of the feasibility of using this method in veterinary breeding practice.

MATERIALS AND METHODS

Animal Material

This study was conducted between August 2021 and February 2022 at the Faculty of Veterinary Practice Farm of Harran University, located in the Eyyubiye district of Şanlıurfa Province, southeast Türkiye, at an altitude of 517 m, 37°07'18.1" N latitude and 38°49'13.0" E longitude. The animal sample comprised 60 Awassi sheep, aged 2-4 years, each weighing mean 53.96±1.09 kg, with a body condition score ranging from 2-3 (1=Extremely weak, 5=Obese) (2.63±0.05), which had previously given birth at least once, and had no genital system conditions. During the study period, the sheep grazed freely on pasture, and were taken into a closed pen for the process of oestrus synchronization, and the collection of blood and colostrum samples. The sheep were fed a mixture of hay (5.2%), clover (32.9%) and milk feed (61.9%) and were provided with fresh water *ad libitum*.

Oestrus Synchronization and Oestrus Tracking

In order to provide a sample during pregnancy and to induce lambing within a certain period of time, progesterone-based oestrus synchronization was applied in the breeding season. Progesterone impregnated vaginal sponges (Medroxyprogesterone acetate, Esponjavet®, Hipra Animal Health, Türkiye) were placed in the vagina to remain in the vagina for 12 days. On the 11th day, 2 ml of PGF2a (Dinoprost tromethamine, Dinolytic®, Zoetis, Türkiye) was administered intramuscularly. On the 12th day after insertion, the vaginal sponges were removed and 500 IU PMSG (PMSG, Oviser®, Hipra Animal Health, and Türkiye) was injected intramuscularly. After the injection of PMSG, oestrus was followed up using teaser rams for 30 minutes at 8 hour-intervals for 3 days, and the sheep showing oestrus

were hand-mated with rams of the same breed, with pre-determined fertility.

Ultrasonographic Examinations and Melatonin Implantation

Ultrasonographic pregnancy examinations of the sheep at 35-45 days following the mating and during the 4 months of melatonin application were performed transrectally and transabdominally using a real-time B-mode ultrasound device (Hasvet 838 ultrasound device, Antalya, Türkiye) with a linear probe at a frequency of 5 MHz.

The ultrasound examinations were all performed by the same researcher, with a record kept of the screen settings of depth, gain, focus, and brightness for all the images. The sheep determined to be pregnant were separated into 2 groups as the melatonin (n=30) and control (n=30) groups. The melatonin group was treated with a subcutaneous implant behind the ear (Melatonin, Regulin®, CEVA, Türkiye), and the control group received 1 ml saline solution subcutaneously.

Collecting Milk Colostrum and Blood Samples

Colostrum samples of 15 mL in total from a single mammary lobe after the teat ducts were emptied were withdrawn into centrifuge tubes (15mL, Isolab®, Germany) following the rules of asepsis and antisepsis before the lambs were suckled within the first hour after birth. The samples were transported to the laboratory on ice, and then centrifuged at 3000 rpm for 10 minutes. The colostrum serum obtained from each sample was transferred to a 2 ml microcentrifuge tube and stored at -80°C until analysis.

In order to determine oxidative stress (TOC and TAC) from the sheep and lambs in the study group, blood samples from the Vena jugularis were withdrawn into tubes containing 5 ml of coagulation activator in accordance with the procedures of asepsis and antisepsis within 1 hour after delivery (before the lambs suckled). The blood samples taken were delivered to the laboratory under cold chain conditions and centrifuged for 10 minutes at 3000 rpm. The obtained serum samples were transferred to 2 ml micro centrifuge tubes and stored at -80°C until the relevant analyses. The sex and birth weight of each lamb born from all the sheep were recorded, with weight recorded according to whether a singleton or twin birth.

Biochemical Analysis

The serum TAS levels of the sheep and lambs in the study groups were determined spectrophotometrically at 660 nm using a commercial kit (Total Antioxidant Status, NN21117A, Rel Assay Diagnostics®, Mega Medicine, Gaziantep, Türkiye). The serum TOS levels were determined spectrophotometrically (Molecular Device SpectraMax M5 Plate Reader, Pleasanton, California, United States) at 530 nm using a commercial kit (Total Oxidant Status, NN211290, Rel Assay Diagnostics®, Mega Medicine, Gaziantep, Türkiye). The oxidative stress index (OSI) was calculated as $OSI = ([TOC \{mmol/L\}] / [TAC \{mmol\} Trolox \text{ equivalent}] / 1 \times 100)$ (14). IgG levels of colostrum serum were evaluated using the ELISA method with a commercial kit (Sheep Immunoglobulin G, IgG, Cat.No. E0019Sh, BT LAB®, Zhejiang, China).

Statistical Analysis

Statistical analysis of the data was performed using the Statistical Package for the Social Sciences software (SPSS for Windows; version 26.0). A 2x2x2 factorial design was established in which group (control and melatonin), number of offspring and sex of offspring were fixed to determine the level of parameters examined in the treatment of mothers with melatonin. Differences between groups were changed to the General Linear Model (GLM) procedure by adding additional coding to the syntax menu. The data in the tables, graphs, and results section were expressed as mean±standard error (SEM) values. A value of $p < 0.05$ was accepted as statistically significant.

RESULTS

Pregnancy and Number of Offspring: There was no pathological conditions that might have affected the general health status of the sheep during the pregnancies. It was observed that the sheep stopped ruminating a few days before the parturition and remained separated from the herd. These sheep were placed in separate compartments (2x2m) and their births were followed up. Birth occurred on 150 ± 2 days of pregnancy, all were by the vaginal route, single (151.10 ± 0.11) or twin (150.82 ± 0.16) without requiring help and no maternal problems were encountered in the postpartum period. As a result of the births, 18 male and 24 female offspring were born from 18 single and 12 twin births in the melatonin

Table 1. A 2×2 ANOVA summary table in which the group, number of offspring and sex of offspring are presented the sheep.

Factors			IgG level	Serum TAC level	Serum TOC level	OSI level	Gestation period (days)
Group	Sex of Offspring	Single and Twin Births	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Control	No female offspring	Single	43.60±0.89	2.07±0.02	14.45±0.04	6.97±0.06	151.22±0.24
		Twin	54.11±1.54	2.08±0.04	14.36±0.8	6.89±0.11	150.33±0.41
	Female offspring	Single	24.39±0.80	2.08±0.02	14.50±0.04	6.95±0.05	150.90±0.21
		Twin	53.58±1.01	2.08±0.02	14.47±0.05	6.93±0.07	150.571±0.27
Melatonin	No female offspring	Single	69.77±0.94	2.31±0.02	12.47±0.04	5.35±0.06	151.375±0.25
		Twin	64.37±1.33	2.34±0.03	12.45±0.06	5.32±0.09	151.25±0.36
	Female offspring	Single	43.12±0.84	2.34±0.02	12.51±0.04	5.34±0.06	150.90±0.22
		Twin	64.11±0.94	2.33±0.02	12.48±0.04	5.34±0.06	151.12±0.25
Main effects and interactions			*P value	*P value	*P value	*P value	*P value
Group			<0.001	<0.001	<0.001	<0.001	>0.05
Sex of offspring			<0.001	>0.05	>0.05	>0.05	>0.05
Single and Twin Birth			<0.001	>0.05	>0.05	>0.05	>0.05
Group x Sex of Offspring			<0.05	>0.05	>0.05	>0.05	>0.05
Group x Single and Twin Birth			<0.001	>0.05	>0.05	>0.05	>0.05
Group x Sex of Offspring x Single and Twin Birth			<0.05	>0.05	>0.05	>0.05	>0.05

Total antioxidant capacity (TAC), total oxidant capacity (TOC), Oxidative Stress Index (OSI), Standard error of the mean (SEM)

group, and 18 male and 22 female offspring were born from 20 single and 10 twin births in the control group.

A 2×2 ANOVA summary table in which the group, number of offspring and sex of offspring are fixed in the sheep and lambs is presented in Table 1 and Table 2.

Colostrum Immunoglobulin G Levels: The colostrum IgG levels were higher in the melatonin group than in the control group ($p<0.001$). According to the sex of offspring born, the colostrum IgG level was higher in the sheep with male offspring at birth than in the group with female offspring at birth ($p<0.001$). According to single and twin births, colostrum IgG levels were higher in sheep giving birth to twins than in sheep giving birth to singletons ($p<0.001$). Colostrum IgG levels were higher in the ewes with singleton birth compared to ewes not giving birth to females in both the melatonin and control groups ($p<0.001$). In the melatonin and control groups, there was no differences in ewes that gave birth to twins according to whether or not there were males or female offspring ($p>0.05$).

Total Antioxidant, Total Oxidant Capacity and Oxidative Stress Index of Blood Serum in Sheep: The

blood serum TAC level was higher in the melatonin group than in the control group ($p<0.001$). The blood serum TOC and OSI levels were higher in the control group than in the melatonin group ($p<0.001$). There was no difference in blood serum TAC, TOC and OSI levels between the sexes of lambs born and single and twin births ($p>0.05$). There was no differences in interaction between the group* lamb sex, group*single and twin births and group* lamb sex*single and twin births ($p>0.05$).

Total Antioxidant, Total Oxidant Capacity and Oxidative Stress Index of Blood Serum in Lambs: The blood serum TAC level was higher in the melatonin group than in the control group ($p<0.001$). The blood serum TAC level was higher in singleton lambs than in twin lambs ($p<0.001$). However, there was no significant difference between the sexes of lambs born ($p>0.05$). There was no difference between the group* lamb sex and the group* lamb sex*single and twin birth interactions ($p>0.05$). The blood serum TOC and OSI levels were higher in the control group than in the melatonin group ($p<0.001$). The blood serum TOC and OSI levels were higher in singleton lambs born

Table 2. A 2x2x2 ANOVA summary table in which the group, number of offspring and sex of offspring are fixed in the lambs.

Factors			Serum TAC level	Serum TOC level	OSI level	Birth weight (gr)
Group	Sex of Offspring	Single and Twin Birth	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Control	Male	Single	1.78±0.01	15.52±0.03	8.70±0.05	4.14±0.71
		Twin	0.94±0.01	7.38±0.03	7.84±0.05	3.76±0.71
	Female	Single	1.77±0.00	15.56±0.03	8.77±0.04	3.95±0.64
		Twin	0.94±0.00	7.39±0.03	7.86±0.04	3.50±0.64
Melatonin	Male	Single	1.99±0.01	12.72±0.03	6.37±0.05	5.73±0.64
		Twin	1.08±0.01	6.16±0.03	5.69±0.05	3.71±0.71
	Female	Single	2.01±0.00	12.74±0.03	6.33±0.04	4.03±0.71
		Twin	1.07±0.00	6.17±0.03	5.71±0.04	3.47±0.64
Main effects and interactions			P value	P value	P value	P value
Group			<0.001	<0.001	<0.001	>0.05
Sex of offspring			>0.05	>0.05	>0.05	>0.05
Single and Twin Birth			<0.001	<0.001	<0.001	>0.05
Group x Sex of Offspring			>0.05	>0.05	>0.05	>0.05
Group x Single and Twin Birth			<0.001	<0.001	<0.001	>0.05
Group x Sex of Offspring x Single and Twin Birth			>0.05	>0.05	>0.05	>0.05

Total antioxidant capacity (TAC), total oxidant capacity (TOC), Oxidative Stress Index (OSI), Standard error of the mean (SEM)

than in twin lambs ($p < 0.001$), with no significant difference between the sexes of lambs born ($p > 0.05$). No difference was determined between the group* lamb sex and the group* lamb sex* single and twin birth interactions ($p > 0.05$).

Parameters Related to Gestation Period in Sheep and Birth Weight in Lambs: In the parameters of gestation period in sheep and birth weight in lambs, there was no significant difference between the groups in terms of the sexes of lambs born compared to single and twin births ($p > 0.05$). No difference was determined between the group* lamb sex, group* single and twin births and group* lamb sex* single and twin births interactions ($p > 0.05$).

DISCUSSION

The melatonin hormone, the effect of which was investigated in this study, is synthesized from the pineal gland as a result of the transmission of photoperiodic signals to the reproductive neuroendocrine axis by the effect created by the day-night change. It is an immunomodulatory (helping to support immune function) factor and is produced by immunocompetent (lymphocytic tissue cells involved in immune reactions) cells. It also works as an antioxidant by removing free radicals and

activating enzymes (15). It is known that melatonin plays a role in the season-related regulation of the immune system, and its effect on IgG production has been shown (16, 17). In a study of mice it was observed that melatonin administered in the evening increased the primary antibody response *in vivo* in erythrocytes after 5 days (16). It has also been reported that increased melatonin levels by artificial darkening in elderly rats may prevent immunosuppression by increasing IgG and IgM levels (17). In the literature reviews of sheep, which constitute the material of the current study, there is limited information on the use of exogenous treatments to improve the quality of colostrum.

It has been stated that since immunoglobulins cannot cross the placental barrier in sheep with epitheliocorial placenta structure, it is important to provide these components from the sheep colostrum to enhance the viability of newborn lambs (18). In a previous study examining the colostrum IgG level in Awassi sheep, the IgG level of the colostrum sample taken after birth was reported to be 6.09 g/dl (19). The IgG level has been reported as 49.50±4.36g/L (20) in colostrum samples of Rasa Aragonesa sheep and as 30.89±0.87g/L in Karagül sheep (21). Other studies have shown IgG levels

in colostrum to be 50-70mg/mL in different sheep breeds (Rambouille, Targhe, Columbia, Finnish hybrids, Lacaun, East Frisian, Suffolk, Lori bakhtyari, Shaul) (22-25). In studies conducted on goats, it has been reported that the postpartum level of colostrum IgG was 50-72mg/mL (26-28).

In the current study, the colostrum IgG levels in Awassi sheep were determined to be 43.92 ± 0.55 mg/mL in the control group, which was similar to the findings of previous studies (22-25). Although studies of other species were not directly related to the current study, they provide an idea in terms of colostrum IgG level monitoring. It is thought that the reason for the difference in the reference range in colostrum IgG levels may be related to factors such as breed, diet, season and number of offspring.

It has been previously reported that IgG levels were higher in the melatonin group in the colostrum sample taken after birth when melatonin was administered as a subcutaneous implant in the 4th month of pregnancy (20). In the current study, as a result of melatonin application in the 4th month of pregnancy, it was found that the IgG level of colostrum was significantly higher in the melatonin group than in the control group, and this was in accordance with the only literature on exogenous melatonin in sheep. In the current study, the increase in the amount of IgG in colostrum after melatonin implantation was probably due to melatonin activating immune system cells either directly through melatonin receptors or indirectly due to changes in steroid hormones (29). Abecia *et al.* (20) reported that colostrum IgG levels were low when the fetus was female in single and multiple offspring ewes in all study groups with and without melatonin. While a significant difference was reported in ewes giving birth to singletons, no significant difference was detected in ewes giving birth to twins. In the present study, a low level of colostrum IgG was measured in the female lambs. In addition, the colostrum IgG level was higher in twins compared to singletons in this study. While there was no difference in colostrum IgG level in twin pregnancies in this study, the low IgG level in single pregnancies in the presence of a female fetus is compatible with the literature. In the current study, according to the sex of the offspring at birth, while there was no difference in twin pregnancies, the incidence of miscarriage of a female fetus in single pregnancies was seen to be consistent with the data in the literature. The possible cause of this condition was thought to be the concentration dilution effect. It has

been reported that female offspring production has a positive effect on milk yield in Florida goats (30) and Churra and Lacaune sheep (31). In the current study, colostrum production was not measured, presumably ewes bearing females will produce the most colostrum, so that even with no differences in IgG production, any molecule diluted in colostrum will have a lower concentration (20). A similar situation has been observed in dairy cows, and those with female calves were reported to produce higher colostrum and milk during lactation than those with male calves, and the total immunoglobulin concentration was higher in males (32).

In the present study, the fact that sheep with female lambs have low IgG levels was found to be similar to the literature references. Milk production can be affected by the sex of offspring through sex-specific fetal hormones, which have been determined to have the potential to affect placental and mammary gland tissue (33). In the current study, sex-specific hormones released by the fetus may have affected colostrum IgG secretion. However, no association between the sex of the offspring and colostrum IgG levels has been reported in other studies (34, 35). Melatonin applications in Assaf and Lacaune sheep did not affect milk production and quality of milk due to colostrum melatonin treatment (36). In another similar study, it was shown that the application of melatonin during pregnancy improved the oxidative stress status of sheep under heat stress, increasing milk production during multiple pregnancies (37).

During pregnancy, both sheep and fetuses are exposed to oxidative stress caused by an increased amount of reactive oxygen species (ROS) (38). It has been stated that measuring antioxidants separately does not fully reflect the antioxidant capacity of the body, and therefore the colorimetric value of TAC, which reflects the total of all antioxidants in the biological system, should be measured for this purpose. In studies conducted in cows, it has been reported that the TAC and TOC levels will vary according to the measurement methods (39) and nutritional differences (40). In the present study, all sheep were under the same care and feeding conditions and an attempt was made to provide a sample using the same analytical method in TAC-TOC measurements.

It is known that application of melatonin implants four times on days 0-40-80 and 120 during pregnancy in sheep increases serum TAC levels and improves redox status, in-

creases the average number of lambs born per sheep, the body weight of the lambs, and milk production (37). It has been reported that TAC values are high in single pregnancies with different antioxidant applications in pregnant sheep (41). In the present study, serum TAC levels were higher in sheep and lambs in the melatonin group ($p < 0.001$), and serum TOC and OSI levels were lower in the melatonin group ($p < 0.001$). This effect, which occurs as a result of the application of melatonin, an antioxidant, is consistent with the literature references. Melatonin increases the TAC level and decreases the TOC levels by stimulating the synthesis of antioxidants such as GPx and reducing by-products of oxidants such as lipid peroxidase (42, 43). In the present study, it was also determined that twin lambs have significantly lower serum TAC levels than single lambs showing compatibility with the limited literature data (41). In addition, the serum TOS and OSI levels in the current study were observed to be significantly lower in twin lambs than in singleton lambs, but it was not possible to evaluate this fully due to the absence of a similar study. Similar studies in the future may help in this regard.

In a study conducted on Holstein cows, it was reported that TOS levels were higher in the group that did not receive prenatal antioxidant applications (44). It has been reported that antioxidant applications in the prenatal period increase serum TAC levels and decrease TOC levels during the postpartum period (45). In another study conducted on cows, the effect of different prenatal antioxidants on TAC was investigated and it was reported that serum TAC levels were significantly higher in the treatment group compared to the control group during the postpartum period (46). In studies conducted on other species, the increase in serum TAC levels and decrease in TOC levels with the use of antioxidants during pregnancy support the results of the current study.

In pregnant ewes in a number of studies, the administration of melatonin has been shown to have variable effects on the live birth range of lambs when given orally (12 mg per day, from day 90 of pregnancy to birth) or implanted (18 or 36 mg, from day 100 to birth) (47-50). In the present study, melatonin implantation in the last period of pregnancy did not affect the birth weight of lambs born in the control and melatonin groups ($p > 0.05$). In a study conducted on Merino sheep, prenatal melatonin was applied, but there was reported to be no difference between the birth weights of single and

twin lambs (50). This suggests that melatonin, which was administered only in the last month of pregnancy, was insufficient to create the expected effect on the weight of the lambs born. It is thought that melatonin-induced growth development potential may have been minimized because the sheep in the current study were fed optimally with appropriate rations throughout pregnancy.

The administration of melatonin to sheep during pregnancy is known to prolong the gestation period by 1-2 days (50). In the present study, no difference was observed between the groups. It can be deduced that this condition, similar to birth weight, is due to the fact that melatonin was given in a more limited time period and was unable to stimulate the desired effects.

CONCLUSIONS

In conclusion, the results of this study demonstrated that the prepartum application of melatonin, an antioxidant, increased the antioxidant defence system by decreasing the TOS levels and increasing the TAS levels in sheep and lambs.

It was determined that melatonin applications as subcutaneous implants in the prepartum period significantly increased the level of colostrum IgG in the melatonin group compared to the control group. It was observed that the colostrum IgG level was higher in the ewes that did not have female offspring at birth in the control group compared to those that received melatonin as subcutaneous implants in the prepartum period. In addition, it was observed that melatonin applications as subcutaneous implants in the prepartum period did not differ in twin birth groups, but differed in single birth groups.

It was concluded that the application of melatonin applied in the last part of the prepartum period had no effect on the duration of labor and birth weight.

In sheep breeding enterprises it is recommended that prepartum antioxidant applications be used to reduce reactive oxygen products and improve the body's antioxidant defence system, to increase the quality of colostrum and to provide adequate immunity to the newborn lambs.

Due to the limited number of studies on melatonin applications in sheep, there is a need for further studies on the subject taking into account factors such as season, breed, dosage, and duration of implementation.

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CONFLICT OF INTEREST STATEMENT

The authors of this article have no conflict of interests to declare.

REFERENCES

1. Yu, H.S. and Reiter, R.J.: Melatonin: Biosynthesis, Physiological Effects, and Clinical Application. CRC Press: Taylor & Francis Group, London, New York, 2020.
2. Durackova, Z.: Some Current Insights into Oxidative Stress. *Physiol. Res.* 59: 459-469, 2010.
3. Bonnefont-Rousselot, D. and Collin, F.: Melatonin: Action as Antioxidant and Potential Applications in Human Disease and Aging. *Toxicol.* 278: 55-67, 2010.
4. Weaver, D.M., Tyler, J.W., Vanmetre, D.C., Hostetler, D.E. and Barrington, G.M.: Passive Transfer of Colostral Immunoglobulins in Calves. *JVIM.* 14: 569-577, 2000.
5. Barrington, G.M., Mcfadden, T.B., Huyler, M.T. and Besser, T.E.: Regulation of Colostrogenesis in Cattle. *Livest. Prod. Sci.* 70: 95-104, 2001.
6. Selk, G.E.: Disease Protection for Baby Calves. *Agric. Na. Resour.* 3358: 1-7, 2003.
7. Butler, J.E.: Synthesis and Distribution of Immunoglobulins. *AVMA.* 163: 795-800, 1973.
8. Selk, G.E.: Management Factors that Affect the Development of Passive Immunity in the Newborn Calf. In: Selk GE, Editor. *Beef Cattle Handbook-2240.* Extension Beef Cattle Resource Committee, 1-7, 1998.
9. Dwyer, C.M.: The Welfare of the Neonatal Lamb. *Small Rumin. Res.* 76: 31-41, 2008.
10. Przybylska, J., Albera, E. and Kankofer, M.: Antioxidants in Bovine Colostrum. *Reprod. Domest. Anim.* 42: 402-409, 2007.
11. Swecker, J.W., Thatcher, C.D., Eversole, D.E., Blodgett, D.J. and Schurig, G.G.: Effect of Selenium Supplementation on Colostral IgG Concentration in Cows Grazing Selenium-Deficient Pastures and on Postsuckle Serum IgG Concentration in Their Calves. *Am. J. Vet. Res.* 56: 450-453, 1995.
12. Godden, S., Mcmartin, S., Feirtag, J., Stabel, J., Bey, R., Goyal, S., Metzger, L., Fetrow, J., Wells, S. and Chester-Jones, H.: Heat-Treatment of Bovine Colostrum. II: Effects of Heating Duration on Pathogen Viability and Immunoglobulin G. *International J. Dairy Sci.* 89: 3476-3483, 2006.
13. Abuelo, A., Hernández, J., Benedito, J.L. and Castillo, C.: Redox Biology in Transition Periods of Dairy Cattle: Role in the Health of Periparturient and Neonatal Animals. *Antioxidants.* 8: 20, 2019.
14. Kosecik, M., Erel, O., Sevinc, E. and Selek, S.: Increased oxidative stress in children exposed 373 to passive smoking. *Int. J. Cardiol.* 100: 61-64, 2005.
15. Yonei, Y., Hattori, A., Tsutsui, K., Okawa, M. and Ishizuka, B.: Effects of Melatonin: Basics Studies and Clinical Applications. *J. Anti-Aging Med.* 7: 85-91, 2010.
16. Maestroni, G.J.M., Conti, A. and Pierpaoli, W.: Role of the Pineal Gland in Immunity: II. Melatonin Enhances the Antibody Response via an Opiatergic Mechanism. *Clinic.Experiment. Immunol.* 68: 384-391, 1987.
17. Demas, G.E. and Nelson, R.J.: Photoperiod and Temperature Interact to Affect Immune Parameters in Adult Male Deer Mice (*Peromyscus maniculatus*). *J. Biol. Rhythms.* 11: 94-102, 1996.
18. Nowak, R. and Poindron, P.: From Birth to Colostrum: Early Steps Leading to Lamb Survival. *Reprod. Nutr. Dev.* 46: 431-446, 2006.
19. Higaki, S., Nagano, M., Katagiri, S. and Takahashi, Y.: Effects of Parity and Litter Size on the Energy Contents and Immunoglobulin G Concentrations of Awassi Ewe Colostrum. *Turkish J. Vet. Anim. Sci.* 37: 109-112, 2013.
20. Abecia, J.A., Garrido, C., Gave, M., Garcia, A.I., Lopez, D., Luis, S., Valares, J.A. and Mata, L.: Exogenous Melatonin and Male Foetuses Improve the Quality of Sheep Colostrum. *J. Anim. Physiol. Anim. Nutr.* 104: 1305-1309, 2020.
21. Ahmad, R., Khan, A., Javed, T.M. and Hussain, I.: The Level of Immunoglobulins in Relation to Neonatal Lamb Mortality in Pak-Karakul Sheep. *Veterinarski Arhiv.* 70: 129-139, 2000.
22. Tabatabaei, S., Nikbakht, G., Vatankhah, M., Sharif, H. and Alidadi, N.: Variation in Colostral Immunoglobulin G Concentration in Fat Tailed Sheep and Evaluation of Methods for Estimation of Colostral Immunoglobulin Content. *Acta Veterinaria Brno.* 82: 271-275, 2013.
23. Gilbert, R.P., Gaskins, C.T., Hillers, J.K., Parker, C.F. and McGuire, T.C.: Genetic and Environmental Factors Affecting Immunoglobulin G1 Concentrations in the Ewe Colostrum and Lamb Serum. *J. Anim. Sci.* 66: 844-863, 1988.
24. Waelchli, R.O., Mulher, C.H., Hassig, M. and Rusch, P.: Immunoglobulin Concentration in Colostrum and Serum of Lamb of Dairy Sheep Breeds. *Vet. Rec.* 135: 16-17, 1994.
25. Dwyer, C.M. and Morgan, C.A.: Maintenance of Body Temperature in the Neonatal Lamb: Effects of Breed, Birth Weight, and Litter Size. *J. Anim. Sci.* 84: 1093-1101, 2006.
26. Ismail, H. and Kon, Y.: Immunological Quantitation of IgG and IgM in Milk and Serum of the Goat at Different Stages of the Reproductive Cycle. *Ann. Biol. Res.* 6: 16-20, 2015.
27. Micusan, V.V. and Borduas, A.G.: Biological Properties of Goat Immunoglobulins G. *Immunol.* 32: 373-381, 1977.
28. Yang, Y.X., Chen, J.P. and Zhang, F.X.: Research on the Chemical Composition of Saanen Goat Colostrum. *International Journal of Dairy Technology.* 62: 500-504, 2009.
29. Hotchkiss, A.K. and Nelson, R.J.: Melatonin and Immune Function: Hype or Hypothesis? *Crit. Rev. Immunol.* 22: 351-371, 2002.
30. Abecia, J.A., Arrebola, F., Sanchez, M., Lopez, M.D. and Palacios, C.: Goats Kidding Females Produce More Milk, Fat, Protein and Lactose than Goats Kidding Males. *Large Anim. Rev.* 24: 143-147, 2008.
31. Abecia, J.A. and Palacios, C.: Ewes Giving Birth to Female Lambs

- Produce More Milk than Ewes Giving Birth to Male Lambs. *Ital. J. Anim. Sci.* 17: 736-739, 2008.
32. Angulo, J., Gómez, L.M., Mahecha, L., Mejía, E., Henao, J. and Mesa, C.: Calf's Sex, Parity and the Hour of Harvest After Calving Affect Colostrum Quality of Dairy Cows Grazing Under High Tropical Conditions. *Trop. Anim. Health Prod.* 47: 699-705, 2015.
 33. Anand-Ivell, R., Hiendleder, S., Viñoles, C., Martin, G.B., Fitzsimmons, C., Eurich, A., Hafen, B. and Ivell, R.: INSL3 in the Ruminant: A Powerful Indicator of Gender- and Genetic-Specific Feto-Maternal Dialogue. *PLoS ONE* 6: e19821, 2011.
 34. Ciupercescu, D.D.: Dynamics of Serum Immunoglobulin Concentrations in Sheep During Pregnancy and Lactation. *Res. Vet. Sci.* 22: 23-27, 1977.
 35. Kaymaz, A.A., Bakirel, U., Çağatay, P. and Tan, H.: The Effects of Serum IgG and Trace Elements-Copper and Zinc on the Development of Kivircik Lambs Following Colostrum Intake. *Acta Vet. Eurasia.* 26: 475-481, 2000.
 36. Abecia, J.A., Forcada, F., Valares, J.A., Palacín, I., Martín, S., Martino, A., Gomez, M.I. and Plalacios, C.: Does Melatonin Treatment During Lactation Influence Milk Production in Lacaune and Assaf Ewes? *SJAR.* 3: 396-401, 2005.
 37. Bouroutzika, E., Kouretas, D., Papadopoulos, S., Veskoukis, A.S., Theodosiadou, E., Makri, S., Paliouras, C., Michailidis, M.L., Caroprese, M. and Valasi, I.: Effects of Melatonin Administration to Pregnant Ewes Under Heat-Stress Conditions, in Redox Status and Reproductive Outcome. *Antioxidants.* 9:266, 2020.
 38. Garrel, C., Fowler, P.A. and Al-Gubory, K.H.: Developmental Changes in Antioxidant Enzymatic Defences Against Oxidative Stress in Sheep Placentomes. *J. Endocrinol.* 205: 107-116, 2010.
 39. Andrei, S., Matei, S., Rugină, D., Bogdan, L. and Ștefănuț, C.: Interrelationships Between the Content of Oxidative Markers, Antioxidative Status, and Somatic Cell Count in Cow's Milk. *Czech J. Anim. Sci.* 61: 407-413, 2016.
 40. Mandevu, P., Castillo, J.B., Steckley, D.J. and Evans, E.: Total Antioxidant Capacity: A Tool for Evaluating the Nutritional Status of Dairy Heifers and Cows. *Can. J. Anim. Sci.* 83: 605-608, 2003.
 41. Sales, F., Peralta, O.A., Narbona, E., McCoard, S., Lira, R., Reyes, M.D.L., Bulnes, A.G. and Parraguez, V.H.: Maternal Supplementation with Antioxidant Vitamins in Sheep Results in Increased Transfer to the Fetus and Improvement of Fetal Antioxidant Status and Development. *Antioxidants.* 8: 59, 2019.
 42. Ozawa, M., Hirabayashi, M. and Kanai, Y.: Developmental Competence and Oxidative State of Mouse Zygotes Heat-Stressed Maternally or *In Vitro*. *Reprod.* 124: 683-689, 2002.
 43. Aréchiga, C.F., Ealy, A.D. and Hansen, P.J.: Evidence That Glutathione is Involved in Thermotolerance of Preimplantation Murine Embryos. *Biol. Reprod.* 52: 1296-1301, 1995.
 44. Kurt, S., Salar, S., Küplülü, Ş. and Baştan, A.: Effects of Prepartally Supplements of Vitamins and Trace Elements on Total Antioxidant and Oxidant Status and Udder Health Parameters on Day 14 Postpartum in Holstein-Friesian Cows. *Kocatepe Vet. J.* 12: 322-328, 2019.
 45. Omur, A., Kirbas, A., Aksu, E., Kandemir, F., Dorman, E., Kaynar, O. and Ucar, O.: Effects of Antioxidant Vitamins (A, D, E) and Trace Elements (Cu, Mn, Se, Zn) on Some Metabolic and Reproductive Profiles in Dairy Cows during Transition Period. *Pol. J. Vet. Sci.* 19: 697-706, 2016.
 46. Abuelo, A., Alves-Nores, V., Hernandez, J., Muino, R., Benedito, J.L. and Castillo, C.: Effect of Parenteral Antioxidant Supplementation During the Dry Period on Postpartum Glucose Tolerance in Dairy Cows. *JVIM.* 30: 892-898, 2016.
 47. Seron-Ferre, M., Reynolds, H., Mendez, N.A., Mondaca, M., Valenzuela, F., Ebensperger, R., Valenzuela, G.J., Herrera, E.A., Llanos, A.J. and Farfan, C.T.: Impact of Maternal Melatonin Suppression on Amount and Functionality of Brown Adipose Tissue (BAT) in the Newborn Sheep. *Front. Endocrinol.* 5: 232, 2015.
 48. Sales, F., Parraguez, V.H., McCoard, S., Cofré, E., Peralta, O.A. and Subiabre, I.: Fetal Brown Fat Deposition is Increased by Melatonin Implants in Sheep. *J. Animal Sci.* 95: 152-153, 2017.
 49. Sales, F., Peralta, O.A., Narbona, E., McCoard, S., González-Bulnes, A. and Parraguez, V.H.: Rapid Communication: Maternal Melatonin Implants Improve Fetal Oxygen Supply and Body Weight at Term in Sheep Pregnancies. *J. Animal Sci.* 97: 839-845, 2019.
 50. Flinn, T., McCarthy, N.L., Swinbourne, A.M., Gatford, K.L., Weaver, A.C., McGrice, H.A., Kelly, J.M., Walker, S.K., Kind, K.L., Kleemann, D.O. and van Wettere, H.E.J.: Supplementing Merino Ewes with Melatonin During the Last Half of Pregnancy Improves Tolerance of Prolonged Parturition and Survival of Second-Born Twin Lambs. *J. Anim. Sci.* 98: 1-35, 2020.