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Original article

Effects of melatonin implantation on the fertility potentials of Kivircik and Charollais ewes and rams during the non-breeding season

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Abstract

This study examined the effect of melatonin implantation during the non-breeding season on the reproductive performance of ewes and the testicular dimensions of rams. In seasonally anestrus Kivircik and Charollais ewes and rams were subjected to melatonin. Estrus response was significantly higher in treated than control ewes of both breeds ($p<0.001$). The pregnancy rate was significantly lower ($p<0.001$) in the control than in the treated animals. The twinning rate was significantly lower in melatonin implanted Kivircik than Charollais ewes ($p<0.05$). The testicular dimensions after 42 days of melatonin treatment increased in both breeds. Scrotal length (SL) increased in Kivircik and Charollais rams ($p<0.01$). The increase in scrotal circumference (SC) was more marked in the Charollais ($P<0.01$) than in the Kivircik rams. There was a large increase in testicular volume (TV) in both Kivircik ($p<0.01$) and Charollais ($p<0.001$) rams. This study shows that melatonin implants can be applied to induce estrus in ewes approximately four months earlier than breeding season. Melatonin implantation in the non-breeding season significantly increased testicular dimensions in Kivircik and Charollais rams thus increasing their reproductive potential.

Key words: ewe, ram, seasonal anestrus, melatonin implantation, fertility, testicular changes

Introduction

Productivity in farm animals, especially in sheep, is limited by sexual seasonality, which in turn is regulated by photoperiod. Among farm animals living in the temperate climate zone, sheep are regarded as a strongly seasonal species. This phenomenon is controlled primarily by cyclic changes of daylight and several other factors, such as temperature, feeding, contact with males, lambing time and lactation duration. Seasonal differences between breeds in reproductive behaviour and performance are due to the level of

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domestication and breeding intensity (Haresign et al. 1990, Rosa et al. 2012). The mating season in Turkey is August-December in some regions, and June-January in others. Generally, September-November is the most suitable time for mating and insemination (Horoz and Kasikci 2003, Uslu et al. 2012). Seasonality is clearly reflected by detectable changes in behaviour, gametogenesis and hormone secretion (Forcada et al. 2002, Chemineau et al. 2008, Egerszegi et al. 2014), and approximately follows the breeding activity of the ewes within a given breed (Zuniga et al. 2002). The importance of the pineal gland and its hormone melatonin in controlling breeding season for ewes has been established. It has been demonstrated that melatonin given by injection, oral administration or vaginal or subcutaneous implantation can advance the breeding season in ewes. Melatonin also affects the ovaries through a luteotrophic action both in vitro (Egerszegi et al. 2014) and in vivo (Abecia et al. 2007). It enhances the ovulation rate by decreasing the atresia of medium and large follicles (Forcada et al. 2002). Seasonal anestrus reduces reproductive efficiency and hinders productivity. Melatonin treatment during the nonbreeding season can reverse the :long-day effect; in rams (Rosa et al. 2012) increasing lamb production after estrus synchronization in ewes or artificial insemination procedures. In mature rams, the insertion of melatonin implants facilitated testicular growth with an elevated testosterone concentration and improved semen characteristics in different breeds (Chemineau et al. 1996, Palacin et al. 2008, Casao et al. 2010, Rosa et al. 2012). The objective of this study was to determine whether melatonin implants have a significant effect on fertility and some testicular characteristics in Kivircik and Charollais ewes and rams during the nonbreeding season.

Materials and Methods

This study was conducted on two different sheep breeds in Catalzeytin-Kastamonu, Turkey (longitude 41º52'35.14" East, latitude 34º13'13.07" North) and was carried out between April and July, when the daylight length increases.

Animals

All procedures described here in were approved by the Ondokuz Mayis University Ethic Committee on Animal Research (approval date/number: December 2013/04-103). In this study, clinically healthy, in seasonally anestrus Kivircik (n = 77) and Charollais (n = 33) ewes, and 4 Kivircik and 4 Charollais rams were treated with a single subcutaneous implant of melatonin (Regulin®), containing 18 mg of melatonin (2-5 years of age). An additional 15 ewes of each breed served as controls i.e. no melatonin or placebo treatment during the experimental season. Experiment groups were formed randomly. The number of offspring produced by the ewes in the experimental season was compared to the performance of the same ewes in the previous season when they were not treated with melatonin. The body condition scores ranged from 3.0 to 4.0 with an average of 3.5 ± 0.4 [using a scale of 1-5; 1 (emaciated) to 5 (obese)] (Ucar et al. 2005). The ewes remained housed throughout the experimental period. Experiments were performed in the non-breeding season in a natural photoperiod environment. The ewes used in this study were housed in indoor shelters during winter and were lambed, as expected, from mid-March onwards. Water and mineral licks were offered ad libitum. The ewes were not milked, but their lambs were allowed to suckle. Prior to the study period, the rams were separated from the flock until re-introduction.

The ewes were isolated from the rams at least one month prior to melatonin implantation. Treatments were initiated on April 17. Each of the Reguline implants (Ceva Animal Health, Turkey) contained 18 mg of melatonin and were placed subcutaneously in the ears. The melatonin implants were not removed. The animals kept as control groups received no hormonal treatment. Evaluation of Reproductive Performance in Ewes On May 22, 35 days after melatonin implantation the ewes were all visually monitored twice a day (morning and evening) for signs of estrus. Estrus was monitored regularly after the introduction of the rams to the flock. Uneasiness, increased occurrence of repeated tail wagging, frequent urination, an abnormal amount of bleating, reddish and swollen vulva and mucus under the tail were all considered true signs of estrus (Ucar et al. 2005). The main criteria for signs of estrus were the attractivity and receptivity of ewes (standing still to be mounted by the ram). Ewes showing estrus were subjected to natural mating. In this study, the ram to ewe ratio was used 1/20 in natural breeding. The estrus response, pregnancy, litter size, twinning and number of live offspring were evaluated in all groups.

Evaluation of testicular characteristics in rams

In this study, four Kivircik and four Charollais rams with known fertility (according to farm records) were chosen for both natural breeding and in order to obtain andrological data. The rams received three implants one week before the ewes (on April 10) were treated and were introduced into each group six weeks later to induce a ram effect and mating. The rams remained in the flock for 30-36 days. To obtain an objective measurement of the effects of the melatonin implants on the reproductive potential of rams, we evaluated changes in testicular and scrotal dimensions.

Measurements of testicular dimension (TD), testis volume (TV), scrotal length (SL) and scrotal circumference (SC) were performed during the melatonin implantation and after 42-45 days of melatonin implantation. Callipers positioned at the widest anteroposterior diameter were used to measure the TD (cm) of the left and right testicles. Callipers were also used to measure the SL (cm) as the distance between the tip of the scrotal sack and its neck. SC (cm) measurements were obtained while the ram was in a standing position by pushing the testicles to the bottom of the scrotum and then measuring the greatest circumference with a flexible metal tape measure. TV was measured using the water displacement method.

Statistical Analysis

In this study, the data are summarized by descriptive statistics in terms of mean and their standard error of means for the pregnancy rate (number of pregnant ewes/total number of ewes), litter size (number of lambs live born/total number of ewes), and twinning rate and number of live offspring (number of lambs born) were calculated after parturition for summarized data. In this study, to objectively determine the effects of melatonin administration, we examined the number of offspring and the survival rate of ewes in the nonbreeding season compared to the number of offspring and the number of survival in previous births by the same ewes. Discrete and threshold characteristics data within and between treatments for the estrus response, pregnancy status, litter size and number of live offspring were compared using the GENMOD procedure integrated with a log link function and the generalized estimation equation technique. Model selections are done via BIC and AKAIKE criterions. Therefore, due to the insignificant interaction effects between main effects, GENMOD model without interactions is fitted as:

$$\mu_m = \beta_{\mathrm{m},0} + X_1\,\beta_{m,i} + X_2\beta_{m,j}$$

Where μ_m is the mean of the mth traits, $\beta_{m,0}$, β_i and $\beta_{m,j}$ are unknown parameter vectors for the effects on mth traits for two breeds and for two treatment groups, and X is a matrix for the breeds and treatment groups. Andrological trait comparisons by breed were performed using the one-way ANOVA (Hardin 2005). All analyses and statistical calculations were executed using SAS statistical suit (2009).

Results

Estrus response in the melatonin treated ewes was 0.97 ± 0.16 (mean ± SEM; Table 1) in Kivircik and 1.00 ± 0.00 for Charollais, and was significantly higher ($p<0.001$) than in the non-treated Kivircik (0.13 ± 0.35) and Charollais (0.20 ± 0.41) controls. Differences between breeds were not significant. A similar pattern of treatment and breed differences were observed for the degree of estrus response. None of the Kivircik and only 0.06 ± 0.25 of the Charollais control ewes became pregnant compared to 0.92 ± 0.26 of the Kivircik and 1.30 ± 1.74 of the Charollais ewes treated with melatonin ($p<0.001$). When the breeds were analysed separately, melatonin implantation increased the fertility rate significantly in both the Kivircik and Charollais ewes ($p<0.05$). The twinning rate, number of live offspring per ewe, and litter size were all significantly higher ($p<0.001$) in melatonin than in control ewes. The twinning rate was significantly lower for melatonin treated Kivircik than in Charollais ewes (0.44 ± 0.01 vs. 0.63 ± 0.06; $p<0.05$). Consequently, the number of live offspring per pregnant ewe was significantly higher ($p<0.05$) in melatonin treated Charollais (1.63 ± 0.10) than in Kivircik (1.37 ± 0.08) ewes. The control animals of both breeds produced no lambs in the experimental season which was significantly ($p<0.001$) less than in the previous breeding season (Kivircik 1.00 ± 0.53; Charollais 0.86 ± 0.61 per ewe). In contrast, the melatonin treated ewes produced significantly ($p<0.05$) more lambs per ewe than in the previous season (Kivircik 1.37 ± 0.62 vs. 0.76 ± 0.77; Charollais (1.63 ± 0.60 vs. 0.84 ± 0.61; Table 1).

The testicular dimensions in both breeds before and after 42 – 45 days of melatonin implantation are presented in Table 2. During this period, the SL of Kivircik rams increased from 12.6 ± 0.95 to 14.1 ± 0.40 cm (NS) and from 19.4 ± 0.85 to 22.8 ± 0.75 cm ($p<0.01$) in the Charollais rams. The increase in SC was more marked in the Charollais ($p<0.01$) than in the Kivircik rams (NS). Both breeds showed increases in TD but the differences were not significant. There was a large increase in TV in both Kivircik (442 ± 14.0 vs. 639 ± 41.5 cm3; $p<0.01$) and Charollais (650 ± 140 vs. 1075 ± 225 cm3; $p<0.001$) rams. SL, SC, TD and TV were smaller for Kivircik than Charollais rams both before and after melatonin implantation. The breed differences were significant ($p<0.05$ and $p<0.001$) except for TD. For example, TD in Charol-

Table 1. The reproductive performance in Kivircik and Charollais ewes implanted with melatonin during the non-breeding season.

Variables	Treatments				P
	Kivircyk		Charollais		
	Control (n=15)	Experiment (n=77)	Control (n=15)	Experiment (n=33)	
Estrus response	0.13 ± 035	0.97 ± 0.16	0.20 ± 0.41	1.00 ± 0.00	P<0.001
Pregnancy rate	0.00 ± 0.00	0.92 ± 0.26	0.06 ± 0.25	1.30 ± 1.74	P<0.001
Twining rate	0.00 ± 0.00	0.44 ± 0.01	0.00 ± 0.00	0.60 ± 0.06	P<0.001
Live offspring per ewe	0.00 ± 0.00	1.37 ± 0.62	0.00 ± 0.00	1.63 ± 0.60	P<0.001
Litter size	0.00 ± 0.00	1.32 ± 0.65	0.00 ± 0.00	1.48 ± 0.66	P<0.001
NOPB	1.00 ± 0.53	0.76 ± 0.77	0.86 ± 0.63	0.84 ± 0.61	P>0.478

NOPB: Number of offspring in a previous birth. Means (±SEM) having different superscripts within the same row are significantly different from each other. *: p<0.05; **: p<0.01; p>0.05: not significant

Table 2. Testicular characteristics in Kivircik and Charollais rams treated with melatonin during the non-breeding season.

Variables	Measuring time	Kivircik (n= 4)	Sign.	Charollais (n= 4)	Sign.
Scrotal length (cm)	1	12.55 ± 0.95		19.35 ± 0.85	
	2	14.10 ± 0.40	NS	22.75 ± 0.75	*
Scrotal circumference (cm)	1	30.95 ± 0.75		34.95 ± 2.55	
	2	32.10 ± 0.90	NS	40.00 ± 3.70	*
Testis diameter (cm)	1	5.60 ± 0.10		8.47 ± 0.22	
	2	6.05 ± 0.15	NS	9.20 ± 0.15	NS
Testis volume (cm^3)	1	442.00 ± 14.00		650.0 ± 140.0	
	2	638.50 ± 41.50	*	1075.0 ± 225.0	**

(1): Measurements during the melatonin implantation, (2): Measurements after 42-45 days of melatonin implantation.
Means (±SEM) having different superscripts within the same row are significantly different from each other.
*: p<0.05, **: p<0.01, NS: not significant (p>0.05)

lais is approximately 1.5 times longer that in Kivircik after 45 d of treatment (9.20 ± 0.15 vs. 6.05 ± 0.15). The difference is not statistically significant but it is large and similar to the trend observed for all the other parameters.

Discussion

In the present study, we investigated the effects of melatonin administration on the reproductive performance in Kivircik and Charollais ewes in the non-breeding season. Our results demonstrated that a pregnancy rate was achieved in the melatonin-treated groups in the non-breeding season. Therefore, melatonin implantation in the non-breeding season significantly increased the fertility rate in both the Kivircik and Charollais ewes. It is well known that ewes are seasonally polyestrus animals and that their sexual activity starts during the autumn months when the days shorten and the heat decreases (Horoz and Kasikci 2003). Breeding is one of the main sources of income for farmers, especially in rural/mountain areas with large grazing/pasture lands in Turkey. The mating season in Turkey is August-December in some regions and June-January in others. Generally, September-November is the most suitable time for mating and insemination. Decreasing day length is thought to stimulate the endocrine changes associated with cyclicity (Emrelli et al. 2003, Horoz and Kasikci 2003, Uslu et al. 2012). Abecia et al. (2007) reported that melatonin can increase fertility and litter size by improving luteal function and embryonic survival, probably by inducing a uterine environment that is more conducive to pregnancy. The authors explain this by the effect of melatonin on the corpus luteum and its ability to increase progesterone levels during the luteal phase and to support embryo development (Haresign 1992, Zuniga et al. 2002, Abecia et al. 2007, Chemineau et al. 2008). In our study, rams were introduced to the flock after six weeks of melatonin and a pregnancy rate of 0.92 ± 0.26 – 1.30 ± 1.74 and a twinning rate of 0.44 ± 0.01 – 0.60 ± 0.06 was achieved. These rates were higher in the Charollais ewes compared to the Kivircik ewes. The pregnancy rate and estrus response was also similar to that reported by Haresign (1992), Horoz and Kasikci (2003) and Padeanu et al (2011). Although the pregnancy rate was lower than that reported by Emrelli et al (2003) and Haresign et al (1990), it was higher than that reported by Abecia et al. (2007) and de Nicolo et al. (2008). This discrepancy in the results for pregnancy and estrus response rates reported by different researchers can be explained by the use of different breeds and ages of animals or by nutritional factors. Surprisingly, we obtained 0.20 ± 0.41 and 0.13 ± 0.35 estrus response rates in the control groups (Charollais and Kivircik ewes, respectively). Accordingly, the control ewes could have been sexually stimulated by the rams (via the combined effects of visual, auditory and olfactory factors), which caused them to enter estrus. In this study, the number of live offspring per ewe was also similar to that reported by Horoz and Kasikci (2003) but was higher than that reported by Abecia et al. (2007) and lower than that reported by Haresign et al. (1990) and Haresign (1992). The litter size differed significantly between the two breed groups, being the highest in the Charollais ewes and the lowest in the Kivircik ewes. The average litter size was also similar to that reported by de Nicolo et al. (2008). However, the pregnancy rate was lower than that reported by Haresign (1992) and Haresign et al (1990) and was higher than that reported by Abecia et al. (2007), Padeanu et al. (2011) and Uslu et al. (2012). This discrepancy in the results reported by different researchers may be due to various factors, such as: (I) the time of year (earlier in this study); (II) route of administration (subcutan or intravaginal); (III) breed type with fat tails (Morkaraman, as used here) or lean tails (Kivircik breed, as used by Horoz and Kasikci, 2003) and (IV) geographic location.

Seasonal changes in reproductive performance were also observed in the rams. It is well known that the testicular size and efficiency of spermatogenesis in rams are at their maximum levels during the breeding season and decrease during the non-breeding season. Seasonal changes in TDs, preceded by the decrease in Luteinizing hormone (LH) and the increase in Follicle stimulating hormone (FSH) secretions, have been described in numerous sheep breeds, including the Suffolk, Texel, Dorset, Il-de-France, British Milk, Soay, Karakul, Chios, Serres Finn and Scottish Blackface breeds (Mandiki et al. 1998, Sarlos et al. 2013). In British Milk rams, TV was the lowest in winter, and then after a continuous increase, it reached the highest value in the autumn. The maximal testicle mass measured in September exceeded the lowest value found in January by 33.5% (Sarlos et al. 2013). Each gram of the testis produces approximately 20 x 106 sperm cells per day, irrespective of size. SC is a good index of sperm production in rams. Therefore, SC and TD are suitable selection criteria to improve reproductive performance. They are also traits that can be easily measured in live animals (Endale et al. 2009). In the present study, SL and SC increased in melatonin-implanted Charollais rams compared to that found before the melatonin implantation ($p<0.05$). SL and SC were also higher in melatonin-implanted Charollais rams compared to those determined in Kivircik rams ($p<0.05$). Testicular diameters increased significantly due to melatonin implantation in both breeds of rams. The TD in Charollais rams was approximately 1.5 times longer than that in Kivircik rams both before and after 45 day of treatment. The differences were not significant but the trend was consistent with that of the other testicular parameters. In this study, the changes in SC and TD in Kivircik rams were similar to those reported by Endale et al. (2009), Egerszegi et al. (2014) and Rosa et al. (2012). However, SC and TD in the melatonin-treated Charollais rams were higher than those reported by Kridli et al. (2006), Kridli et al. (2007) and Egerszegi et al. (2014).

Conclusion

This study shows that melatonin implants can be applied to induce estrus in ewes approximately four months prior to the normal autumn breeding season. Consequently, melatonin implants can be used in out-of-season breeding programs for Kivircik and Charollais ewes in Turkey. Melatonin implantation in the non-breeding season significantly increased the testicular dimensions in Kivircik and Charollais rams thus increasing their reproductive potential.

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