Use of exogenous melatonin in Ouled Djellal ewes and rams during non-breeding season

M. Adnane and K. Miroud*

Abstract

The study aimed to investigate the effect of exogenous melatonin treatment on the ewe's and ram's reproductive activity, out-of-season breeding, usually matching with spring. The eighty four adult ewes and 16 genitor rams of Ouled Djellal breed, taken randomly out of a 176 ovine flock were treated with respectively 1 or 3 melatonin subcutaneous ear implants (Melovine). The 76 non-treated remaining ewes served as the control group. The rams, kept separately from the females, had scrotal perimeters measured before (PS1) and 47 days after (PS2) melatonin treatment, before they were allowed to run with the ewes. The analysis of the lambing rates had not shown any significant difference ($P>0.05$) between the melatonin treated ewes and those of the control group (90.4% vs. 97.3%) which proves that their sexual activity is not under photoperiod control. However, the analysis by the t-test of the paired samples of the ram's scrotal perimeters had shown a significant difference ($P<0.05$) between the measurements before and after melatonin treatment in rams which proves the potential effect of this hormone on daily sperm output. During the study period, no effect on female sexual activity has been noted. In Ouled Djellal sheep breed, melatonin use is not recommended in ewes but it can be useful in rams since it enhances sexual capacity during mating.

Key words: *ovine; non-breeding season; photoperiod; scrotal perimeter; Algeria*

Introduction

Photoperiod is considered to be one of the major factors influencing sexual activity in small ruminants (Malpaux et al., 1999). Ewes ovulate spontaneously and are commonly considered as a species with a seasonal cycle (Thimonier et al., 1984). However, unlike to what is observed during the seasonal anoestrus of

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females, the sexual activity of males weakens but does not cease (Graeme et al., 1994; Kridli et al., 2002; Gundoğan et al., 2003; Oberst et al., 2011) and can be maintained by regular training, or by an adapted diet (Boukhliq and Martin, 1996; Graeme et al., 1999; Santos et al., 2015).

Hormonal treatment with melatonin is a mean of controlling reproduction during anoestrus in ewes and goats as well as in rams; it shortens the anoestrous seasonal period and significantly improves the sexual behaviour and the reproductive capacity of the genitors (Malpaux et al., 1995). It causes a rise in plasma melatonin concentrations for 24 hours during the day, without suppressing the endogenous nocturnal secretion of the pineal hormone. Thus, melatonin treatment brings about a response similar to that obtained during short days by extending the duration of its signal (Malpaux et al., 1997).

Ewes and rams of the Ouled Djellal breed were, out breeding season (in spring), treated with melatonin subcutaneous ear implants in order to determine the possible effects of this hormone on their reproduction.

Materials and methods

Study areas

This study was conducted, in a livestock husbandry demonstration farm in El Khroub (Latitude: 36°15′47″ North; Longitude: 6°41′36″ East; altitude: 630 m), located 13km south of Constantine – Algeria (Figure 1).

According to the Köppen-Geiger classification, the climate of the study site is of type Csa (URL1) (Cs: warm temperate climate with dry summer (Mediterranean), a hot summer, average temperature of the hottest month > 22 °C). Annually in El Khroub, the average temperature is 15.6 °C and the average rainfall is around 540 mm.

Experimental design

During the non-breeding season (in spring), a flock of 160 ewes and16 rams

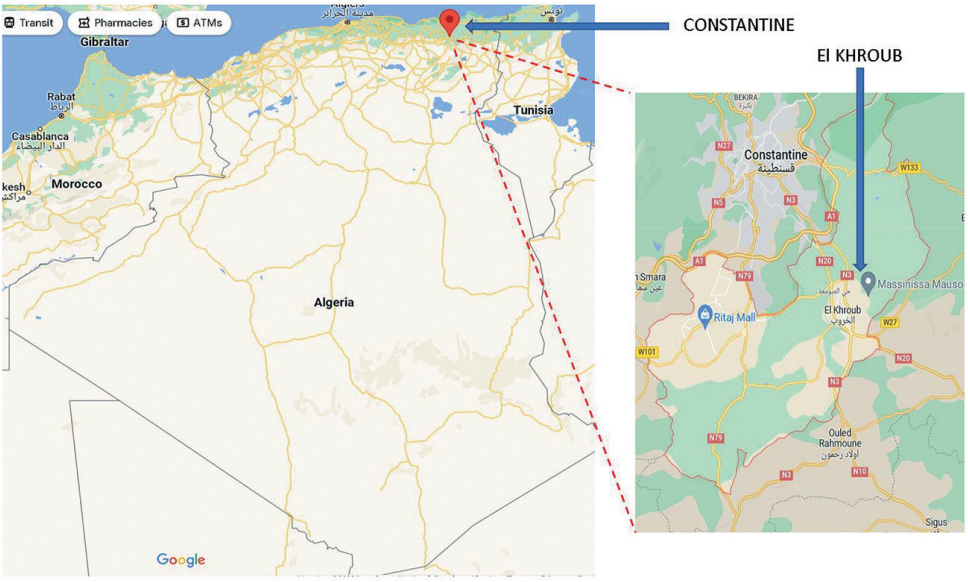

Figure 1. Geographical position of the study area

of the Algerian Ouled Djellal breed was involved in this study. The ewes, 2 to 5 years old, grazed in the morning and afternoon, received a concentrated supplement, a mixture of vetch and oat hay, and had access to the water twice a day.

The rams, aged 3 to 5 years, were kept separately from the females and only fed hay, straw, concentrate and had access to the water. All the animals in the herd were dewormed 2 months prior to the study onset. The unmarked ewes (n=84), taken randomly, received each an ear s/c implant containing 18 mg of melatonin (Melovine®, CEVA animal health, France). The 16 genitor rams, received 3 ears/c implants each *i.e.* 54 mg of melatonin, were released with all the females of the herd 47 days after treatment and removed 36 days later, the equivalent of nearly a 2 oestrous cycle period. The treatment was initiated 40 days before coupling according to the manufacturer's instructions (CEVA animal health, France). The scrotal circumference of the rams before (PS1) and 47 days after treatment (PS2) was measured using a tape measure. The rest of the herd, 76 ewes (160 - 84), marked with an indelible blue paint, did not undergo any treatment and served as the control group.

Ethical statement

The study was carried out in an experimental cattle and sheep breeding unit belonging to the Algerian Ministry of agriculture and Fisheries located in Constantine-Algeria. Prior to the study, we obtained the approval from the Ethics and deontology committee (File number: 02/2022 recorded on the 5th of March2023) of Chadli Bendjedid University, El Tarf-Algeria.

Statistical Analysis

Pearson's chi-square statistical test was used to examine the influence of the melatonin on the rating of lambing of the treated and untreated ewes and the t-test of the paired samples carried out for the two measurements of the scrotal circumference before and after treatment was used to determine the effect of melatonin in rams. Statistical tests were performed using SPSS software (IBM SPSS statistics for windows version 20.0, 2017) and were considered significant at (P<0.05).

Results

Table 1. shows no significant difference in the percentage of lambing (P>0.05) between, respectively, treated and untreated ewes with melatonin (97.3% vs. 90.4%).

Table 2. shows that the mean, standard deviation and standard error of the scrotal circumference measurements of the 16 rams before treatment (PS1) were

Table 1. Analysis by the X^2 test of the relationship between the rate of lambing in treated and untreated ewes

Lambing				
Control group		Treated group		X^2
N=76		N=84		
n	%	n	%	NS
74	97.3	76	90.4	
2	2.6	8	9.5	

lower than those consecutive to melatonin treatment (PS2).

A high correlation of 0.7 corroborated by the level of significance of the value (P<0.05) was recorded (Table 3).

In table 4, p value is below the significance threshold (P<0.05); the averages of the two measurements of the scrotal perimeter of the rams (PS1, PS2) before and after treatment with melatonin were different. The analysis by the t test of the paired samples carried out for the two measurements of the scrotal circumference proves the effect of melatonin.

Discussion

Forcada et al. (2002) reported that for the Mediterranean sheep breeds, melatonin treatment is immediately effective after winter. This could explain why the ewes of Ouled Djellal breed expressed heat and conceived in Spring, matching with non-breeding season, although the ability to mate and conceive all year around, without the use of melatonin, was reported (Adnane et al., 2018).

The photoperiodic nondependent sexual activity of these ewes is therefore not necessarily the result of melatonin exogenous treatment. Our results agree with those of Rosa and Bryant (2003), who reported that breeds bred between 35° N and 35° S tend to reproduce during all the year and that at latitudes above 35° they express a seasonal polyestrian sexual activity initiated by short days, which is the case of the Ouled Djellal breed, and mid- or low-latitude breeds have a seasonal sexual activity and are therefore

Table 2. Statistics for paired samples

		Mean	N	Standard-deviation	Standard-error
Pair 1	PS1 before treatment	31.3	16	2.7	0.7
	PS2 after treatment	37.8	16	3.5	0.9

Table 3. Correlations for paired samples

		N	Correlation	Significance
Pair 1	PS1 before treatment & PS2 after treatment	16	0.7	0.002

Table 4. Paired Samples Test

		Paired differences					t	ddl	Sig. (Bilateral)
		Mean	S.D.	S.E.	95% C.I. Inferior	95% C.I. Superior			
Paire 1	PS1 before treatment – PS2 after treatment	-6.5	2.5	.6	-7.8	-5.1	-10.3	15	0.000

S.D.: Standard deviation; S.E.: Standard error; C.I.: Confidence interval; ddl: degree of freedom; Sig.: Significance level

sensitive to photoperiod variation. They also agreed with some authors, although with other breeds, that are not very seasonal, such as the Fine Tail of the West bred in Tunisia, which is in reality the Algerian Ouled Djellalbreed and the Noire de Thibar (Zaiem et al., 2000) and also those obtained outside North Africa for other breeds whose sexuality is very little affected by season, such as the Rouge de l'Ouest or the Caussenard du Lot or the Australian Corriedale (Chemineau et al., 1991, 1993; Staples et al., 1991).

Adnane et al. (2018-2022) reported respectively 97% and 78.3% lambing rates after allowing untreated rams to run with ewes in spring, which confirms the photoperiodic independent character of the Ouled Djellal ewes since they expressed heat, conceived in the sexual out-breeding season and lambed. These findings are similar to those of Niar et al. (2001), Taherti et al. (2016), Benia et al. (2018) and Benyounes and Lamrani (2013) who reported that Ouled Djellal ewes have continuous sexual activity all year around. On the other hand Berdugo, et al. (2021) showed in the study realize in water buffalo to determine the effect of exogenous melatonin on follicular development parameters in a fixed-timed artificial insemination programme, that there is no effect of melatonin on the ovarian response of buffaloes and suppose that other factors, such as environmental conditions of subtropical areas and species specificities, may have a more significant effect on buffalo endocrinology.

The data in table 4, analysed by the t-test of the paired samples, revealed a significant difference ($P<0.05$) between the scrotal perimeter of rams before and after melatonin treatment.

Melatonin implants in the Ouled Djellal rams in the non-breeding season therefore led to an increase in the size of the testicles. This was also reported by Allaoui (2019) who declared that the use of this hormone is interesting as it makes it possible to increase and stabilize testicular size in rams throughout the breeding period, on the one hand, and enhances the reproductive performance of rams (fertility and fecundity) in natural climb during the spring mating without promoting a better libido (expressed by the plasma testosterone concentration) in rams, on the other hand.

Some studies (Chemineau et al., 1988, 1991, 1993, 1996; Philippe et al., 1996; Casao et al., 2010; Egerszegi et al., 2014; Cevik et al., 2017) stated that in adult rams, the use of melatonin implants facilitates and maintains testicular development in the out-breeding season and further improves sexual behaviour and sperm characteristics in different breeds of sheep. Other studies (DeNicolo et al., 2008; Inmaculada et al., 2008; Luridiana et al., 2015) explain this by the fact that the treatment of rams with melatonin between the middle and the end of spring allows the acceleration of the appearance of seasonal LH peaks causing an increase in the size of testicles. Lincoln and Maeda (1992) found out that the placement of micro-implants of melatonin in the mid-basal area of the hypothalamus in rams is accompanied by a reactivation of the hypothalamic-pituitary-gonadal axis with an increase in the secretion of GnRH and FSH and therefore, an acceleration of testicular growth; similar results were also put forward by Bittman and Karsch (1984), Tamarkin et al. (1985). On the other hand, Misztal et al. (1996) showed that the duration of the reproductive activity of rams treated with melatonin is shorter than in natural photoperiod conditions. A positive effect of exogenous melatonin on the proportions of French. Alpine buck spermatozoa in subpopulations has

also been showed (Vince et al., 2017; Žura Žaja et al., 2018, 2020).

In the present study, the effect of melatonin, although it has led to an increase in SP, cannot on its own explain the non-significant difference between lambing rates following spring mating and conception of treated and untreated ewes.

Conclusion

Ewes of the Ouled Djellal breed expressed sexual activity during the period usually corresponding to seasonal anoestrus (spring), conceived and lambed. In view of the non-significant different lambing rates recorded between untreated and melatonin-treated ewes, it is concluded that the sexual activity of this breed is not photo-dependent. In rams of the same breed, on the other hand, melatonin caused a significant increase in the size of the testicles. Melatonin is therefore more beneficial to rams than to ewes; it could be recommended for rams in order to enhances sexual capacity during mating, since there mating capacity may decrease due to the difference between the scrotal perimeters measured during breeding and no breeding seasons.

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Uporaba egzogenog melatonina u ouled djellal ovaca i ovnova izvan sezone parenja

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Cilj studije bio je istražiti učinak primjene egzogenog melatonina na reproduktivnu aktivnost ovaca i ovnova, izvan sezone parenja, što se obično poklapa s proljećem. Osamdeset i četiri odrasle ovce i 16 rasplodnih ovnova ouled djellal pasmine nasumice je izabrano ih stada od 176 ovaca i primilo je 1 ili 3 potkožna ušna implantata melatonina (Melovine). Preostalih 76 netretiranih ovaca služili su kao kontrolna skupina. Ovnovima, koji su držani odvojeno od ženki, izmjeren je opseg skrotuma prije (PS1) i 47 dana nakon (PS2) primjene melatonina, prije nego su vraćeni među ovce. Analiza stope janjenja nije ukazala na značajnu razliku (P>0,05) između ovaca tretiranih melatoninom i onih iz kontrolne skupine (90,4 % u usporedbi s 97,3 %) što dokazuje da na njihovu spolnu aktivnost ne utječe broj sati svjetla u danu (fotoperiod). Međutim, analiza t-ispitivanja uparenih uzoraka opsega skrotuma ovnova pokazala je značajnu razliku (P<0,05) između mjerenja prije i nakon tretiranja melatoninom u ovnova što dokazuje potencijalni učinak ovog hormona na dnevnu proizvodnju sperme. Tijekom razdoblja studije, nije uočen nikakav učinak na spolnu aktivnost ženki. U ovaca pasmine ouled djellal, uporaba melatonina nije preporučena u ovaca, ali može biti korisna u ovnova, jer povećava spolni kapacitet tijekom parenja.

Ključne riječi: *ovce, izvan sezone parenja, fotoperiod, opseg skrotuma, Alžir*