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Evaluation of the Effects of Melatonin Implants on Testicular and Spermatic Parameters of Rams Raised in Station in the Niayes Zone in Senegal

Bilkiss Vinode Maureen Assani[1, *], Simplice Bosco Ayssiwede[2], Mohamed Sallim Kinnou[1], Félix Victor Latyr Mbengue[2], Ayao Missohou[2], Naceur Slimane[3]

[1]Service de Chirurgie-Reproduction, Ecole Inter-Etats des Sciences et Médecine Vétérinaires (EISMV), Dakar, Senegal

[2]Service de Zootechnie-Alimentation, Ecole Inter-Etats des Sciences et Médecine Vétérinaires (EISMV), Dakar, Senegal

[3]Service des Sciences et Pathologies de Reproduction, Ecole Nationale de Médecine Vétérinaire (ENV) de Sidi-Thabet, Tunis, Tunisia

Email address:

ammaureen@yahoo.fr (Bilkiss Vinode Maureen Assani), ayissimbos@yahoo.fr (Simplice Bosco Ayssiwede), kinnousallim04@gmail.com (Mohamed Sallim Kinnou), felixmbengueg1@gmail.com (Félix Victor Latyr Mbengue), missohou@gmail.com (Ayao Missohou), naceur_s@yahoo.fr (Naceur Slimane)

[*]Corresponding author

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Abstract: The intensification of livestock production in response to the growing demand for animal products is increasingly creating new challenges in terms of control of livestock management and feeding, and especially reproduction. Also, ewes are often considered responsible for infertility problems in a sheep flock, as the reproductive capacity of rams is often taken for granted. However, it is important to have genetically superior males to improve the overall fertility of the herd. And, one way to control the sexual performance of rams and improve their fertilizing capacity, is to have them undergo melatonin treatment. The objective of this study was to evaluate the effects of the use of melatonin implants on testicular and spermatic parameters of rams raised in stations in the Niayes area of Senegal. The experimental herd consisted of 6 rams of well-known body characteristics which were followed for 2 months during which testicular measurements were carried out at the beginning and semen was collected by electroejaculation every 2 weeks. After a break of 2 weeks, the same rams are implanted with melatonin (MELOVINE ®) at a rate of 3 implants/ram, i.e. a dose of 54 mg of melatonin per ram, and the same measurements and semen collections are again carried out for 2 months. Data on antero-posterior testicular diameter, testicular weight, scrotal circumference, and semen analysis (volume, color, appearance, mass and individual motility, concentration, morphology) recorded and processed with Excel were exported to the SPSS software where they were subjected to descriptive statistical analysis and independent sample comparison testing. The results showed that melatonin treatment significantly reduced weight (555±155 g vs. 396±128 g) and antero-posterior diameter (23.42±2.0 cm vs. 20.10±2.26 cm). However, it induced improvements in ejaculate volume, color, and appearance, sperm production, and morphological abnormality rate, and, most significantly, sperm motility (2.1±1.5 vs. 3.3±1.2) and survival rate (54±34% vs. 80±31%) of spermatozoa. Melatonin has therefore improved the sperm characteristics of rams, and is an interesting reproductive management tool for improving the productivity of sheep farms. It can be recommended for use in artificial insemination centres in order to improve the characteristics of the semen doses produced.

Keywords: Melatonin, Rams, Reproduction, Testicular Parameters, Spermatic Characteristics

1. Introduction

Sheep farming is an important source of income for a large part of the population in Senegal. Sheep provide a significant proportion of the animal protein needed by rural and urban populations and play an important socio-cultural role in marriage, baptism, death and other ceremonies. In order to meet

the ever-increasing demand for animal products resulting from the galloping demographics, it was necessary to intensify production. This requires a better knowledge of the zootechnical performance and reproductive characteristics of the animals and environmental factors that affect them in order to establish methods and techniques to ensure a good profitability of the herd [22]. But, like other animal production, the profitability of sheep farms depends largely on the success of the herd's reproduction and the development of methods and techniques to make the most of the animals' reproductive capacities implies a knowledge as rigorous as possible of the reproductive function. Indeed, the recognition of a breed's reproductive characteristics is an essential starting point for improving its productivity.

However, the reproductive control methods currently used by sheep producers to try to improve the productivity of this species are mainly oriented towards females (hormonal treatments based on progestagens, photoperiod program, melatonin). Very little effort is directed at rams because when it comes to infertility, failure to reproduce in a herd, ewes are often singled out, the reproductive capacity of rams being too often taken for granted. Thus, the ewe is often wrongly blamed for the reproductive outcome, whereas the male's share of responsibility must also be taken into account, as Colas pointed out [11]. Augas et al. specify that the role of the ram is paramount on reproductive results, because it acts on both fertility and prolificity (also depending on the ewe's abilities) [4]. Maintaining high fertility by genetically superior males producing a large number of quality sperm, is therefore important for improving the overall fertility of the herd [36]. One way to control the sexual activity of rams is to treat them with melatonin as in ewes.

To this end, studies have effectively shown that certain melatonin treatments have an impact on the parameters related to the reproductive activity of rams. These studies have been carried out, on the one hand, mainly on seasonal breeds in temperate and Mediterranean climates where an improvement in semen quality was observed during the period when naturally a decrease in performance could be observed and, on the other hand, no study has shown that the improvements in indirect measures of reproductive capacity observed with melatonin can really be reflected on the fertility and prolificity of the ewes in mating conditions. In addition, information on body size and testicular traits of various breeds at constant age is of primary importance in selecting genetically superior animals for production and reproduction purposes [41] because testicular development is strongly correlated with reproductive activity [28].

This study investigated the effects of melatonin on testicular growth and semen quality in station-raised rams in the Niayes area of Senegal, a tropical climate country where animals are little or not at all sensitive to photoperiodic variations and where the sexual season is almost permanent.

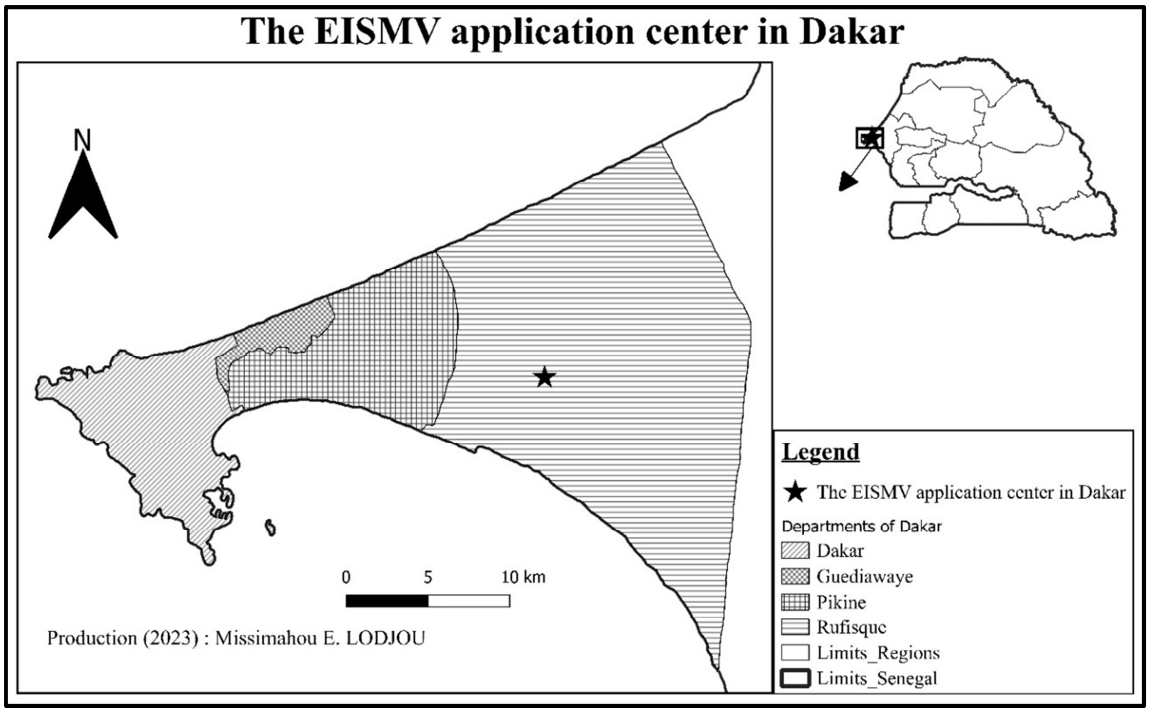

Figure 1. *Map showing the location of the study site, the EISMV application center in the Dakar region.*

2. Materials and Methods

2.1. Study Period and Site

The study was carried out from September 2021 to January 2022 on the application center of the Ecole Inter-Etats des Sciences et Médecine Vétérinaires de Dakar (EISMV) located in Keur Ndiaye Lo about 35 km from Dakar in the commune of Sangalkam, Rufisque department, Dakar region (Figure 1). This region is characterized by a tropical desert climate with two seasons, a dry season (November to June)

and a rainy season (July to October) influenced by the maritime trade wind and the monsoon. The average annual rainfall follows a decreasing gradient from the south to the north of the country. It goes from 1200 mm in the south to 300 mm in the north, with variations from year to year. The temperature varies according to the seasons. It ranges from 17 to 25°C from December to April and from 27 to 30°C from May to November [1].

2.2. Experimental Animals and Experimental Device

The trial involved six (6) Touabire x Ladoum and Touabire x Peul Peul crossbred rams, aged 20 months and weighing between 33 and 42 kg. They were fed a diet composed of peanut bark, commercial concentrated feed (served at a rate of 300 g/ram/day) and trace elements served in the form of licking stones. They were watered with tap water from the Société des Eaux (SDE) *ad libitum*.

Before the start of the trial, rams were vaccinated against the Plague of Small Ruminants and Pasteurellosis, and dewormed (Ivermectin subcutaneously, Oxfendazole *orally* and Cypermethrin *for on*). The internal parasitic treatment was renewed every 2 months and the external one, repeated every 2 weeks. Also, they were treated with oxytetracycline and vitamins after each collection session.

At the beginning of the trial, the body characteristics of the rams were well known, but they were also taken up at the end of the experiment. These same animals were used as control and experimental batches.

2.3. Data Collection

2.3.1. Measurement of Morpho-Biometric Parameters

The assessment of all the morpho-biometric parameters (body and testicles) was carried out using a toise or a metric tape.

The various body parameters (live weight, height at the withers, body length, chest perimeter) were measured at the beginning and at the end of the experiment in order to check the uniformity of the parameters between the phases (without and with melatonin).

The live weight (LW) of each animal was taken using an Iconix FX1® electronic scale with a maximum capacity of 2,000 kg and 100 g of accuracy, tared to the weight of the operator who ensures the restraint of the animal. The height at the withers (HW) was taken using a height gauge placed parallel to and behind the animal's foreleg after the animal was held on a flat surface. The length from the ground to the highest point of the withers is then taken. Body length (BL), also called scapulo-ischial length, was taken using a metric tape and corresponds to the distance from the tip of the shoulder to the tip of the buttock (ischium). The thoracic perimeter (ThP) or thoracic circumference (ThC) or circumference of the thorax was measured using a metric tape by going around the thorax a little behind the withers.

Testicular parameters measured in rams are testicular weight, scrotal circumference, and right and left anteroposterior diameters.

Testicular weight (TW) is obtained on an animal living in a standing position, hindlimbs spread apart using the water displacement method as described by Evans and Robinson [19]:

1. Weigh a container filled with warm water in which the testicles are immersed. This operation is accompanied by the discharge of a certain quantity of water from the container;
2. This partially filled container is then weighed again; the difference between the initial weight and the final weight of the container constitutes the volume of water lost and thus corresponds to the estimated testicular weight.

The scrotal circumference (SC) is taken by measuring the scrotal circumference of the testicles in its widest part after lowering the testicles into the scrotal pocket while taking care to eliminate the void between the two testicles. The antero-posterior testicular diameter (APD) corresponding to the length of the widest part of the antero-posterior axis of the testicle concerned is measured after the testicles have been lowered into the scrotal pouch with slight pressure.

2.3.2. Collection of Semen

The semen was collected through the use of an electro-ejaculator for electrical stimulation series of 05 seconds with a rest time of 02 seconds between each new pulse. An average of two ejaculates per harvest (three minutes apart) per animal was used for semen analysis.

Since the effects of melatonin were felt after at least 2 months, the animals were implanted at the beginning of the experiment at the rate of 3 melatonin implants/ram, i.e. at a dose of 54 mg of melatonin per ram. During the first phase, known as the control phase, which lasted 2 months, the semen collection of the rams took place every 2 weeks, i.e. four (4) semen samples per ram corresponding to 24 semen samples collected during the control phase. After 2 weeks of rest, these same rams enter the second phase, called the melatonin phase, during which they have been removed again every 2 weeks for 2 months. Thus, 4 semen samples were taken per ram also corresponding to 24 semen samples collected during the melatonin phase.

At the end of each collection, the semen is sent directly to the laboratory for quality evaluation analysis, for a total of 48 samples of ram semen analyzed for both phases.

2.3.3. Analysis of Semens Collected from Rams

It focused on the evaluation of parameters such as volume, colour, appearance, mass and individual motility, concentration and percentages of live, dead and abnormal spermatozoa of the collected semens.

Thus, the volume of the ejaculate, expressed in milliliters (ml), was determined directly using the graduations of the conical collection tube immediately after collection without taking into account the foamy part of the ejaculate. The color of the ejaculate is determined by careful observation when reading the volume of the ejaculate. More often whitish, the color of the sperm can be modified for physiological reasons (concentration) but most often pathological. As the appearance of the semen depended on its sperm content, it was subjectively assessed by comparing it to distilled water.

The concentration of the ejaculate (expressed as sperm count per ml - spz/ml), was determined using a calibrated spectrophotometer of the "IMV Technologies" brand. It is the most effective technique because it combines speed and precision. The general principle is to measure the optical density of 3.90 ml of a formolated saline solution (9 ml of 0.9% NaCl solution + 1 ml of 40% formalin) containing 10 µl of pure semen in the spectrophotometer. By measuring this density, the spectrophotometer automatically converts it into the concentration that is read on the device.

The number of spermatozoa per ejaculate was then determined from the measured volume and concentration of the ejaculate according to the following mathematical formula:

Number of spermatozoa in the ejaculate (spz)=Ejaculate concentration (spz/ml) ×Volume of the ejaculate (ml)

The assessment of mass motility was made based on a scale of 0 to 5 described by Baril et al. [5]. To this end, a drop (10 µl) of the collected pure semen is deposited on a slide preheated to 37-38°C. and then examined under a microscope at magnification 10. The overall movement of the spermatozoa is thus observed and according to its speed and amplitude, it is assigned a score of 0 to 5.

The individual motility is determined by observing a drop of pure semen on a preheated slide (37-38°C) at magnification 40 of the microscope, to assess and note according to the scale proposed by the same author the individual movements such as the speed, the trajectory of the movement, the lateral movements, …of the spermatozoa.

Finally, the percentages of live, abnormal and dead spermatozoa were determined after staining the semen and performing a smear. To this end, 10 ul of pure semen are taken and deposited on the edge of a slide. A drop of methylene blue is then added thereto, which is mixed before spreading this mixture over the entire length of the blade using another blade. The smear is allowed to dry for a few seconds and then reading is carried out under a microscope at magnification 40 in order to count the various sperm types. Thus, the live spermatozoa appear transparent while the dead ones absorb the staining. Abnormal spermatozoa are also observed and counted, including:

1. Spermatozoa without tail;
2. Spermatozoa with an abnormality in the head (abnormal acrosome, small or narrow head, pear-shaped enlarged head, etc.);
3. Spermatozoa with an abnormality in the flagella;
4. Spermatozoa with a proximal cytoplasmic droplet;
5. Spermatozoa with a distal cytoplasmic droplet.

The slide is scanned up and down choosing 4 reading fields in which live, dead and abnormal sperm are counted for a total of 150 sperm in the 4 selected fields.

2.4. Data Processing and Statistical Analysis

The various data obtained were recorded and processed with the Microsoft Excel 2010 software and then exported into the IBM Statistical Package for the Social Science (SPSS) software where they were the subject of a descriptive analysis (mean, standard deviation, khi-square test) and of the Student T-test of the independent samples at the significance threshold of 5% to compare the results of the two phases.

3. Results

The morpho-biometric characteristics (bodily and testicular) studied are recorded in Table 1.

Table 1. *Body and testicular parameters obtained in untreated (controls) and melatonin treated rams in stations in the Dakar region.*

Morpho-biometric characteristics	Control phase rams (0 melatonin, *n*=6)	Melatonin-phase rams (*n*=6)	*P*-value (0.05)
Body parameters			
Live weight (LW) (kg)	38.18 ± 2.80	38.10 ± 2.63	0.920
Height at withers (HW) (cm)	75.43 ± 2.98	76.35 ± 2.83	0.282
Body Length (BL) (cm)	67.32 ± 3.86	67.19 ± 3.45	0.900
Thoracic circumference (ThC) (cm)	79.00 ± 3.24	78.73 ± 1.43	0.714
Testicular parameters			
Estimated testicular weight (TW) (g)	554.92 ± 155.21[a]	395.67 ± 127.58[b]	0.000
Scrotal Circumference (SC) (cm)	27.83 ± 2.06	27.17 ± 2.22	0.287
Right antero-posterior diameter, R-APD (cm)	23.47 ± 1.88[a]	20.15 ± 2.27[b]	0.000
Left antero-posterior diameter, L-APD (cm)	23.38 ± 2.05[a]	20.04 ± 2.48[b]	0.000

a, b: the averages with distinct letters are significantly different on the same line (P<0.05)

It appears from the latter that the use of melatonin did not cause any significant modification of the body parameters (live weight, height at the withers, length of the body, chest perimeter) which remained similar in the animals during the two phases.

With respect to testicular parameters, melatonin use resulted in a significant decrease in testicular weight and antero-posterior testicular diameter, unlike scrotal circumference where no significant difference was noted.

The various parameters studied on the semens of the rams (volume, colour, appearance, concentration, number of spermatozoa/ejaculate, mass and individual motility, live, abnormal and dead spermatozoa) in control and melatonin phases are recorded in Table 2. It emerges from the latter that the treatment with melatonin had no harmful effect on these parameters, which for the most part were improved compared with the control.

Table 2. *Spermatic characteristics of semens collected from untreated (controls) and melatonin treated rams in stations in the Dakar region.*

Semen characteristics		**Semen of rams in control phase (0 melatonin, *n*=24)**	**Semen of rams in melatonin phase (*n*=24)**	***P*-value (0.05)**
Volume of ejaculate (ml)		0.61 ± 0.29	0.74 ± 0.5	0.284
	Whitish	61%	48%	
Semen color	White	26%	52%	0.68
	Yellowish	13%	0%	
	Creamy.	43%	48%	
Appearance	Milky	30%	48%	0.15
	Liquid	26%	4%	
Concentration (10^9spz/ml)		3.02 ± 1.47	2.67 ± 1.75	451
Number of Spermatozoa (10^9spz)		1.78 ± 1.16	2.22 ± 2.58	0.453
Mass motility		2.1 ± 1.5[b]	3.3 ± 1.2[a]	0.006
Individual motility		2.8 ± 1.3[b]	3.5 ± 1.2[a]	0.05
Live spermatozoa (%)		54 ± 34[b]	80 ± 31[a]	0.008
Abnormal spermatozoa (%)		15±23	10±17	0.431
Dead spermatozoa (%)		31 ± 31[b]	9 ± 20[a]	0.007

a, b: the averages with distinct letters are significantly different on the same line (P<0.05)

Indeed, melatonin resulted in a non-significant improvement in volume, color, appearance, sperm count/ejaculate, and abnormal sperm.

We recorded a non-significant decrease in semen sperm concentration in melatonin-phase rams.

Moereover, melatonin significantly improved (p<0.05) mass motility by 57% and individual motility by 25% at the limit of significance (p≈0.05).

Furthermore, melatonin significantly improved (p<0.05) sperm survival (80 vs. 54%) by significantly reducing their mortality rate (9 vs. 31%) compared to the control.

4. Discussion

In our study, melatonin use had no significant effect on body parameters. However, Arranz et al. demonstrated in their trial combining a luminous treatment ("long days") with melatonin on antennae and lambs of the "Manech Tête Rousse" breed that the use of melatonin treatment slowed the growth of males during the two months following the pose and this is all the more marked as the melatonin intakes are high [2]. Pool et al., by testing the effect of exogenous melatonin on the advance of the breeding season and the testicular function of Merino and Poll Dorset rams, attributed the recorded variations in live weight (slight increase then slight decrease) to the season and not to the use of this product [35]. Also, Piscine and al., by examining the effect of exogenous melatonin on reproductive endocrinology, sperm quality and production, testicular size and libido in Merino and Poll Dorset rams, showed that this substance has no impact on the libido or body status of the animals [34]. The same is true for Kleemann et al. who did not find any modification of these parameters on the Border Leicester rams as in our study where the rams were already all adults and subjected to a maintenance ration during the test [27].

As for the testicular parameters, our results are contrary to those of Chemineau et al. who had reported a significant increase in testicular weight after about 100 days, accompanied by an improvement in sexual behavior at collection, the quantity and quality of spermatozoa, in the Ile-de-France ram receiving an additional hour of nocturnal illumination for two months (after a fixed dawn) followed by the insertion of a melatonin implant compared to the control rams [9]. Arranz et al. also concluded on antennae and lambs of the "Manech Tête Rousse" breed that the placement of melatonin implants induces a very significant and rapid increase in the size of the testicles two months after the placement [2]. El Bouyahiaoui et al., by studying the effects of the use of exogenous melatonin (MELOVINE ®) in rams of the Rembi and Hamra breeds during the pre-fall breeding period, reported that melatonin slightly stimulated testicular growth [17]. The similarity noted for the scrotal circumference of rams during the two phases is consistent with the results of Rosa et al. and Palacín et al. who reported no difference between the treated and control groups respectively in Charollais and Texel rams and in Rasa Aragonesa rams [38, 33] unlike the Assaf and Manchega rams, Merinos and Poll Dorset rams treated with melatonin which had a significant increase in the circumference of their scrotum [34, 35]. Our results are also consistent with those of Soukhtehzari et al. in Shall rams [40] and Kleemann et al. in Border Leicester rams [27], which recorded a significant decrease in these parameters. The latter had explained this decrease by the insensitivity of the body to the endogenous stimulus of melatonin which had to be secreted naturally without exogenous intake. Furthermore, the significant decrease in testicular weight estimated in the melatonin phase can be explained by the difference in ambient temperature between the 2 phases. Indeed, the temperature at the beginning of the control phase was higher (27-31°C.) than that (16-25°C.) at the beginning of the melatonin effect phase where it could have a start of the thermoregulation via several mechanisms (vasoconstriction, cremaster contraction, rising of the testicles) in order to protect the testicles against the cold. Thus, with this vasoconstriction, there will be a decrease in blood flow and a decrease in heat loss. Also, the contraction of the skeletal striated muscles of the dartos and cremaster during this period would have led to a rise of the testicles; which resulted in a reduction in the volume of water poured and corresponding to the estimated weight of the

testicles in these implanted rams.

The non-significant improvement in volume, colour, appearance, sperm/ejaculate and abnormal sperm count by the use of melatonin in our study is also reported by Faigl et al**.** in Awassi rams [20] or Soukhtehzari et al. in Shall rams (volume) [40], Arranz et al. on antennae and lambs of the Manech Tête Rousse race [2], Piscine et al. in Merino and Poll Dorset rams (colour and appearance) [34], Piscine et al. and Pool et al. in Merino rams (sperm count) [34, 35]. However, Arranz et al. found in the antennae of the Manech Tête Rousse breed, that the semen volumes were 17-18% higher compared to the controls while in the lambs of the same breed, the volumes were similar between the batches [2]. Egerszegi et al. meanwhile, recorded a significant increase of the order of 25% in semen volume and sperm production in Black Racka rams with two melatonin implants [18]. The non-significant increase recorded in our study could be explained by the increase in sexual ardor and improvement in sexual behavior of rams thanks to their familiarity with electro-ejaculation collection and by the variable response to this collection technique as well as a possible retrograde ejaculation in some animals [39].

Regarding semen sperm concentration, its non-significant decrease recorded in rams in the melatonin phase is similar to the results of Arranz et al. which admittedly observed a non-significant improvement in semen sperm concentration compared to the control; therefore, similar concentrations [2]. However, this result is contrary to those of Egerszegi et al. in Black Racka rams [18], Piscine et al. and Pool et al. in Merino and Poll Dorset rams [34, 35] which instead recorded a significant increase in semen concentration in the treated subjects compared to controls.

The significant improvement in mass and individual motility recorded in our study is in perfect agreement with the results obtained on Konya Merinos rams by Kaya et al., Ashrafi et al. and Dai et al. on Sufflock rams [26, 3, 15]. Contrary to our observations, Arranz et al., Piscine et al. and Pool et al. did not find any improvement effect of melatonin on motility during and out of the breeding season [2, 34, 35], whereas Egerszegi et al. in Black Racka rams [18] and Casao et al. in Rasa Aragonesa rams [7], they obtained better results than ours by doing the out of the breeding season experiment. As such, tissue sensitivity to melatonin may probably differ between animals [9, 10, 38]. Reproductive activities are strongly influenced by the seasons in temperate and Mediterranean breeds and therefore more sensitive to the effects of melatonin; this would explain the differences in the results obtained.

In addition, the determination of motility is, at the current state of knowledge, a criterion for sorting ejaculates to be inseminated and breeding animals, because it corresponds to the percentage of live and mobile sperm [12, 16, 5]. The score of 3 corresponds to about 60% mobile sperm for mass motility and at least 60% live sperm for individual motility. Motility is not related to the fertilizing power of sperm, but its evolution gives a vague idea of the proportion of living cells in the semen. Since melatonin is a highly lipophilic molecule, it stabilizes, protects and improves the mitochondrial activity of sperm via several mechanisms. It can reduce oxygen uptake, membrane potential, and production of superoxide anions [31] and can interact with lipid bilayers and thereby stabilize the inner membranes of mitochondria. This then makes it possible to increase the motility by improving the activity of the electron transfer chain [29].

In addition, the overall average rate of dead sperm is below the accepted percentage (≤ 20% of dead and abnormal) for good quality sperm [14]. This improvement in the survival rate obtained in our study could be a guarantee of the success of artificial protrusions or inseminations. The average rate of overall morphological abnormalities obtained in this study is lower than the percentage of 15% when the rams were treated with melatonin; above this rate, the ejaculate is considered to be of poor quality [16, 5], even if this decrease was not significant. Indeed, it is established in rams that only the percentage of abnormalities is negatively correlated with fertility [13].

Even though our results seem to be contrary to those of Piscine et al. and Pool et al. who did not obtain any effect of the treatment with melatonin on the morphology of spermatozoa [34, 35], they still remain in agreement with those of other authors including Kaya et al. and Kleemann et al. [25-27]. Also, many other works justify our results by the fact that:

1. the increase in motility and viability of spermatozoa in the semen in short days would be caused, among other things, by a change in melatonin secretion that increases LH and testosterone levels [43];
2. melatonin has the ability to reduce oxidative stress [37, 24];
3. melatonin stimulates the activities of enzymes involved in the metabolism of reactive oxygen species (ROS) and preserves the integrity of the plasma membrane [23, 15];
4. melatonin increases plasminogen activity in ram sperm [42];
5. melatonin may be a potent anti-apoptotic agent [6, 32];
6. melatonin can effectively protect sperm with antioxidants through the presence and activation of melatonin receptors in sperm [30, 8, 3, 21].

5. Conclusion

Apart from the reduction in the weight and antero-posterior diameter of the testicles (attributable to the entry into a period of freshness), melatonin treatment had no significant adverse effect on most sperm parameters were improved compared to the control. Thus melatonin significantly improved the mass and individual motility, the survival rate of spermatozoa with a very low level of abnormal spermatozoa observed compared to the control. However, motility is an important indicator of semen preservation that is used to measure the effect of storage, because it is essential for the movement of sperm in the female genital tract. In addition, the rate of abnormalities is the only

parameter inversely correlated with fertility. In other words, the higher the rate of abnormalities, the lower the fertility; and the higher the survival rate, the higher would be fertility and prolificity. Melatonin therefore not only preserved but also improved all the fertilizing capacities of the semen of rams in our study. Melatonin is an interesting reproductive management tool for improving the productivity of sheep farms. It can thus be recommended for use in artificial insemination centers in order to improve the characteristics of the semen doses produced. However, it would be interesting to repeat this test during a hot period of the year in order to measure the influence or not of the climate.

Authors' Contributions Statement and Agreement

This work was carried out in collaboration among all authors. All authors read and approved the final report.

Assani B. V. M.: conceptualization, methodology, validation-verification, formal analysis, investigation, resources, data curation, writing-original draft, writing-review and editing, visualization, project administration.

Ayssiwede S. B.: methodology, validation-verification, resources, writing-review and editing, supervision, visualization, project administration.

Kinnou S. M.: formal analysis, investigation.

Mbengue F. V. L.: investigation.

Missohou A.: resources.

Slimane N.: writing-review and editing, supervision.

Conflict of Interests

The authors declare that they have no competing interests.

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