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Testicular and spermatic performances in *Ouled Djellal* rams during the increasing of day length period

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ABSTRACT

The aim of this study was to control sexual activity in 10 mature OuledDjellalrams ranging in age between3 and 9 years during the increasing daylight lengthperiod (time beforemating out of the breeding season). The parameters studied were weight, body condition scores, testicular morphometric characteristics (scrotal circumference, testicular length and diameter as well as the epididymal tail diameter) semen and spermatozoa characteristics (sperm volume and appearance, mass and individual progressive motilities, concentration, percentages of live and abnormal sperm). A battery-operated ejaculator was the equipment employed for semen harvest. Live weights, testicular measurements and semen collection were performed fortnightly during three months (January, February and March). The results show stability of the different studied parameters, indicating a certain stability of the sexual activity in Ouled Djellal rams. The presence of spermatozoa, sperm quantity and quality show that sexual activity in Ouled Djellalrams decrease during the increasing of daylight length.

Keywords: Ram semen, sexual activity, testicular morphometry, *Ouled Djellal* breed.

INTRODUCTION

In Algeria, the sheep population is the largest animal resource of the country. It was estimated at 18.5 million heads in 2004,which almost two thirds are female of the total number. [29].According to the latest statistics, sheep heads reach around 20 million heads [17].*OuledDjellal*is the main dominant local breed and it represents 63% of the Algerian flock. Owing to its good characteristics in arid and semiarid region, it has been introduced into neighboring countries. Thus, it plays a relevant role in the economy according to its importance and is actively involved in the production of red meat [20].

The problem that arises in all sheep owners is to recognize males physically and behaviorally capable to copulate with ejaculation of viable, normal and fertile spermatozoa and able to ensure successful fertilization. The selection of these rams must be accomplished through a deep examination of the reproductive tract and also after collection and examination of semen at rest and in action.

The purpose of the study reported here was to control the sexual activity during the increasing day length in *Ouled Djellal* rams selected only on the basis of purely phenotypic characteristics. Will be considered in this analysis only the aspects related to testicular size and the quantitative and qualitative characteristics of semen harvested from *OuledDjellal* rams during the non-breeding season of seasonal breeds (January, February and March).

MATERIALS AND METHODS

The study was carried out at the Technical Institute of livestock farm (ITELV AinM'lila, Algeria), which is located in the semi-arid area at an altitude of 678 m above sea level, latitude 35 ° 52 'N and longitude 7 ° 6' E.

A total of 10 intact *Ouled Djellal*rams with previous breeding experience, aged between 3 and 9 years was used. They were raised together under the same management and nutrition conditions and exposed to normal changes of day length. Each ram was offered a daily ration consisting of 4 kg of barley hay and 650 g of concentrate supplement. Water available twice daily (morning and afternoon).

Rams were not allowed contact with females, and were introduced in the herd during the mating seasons (two mating seasons are arranged in spring: April- May and autumn: October- November).

A general management protocol including dewormingagainst internal and external parasites, vaccination against sheep pox and enterotoxemia, clinical examination, claws trimming and sanitary measures was adopted.

Rams were weighed by a spring balance designed for small ruminants (200 kg ± 500 g - Marechalle-pesage, France). Scrotal circumference was measured using a tape flexible measuring (Rondo ® model, Kruuse, Switzerland) at the widest scrotal diameter[3;25]. While the testicularlength, testis andepididymaltail diameterswere measured of each testisusing a caliper [34]. The body condition score (BCS) was evaluated subjectively by using a scale from 0 to 5 [13].These measurements were carried out at biweekly intervals from January to March.

Semen was obtained using a battery-operated ejaculator (MedataRam Ejaculator, England). A light microscope equipped with a warm stage (Olympus Corporation, Tokyo, Japan) was also used. The semen collection was performed according to the same rate as body weight and testicular measurements with two (02) collections per ram, at10 minutes rest.

Semen was immediately evaluated for volume andgeneral appearance: color and consistency [18]by direct reading from conical graduated glass tube (4 ± 0.1 mL). The collected samples are then immediately placed in a water bath at 37.5 ± 1°C. Microscopic examination includes the mass and individual motilities, the determination of the concentration by ahaemocytometer slide (Thoma slide, Marienfeld, Germany) [2] and the determination of the percentages of live and morphologically abnormal sperm (eosin/nigrosinstaining technique).

The proportionsof live and abnormal spermwere assessed by examining 200 spermatozoa in five different fields on each slide [2].

The experiment was carried out according to the animal welfare regulations of the Institute of Veterinary Science, University of Constantine 1, Algeria.

Statistical analysis involved the calculation of descriptive statistics (mean, standard deviation, standard error,minimum and maximum), analysis of variance (ANOVA) and the correlation matrix using the Pearson correlation coefficient [32; 37]. Values were considered to be statistically significant when$P<0.05$.

RESULTS

The least-squares means and standard error of testis measurements and semen characteristics at last collection during the three months study are shown in table 1. Differences between the individual rams for testicular parameters and sperm characteristics were significant ($P<0.05$).

The right testicular length increased significantly ($P<0.001$) in *OuledDjellal* rams from 3 to 5 years of age, while it decreased in those older than 5 years. The length of the left testis presented a similar evolution, comparable tothe right one during the study period.

Similar to the testicular length, testicular and epididymal tail diameters of the two gonads and scrotal circumference tended to have the same evolution.

Table 1:Least-squares means and standard error of testis measurements and semen characteristics at the last collection during the three months study

Parameter (Mean ±SEM)	Month		
	January	February	March
Weight (kg)	98.08 ±2.02	99.65±1.27	101±1.55
Body condition scoring (0-5)-	2.68 ±0.08	2.3±0.08	2.4±0.1
Scrotal circumference (cm)	35 ±0.68	32.12±3.25	35.4±0.76
Right testicular length(cm)	9.41 ±0.19	8.92±0.29	8.59±0.18
Left testicular length (cm)	9.46 ±0.23	9.17±0.21	8.54±0.19
Right testicular diameter (cm)	5.84 ±0.15	5.8±0.20	6.02±0.27
Left testicular diameter (cm)	5.93 ±0.1	6.07±0.09	6.14±0.21
Diameter of the right epididymal tail (cm)	2.46 ±0.08	2.51±0.08	2.63±0.08
Diameter of the left epididymal tail (cm)	2.3 ±0.06	2.39±0.07	2.6±0.06
Volume of the ejaculate (mL)	1.31 ±0.06	1.63±0.58	0.91±0.1
Color (1-2)	1.95 ±0.05	1.75±0.13	1.75±0.11
Consistency (1-3)	2.4 ±0.13	2.1±0.22	2.15±0.25
Mass motility (0-5)	2.18 ±0.5	2.1±0.39	3.45±0.46
Individual progressive motility (0-5)	2.63 ±0.39	2.38±0.38	3.3±0.46
Concentration ($\times 10^9$ mL^{-1})	3.07 ±0.48	2.86±0.93	1.41±0.44

Correlation analysis (Table 2) indicated that there were positive and significant correlations between semen characteristics and testicular parameters (diameter of theleft epididymal tailwith individual progressive motility) and especially among testicular parameters (scrotal circumference with left and right testicular lengths, right testicular length with the diameter of the two testis). Testis diameter and length varied differently between the two gonads (right and left) in *OuledDjellal* rams. However, there was a high degree of symmetry between left and right epididymides (table 2). Moreover, asimilar correlation between percentage of live sperm and concentration was obtained.The strongest correlation was observed between individual progressive motility and mass motility.

Table 2: Matrix of correlations between the various studied parameters (testicular morphometry and sperm production at the last collection)

	Weight	BCS	SC	RTL	LTL	RTD	LTD	RED	LED	VOL	COL	CONS	MM	IM	CON
Weight	1														
BCS	**0.66***	1													
SC	0.26	0.2	1												
RTL	0.42	0.24	**0.69***	1											
LTL	0.48	0.48	**0.9*****	**0.74***	1										
RTD	0.31	0.31	0.6	**0.8****	0.63	1									
LTD	0.13	0.13	0.54	**0.69***	**0.64***	**0.91*****	1								
RED	0.56	0.56	0.47	0.42	0.58	0.56	0.55	1							
LED	0.53	0.53	0.39	0.27	0.4	-0.29	0.02	0.1	1						
VOL	0.24	0.24	0.27	0.16	0.47	0.42	0.56	0.17	0.24	1					1
COL	-0.03	-0.03	0.02	-0.04	-0.02	0.16	0.05	0.29	0.45	-0.12	1				
CONS	0.2	0.2	0.07	0.25	0.13	0.29	0.17	0.4	0.38	-0.11	**0.8****	1			
MM	0.05	0.05	-0.18	-0.33	-0.26	0.01	-0.2	-0.00	0.63	-0.04	**0.74***	0.51	1		
IM	0.15	0.15	-0.00	-0.2	-0.02	0.16	-0.01	0.19	**0.66***	0.12	**0.73***	0.63	**0.94*****	1	
CONC	0.32	0.32	0.06	0.3	0.08	0.37	0.27	0.45	0.06	0.15	0.38	0.59	0.11	0.15	1
LS	0.34	0.00	0.00	0.34	0.17	0.4	03	0.06	0.5	0.48	0.4	0.62	0.34	0.41	**0.64***

: Significant correlation marked at P<0.05.; *: Significant correlation marked at P<0.01.; *****: Significant correlation marked at P<0.001. ***BCS**: Body Condition Score. **CS**: Scrotal Circumference,**RLT**: Right Testicular Length, **LTL**: Left Testicular Length, **RTD**: Right Testicular Diameter, **LTD**: Left Testicular Diameter, **RED**: Diameter of the Right Epididymal tail, **LED**: Diameter of the Left Epididymal tail. **VOL**: Volume, **COL**: Color. **CONS**: Consistency. **MM**: Mass Motility. **IM**: Individual progressive Motility,**CONC**: Concentration of the ejaculate and **LS**: Percentage of live sperm.*

In *OuledDjellal*rams,the live sperm percentages were in the range of 37.5 and 91.83%, with amean percentage of 69.92%. While the percentage of total abnormal sperm ranged between 2.52 and 54.12% with a mean percentage of 18.51%.

With a predominance of minor anomalies, a slight decrease in major anomalies, and conversely a slight increase in minor anomalies were observed. Major defects do not exceed 20% of the overall abnormal count.

DISCUSSION

Boucif*et al.*[4]reported that there are significant seasonal variations in scrotal circumference in *OuledDjellal*rams. However,significant monthly variationswere observed in the rams under investigation despitethe short duration of the experiment.

The difference between the individual rams for scrotal circumference ($P<0.05$) reiterates the finding of Colas *et al.*[8]. It should be noted that the rams did not have the same age. The analysis of variance (ANOVA) revealed a significant effect of age on the scrotal circumference ($P<0.001$), which explains this individual differences. However, it exceeded 30 cm in all rams.

In agreement with Hassan *et al.*[21], scrotal circumference has the strongest correlations with testicular length.

The current results agree with those of Webb *et al.* [35], who reported that concentration and live sperm percentage are significantly correlated.
The ANOVA also showed a significant effect of age on the other testicular parameters (testicular length and diameter as well as the diameter of the epididymal tail) ($P<0.001$), which may be the origin of the individual differences observed.

The mean ejaculatevolume obtained during January (1.3±0.06 mL) wasslightly higher than that of 1.03 mL collected from the same breed in the same period [19], but lower than that reported by Aissaoui*et al.*[1](1.62 ± 0.06 mL). Rams used for this experiment had a very wide range of age (from 3 to 9 years), while those of the previous study were between 3 and 5 years.

Rege*et al.*[30] have implicated the age as an important factor of volume variation in sheep. According to Eaton *et al.*[15], the volume of the semen decreases significantly in sheep over 2 years of age. This implies according to David *et al.*[12]a causal relationship between the age and quantitative semen production in rams.

Remarkable differences betweenrams in the ejaculate volume were observed. Other investigators [1; 19]have found the similar results.

Selection on semen appearance especially, color of the ejaculate in *OuledDjellal* rams is likely to be accompanied by more favorable correlated response in other semen traits.

The concentration of the semen collected in almost all the studiedrams was less than that reported by Aissaoui*et al.*[1]and Ghozlane*et al.*[19]in rams of the same breed aged between 3 and 5 years (3 to 5 x 10^9 spermatozoa/mL). This difference seems to be related to the method of collection (electro ejaculation or artificial vagina), becauseartificial vagina resulted in a higher sperm cell density of spermatozoa [5; 14; 26; 28; 31; 33].

In agreement with the report of Karagiannidis*et al.*[23]but in contrary to the reports of David *et al.*[10], David *et al.*[11]andHassan *et al.* [21], no significant positive or negative correlation between the concentration and volume of ejaculate in *OuledDjellal*rams was observed ($P<0.05$).

As expected, among all semen traits, the strongest correlation was that between mass and individual motilities. This result concurs with the report of Rege*et al.*[30].

Generally the percentage of dead sperm recorded in *OuledDjellal* rams (from 8.17 to 62.5%) was relatively high compared to the limits considered by Colas [7]as acceptable for good breeders (between 20 and 30%) and does not allow fertilization as ejaculates with more live sperm [22; 36]. It is well established that this parameter is affected with ambient temperature [9;16; 19]. It is noteworthy to mention that as all collections were performed under conditions of cold temperatures, having adverse effects on spermatozoa, the effect or mechanisms to regulate testicular temperature are found to be insufficient [24].

During the period of increasing day length, *OuledDjellal*rams have a maximum value of abnormal sperm percentage that was very high well above the permissible level of 20 % required for goodram fertility [7; 22].

Actually, the first ejaculates of *OuledDjellal* rams are usually of inferior quality and quantity relative to the second ejaculates.

In agreement with previous studies [1; 6; 7; 8; 27], individual differences of morphologically abnormal sperm production cannot be ignored in *OuledDjellal* rams.

Rege*et al.*[30] proposed that semenproduction and spermatozoa characteristics vary according to age. However, in *OuledDjellal*rams, phenotypic and positive correlations among sperm traits within age category were recorded.

CONCLUSION

Sexual activity among *OuledDjellal*rams is stable during the increasing day length period. This is proved by the stability of the measured parameters (scrotal circumference, ejaculate volume, testicular length and diameter). The sperm concentration during this period was lower than that reported during the breeding season in seasonal breed. Similar findings are attributed to the other semen parameters. Moreover, sperm production is characterized by a higherfrequencyof spermatozoa abnormalities despite the low volume.

Large variability between rams for almost of the studied parameters was noted; typically, values of the semen parameters. It must be recognized that electro-ejaculation tends to yield variable results. However, for the same period, sheep breeds originated from temperate climates in high latitudes have a weaker number of spermatozoa per ejaculate and may even have a temporary interruption of spermproduction. Therefore, it appears from these results that*OuledDjellal*rams suffer from a decline of their sexual activity during the increasing day light length but not a total interruption. Our study allowed us to postulate that *OuledDjellal*ram are year-round breeders but the intensity of sexual activity varies with photoperiod. Thus, it is reasonable to conclude that the sensitivity to light length seems to be lowerthan seasonal rams. It remains to be determined whether this decrease in sperm production can effects ram fertility.

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