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Seasonal variations in reproductive parameters of Ouled Djellal rams in the East of Algeria

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ABSTRACT

This experiment was carried out on Ouled Djellal rams in order to study the effect of season on testicular size, serum testosterone level and semen characteristics for a period of one year. Blood samples were obtained via jugular vein, and semen was collected by using an electro-ejaculator from six fertile male rams (2-4 years of age) on a monthly basis. In this breed, testis parameters varied seasonally showing the highest average values during autumn. However, the variation of body weight, GSI, serum testosterone levels, mass motility, individual motility and sperm concentration were not significant during the sampling period. The results reported show that the lowest and highest levels of semen volume were recorded in winter (0.62±0.11 ml) and in autumn (1.23±0.09 ml) respectively. The pH value and total number in sperm showed a uniform pattern with low scores during breeding season and high scores in non-breeding season. High percentage of live sperm was recorded during the summer and high percentage of abnormal sperm was recorded during winter.

Key words: Algeria, Ouled Djellal rams, Ram, Semen, Testosterone, Testis.

INTRODUCTION

The main factors that limit male reproduction efficiency along the year are testicular size, plasma levels of testosterone hormones and semen quality and quantity. These factors may vary according to different environmental and physiological factors such as breed (Avdi *et al.*, 2004), season, nutritional level, temperature and rainfall (Abdullah *et al.*, 2010).

Ouled Djellal breed is considered one of the most important sheep breed in Algeria. In this country, Ghozlane *et al.*(2005) reported that Ouled Djellal rams expressed a weak seasonal variation in their reproductive variables and Aissaoui *et al.* (2004) found that there is seasonal variation in semen quantity in Algeria. However, no detailed studies of estimations of seasonal variation in testicular size, hormonal concentration and semen quality and quantity have been performed on Ouled Djellal rams. In this context, the fact of knowing the factors affecting variation in semen quality is an essential parameter that can be used to improve this breed in Algeria.

MATERIALS AND METHODS

Animals and research conditions: This study was performed at the Technical Institute of breeding, located in Ain M'Lila, Algeria (35°52' N latitude, 7°6' E longitude). Over a period of one year from June 21st 2013 to July 20th 2014. Data were collected from six sexually mature rams grouped according to age into two batches, each of three rams (batch one with rams of two years; batch two with rams of four years).

These animals were kept under natural photoperiod. Each ram was fed daily 2 kg of hay and 0.8 to 1 kg of commercial concentrate. They also graze for a period of one hour during spring. All rams had free access to water and mineral blocks.

Physical measurements and blood samples: All testicular measurements, body weight and serum testosterone concentration were recorded at monthly intervals, with scrotal circumference being measured using a tape measure, and the combined testes length, testis width and testis thickness determined with the aid of a caliper. Gonadosomatic index (%) was calculated (GSI = [testis weight/body weight] x 100) (Daramola *et al.*, 2015).

A jugular blood sample was collected (08.00 h) from each ram in dry tubes and immediately centrifuged at 1500 × g for 10 min. The harvested plasma was then stored at –20°C until hormone analyses. The concentration of testosterone in plasma was measured in all samples by ICLIA (electro-immunochimiluscence).

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Semen evaluation: Semen collection was attempted from each ram monthly using elecro-ejaculator (09.00 to 12.00 h). The tubes with the freshly collected semen were immediately transferred to the laboratory and immersed into the water bath at 37ºC.

Ejaculated semen volume was recorded after collection using a glass graduated tube. The seminal pH was also directly measured using a pH paper. The mass and individual motility were assessed according to Zamiri *et al.* (2010). The color and consistency of semen was determined according to Fourie *et al.,* (2004) method.

The sperm concentration of each sample was determined by photometer (SpermaCue, Minitub, Germany) at 546 nm wave length. Total number of sperm per ejaculate was calculated by multiplying sperm concentration and ejaculate volume. Furthermore, a semen smear was stained with eosin-nigrosin to determine the live/dead ratio and percentage abnormal sperm by counting at least 200 spermatozoa under oil immersion objective (100 X) random fields (Rege *et al.,* 2000).

Statistical analysis: Data were analyzed using SPSS 21 (SPSS, 2013). In effect, three types of statistical analysis were performed. Because the sample size is small (6 ram) and the normality of the data is no longer assured for all variables. We opted for the analysis of the nonparametric Kruskal-Wallis variance with fixed factors like season and age of rams. The general linear model (GLM) was used to analyze the effects of interactions between variables.

If a factor was significant, the Tukey's multiple comparisons test was used to determine differences between means and probabilities ($p < 0.05$) were considered to be statistically different. All mean values are expressed as the mean ± standard error of mean (SEM). Partial Pearson correlation controlled by the effect of age rams between various physical parameters or seminal characteristics were calculated.

RESULTS AND DISCUSSION

Body weight and GSI: The results of the analysis of body weight during the examination period showed no season × age interactions were detected ($p > 0.05$) for any of the variables measured. The values of BW were significantly dependent on age ($p < 0.05$). Throughout the experiment, rams aged four years were heavier than rams aged two years (Table 1).

However, seasonal variations little marked or not ($p > 0.05$) were observed by different authors and in different breeds: Kridli *et al.* (2007) in Awassi rams and Dorostghoal *et al.* (2009) in Arabic rams. This difference in body weight of sensitivity to changes in photoperiod is explained by differences related to the environment and especially the food level (Abdullah *et al.,* 2010).

Table 1: Effect of season and age on body weight, testicular measurements, testosterone concentration and sperm characteristics (X ± SEM).

Factors	BW(kg)	TW(g)	GSI(%)	LT(cm)	Tl(cm)	TT(cm)	SC(cm)	T(ng/ml)	pH	V(ml)	MM	IM	C(x10^{6}spz/ml)	NTS	PSL	PSA
Season	0,105^{NS}	0,043*	0,130^{NS}	0,049*	0,015*	0,006**	0,013*	0,095^{NS}	0,000***	0,000***	0,698^{NS}	0,315^{NS}	0,170^{NS}	0,017*	0,000***	0,003**
Summer	98.55±2.10	836.11±50.29	0.8±0.00	15.16±0.32	6.21±0.13	6.76±0.12	35.93±0.61	4,24±0.71	6,97±0.04	1,05±0.10	1,21±0.36	1,41±0.40	383,50±50.47	429,75±71.35	60,00±3.77	18,36±3.30
Autumn	96,88±2.26	961,11±56.52	0,9±0.00	15,63±0.27	6,63±0.12	7,11±0.11	36,55±0.53	6,25±1.33	7,52±0.09	1,23±0.09	1,38±0.34	1,44±0.33	290,22±53.16	396,12±99.87	40,75±6.03	13,91±2.28
Winter	91,88±2.16	800,00±43.72	0,8±0.00	14,43±0.30	6,08±0.13	6,66±0.15	32,40±1.82	3,15±0.80	7,68±0.10	0,62±0.11	1,44±0.18	1,77±0.19	332,38±28.92	203,64±35.30	45,33±3.90	31,52±3.43
Spring	94,44±2.49	861,11±41.92	0,9±0.00	15,28±0.34	6,24±0.12	6,50±0.12	35,77±0.51	3,84±0.77	7,93±0.17	1,18±0.08	1,70±0.24	2,16±0.30	453,44±76.02	577,51±120.98	29,88±4.64	24,86±4.02
Age (Year)	0,000***	0.000***	0,003**	0,006**	0,002**	0,000***	0,001***	0,008**	0,357^{NS}	0,041*	0,581^{NS}	0,762^{NS}	0,171^{NS}	0,078^{NS}	0,268^{NS}	0,454^{NS}
2	90.88±1.33	755.55±26.41	0.8±0.00	14.70±0.18	6.09±0.10	6.52±0.08	33.50±0.95	3.14±0.60	7.58±.08	0.93±0.08	1.35±0.21	1.65±0.23	327.88±36.64	329.55±58.92	46.63±3.86	23.44±2.49
4	100.00±1.54	973.61±33030	0.9±0.00	15.56±0.24	6.49±0.08	6.99±0.09	36.82±0.32	5.60±0.69	7.47±0.10	1.11±0.07	1.51±0.19	1.75±0.21	401.88±0.41	473.96±0.68	41.34±3.55	20.88±2.63
Interaction (season* age)	0,459^{NS}	0,329^{NS}	0,499 ^{NS}	0,696^{NS}	0,219^{NS}	0,506^{NS}	0,266^{NS}	0,315^{NS}	0,812^{NS}	0.001***	0,442^{NS}	0,336^{NS}	0,157^{NS}	0,006**	0,765^{NS}	0,873^{NS}

BW (live weight); TW (testicular weight); TL (testis length); Tl (testicle width) TT (testicle thickness); SC (scrotal circumference), T (testosterone concentration); V (volume); C (concentration); MM (mass motility); IM (individual motility); NTS: (total number in sperm); PSL (percentage of live sperm); PSA (percentage of abnormal sperm); NS: Not significant, *: Significant at $P < 0.05$, ** Significant at $P < 0.01$,*** Significant at $P < 0.001$.

The GSI is considered a good indicator of seasonality. This report for these rams is relatively constant during the year (0.8 %). Bonnes *et al.* (2005) indicated that GSI in rams is equal to 0.5 % during the no-breeding season and 2 % during the breeding season.

The GSI, from two years to four years, ranged from 0.8 to 0.9% ($p<0.01$). The high correlation observed in this study between changes in testicular weight with age and body weight was similar to that described for Najdi breeds (Al-kawmani *et al.,* 2014). Gonadosomatic index has been considered as reliable estimate for gonad maturity of any species.

Testicular size: Throughout the experiment, all testicular size was affected ($p < 0.05$) by season of the year. As indicated in Table 1, TW, TL, Tl, TT and SC showed a uniform pattern with low scores during non-breeding season and high scores in breeding season. The lowest average values of these variables were determined at two years of age, while the highest values were measured at four years of age (Table 1). Our results showed a significant effect of age and season on testicular measurements, which were in accordance with results obtained by Focsăneanu *at al.* (2014).

The testes size in rams may be influenced by many factors, and is usually highest during the natural breeding season, which is associated with the impact of the length of the photoperiod on neurohormonal complex mechanisms that regulate reproductive function. The present study show pronounced seasonal fluctuations; the recorded reduction of the testicular size during the winter season was in line with the findings of Kafi *et al.* (2004) on the smallest value of scrotal circumference of rams during the winter.

Testosterone concentration: No differences ($p > 0.05$) were recorded in serum testosterone concentrations between seasons. The average values of testosterone levels in the blood serum ranged from 3.14 ±0.60 ng/ml at two years of age to 5.60±0.69 ng/ml at four years of age, which was significant at $P < 0.01$(Table 1). The obtained results were confirmed in earlier studies by Ungerfeld and Gonzalez-Pensado (2008) showing an increase in the testosterone level with age as well as fluctuations in the determined values.

Seminal characteristics: The chi-square test shows a color and consistency no dependency with seasonal effects. All samples from harvest by electroejaculator recorded a milky to cloudy color (less dense) throughout the study period. The consistency varies only according to age rams, sperm is disorder in rams two years and becomes milky in rams four years ($p<0.05$). However, other studies such as that of Greyling and Grobbelaar (1988) showed that autumn (natural breeding season) recorded a slight increase in semen color for the artificial vagina semen collection, which usually indicated a corresponding higher sperm count (concentration).

In our study, it was observed that the volume of ejaculate ($p<0.001$) and the total number of spermatozoa in the ejaculate ($p<0.01$) increased with age. The best values were recorded in mature rams over four years (Table 1). A similar trend in the values of sperm parameters was found by Kridli *et al.* (2006).

Significant differences ($p<0.05$) were observed for the various sperm measurements, except for assessment of sperm concentration during the recording period. The volume increased during the breeding season. While the pH value, total spermatozoa output percentage showed a uniform pattern with low scores during breeding season and high scores in non-breeding season. The significant seasonal differences in semen characteristics observed in the present study have also been reported for the other breeds of rams under different environmental conditions (Aller *et al.,* 2012). However, Ghozlane *et al.,* (2005) observed no significant seasonal variation in the semen volume of same breed harvest by artificial vagina. Age and weight of animals, sexual stimulation prior to semen collection, method of semen collection and the geographical location may be responsible for the differences.

The results of mass motility and individual motility, percentage of abnormal sperm and percentage of live sperm are not depending on the age of the animals ($p>0.05$). Studies conducted by Focsãneanu *et al.* (2014) and on the influence of age on semen parameters in rams of the breed Turcana Alba, claim the increase of semen mobility and decreasing the percentage of abnormal sperm with age of rams.

In our study, mass and individual motility are not depending on season of the year ($p>0.05$). It is consistent with the results observed in Egyptian breeds (at latitude 31º N) (Taha *et al.,* 2000) and in the Persian Karakul breed (at latitude 20º N) (Kafi *et al.,* 2004). However, in other studies semen samples had a higher mass motility in autumn and differences among breeds were reported (Gündogan, 2007).

A significant effect of season on percentage of abnormal sperm and percentage of live sperm ($0.001>p<0.01$) was found in the current study (Table 1). The highest values of PSA were recorded in winter and spring, with lower values in the summer and autumn seasons, while the PSL increased during the summer. This is consistent with the results obtained in both Suffolk and Ile-de-France rams (Mandiki *et al.,* 1998). The high percentage of sperm abnormalities during the winter and spring seasons can possibly be attributed to the low level of circulating plasma testosterone (Table1) and a reduction in the thickness of the seminiferous tubules and spermatogenic activity (Barkawi *et al.,* 2006). Reproductive activity in the Ouled Djellal is lowly seasonal and closely related to changes in the photoperiod (Ghozlane *et al.*, 2005).

The percentage of minor abnormalities doesn't follow a consistent trend as the percentage of major abnormalities. Throughout the period of the experiment, the percentage of minor abnormalities was still high (11.72 to 26.41%) compared to the percentage of major abnormalities (1.91 to 6.50%) (Table 2).

Different classes of anomalies studied are not influenced by the age of rams. However, Rege *et al.* (2000) found a strong association between age and the different classes studied of abnormalities (head abnormalities, anomalies of the intermediate piece, tail anomalies, detached head, distal cytoplasmic droplet and proximal); they tend to fall between the ages of 6 months and 12 months in Menz and Horro breeds.

For minor abnormalities, PSm2 (normal head no tail), PSm3 (folded or curled tail to the tip) and PSAm1 (distal cytoplasmic droplet) occupy respectively the first, second and third place in order of importance influenced by season, characterized by a higher frequency during dry periods.

Correlation: A weak positive and significant correlation was determined between testosterone level and scrotal circumference ($r = 0.25$; $P < 0.05$) (Table 3). A significant and positive correlation ($r = 0.23$) between these parameters in rams aged 10 to 12 months was confirmed in a study by Fourie *et al.* (2005). Preston *et al.* (2012) found that the variation in the size of the testicles is a powerful predictor of testosterone levels during the mating season. It is possible that bigger testes contain more androgen producing tissues (Leydig cells) or higher testosterone levels stimulate the growth and development of spermatogenic tissues, which affect the size of the testes (Fourie *et al.*, 2005).

Our study showed any relationship between the serum testosterone levels and the body weight of rams ($r = 0.167$).

Various studies have suggested that the scrotal circumference is strongly positively correlated with the body weight of animals at different ages (Duguma *et al.*, 2002).The present study failed to establish any relationship between body weight and scrotal circumference of rams. No relation ($r = 0.23$) between these parameters in rams was found in a study by Kafi *et al.* (2004).

Many authors have presented observations regarding sperm production and its quality in terms of correlations with testicular size and plasma testosterone levels (Kishk *et al.*, 2008). However, in our study all parameters of semen quality and quantity were poorly correlated with body weight and scrotal circumference, while

Table 2: Effect of season and age on major and minor abnormalities (X ± SEM).

Factors	PSAM(%)	PSAM1(%)	PSAM2(%)	PSAM3(%)	PSAM4(%)	PSAM5(%)	PSAm(%)	PSAm1(%)	PSAm2(%)	PSAm3(%)	PSAm4(%)	PSAm5(%)
Saeson	0.063^{NS}	0.131^{NS}	0.100^{NS}	0.110^{NS}	0.399^{NS}	0.332^{NS}	0.001***	0.001***	0.002**	0.004**	/	0.399^{NS}
Summer	4.97±1.43	1.00±0.77	0.13±0.13	3.61±1.09	0.00±0.00	0.22±0.14	13.19±2.28	0.58±0.27	3.77±1.21	8.69±1.95	0.00±0.00	0.13±.13
Autumn	1.91±0.90	0.33±0.19	0.05±0.05	1.33±0.59	0.00±0.00	0.13±.13	11.72±2.10	0.63±0.25	5.61±1.52	5.75±1.77	0.00±0.00	0.00±0.00
Winter	5.02±0.75	0.61±0.18	0.36±0.11	4.02±0.62	0.11±0.11	0.00±0.00	26.41±2.87	3.194±0.72	10.72±1.47	12.47±1.79	0.00±0.00	0.00±0.00
Spring	6.50±1.45	1.94±0.56	1.08±0.58	3.75±.098	0.00±0.00	0.00±0.00	18.27±2.76	2.38±0.65	11.94±2.06	4.19±.80	0.00±0.00	0.00±0.00
Age (Year)	0.529^{NS}	0.784^{NS}	0.965^{NS}	0.249^{NS}	0.321^{NS}	0.892^{NS}	0.421NS	0.817^{NS}	0.906^{NS}	0.337^{NS}	/	0.321^{NS}
2	4.98±0.90	0.903±0.27	0.41±0.19	3.68±0.70	0.05±0.05	0.09±0.07	18.44±1.94	1.76±0.45	8.11±1.22	8.56±1.38	0.00±0.00	0.00±0.00
4	4.22±0.83	1.04±0.43	0.40±0.25	2.68±0.51	0.00±0.00	0.08±0.07	16.36±2.06	1.63±0.36	7.91±1.28	6.98±1.12	0.00±0.00	0.06±.06
Interaction (saeson* age)	0.912^{NS}	0.453^{NS}	0.923^{NS}	0.701^{NS}	0.399^{NS}	0.394^{NS}	0.767NS	0.970^{NS}	0.956^{NS}	0.282^{NS}	/	0.399^{NS}

PSAM (major abnormalities); PSAM1 (proximal cytoplasmic droplet); PSAM 2 (periforme head); PSM 3 (curly tail or tail wrapped around the head); PSAM 4 (deformation of the intermediate piece); PSAM 5 (poor development).
PSAm (minor abnormalities); PSm1 (distal cytoplasmic droplet); PSm2 (detached head); PSm 3 (folded or curled tail to the end); PSAm 4 (small or giant narrow head); PSAm5 (abaxial implantation).
NS: Not significant, ** Significant at P <0.01, *** Significant at P <0.001.

Table 3: Coefficients of partial correlations between size testicular, characteristics sperm and testosterone concentration in rams.

r	BW (Kg)	TW (g)	TL (cm)	Tl (cm)	TT (cm)	SC cm)	T (ng/ml)	pH	V (ml)	MM	IM	C (x10^6 spz/ml)	NTS (x10^6 spz/eja)	PSL (%)	PSA (%)
BW (Kg)	1														
TW (g)	0.27*	1													
TL (cm)	0.140	0.52***	1												
Tl (cm)	0.013	0.65***	0.55***	1											
TT(cm)	0.058	0.50***	0.52***	0.63***	1										
SC (cm)	0.050	0.33**	0.26*	0.50***	0.28*	1									
T (ng/ml)	0.167	0.53***	0.53***	0.48***	0.47***	0.25*	1								
PH	-0.188	0.113	-0.016	0.054	0.041	-0.079	0.129	1							
V (ml)	0.203	0.219	0.28*	0.26*	0.160	0.141	0.197	-0.028	1						
MM	-0.073	-0.113	0.023	-0.024	-0.113	-0.094	-0.231	-0.037	0.29*	1					
IM	-0.127	-0.179	-0.019	-0.111	-0.23*	-0.169	-0.29*	-0.063	0.231	0.93***	1				
C (x10^6 spz/ml)	0.055	-0.084	0.032	-0.121	-0.23*	-0.043	-0.37***	-0.205	0.233	0.66***	0.64***	1			
NTS (x10^6 spz/eja)	0.116	0.069	0.162	0.050	-0.109	0.023	-0.213	-0.170	0.61***	0.62***	0.59***	0.86***	1		
PSL (%)	0.087	-0.055	-0.125	-0.223	-0.174	0.004	-0.081	-0.41***	-0.070	0.045	0.060	0.164	0.113	1	
PSA (%)	-0.196	-0.070	-0.186	-0.146	-0.095	-0.175	-0.198	0.123	-0.217	-0.152	-0.112	-0.027	-0.111	-0.204	1

BW (live weight); TW (testicular weight); TL (testis length); Tl (testicle width) TT (testicle thickness), SC (scrotal circumference); T (testosterone concentration); V (volume); C (concentration); MM (mass motility); IM (individual motility); NTS: (total number in sperm); PSL (percentage of live sperm); PSA (percentage of abnormal sperm); *: Significant at P <0.05, ** Significant at P <0.01, *** Significant at P <0.001.

testosterone concentration was weakly negative correlation only with mass motility and concentration of spermatozoids.

CONCLUSION

During this study, there were variations in the BW, GSI and testicular testis, depending on the age of the animals. Analyzing the evolution of the averagesize of the testis parameters there is an increase with age of the rams, which may be explained by sexual maturation.

In conclusion, these findings show the variable character of serum testosterone caused by various internal and external factors. There is an indisputable relationship between serum testosterone level and all testis parameters, while semen characteristics were poorly to moderately correlated with these parameters. Therefore, testicular measurements and body weight cannot be used as reliable predictors of sperm production.

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