Original Article

Influence of season on stereological and histomorphometric characteristics of testes of Ouled Djellal rams in Algeria

Yamina Belkhiri[1,2*] Souheyla Benbia[1,3] Amal Djaout[2,4]

Abstract

The aim of this study was to investigate the influence of season on testicular morphometry, on ram body weight, testosterone concentration and on stereological structure of the testes in Ouled Djellal rams. Twenty seven rams were randomly selected and the live weight and testicular size were recorded. At the beginning of each season (autumn, winter, spring), among the study group, three rams were randomly selected, slaughtered and their left testes were removed and weighed. For microscopic study, tissue samples were excised from the left testes, fixed in formalin solution and embedded in paraffin and, afterwards, quantitative evaluation was performed. Statistical analysis revealed that the testicular weight and morphometric parameters of the testes including length, width, thickness and circumference were significantly (p<0.001) higher during the breeding season (autumn). The lowest seminiferous tubule diameter (STD) (167.02±6.74µm) was observed in spring and the highest in autumn (212.12±4.94 µm) (p<0.01). Also, tubular tissue volume and height of the germinal epithelium gradually increased during the spring, with the highest values noticed in autumn (p<0.05 and p<0.001, respectively). Seminiferous tubule diameter significantly correlated with scrotal circumference (r=0.54 p<0.001) and with Leydig cell number (r=0.54 p<0.001). Mean serum testosterone concentrations were inversely related to interstitium volume (%) (r= -0.40 p<0.05) but positively significantly related to Leydig cell number (r=0.54 p<0.001). Maximal mean serum testosterone concentrations were observed during autumn (7.81±1.2 ng/ml), whereas minimal concentrations were noticed during spring (3.38±0.83ng/ml) (p<0.05). It can be concluded that the morphometric parameters and stereological structure of the testis exhibit seasonal variations in Ouled Djellal rams in Algeria.

Keywords: Morphometry, Histology, Testis, Season, Ouled Djellal ram

[1]*Biology of Organisms Department, Faculty of Natural and Life Sciences, University of Batna2, 05000, Algeria*
[2]*Laboratory of Animal Productions, Biotechnologies and Health. Institute Agronomic and Veterinary Sciences, Souk-Ahras University, 41000 Algeria*
[3]*Biotechnology's Laboratory of the Bioactive Molecules and the Cellular Physiopathology, University of Batna 2, Algeria*
[4]*Algeria's National Institute for Agricultural Research (INRAA). Setif 19.000, Algeria*
****Correspondence**: y.belkhiri@univ-batna2.dz* (Y. Belkhiri)
Received April 12, 2020.
Accepted July 8, 2020.

Introduction

The fertility of rams indicates a complex relationship between the development of the neuroendocrine system, testosterone concentration, development of individual body parts and sexual maturation (Maksimović *et al.*, 2016). Ram selection for fertilization can be accomplished by taking into account traits such as young age, testes size (length, width) etc.

Influence of season on testicular dimensions (mass and volume) and behaviour proceeded by a decrease of luteinizing hormone and an increase of follicle stimulating hormone secretion, has been described in numerous sheep breeds, including Moghani, Racka and indigenous ram breeds (Mandiki *et al.*, 1998; Sarlós *et al.*, 2013; Egerszegi *et al.*, 2014).

Seasonal variation in the histology of the testis of the ram has been reported previously (Mortimer and Lincoln, 1982). During the non-mating season, there is a decrease in reproductive behavior, efficiency of spermatogenesis and daily production of spermatozoa with reduced diameter of the seminiferous tubules (Gastel *et al.*, 1995) and in the size and activity of the Leydig cells (Mortimer and Lincoln, 1982). These regressive changes correlate with a reduced secretion of FSH and LH by the anterior pituitary gland (Johnson, 1995; Camela *et al.*, 2019).

The Ouled Djellal ram is one of the most important breeds in Algeria. It is also known as the great white Arabian breed which is usually raised in arid and semi-arid regions. This breed is known for its high rusticity and capacity for adaptation to different environments. Very few short-term studies have been carried out on the reproduction of Ouled Djellal rams. A few reports have investigated scrotal and testicular growth, endocrinological profiles and semen quality parameters in association with variation in season in Ouled Djellal breed rams (Belkadi *et al.*, 2017; Belkhiri *et al.*, 2017; Belkhiri *et al.*, 2019). However, there is no data describing the changes in the principal cell types in the Ouled Djellal ram testis (Sertoli, Leydig cells and germ cells), the height of the seminiferous epithelium and the diameter of the seminiferous tubules. In order to select the best spawners during or outside the mating season it is useful to have specific and objective information about morphological and stereological characteristics of Ouled Djellal ram testes and hormonal status. The objective of the study reported here was to investigate the testicular histomorphometrical changes during the different seasons in Ouled Djellal ram breeds in semi-arid zones.

Materials and Methods

Animals and location: The experiment was conducted between September 2018 and May 2019 in Batna region situated in the semi-arid region of the eastern part of Algeria at 968 m of altitude, 35°33′21″ North of latitude and 6°10′26″ East of longitude. The biometeorological factors, relative to ambient temperature and relative humidity values were received from the meteorological station in Batna region (Figure 1 and Table 1).

For the present study, 27 left testes from 27 sexually mature and healthy rams were used. The rams were grouped according to their age into three batches: each one of them included nine rams (batch one with rams of ten months; batch two with rams of twelve months and batch three with rams of fourteen months). The testes were collected from Batna's slaughterhouses over a period of 9 months in the following seasons: autumn or breeding season equivalent to short photoperiods (September to November), winter (December to February), and spring or the non-breeding season equivalent to long photoperiods (March to May). In each season/sampling period, nine animals were randomly selected and slaughtered for histological examination.

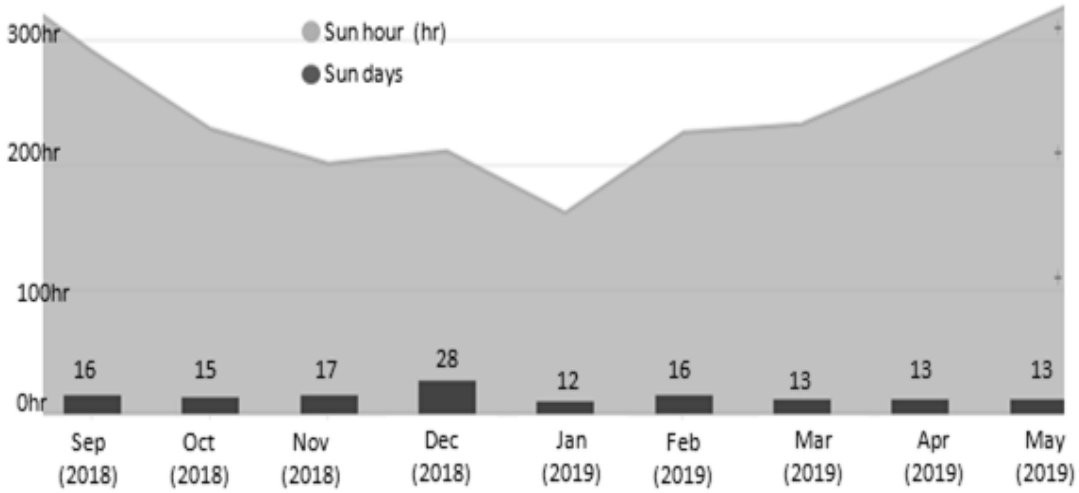

Figure 1 Average sun hour and sun days during the experimental period

Table 1 Average monthly climatological parameters during the experimental period

Months	Average temperature (C°)	Maximum temperature (C°)	Minimum temperature (C°)	Total rainfall (mm)	Average relative humidity (%)
Sep (2018)	22[a]	27[a]	19[a]	20.64[a]	46[a]
Oct (2018)	15[b]	18[b]	11[b]	62.7[b]	60[b]
Nov (2018)	11[c]	14[c]	6[c]	18.7[a]	60[b]
Dec (2018)	9[d]	12[c]	4[c]	7.9[c]	62[b]
Jan (2019)	4[d]	6[d]	0[d]	71.8[b]	74[c]
Feb (2019)	6[d]	9[d]	1[d]	21[a]	62[b]
Mar (2019)	10[cd]	14[c]	4[c]	98.9[bd]	59[b]
Apr (2019)	14[bc]	18[b]	8[b]	62.7[b]	54[d]
May (2019)	17[b]	20[ab]	11[b]	110.5[d]	54[d]

Within column means with different letters (a, b, c, d) differ significantly (p<0.05)

Testes scrotum measurements: Before slaughter, for each animal, scrotal circumference (SC) was assessed with a measuring tape (±1 mm) at the maximum anterior/posterior level of the scrotum with the ram in a standing position (Cevik *et al.*, 2017). Testicular length (TL), testicular width (TWD) and testicular thickness (TT) of both testes were measured by means of sliding calipers, and each male was weighed (BW) using an electronic platform scale.

Blood collection and hormonal analysis: Before slaughter a single blood sample was collected (jugular venipuncture, 10 ml) from each ram and the serum was separated through centrifugation and frozen at - 20°C until analysis. Concentration of testosterone (T) was assessed at 37 °C using Roche Cobas® e 411 Immunoassay (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. In the first incubation stage, 20 µl of the sample were incubated with a biotinylated monoclonal testosterone-specific antibody and 2 bromoestradiol (to release testosterone). In the second stage, streptavidin-coated microparticles and a ruthenylated testosterone derivative were added to the mixture. The reaction mixture was transferred to a measuring cell and the microparticles were magnetically captured on the surface of an electrode. Electrochemiluminescence immunoassay (ECLIA) was measured using photomultiplier and the concentration of testosterone was calculated using calibration curve (Zouei *et al.*, 2018)

Stereological study: Within 20 minutes after slaughter, the scrotum was incised and the paired testicular weight (TW) was recorded using a digital scale. Small pieces were taken from three regions (proximal, middle and distal) of the left testes; immediately fixed in formalin 10% solution, embedded in paraffin, sectioned at 5µm with rotary microtome and stained later with hematoxylin and eosin. The slides were carefully examined for stereological, histological and histomorphometrical assessment.

The TW (g) was directly converted into volume (VT), since the volume density of the testes in mammals is very close to one (Lunstra *et al.*, 2003). The relative volume (Vr) of the seminiferous tubules (the surface occupied by the tubules divided by total surface of the field), was determined using AxioVision software (Carl Zeiss, Thornwood, NY). The total seminiferous tubules volume per testis (VTS) (%) was measured by multiplying Vr by VT. The volume of intertubular tissue (VIT): was estimated by subtracting the volume of seminiferous tubules from the total testicular volume.

The seminiferous tubule diameter (STD) was measured at X 200 magnification, using a digital camera (MICROCAM MA88-500) attached to the ocular of a light microscope (ZEISS, Germany, Axioplan) and connected to a computer. 90 round or nearly round cross-sections of seminiferous tubules were randomly chosen in each ram (30 cross-sections in each region of the left testes) (Dorostghoal *et al.*, 2009). The epithelium height (GEH) was obtained in the same tubules used for tubular diameter measurement (França and Godinho, 2003).

Concerning the assessment of the total length of seminiferous tubules (LST); the seminiferous tubules were assumed to form a single cylinder with a radius r and a volume VTS; using the equation $VTS=\pi r^2$ LST results the equation $LST=VTS/\pi r^2$ (Neves *et al.*, 2002).

The different types of germ cell nuclei and Sertoli cell (SRTL) nucleoli were counted in 10 round or nearly round seminiferous tubule cross sections, chosen at random, for each animal. These counts were corrected for section thickness and nucleus or nucleolus diameter according to França and Godinho (2003). For this purpose, 10 nuclei or nucleoli diameters were measured for each cell type and analysed per animal. Numerical correction (Nc) of spermatogonia (SGN), primary (SPCI) and secondary spermatocytes (SPCII), round (SPDR) and elongated spermatids (SPDA), and Leydig cells (LEY), was performed using the equation: $Nc = COxE/E + D$ where CO is the number of cell nuclei per unit area, E is the average thickness of the section (5 µm), and D is the average nuclear diameter (measured via AxioVision software). The total number of SGN, SPCI, SPCII, SPDR, SPDA, SRTL and LEY per testis were determined by multiplying Nc (for each cell type) by VT. This procedure is more precise for the different types of germ cells and SPDR because they have nuclei with a spherical shape. On the other hand it is less precise for the SPDA and SRTL of rams because these cell nuclei have shapes that are not quite spherical (Hien *et al.*, 2011).

Statistical analysis: All results are given as mean ± standard error of the mean (SEM). Data used in the study was tested for normality before analyses using Shapiro-Wilk test. Data was analysed by the general linear model (GLM) procedure of SPSS (Version 21, 2013). The differences between means were computed for various parameters using ANOVA. A 2 × 2 factorial experimental design was used to examine the effects of age and seasonal variations in the testis size, scrotal circumference and histology, ram body weight and serum testosterone concentrations. Pearson's correlation coefficient was calculated to evaluate the relationship between the traits (BW, TW, SC, T, SRTL, VTS, LEY, DTS and GEH). A value of p≤0.05 was considered statistically significant. Furthermore, according to the level of statistical significance, results of the present study are presented as follows: p<0.05: (*), p<0.01: (**) and p<0.001: (***).

Results

Seasonal effect on testicular morphometry and ram's body weight: The results of the variation of testicular measurements and males' body weight, owing to season and rams' age, are summarized in Table 2.

Table 2 Effect of season on body weight, testicular measurements and serum testosterone concentrations in Ouled Djellal rams (mean ± SEM)

Parameters	Season			Age (months)			Interaction (season* age)
	Autumn	Winter	Spring	10	12	14	
BW (kg)	59.30±0.57^{a}	54.80±0.55^{b}	48.70±0.47^{c}	48.70±0.47^{ns}	56.40±1.09^{ns}	57.70±0.68^{ns}	ns
TW (g)	604.50±20.83^{a}	612.00±10.00^{b}	442.00±3.33^{c}	442.00±3.33^{a}	562.00±6.66^{b}	654.50±4.14^{c}	***
LT (cm)	13.50±0.16^{a}	13.20±0.20^{b}	10.70±003^{c}	10.70±0.03^{a}	12.80±0.06^{b}	13.90±0.03^{c}	**
TWD (cm)	5.60±0.10^{a}	5.70±0.06^{b}	4.50±0.03^{c}	4.50±0.03^{a}	5.40±0.03^{b}	5.90±0.00^{c}	**
TT (cm)	5.55±0.11^{a}	5.00±0.06^{b}	4.50±0.03^{c}	4.50±0.03^{a}	5.00±0.06^{b}	5.55±0.11^{c}	***
SC (cm)	32.40±0.30^{a}	31.60±0.30^{b}	29.35±0.15^{c}	29.35±0.15^{a}	31.10±0.13^{b}	32.90±0.13^{c}	ns
T (ng/ml)	7.81±1.2^{a}	4.68±1.06^{b}	3.38±0.83^{c}	3.38±0.83^{ns}	5.06±0.95^{ns}	7.43±1.37^{ns}	ns

Notes: BW: body weight. TW: testicular weight. TL: testicular length. TWD: testicular width . TT: testicular thickness. SC: scrotal circumference

T: Serum testosterone concentrations

For each factor (season and age), within rows, means with different superscripts differ significantly (p < 0.05).

^{NS}: Not significant. **: Significant at p <0.01. ***: Significant at p <0.001

BW varied seasonally during the experimental period, as the lowest mean values were noticed during spring and the highest values during autumn (p< 0.001). SC significantly (p<0.001) differed among seasons, since the significantly highest value was observed in autumn (32.40±0.30 cm) followed by winter (31.60±0.30 cm), whereas the lowest was observed in spring (29.35±0.15 cm). Similarly, TL, WT and TT of testis differed significantly (p<0.001) among the seasons; they were significantly higher in autumn, but the lowest value was recorded in spring. TW decreased by 27% during the non-breeding season (spring), compared to the breeding season (autumn) There was a significant difference in all testicular measurements (SC, TL, WT, TT, TW) between different age groups (p< 0.001). The lowest average values of these variables were noticed at ten months of age, while the highest values were observed at fourteen months of age.

Seasonal effect on serum testosterone concentrations: Serum testosterone concentrations were significantly affected by season (p<0.05). The highest and lowest mean T were recorded in autumn and spring, viz. 7.81±1.2 ng/ml and 3.38±0.83 ng/ml respectively (Table 2). There was no significant difference among the different age groups (p> 0.05).

Seasonal effect on characteristics of histological examination: The effect of season and age of rams on testicular stereological characteristics is presented in Table 3.

More specifically, VTS was statistically significantly altered throughout seasons, reaching its maximum in autumn, when it constituted about 84.86 % of the testicular parenchyma (p<0.05).

VIT differed significantly between breeding and non-breeding seasons (p<0.001). Actually, its lowest value was observed in autumn, whereas its highest was in winter. The STD, but not the LST, varied significantly during different seasons (p<0.01 and p>0.05, respectively), as the lowest value was measured in spring (167.02±6.74 µm) and the highest value in autumn (212.12±4.94 µm). The STD and the VIT were significantly affected by age (p<0.01 and p< 0.05, respectively).

GEH of testes differed significantly during different seasons (p<0.001), noticing the lowest values in spring (64.31±1.59 µm), while the highest were in autumn (82.72±2.52 µm). However, detailed measurements of seminiferous tubules revealed that not only V and LST but also GEH were unaffected by age (p> 0.05).

The seminiferous epithelium of Ouled Djellal rams is spermatogenically active through the autumn but inactive throughout the rest of the year. By autumn, the major germ cell population has progressed past meiosis with abundant round and early elongating spermatids dominating the seminiferous epithelium. However, no change was recorded among seasons in the number of SRTL nor LEY (p>0.05). On the other hand, a strong effect of age on the number of LEY was recorded, since the lowest value was obtained in the ten month rams' age group, while the greatest value was obtained in the fourteen month rams' age group (p<0.001).

Table 3 Effect of season on testes histological characteristics of Ouled Djellal rams (mean ± SEM)

Parameters	Season			Age (months)			Interaction (season* age)
	Autumn	Winter	Spring	10	12	14	
Total number per testis (x10⁹)							
Sertoli cells	8.59±0.59^{ns}	9.25±0.86^{ns}	8.81±0.92^{ns}	8.81±0.92.^{ns}	8.59±0.79^{ns}	9.25±0.68^{ns}	ns
Spermatogonia	25.57±1.54^{a}	25.16±2.04^{a}	21.97±1.80^{b}	21.97±1.80^{a}	23.836.32±1.48^{b}	26.90±1.95^{c}	ns
Primary spermatocytes	42.25±1.60^{a}	43.21±3.68^{b}	32.89±2.70^{a}	33.22±1.73^{a}	41.92.93±1.16^{b}	43.21±3.68^{c}	ns
Secondary spermatocytes	49.31±2.594.22^{a}	50.18±3.19^{b}	45.49±3.66^{c}	42.49±3.29^{ns}	52.30±2.18^{ns}	50.18±3.19^{ns}	*
Round spermatids	64.86±3.73^{ns}	66.23±5.11^{ns}	62.70±5.06^{ns}	62.70±5.06^{ns}	63.18±4.063^{ns}	67.91±4.73^{ns}	ns
Elongated spermatids	56.03±3.93^{a}	61.17±3.53^{b}	46.13±3.75^{c}	47.97±5.27^{ns}	61.17±3.53^{ns}	54.19±2.24^{ns}	*
Leydig cells	4.87±0.14^{ns}	4.62±.16^{ns}	3.54±0.12^{ns}	3.54±0.12^{a}	4.39±0.12^{b}	5.10±0.07^{c}	ns
Total number per gram of testis (x10⁶)							
Sertoli cells	14.24±0.88^{ns}	15.16±1.46^{ns}	19.95±2.08^{ns}	19.95±2.08^{ns}	15.27±1.33^{ns}	14.13±1.05^{ns}	ns
Spermatogonia	46.20±2.88^{ns}	46.20±3.24^{ns}	46.01±2.47^{ns}	40.05±4.09^{a}	46.41±2.62^{b}	52.17±2.99^{c}	ns
Primary spermatocytes	82.67±4.03^{a}	71.03±4.75^{b}	60.96±2.66^{c}	68.81±2.79^{a}	71.03±4.75^{b}	74.81±6.31^{c}	ns
Secondary spermatocytes	96.81±6.54^{a}	84.51±3.77^{b}	82.64±3.67^{c}	82.84±6.96^{ns}	85.46±3.30^{ns}	95.86±6.96ns	*
Round spermatids	125.69±8.54^{a}	121.39±8.01^{b}	97.96±3.74^{c}	106.58±4.57^{a}	117.06±10.13^{a}	121.39±8.01^{b}	ns
Elongated spermatids	110.65±10.20^{a}	101.45±5.31^{b}	86.15±5.26^{c}	88.73±3.92^{a}	101.45±5.31^{b}	108.07±11.36^{c}	ns
Total seminiferous tubule volume per testis (%)	84.86±0.55^{a}	82.27±0.50b	78.8±1.23c	78.80±1.23^{ns}	83.59±0.59^{ns}	83.53±0.76^{ns}	ns
Length of seminiferous tubules (m)	3209.25±178.90^{ns}	3361.24±255.61^{ns}	3338.80±269.34^{ns}	3338.80±269.34^{ns}	3216.20±215.68^{ns}	3354.28±225.94^{ns}	ns
Interstitium volume (%)	16.77±0.66^{a}	20.92±0.47^{b}	19.09±1.04^{c}	19.09±1.01^{a}	20.22±0.70^{b}	17.47±0.84^{c}	ns
Seminiferous tubuli diameter (µm)	212.12±4.94^{a}	189.04±6.23^{b}	167.02±6.74^{c}	167.02±6.74^{ns}	197.56±9.44^{ns}	203.60±1.25^{ns}	*
Germinal epithelium height of testes (µm)	82.72±2.52^{a}	75.65±2.52^{b}	64.31±1.59^{c}	68.62±2.12^{ns}	71.34±2.47^{ns}	82.76±2.52^{ns}	ns

For each factor (season and age), within rows, means with different superscripts differ significantly ($p < 0.05$)

^{NS}: Not significant. *: Significant at $p < 0.05$

Correlation: The correlation coefficients between BW, TW, SC, T and histological measurements (SRTL, VTS: LEY, STD, GEH) of the Ouled Djellal rams' testes are presented in Tables 4.

SC positively correlated with the BW (r = 0.84; $p < 0.001$), T (r = 0.51; $p < 0.05$), VTS (r = 0.64; $p<0.001$) and STD (r = 0.54; $p<0.01$).

The TW showed significant correlation with the VTS, the LEY and STD (r=0.59; $p<0.001$, r=0.49; $p<0.01$ and r=0.86; $p<0.001$, respectively). However, no significant correlation was observed between TW and LST (r=0.02; $p>0.05$) or SRTL number (r=0.09; $p>0.05$).

No significant correlation was found between T and GEH (r = 0.23; $p>0.05$). In addition, significant but negative correlation was found between STD and SRTL number (r=- 0.42; $p<0.05$). However, positive correlation between STD and GEH (0.56; $p<0.001$) was observed.

Table 4 Coefficients of correlation between BW, TW, SC, T and histological characteristics of Ouled Djellal rams' testes

r	***BW***	***TW***	***SC***	***T***	***SRTL***	***VTS***	***LEY***	***STD***	***GEH***
BW	*1*								
TW	*0.77****	*1*							
SC	*0.84****	*0.94****	*1*						
T	*0.44**	*0.47***	*0.51***	*1*					
SRTL	*0.02*	*0.09*	*0.05*	*0.11*	*1*				
VTS	*0.68****	*0.59****	*0.64****	*0.23*	*0.15*	*1*			
LEY	*0.77****	*0.86****	*0.89****	*0.54****	*-0.09*	*0.60****	*1*		
STD	*0.51***	*0.49***	*0.54***	*0.29*	*-0.42**	*0.19*	*0.55***	*1*	
GEH	*0.81****	*0.78****	*.81****	*0.23*	*-.059*	*0.57****	*0.74****	*0.56****	*1*

Notes: BW: Body weight. TW: Testicular weight. SC: Scrotal circumference. T: Serum testosterone concentrations. SRTL: Sertoli cells. VTS: Total seminiferous tubules volume per testis (%); LEY: Leydig cells. STD: Diameter of seminiferous tubules (µm). GEH: Germinal epithelium height of testes (µm). ^{NS}: Not significant. *: Significant at $p<0.05$. **: Significant at $p<0.01$. ***: Significant at $p<0.001$

Discussion

The present study is the first histomorphometrical investigation of the testis of Ouled Djellal rams, showing basic stereological characteristics of Ouled Djellal ram testis and their changes in different seasons.

Significant differences were observed in the present study, concerning ram' BW among seasons. Similar information has been reported in other studies performed in Greek breed rams (Avdi *et al.*, 2004). However, Aller *et al.,* (2012) observed that body weight decreased during winter in Pampinta and Corriedale rams (4 to 6 years of age).

The present study showed that the time of year significantly affected both testicular dimension and scrotal circumference. Scrotal circumference gave good estimates of testes weight and number of sperm in the testes and epididymides (Hassan *et al.*, 2009). It has been indicated that, during autumn, testes attained their largest volume whereas the seminiferous epithelium attained its maximal activity (Zayed *et al.*, 1995). Testicular volume is a significant criterion in spermatogenesis prediction and evaluation of testicular function in ruminants (Akosman *et al.,* 2013). Because approximately 70% to 80% of the testicular mass consists of seminiferous tubules, any change in testis dimension reflects changes in tissue mass and also spermatogenesis (Cevik *et al.,* 2017).

The present stereological analysis revealed that spermatogenic activity of Ouled Djellal ram testes is seasonal, as it was at its highest during autumn. This fact is confirmed by previous reports that indicate autumn to be the normal mating season (Gastel *et al.*, 1995; Gündoğan, 2007; Dorostghoal *et al.*, 2009). Seasonally, rams regulate spermatogenesis by altering both the number of spermatogonia and germ cell degeneration.

According to Hochereau-de Reviers *et al.,* (1985) no differences were found in SRTL numbers between Soay rams subjected to short photoperiod and rams exposed to long photoperiod. In contrast, Johnson and Thompson (1987) indicated that the number of SRTL in the testes of stallions were maximum in the breeding season and minimum in the non-breeding season. However, it should be emphasized that the season or photoperiod changes testicular structure and function. These changes are much more conspicuous in horses than in rams, the ram being an animal that shows active spermatogenesis throughout the year, including the non- breeding season (Johnson, 1995). Quantitative studies of SRTL in seasonally breeding animals other than the rams have provided variable results. It is not clear whether there are generalized differences in SRTL number in seasonally breeding mammalian species or if the responses are species-specific. Our results indicated that no significant correlation between total numbers of SRTL and TW were present, suggesting that this cell population may not significantly contribute to the weight of this organ (Petersen *et al.,* 2015).

The total number of specific types of germ cells changed owing to the photoperiod in this study. This fact could be the result of a decline in LH and FSH concentrations during long days, influencing the functional activity of the LEY and SRTL (Kliesch *et al.,* 1991). In fact, in mammals, only 2 or 3 of 10 spermatozoa are produced from differentiated type A1 SGN, the highest level of cell degeneration occurs during the spermatogonial proliferative phase and during meiosis (França and Godinho, 2003).

The STD has been widely considered as a reliable parameter for the evaluation of spermatogenic ability (Gastel *et al.*, 1995). The present study indicated that the largest STD was found during autumn, concomitant with large SC, GEH of testes and highest T output. The increase in diameter is due to the proliferation of developing germ cells within the seminiferous epithelium, which causes the tubule to distend. Mean STD decreased during spring. Our results are consistent with previous reports in bucks (Delgadillo *et al.,* 1995) and rams (Dorostghoal *et al.,* 2009). The magnitude of this decrease between breeding and non-

breeding seasons (21.27%) is comparable to that of Soay (25%) (Hochereau-de Reviers *et al.,* 1985) rams and lower than that shown in Corriedale (34%) (Gastel *et al.,* 1995) rams. Moreover, the correlation found in the present study between STD and SC could be explained by the fact that tubular tissue constitutes 78-84% of testicular tissue (Wrobel *et al.,* 1995).

The GEH was also changed by the photoperiod. This is very similar to the changes observed by Dorostghoal *et al.,* (2009). Rams' testicular seminiferous epithelial height varies slightly during the season, being dependent upon the composition of cellular associations and volume changes of SRTL (Wrobel *et al.,* 1995). Since it is a flexible and irregular structure, its simple direct measurement will not lead to an unbiased estimation. Some factors such as orientation of tissue and cutting angle will influence the height (Goodarzi *et al.,* 2018).

In the Ouled Djellal breed, seminiferous tubules occupied about 82% of the testicular parenchyma during winter, according to the findings of present study; representing the same percentage as in Merino or Merino x Suffolk breeds (Wrobel *et al.,* 1995). Interstitial tissue represents 16.77 to 20.92% of testis volume depending on the season. Our histomorphometrical results showed a moderate positive correlation between the GEH and STD, ($r=0.56$, $p<0.001$) which indicates an increase in epithelium height with a consequent increase in STD. On the other hand, an increase in epithelium volume caused a decrease in interstitium size. This increase in STD and GEH is a continuous process that may be caused by a progressively increasing germ population.

The present study indicated that the period of highest T seen in Ouled Djellal rams corresponds to the natural breeding season (autumn). Seasonal variations in serum T found in this study are in accordance with previous reports for Daglic and Chios breeds (Gündoğan, 2007). In the present experiment, there was a significant correlation of T with LEY numbers ($r=0.54$; $P<0.001$). Johnson (1995) showed that testosterone levels are related to the volume of LEY cytoplasm, in particular, to the volume of smooth endoplasmic reticulum, which constitutes most of the LEY cytoplasm but not necessarily to LEY numbers or nuclear volume. However, no change in LEY numbers between seasons was revealed ($P>0.05$). It is, therefore, possible that seasonal changes in T in this study are the result of an increase in LH and FSH concentrations during short days which influences the functional activity of the LEY (Camela *et al.*, 2019). The slow seasonal increase in LH release was shown to be necessary to upregulate the number of testicular LH receptors to result in increased testosterone secretion (Barenton and Pelletier, 1983). The detected lack of correlation between the numbers of LEY and SRTL in the present study can be explained by the substantially different functions of these two cell types (Petersen *et al.,* 2015). On the other hand, as T were compared between the different age groups there was no significant difference. Such a result could be attributed to the fact that all the rams in this study had already reached the age of puberty as it is determined by the onset of active spermatogenesis.

In conclusion, the seasonal variation of testicular morphology and histomorphometry in Ouled Djellal rams, showed a testicular cycle characterized by significantly high scores of testicular weight and scrotal circumference during the non-breeding season and low values in the breeding season. The rise in scrotal circumference was associated with an increase in the seminiferous tubule diameter and volume germinal epithelium height and testosterone concentrations. However, there were no change in Leydig cell and Sertoli cell numbers between seasons. In order to select the best spawners during or outside the mating season, it is useful to have specific and objective information about morphological and stereological characteristics of the Ouled Djellal ram testis and hormonal status. Moreover function of the testis is a good tool for the prediction of the fertility of rams.

Acknowledgements

This work was supported by thr Directorate-General for Scientific Research and Technological Development (DGSRTD), Algeria.

Disclosure statement: No potential conflict of interest was reported by the authors.

References

Akosman MS, Lenger OF and Demirel HH 2013. Morphological, Stereological and Histometrical Assessment of the Testicular Parameters between Holstein and Simmental Bulls. Int J Morphol. 31: 1076–1080.

Aller JF, Aguilar D, Vera T, Almeida GP and Alberio RH 2012. Seasonal variation in sexual behavior, plasma testosterone and semen characteristics of Argentine Pampinta and Corriedale rams. Spanish J Agric Res. 10(2):345.

Avdi M, Banos G, Stefos K and Chemineau P 2004. Seasonal variation in testicular volume and sexual behavior of Chios and Serres rams. Theriogenology. 62(1–2):275–282.

Barenton B and Pelletier J 1983. Seasonal Changes in Testicular Gonadotropin Receptors and Steroid Content in the Ram. Endocrinology. 112(4): 1441-6.

Belkadi S, Safsaf B, Heleili N, Tlidjane M, Belkacem L and Oucheriah Y 2017. Seasonal influence on sperm parameters, scrotal measurements, and serum testosterone in Ouled Djellal breed rams in Algeria. Vet World. 10(12):1486–1492.

Belkhiri Y, Bouzebda-Afri F, Bouzebda Z, Mouffok CE and Djaout A 2017. Age And Seasonal Effects On Sexual Parameters in Mature Rams Used in Artificial Insemination Centre (Algeria). Glob Vet. 18 (1): 31-40.

Belkhiri Y, Bouzebda-Afri F, Bouzebda Z, Mouffok C and Djaout A 2019. Seasonal variations in reproductive parameters of Ouled Djellal rams in the East of Algeria. Indian J Anim Res. 53(11):1407–1413.

Camela ESC, Nociti RP, Santos VJC, Macente BI, Murawski M, Vicente WRR, Bartlewski PM and Oliveira MEF 2019. Changes in testicular size,

echotexture, and arterial blood flow associated with the attainment of puberty in Dorper rams raised in a subtropical climate. Reprod Domest Anim. 54(2):131–137.

Cevik M, Yilmazer C and Kocyigit A 2017. Comparison of sexual performance and testicular characteristics of melatonin treated Kivircik and Charollais rams during the non-breeding season. Arq Bras Med Vet Zootec. 69(2):278–284.

Delgadillo JA, Reviers MH de, Daveau A and Chemineau P 1995. Effect of short photoperiodic cycles on the male genital tract and testicular parameters in male goats (Capra hircus). Reprod Nutr Dev. 35(5):549–558.

Dorostghoal M, Erfani N and Goorani S 2009. Stereological study of Arabian ram testis during different seasons. Iran J Vet Res. 10(4):360–366.

Egerszegi I, Sarlós P, Rátky J, Solti L, Faigl V, Kulcsár M, Cseh S 2014. Effect of melatonin treatment on semen parameters and endocrine function in Black Racka rams out of the breeding season. Small Rumin Res . 116(2–3):192–198.

França LR and Godinho CL 2003. Testis Morphometry, Seminiferous Epithelium Cycle Length, and Daily Sperm Production in Domestic Cats (Felis catus). Biol Reprod. 68(5):1554–1561.

Gastel T, Bielli A, Perez R, Lopez A, Castrillejo A, Tagle R, Franco J, Laborde D, Forsberg M and Rodriguez-Martinez H 1995. Seasonal variations in testicular morphology in Uruguayan Corriedale rams. Anim Reprod Sci. 40(1–2):59–75.

Goodarzi N, Soroor MEN, Rahimi-Feyli P and Kazemi S 2018. Testicular stereology of lambs supplemented with organic and inorganic zinc. Bulg J Vet Med. 21(3):301–312.

Gündoğan M 2007. Seasonal variation in serum testosterone, T3 and andrological parameters of two Turkish sheep breeds. Small Rumin Res. 67(2–3):312–316.

Hassan MR, Pervage S, Ershaduzzaman M and Talukder MAI 2009. Influence of age on the spermiogramic parameters of native sheep Influence of age on the spermiogramic parameters of native sheep. J Bangladesh Agril Univ. 7(2): 301–304.

Hien OC, Diarra B, Brillard JP, Boly H and Sawadogo L 2011. Effects of improving health status on testicular development of guinea fowl (numida meleagris) reared under natural photoperiod in the sudanian zone of Burkina Faso. Int J Poult Sci. 10(2):113–119.

Hochereau-de Reviers MT, Perreau C and Lincolm GA 1985. Photoperiodic variations of somatic and germ cell populations in the Soay ram testis. J Reprod Fertil. 74(2):329–334.

Johnson L 1995. Efficiency of spermatogenesis. Microsc Res Tech. 32(5):385–422.

Johnson L and Thompson D 1987. Effect of seasonal changes in Leydig cell number on the volume of smooth endoplasmic reticulum in Leydig cells and intratesticular testosterone content in stallions. J Reprod Fertil. 81(1):227–232.

Kliesch S, Schweiferl B, Niklowitz P, Nieschlag E and Bergmann M 1991. The influence of LH and / or FSH on Leydig and Sertoli cell morphology after testicular involution in the Djungarian hamster , Phodopus sungorus , induced by hypophysectomy or short photoperiods . Andrologia. 23(2): 99-107.

Lunstra DD, Wise TH and Ford JJ 2003. Sertoli Cells in the Boar Testis: Changes During Development and Compensatory Hypertrophy after Hemicastration at Different Ages. Biol Reprod. 68(1):140–150.

Maksimović N, Hristov S, Stanković B and Petrović MP 2016. Investigation of serum testosterone level , scrotal circumference , body mass , semen characteristics. Turk J Vet Anim Sci. 40: 53-59.

Mandiki SNM, Derycke G, Bister JL and Paquay R 1998. Influence of season and age on sexual maturation parameters in Texel, Suffolk and Ile-de-France rams 2. Circulating concentrations of follicle stimulating hormone, luteinizing hormone, prolactine and testosterone. Small Rumin Res. 28(1):81–88.

Mortimer D and Lincoln GA 1982. Ultrastructural study of regressed and reactivated testes from Soay rams. J Reprod Fertil. 64(2):437–442.

Neves ES, Chiarini-Garcia H and França LR 2002. Comparative Testis Morphometry and Seminiferous Epithelium Cycle Length in Donkeys and Mules1. Biol Reprod. 67(1):247–255.

Petersen PM, Seierøe K and Pakkenberg B 2015. The total number of Leydig and Sertoli cells in the testes of men across various age groups - a stereological study. J Anat. 226(2):175–179.

Sarlós P, Egerszegi I, Balogh O, Molnár A, Cseh S and Rátky J 2013. Seasonal changes of scrotal circumference, blood plasma testosterone concentration and semen characteristics in Racka rams. Small Rumin Res. 111(1–3):90–95.

Wrobel KH, Reichold J and Schimmel M 1995. Quantitative morphology of the ovine seminiferous epithelium. Ann Anat. 177(1):19–32.

Zayed AE, Hifny A, Abou-Elmagd A and Wrobel KH 1995. Seasonal changes in the intertubular tissue of the camel testis (Camelus dromedarius). Ann Anat. 177(3):199–212.

Zouei N, Shojaee S, Mohebali M and Keshavarz H 2018. The association of latent toxoplasmosis and level of serum testosterone in humans. BMC Res Notes. 11:365.