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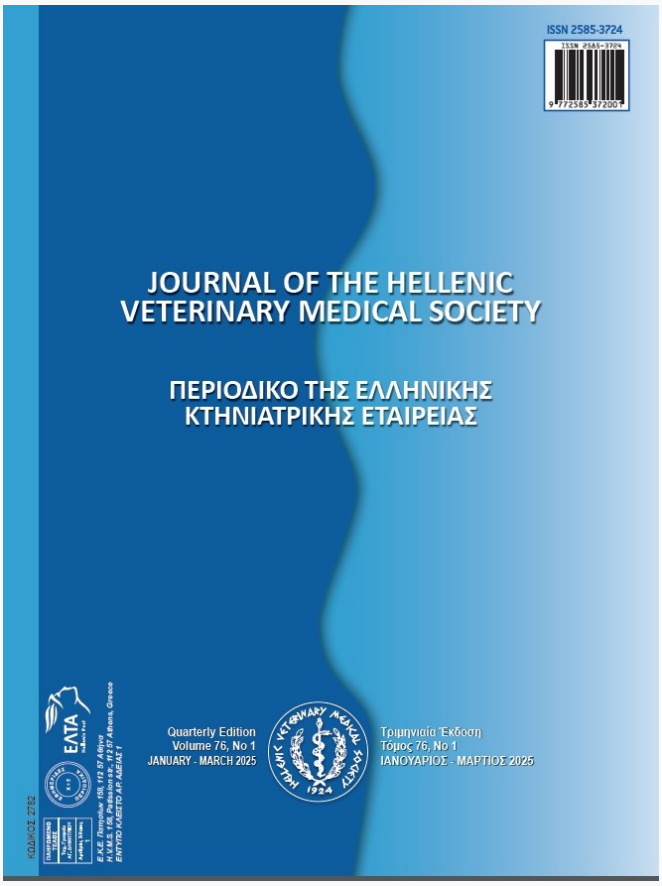


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# Seasonal changes in the histomorphological and spermatogenic activity controlled by proliferation and apoptosis of germ cells of the bull testis in a semi-arid environment (Algeria)

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**ABSTRACT :** The present study was carried out to investigate the spermatogenic activity of mature bull local breed and effect of season's changes on the testes activity and main histomorphological parameters. At the beginning of each season (autumn, winter, spring, summer), among the study group, nine (09) bulls were randomly selected and slaughtered and their left testes were processed by histological and histometric methods. Statistical analysis revealed that the total numbers of Leydig, Sertoli and germ cells per testis including primary and secondary spermatocytes, round and elongated spermatids were significantly higher during the breeding season (autumn). The diameter of the seminiferous tubules and the height of germinal epithelium demonstrated higher ($p<0.05$) significant in autumn and winter. The increase in spermatogenesis process led to an increase the diameter and height of seminiferous. However, the Length of seminiferous tubules did not change ($P>0.05$) due to season. A positive linear relationship ($P<0.05$) was found between Sertoli cell efficiency (Y) and the season (X), which was characterized by the equation $Y= -1.322 X+7.481$ and a coefficient of correlation (R) of 0.323. A significant positive correlation was seen between the total number of Sertoli cells and spermatogonia ($r = 0.998$; $P <0.01$). Histomorphometric studies of reproductive organs could contribute to better develop the reproductive potential in this species. It is therefore recommended that season should be given urgent attention in any bull management system employed in Algeria to obtain the best semen quality.

***Key words*:** histomorphometry; spermatogenic activity; testis; season; bulls.

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## INTRODUCTION

Adult males of many mammals with seasonal variation in reproduction show annual cycles of testicular involution and recrudescence. However, the degree to which males of different species react to changes in season varies. Domestic cattle can breed successfully at any time of the year and, therefore, are not considered to be seasonal breeders in the strictest sense. However, research into the effects of season on bovine bull fertility indicated that there were slight seasonal changes in gonadotropin secretion, gonadal hormone production, volume and total number of sperm produced and sperm morphology (Barth & Waldner, 2002).

The major contributor to the reproductive characteristics is environment. To comprehend the impact of the environment on reproductive characteristics, it is necessary to understand the biological significance of the term 'environment', which could be defined as all factors surrounding the animal, which include several factors, such as ambient temperature, nutrition, humidity, day length, food quality, and management practice (Mathevon et al., 1998).

Testicular morphofunctional evaluation is known to elucidate physiological processes from the perspective of form and function. The quantification of testicular histology is a valuable tool for evaluating the sperm capacity of animals under normal, pathological, or experimental conditions (Rocha et al., 2022). In this regard, knowledge of testicular histo-morphology is mandatory to evaluate the influence that different factors, such as hormonal fluctuations of the photo-neuroendocrine circuit may have on reproductive efficiency which may be a result of seasonal variation (Yasuo et al., 2006).

Histological evaluation is a useful tool to quantify and qualify spermatogenesis in bulls. Quantification can be assessed by morphometric analysis of different measures, the purpose of which is to compare structural changes in cells and tissues, and the density of parenchymal structures (Micklem & Sanderson, 2001).

The seasonal fluctuations in the reproductive efficiency in bulls have already been evaluated based on testicular biometry parameters, *testicular histometry characteristics*, sexual behavior and sperm production and quality (Arrighi et al., 2010) (Genedy et al., 2019). However, there is a lack of information on basic physiology in local bulls in Algeria. Regarding reproduction, some effort has been made to investigate the relationship between age and reproductive efficiency in local breeds bulls (Belkhiri et al., 2021), but definitive information on seasonal testicular function does not exist. Thus, the detailed changes in apoptosis of male germ cells in the testis during the seasonal reproductive cycle, and the resulting regulation of spermatogenesis in the testes of local breed bull have not been studied. The objective of the study reported here was to investigate the testicular histomorphometrical and spermatogenic activity changes during the different seasons in local bull breeds in semi-arid zones in Algeria.

## MATERIALS AND METHODS

### Location and Climate conditions

The experiment was conducted between March 2019 to February 2020 at Batna region situated in eastern Algeria at 968 m of altitude, 35°33′21″ North of latitude and 6°10′26″ East of longitude. The cli-

**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 (%) |
|---|---|---|---|---|---|
| **Mar** | 9.7$^{cd}$ | 16.1$^{c}$ | 2$^{c}$ | 124$^{bd}$ | 72$^{b}$ |
| **Apr** | 13.3$^{bc}$ | 20.9$^{b}$ | 5.6$^{b}$ | 76$^{b}$ | 66$^{d}$ |
| **May** | 15.7$^{b}$ | 23.4$^{ab}$ | 7.3$^{b}$ | 106$^{d}$ | 62$^{d}$ |
| **Jun** | 26.1$^{a}$ | 36.1$^{a}$ | 16.1$^{a}$ | 8$^{c}$ | 38$^{a}$ |
| **July** | 27.9$^{a}$ | 38$^{a}$ | 17.8$^{a}$ | 16$^{a}$ | 36$^{a}$ |
| **August** | 27.6$^{a}$ | 36.9$^{a}$ | 18.3$^{a}$ | 61$^{b}$ | 47$^{a}$ |
| **Sep** | 22.9$^{a}$ | 30.6$^{a}$ | 15.2$^{a}$ | 80$^{a}$ | 64$^{a}$ |
| **Oct** | 17.3$^{b}$ | 25.2$^{b}$ | 9.4$^{b}$ | 36$^{b}$ | 73$^{b}$ |
| **Nov** | 10.3$^{c}$ | 15.5$^{c}$ | 5.1$^{c}$ | 138$^{a}$ | 82$^{b}$ |
| **Dec** | 9.1$^{d}$ | 15.3$^{c}$ | 2.9$^{c}$ | 50$^{c}$ | 76$^{b}$ |
| **Jan** | 6.9$^{d}$ | 13.7$^{d}$ | 0$^{d}$ | 20$^{b}$ | 74$^{c}$ |
| **Feb** | 9.3$^{d}$ | 19.1$^{d}$ | -0.5$^{d}$ | 1$^{a}$ | 67$^{b}$ |

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

mate of the Batna region where the study was conducted is considered semi-arid. The climate data for each month from Mar 2019 to Feb 2020 was accessed from the Bureau of Biometeorology and is available in Table 1. Monthly average temperatures exceed 36˚C from June to August, and then dropped to -0.5 °C in February. The relative humidity all year around generally sat between 36 to 82%, with very few days dropping below this value.

**General procedures**

For the present study, 36 left testes from 36 sexually mature and healthy bulls were used. Testes were collected from Batna's slaughterhouses over 12 months in the following seasons: autumn or breeding season equivalent to short photoperiods (September to November)(n=9), winter (December to February) (n=9), spring or non-breeding season equivalent to long photoperiods (March to May) (n=9) and summer season (Jun to August) (n=9).

After slaughtering the right testes were collected and placed in a plastic box which contained ice until transporting within 45 min to the laboratory, the tissue which surrounds the testes and the epididymis was removed by scissors and scalpel. Testes weight was taken by using digital electrical balance.

The testes tissue samples were cut into 5 $mm^3$ and fixed in buffered 10 % formalin for 24 h. Then the samples dehydrated in upgraded series ethanol, clarified in xylene, and finally embedded in paraffin wax. Serial paraffin sections (5µm thickness) were deparaffinized in xylene and rehydrated in decreasing concentrations of ethanol, stained with Hematoxylin Eosin.

The mean seminiferous tubule diameter (STD) was measured at 200x magnification using a digital camera (MICROCAM MA88-500) attached to the eyepiece of a light microscope (ZEISS, Germany, Axioplan) and connected to a computer. Ten tubule cross-sections, made as circular as possible, were chosen randomly and measured for each testis using AxioVision Rel 4.6 (Carl Zeiss, Thornwood, NY). These sections were also used to measure the germinal epithelium height (GEH), which was measured from the basal membrane to the tubular lumen. All the morphometric measurements of the testicular tubules were taken at stage VIII.

The total seminiferous tubules volume per testis (VTS) (%) and volume of the intertubular tissue (VIT): were obtained by the method described by (Belkhiri et al., 2021). Concerning the assessment of 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=πr2 LST (Sarma & Devi, 2017).

For each histological slide, ten transverse sections of seminiferous tubules were also evaluated at 600X magnification to obtain the following variables: seminiferous epithelium cell population (spermatogonia, primary and secondary spermatocytes, round and elongated spermatids), Leydig cells, and Sertoli cell nucleoli and efficiency of the spermatogenesis, which were estimated in each animal according to the methodology described by Segatelli et al. (2004). The count obtained for each cell type was corrected to the average nuclear diameter and thickness of the cut, using the formula modified by França and Godinho (2003).

The following ratios were obtained from the corrected counts (Pintus et al., 2015):

- Primary spermatocytes / spermatogonia, to estimate the coefficient of efficiency of spermatogonial mitosis;
- Round spermatids/spermatogonia, to obtain the overall rate of spermatogenesis;
- Round spermatids /primary spermatocytes, to obtain the rate of germ cell loss during meiosis (meiotic index);
- Elongated spermatids/ round spermatids, to estimate the post-meiotic germ loss
- Round spermatids/Sertoli cell nucleoli, to estimate the Sertoli efficiency;

**Statistical Analysis**

Data were analyzed using SPSS 20.0 (SPSS Inc, Chicago, IL, USA) and expressed as the mean ± standard error of mean (S.E.M). The Kolmogorov-Smirnov and Levene's tests were used to check data normality and homogeneity of variance, respectively. Simple one-way ANOVA was used to study the effect of season on testicular morphometric and stereological parameters and Tukey's post hoc test was used to differentiate between significant means. Differences with values of $< 0.05$ were considered to be statistically significant. The Pearson's correlation coefficients were used to assess the association between

the parameter's studies. The regression equations employed were:

$Y= b_0 + b\ X$ (linear model)

$Y= = b_0 + b_1\ X + b_2\ X^2$ (quadratic model)

$Y= b_0 + b_1\ X + b_2\ X^2 + b_3\ X^3$ (cubic model)

$Y= b_0\ X^{b1}$ (Power Model)

Y = dependent variables

b0 = the intercept

X = independent variables

$b_1$, $b_2$ and $b_2$ = regression coefficients

e = random error.

## RESULTS

### Seasonal effect on histomorphometric testicular parameters

The effect of season on testicular histomorphometrical characteristics is presented in Table 2 and Figure 1. The seminiferous epithelium of bulls is spermatogenically active through the autumn and the winter. By autumn, the major germ cell population has progressed past meiosis with abundant round and elongating spermatids dominating the seminiferous epithelium. However, no change was recorded among seasons in the number of spermatogonia ($p>0.05$). On the other hand, a strong effect of season on the number of Sertoli cells was recorded, since the greatest value was obtained in autumn, while the lowest value was obtained in the summer ($p<0.05$).

Significant seasonal effect ($P<0.01$) on Leydig cells number differences was also evident. Autumn season had the highest value ($7.59\pm0.29x10^9$) followed by the winter ($7.17\pm0.31x10^9$) then spring ($6.22\pm0.29x10^9$) while least value was recorded during the summer ($5.093\pm0.27x10^9$).

The relationship between season (X) and the different germ cells number (Y) is shown in **Table 3**. A positive relationship was found between season and the number of Sertoli cells. This relationship was characterized by the equation $Y=0.051X^2 - 0.133X+5.628$, $R^2=0.29$ ($P<0.05$) where X equals season and Y denotes the number of Sertoli cells per paired testes in billions. A third-order polynomial (cubic) relation between Leydig cells number and the season of bulls (Table 3).

**Table 2.** Effect of season on number (Mean ± SE) of different cells per testis and per gram of testes of male bulls.

| Season | Paramaters | | | | | | |
|---|---|---|---|---|---|---|---|
| | Sertoli cells | Spermatogonia | Primary spermatocytes | Secondary spermatocytes | Round spermatids | Elongated spermatids | Leydig cells |
| | | | | Total number per testis ($x10^9$) | | | |
| **Autumn** | 5.79±0.44* | 5.57±0.43 $^{NS}$ | 12.80±0.99 * | 12.12±1.00* | 34.01±2.85* | 42.95±5.92*** | 7.59±0.29** |
| **Winter** | 5.30±0.48* | 5.12±0.48 $^{NS}$ | 12.09±.91* | 12.11±0.87* | 32.98±2.60* | 42.43±5.61*** | 7.17±0.31** |
| **Spring** | 4.65±0.48* | 4.46±0.48 $^{NS}$ | 11.55±0.99* | 10.71±0.96* | 30.43±2.85* | 32.73±5.12*** | 6.22±0.29** |
| **Summer** | 5.17±0.51* | 4.29±0.50 $^{NS}$ | 11.37±1.05* | 11.33±0.95* | 28.89±3.01* | 19.51±5.78*** | 5.09±0.27** |
| | | | | Total number per gram of testis ($x10^6$) | | | |
| **Autumn** | 4.15±0.29 * | 3.82±0.29$^{NS}$ | 9.28±0.78** | 9.27±0.66** | 22.27±1.79*** | 32.51±3.70*** | 5.31±0.14** |
| **Winter** | 3.96±0.27* | 3.17±0.25 $^{NS}$ | 8.38±0.78** | 8.21±0.62** | 24.26±1.70*** | 28.40±3.51*** | 4.92±0.14** |
| **Spring** | 3.35±0.27* | 3.26±0.27 $^{NS}$ | 8.82±0.82** | 6.96±0.57** | 18.98±1.55*** | 18.80±3.21*** | 3.69±0.12** |
| **Summer** | 3.29±0.25 * | 3.52±0.27 $^{NS}$ | 6.94±0.71** | 7.68±0.62** | 21.46±1.70*** | 13.98±3.51*** | 3.84±0.14** |

Note: NS: Not significant. *: Significant at P <0.05. **: Significant at P <0.01. ***: Significant at P <0.001.

**Table 3.** Relationship between the different cells in testis and season.

| Dependent variable (Y) | Regression equation (X=Season) | $R^2$ |
|---|---|---|
| **Sertoli cells** | $Y=0.051X^2-0.133X+5.628$ | 0.29* |
| **Spermatogonia** | $Y=0.409X^3-3.026X^2+6.758X+0.798$ | 0.17$^{ns}$ |
| **Primary spermatocytes** | $Y=0.825X^3 - 5.553X^2 +10.456\ X +7.714$ | 0.20* |
| **Secondary spermatocytes** | $Y= 0.900X^2-4.378\ X+16.568$ | 0.15* |
| **Round spermatids** | $Y= 2.238\ X^2 -11.165X +44.458$ | 0.15* |
| **Elongated spermatids** | $Y= -10.10X^3 +76.767\ X^2 - 166.500X+129.133$ | 0.33** |
| **Leydig cells** | $Y= 0.141X^3- 0.384\ X^2 - 0.913X+6.081$ | 0.69*** |

$^{NS}$: Not significant. *: Significant at P <0.05. **: Significant at P <0.01. ***: Significant at P <0.001.

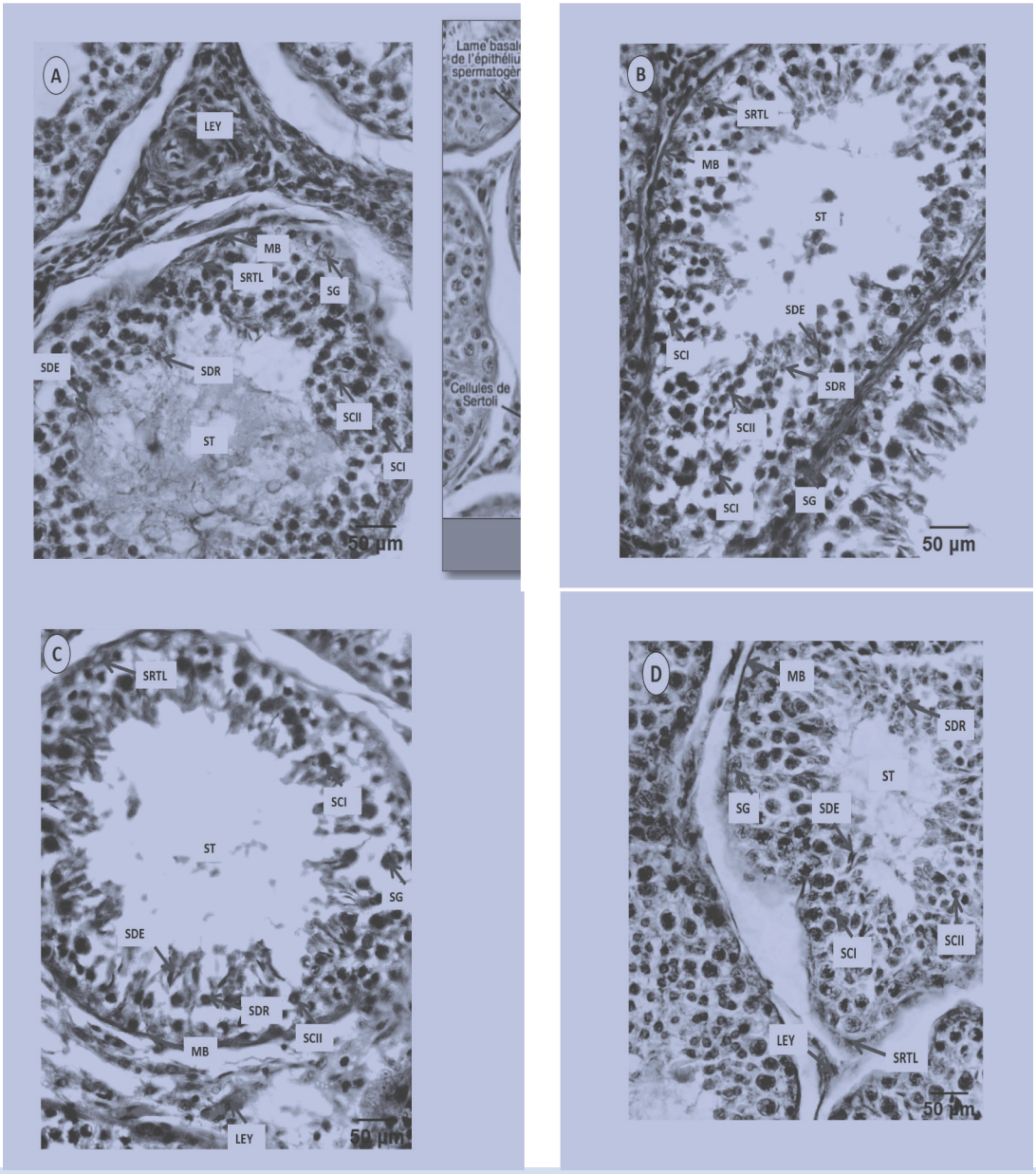


**Figure 1.** Morphological evaluations of testicular tissues during autumn (A), winter (B), spring (C), and summer (D). Different structures were found: spermatogonia (SG), primary spermatocyte (SCI), secondary spermatocyte (SCII), round spermatid (SDR), elongated spermatid (SDE), Sertoli cell (SRTL), Leydig cells (LEY), seminiferous tubule (ST), basal membrane (MB) (Haematoxylin and Eosin 400X).

**Seasonal effect on morphometric testicular parameters**

The changes in total seminiferous tubule volume, total length and diameter of the seminiferous tubules, interstitium volume and seminiferous epithelial height expressed by seasons in local bulls are shown in Table 4.

More specifically, total seminiferous tubule volume was statistically significantly altered throughout seasons, reaching its maximum in autumn, when it constituted about 89.50 % of the testicular parenchyma ($p<0.01$).

Interstitium volume differed significantly between breeding and non-breeding seasons ($p<0.01$). Its lowest value was observed in autumn (12.54±0.94 %) and winter (15.22±0.89), whereas its highest was in summer (20.34±0.86 %).

The diameter of seminiferous tubules ($p<0.01$) increased significantly in autumn and winter these increase in diameter of seminiferous tubules a companied by an increase in height of seminiferous tubules in the same seasons ($p<0.05$). However, the mean

seminiferous tubule length per testis was not significantly (p>0.05) affect by season.

The relationship of season with morphometric testicular parameters of bulls is presented in Table 5. The increase of seminiferous tubule volume in bulls is described by the third-order polynomial (cubic) relation with the season ($Y=1.275X^3-6.742X^2+7.835X+82.406$). The coefficient of correlation (R) between these parameters was 0.532 (P<0.001).

The mean germinal epithelium height of testes evolves according to cubic relation (Y= 2.584X3-11.193X2+4.154X +86.611) with the season. The estimated regression equation showed high coefficient of determination (R = 0.720, p<0.001).

**Seasonal effect on efficiency of the spermatogenesis**

Data corresponding to values obtained for the efficiency of the spermatogenesis are presented in Table 6.

The results indicated that the coefficient of efficiency of spermatogonial mitosis and meiotic index were significantly (p <0.05) higher in the autumn season than in summer season. The change of the efficiency of spermatogonial mitosis and meiotic index followed the same seasonal trend that was seen in germ cell numbers in a testis as listed in Table 2.

The overall spermatogenic yields (ratio between round spermatids and spermatogonia) that was significantly higher (p < 0.01) in the autumn compared to the summer. This ratio recorded in the present study in bulls ranged from 7.81±0.55 in autumn to 5.86±0.58 in summer.

The ratio of elongated spermatids to round spermatids (post-meiotic germ loss) was different (p < 0.05), so that the highest and the smallest values occurred in autumn and summer, respectively (Table 6). Again, the efficiency of Sertoli cells (ratio of round spermatid and Sertoli cell nuclei) showed a significant (P < 0.01) increase between seasons.

**Table 4.** Effect of season on morphometrical values (Mean ± SE) of seminiferous tubules of testes in male bulls.

| Season | Paramaters | | | | |
|---|---|---|---|---|---|
| | Total seminiferous tubule volume per testis (%) | Seminiferous tubuli diameter (µm) | Interstitium volume (%) | Length of seminiferous tubules per testicle (m) | Germinal epithelium height of testes (µm) |
| Autumn | 89.50±1.88** | 221.97±7.83** | 12.54±0.94** | 3374.32±251.81ns | 87.46±0.94* |
| Winter | 82.15±1.78** | 215.89±6.48** | 15.22±0.89** | 3215.31±238.89ns | 84.77±0.89* |
| Spring | 70.81±1.78** | 201.96±6.48** | 18.69±0.98** | 3247.79±238.89ns | 81.30±0.89* |
| Summer | 68.09±1.63** | 199.45±5.91** | 20.34±0.86** | 3218.37±218.07ns | 79.65±0.82* |

Note: NS: *: Significant at P <0.05. **: Significant at P <0.01. ***: Significant at P <0.001.

**Table 5.** Relationship between the morphometric data and season.

| Dependent variable (Y) | Regression equation (X= Season) | $R^2$ |
|---|---|---|
| Total seminiferous tubule volume per testis (%) | $Y=1.275X^3- 6.742X^2+7.835X +82.406$ | 0.532*** |
| Seminiferous tubule diameter (µm) | $Y= 9.185X^2 - 44.412X +251.855$ | 0.559** |
| Length of seminiferous tubules per testicle (m) | $Y=107.880X^3- 728.235X^2+1462.022X +2373.649$ | $0.031^{NS}$ |
| Interstitium volume (%) | $Y= -1.275X^3 + 6.742X^2 -7.835X+17.594$ | 0.540*** |
| Germinal epithelium height of testes (µm) | $Y= 2.584X^3- 11.193X^2+4.154X +86.611$ | 0.720*** |

NS: Not significant. *: Significant at P <0.05. **: Significant at P <0.01. ***: Significant at P <0.001.

**Table 6.** Effect of season on efficiency of the spermatogenesis (Mean ± SE) in bulls (mean ± SEM).

| Season | Parameters | | | | |
|---|---|---|---|---|---|
| | Sertoli cell efficiency | Post-meiotic germ loss | Overall Spermatogenesis Yield | Meiotic index | Efficiency Coefficient |
| Autumn | 2.90±0.23* | 2.83±0.18* | 7.81±0.55** | 0.93±0.12* | 7.59±0.53** |
| Winter | 2.55±0.23* | 2.72±0.15* | 6.43±0.51** | 0.85±0.18* | 6.26±0.53** |
| Spring | 2.37±0.21* | 2.70±0.17* | 6.54±0.55** | 0.65±0.11* | 6.17±0.48** |
| Summer | 2.27±0.24* | 2.43±0.17* | 5.86±0.58** | 0.43±0.14* | 5.66±0.55* |

*: Significant at P <0.05. **: Significant at P <0.01. ***: Significant at P <0.001.

**Table 7.** Relationship between the efficiency of the spermatogenesis and season.

| Dependent variable (Y) | Regression equation (X= Season) | $R^2$ |
|---|---|---|
| **Efficiency Coefficient** | $Y= 2.803X^{-0.190}$ | 0.296* |
| **Meiotic index** | $Y= 0.030X^3+0.248X^2-0.607X+3.093$ | 0.359* |
| **Overall Spermatogenesis Yield** | $Y=7.414\ X^{-0.191}$ | 0.225* |
| **Post-meiotic germ loss** | $Y= 0.237X^3 - 1.072X^2+1.977$ | 0.250* |
| **Sertoli cell efficiency** | Y= 1.322x+7.481 | 0.223* |

*: Significant at P <0.05.

The relationship of season to efficiency of the spermatogenesis in bulls is presented in Table 6. The estimated coefficient of correlation between the efficiency of the spermatogenesis (y) and the season (X) showed a positive and significant ($p<0.05$) correlation ($r=0.296$) between the efficiency coefficient and season characterized by the equation $Y= 2.803X^{-0.190}$. Meiotic index was also positively and significantly ($p<0.05$), correlated ($r=0.359$) to season with a regression equation $Y= 0.030X^3+0.248X^2-0.607X+3.093$. Similar significant correlation relationship with the season (X) existed between spermatogenesis yield $Y=7.414\ X^{-0.191}$, $r=0.225$ ($p<0.05$); post meiotic index $Y= 0.237X^3 - 1.072X^2+1.977$, $r=0.250$ ($p<0.05$). The efficiency of Sertoli cells showed a linear relationship with the season, characterized by the equation $Y = 1.322x + 7.481$, where $r = 0.223$.

## Correlation

The correlation coefficients between histological measurements of the Ouled Djellal rams' testes are presented in Table 4.

A significant positive correlation was seen between the total number of Sertoli cells and spermatogonia ($r = 0.998$; $P = 0.01$). The analysis of the correlation coefficients shows negative correlations between VI and other parameters such as VTS, GEH, LEY and SRTL, ranged between -0.349 and -0.989. However, positive correlation between STD and GEH (0.509; $p<0.01$) was observed. A positive correlation was found between total seminiferous tubule volume and number of Sertoli cells and a coefficient of correlation of 0.549 ($P<0.01$). In addition, there were a high positive correlations between Sertoli cells and diameter of seminiferous tubules ($r=0.531$; $p<0.01$).

## DISCUSSION

The quantitative estimation of the germ cells is an important precondition to characterize the state of as well as the changes in this cell population and their regulation. Such a quantitative determination of the germ cells in bulls was performed in this study; to our knowledge, this study is the first to estimate the spermatogenic activity and to perform a detailed histo-morphometric evaluation of the testis structure

**Table 8: Coefficients of correlation between histological characteristics of bulls' testes.**

| r | SRTL | SG | SCI | SCII | SDR | SDE | STD | VTS | GEH | LTS | LEY | VI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SRTL | 1 | | | | | | | | | | | |
| SG | 0.998** | 1 | | | | | | | | | | |
| SCI | 0.400** | 0.387* | 1 | | | | | | | | | |
| SCII | 0.515** | 0.496** | 0.475** | 1 | | | | | | | | |
| SDR | 0.392* | 0.253 | 0.689*** | 0.327* | 1 | | | | | | | |
| SDA | 0.498** | 0.089 | 0.364* | 0.285 | 0.422** | 1 | | | | | | |
| STD | 0.531** | 0.321* | 0.412** | 0.670*** | 0.590** | 0.524** | 1 | | | | | |
| VTS | 0.549** | 0.343* | 0.336* | 0.495** | 0.265 | 0.414** | .228 | 1 | | | | |
| GEH | 0.012 | 0.017 | 0.073 | 0.231 | 0.147 | 0.464** | 0.509** | 0.638** | 1 | | | |
| LTS | 0.424** | 0.403** | 0.567** | 0.751*** | 0.553** | 0.409** | -0.402** | 0.246 | 0.097 | 1 | | |
| LEY | 0.044 | 0.047 | 0.115 | 0.231 | 0.162 | 0.566** | 0.602** | 0.662** | 0.778*** | 0.032 | 1 | |
| VI | -0.349* | -0.343* | -0.336* | -0.495** | -0.265 | -0.414** | -0.228 | -0.98*** | -0.638** | -0.246 | -0.662** | 1 |

Notes: SRTL: Sertoli cells; SG: Spermatogonia; SCI: Primary spermatocytes; SCII: Secondary spermatocytes; SDR: Round spermatids; SDE: Elongated spermatids; STD: Diameter of seminiferous tubules (µm); VTS: Total seminiferous tubule volume (%); GEH: Germinal epithelium height of testes (µm); LTS: Length of seminiferous tubules (m); LEY: Leydig cells; VI: Interstitium volume (%).

*: Significant at P <0.05. **: Significant at P <0.01. ***: Significant at P <0.001.

over 12 consecutive months in the local bulls in a semi-arid environment in Algeria.

In this study, we detected a definite seasonal change in spermatogenic activity with full spermatogenesis, including from primary spermatocytes to elongated spermatids in the seminiferous tubules in autumn in a local breed bull. In contrast, the division of germ cells decreased in summer in this animal. The typical quantitative fluctuation of the different testicular cells in this study corresponds to that described in several, mostly histological studies of seasonal changes in bulls indigenous in Nigeria (Kumi-Diaka & Zemjanis, 1978). The degree to which spermatogenesis is not completed and testicular regression occurs varies between species. In non-seasonal breeders such as the domestic bovine bull, these changes are small and have no great effect on reproductive potential (Lincoln, 1981). The efficiency of spermatogenesis is directly associated with the ability of germ cells to multiply and differentiate. Several physical, chemical, and endocrine factors affect the testicular structure, which can induce mainly the degeneration of the seminiferous epithelium (Foster, 2016).

The present study showed that the total number of Sertoli cells was significantly higher in the breeding season than in the non-breeding season. This was partly similar to the findings of (Johnson & Thompson, 1987) in stallions who reported that Sertoli cell population can affected by season. However, Mesher (2010) reported that the seminiferous epithelium consists of two types of cells; non-dividing supporting (sustentacular) cells (Sertoli cells) and proliferative cells of the spermatogenic lineage. Therefore, changes in the Sertoli cell number not only affect the magnitude of sperm production, but also sperm function (Holsberger & Cooke, 2005). Sertoli cells are crucial for various processes, with their most pronounced effect being on the elongation of spermatids and the formation of sperm tails (Griswold, 2005). The Sertoli cell has become increasingly recognized for important contributions to the support of spermatogenesis, and recent studies with the human (Johnson et al., 1984) and young beef bull have revealed large, positive correlations between the total number of Sertoli cells and daily sperm production (Berndtson et al., 1987). The number of Sertoli cells is important because it is related to the number of spermatogonia and spermatids in rams (Hochereau-de Reviers et al., 1985), bulls (Berndtson et al., 1987), rats (Orth et al., 1988), humans (Johnson et al., 1984), and horses (Johnson et al., 1986). This study supports their findings. A positive relationship was found between the number of Sertoli cells and total seminiferous tubule volume, with a correlation coefficient of 0.549 ($p < 0.01$). Thus, it was concluded that the seminiferous tubules containing a larger total number of Sertoli cells were bulkier than the seminiferous tubules with fewer Sertoli cells. Larger testes would contain a greater volume of seminiferous tubules, characterized by either greater total tubular length and(or) diameter; the total physical area over which the blood testis barrier was required would also be greater in such testes (Berndtson et al., 1987).

It is known that the organization of interstitial tissue associated with the lymphatic system changes over the reproductive cycle, including the number of Leydig cell population (Abd-elaziz et al., 2012). Leydig cells of the bull have an abundant smooth endoplasmic reticulum, Golgi complex, mitochondria with tubular cristae, and lipid droplets. Our observations revealed that the number of Leydig cells during autumn and winter season are very abundant. Furthermore, several studies has been reported that an increase in Leydig cells number in the seasonal species, such as Iraqi Bull Buffaloes (Ibrahim et al., 2013), camels (Zayed et al., 1995)and hamsters (Hikim & Swerdloff, 1999) during the breeding season. Seasonal changes in Leydig cell number are responsible for seasonal changes in the volume of smooth endoplasmic reticulum in Leydig cells and in intratesticular testosterone content in stallions (Johnson & Thompson, 1987). In the roe deer, (Hombach-Klonisch et al., 2004) proposed a model in which the total number of interstitial cells remains constant, but a number of Leydig cells dedifferentiate during testis regression and then differentiate again during the recrudescence period.

Our findings revealed that the increase in the total seminiferous tubule volume during autumn corresponded with a decreased of the interstitium volume in the same season. The current results were in agreement with those reported by Ibrahim et al. (2013),in Iraqi Bull Buffaloes and (Arrighi et al., 2010) in Buffalo Bulls. An increase in testosterone, FSH and LH hormones caused an increase in seminiferous tubule volume (Al-Sahaf & Ibrahim, 2012), epithelial thickness and a decreased in intertubular spaces (O'shaughnessy & Sheffield, 1990). This might be attributed to cellular proliferation, secretion of tubular fluid, and appearance of the tubular lumen. In general,

there is 70 to 90% of the seminiferous tubule volume in mammals (França and Russel, 1998) and the observed seminiferous tubules volume (68.09±1.63 % to 89.50±1.88%) is situated around the mean range observed for mammals.

The findings in the present study show that the height of the seminiferous tubules was significant during autumn. It has been accepted that species whose testes have a high proportion of seminiferous epithelium produce more sperm per unit mass (Hess and França, 2005). This superiority may have resulted not only from the epithelium density but also a combination of higher Sertoli cell support for germ cells, a greater number of Sertoli cells per gram of testis, as well as higher seminiferous tubule volume density (Okpe & Ezeasor, 2016). The values obtained for the height of the seminiferous tubule in this study are slightly less than those reported for goats by Leal et al. (2004), but higher than those reported by Da Silva et al. (2015) in Bos indicus (Nellore) bulls.

Seasonal changes in the seminiferous tubular diameter, an indicator of the functional state of the spermatogenic testicular parenchyma, were similar to the changes in height of seminiferous tubules, i.e. a peak in autumn and winter followed by a sharp decline during spring and summer. These results are in agreement with Ibrahim et al. (2013) in Iraqi Bull Buffaloes and Arrighi et al. (2010) in Buffalo Bulls. This may indicate a high level of maturation and fluid production by the Sertoli cells of the testes, because seminiferous tubule diameter has been positively correlated with testicular maturation and spermatogenesis (Meisami et al., 1994). However, França and Russel (1998) reported that the non-seasonal animals would not exhibit significant variation in tubular diameter with season after sexual maturity. Germ cell proliferation is the main factor responsible for the seminiferous tubule growth in diameter (Iczkowski et al., 1991).

Our results indicated that a significant correlation between STD and Sertoli cells number were present, suggesting that the seminiferous tubules containing a larger total number of Sertoli cells were larger than the seminiferous tubules with fewer Sertoli cells. Obviously, larger testes would contain a greater volume of seminiferous tubules, characterized by either greater total tubular diameter; the total physical area over which the blood-testis barrier was required would also be greater in such testes (Berndtson et al., 1987). Smaller testicular volumes, together with minor values of tubular diameters might indicate a decrease in spermatogenesis (Al-Sahaf & Ibrahim, 2012).

The total length of the seminiferous tubules corresponds to the total tubular volume divided by the cross-sectional area of the tubule. Therefore, animals with a larger volume percent of seminiferous tubule would have longer seminiferous tubules (Orlu & Egbunike, 2010). This deduction disagrees with our finding, as the length of seminiferous tubules did not decline between bulls subjected to a short photoperiod and local breed bulls exposed to a long photoperiod. Currently, no studies are available on the modifications of seminiferous tubule length with season in bulls, as there are for rams or mice. This increase in tubule length with season may be a characteristic of this species. The mean value obtained for length of the seminiferous tubule per testicular in this study was 3 263,855 ±236,915 m which was a lower than that observed by Andreussi et al. (2014) in Nelore cattle (5639.6 ± 891.7 m) and Gir bulls (4564.8 ± 325.2 m).

The literature suggests that stage frequencies grouped in premeiotic and postmeiotic phases of spermatogenesis might be phylogenetically determined among members of the same mammalian family (Neves et al., 2002). Using the efficiency coefficient of spermatogonial mitoses (primary spermatocytes / spermatogonia), it is possible to quantify the losses (apoptosis) that occur during the spermatogonial phase. Spontaneous germ cell apoptosis is common in normal testes and is an important factor involved in regulating germ cell development and sperm output (Hikim & Swerdloff, 1999). In the local breed bull, there was a seasonal variation in the efficiency of spermatogonial mitosis: this ratio was very low during summer, and increased during the sexual season ($P < 0.05$). Therefore, there is evidence that the photoperiod regulates germ cell apoptosis in this specie. However, further work on this aspect is required as no available literature could be traced to compare with the present findings. Overall, germ cell apoptosis results in the loss of up to 75% of the potential number of mature spermatozoa that can be produced by one differentiated type A1 spermatogonia (Hikim & Swerdloff, 1999). The cell loss that occurs naturally in the spermatogenic process is probably due to density dependent degeneration, where apoptosis is the homeostatic mechanism used to limit germ cells to a number that can be supported by available Sertoli cells (De Rooij & Janssen, 1987). Apoptosis increases when there are lesser androgen concentrations in the

hormonal milieu of the testes (Lee et al., 1999). It is common for losses to occur during spermatogenesis, mainly due to hormonal fluctuations in serum gonadotropin levels, with FSH secretion being the most important factor (Young, 2001).

Again, the maximal percentages of round spermatid and spermayocytes in the present work were found during the breeding season (autumn), indicating intensive meiotic activity. During summer, testicular tissue exhibited only minimal meiotic activity. Meiotic divisions account for a 13% loss of potential production in mice, 2% in Sprague-Dawley rats, 27% in Sherman rats, 25% in rabbits, and 6% to 15% in stallions, depending on season. In humans, a 30% to 40% loss from germ cell degeneration occurs during meiotic divisions. (Johnson, 1985). The germ cell loss (apoptosis) occurs mainly during meiosis, through the elimination of germ cells that are defective or carry DNA mutations, and during the spermatogonial phase in a process known as cell-density regulation (Weinbauer et al., 1998; Young, 2001). Testosterone also plays an essential role in preventing apoptotic cell death in androgen-dependent tissues (Hikim & Swerdloff, 1999). In the absence of testosterone, the Sertoli cell barrier is compromised, germ cells do not complete meiosis, immature germ cells are prematurely displaced from Sertoli cells, and mature sperm are not released (Walker & Cheng, 2005). The interruption of any of these testosterone-dependent steps results in failure of spermatogenesis, causing infertility (Kerr et al., 1993). The level of apoptosis during meiosis is inversely related to both the proliferation and the testosterone concentration in roe deer testis during the annual cycle (Blottner et al., 1995).

The general yield of spermatogenesis (round spermatid/spermatogonia) is a measure of the overall efficiency of the spermatogenic process. The present study indicated that the period of highest yield of spermatogenesis seen in local bull breed corresponds to the natural breeding season (autumn). However, no such reports were found available in the literature to compare the present findings. High levels of FSH and testosterone are necessary for the maintenance of spermatogenesis (Okpe & Ezeasor, 2016).

The expected ratio of elongated spermatids to round spermatids is theoretically 1.0 because spermiogenesis does not involve further cellular divisions (Luetjens et al., 2005). It was observed that this ratio was only 0.43±0.14 in the summer and was significantly lower compared to the autumn (0.93±0.12). In contrast, Pintus et al. (2015) indicated that the ES/RS ratio is significantly lower during the breeding season in red deer. The lowest ratio of elongated spermatids to round spermatids suggests that a greater selective spermiogenesis during the breeding season may be useful to minimize low-quality sperm cells in the ejaculate (Belkhiri et al., 2021). During mammalian spermatogenesis, more than half of the differentiating spermatogenic cells undergo apoptosis before maturing into spermatozoa and are phagocytosed by Sertoli cells (Nakanishi & Shiratsuchi, 2004). It has been suggested that the phagocytic clearance of apoptotic spermatogenic cells is necessary for the progress of spermatogenesis and efficient sperm production. Additionally, it may provide energy substrates to the Sertoli cells (Snook, 2005).

The efficiency of Sertoli cells changed due to the photoperiod in this study ($p < 0.01$). However, further work on this aspect is required as no available literature could be found to compare with the present findings. According to França and Russell (1998), the round spermatids/Sertoli cells ratio is the most important index for estimating sperm efficiency in animals. When the relationship between these factors is strong, sperm production is also high. The number of Sertoli cells per testicle is the main factor determining sperm production and testicular size (De Franca et al., 1995).

## CONCLUSION

It can be concluded that the increase observed in the morphometric parameters and stereological structure of the testis during autumn may indicate local breed bull changes in reproductive potential about changing seasons. In addition, our results of germ cell degeneration occur throughout spermatogenesis but is greater during meiosis and can vary with season.

In order to select the best males during or outside the mating season, it is useful to have specific and objective information about histomorphological and spermatogenic activity of the local bull testis. Moreover function of the testis is a good tool for the assessment of the reproductive state and potential sperm production capacity in the local bull.

## CONFLICT OF INTERESTS

The author declares that there is no conflict of interest regarding the publication of this paper.

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