

## **Study of testis volume density and relative weight of tissue components of seminiferous tubules in different ages for local bulls in Sulaimani province**

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### **Abstract:**

The present study is carried out to investigate the reproductive activity of mature Local bull and effect of age's changes on the testes activity by using Testis Volume Density (VD%) and Relative Weight (RW, g/kg body weight) of Tissue components of seminiferous tubules. Seventy-two testes of the healthy bull (age: 1-4year) were taken from the Sulaimani slaughterhouse in November 2016. The length and width of the right testis were measured after slaughtering, and testes weights were also taken, then the epididymis carefully dissected. The diameters of testicular seminiferous tubules were also measured. The present study demonstrated a significant increased ( $p<0.05$ ) in weight ( $221.5\pm 6.4\text{gm}$ ), length ( $15.00\pm 0.57\text{cm}$ ), width ( $6.33\pm 0.33\text{cm}$ ) and testes circumference ( $19.00\pm 0.01\text{cm}$ ) in age 4<sup>th</sup>-year-old compared to other ages. The diameter of epididymis ( $p<0.05$ ) increased significantly in 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> age old for total length and head of epididymis (Caput) compared to 1<sup>st</sup>-year. The diameter of the seminiferous tubules demonstrated higher ( $p<0.05$ ) significant in thickness at 4th ages. The increase in spermatogenesis process leads to increase the diameter and thickness of seminiferous. The results showed that the degenerative changes in germ cells are associated with histomorphometric variations, including the density of the structures that comprise the testicular parenchyma, and are they representative in bulls with poor semen quality at an early age. In addition, the unsatisfactory bulls for reproduction showed reduced meiotic potential at 1<sup>st</sup>-year compared to that of satisfactory reproductive bulls, especially at 3 and 4 years old in Sulaimani province.

**Key words: Local bulls, Testes, Volume Density, Relative Weight, Seminiferous Diameter.**

## دراسة الكثافة الحجمية للخصية و أوزان المكونات النسيجية النسبية للنبيبات المنوية في أعمار مختلفة للثيران المحلية في محافظة السليمانية

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المستخلص :

الخصية و ذلك باستخدام مقاييس الكثافة الحجمية للخصية (Volume Density%) والوزن النسبي (Relative Weight) غم. كغم<sup>-1</sup> وزن الجسم) لمكونات أنسجة النبيبات المنوية . أخذت (72) أثنان وسبعون خصية من ثيران ناضجة جنسياً ذات صحة جيدة (بعمر: 1-4 سنة) من مسلخ السليمانية خلال شهر نوفمبر 2016. حيث تم قياس كل من طول وعرض الخصية اليمنى ، أوزان الخصيتين و طول أجزاء البربخ بعناية. و قيست أقطار النبيبات المنوية في مقاطع نسيجية لعينات الخصية أظهرت نتائج الدراسة زيادة معنوية ( $p < 0.05$ ) في وزن الخصيتين ( $6.4 \pm 221.5$  غم) و طول الخصيتين ( $0.57 \pm 15.00$  سم) عرض الخصيتين ( $0.33 \pm 6.33$  سم) و محيط الخصيتين ( $0.01 \pm 19.00$  سم) للثيران التي كانت في عمر أربع سنوات مقارنة بالأعمار الأخرى و كذلك سجلت ارتفاع معنوي ( $p < 0.05$ ) في طول أجزاء البربخ عند الأعمار 2 و 3 و 4 سنوات و خصوصاً لكل من الطول الكلي للبربخ و رأس البربخ (Caput) وذلك مقارنة بالسنة الأولى. و أظهرت نتائج قطر النبيبات المنوية أعلى سمك ( $p < 0.05$ ) في عمر أربع سنوات إذ دلت النتائج أن الزيادة في قطر و سمك الخلايا الجرثومية لها تأثير معنوي في عملية تكوين النطف و أن التغيرات الغير نشطة في الخلايا الجرثومية مرتبطة بالتغيرات النسيجية بما في ذلك كثافة الأنسجة داخل النبيبات المنوية في الخصيتين والتي كانت دليل على رداءة نوعية السائل المنوي في أعمار مبكرة للثيران و بذلك نستنتج أن الخلايا المكونة للنطف تكون قليلة الإنتاج في السنة الأولى مقارنةً مع عمر 3 و 4 سنوات للثيران المحلية في محافظة السليمانية.

الكلمات المفتاحية : ثيران محلية ، الخصيتين ، الكثافة الحجمية ، النسبة الوزنية ، قطر النبيبات المنوية.

### Introduction:

In many mammalian species, libido and testicular function of adult male, which are thought to be mediated through alterations in gonadotrophin secretion from the pituitary gland. The testes development and function in fetuses are known to be critically depends on circulating fetal gonadotrophin concentration. The weight of the fetal testes and the length of scrotum increased steadily with an increasing in the fetal Crown Rump Length (CRL). Histological sections of testes showed the fibroblast

layer density and connective tissue capsules as well as the appearance of seminiferous tubules containing a few spermatogonia cells and small Leydig cells at early stages. With an increasing in Crown Rump Length (CRL), the seminiferous tubules filled with different types of spermatogonia cells, spermatocytes cells, increasing in number and size of Sertoli and Leydig cells (5). And the testis is capable of producing various androgen in fetus and then after birth in seminiferous sections or in the epididymis. The first appearance of spermatozoa in testes at about 34 days after birth in sheep (24) and at 16 weeks old kid's sperms appeared in semen fluid (19). The seminiferous cords that become tubules occupy 44% of the testicular parenchyma at 3 months and 81% at 8 months (10) The measurements of testis and live weight, were positively and significantly correlated with each other, and the strong correlation between body weight and testicular measurements were recorded in growing lambs (25). An initial rise in follicle stimulating hormone (FSH) from age 3 to 5 months promoted a proliferation in Sertoli cells, a lengthening of the seminiferous tubules, and an increase in tubule diameter. At the same time, there is a rise in interstitial cell-stimulating hormone secretion caused increasing of testosterone production by the Leydig cells. FSH and interstitial cell-stimulating hormone remain low from 5 to 8 months of age, and rising again with the onset of puberty (10). In the yearling bull, poor health or environmental conditions that interfere with the growth of calves during this critical period are speculated to delay puberty and reduce testis size. The testis growth is very rapid and almost linear from 7 to 12 months of age and declines after 12 months. Between age 7 and 12 months, the scrotal circumference increases at a rate of 0.06–0.07 cm/day (9; 12). Puberty varies from 231 to 371 days, and the pubertal bulls produce a high number of sperm with a variety of abnormalities (2; 20; 30). Studies at the Western College of Veterinary Medicine (WCVM) indicate that approximately 33% and 60% of beef bulls produce satisfactory quality semen at 12 and 14 months of age, respectively, and most bulls will have satisfactory semen quality and at 16 months of age are considered mature (6; 11). Bulls with a high probability of having large testes at age 1 year may be selected as early as the time of weaning; however, culling due to small testes at weaning is not a safe practice since a large proportion of bulls with less than average size testis at weaning will have adequate testis size at 1 year. This study aimed to evaluate useful parameters to determine the relationship between gonadic physiology and reproductive variation between the four ages (1, 2, 3 and 4) years. A morpho- and histometric approach was used, as methods allow for objective and quantitative evaluations of spermatogenesis, such as the gonadosomatic index, volumetric proportion of the testicular parenchyma components, tubule diameter and cross-sectional tubular area, height of the seminiferous epithelial and tubule length in local bulls.

## Materials and Methods:

Seventy-two (72) testes of thirty-six healthy mature Kurdi bulls, aged 1- 4 years, (18 testis.age<sup>-1</sup>) were obtained from the Sulaimani slaughterhouse in November 2016, 3 visits/week. After slaughtering the testes were collected and placed in a plastic box which contained ice until transporting within 2 h to the laboratory of college of Agricultural Sciences/Animal Science department, the tissue which surrounds the testes and the epididymis was removed by scissors and scalpel. Testes weight were taken by using digital electrical balance (Sartorius, Germany) and length and width were measured by the (Vernier caliper). Histological sections were prepared according to Luna (21). The diameters of the testicular seminiferous tubules were measured in 5 cross-sections per sample. Diameters of seminiferous tubules were calculating by measuring the horizontal and vertical diameters of the 5 tubules which selected randomly for each sample by using (OPTIC Educator) microscope and the lens was (400X). When match with stage micrometer by different levels, we calculating these diameters and the height of principal cells was magnification the means of measured from the basement to the apical membrane in cross section of 5 tubules (7) (Figure-1). Visopan Screen Microscope (VSM) was used to calculate Volume Density (VD%) and the relative weight (RW, gm.kg<sup>-1</sup> body weight) of testicular parameters following Weible (29) procedure. Weible Grid Transparency (WGT) comprising of 228 points were imposed on the screen of Visopan microscope (400X), to calculate the number of points that hit a particular component. VD% of any component was calculated by dividing the number of points of that component, on the total points in the reference area (228points). The RW of each component was calculated by multiplying VD % by the relative testicular weight (TW\BW). In the somniferous tubule, spermatogonia, spermatocyte, spermatid ,sperm total spermatogenic cells TSPERGE, basement membrane, Sertoli cells, Lumen and tubule vacuoles were considered. Leydig and Myoid cells, interstitial spaces and blood vessels were also examined in the interstitial. By using the same screen microscope, tubules and lumen diameter and germinal epithelial cell high were calculated by examining about round 5 tubules.

Dat were analyzed statistically by a completely randomized design in one-way ANOVA. Group differences were determined using Duncan Test at ( $p \leq 0.05$ ) (13; 27). In the statistical analyzed, XLStat-Pro 7.5.2 software was used.

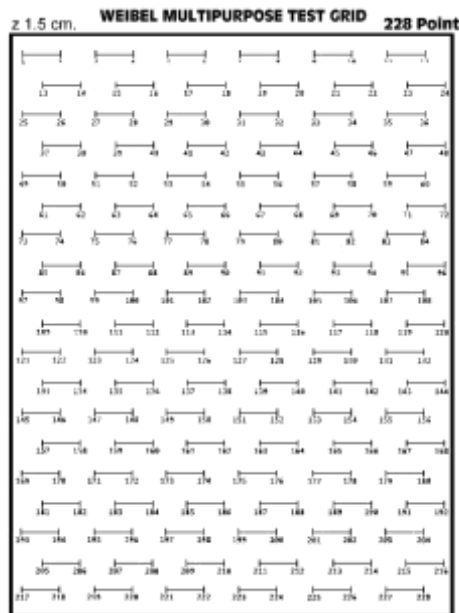


Figure1: Weibel test Grid to measure testicular parenchyma components in 228 point.

### Results and Discussion:

In the present study, the results of the testicular measurement showed differences in correlation with ages, even if accompanied by remarkable variations between individuals. The results of testicular weight are demonstrated in Table (1). The table showed that testicular weight increased ( $p < 0.05$ ) significantly with age, 4<sup>th</sup>-year-old (221.5gm) compared to other ages (135.1, 153.6 and 154.5) for 1st, 2nd and 3rd years respectively. Testicular length, width, and circumference increased ( $p < 0.05$ ) significantly in 4th years (15.00cm, 6.33cm and 19.00cm, respectively) compared to other ages. The increment of testis's weight, length and width in the 4<sup>th</sup> years old indicated to an increase in the physiological activity of the testes in such age, an activity of seminiferous tubules and sperm production in the testes like the morphometric measures of testes in different seasons increased in mating seasons (late autumn-winter) and a decreased in non-mating seasons in bull (3; 14; 18). An initial rise in Follicle Stimulating Hormone (FSH) caused in a proliferation of Sertoli cells, a lengthening of the seminiferous tubules and an increase in tubule diameter. At the same time, the interstitial cell-stimulating hormone rises in secretion caused elevation of testosterone production by the Leydic cells (8) The weight of the testis was one of the markers of a possible alteration in androgen status; a decrease in testicular weight is most likely due to a decrease in the level of serum testosterone (26).

**Table 1: Comparison between different ages in the Live Body Weight and Testes parameters of Local bull (Mean±SE).**

Age (year)	LBW (kg)	Testes Parameters			
		Testes weight (gm)	Testes length (cm)	Testes width (cm)	Testes Circumference (cm)
1 <sup>st</sup>	288.1±8.0 <sup>d</sup>	135.1±1.9 <sup>c</sup>	11.00±0.57 <sup>b</sup>	5.33±0.33 <sup>b</sup>	15.66±0.33 <sup>b</sup>
2 <sup>nd</sup>	325.5±17.3 <sup>c</sup>	153.6±6.4 <sup>b</sup>	11.00±0.57 <sup>b</sup>	5.33±0.33 <sup>b</sup>	15.66±0.33 <sup>b</sup>
3 <sup>rd</sup>	391.5±5.0 <sup>b</sup>	154.5±5.6 <sup>b</sup>	12.66±0.88 <sup>b</sup>	4.33±0.33 <sup>b</sup>	14.00±1.52 <sup>b</sup>
4 <sup>th</sup>	502.8±13.9 <sup>a</sup>	221.5±6.4 <sup>a</sup>	15.00±0.57 <sup>a</sup>	6.33±0.33 <sup>a</sup>	19.00±0.01 <sup>a</sup>

- Small different letters indicated a significant difference between ages (P<0.05).

The results of Table (2) demonstrated that diameter of Epididymis (p<0.05) increased significantly in 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> age old of total length and head of Epididymis (Caput) compared to 1<sup>st</sup> year (21.33±0.23 cm ; 3.33±0.23 cm) And there is no significant differences (p>0.05) in the body (Corpus) and tail of Epididymis (Cauda) The results of Epididymis revealed an increase in the activity of the testes in moderate ages and this activity is regulated by the increases testosterone hormone levels in these ages (28).

**Table 2: Comparison between different ages in the Epididymis parameters of Local bull (Mean±SE).**

Age (year)	Epididymis Parameter (cm)			
	Total length	Head (Caput)	Body (Corpus)	Tail (Cauda)
1 <sup>st</sup>	21.33±0.23 <sup>b</sup>	3.33±0.23 <sup>b</sup>	8.33±0.13 <sup>a</sup>	8.38±0.31 <sup>a</sup>
2 <sup>nd</sup>	23.00±1.52 <sup>a</sup>	4.67±0.67 <sup>ab</sup>	8.60±0.17 <sup>a</sup>	9.77±1.44 <sup>a</sup>
3 <sup>rd</sup>	23.33±1.85 <sup>a</sup>	5.33±0.60 <sup>a</sup>	8.32±0.34 <sup>a</sup>	9.62±0.39 <sup>a</sup>
4 <sup>th</sup>	23.70±0.35 <sup>a</sup>	5.67±0.34 <sup>a</sup>	8.69±0.39 <sup>a</sup>	9.31±0.79 <sup>a</sup>

-Small different letters indicated a significant difference between ages (p<0.05).

The results of Table (3) demonstrated that diameter of seminiferous tubules (p<0.05)increased significantly in 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> age in Germ cell thickness and tubules Circumference of Local bull, which were (2.21±0.02mm × 10<sup>-1</sup>) at age 4<sup>th</sup>-years-old. These increase in diameter of seminiferous tubules a companied by an increase in thickness of seminiferous tubules in these ages (Fig: 1, 2, 3, 4).

**Table 3: Comparison between different ages in the Seminiferous diameters of Local bull (Mean±SE) .**

Age (year)	Seminiferous diameters (mm × 10 <sup>-1</sup> )			
	Tubules Diameter	Tubules lumen	Germ cell Thickness	Tubules Circumference
1 <sup>st</sup>	4.92±0.51 <sup>a</sup>	2.13±0.41 <sup>a</sup>	1.73±0.07 <sup>b</sup>	19.45±3.63 <sup>b</sup>
2 <sup>nd</sup>	6.05±0.27 <sup>a</sup>	1.77±0.26 <sup>a</sup>	1.49±0.06 <sup>c</sup>	26.62±2.85 <sup>a</sup>
3 <sup>rd</sup>	5.89±0.47 <sup>a</sup>	2.23±0.61 <sup>a</sup>	1.79±0.07 <sup>b</sup>	25.21±2.66 <sup>a</sup>
4 <sup>th</sup>	6.03±0.10 <sup>a</sup>	1.95±0.85 <sup>a</sup>	2.21±0.02 <sup>a</sup>	24.76±4.00 <sup>a</sup>

- Small different letters indicated a significant difference between ages (p<0.05).

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Comparison between the ages in the number of germinal epithelial cells (x10<sup>6</sup>) and volume density (%) of seminiferous parameters in local bulls and their respective P-values are summarized in Table (4) and (5). There was a statistically significant difference after age 2<sup>nd</sup> year for germinal epithelial number and volume density of spermatogonia, spermatide and sperm, but Sertoli cells significantly differences at the 1<sup>st</sup> and 4<sup>th</sup> years compared to 2<sup>nd</sup>-year. However, the interstitium cells were higher significantly (p<0.05) at 2<sup>nd</sup> years old of local bulls, which record (21.66±1.25 x 10<sup>6</sup>) and (9.50±0.55 %) for total interstitium number and volume density respectively. However, TSPERCEC significantly increased at 4<sup>th</sup> years (112.33±3.09 x10<sup>6</sup>; 49.26±1.35 %). The spermatogenesis efficiency is directly associated with the ability of germ cells to multiply and differentiate, while several physical, chemical, and endocrine factors affect the testicular structure, which can induce mainly the seminiferous epithelial degeneration (15). The results clearly show that changes in the organization of the testicular parenchyma affect the tubular density, particularly in the germinal epithelial and during meiotic differentiation. Transformations in tubular and interstitial structures of the testes are widely found in bulls with testicular degeneration; however, the primary etiology is not always recognized (23). Regardless of the cause, classic literature reports the occurrence of these conditions in epidemiological surves in slaughtered

**Table 4: Comparison between different ages in the number ( $\times 10^6$ ) of the different germinal epithelial cells of Local bull (Mean $\pm$ S.D.).**

Number of germinal epithelial cells ( $\times 10^6$ )	Age (year)			
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>
Spermatogonia	14.50 $\pm$ 1.14 <sup>c</sup>	23.33 $\pm$ 0.76 <sup>b</sup>	27.50 $\pm$ 0.42 <sup>a</sup>	26.16 $\pm$ 1.24 <sup>a</sup>
Spermatocyte	19.83 $\pm$ 0.65 <sup>c</sup>	25.00 $\pm$ 0.36 <sup>b</sup>	27.33 $\pm$ 0.71 <sup>a</sup>	27.83 $\pm$ 0.79 <sup>a</sup>
Spermatide	7.16 $\pm$ 0.60 <sup>d</sup>	14.83 $\pm$ 1.01 <sup>c</sup>	20.66 $\pm$ 1.05 <sup>b</sup>	26.83 $\pm$ 0.79 <sup>a</sup>
Sperm	13.33 $\pm$ 0.66 <sup>d</sup>	16.50 $\pm$ 0.42 <sup>c</sup>	23.33 $\pm$ 1.17 <sup>b</sup>	31.50 $\pm$ 1.02 <sup>a</sup>
Sertoli cells	20.83 $\pm$ 0.91 <sup>a</sup>	16.66 $\pm$ 0.49 <sup>b</sup>	19.00 $\pm$ 1.12 <sup>ab</sup>	21.83 $\pm$ 1.19 <sup>a</sup>
Basement membrane	21.33 $\pm$ 1.11 <sup>a</sup>	12.33 $\pm$ 0.49 <sup>c</sup>	13.66 $\pm$ 0.55 <sup>c</sup>	17.83 $\pm$ 0.70 <sup>b</sup>
Vacules	106.00 $\pm$ 2.29 <sup>a</sup>	86.66 $\pm$ 1.92 <sup>b</sup>	75.50 $\pm$ 1.33 <sup>c</sup>	56.33 $\pm$ 3.28 <sup>d</sup>
S.T. Lumen*	11.16 $\pm$ 0.91 <sup>a</sup>	11.00 $\pm$ 0.36 <sup>a</sup>	7.33 $\pm$ 0.66 <sup>b</sup>	4.66 $\pm$ 0.88 <sup>c</sup>
Leydic cells	4.50 $\pm$ 0.61 <sup>b</sup>	9.83 $\pm$ 0.60 <sup>a</sup>	5.33 $\pm$ 0.61 <sup>b</sup>	5.00 $\pm$ 0.73 <sup>b</sup>
Myiod cells	2.66 $\pm$ 0.42 <sup>b</sup>	4.66 $\pm$ 0.42 <sup>a</sup>	2.83 $\pm$ 0.47 <sup>b</sup>	3.66 $\pm$ 0.66 <sup>ab</sup>
Blood vessels	1.83 $\pm$ 0.30 <sup>ab</sup>	2.00 $\pm$ 0.25 <sup>a</sup>	1.00 $\pm$ 0.25 <sup>b</sup>	1.83 $\pm$ 0.30 <sup>ab</sup>
Interstitial space	4.83 $\pm$ 0.60 <sup>a</sup>	5.16 $\pm$ 0.54 <sup>a</sup>	4.50 $\pm$ 0.34 <sup>a</sup>	4.50 $\pm$ 0.42 <sup>a</sup>
TSPERGE* <sup>c</sup>	54.83 $\pm$ 1.10 <sup>d</sup>	79.66 $\pm$ 1.85 <sup>c</sup>	98.83 $\pm$ 1.77 <sup>b</sup>	112.33 $\pm$ 3.09 <sup>a</sup>
T.S.Tubules* <sup>a</sup>	214.16 $\pm$ 1.47 <sup>a</sup>	206.33 $\pm$ 1.25 <sup>b</sup>	214.33 $\pm$ 1.02 <sup>a</sup>	213.00 $\pm$ 1.71 <sup>a</sup>
T. Interstitium* <sup>b</sup>	13.83 $\pm$ 1.47 <sup>b</sup>	21.66 $\pm$ 1.25 <sup>a</sup>	13.66 $\pm$ 1.02 <sup>b</sup>	15.00 $\pm$ 1.71 <sup>b</sup>
ST:Int. ratio** <sup>d</sup>	0.25 $\pm$ 0.006 <sup>d</sup>	0.38 $\pm$ 0.009 <sup>c</sup>	0.46 $\pm$ 0.008 <sup>b</sup>	0.52 $\pm$ 0.014 <sup>a</sup>

- Small different letters indicated a significant difference between ages (P<0.05).

\* TSPERGE=Total Spermatogenic Cells, T.S.Tubules= Total Seminiferous Tubules, T. Interstitium = Total Interstitium.

\*\* ST:Int. ratio=Seminiferous Tubules : Total Interstitium.

**Table 5: Comparison between different ages in the Volume Density percent (VD%) oSeminiferous parameters of Local bull (Mean $\pm$ SE).**

Volume Density ( % )	Age (year)			
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>
Spermatogonia	6.36 $\pm$ 0.50 <sup>c</sup>	10.23 $\pm$ 0.30 <sup>b</sup>	11.47 $\pm$ 0.18 <sup>a</sup>	12.06 $\pm$ 0.54 <sup>a</sup>
Spermatocyte	8.69 $\pm$ 0.28 <sup>a</sup>	10.96 $\pm$ 0.16 <sup>a</sup>	11.98 $\pm$ 0.31 <sup>a</sup>	12.20 $\pm$ 0.34 <sup>a</sup>
Spermatide	3.14 $\pm$ 0.26 <sup>d</sup>	6.50 $\pm$ 0.44 <sup>c</sup>	9.06 $\pm$ 0.46 <sup>b</sup>	11.76 $\pm$ 0.34 <sup>a</sup>
Sperm	5.84 $\pm$ 0.29 <sup>d</sup>	7.23 $\pm$ 0.18 <sup>c</sup>	10.23 $\pm$ 0.51 <sup>b</sup>	13.81 $\pm$ 0.44 <sup>a</sup>
Sertoli cells	9.13 $\pm$ 0.39 <sup>a</sup>	7.31 $\pm$ 0.21 <sup>b</sup>	8.33 $\pm$ 0.49 <sup>ab</sup>	9.57 $\pm$ 0.52 <sup>a</sup>
Basement membrane	9.35 $\pm$ 0.48 <sup>a</sup>	5.40 $\pm$ 0.21 <sup>c</sup>	5.99 $\pm$ 0.24 <sup>c</sup>	7.82 $\pm$ 0.30 <sup>b</sup>
Vacules	46.49 $\pm$ 1.00 <sup>a</sup>	38.01 $\pm$ 0.84 <sup>b</sup>	33.11 $\pm$ 0.58 <sup>c</sup>	33.11 $\pm$ 1.44 <sup>d</sup>
S.T. Lumen* <sup>a</sup>	4.89 $\pm$ 0.39 <sup>a</sup>	4.82 $\pm$ 0.16 <sup>a</sup>	3.21 $\pm$ 0.29 <sup>b</sup>	2.04 $\pm$ 0.38 <sup>c</sup>
Leydic cells	1.97 $\pm$ 0.27 <sup>b</sup>	4.31 $\pm$ 0.26 <sup>a</sup>	2.33 $\pm$ 0.27 <sup>b</sup>	2.19 $\pm$ 0.32 <sup>b</sup>
Myiod cells	1.17 $\pm$ 0.18 <sup>b</sup>	2.04 $\pm$ 0.19 <sup>a</sup>	1.24 $\pm$ 0.20 <sup>b</sup>	1.60 $\pm$ 0.29 <sup>ab</sup>
Blood vessels	0.80 $\pm$ 0.13 <sup>ab</sup>	0.87 $\pm$ 0.11 <sup>a</sup>	0.43 $\pm$ 0.11 <sup>b</sup>	0.80 $\pm$ 0.11 <sup>ab</sup>



Interstitial space	2.12±0.26 <sup>a</sup>	2.26±0.23 <sup>a</sup>	1.97±0.15 <sup>a</sup>	1.97±0.18 <sup>a</sup>
TSPERGE* <sup>c</sup>	24.05±0.48 <sup>d</sup>	34.94±0.81 <sup>c</sup>	43.34±0.78 <sup>b</sup>	49.26±1.35 <sup>a</sup>
T.S.Tubules* <sup>a</sup>	93.93±0.64 <sup>a</sup>	90.49±0.55 <sup>b</sup>	94.00±0.44 <sup>a</sup>	93.421±0.75 <sup>a</sup>
T. interstium* <sup>b</sup>	6.06±0.64 <sup>b</sup>	9.50±0.55 <sup>a</sup>	5.99±0.44 <sup>b</sup>	6.57±0.75 <sup>b</sup>
ST:Int. ratio <sup>a</sup>	16.47±1.92 <sup>a</sup>	16.19±0.62 <sup>a</sup>	9.70±1.37 <sup>b</sup>	15.26±1.89 <sup>a</sup>

- Small different letters indicated a significant difference between ages (P<0.05).

\* TSPERGE\* = Total Spermatogenic Cells, T.S.Tubules = Total Seminiferous Tubules, T. Interstitium = Total Interstitium.

\*\* ST:Int. ratio = Seminiferous Tubules : Total Interstitium.

**Table 6:** Showed the comparison between ages 1, 2, 3, 4 years in the relative weight RW (gm.kg<sup>-1</sup> LBW) for seminiferous parameters of local bulls. During the rutting age, the RW was recorded the significant decreasing in spermatogonia, spermatocyte, spermatids and sperm (2.98±0.23, 4.09±0.20, 1.47±0.12 and 2.73±0.09 (gm.kg<sup>-1</sup>) respectively). But basement membrane, vacuoles, and seminiferous tubules lumen significantly difference at age 1 year. Therefore, TSPERGE\* significantly higher at age 4, 3 and 2 years respectively, compared to age 1 year old. Total Seminiferous tubules (T.S. Tubules) were higher significantly (44.18±1.05 gm.kg<sup>-1</sup> BW) at 1 year old, compared to age 3 years old which were (37.16±1.66 gm.kg<sup>-1</sup> BW). However, ST: Int. ratio showed significantly higher at 1-year-old (7.75±0.91 gm.kg<sup>-1</sup> BW) in comparison with 2<sup>nd</sup> years old (4.70±0.57 gm.kg<sup>-1</sup> BW) (Table 6).

**Table 6: Comparison between different ages in the Relative Weight (RW) Seminiferous parameters of Local bull (Mean±SE).**

Relative Weight (gm.kg <sup>-1</sup> LBW )	Age (year)			
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>
Spermatogonia	2.98±0.23 <sup>b</sup>	4.85±0.16 <sup>a</sup>	4.78±0.27 <sup>a</sup>	5.06±0.25 <sup>a</sup>
Spermatocyte	4.09±0.20 <sup>b</sup>	5.23±0.30 <sup>a</sup>	4.74±0.24 <sup>ab</sup>	5.38±0.18 <sup>a</sup>
Spermatide	1.47±0.12 <sup>c</sup>	3.08±0.20 <sup>b</sup>	3.56±0.18 <sup>b</sup>	5.18±0.15 <sup>a</sup>
Sperm	2.73±0.09 <sup>d</sup>	3.45±0.22 <sup>c</sup>	4.02±0.20 <sup>b</sup>	6.08±0.19 <sup>a</sup>
Sertoli cells	4.31±0.27 <sup>a</sup>	3.50±0.28 <sup>ab</sup>	3.30±0.25 <sup>b</sup>	4.24±0.32 <sup>a</sup>
Basement membrane	4.41±0.30 <sup>a</sup>	2.59±0.21 <sup>c</sup>	2.36±0.12 <sup>c</sup>	3.43±0.08 <sup>b</sup>
Vacuoles	21.85±0.56 <sup>a</sup>	18.30±1.66 <sup>b</sup>	13.09±0.65 <sup>c</sup>	11.00±1.01 <sup>c</sup>
S.T. Lumen*	2.29±0.17 <sup>a</sup>	2.32±0.21 <sup>a</sup>	1.28±0.15 <sup>b</sup>	0.90±0.17 <sup>b</sup>
Leydic cells	0.92±0.12 <sup>b</sup>	2.04±0.11 <sup>a</sup>	0.93±0.12 <sup>b</sup>	0.96±0.14 <sup>b</sup>
Myiod cells	0.54±0.08 <sup>b</sup>	0.95±0.04 <sup>a</sup>	0.50±0.09 <sup>b</sup>	0.69±0.11 <sup>ab</sup>
Blood vessels	0.37±0.06 <sup>a</sup>	0.41±0.05 <sup>a</sup>	0.16±0.04 <sup>b</sup>	0.35±0.05 <sup>a</sup>
Interstitial space	1.00±0.13 <sup>a</sup>	1.08±0.13 <sup>a</sup>	0.77±0.06 <sup>a</sup>	0.85±0.05 <sup>a</sup>
TSPERGE* <sup>c</sup>	11.30±0.25 <sup>c</sup>	16.62±0.77 <sup>b</sup>	17.11±0.71 <sup>b</sup>	21.71±0.67 <sup>a</sup>
T.S.Tubules* <sup>a</sup>	44.18±1.05 <sup>a</sup>	43.35±3.05 <sup>ab</sup>	37.16±1.66 <sup>b</sup>	41.31±1.72 <sup>ab</sup>
T. interstium* <sup>b</sup>	2.84±0.29 <sup>b</sup>	4.49±0.23 <sup>a</sup>	2.37±0.21 <sup>b</sup>	2.87±0.29 <sup>b</sup>

ST:Int. ratio**	7.75±0.91 <sup>a</sup>	4.70±0.57 <sup>b</sup>	6.36±0.51 <sup>ab</sup>	6.83±1.04 <sup>ab</sup>
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- Small different letters that indicated a significant difference between ages (P<0.05).

\* TSPERGECE=Total Spermatogenic Cells, T.S.Tubules= Total Seminiferous Tubules, T. Interstitium= Total Interstitium.

\*\* ST:Int. ratio = Seminiferous Tubules : Total Interstitium.

The results of this study demonstrated the increase in testes activity corresponded with an increase of histological measurement of testes that increased significantly in moderate ages, this increase in diameter and thickness affected by an increase in the spermatogenesis process and sperm concentration of local bulls. The increases in spermatogenesis process lead to increase the seminiferous tubules diameter and thickness, these results of these bulls are in agreement with (7) in goats. In the current experiment, a comparison of local bull reproductive organs collected in mating and non- mating seasons showed that morphological integrity was conserved either at testicular levels. Smaller testicular volumes, together with minor values of tubular diameters might indicate a decrease in spermatogenesis (4). The study revealed moderate morphological alteration between ages. At 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> ages it showed moderate spermatogenesis and sperm in the lumina of seminiferous tubules and moderate proliferation of Leydic cells in the interstitial tissues between seminiferous tubules as shown in Fig. 1, 2, 3 and 4.

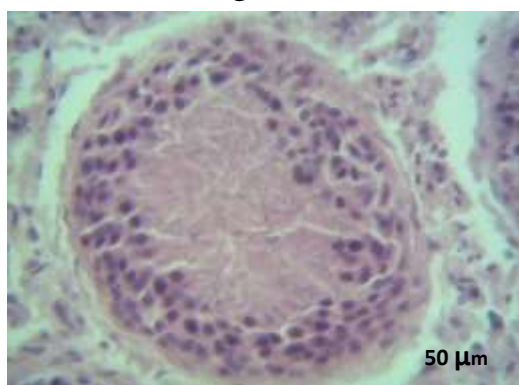


Figure-1: Tubules from local bull testes at age (1) year old. (H&E stain)

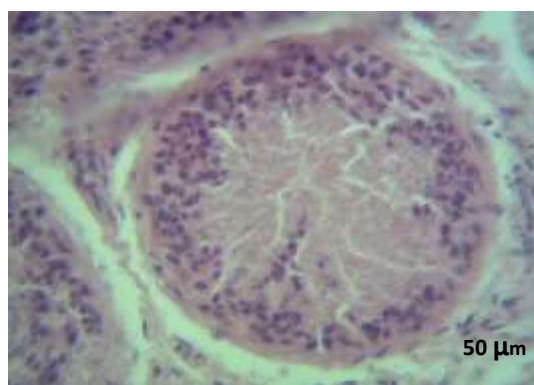


Figure-2: Tubules from local bull testes at age (2) years old. (H&E stain)

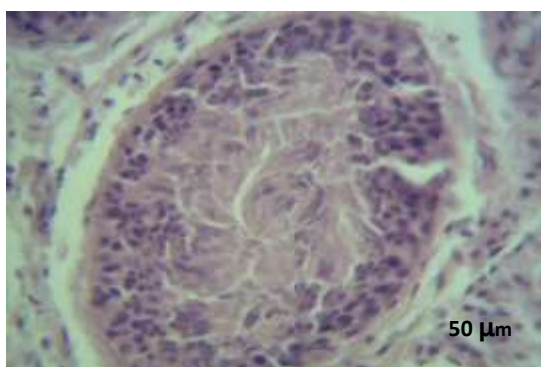


Figure-3: Tubules from local bull testes at age (3) years old. (H&E stain)

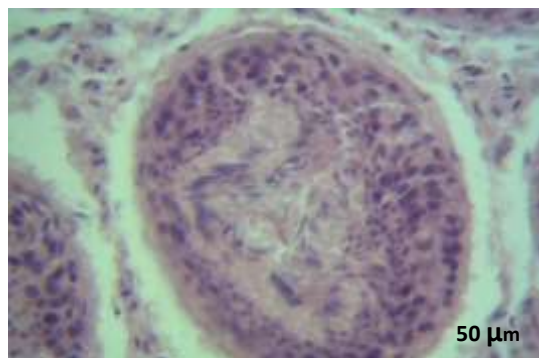


Figure-4: Tubules from local bull testes at age (4) years old. (H&E)

The variation changes in reproductive organs were noticed in most parts of the male reproductive tract in the roe deer (17), demonstrating the interplay of the morphological state of all components of the reproductive tract and the production of spermatozoa, semen plasma and testosterone and in the bull by studying the seasonal variation on the reproductive system of bull (3; 18). Present results of local bulls were in agreement with (19), which demonstrated that are not seasonal animals, because they are capable of being inseminated in around a year but, the male reproductive organs effects by temperature in hot months (summer) The weight organs significantly of the male genital system decreased and led to the disturbance in reproductive activity of the male, but it is a companied by disturbance of the male genital system in hot months. As well as, the level of nutrition may effect on the reproductive activity of the animal (19) It can be concluded in this study that the increased the weight, length, and width of testes in the 4<sup>th</sup> years old indicated an increase in the physiological activity of the testes in such age and an increased in activity of seminiferous tubules and sperm production in the testes like the morph metric measures of testes in different seasons, especially on November (Autumn seasons), that indicated an increase in mating seasons. This study demonstrated the increase in activity of testes corresponded with an increase of histological measurement of testes that increased significantly in moderate ages, this increase in diameter and thickness affected by an increase in the spermatogenesis process and sperm concentration production.

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