Research Article

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Histological and Biometrical Study of The Adult Local Bull Testis in Duhok Province

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Abstract

The purpose of this research was to assess some histological parameters and activity between the left and right testis of ten adult local bulls that were collected from the Duhok slaughterhouse /Iraq. The left and right testis specimens were routinely processed and stained with hematoxylin and eosin. The tunica albuginea is a thick sheet of dense, fibrous connective tissue that covers testicular tissue. The mean thickness of the tunics of an adult local bull differed significantly ($p \leq 0.05$) between the left and right testis. Thin septa that extended from the tunica albuginea to the mediastinum testis separated the parenchyma into lobules. Tortuous seminiferous tubules were found in each lobule. The mean diameter of the rounded seminiferous tubule of the left testicle was substantially ($P<0.05$) larger than that the right testicle. Tubular differential index (TDI) and spermatogenesis index (SPI) were found substantially ($P<0.05$) higher in the left testicle than the right. Sertoli cells are roughly pyramidal in shape, with an oval nucleus. The spermatogonia were circular or cuboidal in shape and possessed a round, dark nuclei. The large spherical nuclei and granular chromatin aggregates were found in primary spermatocytes. The spherical spermatids were small and elongated spermatids were developed. Some seminiferous tubules contain both spermatids and spermatozoa. Either narrow strands of interstitial tissue among two seminiferous tubules or massive triangular and quadrangular interstitial spaces separate up to three tubules. It contains only blood capillaries, a few Leydig cells, and some fibroblast cells, or extensive vessels (blood and lymph), as well as many Leydig cells. Within inter tubular spaces, Leydig cells were found as singular or in clusters. It was concluded that the thickness of the tunica albuginea, the diameter of the seminiferous tubules, the tubular differential index, and the spermatogenesis index were higher in the left testis of local adult bulls than the right testis.

Key words: Testicle, seminiferous tubules, Leydig cells, local adult bulls.

Introduction

The testis is a primary organ that produces testosterone and spermatozoa and so provides both endocrine and exocrine roles. The parenchyma of the testicle is made up of seminiferous tubules, which produce spermatozoa for the continuation of spermatogenesis, and Leydig cells, which release testosterone for the male sexuality (1).The testicles of most mammals, including stallions, rams, bulls, llamas, deer, and boars are complicated tubular organs that are supported by the tunica albuginea, a dense, irregular connective tissue capsule. White, reticular, and elastic fibers, as well as fibroblasts and a few vessels, abound in this capsule (2, 3, 4). Connective tissue septa that run from the tunica albuginea into the testis possess white, reticular, and elastic fibers as well as blood vessels that separate the testis into trabeculae, and these septa are thin in bovines and thick in canines, stallions, and boars. The septa may be fused with the testicular mediastinum connective tissue in the central region (5,6).

The testicular septa separate the organs into lobule testis, which are pyramidal compartments. One to four convoluted seminiferous tubules make up each lobule (7, 8). The seminiferous tubule had an external lamina propria composed of white and reticular fibers as well as myoid cells, as well as different stages of spermatogenic cells and Sertoli cells in domestic animals (9). Sertoli cells were easily distinguished by fewer tall columnar cells with irregular and evident nuclei radially from the basal laminae to the seminiferous tubule lumen (10). Sperm is produced by spermatogenic cells. Small round or cuboidal cells with dark spherical nuclei near the basal membrane are the most immature spermatogenic cells. Primary spermatocytes were the larger spermatogenic cells, with the most intensively recolored nucleus, and secondary spermatocytes were infrequently seen. The round spermatids had the smallest focal circular nucleus of all the spermatogenic configurations. Prolonged spermatids are characterized by small, oval to the extended nucleus, dark heads, lengthy black- out tails that pass into the lumen (9,10,11,12,13). A valuable tool for quantifying and qualifying spermatogenesis in bulls is histological examination (14). The objective of this research is to compare some histological parameters in the left and right adult local bull testicle.

Materials and methods

The research was performed on healthy adult local bulls' ten paired testis. All of the samples were taken as soon as the animals were slaughtered at the local abattoir in Duhok. Small sections of testicular tissue from various regions (two extremities and the center wider part) were placed in a specific container and preserved in formalin (10%) for one week at room temperature. Following fixation, the materials were sent to the laboratory using a standard histology procedure. Each sample had been dehydrated in seven serial stages in various concentrations of ethanol, twice in xylene, cleared for one hour each time, and finally

impregnated by two runs in heated paraffin wax, each step taking two hours (7). Using a tissue processor that is digital following that, a semi-digital embedding center with unique molds for manufacturing wax blocks is used. Finally, using a semi-digital rotary microtome, sectioning is measured at a 6-micrometer thickness, and each slide is stained with Harris hematoxylin and Eosin stain to demonstrate the overall histological features. Following that, histological data were collected.

1-The spermatogenesis index (SPI) and the tubular differential index (TDI) were used to assess testicular activity. Between the left and right testis, the mean SPI and TDI were compared. Every slide was divided into five sections. For each section, 25 seminiferous tubules were examined for SPI and TDI. The SPI was calculated by comparing the number of seminiferous tubules with spermatozoid, which is considered a positive SPI, to the number of seminiferous tubules without spermatozoid, which is a negative SPI. To calculate the TDI, seminiferous tubules with fewer than four rows of different stages of spermatogenic cells were considered negative. The seminiferous tubules had more than four rows, which is the positive TDI. The numbers of positive and negative seminiferous tubules were calculated.

2- The stained sections of testicles were examined using a light microscope with a different objective power. The tunica albuginea thickness and the seminiferous tubules' diameter were measured using an ocular stage micrometer. The more circular seminiferous tubules were chosen to measure the diameter of the seminiferous tubule. The seminiferous epithelium height was measured in the seminiferous tubules that contain more than the four layers of spermatogenic cells. The distance was measured from the luminal border to the basal lamina; in each cross-section, two measurements were taken and the mean was recorded.

3- The average diameter of spermatogonia, primary spermatocytes, Leydig cells, Sertoli, and elongated spermatids were measured by using an ocular micrometer at a high magnification. All data from the left and right testicle was compared by using a T-test for the student and the analysis of variance method (ANOVA) (15).

Results

Under a light microscope, the testicular tunica albuginea, a dense irregular connective tissue thick capsule, was seen in the ten paired testis of adult bulls (Fig. 1). The testis was surrounded by a thick tunica albuginea that was dense white and filled with fibroblasts as well as reticular, smooth muscle, and elastic fibers. The Tunica Vasculosa was a part of the testicular capsule that contained a lot of vascular connective tissue and major blood arteries that ran tortuously across the entire testicular surface. The connective tissue trabeculae in the inner layer of tunica albuginea are also continuous. As a result, unlike in other species, the adult bovine testis does not have the obvious pyramidal compartments known as lobule testis. The tunica albuginea of

local adult bulls' left and right testes had mean thicknesses of 1127.5 ± 99.7653 μm and 807.5 ± 71.0414 μm, respectively (Table 2). The left testicle tunica albuginea was statistically larger (P<0.05) than the right testicle tunica albuginea.

The convoluted seminiferous tubules, where the spermatozoa are produced, make up the majority of the testicular parenchyma (Fig. 2). Tubules are generally two-ended, convoluted loops with specific terminal portions on both ends that enter into the rete testis. The seminiferous tubules of adult bulls are bordered by a unique lamina propria. Two types of differentiated cells line the lamina propria: non-proliferating Sertoli cells and highly proliferating spermatogenic cells (Fig.4). The average diameter of the rounded seminiferous tubule of local adult bulls' left and right testes was 288.75 ± 21.1711 μm and 260 ± 23.6039 μm, respectively (Table 2). The left testicle rounded seminiferous tubule had a considerably larger mean diameter (P<0.05) than the right testicle. The average height of the seminiferous epithelium in the left and right testicles of local adult bulls was 77.81 ± 8.0664 μm and 71.25 ± 10 μm, respectively (Table 2). The height of the seminiferous epithelium in the left and right testes did not differ significantly.

The left and right testicles had mean SPI 79.8 ± 7.5631 and 63.2 ± 4.8166, respectively (Table 1). The left testicle had a significantly higher SPI (P<0.05) than the right testicle. The left and right testicles had mean TDI 92.2 ± 5.933 and 81.8 ± 5.2154, respectively (Table 1). The left testicle had a substantially higher mean TDI (P<0.05) than the right testicle.

The Sertoli cells are easily distinguishable cells. Sertoli cells are large cells with variable shapes; they can be identified most reliably by being the largest columnar cell still attached to the basal lamina and extending the apical lumen of the cross-sectioned seminiferous tubule. When compared to its neighboring cell, the Sertoli cell can also be recognized by its lighter, circular or oval nucleus (Fig.5). The mean diameter of the Sertoli cell of the left and right testis of local adult bulls was 11 ± 0.9258 μm and 10.25 ± 1.0351μm, respectively (Table 2). The diameter of the Sertoli cell did not differ significantly between the left and right testis.

The spermatogenic cells were organized into four to eight layers between the lumenal border of the seminiferous tubules and Sertoli cells, and the area between the basal lamina and the lumen of the tubules was occupied by these four to eight layers. The spermatogonia, or basic germ cells, were circular or cuboidal in shape and possessed round, dark nuclei (Fig.5).The mean diameter of spermatogonia of the left and right testicle of local adult bulls was 6.44 ± 0.4393 μm and 6.6 ± 0.4183 μm, respectively (Table 2). The left and right testicles did not differ significantly in the diameter of spermatogonia.

On the inner side of the spermatogonia, the primary spermatocytes were found. Their nuclei were circular in shape, and the cells were large. These spermatogenic cells nuclei

were larger than those of other sperm cells. (Fig.5). Primary spermatocytes in the left and right testis of local adult bulls had a diameter of 7.4 ± 0.5477 μm and 7.6 ± 0.4183 μm, respectively (Table 2). In terms of primary spermatocyte diameter, there was no significant variation between the left and right testes.

Secondary spermatocytes are smaller than primary spermatocytes and have lower nuclear chromatin density. Spermatids, which are smaller cells than primary and secondary spermatocytes, lie near to the lumen of seminiferous tubules, and the staining affinity of spermatids was lower than that of spermatogonia, primary, and secondary spermatocytes. (Fig.5).The mature one has elongated, darker heads and a prolonged tail that extends into the lumen of the seminiferous tubule. Some seminiferous tubules contain both spermatids and spermatozoa. The mean diameter of the elongated spermatid of the left and right testicles of local adult bulls was 6.3 ± 0.8367μm and 6.1 ± 0.5477 μm, respectively (Table 2). The diameter of the elongated spermatids did not differ significantly between the left and right testicles.

The interstitial tissue of the adult bull testicle is a well-organized testicular structure. Some of them were narrow strands entrapped between two seminiferous tubules; the others were large triangular and quadrangular areas between three to four tubules that make up this structure (Fig.6). The components of these two interstitial forms differ, with the former consisting only of blood capillaries, a few Leydig cells, and some fibroblast, whereas the latter includes major vessels (blood and lymph) as well as many Leydig cells. Leydig cells appear in singular or groups of various sizes, with round nuclei that contain a tiny portion of peripherally dispersed heterochromatin with one or two notable nucleoli, and their distribution appears to be random. Some are per vascular, while others are unattached to the vasculature, and still others are related to the tubules' lamina propria (Fig.6). The mean diameter of the Leydig cell of the left and right testicle of local adult bulls was 5.9 ± 0.9944μm and 5.6 ± 0.7379 μm, respectively (Table 2).The mean diameter of the Leydig cells did not differ significantly between the left and right testicles.

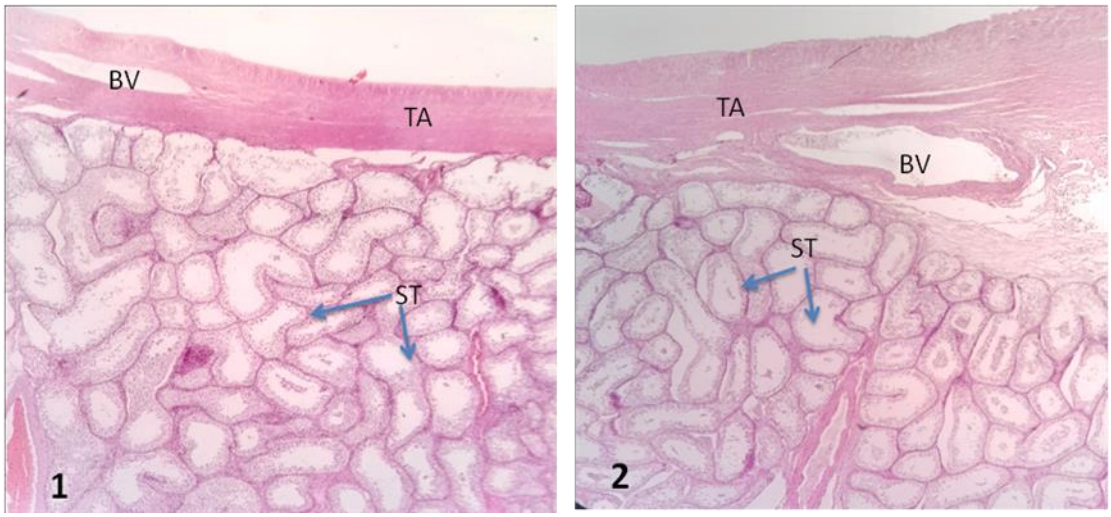

Figure (1): Micrograph of the adult bull testicle showing thickness of (TA)- Tunica albuginea in the (1)- Right testicle. (2)- Left testis testicle. (BV)- Blood vessels of tunica vasculosa. (ST) - Seminiferous tubules. ((H&E Staining × 4).

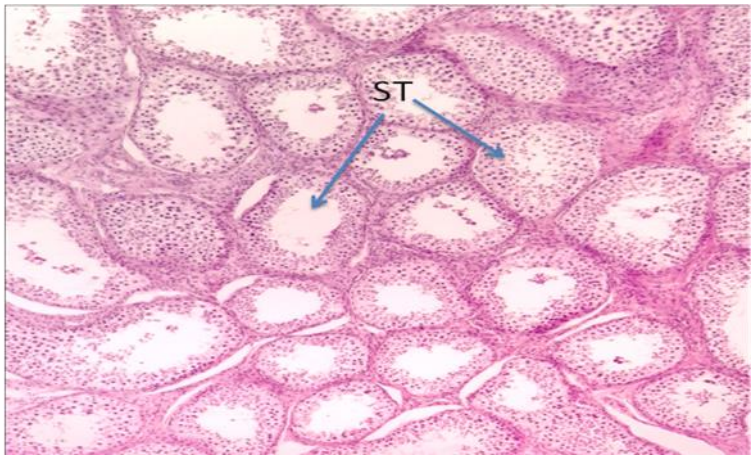

Figure (2): Micrograph of an adult bull testicle reveals that the majority of the parenchyma of the testicle is made up of convoluted seminiferous tubules (H&E Staining × 10).

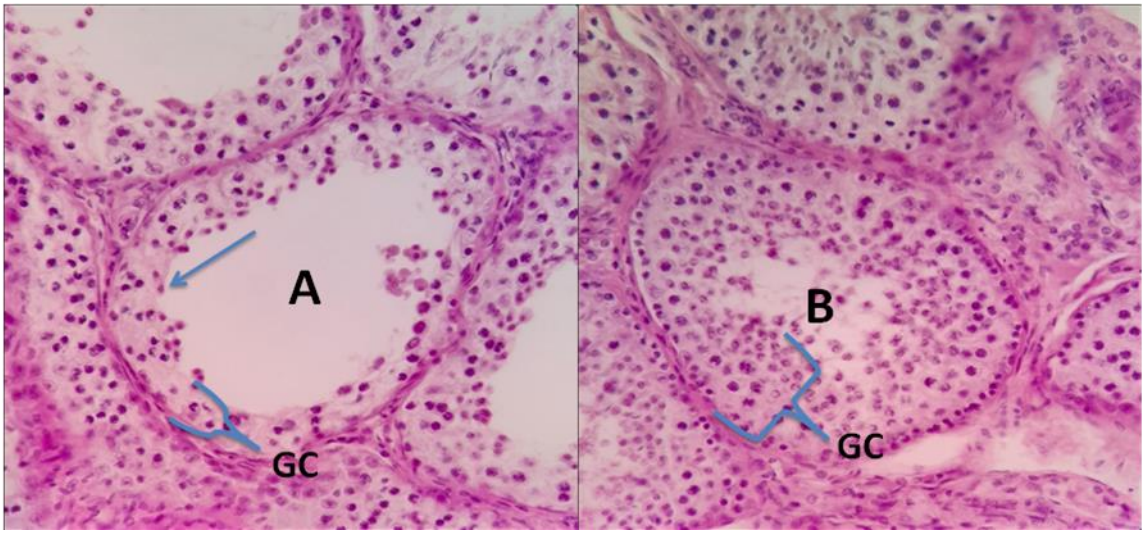

Figure (3): Micrograph of the Seminiferous tubule: (A) - Seminiferous tubule containing fewer than four stages of developing spermatogenic cells (negative TDI). (B)-Sseminiferous tubule that contains more than four stages of developing spermatogenic cells (positive TDI). (GC)- Germinal cells. (H&E Staining × 40).

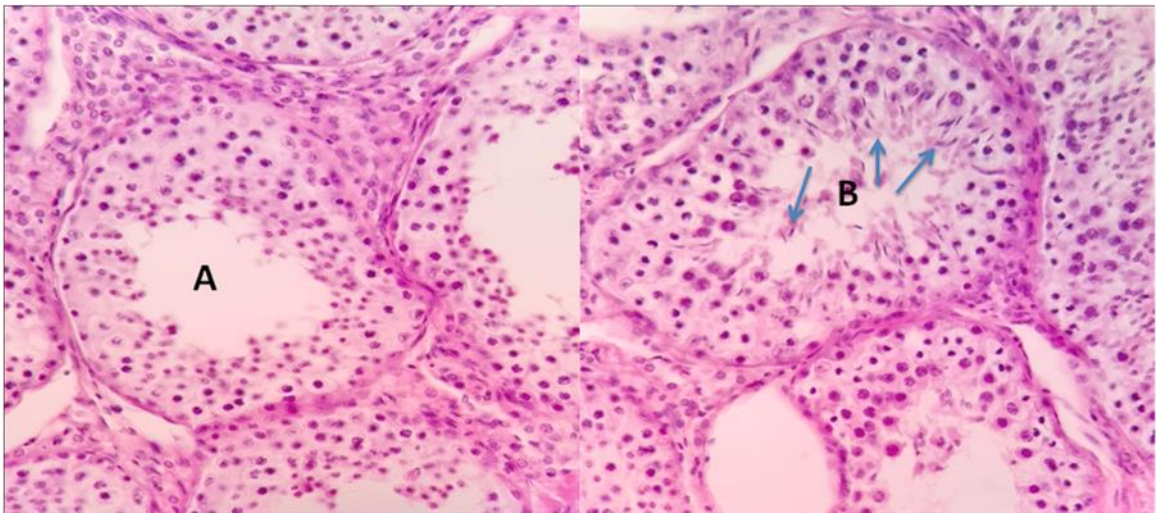

Figure (4): Micrograph of a seminiferous tubule of the bull showing. (A)- Seminiferous tubules without spermatozoids (negative SPI). (B)- Seminiferous tubules that contain spermatozoids (arrow) (positive SPI). (H&E Staining × 40).

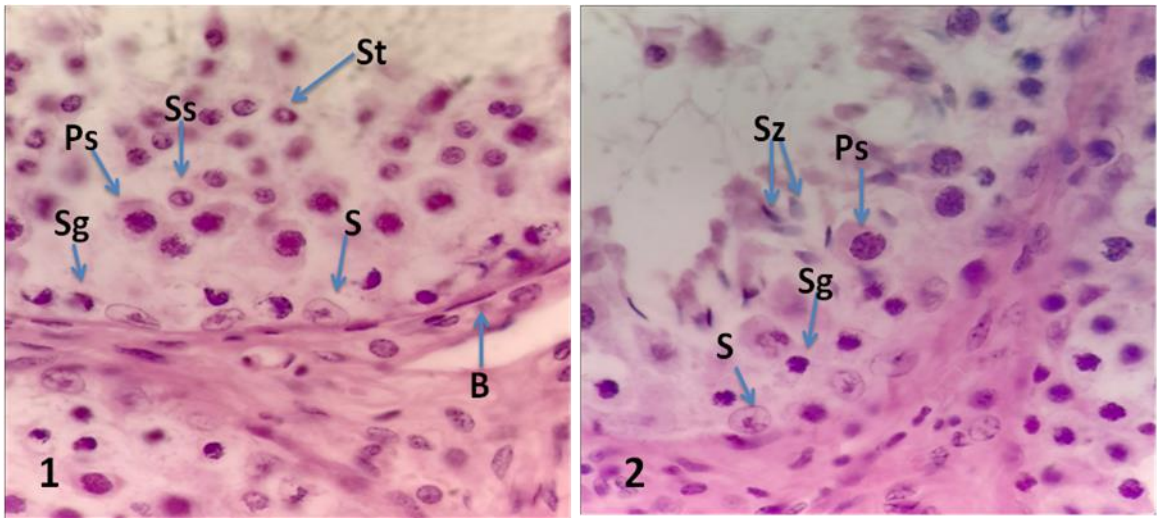

Figure (5): Micrograph of the seminiferous tubule of bull testicle showing the (1-B) - Basement membrane of seminiferous tubules. (1, 2-S) - Sertoli cells. (1, 2- Sg) - Spermatogonia, (1, 2- Ps) - Primary spermatocyte. (1-Ss) - Secondary spermatocyte. (1-St)-Spermatid. (2-Sz)-Spermatozoa. (H&E Staining × 100).

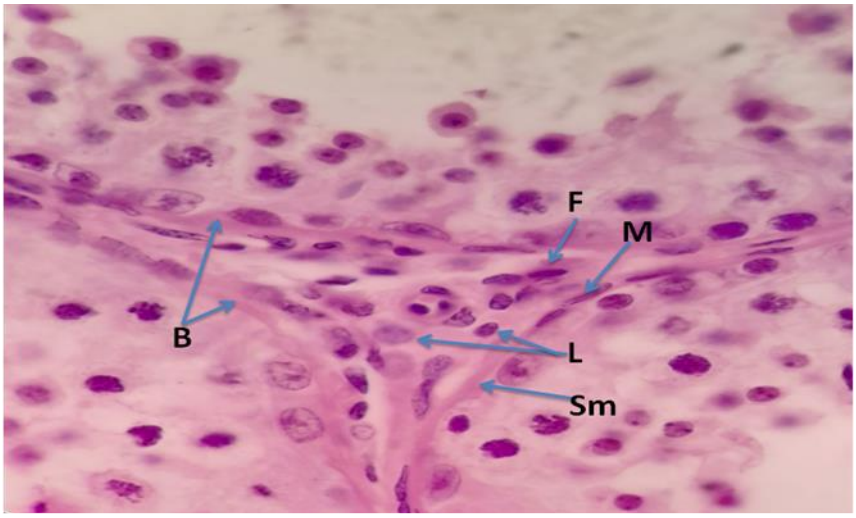

Figure (6): Micrograph of the interstitial tissue of the bull testis showing (B) - basement membrane. (M)- Myoid cell. (F)- Fibroblast. (Sm)-Smooth muscle. (L)- Leydig cell.(H&E Staining × 100)..

Table (1): The spermatogenesis index (SPI) and tubular differential index (TDI) of the left and right adult local bull testicle (Mean ± SD).

Testes/Activity index	Left testis	Right testis
SPI	79.8 ± 7.5631[a]	63.2 ± 4.8166[b]
TDI	92.2 ± 5.933[a]	81.8 ± 5.2154[b]

a, b, : Means (±SD) within a row, indicating that distinct superscript letters are substantially varied (P≤0.05).

Table 2: The biometric features of the left and right testis in an adult local bull (Mean ± SD).

Biometrics	Left testis	Right testis
Thickness of the tunica albuginea (μm)	1127.5 ± 99.7653[a]	807.5 ± 71.0414[b]
Diameter of rounded seminiferous tubules (μm)	288.75 ± 21.1711[a]	260 ± 23.6039[b]
Seminiferous epithelium height (μm)	77.81 ± 8.0664[a]	71.25 ± 10[a]
Diameter of Sertoli cells (μm)	11 ± 0.9258[a]	10.25 ± 1.0351[a]
Diameter of leydig cells (μm)	5.9 ± 0.9944[a]	5.6 ± 0.7379[a]
Diameter of Spermatogonia (μm)	6.44 ± 0.4393[a]	6.6 ± 0.4183[a]
Diameter of primary spermatocytes (μm)	7.4 ± 0.5477[a]	7.6 ± 0.4183[a]
Diameter of elongated spermatid (μm)	6.3 ± 0.8367[a]	6.1 ± 0.5477[a]

a, b, : Means (±SD) within a row, indicating that distinct superscript letters are substantially different (P≤0.05).

Discussion

The tunica albuginea is a thick, fibrous capsule that covers an adult bovine testis. It is divided into two layers: the tunica fibrosa outer layer and the tunica vasculosa inner layer. The latter was most noticeable in bulls, whose massive blood vessels cross the majority of the surface of their testis in a torturous fashion. Bovine tunica albuginea consists of fibroblasts, collagen fiber bundles, and a few elastic fibers. A similar finding was reported by (16, 17). Smooth muscle cells are found in significant numbers in some species (horse, boar, and ram) (18, 10). The testicular capsule is hypothesized to play an important function in controlling interstitial pressure inside the testes and influencing sperm motility in

species with smooth muscle (19). The inner layer of the tunica albuginea is composed of inconspicuous connective tissue trabeculae that only surround the major intratesticular blood vessels. As a result, unlike in other animals, the adult bovine testicle does not have distinct lobules. A similar discovery was reported by (9).

The tunica albuginea thickness differed between the left and right testicles. The tunica albuginea thickness of the bulls was measured in this study and was consistent with research by (10) in bulls, while higher than that observed by (20) in rams and bucks. According to the findings of this study, the tunica albuginea was thicker in the left testicle than the right testicle, and it was substantially ($P<0.05$) thicker in the left testicle than the right testicle. There were no reports in the available literature about the thickness of the tunica albuginea of the testicles of bulls of various testes of the same animal. This finding shows that variations in tunica albuginea thickness may be attributable to testes of varying activeness. After puberty, the thickness of the tunica albuginea decreases as the bull reaches maturity (10).

The parenchyma of the testicles was mainly composed of seminiferous tubules. The tubules were convoluted double loops with a circular and rectangular pattern that varied in frequency due to the tubules' complicated twisting at various angles and levels. These results resembled those of (17, 1). The lamina propria and a lining of Sertoli cells and spermatogenic cells formed the walls of the seminiferous tubules.. This result is in agreement with (21, 18).

The mean diameter of the seminiferous tubules of the left and right testicles of local adult bulls was (288.75± 21.1711 μm and 260 ± 23.6039 μm, respectively) in this study, which was comparable to the finding of (22) that was discovered in adult Holstein bulls (269.0 μm). Greater than Nelore bulls (232.7 μm) (23), Shorthorn bulls (210.4 μm) (24), and non-breed zebu animals (197.6 μm) (25).The variations in the results obtained from the research could be due to the breeds' genetic improvement, i.e., choosing animals with higher spermatozoa production. Differences in methodology should also be considered, particularly in terms of the specimen utilized and the adjustment of estimates related to structural shrinkage of the tissue that happens throughout the preparation of the material. Working with paraffin inclusions, for example, can result in 15% shrinkage, according to (26). Moreover, the result may be affected by the use of sexually immature animals (27).

The left testis had a significantly larger mean diameter of seminiferous tubules ($P<0.05$) than the right testis. These findings resembled those of (10). This finding implied that bulls with larger seminiferous tubules had greater reproductive capacity than bulls with smaller seminiferous tubules (28).The ability to produce sperm is determined by the thickness of the sperm-producing epithelium (29). The seminiferous epithelium height in the local adult bull was similar to that observed in (30) and Nelore

bulls (73.2 μm) (31). (25) Reported lower values (53.4 μm) in zebu bulls without breed characterization.

The spermatogenesis index (SPI) is a useful method to predict testicular activity. Due to the high number of SPI, there was a high percentage of spermatozoid synthesis, which resulted in a high rate of testis activity. The results of this study's histological analysis (SPI) revealed that the left testicles of local bulls were significantly more active than the right testicles ($P<0.05$). Another notable finding for estimating testes activity is the tubular differential index (TDI). Seminiferous tubules with over four layers of germinal cells were sites of active spermatozoid synthesis in the TDI model. The histology results of this study's TDI appeared to suggest that the left testicles were considerably more active than the right testicles ($P<0.05$). In general, the left testes of local adult bulls were not only substantially ($P<0.05$) larger in diameter of seminiferous tubules than the right testicles, but they are also noticeably more active (SPI and TDI) than the right testicles. The causes of testicular function laterality are unknown. A number of factors could have an effect on them. Hormonal, blood vessel distribution patterns, genetic, and many other factors may all be involved. Additional research is needed to discover the various causes and their effects on the left and right testes. This finding is comparable to that found in Ghezel Ram by (32). In agreement with (10) who reported that the cross-sectional of seminiferous tubules' length and breadth were shown to be greater in the left testis of bulls. Sertoli cells were irregularly taller shaped cells near the basal lamina of the tubule. Other than the nucleus, the cells seemed to be clear. The nuclei were pear-shaped and oval. The position of the nuclei in different Sertoli cells varied. These results were in agreement with those of (18, 33, 17, 1). Between the Sertoli cells and the lumen of the seminiferous tubules, the spermatogenic cells were arranged into four to eight rows, with four to eight rows covering the gap between the lamina propria and the tubule lumen. This result was consistent with prior observations (18, 10).

Spermatogonia are the basic germ cells that eventually produce all spermatozoa. They had spherical or cuboidal nuclei and were spherical in appearance. The largest cells were the primary spermatocytes, with round or spherical nuclei. These spermatogenic cells' nuclei were larger than other spermatogenic cells' nuclei, while secondary spermatocytes were smaller. The spermatids were easily identified by their tiny size and position, which were observed in the tubules' lumen. These results were comparable to those of a previous study (18, 1, 9). Interstitial tissue is defined as the tissue that fills the gap between seminiferous tubules and contains, blood and lymph vessels and nerves as well as steroid-secreting cells (34, 19). According to the current strategy, bull interstitial tissue seems to be composed of either narrow strands trapped between two neighboring seminiferous tubules or massive triangular and quadrangular portions communicating more than three tubules. The difference

between these two types of interstitial space is due to their composition. The first one contains a few Leydig cells, with some Fibroblast and only blood capillaries, whereas the latter contains many Leydig cells and major vessels (blood and lymph). These findings were similar to those of (9, 10). Bovine Leydig cells were found in groups or cord of various shapes and sizes with round nuclei and granular cytoplasm. These cells seem to be distributed randomly, with some being perivascular, others related to vessels. These results were consistent with those of (35, 36, 37, 38).

Conclusion

This study showed that the left testis was higher in some histological parameters and testicular activity than the right testis in local adult bulls.

Acknowledgments

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دراسة نسجية ونسجية قياسية لخصية الثور المحلي البالغ في محافظة دهوك

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الخلاصة

الهدف من الدراسة هو تقييم بعض القياسات النسجية والنشاط بين الخصيتين اليمنى واليسرى لعشرة ثيران محلية بالغة والتي تم جمعها من مجزرة دهوك / العراق. تم معالجة عينات الخصيتين اليمنى واليسرى بشكل روتيني وصبغها بالهيماتوكسيلين والأيوسين. الغلالة البيضاء هي طبقة سميكة من النسيج الضام الليفي الكثيف الذي يغطي أنسجة الخصية. اختلف متوسط سمك هذه الطبقة للثور المحلي البالغ اختلافًا معنويا ($p\leq0.05$) بين الخصيتين اليمنى واليسرى. تمد حويجزات رقيقة من الغلالة البيضاء الى المنصف الخصوي حيث تفصل متن الخصية الى فصيصات. وجدت نبيبات منوية ملتوية في كل فص. كان متوسط قطر النبيبات المنوية المستديرة للخصية اليسرى أكبر من الخصية اليمنى ($P <0.05$). كان مؤشرالتفاضل الأنبوبي (TDI) ومؤشر تكوين الحيوانات المنوية (SPI) أعلى في الخصية اليسرى من اليمنى وذواختلاف معنوي ($p\leq0.05$). خلايا سيرتولي هرمية الشكل تقريبًا ولها نواة بيضاوية. كانت الخلايا المنوية البدائية دائرية أو مكعبة الشكل ولها نوى دائرية داكنة. تم العثور على النوى الكروية الكبيرة ذات الصبغين الحبيبي في الخلايا المنوية الأولية. كانت المبيدات المنوية أما صغيرة وكروية الشكل أو ممدودة واكثر تطورا من الاولى. تحتوي بعض النبيبات المنوية على كل من خلايا المبيدات المنوية والحيوانات المنوية. النسيج الخلالي بين النبيبات المنوية يتكون اما من خيوط ضيقة بين نبيبين منويين أومن مسافات كبيرة مثلثة ورباعية الشكل تفصل ما بين ثلاثة أنابيب او اكثر. يحتوي النسيج الخلالي مابين النبيبات المنوي على شعيرات دموية و خلايا ليديج وبعض الأورمات الليفية أو يحتوي على أوعية دموية ولمفاوية فضلا عن خلايا ليديج. وجدت خلايا ليديج ضمن النسيج الخلالي مابين النبيبات بشكل أنفرادي أو بشكل مجاميع من الخلايا.استنتج من الدراسة أن سمك الغلالة البيضاء ، وقطر الأنابيب المنوية ، و مؤشر التفاضل الأنبوبي ، ومؤشر تكوين الحيوانات المنوية كانت أعلى في الخصية اليسرى للثيران البالغة المحلية من الخصية اليمنى.

الكلمات الدالة: الخصية ، النبيبات المنوية ، خلايا ليديج ، الثيران البالغة المحلية.