

## Research article

## Microscopic and biometric investigations of the testes in adult ram and adult buck in Al-Najaf Al-Ashraf Province

Waleed Jaleel abed Al-Kelaby

Aqeel Mohsin Mahdi Al-Mahmodi

Ali Abdulkareem Jabbar Al-Ramahy

*Department of Anatomy and Histology, Faculty of Veterinary Medicine, University of Kufa, Iraq*Corresponding Author Email: [Waleedj.abed@uokufa.edu.iq](mailto:Waleedj.abed@uokufa.edu.iq)Co-Authors Email: [Aqeelm.mahdi@uokufa.edu.iq](mailto:Aqeelm.mahdi@uokufa.edu.iq) [ali.kareem1976@yahoo.com](mailto:ali.kareem1976@yahoo.com)

(Received 2/9/2017, Accepted 29/11/2017)

**Abstract**

*Microscopic and biometric exploration of the testis in ten of the healthy mature rams and buck choose from AL-Najaf slaughter house. Which anticipation to support the future studies and clinical purposes of the biology of the reproductive system. Testicular histological technique civilized then. Microstructures, histodimensions, were recorded then discussed between two target species. Testicular surrounded by thick sheet of dense irregular connective tissue, tunica albuginea. The last secured by a visceral layer of the tunica vaginalis. The mean thickness in these tunics of an adult ram and buck differed significantly ( $p \leq 0.05$ ). The parenchyma was subdivide into lobules by septae extend from mediastinum testis toward tunica albuginea. Each lobule of the two target species contain tortuous seminiferous tubule. Interstitial connective tissue surround each tubule contain blood vessels, fibroblasts loose connective tissue cells, and leydig cells. The mean number, diameter of leydig cells and their nuclei in microscopic fields in ram and buck differed significantly ( $p \leq 0.05$ ). Sertoli cells pyramidal shaped with ovoid nuclei mean diameter of Sertoli cells in ram and buck differed significantly ( $P \leq 0.05$ ). Spermatogonia located near the basal membrane, Mean diameter of Spermatogonia, in ram and buck differed significantly ( $p \leq 0.05$ ). Large primary spermatocytes with large rounded nuclei with coarse clumps of chromatin mean diameter in two species differed significantly ( $p \leq 0.05$ ). Secondary spermatocytes smaller than primary. Small spermatids were rounded. Mature one characterized to elongate. Seminiferous tubule continuous as straight tubules with rete testis tubules lined by simple squamous epithelium. The later end with ductuli efferentes lined with tall ciliated columnar epithelium. Which continued into ductus epididymis lined with ciliated pseudostratified columnar epithelium.*

**Key words:** Ram, Testes, Buck, Tunica albuginea, Spermatogonia, Sertoli cells.**Introduction**

Reproductive organs are the most dynamic organs in all animals (1). The testicles are compound tubular organs, which display both endocrine and cytogenic capacities essential for generation of spermatozoa and testosterone (2). The testicles in most mammals, such as stallion, ram, bulls, llamas, vicuna, deer and boar are complex tubular organs that are contributed by a thick capsule of dense irregular connective tissue, the tunica albuginea. This capsule is rich in white, reticular and elastic

fibers together with fibroblasts and few vessels. Tunica vaginalis composed of mesothelium and underlying connective that mixed with tunica albuginea. (3,4,5,6,7) Testicular capsule comprising three layer external instinctive layer of tunica vaginalis, center tunica albugenia and inward tunica vasculosa contained numerous blood vessels in boar (8, 9). On the other, hand the tunica vasculosa situated in the centerpiece of the capsule in stallion and hog (10). Septae of the connective tissue extends from the tunica

albuginea into the testis, contain white, reticular and elastic fibers along with blood vessels dividing the testis into lobules, and these septae are thin in ruminants and thick in carnivores, stallions, and boar. Centrally, the septae may be combined with the loose connective tissue of the mediastinum testis. (3,4,5,8). Within each lobule there are convoluted seminiferous tubules separated by tissue contained various interstitial endocrine cells of testis Leydig cells polygonal, granular cytoplasm, and spherical nucleus, arranged in large clusters situated in close region to the arteriole and vessels nerve fibers, blood and lymph vessels in bull, ram, horse and in boar (11,12,13,14,8). In domestic animals each seminiferous tubules involved external lamina propria consist of white and reticular fibers and myoid cells, and stratified epithelium of spermatogenic cells and Sertoli cells easily recognizable by were fewer tall columnar cells irregular and clear nuclei set radially from basal laminae to seminiferous tubule lumen. The spermatogenic cells give rise to the sperm. Spermatogonia, the most immature spermatogenic cells, are small round or cuboidal cells with dull round nuclei situated close to the basal membrane. The primary spermatocytes were biggest among spermatogenic cells with greatest thickly recolored nucleus and the littler spermatocytes secondary were occasionally watched. The round spermatids were littlest among spermatogenic arrangement with huge focal circular nucleus. Extended spermatids are described by little, oval to lengthened nucleus, dim heads, long black out tails that undertaking into the lumen (10, 12, 11, 15, 13, 14, 8). In ruminant and horse, elongated spermatids are eventually released from the seminiferous epithelium as spermatozoa. Different groups of sperm cell development occur inside the epithelium of a seminiferous tubule continuous as straight tubules lined with simple cuboidal epithelium then a network of anastomosis channels that form the rete testis were lined by cuboidal

epithelium with some patches of stratified epithelium which encompassed by mediastinum testis it is loose connective tissue then joined to the epididymis duct by efferent ducts. (3,4,5, 6,7,16,17).

## Materials and Methods

### Ethical approval

The Animal Ethical Committee of Veterinary Medicine College, University of Al-Qadisiyah, Iraq, has approved the present study under permission No: 273

Testis of ten healthy adult rams and bucks aged one year chosen from the AL-Najaf slaughterhouse randomly. After orchietomy the left and right testis obtained of each animal, then washed by normal saline solution (0.9% NaCl), after organ cleaned and fragments to three region (two extremities and middle wider part) put in the special casket were fixed immediately in neutral buffer formalin (NBF10%) at room temperature. The routine histological technique had been done: Dehydration of each sample by seven serial stages in deferent concentrations of ethanol, two time in xylene, one hour for each time for clearing, eventually, impregnation samples by two runs in melted paraffin wax (58-60 °C) two hours for each step. By using digital Tissue Processor. After, by using Semi digital embedding center contain special molds for make wax blocks. Finally, Sectioning measures 5-micrometer thickness by using Semi digital rotary microtome, then each slide stained by following

1-To reveal the general histological structures. Used Harris hematoxylin and Eosin stain

2-To description the general histological structures, Weigerts iron hematoxylin stain

3-Periodic acid-shiff (PAS) Stain to demonstrate type of secretions.

Then following histological data were recorded

1-Mean thickness of the tunica albuginea.

2-Mean number of seminiferous tubules in each testicular lobule.

3-Mean diameter of rounded seminiferous tubules.

4-Mean diameter of Spermatogonia, primary spermatocytes, Leydig cells, Sertoli and spermatids.

### Statistical analysis

### Results

Microscopical examinations of the ten testis for each species notice the testicular tunica albuginea thick capsule of dense irregular connective tissue Figures (1), (2). Thick tunica albuginea encompassed the testis it was dense white with fibroblasts and few of reticular, smooth muscle, and elastic fibers mean thickness of the tunica albuginea in ram and buck were ( $240 \pm 0.9 \mu\text{m}$ ) and ( $220 \pm 1.78 \mu\text{m}$ ) respectively (Table, 1). Tunica Vasculosa was part of capsule, which have vessels. Tunica vaginalis consists of mesothelium and underlying connective tissue that blends with tunica albuginea. Connective tissue extends inward from the tunica Vasculosa into the testis to form interstitial connective tissue surround, binds, and supports the seminiferous tubules. Interstitial connective tissue contain blood vessels, fibroblasts loose connective tissue cells, and interstitial cells of leydig which multilateral Figures (3), (4). The mean number in microscopic fields in same animals were ( $3.5 \pm 1.6$ ) and ( $2.9 \pm 0.59$ ) respectively Table (1). Mediastinum testis was connective tissue rich with white fibers bundles. Thin fibrous septae extend from mediastinum testis toward tunica albuginea partially or entirely dividing the testis into partitions Figures (1), (2). Mean diameter of rounded seminiferous tubules in ram and buck were ( $264 \pm 2.9 \mu\text{m}$ ) and ( $245 \pm 1.9 \mu\text{m}$ ) respectively Table (1). Seminiferous tubules lined from several cells spermatogenic, and Sertoli cells Figures (3), (4). The latter appeared pyramidal with oviform nuclei. Basal area of Sertoli cells contain Spermatogonia and primary spermatocytes, while spermatids and spermatozoa occupied the apical regions of these cells. Mean

Testis data to the Statistical Package of Social Sciences (SPACE). T-test for the student and analysis of the variance method (ANOVA). The level of significant variation in mean values between the ram and the testis was completed. Values ( $P \leq 0.05$ ) were considered significant

diameter of Sertoli cells in ram and buck were ( $13.3 \pm 1.9 \mu\text{m}$ ) and ( $12.5 \pm 0.9 \mu\text{m}$ ) respectively (Table, 1). Spermatogonia were round cells with round or ovoid dark nucleus Figures (3), (4). Mean diameter of Spermatogonia, in ram and buck were ( $10.3 \pm 1.7 \mu\text{m}$ ) and ( $9.9 \pm 1.5 \mu\text{m}$ ) respectively Table (1). Improvement stages of the primary spermatocytes above single layer of Spermatogonia large cells with large circular nuclei with coarse clumps or thin threat of chromatin mean diameter of primary spermatocytes in ram and buck were ( $16.6 \pm 1.9 \mu\text{m}$ ) and ( $15.8 \pm 1.3 \mu\text{m}$ ) respectively Table (1). Secondary spermatocytes smaller than primary and less dens nuclear chromatin. Spermatids close to seminiferous tubules lumen, which are smaller cells in comparison primary and secondary spermatocytes Figures (3), (4). Mature one characterized to elongated, dark heads, long tail that project into the lumen of seminiferous tubule. In both species Seminiferous tubule terminate in the straight tubules are short slim duct lined via cuboidal or low columnar epithelium and continuous with rete testis of the mediastinum a network of anastomosis channels wide lumina that are lined by a simple squamous epithelium to low cuboidal. Rete testis terminate in wide lumen of ductuli efferentes lined with tall ciliated columnar epithelium that alternate to groups of shorter nonciliated cuboidal epithelium. Under the basement membrane was a slim layer of connective tissue have a thin smooth muscle layer. Ductuli efferentes terminate in the ductus epididymis that lined by pseudostratified columnar, ciliated epithelium.

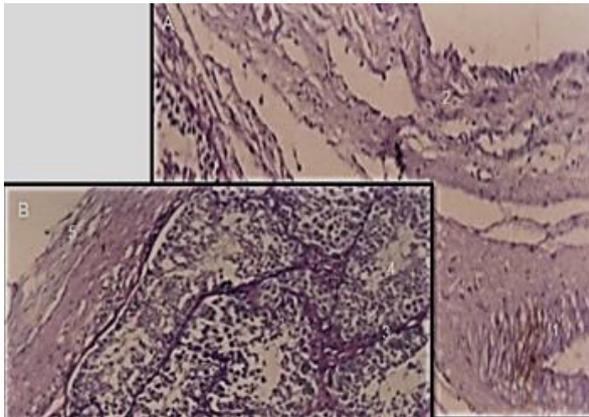


Figure (1): Demonstrate the histological structure of Ram testis).

A-1-Blood vessels, 2- capsule.  
B-3- basement membranes. 4-seminiferous tubules 5- connective tissue septa PAS stain, 40x

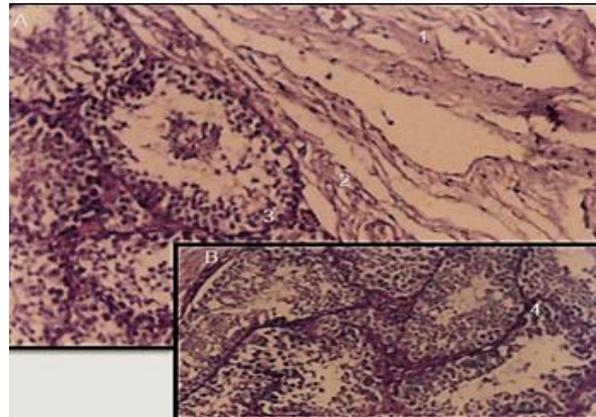


Figure (2): Demonstrate the histological structure of Buck testis

A-1- capsule 2- connective tissue septa 3- seminiferous tubules  
B- 4- basement membranes. PAS stain, 10x.

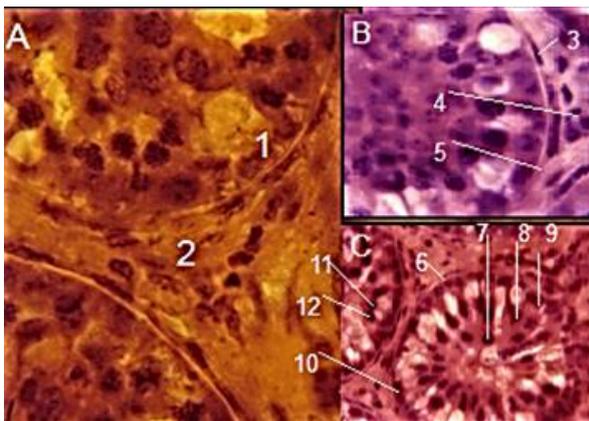


Figure 3 Demonstrate the histological structure of rams testis

A-1-Seminiferous tubules. 2- Interstitial tissue Weigerts iron hematoxylin stain(x100) .B-3-Myoid cell 4-leydig cell. 5- Basement membrane Harris hematoxylin stain(x100). C- 6-Spermatogonia. 7- Nine stage. 8- Sertoli cell 9-permatogonia. 10-Spermatogonia. 11- Seven stage of Spermatid. 12- Six stage of Spermatid P.A.S. Stain(x100).

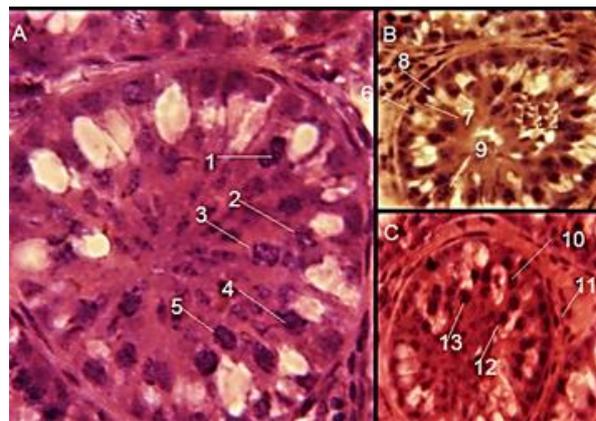


Figure 4 Demonstrate the histological structure of Buck testis

A-1-Zygotene phase. 2- First stage of Spermatid. 3-Four stage of Spermatid 4-Ten stage of Spermatid. 5-fourteen stage of Spermatid. Harris hematoxylin stain (x100). B-6-A-spermatogonia. 7-1-spermatogonia. 8-B-spermatogonia 9-pachytene phase. Weigart iron hematoxylin (x100).C- 10-five stage of Spermatid. 11-myiod cell. 12-eight stage of Spermatid. 13-fifteen stage of Spermatid. P.A.S. Stain (x100).

Table (1): The Biometric characteristics of the Testis in adult Ram and Buck  $\mu\text{m}$  (Mean  $\pm$  SD)

Biometrics	Ram	Buck
Thickness of the tunica albuginea ( $\mu\text{m}$ )	240 $\pm$ 0.9 <sup>a</sup>	220 $\pm$ 1.78 <sup>b</sup>
Diameter leydig nuclei ( $\mu\text{m}$ )	4.6 $\pm$ 1.9 <sup>a</sup>	3.9 $\pm$ 1.4 <sup>b</sup>
Number of leydig	3.5 $\pm$ 1.6 <sup>a</sup>	2.9 $\pm$ 0.59 <sup>b</sup>
Diameter of rounded seminiferous tubules ( $\mu\text{m}$ )	264 $\pm$ 2.9 <sup>a</sup>	245 $\pm$ 1.9 <sup>b</sup>
Diameter of Sertoli cells ( $\mu\text{m}$ )	13.3 $\pm$ 1.9 <sup>a</sup>	12.5 $\pm$ 0.9 <sup>b</sup>
Diameter of Spermatogonia( $\mu\text{m}$ )	10.3 $\pm$ 1.7 <sup>a</sup>	9.9 $\pm$ 1.5 <sup>b</sup>
Diameter of primary spermatocytes ( $\mu\text{m}$ )	16.6 $\pm$ 1.9 <sup>a</sup>	15.8 $\pm$ 1.3 <sup>b</sup>

a and b Mean on the same row with different superscript differ significantly (P $\leq$ 0.05).

## Discussion

In present study for histo-structures and biometrical greatly important in determent infertility animals competency. Increased and decreased these parameters play important role in sperms production and physiological status of animals. When was higher infertility animal's competency then inters these animals in hereditary ameliorating (13). Number and diameter of seminiferous tubules in ram in this study were more than in the buck significantly ( $P \leq 0.05$ ), a consistency with (18) in the types of bulls. Thickness of the sperms production epithelium refers to the capability for sperm production (19). Characteristics of spermatogonia and primary spermatocytes coordinators with (20, 21) opinions in goat, ram, and deer, respectively. Whereas incongruity these finding with (8) in boar. General appearance of the Sertoli cells in our studies fully confirmed with (22, 23) in man and buffalo, respectively. While describing of these cells in pigs, domestic animals, bull, deer, ram, and horse was fewer tall columnar cells (8, 11, 15, 13, 14). Sertoli cells supporting cells in function when sexual

period begin these cells become large and indent on free surface for executed the nutrition and phagocytic functions (22). Interstitial cells of leydig well suited with (24, 20) in camel and goat. Thickness of the testicular capsule, interstitial tissues, and septae and they components in target animals compatible with (25, 26, 2, 8) in rabbits, cattle, goat, and boar. However, the testicular lobulations were imperceptible in horse (14). Essential ingredient of mediastinum of testes in adult buck in our study compatible with (27) in adult buck, while in pre-pubertal commutating connective tissue of mediastinum rich in reticulin, and in pubertal period contain elastin, reticulin. In this study of the rams and the bucks was incongruity with what the (5) in terms of the possibility that the epithelium lining of the straight tubules was squamous, the rete testes was cuboidal epithelium or bistratified cuboidal, Ductuli efferentes simple columnar or a pseudostratified epithelium with some ciliated cells in the bull, While consistent with what he said about the tissue specifications of the ductus epididymis.

## References

- 1-Robinson JE, Karsch FJ. Photoperiodic history and a changing melatonin pattern can determine the neuroendocrine response of the ewe today length. *J. Reprod. Fertil.* (1987); (80) pp159-165
- 2-Hafez RSE. Reproduction in farm animals. 7<sup>th</sup> (ed.): Lea and Febiger Philadelphia (2000); pp 324-506
- 3-Mahmud MA, Onu JE, Shehu SA, Umaru MA, Danmaigoro A, Bello A. Comparative Gross and Histological studies on testis of one-humped camel bull, UDA Ram and Red sokoto Buck. *Int. J. multidisciplinary research and inform. (IJMRI)*, (2015);1(1) pp 81-84.
- 4-Rodrigues A, Rojas MA, Bustos-obrego E, Urquieta B, Regadera J. Distribution of Keratins, Vimentin, and Actin in the Testis of Two Sout American Camelids: Vicuna (*Vicugna vicugna*) and Llama (*Lama glama*) .An Immunohistochemical Study. *ANAT. RECO.* (1999); (254) pp 330-335.
- 5-Bacha WJ, Bacha LM (2000) Color Atlas of Veterinary Histology 2nd (ed.): Lippincott Williams & Wilkins. PP: 203-205
- 6-Kuwar RB, Jha CB, Saxena AK, Bhattacharya S. (Effect of phosphamidon on the testes of albino rats: a histological study. *Nepal. Med. Coll. J.* (2006); 8(4) pp 224-6
- 7-Odaba Ö, Kanter M. Histological investigation of testicular and accessory sex glands in Ram lambs immunized against recombinant GnRH fusion proteins. *Eur. J. Gen Med* (2008); 5(1) pp21-26
- 8-Reddy DV, Rajendranath N, Pramod KD, Raghavender KBP. Microanatomical studies on the testis of domestic pig (*Sus scrofa domestica*). *Inter. J. of Scie. , Enviro. and Technol.* (2016) ;5(4) pp 2227 - 2231
- 9-Ohanian C, Rodriguez H, Piriz H, Martino I, Rieppi G, Garofalo EG, Roca RA. Studies on the contractile activity and ultrastructure of the boar testicular capsule. *J. of Repro. and Ferti.* (1979); (57) pp 79-85.
- 10-Trautmann A, Fiebiger J. Fundamentals of the histology of domestic animals. Revised by Hobel R E, Biberstein EL. Printed in United States of America by George banta publishing company, Menasha, Winconsin. (1952); pp 258-269.
- 11-Gofur MR, Khan MZI, Karim MR, Islam MN. Histomorphology and histochemistry of testis of

- indigenous bull (*Bos indicus*) of Bangladesh. *Bangladesh J. of Vet. Med.* (2008); (6) pp67-74.
- 12-Ahmed AE. Morphological, Glycohistochemical, and Immunohistochemical Studies on the Embryonic and Adult Bovine Testis. PhD. Thesis Vet. Med. Ludwig-Maximilians-Univ. (2005).
- 13-Kishore PVS, Geetha Ramesh and Sabiha Hayath Basha (2007b): Intertubular tissue in the testis of ram –A postnatal histological study. *Indian J. of Vet. Anat.* 19 (2) pp 7-10.
- 14-Shukla P, Bhardwaj RL, Rajesh R. Histomorphology and micrometry of testis of chamurthi horse. *Indian J. of Vet. Anat.* (2013); 25 (1) pp36-38.
- 15-Moonjit P, Adcharatt S. Histological structure of testes and ductus epididymis of Rusa deer (*Cervus timorensis*). *Kasetsart J. Nat. Sci.* (2007); (41) pp 86-90.
- 16-Swunnsrne EE. Structural composition of shorthorn Bull Testes and daily spermatozoa production as determined by quantitative testicular histology. *Can. J. Animal Sci.* (1966); (46) pp 108-119
- 17-Elzoghby EMA, Sosa GA, Mona NAH. Postnatal development of the sheep testis. *Benha Vet. Med* (2014); (2) 26 pp186-190
- 18-Tripathi UK, Chhillar S, Kumaresan A, Aslam MKM, Rajak SK, Nayak S, Manimaran A, Mohanty TK, and Yadav S. Morphometric evaluation of seminiferous tubule and proportionate numerical analysis of Sertoli and spermatogenic cells indicate differences between crossbred and purebred bulls, *Vet. World* (2015); 8(5) pp 645-650.
- 19-Riviers D, Williams MJB. Testes development and production of spermatozoa in the cockerels (*Gallus domesticus*). *Reprod. Bio. of Poultry.* Longman, Harlow, (1984); pp.183- 202.
- 20-Mohammed AHS, Kadium DAH, Ebed AK. Some morphometric and histological description of the seminiferous, striaghted and rete testis tubules in the testis of indogenous male goats (two years old). *Kufa J. For Vet. Med. Scie.* (2011); (1) 2 pp 19-29
- 21-Reichold WJ, Schimmel M. Quantitative morphology of the ovine seminiferous epithelium. *Anat-Anz* (1995); ( 177) pp19-32.
- 22-Pawar H, Worble K. The Sertoli cells of water buffalo (*Bubalus bubalus*) during the Spermatogenic cycle. *Cell. Tissue-Res.* (1991); (265) pp43-50.
- 23-Keer J. Functional cytology of human testis. *Baillieres Clin. Endocrinol. Metab.* (1992); (6)235-250.
- 24-Zayed A, Hifny A, Abou-Elmaged A, Worble K. Seasonal changes in the intertubular tissue of camel testis (*Camelus dromrdarius*). *Ann.Anat.*, (1995);177:199-212.
- 25-Ball PJH, Peters AR. *Reproduction in Cattle.* 3<sup>ed</sup> (ed). Black Well (2004); pp 13-15
- 26-Al-Zobaidy A. Anatomical and Histological study of testis and epididymis of Rabbits pre and post pubertal. MSc. thesis. Vet. Med. collage, University of Al-Qadisiyah (2009).
- 27-Archana Pathak RS, Katiyar2, DN Sharma December. Anatomy of the Mediastinum Testis of Goat (*Capra hircus*) - A Temporal Study. *Indian J. of Vet. Anat.* (2014); 26 (2) pp 115-117