



## Histological development study of testis and epididymis of Awassi sheep *Ovis Aries* at postnatal stages

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### Abstract

**Objective:** The purpose of this study was to provide a light on the rams' testicular structure at various ages.

**Materials and method:** 18 rams were used (6 prepuberty age 1-5 month; 6 Sexual puberty age 6-8 month and 6 Sexual maturity age 9-12 month).

**Results:** In each ages, a thick capsule encased the two testis. Tubules and interstitial tissue make up each testicular lobule. Multiple testicular cords were seen in the testicular parenchyma of aged 1-3 months. By the fourth month, there were very few cords. Not spermatogenesis started until the fifth month, the tunica albuginea becomes organized into outer and interior layers. The Sertoli cell was oval in shape had a euchromatic pyramidal nucleus; in the connective tissue separating adjacent tubules. The cuboidal epithelium line the rete testis had an acidic cytoplasm and spherical nucleus. Efferent ductules connected the cords to the ductus epididymis. At the 6–8 months, the testis began to mature, spermatogenesis, and some spermatozoa could observe inside the tubules. The excurrent duct system of the testis became well-organized in 9–12 months when well-developed spermatozoa were present in the lumen of seminiferous tubules. It included rete testis channels, straight tubules, and the segment of seminiferous tubules. Simple cuboidal epithelium bordered the straight tubules. Located on the basement membrane, the spermatogonia are tiny, spherical cells with black nuclei that responded to PAS. Primary spermatocytes are larger cells with often different chromatin. Because of their rapid second meiotic division, secondary spermatocytes are uncommon. Round, pale-nucleated cells called spermatids are gathered near the seminiferous tubule's lumen. Leydig cell was polymorphic with ovoid nucleus, the epididymis lined by pseudostratified epithelium with stereocilia.

**Conclusion:** At 9 to 12 months of age, the testis's structure resembled that found in other ruminant species; the seminiferous tubules have a large lumen and adult sperm.

**Keywords:** Rete testis, Convolutd Seminiferous Tubules, Epididymis, Leydig cell.

### Introduction

In males, the first ejaculation accompanied sperm release is seen as a sign of puberty. Puberty is influenced by a wide range of environmental factors, including diet and illnesses. Understanding the onset of puberty and sexual development is essential for effective reproductive management of domestic animals [1-3]. Sexual maturation is essential for choosing males within a breed, and its timing can have a substantial impact on an individual's reproductive effectiveness. Early sexual maturity improves the selection process by reducing the generation interval enabling early progeny testing. The age at which male sheep reach sexual puberty varies depending on age, season, breed, body weight, testicular development, and the increased effectiveness of endocrine gland secretions. It is noteworthy in this regard that the majority of sheep breeds are seasonal producers [4-8]. During the early postnatal stage in lambs, gonocytes centrifuge from their central location toward the base membrane, where they develop into stem cells and produce spermatogonia [9, 10]. Growth factors, cytokines, and gonadotropins interact intricately to control testicular development and function, as well as the hypothalamo hypophyseal gonadal axis. The start of spermatogenesis, which results in the creation of sperm as a result of the gradual rise in luteinizing hormone pulses, signifies the beginning of the prepubescent stage. In males, spermatogenesis is a continuous, methodical process that does not occur simultaneously in every seminiferous tubule [11, 14]. Collagen fibers which are organized in connective

tissue design parallel to one another make up the thick tunica albuginea of the testis, giving the tissue greater strength and less flexibility. This thickens on the dorsal side, dividing the organ into pieces called testicular lobes and creating the mediastinum testis [15]. Beyond that, testicular arteries and veins pierce this capsule, revealing a vascularized structure called the visceral part of tunica vaginalis, made up of blood vessels and connective tissue that contains fibroblasts [16, 18]. Determining the start of maturity in the ruminants requires knowledge of the several changes that take place in the testis' and its duct system's development. Depending on the reproductive organs' functional activity at different ages, the male animal goes through several androgenic phases. The accessible method for choosing a man for reproduction is to describe puberty and the associated alterations in the reproductive organs [19-22]. This research aims to provide insight into the ram's testicles as this is essential for providing data for other investigations.

### Materials and methods

**Animals:** 18 healthy rams were used in the study. They were split into three groups: six pre-puberty rams, aged 1–5 months; six sexual puberty rams, aged 6–8 months; and six sexual maturity rams, aged 9–12 months. The animals were given an overdose of ketamine and xylazine, and then they were killed. Their testes were directly removed by castration from a Tikrit city slaughterhouse when they reached adult age, and the testis taken for histological analysis [12].

**Histological methods:** many stains used after xylene for cleaning, paraffin for the an embedding, alcohol for dehydration, and a rotary microtome for sectioning [23].

**Statistical analysis:** A one way analysis of a variance (ANOVA) tests was used to examine the study at the 5% significant levels. Data were processed and controlled using social science statistical tools [24].

### Results and discussion

The current study found that the parenchyma of the testis at one month age had multiple solid cords, at two -three month ages, the number of solid cords decreased and was replaced by testicular cords; there was no spermatogenesis; and within these cords were three different types of cells: the first type rested on the basement membrane of the cords and had oval, darkly stained nuclei; the second type was in the lumen of the cords and had a spherical, centrally nucleus and acidophilic cytoplasm; the third type of cells was the sertoli cell, an elongated pyramidal cell with acidophilic cytoplasm and ovoid nuclei; and interstitial cells in the connective tissues between the cords (Fig.1), This conclusion is consistent with [17] in lamb, but it contradicted [25, 26] in goat, where it was discovered that the epithelium covering the seminiferous tubule was stratified at three months of age, and that kids with high body weight had spermatid-containing seminiferous tubules. Seminiferous tubules produce spermatozoa, Leydig cell produce testosterone in sexuality age [2, 24]. [27] note that because to the animals' distinct and constrained breeding season, which varies based on their geographic and genetic composition, ecological site, management techniques, and weather patterns all have an impact on these seasonal changes. Spermatogenesis is highest during the breeding season. Researchers agree that the season has a noticeable impact on both the appearance of the testicular tissue and activity of these cells. Sex hormones are essential for the completion of sexual development in male. The initiation of sexual desire is mostly dependent on testosterone, and variations in testosterone levels during the postnatal period can significantly affect the spermatogenesis process [2, 4].

Compared to the first month, there were much fewer solid cords at 4 months of age, and the majority of the cords contained lumina, the process of spermatogenesis begins when gonocytes undergo the mitosis and produce spermatogonia. When the intercellular substance of sexual cords liquates and the core cells break down, lumen of seminiferous tubules emerges (Fig.2). This foundation resembled that of the Black goats [28]. Parallel to the synthesis of fluid by the sertoli cell and the movement of fluid through interstitial tissue by the sertoli cells, lumen development mostly takes place after the blood test is placed. Each breed of lamb may have a different spermatogenesis start time. Elevated FSH levels promote the conversion of gonocytes into spermatogonia by acting through FSH receptor on the Sertoli cell [29, 30]. It has been suggested that testosterone in bull can cause Sertoli cell to differentiate [4]. The findings supported those of [5, 31, 32], who found sertoli cell might be identified by their distinct smaller size, irregular nuclei, and less tall columnar cells. Tunica albuginea more arranged into two layers during the fifth month; the inner layer included blood supplies, while the outer layer was fibrous. No spermatogenesis is started until this age, and the testicular cords looked like those from

the fourth month. The Sertoli cell had a pyramidal shape, an acidophilic cytoplasm, and a euchromatic nucleus. The mediastinum contained the rete testis cords, which were irregular tubules of different widths bordered with cuboidal epithelium that had an acidophilic cytoplasm and a central, spherical nucleus (Fig.3), many spermatocytes were found in every tubule at five months of age showed that when the majority of the seminiferous tubules were occupied by immature germ cell At this point, the testis' histological structure is similar to that of the bull's described by [3, 33].

At the 6–8 month, the testis matured, spermatogenesis began, and some spermatozoa were inside the seminiferous tubules. The Leydig cell that made up the interstitial tissue between seminiferous tubules were polymorphic and had an oval, eccentric nucleus (Fig.4). Similar to [6, 34, 35, 36], secondary spermatocytes first emerged between 7 and 9 months of age. The transformation of primary spermatocytes become secondary spermatocytes requires the luteinizing hormone - release of testosterone, which may have been promoted by the an enhanced luteinizing hormone receptor activity. According to histological investigations, these findings demonstrated the presence of spermatozoa in epididymal lumen [33, 37, 38]. In sheep, on the other hand, showed signs of puberty at 21 weeks of age, with spermatozoa in the different segments of epididymis [16, 39]. Lambs' sexual development is influenced by their environment; nutrition and the season of birth affects how long it takes for them to reach the sexual maturity [33-35].

The excurrent duct system of the testis became well-organized in the 9–12 month stage when well-developed spermatozoa in the lumen of the seminiferous tubules. It included straight tubules, rete testis channels lined with cuboidal epithelium, and the segment of seminiferous tubules, Collagen fibers and fibroblasts in the tunica albuginea, a capsule that makes up the testicle. The tubules seminiferous also have established interstitial tissue, vascular compartments containing Leydig cell, and visible support cells or sertoli with uneven shapes. Sperm cells that have grown as a consequence of spermatogenesis will approach the lumen of seminiferous tubules. The sperm cells will travel via the tissues of the rete testis and ductuli efferentes before arriving at the epididymis, where they will start to grow [37]. Spermatogonia were the first cells in the spermatogenic lineage. Primary spermatocytes, which are bigger cells with often distinct chromatin, are created during mitosis in the spermatogonia. Secondary spermatocytes are produced by the initial meiotic division of the larger main spermatocytes. Due to their rapid second meiosis and consequent haploid spermatid production, secondary spermatocytes are seen. Spermatids were spherical cells with a pale nucleus that were gathered near the seminiferous tubule's lumen [36]. also outlined in sheep spermatogenesis. Spermatic cords have a smaller diameter before puberty and a larger diameter during the sexual development (Table 1).

The epididymal ducts were lined with columnar pseudostratified with stereocilia. Along the efferent ductules, columnar ciliated cells proliferated toward the epididymal duct, exhibiting indications of apocrine secretion. Near the epididymal duct junction, a few tall, thin, black cells appeared in the epithelium. A smooth muscle surrounded efferent ductules, the frequency of these layers increased as one got closer to the epididymal duct. The subepithelial connective tissues exhibited alkaline

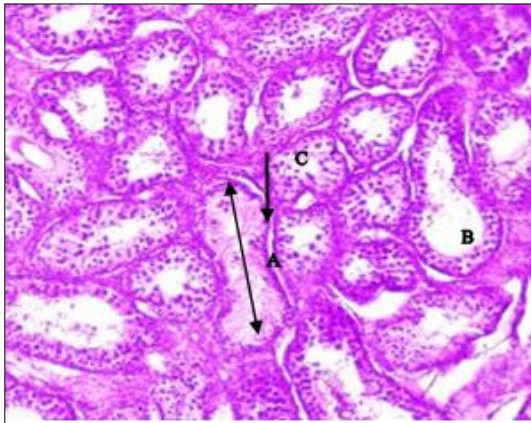
phosphatase enzyme reactivity. Throughout the whole epithelium, phosphatase enzymes exhibited high granular activity (Fig.5). The epididymis of mammals is thought to be an essential part of extra-testicular sperm route. It is a

tightly wound tube that forms a dense mass along the testicle's lateral side. Along with being the primary location for the sperm maturation, it is also where the sperm gain motility [6, 8, 9].

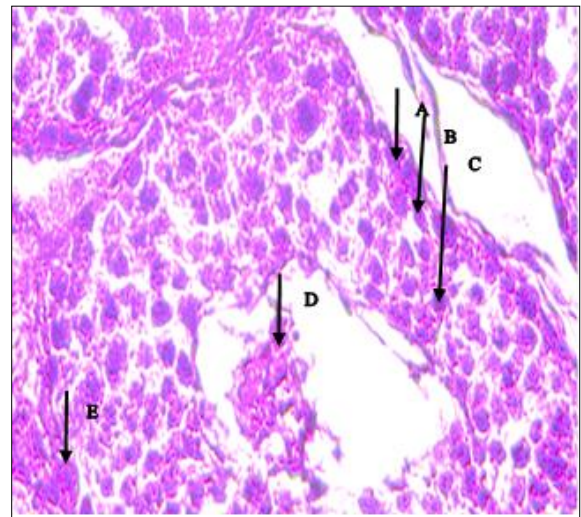
**Table 1:** Measurements each of testis and epididymis of ram,  $\mu\text{m}$

Measure Ages	Capsule of testis		Diameter of convoluted Seminiferous		Diameter efferent ductules	Diameter duct of epididymis
	Left	Right	Left	Right		
1-5 month	72.3 $\pm$ 0.2A	67.1 $\pm$ 0.3A	130.4 $\pm$ 0.3A	118.1 $\pm$ 0.2A	77.7 $\pm$ 0.3A	82.6 $\pm$ 0.1A
6-8 month	87.6 $\pm$ 1.1A	81.3 $\pm$ 0.2A	163.2 $\pm$ 0.4A	151.6 $\pm$ 0.1A	98.5 $\pm$ 0.5A	109.3 $\pm$ 0.4A
9-12 month	96.8 $\pm$ 0.1A	89.1 $\pm$ 0.1A	190.3 $\pm$ 0.1A	178.4 $\pm$ 0.3A	112.6 $\pm$ 0.8A	127.2 $\pm$ 0.5A

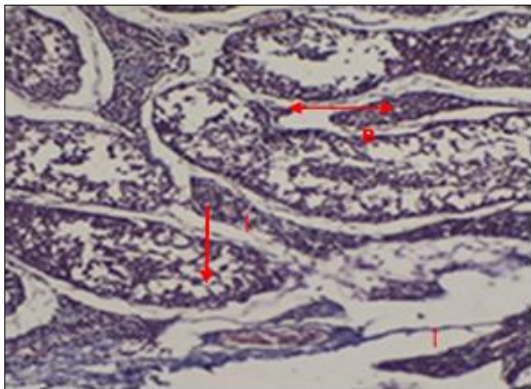
Values with capital letters in same columns denote to significant differences ( $P>0.05$ )



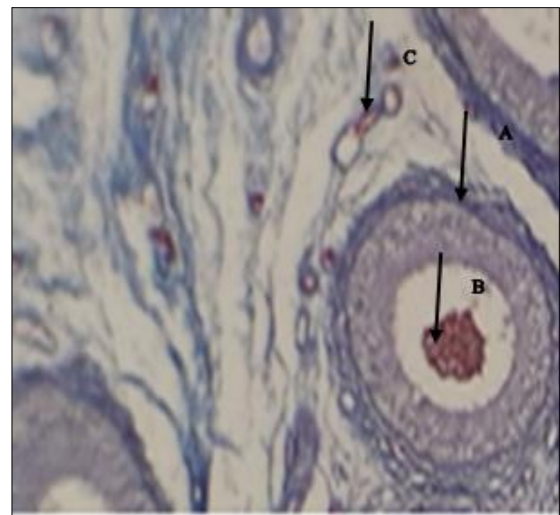
**Fig 1:** section of testis, seminiferous tubules (A), lumen of seminiferous tubules (B), connective tissue (C), H@E,4X.



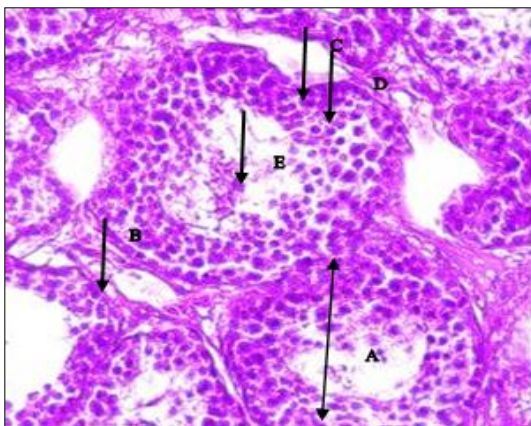
**Fig 4:** section of testis; primary spermatocyte (A), secondary spermatocyte (B), spermatids (C), adult Sperm (D), sertoli cell (E), Van gesion,400X.



**Fig 2:** section of testis; basement membrane (A), lumen of seminiferous tubules (B), connective tissue (C), Mallory 10X.



**Fig 5:** section of epididymis; basement membrane (A), sperm (B), blood vessel (C), Mallory,10X.



**Fig 3:** section of testis; seminiferous tubules (A), primary spermatocyte (B), secondary spermatocyte (C), spermatids (D), adult Sperm (E), H@E,20X.

**Conclusion**

Prepuberty, puberty, and maturity are the three phases of testis development. The hard rete cords first appeared in the mediastinum at pre-puberty phases, they had reached full maturity at puberty and maturity phases. Spermatogenesis is not started at Prepuberty ages, and the testicular parenchyma is made up solely of the spermatic cords that are coated contain prespermatogonia and sertoli cell. These cords are known as solid cords because lumens of cords are filled

with basophilic material, the bulk of the testis is filled with firm cords, which diminish in quantity as ram age, stages of spermatogenesis enabling the formation of spermatozoa are began at the age of 9–12 months, which is also the age of maturity. More spermatozoa are then produced by the testis.

### Funding

The author funds this study alone rather than through outside financing.

### Availability of data and materials

Data for the current study is available upon an adequate request.

### Competing interest

None of the writers have any conflicts of interest.

### Ethical consideration

Study conduct at Tikrit University in Iraq had been conducted in accordance with ethical standards.

### Author contributions

Every author makes an equal contribution.

### Acknowledgements

We would especially want to thank the Histology Department at Tikrit University's Veterinary Medicine College in Iraq for their hard work and dedication to our project.

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