Histological and anatomical study of the White Rooster of testis, epididymis and ductus deferens.

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Abstract

This study was carried out to investigate the histological and anatomical description of the male genital tract in Iranian Native White Roosters (IWR). The seminiferous tubules had a dense and compact organization. An average of 12 layers of cells was seen in the seminiferous tubules epithelium. The spermatogonal cell layer showed some variation between different birds in 1-3 layers; however, the spermatocyte, secondary spermatocytes and spermatids were arranged in 2, 3-4 and 3-5 layers, respectively. The testicles were oval in shape, placed on the left and right sides of midline, situated on the caudal aspect of the lungs and the ventral aspect of either the right or left kidneys, and the visceral surfaces of the left and right lobes of the liver. The epididymis was not divided into recognizable parts and was frontally attached to the corresponding testis on its dorsomedial aspect continuous with the ductus deferens. The ductuli efferentis were moderately large with pseudostratified columnar epithelium lined with three cell types, including ciliated, non-ciliated and basal cells. The connective ducts were covered with scare ciliated pseudostratified columnar epithelium. The epithelium of the epididymis was pseudostratified and columnar, embedded in a loose connective tissue. The proximal part of the ductus deferens was covered with pseudostratified columnar epithelium, which was continuous with simple cuboidal epithelium towards the distal portion. Anatomical studies revealed a large sac-like accessory process that consisted of two non-discrete parts in the frontoventricular region of the testes between the epididymis and the ductus deferens, which was closely related ventrally to the epididymis and laterally to the kidneys. This organ was lined with stratified cuboidal epithelium with thick clear mucosal muscle. The tunica sub mucosa was evident, and the outer layer was composed of a fibrous capsule with a well-developed vascular supply. This suggested that the role of the sac-like accessory process was as an organ to supply sperm, equivalent to the tail of the epididymis in mammals.

Introduction

Studies on the development of the genital system of the domestic fowl and variation in their gonadal size from hatching to sexual maturity have been of great interest to those in the poultry industry and other investigators (Parker *et al*., 1942; Bennet, 1947). Macroscopic anatomical aspects of the male fowl reproductive tract have been described by Kaupp (1915), Gray (1937), Parker *et al* and Lake, (1957), Marvan (1969), Lake (1971), Tingari (1971), Amer and Chain (1975), King (1986), while microscopic aspects were investigated by Aire (1979, 1980 and 1982). In these investigations, Aire successfully described the histology of the epididymis in the fowl *(Gallus domesticus)*, guinea fowl *(Numiela moleagris)*, quail *(Coturix coturinx Japonica)* and duck *(Anas Platyrhynchos)*. There are also reports on the microanatomy of male fowls *(Gallus domesticus)*, testicular tunica albuginea (Aire, 1979), seminiferous tubules (Marvan, 1969), rete testis (Aire, 1982; Tingari, 1980), ductus deferens and epididymis (Marvan, 1969; Lake, 1972; Aire, 1980), cloaca (Marvan, 1969), and testicular interstitial tissue (Amre *et al*., 1970). Mercadante *et al*. (1982) carried out investigations on the analyses of the anatomy of the genital organs of the male pigeon *(Colombia livia)*, including the testis, epididymis, ductus deferens and the copulatory organ.

According to Lake (1971), the pampiniform plexus, which is typical in mammalian species, does not exist in birds.

Until the 1950s, a few studies have reported that there is a correlation between testicular growth and the body weight of fowls (Kumaran *et al*., 1949), however, Marvan (1979), Tingari *et al*. (1980) and Aire (1982) have also tried to establish a correlation between the sexual maturity of fowls, testicular development, testicular weight and the age of birds. Artoni (1993) described the testicular microanatomy and morphometry in quails *(Coturnix coturnix japonica)* and established the annual testicular cycle in this bird. Hess et al. (1976) described the ductus succession from the seminiferous tubule to the ductus deferens papilla, as well as the microanatomy of the epididymal region and the ductus deferens in the turkey *(Meleagris gallopava)*. Wyandotte reported the growth of the testis with the use of organ weight and histological analyses through measurement of the diameter of the seminiferous tubules. Vehrencamp (1982) carried out measurements of the diameters of the seminiferous tubules of Crothophaga sulcirostris and correlated these parameters with age.

The aim of this study was to investigate microanatomical and macroanatomical features of the reproductive organs of the Iranian white rooster, which is one of the most reared birds in the world.

Materials and Methods

Ten mature and apparently healthy Iranian Native White Roosters were used in this study. Initially, the animals were anesthetized with ketamine and euthanized using of CO2 gas. The reproductive organs, including the testis, ductus deferens and epididymis of the birds were dissected and placed into 10% formalin solution for fixation.

Preparation for light microscopy

Specimens were processed through paraffin embedding and cut into 5- 7 µm sections, stained with the iron-Weigert and hematoxylin and eosin techniques. In this study, semiserial sectioning was adopted.

For the quantification of cells and their dimensions, we used a morphometric lens device (Olympus, Germany). The dimensions were expressed in µm. We adopted the technique of Ozen *et al*. (2007), for morphometric studies.

Statistical analyses

Data were analyzed using two ways Analysis of Variance followed by Duncan's multiple range test. The SPSS software (version 15) was used.

Results

This histological study revealed that the tunica albuginea of testes is made up of collagenous connective tissue. The course of the testicular arterial branches passes along those of anastomosing veins, which constituted the vascular layer of this connective tissue (Figure 1). The seminiferous tubules were found to have a dense and compact organization within the testicular tissue. The spermatogenic lineage, including spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa were observed in the epithelium of seminiferous tubules. Along these cells, sustanticular (Sertoli) cells, also observed. On average, 12 layers of cells were seen in the epithelium of seminiferous tubules. The spermatogonal cell layer showed some variation between different birds and these cells were arranged in between one and three layers' however, the spermatocytes (A and B), secondary spermatocytes and spermatids were arranged in 2, 3-4 and 3-5 layers respectively. The interstitium of the testes was made up of loose connective tissue, which contained Leydig cells in close vicinity to the arterioles and capillaries. The mode of distribution of these cells was not uniform. A relatively high population of mononuclear immune cells, fibroblasts and fibrocytes were observed in close proximity to the blood vessels (Figure 2 A, B and C). The iron-Weigert staining technique revealed myofibroblasts around the seminiferous tubules (Tables 1 and 2).

The right and left testicles were found to be oval in shape, and placed to the left and right sides of midline.

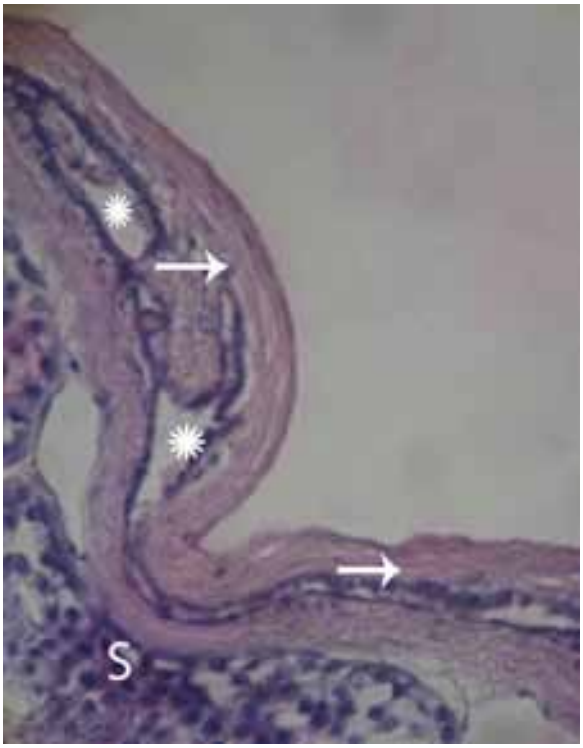


Figure 1: Tunica albuginea, note the arteriole branches (*) between intensive collagen fibers (arrow), S seminiferous tubule. Hematoxiline and eosin staining. X100.

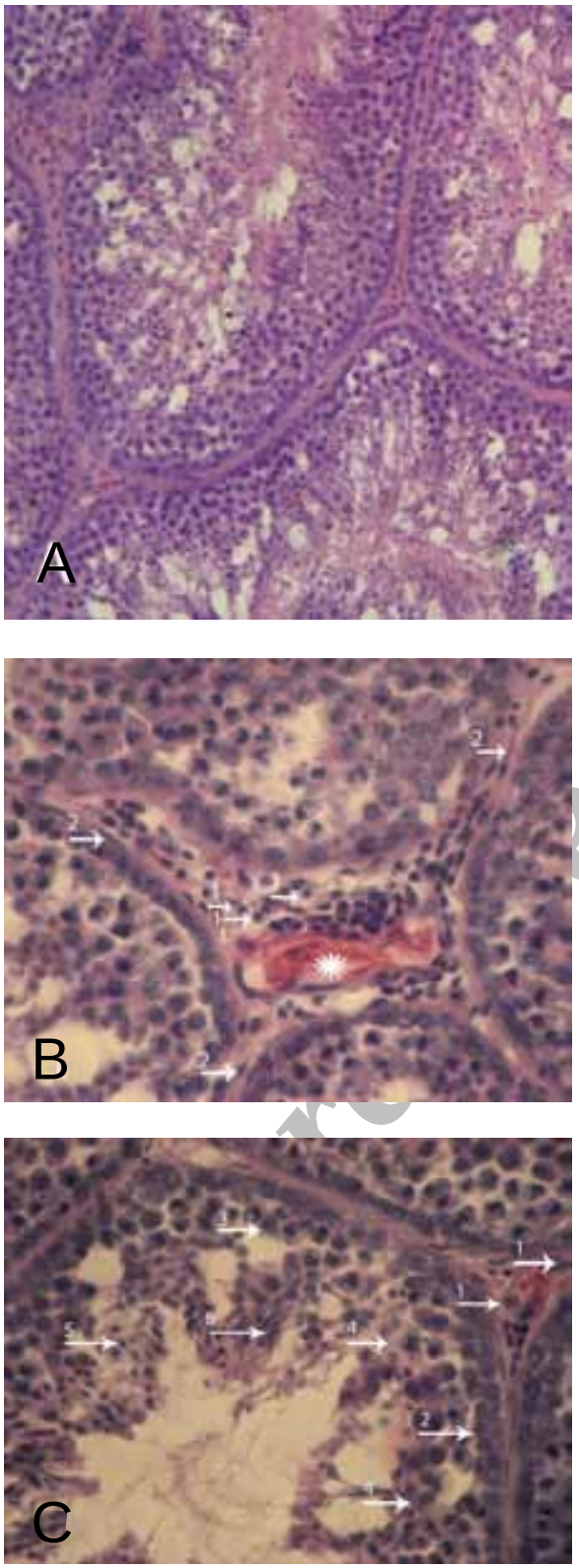


Figure 2 : compactly seminiferous tubules A , note figure B myoid cells (2 arrows), blood vessels 1 (*) ,leydic cells (1),figure C indicating leydic cells (1), spermatogonea cells (2) , sertoli cell(3), spermatocytes(4), spermatid (5) and spermatozoids (6). Hematoxiline and eosin .X100 (A) .X400 (B, C).

Table 1: comparative morphometric analysis of testis of IWR (mean±SD)

Parameters	Morphometry (µm)
Seminiferous tubule diameter	162.07±4.74
Seminiferous tubule Epithelium	96.73± 2.83
Tunica albuginea	12± 0.35
Interstitial connective tissue	12.54±0.63

Table 2: Comparative cells number and percent of seminiferous tubules of IWR (mean±SD).

Parameters	Cells	Cell percentage
Spermatogonia	184±25.573	9.25 ± 0.003
Spermatocyte I	364 ± 48.843	18.31 ± 0.005
Spermatocyte II	728± 97.685	36.63 ± 0.010
Spermatid	717.5 ± 133.475	36.12 ± 0.008

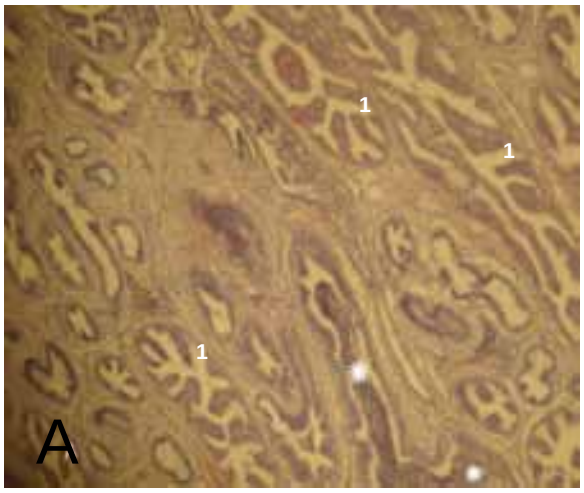
Table 3: Length, width and thickness of right and left testis of IWR (mean±SD).

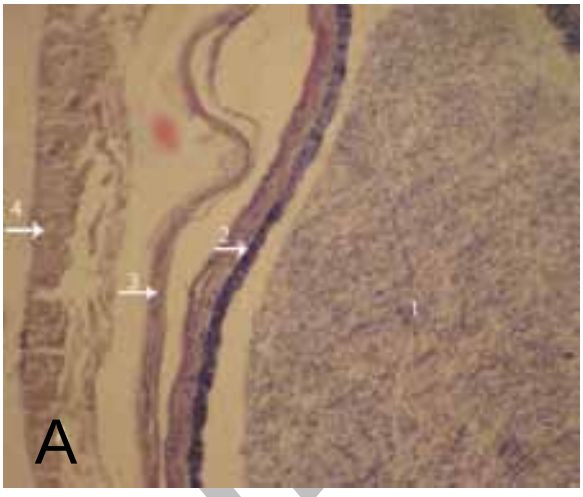
Parameters	Length (µm)	Width (µm)	Thickness (µm)
Right testis	4.9±0.14	2.10 ±0.08	2.20 ±0.09
Left testis	4.43±0.18	10.94±0.08	2.15±0.09

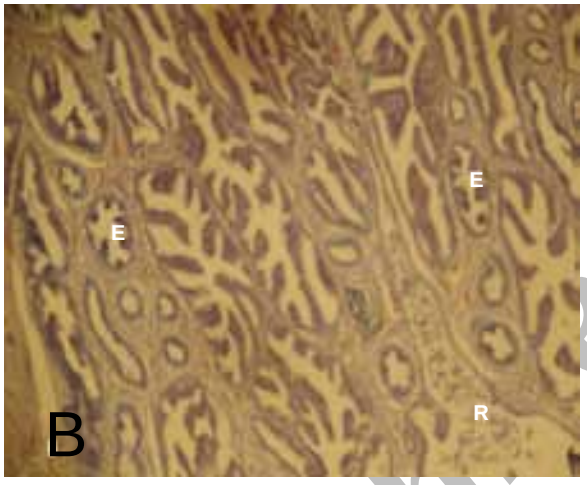
The topographic relationship of these organs was as follows: they were situated on the caudal aspect of the lungs and the ventral aspect of the right and left kidneys, and the visceral surfaces of the left and right lobes of liver. The macromorphometric evaluation of the right and left testes did not reveal significant differences; however, in some cases the right testis had relatively predominant dimensions (Table 3).

Histological investigations showed that the epididymis had no discrete compartments. Instead, its body (without head, body and tail regions) was proximally attached to the corresponding testis on the dorsomedial aspect and continuous with the ductus deferens. The latter organ ran on median line of body dorsomedial to the testis and its mean length was 3 cm. Other structures were evident, including the rete testis, ductuli efferentis, connecting the ductules and ductus epididymis. The ductuli efferentis were connected to the rete testis and ended at connecting ductules. The connecting ductules were connected to the ductus epididymis at several places. The ductus epididymis continued with a highly convoluted ductus deferentis. The epithelium of the rete testis was simple squamous or low cuboidal, but abrupt changes into simple columnar were found at the beginning of the ductuli efferentis. The ductuli efferentis were moderately large, with average diameter of 98-103 µm. Their epithelial type was pseudostratified columnar and the height of the cells was 19-21 µm. The epithelium was lined with three cell types, including ciliated, non-ciliated and basal cells. Intraepithelial mononuclear immune cells, such as lymphocytes, were observed in the ductuli efferentis and the epididymis. The evidence of secretary activity was revealed by the observation of blebbings on the surface of cells.

The connective ducts that were the smallest among the ductal system were covered with pseudostratified







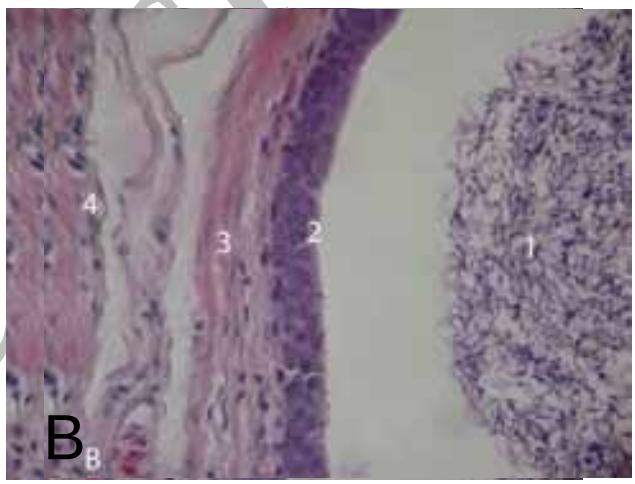


Figure 4: low magnification of ductus deferens figure A, note sperms in lumen (1), pseudostratified columnar epithelium (2), mucosal muscle (3), tunica mascuaris (4). High magnification figure B. Hematoxiline and eosin. X 100 figure A. X400 figure B.

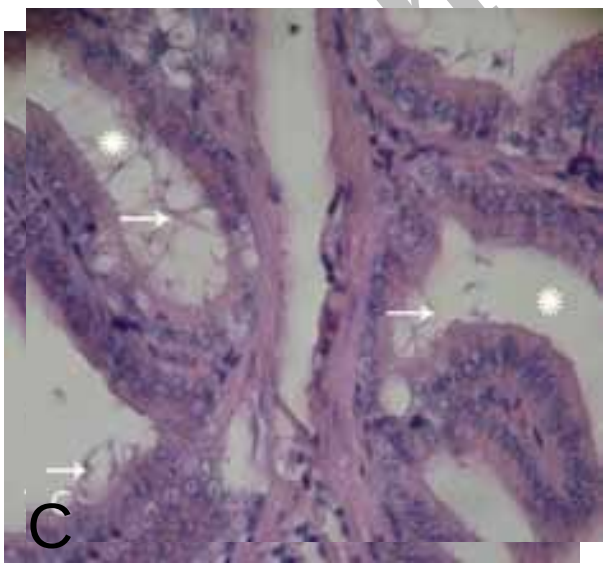


Figure 3: epididymis A, low maginification. Note (stars) showing connective duct. Figure B, note epididymal ducts (*E*), rete testis *(R)*. Figure C, higher magnification of ductuli efferent highly foldes columnar epithelium, (stars) lumen, ciliated cells (*arrows*). Hematoxiline and eosin. X 40 (A). X100 (B). X 400 (C).

Table 4: Mophometry of ductus deferens various layers (mean±SD).

Parameter	µm
Tunica Mucosa	21.56 ± 0.63
Tunica sub mucosa	89.57±2.62
Tunica masculais	188.1±5.51

columnar epithelium. Some cilia were observed at the top of those cells, but these were very scarce. The histological features of the epithelium were similar to those of the connective ducts, but their diameter was comparatively wider. The epithelium of the epididymis was pseudostratified columnar and it was embedded in a loose connective tissue. Its diameter was also smaller than the efferent ductules (Figures 3 A, B and C).

Our histological study revealed that the ductus deferens was covered with pseudostratified epithelium with mean height of 22 µm. The wall of the ductus deferens was made up of loose connective tissue. The

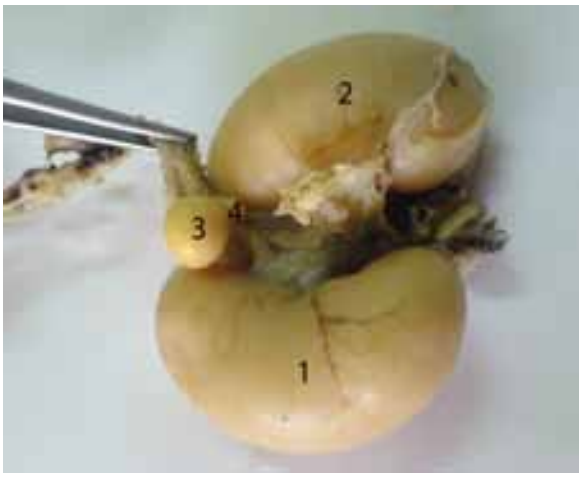


Figure 5: Macroscopic view of testes. Note elongated testes (1, 2) and sac – like accessory process (3, 4) located between epididymis and ductus deferens. Note two not discrete compartment dorso caudally to the testes.

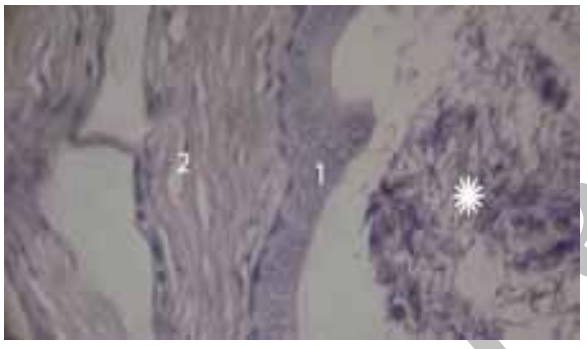


Figure 6: Sac like assecorry organ, stratified coboidal epithelium (1), dense connective tissue (2) and sperm content (*). Hematoxiline and eosin .X400 (A).

Table 5: Comparative morphometric analysis of various layers of sac – like accessory process (mean±SD).

Parameters	Morphometry (µm)
Epithelium	26.31±4.628
Mucosal muscularis	33.02±11.008
Tunica mucosa	108.39±13.161
Tunica serosa	43.35±7.607
Fold	120.55±26.923

population of basal cells in the epithelium was higher in the ductus deferens than the epididymis. Distally, the epithelium of the ductus deferens gradually changed to a simple cuboidal type and the thickness of the wall increased accordingly because of an increase in the amount of muscular tissue. The terminal portion of the ductus deferens, the so-called ejaculatory duct, was opened into the urodeum via a small, round erectile papilla. The lamina propria was located on the other perimeter of the epithelium, in which numerous blood-filled sinuses was seen. A well-developed muscular layer was present in the wall of the ductus deferens. Intraepithelial lymphocytes were observed in this region too. In some regions of ductus deferens, a mucosal fold was seen, but at the terminal portion, theses folds were completely lost. Consequently, the diameter of the ductus deferens increased drastically at these points. Inside the ductus deferens, spermatozoa were frequently observed (Figures 4 A and B).

The internal epithelium of the papilla was identical to that of the receptaculum, being pseudostratified columnar type which was composed of light and dark columnar and basal cells. The data regarding to the morphometric parameters of the ductus deferens is depicted in Table 4. Our anatomical study revealed a moderately large sac-like accessory process in the frontoventricular region of the testes, between the epididymis and ductus deferens. It was closely related ventrally to the epididymis and laterally to the kidneys. This process consisted of two non-discrete parts. The epithelium of the organ was composed of stratified cuboidal cells with thick clear mucosal muscle (Figure 5 and 6). The tunica submucosal layer was evident, and the outer layer was made up of a fibrous capsule with well-developed arterioles and capillary supply (Table 5).

Discussion

The entire reproductive systems of the birds are necessary for breeding, but the testes, epididymis and ductus deferens are the most important functional regions. The male reproductive system in male birds consists of the testes, epididymis, ductus deferens, ejaculatory region and mating organ. Lately, researchers have taken into consideration studies on birds since they represent an excellent nutritional source. There are several classical descriptions of the male reproductive tract, which aim at establishing a correlation with shape, testicular size, age and sexual maturity. However, roosters such as game and fantastic birds have not been sufficiently investigated anatomically and histologically. Therefore, this present study aimed to investigate the reproductive system in Iranian white roosters.

The tunica albuginea is a solid capsule of dense irregular connective tissue. It consists predominantly of collagen fibers, a few elastic fibers, and myofibroblasts that meander along the branches of the testicular artery; a network of anastomosing veins constitutes the vascular layer of the tunica albuginea (Dellman *et al*., 2006; Ozegbel *et al*., 2007). Similarly to the findings of Ozegbel *et al*., our results showed that the tunica albuginea of the Iranian white rooster contained more collagen fibers than smooth muscle cells, which is similar to the ostrich and emu. There were advanced branches of the vascular tree among the collagen fibers of the tunica albuginea. Histological observations showed that there were considerable nervous trunks near to the arteriolar branches located in the subcapsular region. In comparison to other birds,

these nerve trunks were very advanced. The intertubular space of the testis contains loose connective tissue, blood and lymph vessels, fibrocytes, free mononuclear cells and interstitial endocrine (leydig) cells (Stechell *et al*., 1980), but there are no connective trabecules to divide the testis into lobules (Rezaian, 2006). In the light of the findings of Rezaian and Stechell, our study demonstrated the high density of the seminiferous tubules, while, in some cases, the interstitial connective tissue between seminiferous tubules was extensive.

The convoluted seminiferous tubules *(Tubui seminiferi convuluti)* in most animals are tortuous two–ended loops with a diameter that ranges between 150 and 300 µm. They are lined by stratified spermatogenic epithelium (germinal epithelium), surrounded by the lamina propria and connected at both ends to straight testicular tubules by a specialized terminal segment (Dellman *et al*., 2006; Lake, 1957; Lake, 1971). In this study, the seminiferous tubules diameter was evaluated 162 µm in diameter with highly active spermatogenic epithelium.

Normal turkey testes are long (6-7 cm), soft to the touch and have gross characteristic similar to those described for the chicken testis (Tingari, 1971); in this study, the testes were 4-5 cm in length. A comparative analysis of the length of the right and left testis showed that, up to 24 weeks of age, the left testis was longer in most cases. Our results identified that testes are same in length, while the left testis wide (2.10 ±0.08 cm) was significantly less than the right (10.94 ± 0.08 cm).

Light microscopy confirmed the presence of a large number of ducts located posteriomedial to each testis. The intracapsular rete testis consisted of a rather dilated series of tubules embedded within the connective tissue of the tunica albuginea inside the testis (Barker and Marion, 1980). The high volume of connective tissue in the domestic fowl epididymis is largely attributable to the large number of mononuclear cells and lymphoid nodes scattered erratically in the periductal tissues throughout the epididymal region in the quail and guinea fowl (Aire, 1978). In similar findings to those of Aire and Barker, a high degree of separation of the ducts was seen embedded in the connective tissue and, furthermore, numerous mononuclear immune cells were scattered in the connective tissue. Additionally, interepithelial lymphocytes were presented in the lining epithelium of the ducts.

In the histological part of this study, moderate scattering of nervous trunks were demonstrated in the connective tissue. There were no significant interspecies differences in the proportion of the rete testis and blood vessels (Budras and Sauer, 1975; Aire, 1978). Barker and Marion (1980) reported that in quelea, the intracapsular rete testis were lined by a simple low cuboidal or squamous epithelium, which corroborated previous findings with regards to the Iranian White Rooster that showed the rete testis was lined by simple squamous and cuboidal epithelium, which changes abruptly into columnar cells at the beginning of the ductuli efferentes. The epithelium of the rete testis in turkeys is 1.5 – 12 µm in height. In this study, light microscopic analysis showed that epithelium was 1.8 – 15 µm in height. Most of the epididymis is made up of efferent ductules in chickens (Tingari *et al*., 1971), in the Japanese quail (Aire, 1979) and in avians (Aire, 1978). These authors reported that cross-sections of the efferent ductules appeared to show that these were the commonest and largest ducts in the epididymal region. Our results agree with those, which suggest that the efferent ductules play a particularly critical and important role in the epididymal region of birds. The testes of the birds contain large amounts of fluid, and the absorption of the larger part of this fluid would be an important function for highly developed efferent ductules to perform (Lake, 1957; Aire, 1978). We believe that the data from our anatomical study supports the hypothesis that this is one of the functions of the efferent ductules in Iranian White Roosters.

The area taken up by the ductus epididymis in the chicken was significantly higher than in the quail and guinea–fowl (Aire, 1979). Tingari (1971) observed that the chicken has a tortoise-shell shaped ductus epididymis. In this study, the proportion of the ductus epididymis was almost the same as the results of studies in chickens. The epididymis of the cock is divided into a main part and an appendix epididymis (Budras and Sauer, 2004), while in the present study there were no discrete compartments. Several works agree with our results with regards to the epididymis size and position and the ductus deferens site and tortuousity (Lake, 1957).

In the histological study of the ductus deferens in the turkey, the epithelium is pseudostratified columnar. Outside the epithelial lining, there was a thick layer of smooth muscle and the lamina propria included blood vessels (Parker *et al*., 1942; Aire, 1979). In corroborating those reports, the ductus deferens of Iranian white roosters was shown to be lined by pseudostratified columnar epithelium with a high distribution of mononuclear cells intraepithelially. Unlike findings in the turkey, the lamina propria was very thin and there was an advanced mucosal muscle covering the lamina propria and dens connective tissue presentation demonstrated in the ductus deferens of the Iranian white rooster. The tunica mascularis was only found to have a single layer in the ductus deferens. In comparison with other wild and domestic birds, our observations demonstrated the presence of sac-like accessory process with a wide lumen and high concentration of spermatozoa and stratified cuboidal epithelium lined with basophilic basal layer cells. There was also a wide layer of mucosal muscle that suggested that the ductus

deferens also had a role in conducting fluid produced by the testis and epididymis to the ductus deferens. Therefore, the sac-like accessory process may act as an organ to supply sperms, in an analogous manner to the function of the tail of the epididymis in mammalians.

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