



■ Author(s)

Althnaian TA¹  <https://orcid.org/0000-0002-7025-1847>

¹ Department of Anatomy, College of Veterinary Medicine, King Faisal University, P.O. Box 400, Al-Ahsa 31982, Saudi Arabia.

■ Mail Address

Corresponding author e-mail address
Thnaian A. Althnaian
Department of Anatomy, College of Veterinary Medicine, King Faisal University, P.O. Box 400, Al-Ahsa 31982, Saudi Arabia.
Phone: +966558122112
Email: talthnaian@kfu.edu.sa

■ Keywords

Al-Ahsa rooster, testis, epididymis, vas deferens, morphology.



Morphological Studies on the Testis, Epididymis and Vas Deferens of Al-Ahsa Native Rooster

ABSTRACT

This study was carried out to investigate the morphological and histological structures of the testis, epididymis and vas deferens of the Al-Ahsa native rooster (ANR).

There were two types of ANR; the brown feather one with light yellow shank and the black feather one with grey or dark grey shank. Their body weight was 1840.88 ± 92.13 g and 1555.66 ± 82.83 g, respectively. The morphology of the testes showed that the black rooster has larger testes than the brown rooster and there was asymmetry in size between the right and left testis in both. They were grey yellowish in color and oval-shaped, situated in the abdomen dorsal to the proventriculus, the liver and the gizzard, cranial to the lungs, caudal to the abdominal sac and ventral to the kidneys. The histology of the testes revealed the capsule, the different cells of the lining epithelium of the seminiferous tubules and the interstitial tissue. The morphology of the epididymis was revealed pseudostratified columnar epithelium, light brown in color with c to L-shaped, located cranial to the testis and extended caudally to continue with vas deferens. The latter has columnar epithelium, light grey in color, run caudally medial to the kidneys and opened in the cloaca.

INTRODUCTION

Native chickens play a major role in the rural sector aspects of smallholder communities in Saudi Arabia. Previous studies had been done on the native chicken breed because they were resistant to a wide range of infectious diseases and high environmental temperature (Al-Yousef, 2007; Al-Moshawah, 2015; Fathi *et al.*, 2017).

The morphological structures of the male fowl reproductive tract have been reported for a long time by Kaupp (1915), Gray (1937), Burrows & Quinn (1937) and other authors. Recently, Maruch *et al.* (1998), Madekurozwa *et al.* (2002), Simões *et al.* (2004), Aire & Ozegbe, (2007), Razi *et al.* (2010) have described the anatomical and histological structures of the testis, epididymis and vas deferens in many species of birds.

However, there were scarce information on the morphological structure of the local Saudi chicken. Therefore, the aim of this study was to investigate morphological and histological structures of the testis, epididymis and vas deferens of the Al-Ahsa native rooster (ANR) at the Kingdom of Saudi Arabia related to their morphological features.

MATERIAL AND METHODS

The study was carried out in healthy fowls, ANR, which was obtained from the Poultry Unit at the Research Station, King Faisal University,



Al-Ahsa, Kingdom of Saudi Arabia. All sampling and experimental measurement were approved by the King Faisal University Animal Care and Use Committee (KFU-ACUC).

For morphological measurements, a digital balance was used for weighing 35 roosters and their organs which include testis, epididymis and vas deferens. The length of these organs was measured in 35 birds and the mean \pm SD was calculated. The data were analyzed using SPSS software (version 15).

For histological study, tissue samples from testis, epididymis and vas deferens of these roosters were fixed in 10% neutral buffered formalin. The tissues were processed routinely as described by Bancroft & Stevens (1990) and embedded in paraffin wax. Sections of about 5 μ m thick were cut by a rotary microtome. Then, Haematoxylin and Eosin (H&E), and trichrome stains were used for the general histology. The histological slides were visualized with a light microscope (Microscope Leica DM6000 B, Germany).

RESULTS

These studies were done in two types of ANR. The mean of the total body weight and their morphological

appearance were recorded in Table 1. The first type was characterized by brown feather, light yellow shank, and single, single opened or duple opened comb with bodyweight 1840.88 ± 92.13 gram (Fig.1a). Whereas the second type features were; black feather, grey, or dark grey shank with a presence of three different shapes of a comb (single, single opened, or duple opened) and weighing 1555.66 ± 82.83 gram (Fig.1b).



Figure 1a – The brown feather Al-Ahsa native rooster; has a light yellow shank and duple opened comb.

Table 1 – Showing the mean \pm SD of total body weight and classification of the brown and black Al-Hasa native rooster chicken according to comb type and shank color in 15 brown and 20 black Rooster.

	The mean \pm SD of total body weight	Comb Type			Shank Color		
		No. of Single comb	No. of Single Opened comb	No. of Duple Opened comb	No. of Grey	No. of Dark Grey	No. of Light Yellow
Brown	1840.88 ± 92.13	5	3	7	0	0	15
Black	1555.66 ± 82.83	4	6	10	6	14	0
Total		9	9	17	6	14	15



Figure 1b – The black feather Al-Ahsa native rooster; has a grey shank and single comb.

The means of the length, width, and thickness of the right and left testes, epididymis, and vas deferens

in the brown and black ANR were shown in Table 2. While the mean weight of the testis and epididymis of both native roosters was recorded in Table 3.

Generally, the black rooster has larger testes than the brown one. Moreover, in both types, there was a difference in size between the right and left testis with the left being the biggest (Table 2). The morphology of the testes showed shining fading grey-yellowish color and oval shape. They were located in the abdomen on the left and right sides of the midline; their cranial pole was placed caudally to the lungs, while the caudal pole was related to the abdominal sac. Their dorsal surface presented ventrally to the right and left kidneys. Whereas, their visceral surfaces were placed on the proventriculus, the left and right lobes of the liver, and gizzard from cranial to caudal respectively (Fig. 2).



Figure 2 – The testis (T) is located dorsal to the proventriculus (P), the left and right lobes of the liver (L) and gizzard (G) from cranial to caudal, respectively.

Table 2 – The mean \pm SD of the measurements of testis, epididymis and vas deferens of Al -Hasa native 35 brown and black rooster chicken.

Color	Sample	(Right)			(Left)		
		Length	Width	Thickness	Length	Width	Thickness
Brown	(Testis)	3.65 \pm 0.58	2.14 \pm 0.28	1.86 \pm 0.25	3.78 \pm 0.40	2.30 \pm 0.31	2.02 \pm 0.32
Black	(Testis)	4.23 \pm 0.27	2.31 \pm 0.23	1.93 \pm 0.18	4.07 \pm 0.36	2.29 \pm 0.28	2.04 \pm 0.18
Brown	(Epididymis)	2.18 \pm 0.13	0.42 \pm 0.08	0.38 \pm 0.09	1.87 \pm 0.29	0.51 \pm 0.09	0.37 \pm 0.08
Black	(Epididymis)	1.65 \pm 0.16	0.48 \pm 0.09	0.40 \pm 0.09	1.78 \pm 0.19	0.45 \pm 0.07	0.42 \pm 0.08
Brown	(Vas Deferens)	9.40 \pm 0.83	0.28 \pm 0.07	0.25 \pm 0.05	9.63 \pm 0.95	0.22 \pm 0.09	0.23 \pm 0.06
Black	(Vas Deferens)	10.09 \pm 0.65	0.28 \pm 0.07	0.30 \pm 0.09	9.95 \pm 0.72	0.29 \pm 0.06	0.35 \pm 0.07



Figure 3 – The vas deferens (V) is running caudally medial to the kidneys (K) and opened in the cloaca. Testis (T).

The epididymis in the brown rooster was large than that of the black rooster (Table 2), it was light brown in color and c to L-shaped. The cranial part of it was attached to the adrenal gland, while the caudal part is thick and continued with vas deferens. There were no significant differences in the length of the vas deferens in brown and black roosters and also between the right and left ones (Table 2). The vas deferens was light grey in color, run caudally medial to the kidneys, and opened dorsolateral in the cloaca (Fig. 3).

The histological structure of the testis showed that the tunica albuginea consisted of connective tissue collagen fibers and contained blood vessels, which formed the vascular layer (Fig. 4).

Table 3 – The mean \pm SD of the testis, epididymis weight of Al -Hasa native 35 brown and black rooster chicken.

Color	Testis		Epididymis	
	Right	Left	Right	Left
Brown	11.89 \pm 0.88	9.60 \pm 0.60	0.26 \pm 0.09	0.23 \pm 0.08
Black	11.03 \pm 0.84	10.18 \pm 0.82	0.24 \pm 0.08	0.22 \pm 0.07

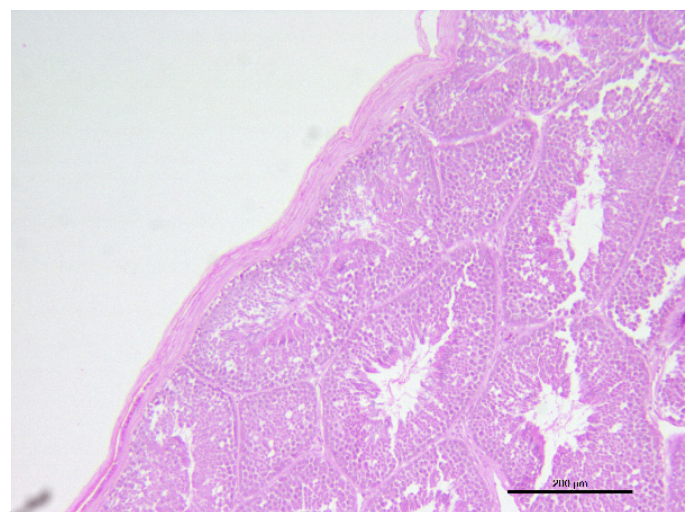


Figure 4 – The tunica albuginea (TA) consisted of collagen fibers and contained blood vessels (bv). (Bar= 200 μ m).

In both roosters, the seminiferous tubules were different in shape and size and filled the parenchyma of the testis. The seminiferous tubule was lined by 9- 12



layers of epithelial cells which include; spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids, and spermatozoa from the basement to the lumen of the tubule, respectively (Figs. 4, 5). The primary spermatocyte was the largest cell, whereas the spermatid was the smallest cell. Sertoli cells were located between primary spermatocytes, they have eccentric round nuclei (Fig. 5). The interstitial tissue of the testis was formed of connective tissue that contained Leydig' cells, which had oval nuclei, near the blood capillaries (Fig. 5).

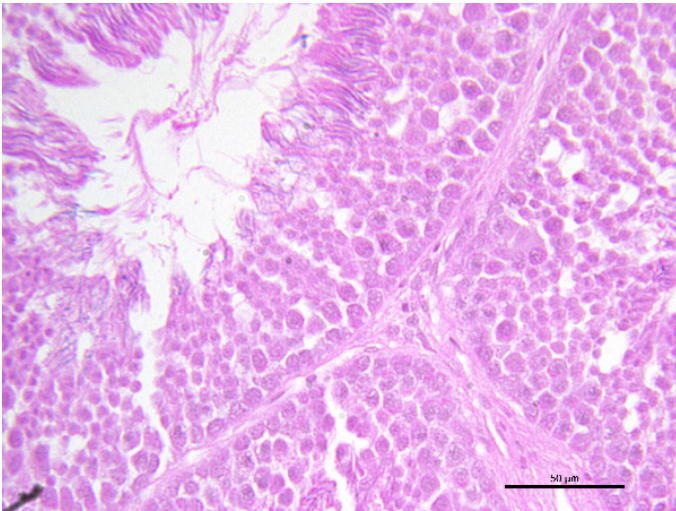


Figure 5 – The seminiferous tubule is lined by 9- 12 layers of epithelial cells which include; spermatogonia (1), primary spermatocytes (2), secondary spermatocytes (3), spermatids (4) and spermatozoa (5). Sertoli cells (S) between primary spermatocytes have eccentric round nuclei (Fig. 5). The interstitial tissue (IT) contained Leydig' cells (L). (Bar= 50 µm).

Histologically, the epididymal ducts were varied in shape and size. They had folded pseudostratified columnar epithelium, their lumen filled with spermatozoa. These ducts were surrounded by loose connective tissue (Fig. 6).

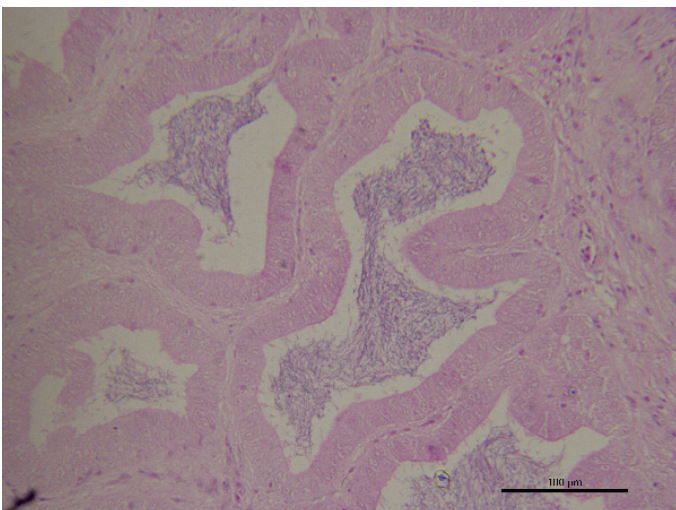


Figure 6 – The epididymal ducts are varied in shape and size, lined by pseudostratified columnar epithelium, their lumen filled with spermatozoa (sp). (Bar= 100 µm).

The microscopic structure of the vas deferens revealed irregular lumen lined with columnar epithelium, a loose connective tissue in the lamina propria, an incomplete inner longitudinal layer of smooth muscle, whereas the outer layer was completely circular. The tunica serosa had loose connective tissue that contained blood vessels (Fig. 7).

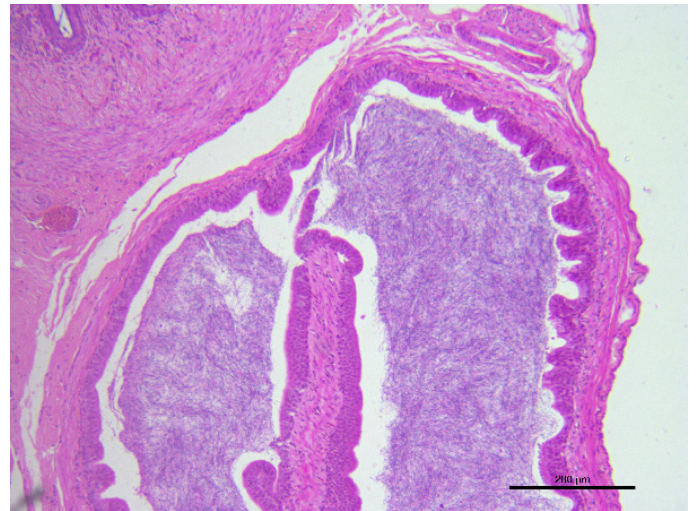


Figure 7 – The vas deferens showing irregular lumen lining with columnar epithelium. (Bar= 200 µm).

DISCUSSION

The male reproductive system of chicken consists of the testes, epididymis, ductus deferens, ejaculatory region and mating organ. There are several studies in the morphological and histological structures of the testes, epididymis and ductus deferens of the rooster for the importance of these organs. However, there is a paucity in the literature about the study of the local breed roosters in Saudi Arabia.

The present study has represented two types of ANR breeds; the first one has a brown feather, light yellow shank and single, single opened or duple opened comb with high body weight (1840.88 ± 92.13 gram), whereas the other has a black feather, grey or dark grey shank with three shapes of a comb (single, single opened or duple opened) and less weighing (1555.66 ± 82.83 gram). The second type of chicken was described by Fathi *et al.* (2017) as one of the Saudi native chicken breeds which adapted to hard environmental conditions with importance in the rural households and small poultry farmers.

In this study, the testis in the black rooster is larger than in the brown rooster. Besides that, the size of the right testis is larger than the left, whereas the brown rooster has a larger left testis. The asymmetry of the testicular size in this research is confirmed by previous



studies in adulthood of some bird's species (Yu, 1998; Gunn *et al.*, 2008; Calhim & Montgomerie, 2015; Abdul-Rahman *et al.*, 2018), but different from that mentioned in chicken by Hocking (1992).

The testes of ANR were shining fading grey-yellowish in color and oval-shaped. They were located in the abdominal cavity, visible after removing the proventriculus, the liver and the gizzard. They lay between the lungs cranially, the abdominal sac caudally and ventral to the kidneys. The same position of the testes in this study has been described in birds by Frey & Goymann (2009) and Razi *et al.* (2010). The histological structure of the testes in this study showed that the germinal epithelium that lined the seminiferous tubules have cells in different sizes and shapes, the interstitial tissue included Leydig' cells. These findings are in agreement with that reported by Razi *et al.* (2010) and Sun *et al.* (2019).

The epididymis of ANR in this research has c to L-shaped with brown color, near the adrenal gland cranially and continuous caudally with vas deferens which was light greyish, pass medial to the kidneys and extended caudally until opened dorsolateral in the cloaca. Whereas the lining epithelium of the epididymis and vas deferens is the pseudostratified columnar and columnar epithelium, respectively. The morphology and histology of both epididymis and vas deferens were confirmed with that mention by former authors in avian (Bull *et al.*, 2007; Razi *et al.*, 2010; Saleem *et al.*, 2017; Khatun & Das, 2019).

ACKNOWLEDGEMENTS

The author would like to thank king Faisal University, and the deanship of research for their financial support, grant number NA000149.

REFERENCES

- Abdul-Rahmana I, Obeseb FY, Robinsonc JE. Testis size and asymmetry in the Guinea Fowl (*Numida Meleagris*):a test of the compensation hypothesis. *Avian Biology Research* 2018;11(2) 123-131.
- Aire TA, Ozegbe PC. The testicular capsule and peritubular tissue of birds:Morphometry, histology, ultrastructure and immunohistochemistry. *Journal of Anatomy* 2007;210(6):731-740.
- Al-Moshawah A. Determination of immunogenetics in local breeds of chickens [thesis]. Buraydah (SA): Qassim University; 2015.
- Al-Yousef YM. A survey study on the distribution of Saudi baladi chickens and their characteristics. *International Journal of Poultry Science* 2007;6(4):289-292.
- Bancroft JD, Stevens A. Theory and practice of histological techniques. 3rd ed. Edinburgh: Churchill Livingstone; 1990.
- Bull ML, Martins MRFB, Cesário MD, Padovani CR, Mendes AA. Anatomical study on domestical fowl (*Gallus domesticus*) reproductive system. *International Journal of Morphology* 2007;25(4):709-16.
- Burrows WH, Quinn JP. The collection of spermatozoa from the domestic fowl and turkey. *Poultry Science* 1937;16(1):19-24.
- Calhim S, Montgomerie R. Testis asymmetry in birds:the influences of sexual and natural selection. *Journal of Avian Biology* 2015;45:175-85.
- Fathi MM, AL-Homidan I, Abo-Emera OK, AL-Moshawah A. Characterisation of saudi native chicken breeds:a case study of morphological and productive traits. *World's Poultry Science Journal* 2017;73:916-27.
- Frey R, Goymann W. A single functional testis and long deferent duct papillae: the peculiar male reproductive tract of the classically polyandrous, sex-role reversed Black Coucal (*Centropus grillii*). *Journal of Ornithology* 2009;150(4):827-38.
- Gray JC. The anatomy of the male genital ducts in the fowl. *Journal of Morphology* 1937;60:393-405.
- Gunn MR, Champion Z, Casey ME, Teal P, Casey PJ. Testicular and spermatozoan parameters in the pukeko (*Porphyrio porphyrio melanotus*). *Animal Reproduction Science* 2008;109:330-42.
- Hocking PM. Bilateral testicular asymmetry and supernumerary testis in the domestic fowl (*Gallus domesticus*). *British Poultry Science* 1992;33:455-60.
- Kaupp BF. Male reproductive organs of birds. *American Journal of Veterinary Medicine* 1915;10:461-4.
- Khatun P, Das SK. Gross anatomy of epididymis and ductus deferens of adult khaki campbell duck (*Anas platyrhynchos domesticus*) in Bangladesh. *Journal of Bioscience and Agriculture Research* 2019;22(01):18059.
- Madekurozwa MC, Chabvepi TS, Matema S, Teerds KJ. Relationship between seasonal changes in spermatogenesis in the juvenile ostrich (*Stuthio camelus*) and the presence of the LH receptor and 3 β -hydroxysteroid dehydrogenase. *Reproduction* 2002;123:735-42.
- Maruch SM, Ribeiro M, Teles MEO. Morphological and histological aspects of the epididymal region and ductus deferens of columbina talpacoti (*Temmncck*)(Columbidae, Columbiformes). *Revista Brasileira de Zoologia* 1998;15(2):365-73.
- Razi M, Hassanzadeh SH, Najafi GR, Feyzi S, Amin M, Moshtagion M, et al. Histological and anatomical study of the White Rooster of testis, epididymis and ductus deferens. *International Journal of Veterinary Research* 2010;4(4):229-36.
- Saleem R, Singh B, Khan IM, Singh I, Bharti SK. Gross and biometrical studies on male reproductive system of adult local fowl of uttarakhand (Uttara Fowl). *International Journal of Pure and Applied Bioscience* 2017;5(3):634-8.
- Simões K, Orsi AM, Artoni SMB, da Cruz C, Schimming BC, Pinheiro PFF. Structural features of the epididymal region of the domestic duck (*Anas platyrhynchos*). *Brazilian Journal of Veterinary Research and Animal Science* 2004;41:92-7.
- Sun Y, Xue F, Li Y, Fu L, Bai H, Ma H, et al. Differences in semen quality, testicular histomorphology, fertility, reproductive hormone levels, and expression of candidate genes according to sperm motility in Beijing-You chickens. *Poultry Science* 2019;98:4182-9.
- Yu ZH. Asymmetrical testicular weights in mammals, birds, reptiles and amphibian. *International Journal of Andrology* 1998;21(1):53-5.

