Comparative Histomorphological and Morphometric Analysis of the Testes in *Agama* Lizard and Albino Wistar Rat

Original Article

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ABSTRACT

Introduction: Comparative Anatomy is the study of structural relationships and modifications in anatomy of structures in different organisms. The current research was aimed at finding the gross, morphological and histological differences and similarities in the testes of the *Agama* Lizard and Albino Rat.

Materials and Methods: Five adult, healthy Albino rats and *Agama* lizards were obtained for the study. The animals were sacrificed and an incision was made to expose the testes which were removed and morphologically examined. Several parameters were measured and the results analyzed.

Results: Gross assessment revealed that the testes were found in different locations in these species. Testicular length was significantly ($p<0.05$) increased in the left testicle of the *Agama* lizard. Testicular volume was significantly ($p<0.05$) increased in the right testes in Albino rats. The reverse was the case in the volume of testes in the *Agama* lizard.

Conclusion: Histomorphological assessment revealed that the germinal layer and seminiferous tubular area of the *Agama* lizard was thicker than in the Albino rat. In the *Agama* lizard, there was reduced interstitial space and the basement membrane of the seminiferous tubules closely abuted each other. The other structures were analogous and had similar anatomical and histological appearance and performed the same physiological functions.

Key Words: *Agama* lizard, Albino rats, comparative anatomy, histo-morphology, testes.

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INTRODUCTION

Comparative Anatomy is the study of structural similarities and differences in anatomy of different organisms. The knowledge of the natural relationship and ancestral history of animals can only be gained by comparative study of body parts (comparative anatomy) and their mode of development (embryology or ontology). It is a field that is closely related to evolutionary biology and phylogeny[1]. Comparative anatomy has provided evidence of common descent in organisms and has assisted in the classification of animals by indicating if organisms share a common ancestor[2]. Though random mutations and natural selection, each organism's anatomical structures gradually adapted to suit their respective habitats[3]. The roles for development of special characteristics which differ significantly from general homology as listed by Von Baer[4]. These homologous structures are similar in different species because the species have common descent and have evolved, usually divergently, from a shared ancestor. However, these structures may or may not perform the same function[4].

The testes have an ellipsoid shape and enclosed in a scrotal sac whose location is important for maintaining testicular temperature, approximately 1.5 to 2.5 °C below body temperature, required for spermatogenesis (sperm production). The testes works best at slightly core body temperature, spermatogenesis is less efficient at lower and higher temperature, and this is presumably why testes are located outside the body[5-6].

The testes (testicles) are the male gonads paired ovoid reproductive glands that produce sperm (spermatozoa) and male hormones primarily testosterone[7]. The testes are paired, ovoid male reproductive organs that sits in scrotum, separated from its mate by a scrotal septum[8]. Smooth to palpation, the testes sits obliquely with its long axis mostly vertical with a slight anterior and lateral slant to the superior pole. Superiorly, it is suspended by the spermatic cord, with the left testis often siting lower than the right testis. Inferiorly, the testis is anchored to the scrotum by the scrotal ligament, a remnant of the gubernaculum[9]. The surface of each testis is covered by the visceral layer of the tunica vaginalis, except where the testis attaches to the epididymis and spermatic cord. The tunica vaginalis

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is a closed peritoneal sac partially surrounding the testis, which represents the closed off distal part of the embryonic processus vaginalis[7].

The visceral layer of the tunica vaginalis is closely applied to the testis, epididymis and inferior part of the ductus deferens. The sinus of the epididymis is between the body of the epididymis and the postero-lateral surface of the testis. The parietal layer of the tunica vaginalis, adjacent to the internal spermatic fascia, is more extensive than the visceral layer and extends superiorly for a short distance unto the distal part of the spermatic cord. The small amount of fluid in the cavity of the tunica vaginalis separates the visceral and parietal layers, allowing the testes to move freely in the scrotum. The testes have a tough fibrous outer surface, the tunica albuginea, which thickens into a ridge on its anterior and posterior aspect as the mediastinum of the testes[7]. From this internal ridge, fibrous septa extend inward between lobules of minute but long and highly coiled seminiferous tubules in which the sperms are produced. The seminiferous tubules are joined by straight tubules to the rete testes, a network of canals in the mediastinum of the testes[7].

Agama (from SrananTango meaning Lizard) is the name of a genus of small-to-moderate sized, long tailed, insectivores and also is one of their common names. *Agama* includes at least 37 species in Africa, especially sub-Saharan Africa, where most regions are home to at least one species. The various species differs in size, ranging from about 12 to 30cm (5 inch to 1 feet) in length, when fully grown[10]. The Albino rat (Rattus Norvegicus) are mammals and the species has red eyes and white fur and these rats serve as an important animal model for research in various fields of medicine and science. The Wistar rats encompass a number of rat strains derived from a common lineage over the past fifty years and is considered as the right choice in establishing mammalian standards and is established as an ideal standardized mammal[11, 12].

Reptiles are poikilothermic animals with testes located in the abdominal and pelvic cavities while albino rats are endothermic animals with testes outside body in a pouch of skin[13]. Environmental temperature and relative humidity may affect the structure of testes and since it exists in different locations in the two species, the current research was aimed at finding out the morphological/histological difference and similarities in the testes of these animals and evaluating the morphological differences/similarities in testes of male *Agama* lizard and albino rats.

MATERIALS AND METHODS

Materials Used

The following materials were used in this study; five (5) male Wistar albino rats, five (5) male *Agama* lizard, dissecting set, hand gloves, razor blades, rat cage, lizard cage, pins and clip board, specimen bottles, glass slides, cover slip, refrigerator, rotatory microtome, hot air oven, choke, alcohol, staining trough, paraffin wax, Haematoxylin, Eosin, water bath, weighing balance, meter rule, thread, light microscope, Bouin's solution, 10% formalin, and distilled water.

Experimental Animals

Five adult healthy Wistar Albino rats and five *Agama* lizards were obtained for the study. The animals were kept in plastic cages in the experimental room of the animal house to acclimatize for one week prior to the time of sacrifice under standard animal house conditions. The animals were fed with standard laboratory diet (grower feeds) and water ad libitum.

Animal Sacrifice

The animals were sacrificed after being anaesthetized by inhalation of chloroform. They were pinned supine on a wooden board and an incision was made in the abdomino-pelvic region to expose the abdominal and pelvic viscera. The testes of each animal were removed and morphologically examined.

Tissue Preparation

The testes was fixed in Bouin's fluid for a period of 24 hours. Thereafter, the tissues were trimmed and conveyed through a series of solvents as per schedule for dehydration, clearing and paraffin infiltration. The tissues underwent normal histological procedures which included dehydration in ascending grades of alcohol (50, 70, 80, 95, and 100%), clearing using pure xylene followed by impregnation in molten paraffin wax, and sectioning with a rotatory microtome. 5µm sections were obtained and fixed on a clean albumenized surface of the slide of glass for Haematoxylin and Eosin staining.

Histomorphometric Analysis

The following parameters were measured and determined:

Total body Weight

Right Testes Weight

Left Testes Weight

Right Testes Length

Left Testes Length

Right Testes Width

Left Testes Width

Right Testes Volume

Left Testes Volume

Right Lumen Diameter

Left Lumen Diameter

Right Germinal Layer Thickness

Left Germinal Layer Thickness

Sertoli Cell Area

Seminiferous Tubular Lumen Diameter

Gonadal Index (GI)

Total body weight of the animals were taken at the onset of the experiment, before acclimatization and again just before the experimental study using a weighing scale. Testicular length was measured between the superior and inferior poles of the right and left testes, the width was measured across the widest part of the horizontal axis of the testes using a vernier caliper with an accuracy of 0.002mm. The area of the Sertoli cell was determined by using the freehand tool of image J application. The weight was measured using a digital weighing scale with an accuracy of 0.001g. Testicular volume was measured using the formula for the volume of an ellipsoid: $4/3\pi ab^2$, where a=1/2 the longest axis and b=1/2 the shortest axis. Gonadal index (GI) is determined using the formula – gonadal weight/body weight X 100.

Lumen diameter, germinal layer thickness and seminiferous tubular lumen diameter was measured on the micrographs obtained using the freehand line tool on Image J software Wayne Rasband National Institutes of Health, USA, Java 18.0_112). The images of the histological sections were obtained using an Amscope light microscope (MBJX-ISCOPE, Los Angeles) fitted with a digital camera (M500, X 64, version 3.7). Images of the histological sections were obtained using 4X, 10X and 40X objective lenses.

An ocular micrometer which was previously standardized with a stage micrometer was also used to measure areas of interest in the histological slides for morphometric analysis. The stage micrometer was used to calibrate imageJ using the same objective and pixel resolution as the micrographs being measured. The parameters and measurements were as adopted and reported by (14).

Statistical Analysis

The numerical data were obtained were presented as the mean and standard error of mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA). The analysis was carried out using Graph-Pad Instat 3. $P<0.05$ was considered as significant.

RESULTS

Gross Observations::

Dissection of the two species revealed different locations of the testes in the Albino rat and *Agama* lizard. The testes was located in the scrotal sac in the albino rat and was exposed when the thin skin of the sac was dissected (Figure 1). In the *Agama* Lizard, the testes were situated in the abdominal cavity and was exposed when the abdomen was dissected. It was located in the posterior wall of the abdomino-pelvic cavity just below the abdominal viscera (Figure 2). In both species, the left testes was located lower than the right testes (Figures 1 and 2). The location of the testes within the abdominal cavity in the *Agama* lizard could be explained by the function of the testes for spermatogenesis. The reptile as a poikilothermic specie requires a specific temperature for spermatogenesis compared to the Albino rat which is a mammal also requires a specific temperature for spermatogenesis and hence, the testes were located outside the abdominal cavity and suspended in the scrotal sac. These results corresponded to findings by[6, 15,16].

The testes in the Albino rats were oval in shape and the testicular blood vessels were observed to be radiating in a zig zag manner on the anterior and posterior surfaces as observed under the enclosing capsule (Figure 3). The testes of the *Agama* lizard on the other hand was also oval in shape, however, it was more rounded when compared to the testes in Albino rats. The testicular vessels were brached, rather than zig zag as observed in the testes in Albino rat (Figure 4). The testes in the Albino rats were paler in colour compared to the testes in the *Agama* lizard Figures 1-2).

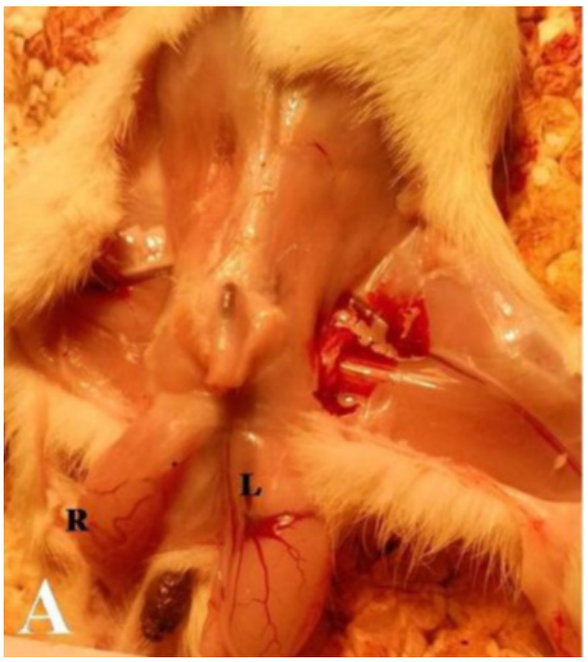

Fig. 1: showing the testes exposed from the scrotal sac in the albino rat. The right testes is labelled R and the left one L.

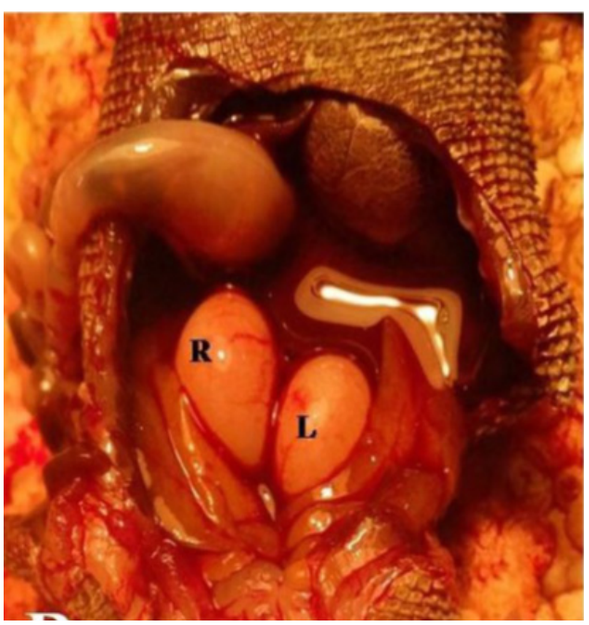

Fig. 2: showing the testes exposed within the abdominal cavity of the *Agama* lizard. The right testes is labelled R and the left one L.

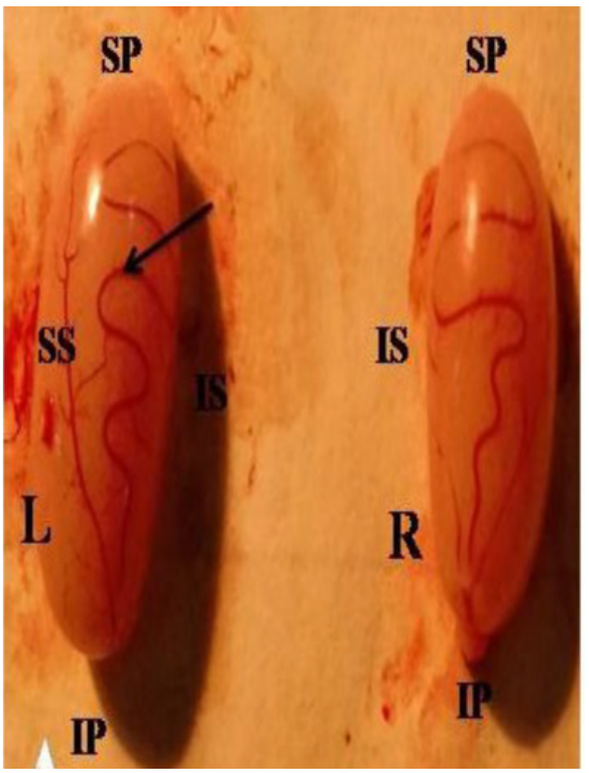

Fig. 3: showing the superior pole (SP), Inferior pole (IP), Superior Surface (SS) and Inferior Surface (IS) on the left and right testes in the Albino rats in anatomical position. The arrow indicates a testicular vessel running in a convoluted course.

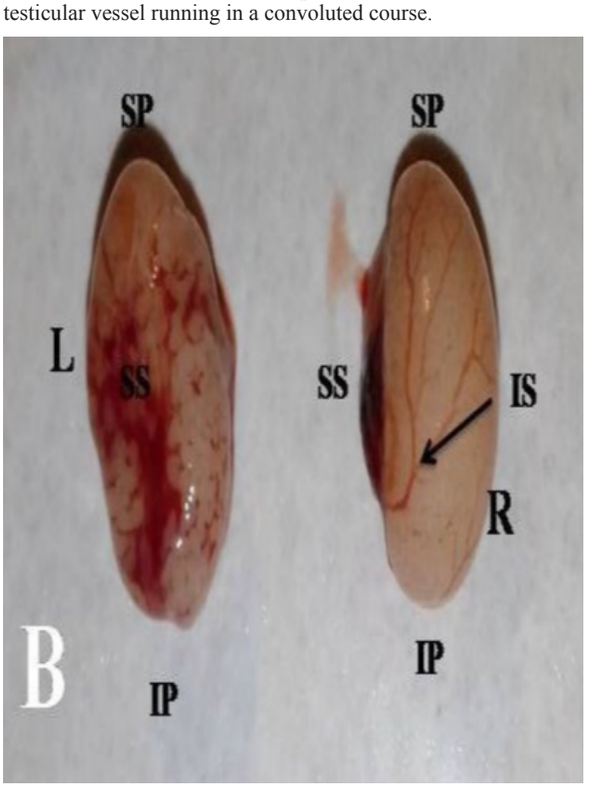

Fig. 4: demonstrating the superior pole (SP), Inferior pole (IP), Superior Surface (SS) and Inferior Surface (IS) on the left and right testes in the *Agama* lizard in anatomical position. The arrow indicates a testicular vessel branching out.

Histomorphological Analysis::

Table 1 shows the gonadal index which was a percentage of the testicular size compared to the total body weigth and from the results obtained, the weight of the testes in the rat were comparatively bigger compared to the weight in the *Agama* Lizard.

The results obtained after measuring the testicular weight in both the *Agama* lizard and the Albino rats showed that the right testes in the *Agama* lizard and Albino rats were slightly heavier than the left testes, however, the measurement of testicular length revealed that the right testis in *Agama* Lizard was significantly ($p<0.05$) longer on the right when compared to the left. The widest point in the left testes of the *Agama* Lizard was also significantly ($p<0.05$) wider than the right testes (Table 2)

Agama lizards are reported to be reproductively active during a period of decreasing ambient temperature, photoperiod and rainfall. Peak spermatogenesis in lizards occurs during late winter to early summer at which times, testicular activities were maximal.[14] reported that testicular weight and volume of lizards testes were at the maximum in May and regularly decreased in June, reducing into a smaller size and no sperm cells seen in the seminiferous tubules in July. The size remains small until September and October where the size, volume and weight of the testes increase until subsequent June when the size would decrease again[14]. The lizards used for this research work were caught in early August and the testicular sizes and weight were comparatively larger when compared to the study conducted by[14] where the mean weight, length, width and volume of the testes were 0.053g, 5.15mm, 3.6mm and 48.6mm3 compared to the present study where the same parameters in the right testes are recorded as 4.1g, 9.4mm, 4.7mm and 46.7mm3.[17] measured testicular weight to range between 37.3mg/100g and 42.9mg/100g in the regressive and regenerative stages of reproduction.[15] on the other hand determined the mean left testicular weight to be 200.92±113.9mg with a maximal increase from the end of March through to the beginning of April (be 298 mg – 340mg). The weight of the testes were found to be lowest at the end of the rainy season in October.

The mean testicular length in Albino rats were 18.8mm and 17.6mm for the left testis and 4.1mm and 4.0mm for the testicular width which correlated with studies conducted by[18] where the same parameters were recorded as 1.89 and 0.91cm for the right testes and 1.88cm and 0.91cm for the left testes[18]. The volume of the right testis was significantly ($p<0.05$) increased in the right testes compared to the left whereas, the reverse was the case for the *Agama* lizard where the left testis had a significantly ($p<0.05$) increased volume compared to the right.

The thickness of the germinal layer is considered to be the layer on the seminiferous tubule that bears the germinal cells and its supporting cells before mature spermatozoa migrates towards the lumen. The thickness of this layer in the Albino rat was found to be 16.1±0.6µm in the right and 12.6±0.6µm in the left testis. The corresponding thickness was recorded as 16.1±0.9µm and 20.6±1.3µm revealing that the thickness of the germinal layer in the *Agama* lizard was comparatively thicker than in Albino rats (Table 2).

The lumen of the seminiferous tubules on the other hand was wider in Albino rats (38.9±2.5µµm and 41.6±4.0µm in the right and left testes) than in the *Agama* lizard (19.9±1.8µm and 21.9±1.7µm) in the corresponding testes. The area of the seminiferous tubules were significantly ($p<0.05$) increased (20.9±0.7 mm2 and 27.5±2.0 mm2) in the *Agama* lizard as the tubules were ovoid and closely spaced out with limited interstitial space (Figure 5) when compared to the micrograph of the Albino rat where the tubules were rounded and there was interstitial spaces available (Figure 6). The area of the seminiferous tubules in Albino rats were 13.9.9±1.5 mm2 and 12.1±0.4 mm2 in the right and left testes. The area of the Sertoli cell was found to be greater in Albino rats (3.1±0.4µm2 and 3.0±0.3µm2) compared to the *Agama* lizard where the measurement was (1.81±0.8µm2 and 1.9±0.2µm2).

(18) recorded the mean long and short diameters of the seminiferous tubules in Albino rats were 219.19±4.22 µm and 132.92±2.84 µm and 273.23±7.52 µm and 169.55±6.47 µm in the peripheral and central region of the testis respectively. The average height of the epithelium in peripheral and central seminiferous tubules were measured and found to be 11.40±0.93 and 19.82±1.48 µm in the peripheral and central region respectively[19] documented the height of the seminiferous tubules in the *Agama* lizard as 7.17+0.37µm. the diameter of the seminiferous tubule was recorded as 7.17+0.37µm.

Histological Observations

In Albino rats, Interstitial tissue was found in between the seminiferous tubules. the interstitial tissue filled the intertubular space and consisted of loose meshwork of connective tissue with the fibroblasts, Leydig cells along with arterioles, venules and capillaries (Figure 8). Similar observations have been recorded by[19]. In the *Agama* lizard, there was reduced interstitial space and the basement membrane of the seminiferous tubules closely abuted together (Figure 7). The findings from the micrograph corresponded with findings of[18].

In both *Agama* lizard and Albino rat, the individual tubules consisted of a basement membrane formed by laminated connective tissue which was darkly staining covered by an external layer of flattened epitheloid cells (Figures 7-10). Within the basement membrane were

epithelial cells which were arranged in several irregular layers which were differentiated and separated into germinal cells as they advance toward the lumen. Also, interspersed in the layer are Sertoli cells, which project inward from the basement membrane to the lumen and provide support to the developing sperm cells. Spermatogonia type A was observed in both micrograph characterised by its large, round nucleus as well as condensed nuclei. This cell type differed from spermatogonia type B which had dispersed chromatin, central nucleoli and no nuclear vacuole. Type B spermatogonia differentiated in both species to form mature spermatozoa which were located close to the lumen of the tubule. The mature spermatozoa (S4) in the micrographs representing the *Agama* lizard were darkly stained when compared to the mature spermatozoa in albino rats. Sertoli cells were identified in both species supporting the germinal cells (Figures 9-10). These structures are analogous and also present in other studies conducted[15,18-19] and perform the same physiological function in both species[8].

Table 1: Showing Gonadal Index (GI) and Mean Body Weight in Male Albino Rats and Male *Agama* Lizards

Group	Mean Body Weight (g)	Gonadal Index
Rats	178.26±11.80	3.7
Lizards	106.78±4.95	3.3

All values are expressed as Mean ± SEM. The gonadal index (GI) indicated is a sum for both testes.

Table 2: Showing Morphometric Parameters in Testes of Albino Rats and *Agama* Lizards

Parameter	Animal	Right Testes	Left Testes
Testicular Weight (g)	Albino Rat	12.2±0.05	11.4 ±0.08
	Agama Lizard	4.1±0.06	3.0±0.05
Testicular Length (mm)	Albino Rat	18.8±0.07	17.6±0.07
	Agama Lizard	$9.4±0.09^{a}$	$12.4±0.07^{b}$
Testicular Width (mm)	Albino Rat	4.1±0.01	4.0±0.04
	Agama Lizard	4.7±0.01	5.8±0.01
Testicular Volume (mm^3)	Albino Rat	$92.3±0.4^{a}$	$80.9±0.6^{b}$
	Agama Lizard	$46.2±0.4^{a}$	$64.2±0.8^{b}$
Germinal Layer Thickness (µm)	Albino Rat	16.14±0.6	12.6±0.6
	Agama Lizard	16.1±0.9	20.6±1.3
Seminiferous Tubular Lumen diameter (µm)	Albino Rat	38.9±2.5	41.6±4.0
	Agama Lizard	19.9±1.8	21.9±1.7
Seminiferous Tubule Area (mm^2)	Albino Rat	13.9.9±1.5	12.1±0.4
	Agama Lizard	$20.9±0.7^{a}$	$27.5±2.0^{b}$
Area of Sertoli Cell (mm^2)	Albino Rat	3.1±0.4	3.0±0.3
	Agama Lizard	1.8±0.08	1.9±0.02

All values are expressed as Mean ± SEM. Values in the same row with different superscripts are significantly different

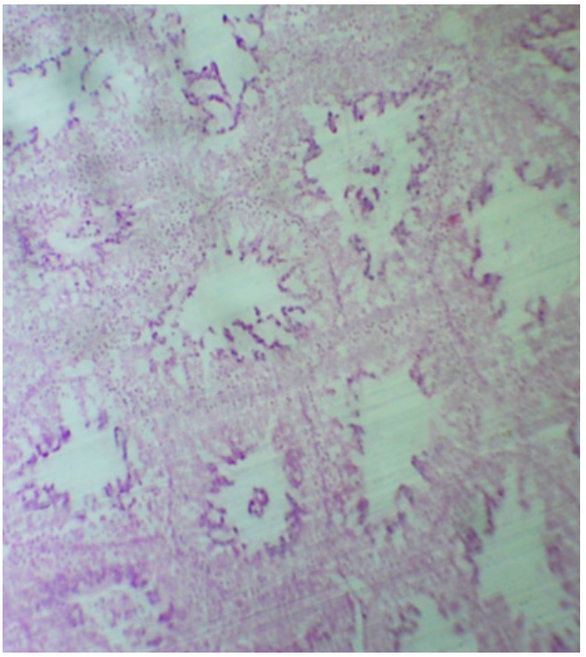

Fig. 5: showing a photomicrograph of the testis of the *Agama* lizard showing rounded seminiferous tubules (black arrow) with germinal cells and spermatozoa which surrounds a central lumen (red arrow) H & E X40

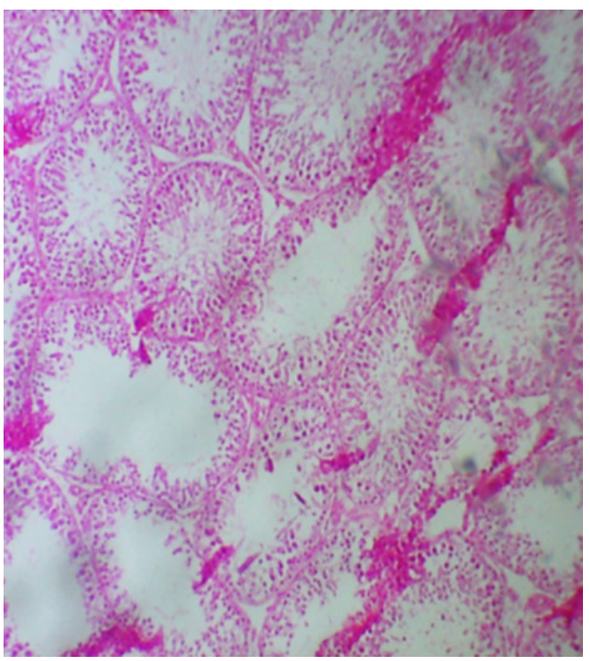

Fig. 6: showing a photomicrograph of the testis of the Albino rat showing seminiferous tubules (black arrow) lying on a supporting basement membrane with a central lumen (red arrow). H & E X40

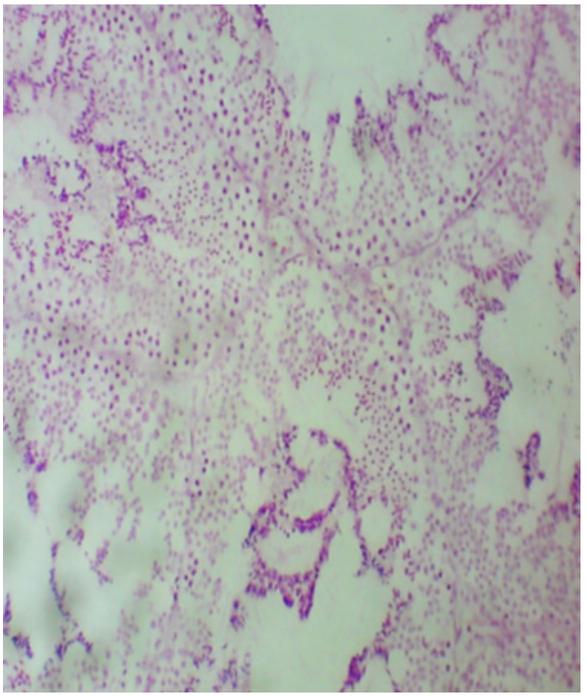

Fig. 7: showing the supporting basement membrane (Black arrow) of the tubules in the *Agama* lizard and connective tissue in the interstitial spaces (red arrow) were less defined in the *Agama* lizard. H & E X100

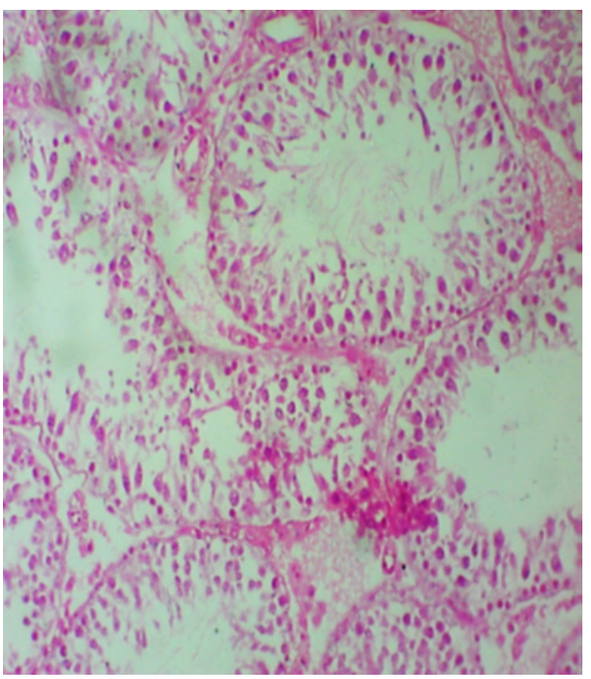

Fig. 8: showing the supporting basement membrane (black arrow) with germinal cells. The blood vessels (red arrow) in the interstitial space is prominent in the Albino rats H & E X100

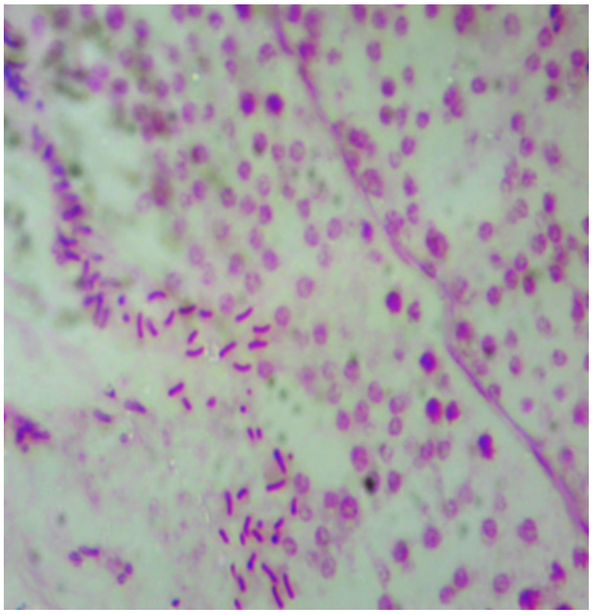

Fig. 9: shows a higher magnification of the cells lining the seminiferous tubules in the *Agama* Lizard. The mature spermatozoa (S4) have darkly staining tails (black arrows). Sertoli cells (brown arrow), Spermatogonia type B (red arrow) and spermatogonia type A (yellow arrow) are found on the germinal epithelia. Myoepithelial cells are also observed (green arrow). H & E X400

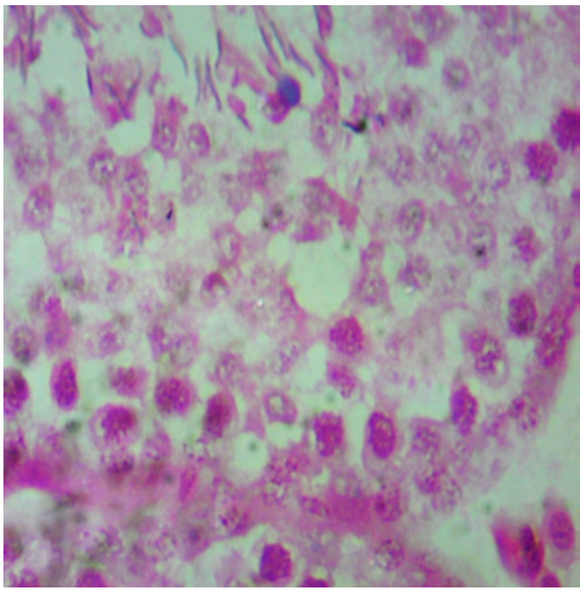

Fig. 10: shows a higher magnification of the germinal epithelium of the seminiferous tubules in the Albino rat. The mature spermatozoa (S4) have tails extending towards the lumen (black arrows). Sertoli cells (brown arrow), Spermatogonia type B (red arrow) and spermatogonia type A (yellow arrow) are displayed. Myoepithelial cells (green arrow) are observed and Leydig cells in the interstitial space are represented by a blue arrow H & E X400

CONCLUSION

The present study compared gross, morphological and histological parameters in the testes in the *Agama* lizard and albino rats. The gross appearance of the testes were similar in both species as they were both oval although it was wider in the *Agama* lizard. Testicular weight, length and volume were greater in the albino rat. Histomorphological assessment revealed that the germinal layer and seminiferous tubular area of the *Agama* lizard was thicker than in the Albino rat. The luminal area and sertoli cell area however was wider in Albino rats. The testes were found in different locations in these species: in the rats, the testis were situated ventrolateral to the anus with its curved surface directed caudally housed in scrotal sacs while in the *Agama* lizard, the testes were located in the abdominal cavity below the kidneys. In spite of the histological and morphological differences observed in the current study, the testes carried out the function of spermatogenesis efficiently in both species by producing mature sperm cells and hormones for reproductive purposes.

CONFLICT OF INTEREST

There are no conflicts of interest.

ETHICAL COMMITTEE APPROVAL

Ethics committee approval was received for this study from the Departmment of Human Anatomy Ethical Committee, University of Maiduguri (Code No. UM/HA/UGR 18.19-003).

AUTHOUR CONTRIBUTIONS

Concept: N.J.M, H. B. I., Design: N.J.M, M.O.O.A., Supervision: H.I.B., M.O.O.A., N.I.D., Funding: N.J.M. Resources: N..J. M, M.O.O.A., Materials: N.J.M, M.O.O.A. N.I.D., Data Collection and Processing: N.J.M, N.I.D., M.O.O.A., Analysis and/or Interpretation: N.J.M, N.I.D., M.O.O.A., Literature Search: N.J.M, H.I.B., Writing Manuscript: N.J.M., M.O.O.A., Critical Review: N.J.M, N.I.D., H.B.I., M.O.O.A.

FINANCIAL DISCLOSURE

The Authours declare that the work was privately funded and received no financial contributions.

REFERENCES

1. Gaucher EA, Krafzer JT, Randall RN, (2010). Deep Phylogeny-how a Tree can help Characterize early life on Earth Cold Spring Harber Perspective in Biology. 2(1): 9002238 pp. a002-238.

2. Hardison RC (2003) Comparative Genomic PLOS Biology 1(2): E58. doi:10.1371/journal.

3. Campbell NA and Reece JB (2002). Biology. (6th ed.). Son Francisco, CA: Benjamin Cummings. Pp. 438-439

4. Kardong KV (2015). Vertebrate Comparative Anatomy: Functional Evolution. New York: McGraw-Hill Education. Pp. 16-16

5. Ganong WF (2005) The Male Reproductive System (22nd ed.) McGraw Hill Companies, Pp. 107-109

6. Ben Team (2019). Do reptiles have testicles, cited on http://www.mom.me/repiles-testicles on 27/05/2019

7. Moore KL, Arthur FD, Anne MRA (2014). Clinically Oriented Anatomy: Testes. (7th ed.). Wolters Kluwer/Wilkins and Williams Publisher. Pp. 206-210

8. Snell RS (2000). Structures of Anterior Abdominal wall: Scrutom, Testes, and Epididymis. Clinical Anatomy for Medical Student (6th ed.) Philadelphia Pp. 153

9. Swartz MH (2006). Male Genitalia and Hernia, Textbook of Physical Diagnosis: history and Examination (5th ed.). Philadelphia. Cited 10th July, 2019 at www.medscape.com

10. Burton M and Burton R (1974). The Funk and Wagnals Wildlife Encyclopedia 1. New York

11. Clause BT (1993) The Wistar Rat as the right Choice: Establishing Mammalian Standards and the Ideal of a Standardized Mammal, Journal of History and Biology 26(2) pp 329-349.

12. McCormick DL (2017) Preclinical Evaluation of Carcinogenicity Using Standard Bred and Genetically Engineered Rodent Models, A Comprehensive Guide to Toxicology in Non-Clinical Drug Development, Second Edition pp 273-292

13. Sarkar H, Arya S, Rai U, Majumda SS (2016). A study of Differential Expression of Testicular Genes in Various Reproductive Phases of Hemidactylus flaviviridis (wall Lizard) to Derive Their Association with Onset of Spermatogenesis and Its Relevance to Mammals. PLOS ONE 11(3)

14. Soheila R. and Hojati V (2015). The male reproductive cycle of the spotted Toad Head Agama Lizard, Phrynocephalus Maculatus in Iran. International Journal of Zoology Vol. 2015 (5)

15. Ejere VC and Adegoke JA (2005) Seasonal Testicular Histology and Reproductive Cycle of the Rainbow Lizard, Agama Agama Agama, L, (Agamidae, Reptilia) in Ile-Ife, South Western Nigeria, Animal Research International 2(3): 393 – 398

16. Ortis MA, Boretto JM and Ibarguengoytia, NR (2019). Reproductive Biology of a viviparous lizard (Mobuyadersivittata) from the subtropical wet checo of Argentive. Geographical variations in response to local environmental pressure Anais of Da Academic Brasileira De Ciencias 91(1)

17. Gaitonde SG and Gouder BYM (1985) Spermatogenic and steroidogenic activity in the Testis of the Lizard, Calotes versicolor, treated with Mammalian Gonadotropins and Testosterone Italian Journal of Zoology 52:393-405

18. Kumar and Nagar (2014), Histomorphometric study of Testis in Deltametrin treated Albino Rats, Toxicology Reports 1 (2014) 401-410.

19. Ozegbe PC and Omirinde JO (2012) Comparative morphophysiological evaluation of the testis of adult Wistar rats fed low protein-energy diet and dosed with aqueous extracts of Cuscuta australis Nigerian. Journal of Physiological Sciences. 27(2012) 149 –155