Original Article

Sahel Journal of Veterinary Sciences

Sahel J. Vet. Sci. Vol. 17, No. 2, pp. 19-25 (2020)

Article History
Received: 4th March, 2020
Revised: 3rd June, 2020
Accepted: 7th June, 2020
Published: 30th June, 2020

Structure of the Leydig Cell in the African Sideneck Turtle (*Pelusios castaneus*)

Olukole, S. G. and Oke, B. O.

Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria.

* *Author for Correspondence: sg.olukole@mail.ui.edu.ng; +2348033574752*

Abstract
The African sideneck turtle (*Pelusios castaneus*) is a freshwater turtle of West African origin used in traditional medicine with little consumption as meat. There have been documentations on the reproductive biology of the turtle with no report on the structure of the Leydig cell of the animal. We described the structure of the Leydig cell of the adult African sideneck turtle using histology, microstereology and transmission electron microscopy. The Leydig cell of the African sideneck turtle were elliptical in shape when found proximal to blood vessels and elongated at other points within the testicular interstitium. Leydig cells occurred in cords or clusters of varying sizes and numbers (3-5 cells) that appear to be random in distribution possessing round to ovoid nuclei containing small amount of peripherally disposed heterochromatin with prominent nucleoli. The seminiferous tubules of the turtle occupied about 85% of the total testicular parenchyma while the interstitium occupied 15% of it. Of this 15%, the Leydig cell occupied about 10% while the stromal elements, inclusive of blood vessels occupied the remaining 5%. The cytoplasm of the Leydig cell was about 85% of the entire cell and comprised microfilaments, lipid droplets, smooth and rough endoplasmic reticula as well as numerous mitochondria. In conclusion, the histological and ultrastructural features of the Leydig cell of the African sideneck turtle bear close similarities with those of other reptiles with little variations. These variations include interstitial location as well as in relation to blood vessels within the testicular interstitium. Information made available by this study is expected to be useful in the comparative anatomy of the Leydig cell of turtles and reptiles.

Keywords: African sideneck turtle; Leydig cell; Testicular interstitium; Reptiles.

Introduction

The distribution of African sideneck turtle (P*elusios castaneus*) ranges from Guinea and Senegal to Northwestern Angola and the Central African Republic including the Sao Tome Islands (Kirkpatrick, 1995; Olukole and Oke, 2018, 2020). The reproductive biology of the turtle has been the focus of our investigations in recent times (Olukole *et al*., 2013; Olukole *et al*., 2014a; Olukole and Oke, 2018; Olukole *et al*., 2018a). The animal is important in trado-medical practices especially in Southwestern Nigeria with only few human populations consuming its meat (Olukole *et al*., 2018b).

Reptiles have been described as the first vertebrates to be successfully adapted to life on land (Ashanbhag, 2002). Their adaptation strategies include the ability to survive in water and come on land for reproduction, especially to lay eggs. Leydig cells are large polyhedral cells of the interstitial tissue of the testis, named after the German Scientist, Franz Leydig in 1850 (Mendis-Handagama and Ariyaratne, 2005). Leydig cells produce testosterone responsible for the normal functioning of the male reproductive as well as accessory sex organs. Leydig cells have been reported to be resident within the testicular capsule as circum-testicular tunic beneath the tunica albuginea in the lizard Agama tuberculate and absent in the lizard, Lygosoma himalyanum (Ashanbhag, 2002).

Previous investigations from our laboratory on the reproductive biology of this turtle had been on Sperm morphological characteristics and morphometry (Olukole *et al*., 2013); gross and microanatomy of the male reproductive organs (Olukole *et al*., 2014a), seasonal spermatogenic cycle (Olukole *et al*., 2014b); gonadal and extragonal spermatozoa reserves (Olukole *et al*., 2018a); spermiogenesis and acrosomal vesicle formation (Olukole *et al*., 2018b); Sertoli cell morphology (Olukole and Oke, 2018) as well as the ultrastructural features of the epididymis (Olukole and Oke, 2020). Unlike turtles, extensive literature describing morphology of the Leydig cells exist on mammals and birds (Oke *et al*., 1984; Russell, 1996; Aire, 1997; Bacha and Bacha, 2000; Mendis-Handagama and Ariyaratne, 2005; Ali *et al*., 2015). There are, however, a good number of literature on the testicular ultrastructure of reptiles with no detailed information on the Leydig cell (Mahmoud *et al*., 1985; Dubois *et al*., 1988; Meylan *et al*., 2002; Al-Dokhi *et al*., 2004). An extensive webometric literature search revealed

that little is known about the structure of the Leydig cell of turtles while there is no information on the structure of the Leydig cell of the African sideneck turtle. This study was therefore designed to investigate the structure of the Leydig cell to partly fill its existing lacuna in the literature.

Materials and Methods

Experimental Animals

The study was carried out using eight adult male African sideneck turtles (*Pelusios castaneus*) with an average bodyweight of 0.65 kg, collected from river drainages in Ibadan, Nigeria. The turtles were sampled in August and September, a period of peak spermiogenesis (Olukole *et al*., 2014b). To determine adulthood in the turtles, carapacial and plastral characteristics of the turtle (Kirkpatrick,1995), were utilized. Anaesthesia was achieved with an intramuscular injection of ketamine-HCl (Sigma, St. Louis, MO, USA) (25 mg/kg body-weight) before the turtles were sacrificed by cervical decapitation. Testicular samples were removed after separating the plastron from the carapace. Procedures adopted in the study were approved by the University of Ibadan Animal Care and Use Research Ethics Committee (UIACUREC: 12/13/05).

Light Microscopy (LM)

Testicular samples were fixed in buffered neutral formalin and embedded in paraffin blocks. Sections 2-4 µm thick were stained with Haematoxylin and Eosin, Periodic Acid Schiff (PAS) as well as Masson's Trichrome (MT) as described by Rao and Shaad (1985). The slides were then studied under a light microscope (Olympus BX63 with a DP72 camera).

Transmission Electron Microscopy (TEM)

Testicular tissues were fixed in glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) for 4 hours at 4 0 C as previously described by Olukole and Oke (2018). Briefly, testicular samples were thoroughly washed in the same buffer and then post-fixed in 1% osmium tetroxide. They were then dehydrated in a graded series of ethanol solutions followed by clearance with propylene oxide, infiltrated with a 1:1 solution of propylene oxide:epoxy resin and then 1:2 solution of propylene oxide:epoxy resin. They were subsequently placed in 100% epoxy resin for 36 hours under vacuum. Embedding was done in fresh epoxy resin and cured at 60 0 C for 48 h. Toluidine blue was used to stain semi-thin sections of tissues and observed under the light microscope (Olympus BX63 with a DP72 camera). Ultra-thin sections (70-80 nm) were cut with a diamond knife on an ultramicrotome (Ultracut- Reichert, Austria). The sections were then double-stained with uranyl acetate and lead acetate. The copper grids were examined under a transmission electron microscope (Philips CM 10 TEM) operating at 80 kv. Representative micrographs of different sections of the testis were taken using a Gatan 785 Erlangshen digital camera (Gatan Inc., Warrendale, PA). Analysis and assembling of composite micrographs were carried out using Adobe Photoshop CS5 (Adobe Systems, San Jose, CA). Morphometric analyses of testicular interstitial elements were performed with the aid of GIMP 2 Software according to the method of Franca and Godinho (2002).

Results

The testicular interstitium of *P. castaneus* is a highly organized structure consisting of either narrow strands wedged between two seminiferous tubules or large tri- and quadrangular interstices among three to four seminiferous tubules (Figure 1 A-B). The interstitium stained densely pinkish-red with PAS, indicative of an acidic nature with the Leydig cells well outlined (Figure 1C). The interstitium was also MT-positive, showing the presence of collagen fibres (Figure 1D).

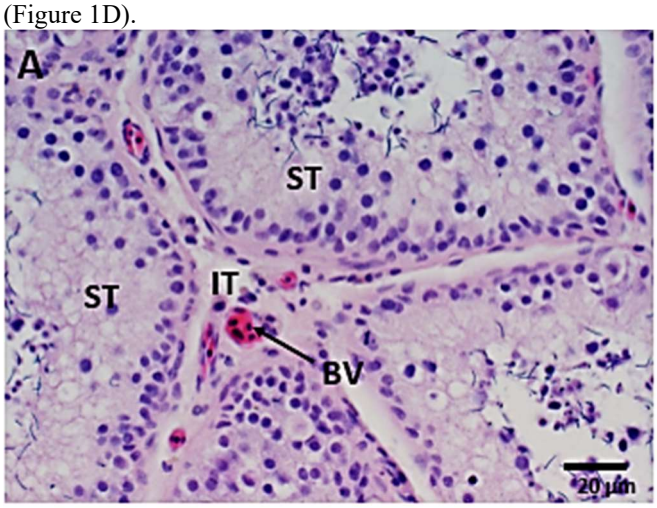

Figure 1A: Photomicrograph of the testicular tissue of *P. castaneus* showing the interstitial Tissue (IT), Seminiferous tubules (ST); BV: Blood Vessel (H&E).

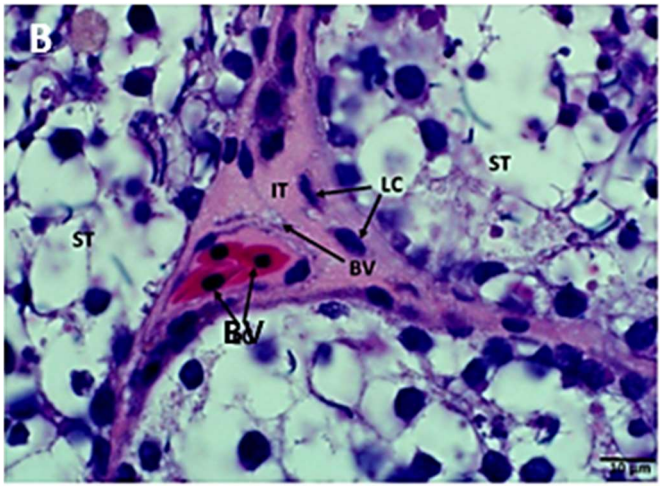

Figure 1B: Photomicrograph of the testicular tissue of *P. castaneus* showing the interstitial tissue: Large triangular tissue bounded by three seminiferous tubules (ST); BV: Blood Vessel; IT: Interstitial Tissue; LC: Leydig Cell (H&E).

The interstitial tissue surrounded by the seminiferous tubules constitutes a boundary tissue, the tunica propria (Figure 2A and B). Leydig cells as well as blood vessels were seen within the interstitium, particularly between the peritubular myoid cells and the lymphatic endothelium of the boundary tissue. The tunica propria was composed of a basal lamina, collagen fibres, myoid cells and fibroblasts (Figure 2B). Mature Leydig cells of *P. castaneus* were elliptical in shape when found near blood vessels but elongated when found elsewhere within the testicular interstitium. Leydig cells

occurred in cords of about three to five cells or clusters of varying sizes that appeared to be random in distribution.

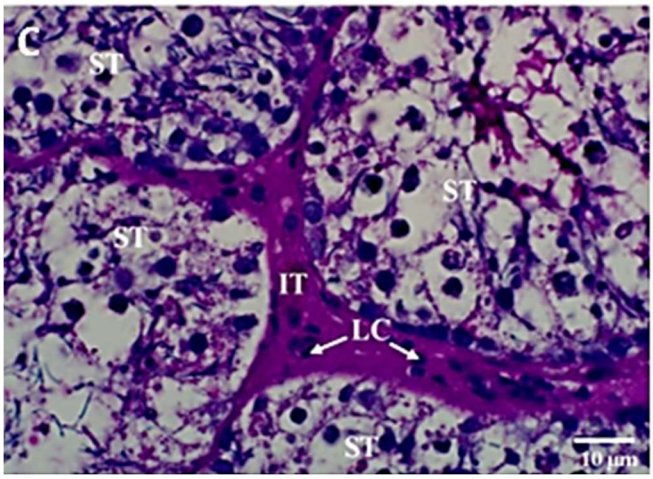

Figure 1C: Photomicrograph of the testicular tissue of *P. castaneus* showing the interstitial tissue with densely-stained pinkish-red interstitium (IT) populated by Leydig cells (LC) well outlined; ST: Seminiferous Tubule (PAS).

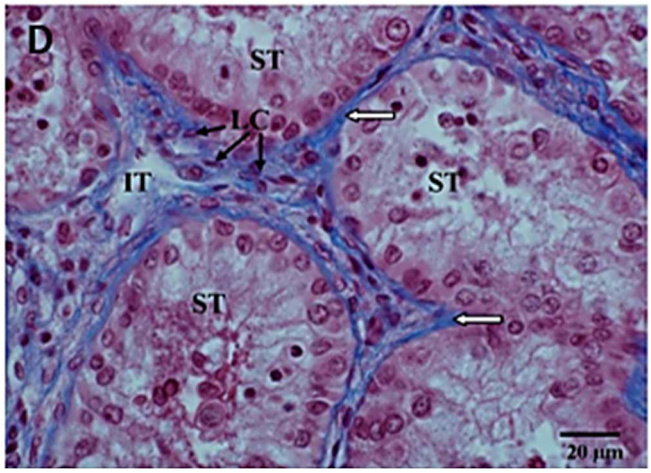

Figure 1D: Photomicrograph of the testicular tissue of *P. castaneus* showing the interstitial tissue (IT), seminiferous tubules (ST); LC: Leydig Cell (White arrows). Masson's Trichrome- Positive basement membrane of seminiferous tubule (MT).

They possess round to ovoid nuclei containing small amount of peripherally disposed heterochromatin with prominent nucleoli (Figure 2C). The cytoplasm of the Leydig cell was composed of microfilaments, numerous lipid droplets of varying sizes (ranging from 0.2 to 0.4µm), smooth endoplasmic reticulum, a few rough endoplasmic reticulum, numerous mitochondria and Golgi complex (Figure 2D).
The cytoplasmic matrix of the Leydig cell was basically composed of free ribosomes (Figure 3 A). The nuclei of Leydig cells were round or ovoid in shape, with little chromatin marginations. Their nuclear membranes exhibited nuclear pores (Figure 3 B) while adjacent Leydig cells were joined by tight junctions (Figure 3 C). Myoid cells in the interstitium lined the seminiferous tubules and bounded on the interstitial side by the lymphatic endothelium of lymphatic vessels or lymphatic sinusoids.

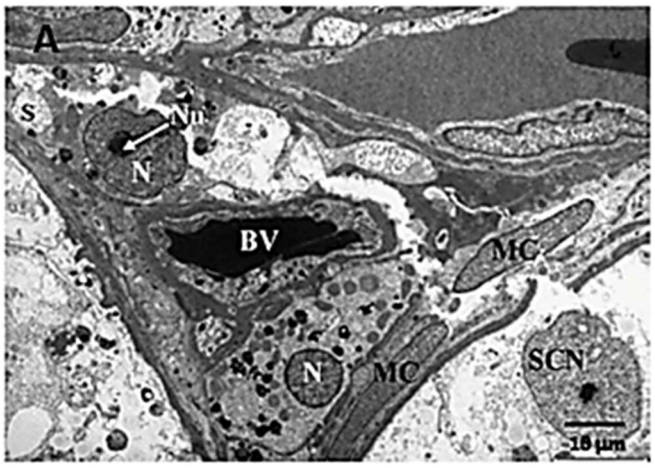

Figure 2A: Transmission Electron Micrograph of the testicular interstitial tissue of *P. castaneus* showing a large centrally placed Blood Vessel (BV), Leydig cell nucleus and nucleolus (N and Nu, respectively) and the surrounding boundary tissue, the tunica propria. S: Sinusoid; MC: Myoid Cell nucleus; SCN: Sertoli Cell Nucleus.

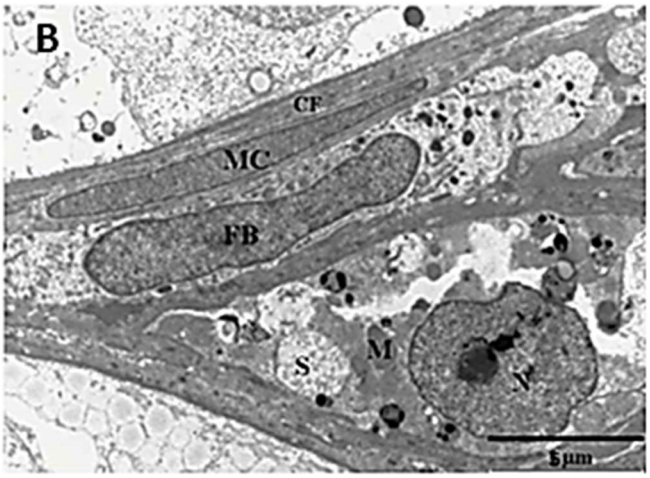

Figure 2B: Transmission Electron Micrograph of the testicular interstitial tissue of *P. castaneus* showing the tunica propria is composed of Collagen Fibres (CF), Myoid Cell (MC) and Fibroblast (FB); S: Sinusoid; M: Mitochondria; N: Nucleus of Leydig cell.

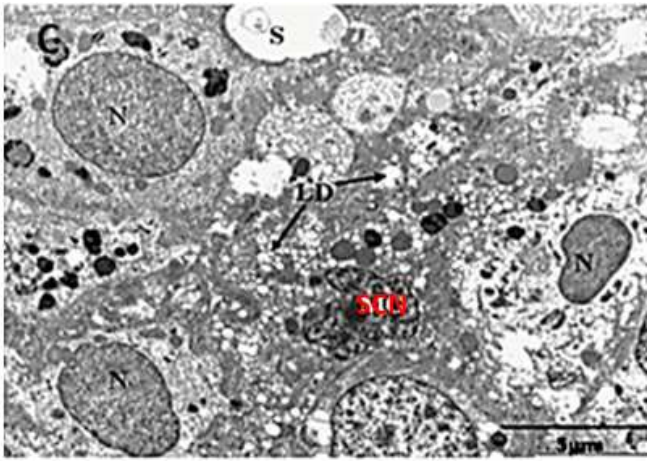

Figure 2C: Transmission Electron Micrograph of the testicular interstitial tissue of *P. castaneus* showing Leydig

cell nucleus (N); MG: Macrophage, S: Sinusoid and Lipid Droplets (LD).

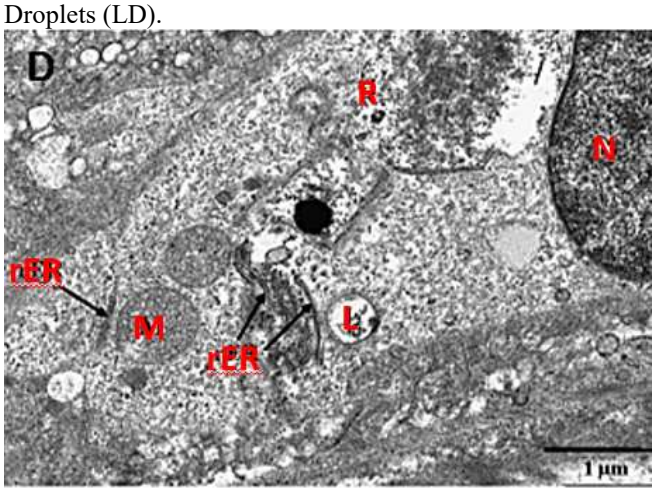

Figure 2D: Transmission Electron Micrograph of the testicular interstitial tissue of *P. castaneus* showing cytoplasm of the Leydig cell. N: Nucleus; R: free ribosomes; rER: Rough endoplasmic reticulum; L: Lysosome; M: Mitochondrion.

Myoid cell nucleus was covered by cytoplasm pitted with numerous micropinocytic vesicles on both the tubular and interstitial surfaces, both of which were covered by basal lamina (Figure 3 D).

Morphometric analysis of sections revealed that the seminiferous tubules occupied about 85% of the total testicular parenchyma while the interstitium occupied 15% of it (Table 1). Of this 15%, the Leydig cell occupied about 10% while the stromal elements, inclusive of blood vessels occupied the remaining 5%. Leydig cell comprised 85% cytoplasm and about 15% nucleus. The average diameter of the nucleus and nucleolus of Leydig cell were 7.5µm and 1.8 µm, respectively (Table 1).

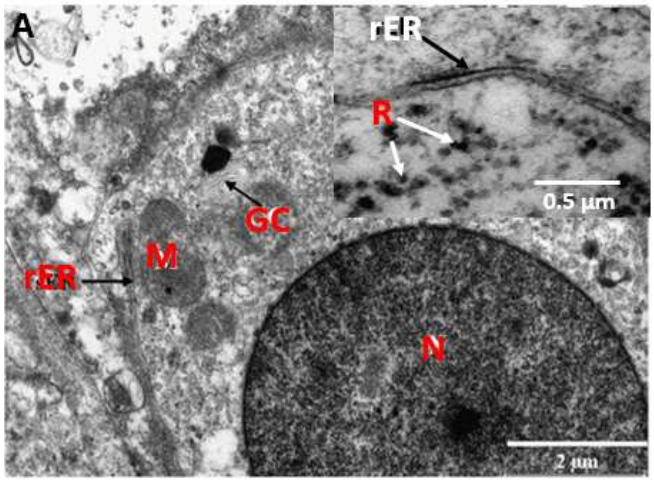

Figure 3A: Transmission Electron Micrograph of the testicular interstitial tissue of *P. castaneus* showing supranuclear region of the cytoplasm of the Leydig cell of *P. castaneus*. Free ribosomes (R), smooth endoplasmic reticulum (sER), mitochondria (M) and Golgi complex (GC); N: Nucleus. Inset: rER: Rough Endoplasmic Reticulum; R: Ribosomes.

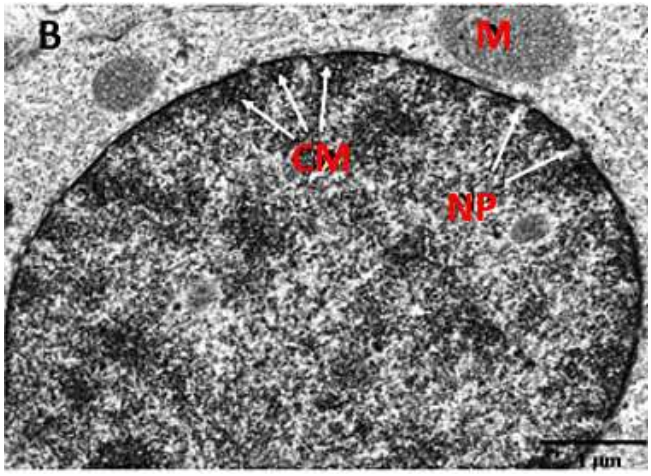

Figure 3B: Transmission Electron Micrograph of the testicular interstitial tissue of *P. castaneus* showing heterochromatic nucleus of the Leydig cell of the *P. castaneus*. CM: chromatin margination; NP: Nuclear pore; M: Mitochondria.

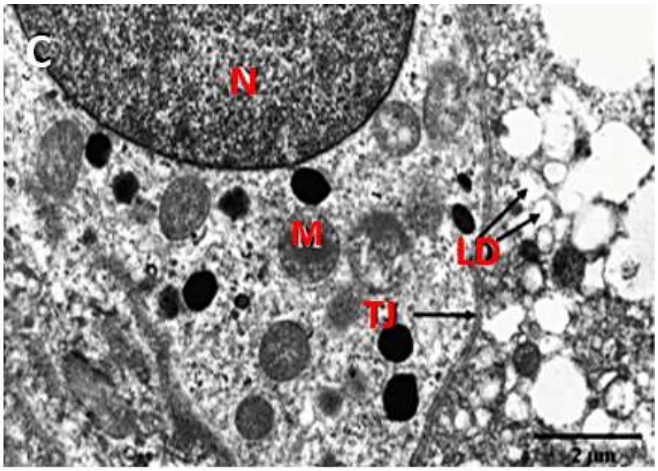

Figure 3C: Transmission Electron Micrograph of the testicular interstitial tissue of *P. castaneus* showing Tight junction (TJ) between adjacent Leydig cells; M: Mitochondria; LD: Lipid droplets; N: Nucleus.

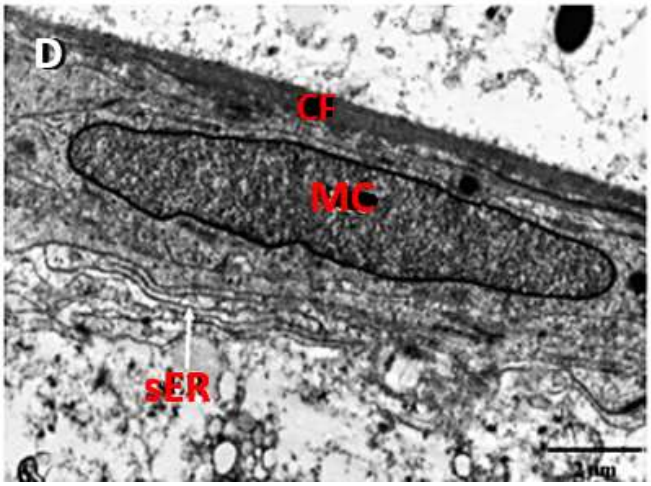

Figure 3D: Transmission Electron Micrograph of the testicular interstitial tissue of *P. castaneus* showing testicular myoid cell (MC) of *P. castaneus* showing its nucleus covered by cytoplasm, smooth endoplasmic reticulum (sER) on its interstitial surface and collagen fibres (CF) on its tubular surface.

Table 1: Morphometric parameters of the testicular tissue and Leydig cell of the African sideneck turtle

Parameters	**Mean ±SD (n=8)**
Volumetric rate (%)	
Seminiferous tubules	85.01 ± 3.9
Testicular interstitium	14.99 ±4.28
Leydig cells	10.02 ± 1.7
Stroma	4.97 ± 0.9
Leydig Cell	
Proportion of cell occupied by nucleus (%)	15.21 ± 1.97
Proportion of cell occupied by cytoplasm (%)	84.79 ± 4.3
Diameter of nucleus (µm)	7.51 ± 1.8
Diameter of nucleolus (µm)	1.8 ± 0.26

Discussion

The testicular interstitial tissue has been shown to play very important roles in the physiology of the testis, providing mechanical support to the seminiferous tubules thereby participating in the regulation of spermiogenesis and Sertoli cell functions apart from its major role in the production of testosterone by the Leydig cell (Losinno *et al*., 2012; Domke, 2018). The histological features of the interstitial tissue of the African sideneck turtle are similar to those earlier reported in other chelonians (McPherson & Marion, 1981; Rao & Shaad, 1985; Meylan *et al*., 2002; Gribbins *et al*., 2008). The positive reactions of the testicular interstitium to MT and PAS implies that the region is rich in collagen fibres and carbohydrate, respectively. However, the interstitial location of the Leydig cell of the turtle is at variance with those of a few reptiles including the lizard *Agama tuberculate,* in which the Leydig cell is located within the testicular capsule (Ashanbhag, 2002). A number of variations in the structure and location of reproductive organs has been reported within the reptilian class (Ashanbhag, 2002; Gribbins *et al*., 2008).

The structure and composition of the tunica propria of the testicular interstitium of the African sideneck turtle are also consistent with those reported in reptile and mammals (Meylan *et al*., 2002; Al-Dokhi *et al*., 2004; Young *et al*., 2006). The tunica albuginea of reptiles and mammals are composed of dense connective tissue containing blood vessels, fibrocytes and collagen fibres (Bacha and Bacha, 2000; Al-Dokhi *et al*., 2004; Young *et al*., 2006). The histological and ultrastructural features of the Leydig cell of the turtle in this study show that the Leydig cell bears similar features both in mammals and reptiles. In the present study, the shape of the Leydig cell varied with its proximity with other interstitial tissue structures. For example, at points where Leydig cells where found near blood vessels, they presented elliptical shape and elongated elsewhere. In the collared peccary (*Tayassu tajacu*), Leydig cells had been reported to vary in shape (elongated to elliptical) based on their proximity to blood vessels within the testicular interstitium (Costa *et al*., 2007).

The peripherally disposed heterochromatin with prominent nucleoli status of the nucleus of Leydig cells observed in this study has been reported as a universal characteristic of Leydig cells (De Kretser and Kerr, 1994). However, chromatin margination and its relationship with nuclear pores of the nuclear envelope observed in this study has not been reported in any reptile.

Abundance of lipid droplets have been reported in the cytoplasm of Leydig cells of lizards and have been linked to the production of steroid in reptiles (Dufaure, 1968). The presence of numerous mitochondria and lipid droplets as well as well-developed smooth endoplasmic reticulum in the cytoplasm of Leydig cells have been described as indicators of steroidogenesis in turtles (Mahmoud *et al*., 1985; Dubois *et al*., 1988). It is assumed that the accumulation of lipid droplets in the cytoplasm of Leydig cells of the African sidenck turtle would have a direct impact on testosterone production and spermiogenesis. An inverse relationship between the size of Leydig cells and active spermiogenesis have been reported in the testis of the lizard *Tropidurus itambere* (Ferreira and Dolder, 2003). However, this relationship could not be established or otherwise in the present study since the turtles were sampled during active spermiogenesis.

The volumetric rates of testicular interstitial elements observed in this study bear a close range with those reported for the collared peccary (Costa *et al*., 2007) as well as those reported in most mammals (Russell, 1996). The volumetric rate of the Leydig cell of the African sideneck turtle is similar to those reported in the West African Dwarf bucks (Oke *et al*., 1984). Volume densities of the seminiferous tubules of 92 and 90%, respectively had been reported in the Gerbil (Sagatelli *et al*., 2004) and domestic cat (Franca and Godinho, 2002). However, the proportion of Leydig cell occupied by cytoplasm in the African sideneck turtle is higher than the 70% earlier reported in wild boars (Almeida, 2002).

Conclusions

In conclusion, the histological and ultrastructural features of the Leydig cell of the African sideneck turtle bear close similarities with those of other reptiles with little variations. These variations include interstitial location as well as variations in Leydig cell shape in relation to blood vessels within the testicular interstitium. The study provides

information useful in the comparative anatomy of the Leydig cell of turtles and reptiles generally.

Acknowledgement
We acknowledge the technical support received from Dr Lizette du Plessis of Electron-Microscopy Unit, Department of Veterinary Anatomy and Physiology, Faculty of Veterinary Science, University of Pretoria, South Africa.

Conflict of Interest
The authors declare that they do not have any conflict of interest.

Grants
The University of Ibadan, Senate Research Grants (SRG/FVM/2010/4A and SRG/FVM/2010/1B) to BOO partly supported this study.

Authors' Contributions
Both authors executed and jointly wrote the manuscript.

References

Aire, T.A. (1997). The structure of the interstitial tissue of the active and resting avian testis. Onderstepoort J. Vet. Res., 64(4):291-9.

Ali, M.Z., Qureshi, A.S., Rehan, S., Akbar, S.Z. and Manzoor, A. (2015). Seasonal variations in histomorphology of testes and bursa, immune parameters and serum testosterone concentration in male guinea fowl (*Numida meleagris*). Pak. Vet. J., 35(1):88-92.

Al-Dokhi, O.A., Al-Onazee, Y.Z. and Mubarak, M. (2004). Light and electron microscopy of the testicular tissue of the Snake *Eryx jayakari* (Squamata, Reptilia) with a reference to the dividing germ cells. J. Biol. Sci. 4(3): 345- 351.

Almeida, F.F.L. (2002). Estrutura e função testiculares em javalis (*Sus scrofa scrofa*) sexualmente maduros. 2002. 65 f. Dissertação (Mestrado) - Escola de Veterinária, Universidade Federal de Minas Gerais, Belo Horizonte, 2002.

Ashanbhag, B. (2002). Reproductive Biology of Indian Reptiles. Proc. Indian Natn. Sci Acad. (PINSA) B68, 6: 497-528.

Bacha, W.J. and Bacha, L.M. (2000). Color Atlas of Veterinary Histology. Lippincott William and Wilikins, USA, 219 pp.

Costa, D. S., Silva J.F.S. and Silveira, L. S. (2007). Morphometry of Leydig cells in the collared peccary (*Tayassu tajacu*). Braz. J. Vet. Res. Anim. Sci., Sao Paulo, 44(5):384-389.

De Kretser, D.M. and Kerr, J.B. (1994). The cytology of the testis In: Knobil, E., Neill, J.D. The physiology of reproduction. 2ed. New York: Raven Press, p.1177-1290.

Domke, L.M. (2018). Molecular and ultrastructural characteristics of adhering junctions and cytoskeletons in cells of the seminiferous tubules and the peritubular walls of mammalian testes. PhD Thesis. Combined Faculties for the Natural Sciences and for Mathematics, University of Heidelberg, Germany

Dubois, W., Pudney, J. and Callard, I.P. (1988). The annual testicular cycle in the turtle *Chrysemys picta* A histochemical and electron microscopic study. Gen. Comp. Endocrinol., 71:191-204.

Dufaure, J.P. (1968) L'ultrastructure des cellules interstitielles du testicule adulte chez deux Reptiles Lacertilies: le lézard vivipare (*Lacerta vivipara* Jacquin) et l'orvet (Anguis fragilis L.). C R Acad. Sci. Paris 267: 883–885.

Ferreira, A. and Dolder, H. (2003). Cytochemical study of spermiogenesis and mature spermatozoa in the lizard *Tropidurus itambere* (Reptilia, Squamata). Acta Histochem. 105(4) 339–352.

Franca, L.R. and Godinho, C.L. (2002). Testis morphology, seminiferous epithelium cycle length and daily sperm production in domestic cat (*Felis catus*). Biol. Reprod. 68:1554–1561.

Gribbins, K.M., Rheubert, J.L., Collier, M.H., Siegel, D.S. and Sever, D.M. (2008). Histological analysis of spermatogenesis and the germ cell development strategy within the testis of the male Western Cottonmouth Snake, *Agkistrodon piscivorus leucostoma*. Ann. Anat. 190: 461—476.

Kirkpatrick, D.T. (1995). An Essay on Taxonomy and the Genus *Pelusios*. Available at: http://www.unc.edu/~dtkirkpa/stuff/pel.html. Accessed 22 February 2013.

Losinno, A.D., Morales, A., Fernandez, D., Lopez, L.A. (2012). Peritubular myoid cells from rat seminiferous tubules contain actin and myosin filaments distributed in two independent layers. Biol. Reprod. 86:150 151-158.

Mahmoud, I.Y., Cyrus, R.V., Bennett, T.M, Woller, M.J. and Montag, D.M. (1985). Ultrastructural changes in testes of the snapping turtle *Chelydru serpentina* in relation to plasma testosterone, 5-3B-hydroxysteroid dehydrogenase, and cholesterol. Gen. Comp. Endocrinol., 57:454-464.

Mcpherson, R.J. and Marion, K.R. (1981). Seasonal testicular cycle of the stinkpot turtle (*Sternotherus odoratus*) in central Alabama. Herpetologica 37:33-40.

Mendis-Handagama S.M.L. and Ariyaratne, H.B.S. (2005). Leydig cells, thyroid hormones and steroidogenesis. Indian J. Exp. Biol. 43: 939-962.

Meylan, P.A., Sculer, R. and Moler P. (2002). Spermatogenic Cycle of the Florida Softshell Turtle, *Apalone ferox.* Copeia (3): 779–786.

Oke, B.O., Ugwuegbu, S.O. and Akusu, M.O. (1984). Morphometric study of the testis of the West African Dwarf goat. Bul.l Anim. Health Prod. Afri. 32:57–60.

Olukole S.G., Okusanya, B.O., Oyeyemi, M.O. and Oke, B.O (2013): Sperm morphological characteristics and morphometry in the African sideneck turtle (*Pelusios castaneus).* Asian J Exp. Biol. Sci. 4(4): 607-612.

Olukole, S.G., Oyeyemi, M.O. and Oke, B.O. (2014a): Anatomy of the male reproductive organs of the

African sideneck turtle (*Pelusios castaneus*). Anat J. Afri. 3(3) 380-385

Olukole, S.G., Oyeyemi, M.O. and Oke, B.O. (2014b): Spermatogenic cycle of the African sideneck turtle, *Pelusios castaneus* (Schweigger, 1812) (Reptilia: Testudines), Ital. J. Zool. 81(1) 25-31.

Olukole, S.G. and Oke, B.O. (2018). Sertoli cell junctions during active spermiogenesis in the African sideneck turtle (*Pelusios castaneus*): Implications for the blood-testis barrier. Int J. Vet. Sci. 7 (2): 106-112.

Olukole S.G., Onwuama, K.T., Oyeyemi, M.O. and Oke, B.O. (2018a). Gonadal and extragonadal sperm reserves in African sideneck turtle (*Pelusios castaneus*). Savannah Vet. J. 1(1): 10-13.

Olukole, S.G., Madekurozwa, M-C., and Oke, B.O. (2018b): Spermiogenesis in the African sideneck turtle (*Pelusios castaneus*): Acrosomal vesicle formation and nuclear morphogenesis. J. King Saud Univ. - Science 30: 359-366.

Olukole S.G. and Oke, B.O. (2020). Morphophysiology of the epididymis of the African sideneck turtle (*Pelusios castaneus*): Histological, microstereological and ultrastructural approach. Sahel J. Vet. Sci. 17(1): 17-25.

Rao, R.J. and Shaad, F.U. (1985). Sexual cycle of the male freshwater turtle, *Trionyx gangeticus (Cuvier)* Herpetologica 41(4): 433-437.

Russell, L.D. (1996). Mammalian Leydig cell structure. In: Payne, A.H., Hardy, M.P., Russell, L.D. The Leydig cell. Vienna, IL: Cache River Press. p.43-96.

Sagatelli, T.M., Franca, L.R., Pinheiro, P.F.F., Alemida, C.C.D., Martinez, M. and Martinez, F.E. (2004). Spermatogenic cycle length and spermatogenic efficiency in the Gerbill (*Meriones unguiculatus*). J Androl. 25:210–222.

Young, B., Lowe, J.S., Stevens, A. and Heath, J.W. (2006). Wheater's Functional Histology: A text and colour atlas. Fifth Edition, Churchhill Livingstone, Elsevier Limited. Pg 346-358.