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Research Articles

Comparative Histomorphologic Evaluation of the Testicular Apparatus of Vertebrates

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SUMMARY
Objectives: The testis, the reproductive organ of male vertebrates, shares common features across the Amniotes, Phylum. While variations are well circumscribed, only sparse documentation on comparative histomorphology of the vertebrate testes exists and these are focused mainly on within-class interspecies Phylogeny characterization.
Methods: Ethical clearance for this comparative histologic observational study was obtained from the HREC of the Department of Human Anatomy and Cell Biology, Delta State University, Abraka (No. DELSU/CHS/ANA/118). The research was conducted in accordance with the guidelines for the care and use of animals for research.
Results: The testes of the studied vertebrates all possessed capsules which was thin in non-amniotes and thicker in amniotes. Seminiferous tubules were common to all classes though cystic patterns were peculiar to fish and amphibians. It was revealed that fish had the least developed tubular system and number of germ cells per tubule while germ cells were most abundant in birds. Sertoli cells were present in all the seminiferous tubules of the vertebrate classes.
Conclusion: The reported histomorphologic features in the studied vertebrates strongly demonstrate testicular characteristics that are conserved within the group despite differences in class habitat. The implication is the probable existence of a tightly regulated genetic control of male gonads within the phylum. The findings suggest that the observed differences arose from possible adaptational changes owing to evolutionary pressures within the different environments to which the animal class found itself.

INTRODUCTION

The testes are the reproductive organ of male vertebrates, displaying conserved features across the phylum, albeit apparent variations.[1] The germ cells have earlier been shown to originate from the wall of the yolk sac in early embryonic life and migrate to the final position as observed in the adult.[2] The testes not only accommodates the germ cells but is also the location for Sustentacular cells of Sertoli and interstitial cells of Leydig which are instrumental to development of male phenotypic characteristics through its production of the hormone, testosterone, the most potent natural androgen.[3] The male sex cells, the spermatozoa are generated in the seminiferous tubules of the testes through empirical processes of mitosis and meiosis, from their precursors; spermatogonia, spermatocytes and spermatids.[4]

The presence of a vertebral column derived from a notochord is the anatomical basis for taxonomical designation of the vertebrate phylum.[5] Despite this long accepted characteristic, vertebrates share other features peculiar to the group. A copious literature search scarcely revealed the effects of adaptational and/or evolutional factors in relatedness vis-à-vis variations existing between and within vertebrates.[5-10]

The thrust of this study therefore was to demonstrate the features of the testes in vertebrates and provide a detailed histologic description of the testes of the different classes. The observed ontogenic patterns will definitely provide an explanation for the relatedness of members of the vertebrate phylum. The possible evolutionary, adaptational factors involved and the histomorphologic variations existing in the vertebrate phylum was also established.

METHODS

Permission for this comparative histologic observational study was sought and received from the research and ethics committee of the department of Human Anatomy and Cell Biology, Faculty of Basic Medical Sciences, College of Health Sciences, Delta State University, Abraka (Number DELSU/CHS/ANA/118). The research was conducted in accordance with the guidelines for the care and use of animals for research.[11-12]

Five animals were utilised for the study (one from each vertebrate class). The animals included: common cat fish (*Clarias gariepinus* - fish); the toad (*Bufobufo* - amphibian); the agama lizard (*Agama agama* - reptile); the house hold chicken (Gallus domesticus - bird) and the Wistar rat(*Rattus norvegicus*- mammal).

Following euthanasia, the testes of each animal were dissected out and preserved in 10% formal saline for about 48 hours. The samples were then processed using standard procedures involving progressive dehydration from 75% to absolute alcohol, emersion in xylene, embedding in wax, processing through sectioning (3-4μm) and staining with haematoxylin and eosin.[13] The histology slides obtained were then analysed and interpreted with the aid of a Scopetek DCM 500, 5.0 mega pixel Microscope Camera and computer.

RESULTS

Sections of the testes of a fish show a capsule (Figures 1), 1-2 layer thick of dense connective tissue in which are some duct-like structures lined by pseudostratified ciliated epithelium (Figure 2). The capsule encloses several diverse shaped cystic structures lined by moderately sized germ cells with round to oval centrally disposed bland nuclei and pale cytoplasmic borders (Figure 3). These stem cells give rise to about four to five generation of cells with smaller size as they approach the luminal aspect of the cyst (Table 1). The cells are held together by extensive cytoplasmic processes of Sertoli cells which possess a small round to oval nuclei, a pattern suggestive of a monoclonal origin. Each cystic structure is separated by a fibrovascular connective tissue stroma in which are few pale interstitial cells with vesicular nuclei disposed in clusters. These cells are relatively large with pale nuclei.

Sections of toad testes showing several variably sized cystic structures lined by flattened epithelial cells with bland spindle shaped nuclei (Figure 4). The cysts invest germs cells of varying stages of maturity, disposed in clusters invested by cytoplasmic processes of Sertoli cells which possess round to oval pale nuclei with prominent nucleoli (Figure 5). Corresponding with Sertoli cells, about 4-6 clusters of cells are present in each cyst 4-6 layers thick (Table 1). The terminally differentiated cells are flagellated and invested in cytoplasmic processes forming pseudo-rosettes. Pale cells are intermixed in the intervening fibrovascular connective tissue stroma (Figure 6). These Leydig cells possess vesicular variably shaped nuclei. There is a 1 to 2 cell layer of fibroconnective tissue stroma investing the testes.

Sections showing several seminiferous tubules of varying sizes in a lizard testis (figure 7). Each tubule is composed of germinal epithelium at different stages of development (figure 8). The basal spermatogonia are relatively small with dense nuclei and scant cytoplasm. This gives rise to the slightly larger primary spermatocytes, spermatids and flagellated spermatozoa which are in the luminal aspect of tubule. The cells occur in columns with about 15-20 columns, corresponding with Sertoli cells, per tubule and 10-15 cells per column (Figure 9, Table 1). The intervening fibrovascular stroma contains moderately sized Leydig cells with squamoid appearance and vesicular nuclei. There is a 2-3 cell layer dense fibroconnective tissue capsule investing the testes.

Sections of bird testes show several variably sized seminiferous tubules disposed within a fibrovascular stroma in which are several moderately sized interstitial cells (Figure 10). These Leydig cells possess pale cytoplasm with moderate sized nuclei and prominent nucleoli. Within the tubule are germ cells at different stages of development (Figure 11). The cells are arranged in columns, coinciding with the support cells (Sertoli cells) with each tubule containing 20-25 of such columns and each column consisting of 5-10 cells (Table 1). The basal cells, spermatogonia display nucleopleomorphism with mild vesiculation and scant cytoplasm. 7-8 generations of cells have eosinophilic cytoplasm with evidence of mitotic changes occurring (Figure 12). The spermatids, small cells with small sized nuclei and eosinophilic cytoplasm are present with flagella tails disposed in the luminal aspect of the tubule.

Sections of rat testes indicate several variably sized seminiferous tubules lined by germ cells at different stages of maturation (Figure 13). Spermatogonia, small sized cells with oblong, densely coloured nuclei line the basal aspect of each seminiferous tubule (Figure 14). Primary spermatocytes follow these stem cells and they are committed to production of spermatids. The primary spermatocytes are moderately large cells with moderately abundant eosinophilic nucleus and with chromatin in mitosis. Spermatids are relatively smaller flagellated cells held together by cytoplasmic processes. From the spermatogonia to the terminally differentiated spermatozoa, the cell layer is about 6-10 layer thick in most tubules (Figure 15, Table 1). Interstitial cells of Leydig are present in the intervening fibromuscular connective tissue stroma. Blood vessels are also seen. The testis is invested in a dense regular 2-3 cell layer thick connective tissue capsule.

Table 1: Vertebrate testes; Comparison of Microanatomic Structure

S/N	Vertebrate	Capsule	Interstitium	Seminiferous Tubule Pattern	Cell location	Cell arrangement	Flagella -
1.	Fish	Thin	Leydig cells	Cystic	Central	Singles/clusters	+
2.	Amphibian	Thin	Leydig cells	Cystic	Peripheral	Clusters 4-6	+
3.	Reptile	Moderate	Leydig cells	Small tubules	Peripheral	Columns 10-15	+
4.	Bird	Thick	Leydig cells	Polygonal tubules	Peripheral	Columns 5-10	+
5.	Mammal	Thick	Leydig cells	Round to Oval tubules	Peripheral	Columns 6-10	+

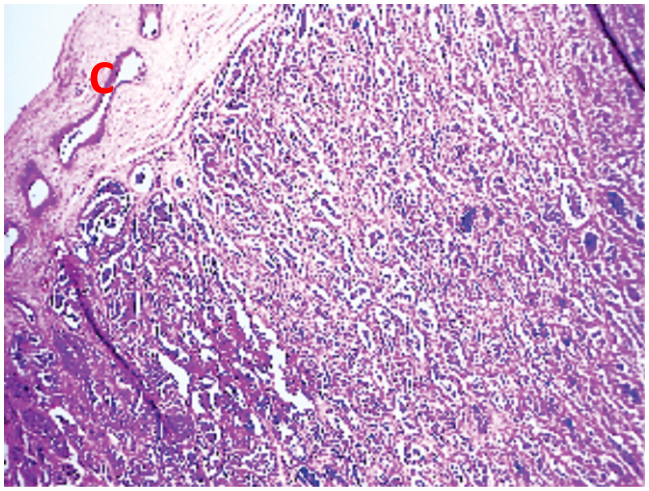

Figure 1: Photomicrographic section of the testes of cat-fish *Clarias gariepinus* showing the Capsule C. (H&E stain, mag x40).

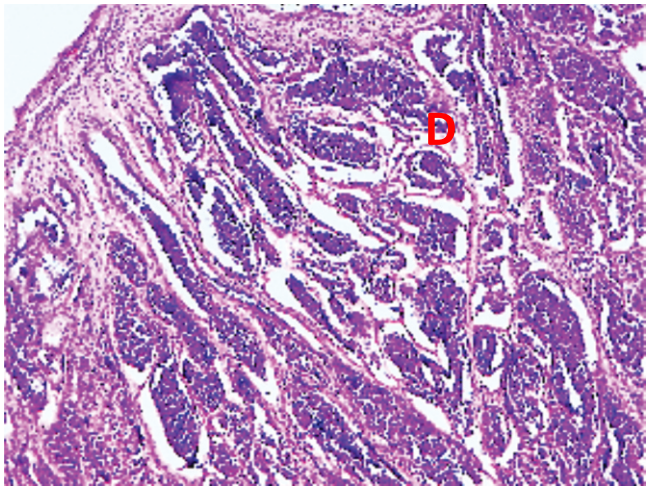

Figure 2: Photomicrographic section of the testes of cat-fish *Clarias gariepinus* showing duct-like structures, D, lined by pseudostratified ciliated epithelium (H&E stain, mag x100).

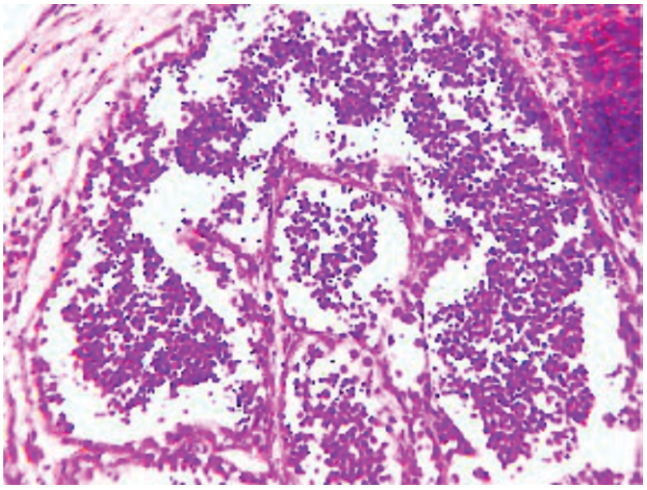

Figure 3: Photomicrographic section of the testes of cat-fish *Clarias gariepinus* showing the capsule enclosing several diverse shaped cystic structures, Cy, and lined by moderately sized germ cells with round to oval centrally- disposed bland nuclei and pale cytoplasmic borders (H&E stain, mag x400).

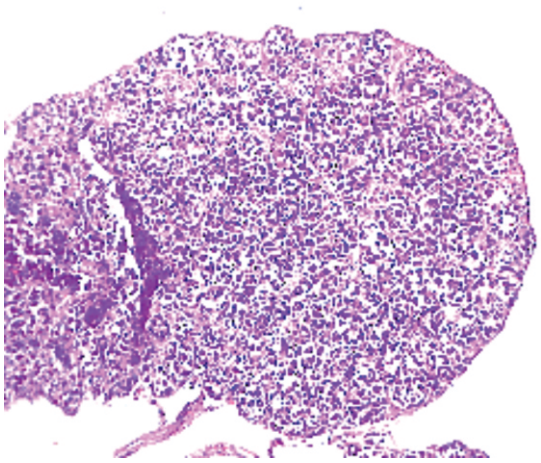

Figure 4: Photomicrographic section of the testes of the amphibian toad *Bufobufo* showing several variably sized cystic structures lined by flattened epithelial cells with bland spindle shaped nuclei (H&E stain, mag x40).

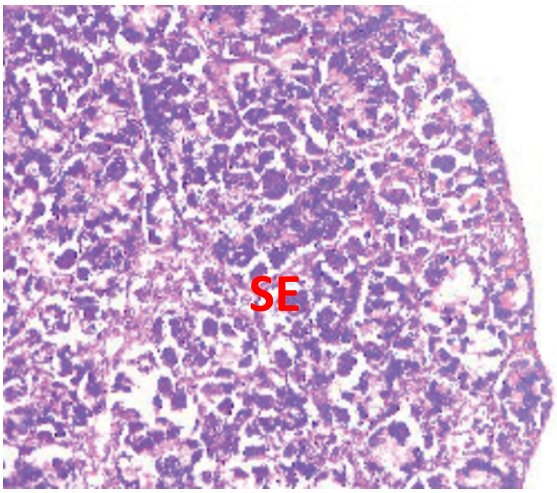

Figure **5:** Photomicrographic section of the testes of amphibian toad *Bufobufo*. The cysts, Cy, invest germs cells of varying stages of maturity, disposed in clusters invested by cytoplasmic processes of Sertoli cells, SE. Corresponding with Sertoli cells, about 4-6 clusters of cells are present in each cyst 4-6 layers thick (H&E stain, mag x100).

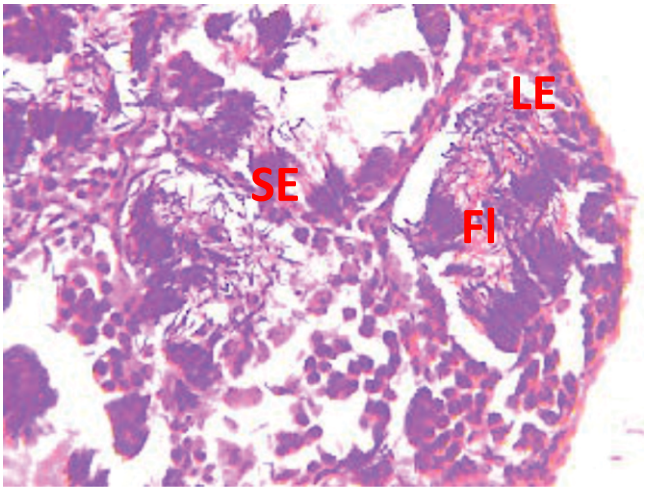

Figure 6: Photomicrographic section of the testes of amphibian toad *Bufobufo*. Pale cells, LE, are intermixed in the intervening fibrovascular connective tissue stroma. Flagellated cells, Fl, are also seen (H&E stain, mag x400).

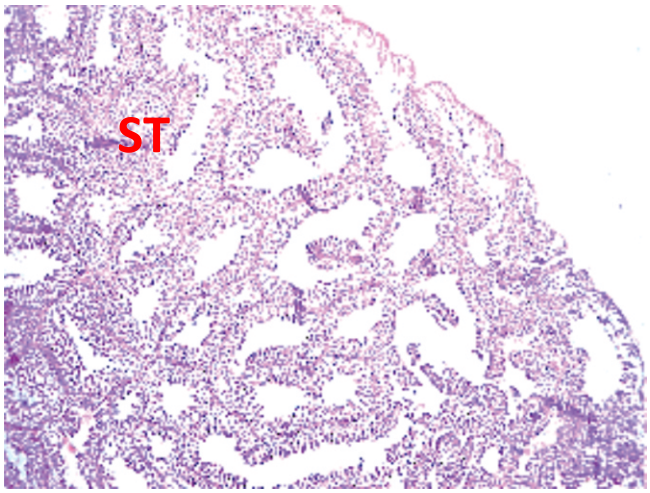

Figure 7: Photomicrographic section showing several seminiferous tubules, ST, of varying sizes in a reptilian lizard *Agama agama*, testis (H&E stain, mag x40).

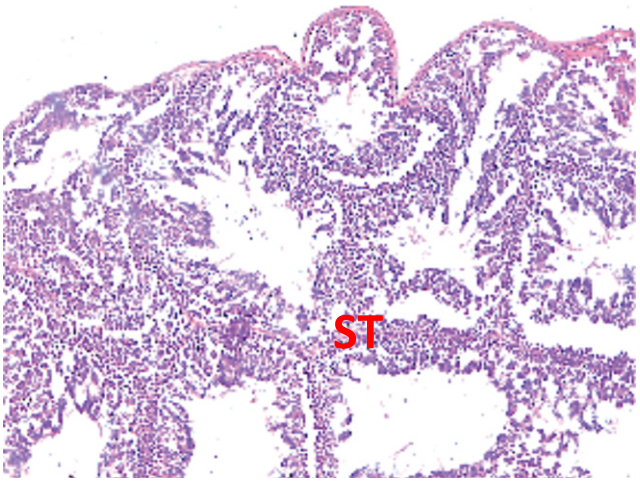

Figure 8: Photomicrographic section of the testes of reptilian lizard *Agama agama*. Each tubule is composed of germinal epithelia at different stages of development. The basal spermatogonia are relatively small with dense nuclei and scant cytoplasm (H&E stain, mag x100).

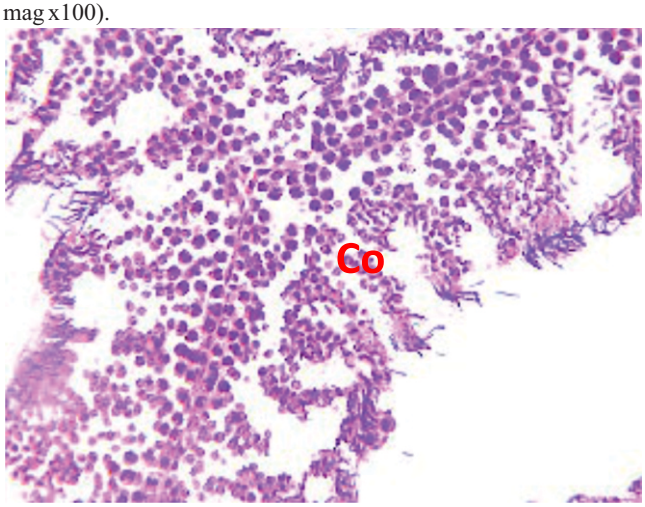

Figure 9: Photomicrographic section of the testes of reptilian lizard *Agama agama*. The intervening fibrovascular stroma contains moderately sized Leydig cells with squamoid appearance and vesicular nuclei. There is a 2-3 cell layer dense fibroconnective tissue capsule investing the testes. There is a 2-3 cell layer dense fibroconnective tissue capsule investing the testes (H&E stain, mag x400).

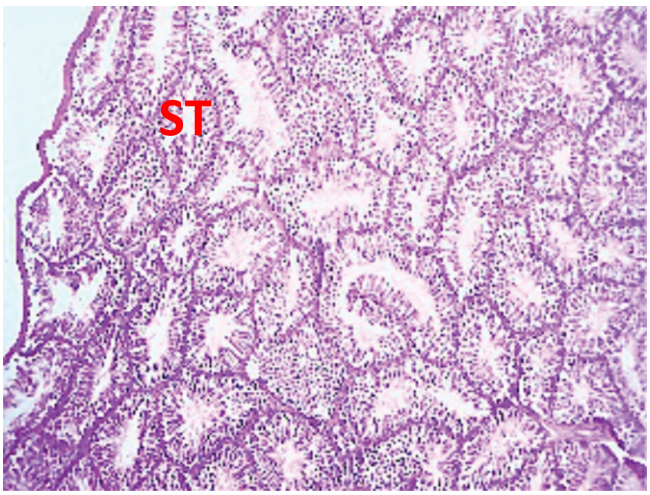

Figure 10: Photomicrographic section of bird, domestic fowl *Gallus domesticus*, testes showing several variably sized seminiferous tubules disposed within a fibrovascular stroma in which are several moderately sized interstitial cells (H&E stain, mag x40).

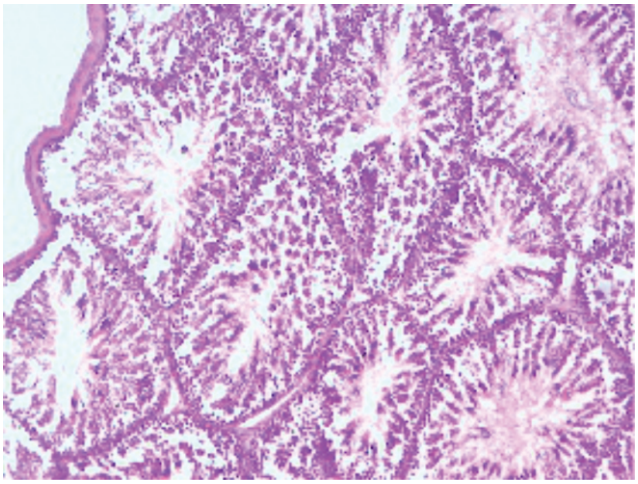

Figure 11: Photomicrographic section of the testes of bird, domestic fowl *Gallus domesticus*. Within the tubules are germ cells at different stages of development (H&E stain, mag x100).

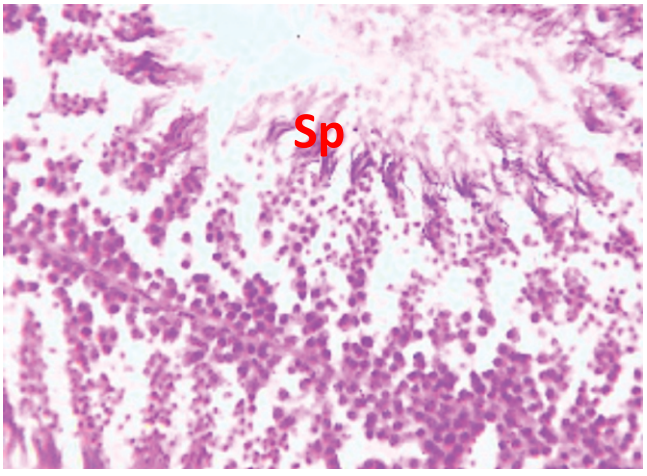

Figure 12: Photomicrographic section of the testes of bird, domestic fowl *Gallus domesticus*. The cells are arranged in columns, coinciding with the support cells (Sertoli cells) with each tubule containing 20-25 of such columns and each column consisting of 5-10 cells. The spermatids, small cells with small sized nuclei and eosinophilic cytoplasm are present with flagella tails disposed in the luminal aspect of the tubule (H&E stain, mag x400).

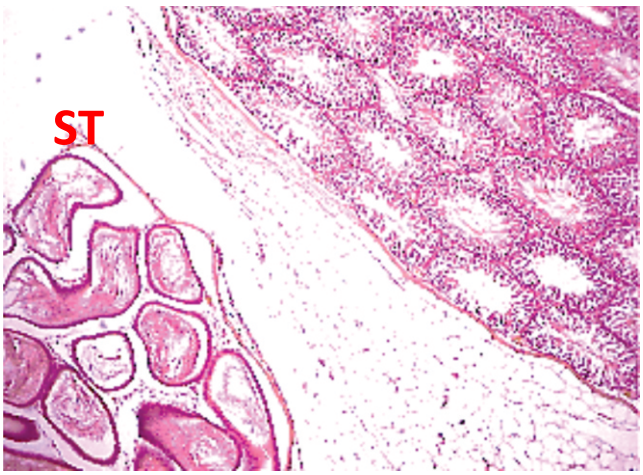

Figure 13: Photomicrographic section of mammalian Wistar rat, *Rattus norvegicus* testes indicating several variably sized seminiferous tubules, ST, lined by germ cells at different stages of maturation. At the lower left corner are sections of epididymis (H&E stain, mag x40).

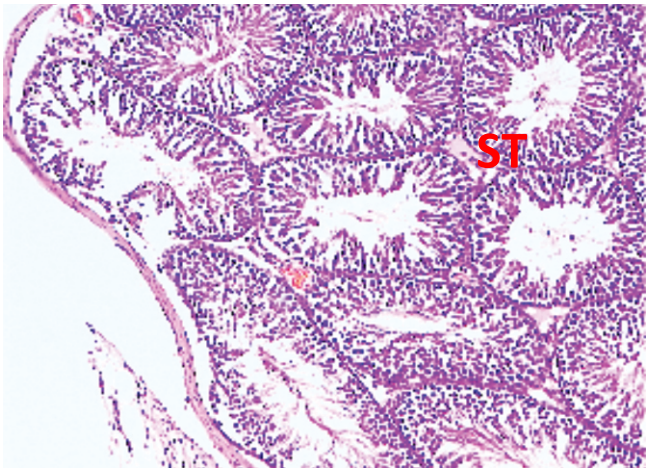

Figure 14: Photomicrographic section of the testes of mammalian Wistar rat, *Rattus norvegicus.* Spermatogonia, small sized cells with oblong, densely coloured nuclei line the basal aspect of each seminiferous tubule. Spermatids are relatively smaller flagellated cells held together by cytoplasmicprocesses. (H&Estain,magx100)

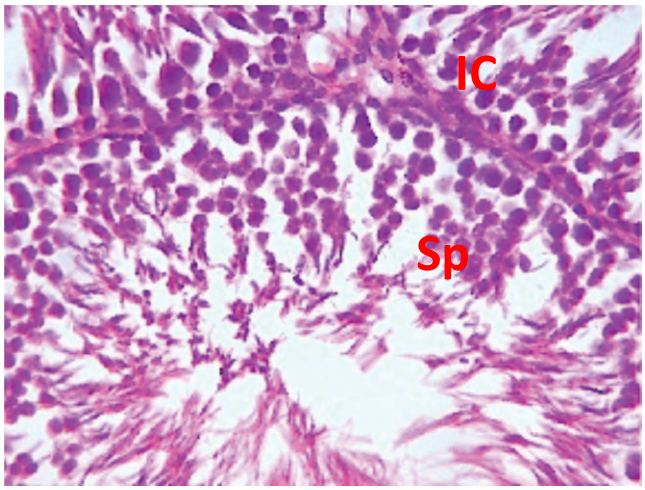

Figure 15: Photomicrographic section of the testes of mammalian Wistar rat, *Rattus norvegicus*. From the spermatogonia to the terminally differentiated spermatozoa, Sp, the cell layer is about 6-10 layer thick in most tubules. Interstitial cells, IC, of Leydig are present in the intervening fibromuscular connective tissue stroma. Blood vessels are also seen. The testis is invested in a dense regular 2-3 cell layer thick connective tissue capsule (H&E stain, mag x400).

DISCUSSION

This study has elucidated the structural relationships and variations existing between and within the male reproductive testes in vertebrates. The observations are strongly suggestive of interplay between both adaptive and evolutionary factors in fashioning of ontogenic patterns of testicular morphology in the studied vertebrate subphylum. Assessment of fish testes revealed that they possessed the most primitive form of this male sex organ in the group. The lobulocystic patterns observed in this study was not new. Several authorities have affirmed that some fish species displayed cystic lobular arrangement - a feature of primitive gonads.[7,14-17] It has been further suggested that some fish species, teleosts, maintained testicular cycles in which two sets of tubules were present in the same fish, some lobules containing spermatogenic cells while others were clustered with differentiated/specialised spermatozoa.[16,18-20]

Only spermatogenic cells, spermatogonia and spermatocytes were described in this study. It is likely that the fish analysed was at the period of the testicular cycle identified by Ahmed *et al*. as spermatogenic testis in which most of the luminal cells were in the developing mitotic phase of the cell cycle unlike the pubertal testis in which the testes was in the meiotic phase.[7,21] Another important observation was the clustering of cells within each cyst. Cell clusters were invested in cytoplasmic processes of Sertoli cells which have been shown to provide nutrition for the spermatogenic cells.[2]

A thin fibroconnective tissue capsule was noted encasing the fish testes. The importance of this is not farfetched especially as the mechanism of release of the germ cells from the male sex organ is by rupturing of the cystic tubules into the surrounding fluid medium and migration of the spermatozoa to the eggs hitherto laid by the female fish. The toad, amphibian was also observed to possess a similar thin fibroconnective tissue capsule in which the testes were invested. Some authors have confirmed that most amphibians possessed extensive seminiferous tubules in their testes, each containing clusters of clonal germ cells whose nutrition is provided by a Sertoli cell. [22-23] As was demonstrated in the index study, most clones were composed of columns of between 4-6 cells of the spermatogonic series like Rastogi *et al*. and Takamure *et al*. had previously confirmed.[22-25]

The existence of monoclonal progenies of spermatogonia in each tubule had earlier been identified [26-28] and the current study further demonstrated an extensively populated seminiferous tubule, with more flagellated spermatogonia in the toad than in the fish. Though both the fish and toad possessed cystic structures for generating germ cells, several factors had the tendency of affecting amphibians more. Rastogi *et al*., and Loumbourdis and Kyrie corroborated this fact when they affirmed the influence of environmental fluctuations on the testes of toads.[29,30] The fish on the other hand inhabit a more stable aquatic environment which is indispensable in the dispersal of spermatozoa for effective fertilization.

The interstitial cells described in this study were not new. A prior investigation by Fraile *et al*.[31] had shown a parenchymal to stromal ratio of 7:1 which was in keeping with the finding in this study even amongst vertebrates of higher classes. This apparent conservation of interstitial/stromal tissue also extended specifically to the

Leydig cell morphology which also clearly exhibited close similarity in the vertebrate phylum.

Reptiles, unlike fish and amphibians, are the first in the series of amniotes. This group of vertebrates are adapted to the terrestrial environment by developing seminiferous tubules with numerous germ cells and further developed Sertoli cells which are retained following release of the mature germ cells from the tubules.[6,32-34]

Generally, the structure of the seminiferous tubule could clearly be differentiated from those of fish and amphibians. They were larger and exhibited a close association between each germ cell cluster and Sertoli cell like in mammals and birds. Russel *et al*. had previously emphasised this spatial arrangement.[35] The significance of this pattern has been ascribed to internal fertilization, an evolutionary sophistication of amniotes.[36-37] Despite the inclination to the mammalian organisation, the cluster/tubular inconsistency of the amphibians were however retained.[38-44] The interstitial cells were not different from those of the fish and amphibians further corroborating an earlier reports that the Leydig cell morphology was conserved throughout the vertebrate phylum.[45-46]

The seminiferous tubules of birds were distinctly polypoid in shape with prominent interstitial cells of Leydig. The observed abundant Leydig interstitial cells observed could have been a result of seasonal changes in the reproductive cycle of the bird studied. Rosenstrauch *et al*. [47] revealed that interstitial cells were more prominent during breeding than in non-breeding states. The specific features of these cells, prominent nucleoli within a vesicular nucleus in a squamoid cell with granulated cytoplasm, as seen in other lower vertebrates, remained unaltered.

Germ cells at different stages of maturation lined the tubules. Each column was closely associated with its own sustentacular cell. Paramount was the multiple stages/generation of differentiation of the germ cells unlike in the reptiles, Amphibians or fish. Also, several of such columns per tubule were observed. Pudney[48] opined that this was necessary for adequate reservoir for subsequent spermatogenic activity following ejaculation.

Another striking feature of the testes in the bird studied was the dense fibroconnective tissue investing the male gonad. This dense irregular connective tissue was observed to extend deep between and within the seminiferous tubules, separating them into clusters, albeit less intense than the thickness of the overlying capsule. Aire and Ozegbe [49] explained that myoid tissue present in bird capsule was of significant importance in aiding the propulsion of fluid content of the testes to the epididymis. Cortical and medullary septa from the capsule were however not present.[50-52]

Similarly, the testes of the mammal,(rat), was observed to be enclosed by a similar dense irregular connective tissue capsule which displayed extensions into the testicular parenchyma carrying neurovascular bundles providing nourishment. Unlike the polygonal disposition of the bird seminiferous tubules, those of rats were round to oval in shape and surrounded by interstitial cells of Leydig. These cells, as has earlier been described were not different from those of the other vertebrates in the phylum.

In conclusion, the reported histomorphologic features in the studied vertebrates have strongly suggested characteristics that are conserved within the group despite differences in class habitat. The implication of this is that there exists a genetic control of male gonads within the phylum which is tightly regulated. Also, the existence of differences, which has most likely developed from adaptational changes owing to the different environments to which the animal class finds itself, has been established.

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Declaration

We declare no conflict of interest.

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