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Morphological and Histomorphological Structure of Testes of the Catfish *"Clarias Gariepinus"* From Egypt

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Abstract: Knowledge of the normal reproductive biology of catfish is of a great importance not only for better understanding of the theory of fish development, but also to be used as a baseline for understanding the pathological changes results form exposure to harmful toxicants. Thus, the aim of the current study was to elucidate the gross anatomical and light microscopic features of the testes of the catfish "*Clarias gariepinus*" from Egypt. The present study was carried out on 36 mature male catfish (*Clarias gariepinus)* collected monthly during the spawning period (April-September) throughout the Nile River, crossing Sohag city in Egypt, in the year 2011. Samples were processed for light and electron microscopy. Each testis consisted of two regions, a fringed outer lateral region and a smooth sagittal-medial region. Histological examination revealed that the testis was covered with a highly vascular connective tissue capsule sending septa dividing the testis into seminiferous lobules separated by interstitial connective tissue containing steroid secreting Leydig cells. Seminiferous lobules were made up of spermatogenic cells and Sertoli cells; the spermatogenic cells located within cysts formed by the cytoplasmic projections of the Sertoli cells. According to the maturation stages, the seminiferous lobules were classified into three types. Spermatogenic seminiferous lobules were lined with different spermatogenic cells but had few or no spermatozoa. Pubertal seminiferous lobules were packed with spermatozoa with few spermatogenic cells. Spent seminiferous lobules contained remnants of spermatozoa and spermatogenic cells. In conclusion, the testis of catfish *Clarias gariepinus* from Egypt was similar in structure to other catfish and teleost species.

Key words: Catfish, *Clarias gariepinus*, testis, histology

INTRODUCTION

Catfish (Order siluriformes) is a diverse and widespread fresh water group of Ostariophysan fish (Wright, 2009). Catfish is the most diverse fish order and the second or third diverse order among vertebrates; it represents 1 to 10 of the fish and 1 to 20 of the vertebrates (Van Dyk and Pieterse, 2008). Many species of catfish including the sharp tooth catfish *Clarias gariepinus* (*C. gariepinus*) have been introduced into aquaculture (Teugels, 1996). Understanding the biological processes especially the reproductive biology is the most important factor to improve the catfish culture.

The testes of sexually mature catfish are paired elongated structures located dorsally in the body cavity and the left testis is usually longer than the right one (Van Dyk and Pieterse, 2008). The testes of most catfish species have digitiform projections or fringes, but some species have no fringes (Loir *et al*., 1989). Similar to mammals (Ahmed *et al*., 2012) and birds (Friedlander *et al*., 1992), the fish testis parenchyma is formed from two main compartments; seminiferous lobules or lobules and interstitial tissue (Schulz *et al*., 2010). The seminiferous lobules are separated from each other by connective tissue septae that continue with the covering tunica albuginea (Meisner *et al*., 2000). The seminiferous lobules include somatic Sertoli cells and germ or spermatogenic cells that are usually present in cysts (Schulz *et al*., 2010). In most families of catfish, spermatogenic cells are present along the entire length of the testis (Chaves-Pozo *et al*., 2005), whereas in few other families seminal vesicles are present in the caudal region to store spermatozoa or to have secretory activity (Verreth *et al*., 1993). The fish spermatogenic cells, like in other species, include primary and secondary spermatogonia, primary and secondary spermatocytes, spermatids and spermatozoa. Unlike mammals and birds, each spermatogenic cell type present as group within

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cysts made by the processes of the Sertoli cells (Van Dyk and Pieterse, 2008). The Sertoli cells are elongated and have light oval nucleus with distinct nucleolus and their proliferation has been mentioned as the main factor of increasing testis size in fish (Schulz *et al*., 2010). Spermatogonia are large cells with vesicular nucleus, the primary spermatogonia present as one per cyst, while the secondary spermatogonia are smaller and usually observed as two or four per cyst. Spermatocytes are smaller than spermatogonia and present as primary or secondary spermatocytes. Primary spermatocytes form large cysts and the cells have nucleus with indistinct boundaries and the secondary spermatocytes are smaller with irregular nucleus. Spermatids are smaller than spermatocytes and they have highly basophilic nucleus and scant cytoplasm. The spermatozoa are the smallest among the spermatogenic cells, have head with no acrosome and long flagella and located free in the lumen (Santos *et al*., 2001). The interstitial tissue contains sex hormone secreting Leydig cells within a vascular connective tissue containing macrophages and mast cells (Nobrega and Quagio-Grassiotto, 2007). The seminifrous lobules are continuous with a duct system and the spermatic duct of right and left testes joined together at their caudal portions, forming the common spermatic duct, which open into the genital papillae, situated caudally to the anal opening (Lopes *et al*., 2004). The Leydig cells occurs in groups and with electron microscopy appear with vesicular nucleus and cytoplasm containing well developed smooth endoplasmic reticulum and mitochondria with tubular cisternae (Santos *et al*., 2001). Breeding in catfish is seasonal and the level of testicular activity and spermatogenesis depends mostly on the light period and temperature (Garg and Sundararaj, 1985). The current study investigates the morphological and histological changes in the testes of the *C. gariepinus* collected monthly from the Nile River, crossing Sohag city in Egypt during the spawning season in the period between April and September 2011.

MATERIALS AND METHODS

Fish: For the current study 36 male catfish *C. gariepinus* were used. At least 6 sexually mature fish with weight 500±100 g and 42±5 cm standard length were collected monthly from the Nile River, crossing Sohag city in Egypt, during the spawning season (April-September) in the year 2011. Fish were sacrificed by serving the spinal cord anterior to the dorsal fin and the testes were dissected out using sharp scalpel blades and clean dissection tools.

Gross-morphometric measurements: Testicular weight, length and diameter were measured and the Gonado-Somatic Index (GSI) was calculated from the equation:

$$GSI = \frac{\text{Gonad weight}}{\text{Total body weight}} \times 100$$

Histological and histomorphometrical studies: Specimens included all parts of the testis from anterior through middle to posterior parts were rapidly fixed with either 10% buffered formalin or Bouin's solution and embedded in paraffin wax. Paraffin sections were stained with hematoxylin and eosin, Crossman's trichrome and Periodic Acid Schiff's reagent (PAS) and examined with light microscopy. Thickness of the tunica albuginea, diameter of the seminiferous lobules, volume percentage of seminiferous lobule and volume percentage of interstitial tissue were measured using Leica Q500 image analysis system (Leica Microsystems, Wetzlar, Germany).

Electron microscopy: Specimens were fixed with 2.5% buffered glutaraldehyde, post fixed in 1% osmium tetroxide and embedded in epoxy resin mixture. Ultrathin sections were obtained using Leica microtome (Leica Microsystems, Wetzlar, Germany). Sections were stained with uranyl acetate and lead citrate and examined with JIOL 1010 electron microscope (Peabody, USA).

RESULTS

Morphological structure of testes of *C. gariepinus*: The testes of mature *C. gariepinus* were elongated flattened paired organs located in the caudal part of the body cavity and attached to the dorsal surface by the mesorchium. Left testes were longer than the right one in all samples examined. The right and left testes were completely separated from each other but they became closely connected at the most caudal parts. Each testis contained 2 regions; outer and sagittal-medial. The lateral region had digitiform projections or fringes along its length, while the medial one was smooth with no fringes (Fig. 1a). Testis mean weight, length and diameter were 2.56±0.33 g, 5.97±0.20 cm and 6.47±0.34 mm for the left testis and 1.74±0.43 g, 5.40±0.07 cm and 6.47±0.34 for the right testis, respectively. The testicular GSI was 0.85±0.09%.

Histological structure of the testes of *C. gariepinus*: The testis was covered on its exterior with a vascular connective tissue tunica albuginea containing smooth

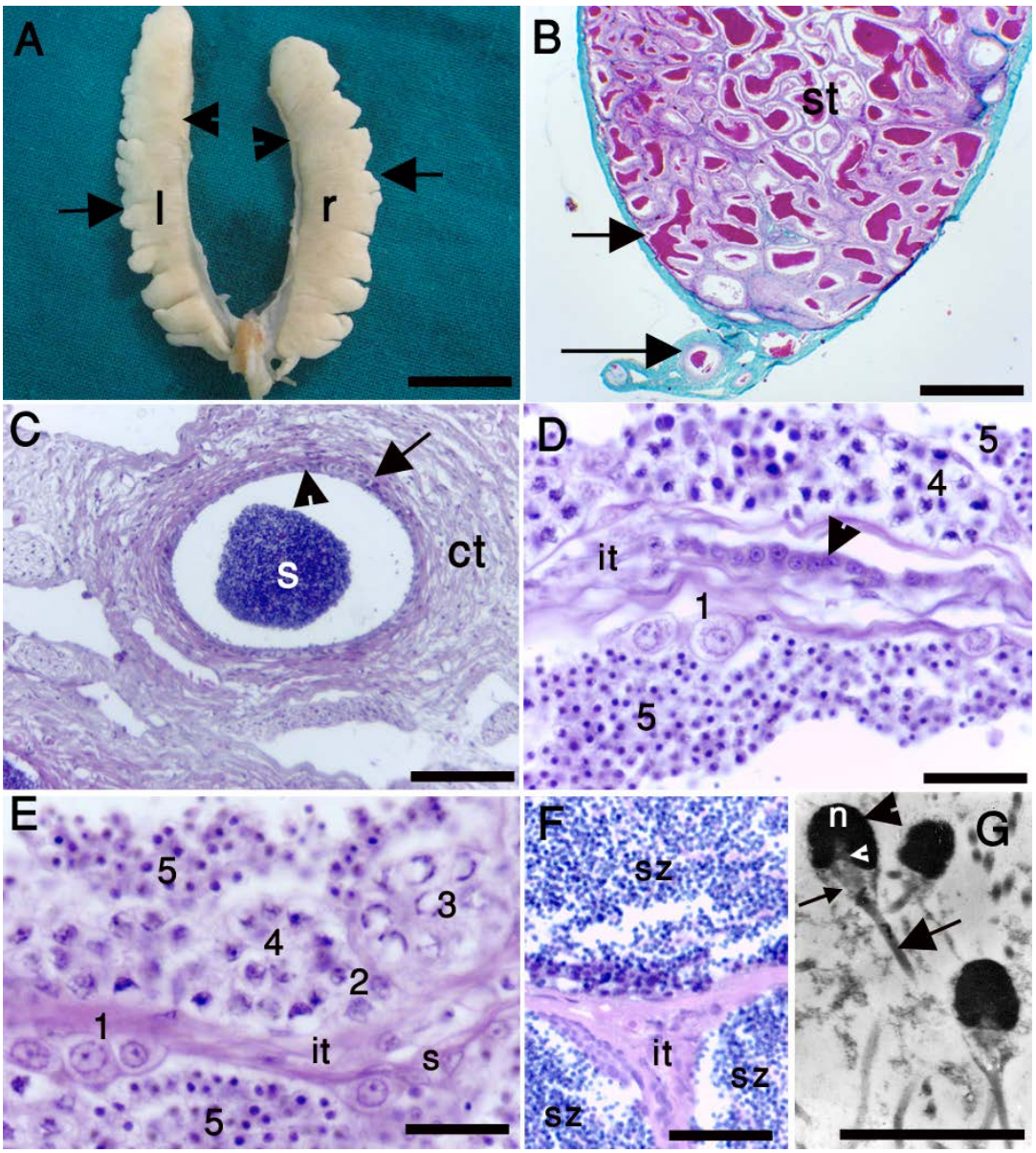

Fig. 1(a-g): Morphology and histology of the testis of the catfish *C. gariepinus* from Egypt, (A) Gross structure of the testis of the catfish *C. gariepinus*. Note right testis (r), left testis (l), fringed lateral surface (arrows) and smooth sagittal-medial surface. B-F: Paraffin section from the testis of the catfish *C. gariepinus* stained with Crossman's trichrome (B) and H and E (C-F), (b) Short arrow indicates tunica albuginea and long arrow indicate spermatic duct. Note seminiferous lobules (st), (c) Arrowhead indicates cuboidal epithelium and arrow indicates smooth muscle fibers of the spermatic duct. The duct contains sperms (s) and is surrounded by connective tissue (ct), (d) Arrowhead indicates clusters of interstitial Leydig cells within interstitial tissue (it), Note primary spermatogonia (1), secondary spermatocytes (4) and spermatids (5), (e) Spermatogenic cells; primary spermatogonia (1), secondary spermatogonia (2), primary spermatocytes (3), secondary spermatocytes (4), spermatids (5) and interstitial tissue (it), (f) Spermatozoa (SZ) and interstitial tissue (it) and (g) Head (arrowhead) with nucleus (n) and (nuclear fossa) small white arrowhead, midpiece (small arrow) and flagellum (big arrow) of the spermatozoa. Bars = 10 mm (A), 200 μm (B), 20 μm (C, F), 8 μm (D, E) and 0.5 20 μm (G)

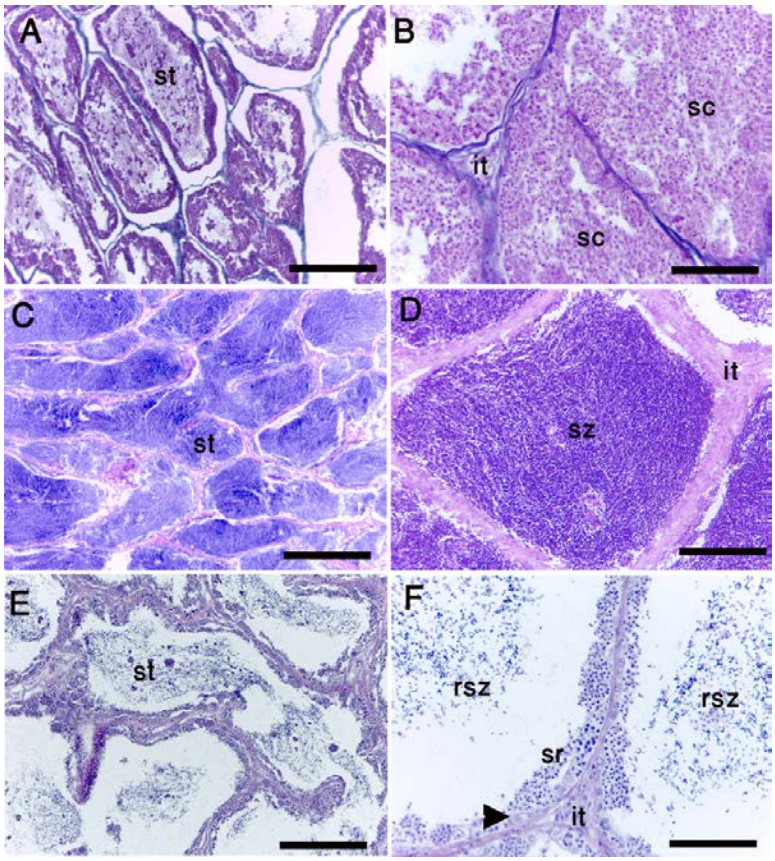

Fig. 2(A-F): Types of seminiferous lobules of the testis of the catfish *C. gariepinus* from Egypt Paraffin section from the testis of the catfish *C. gariepinus* during the spawning season stained with PAS (A, B), h and e (C, D, E, F). Note seminiferous lobules (ST), interstitial tissue (it) spermatogenic cells (sc) and remnants of spermatozoa (rsz). Bars = 80 μm (A, C, E) and 20 μm (B, D, F)

muscle and elastic fibers (Fig. 1b). The periphery of testis had a spermatic duct lined with cuboidal to columnar epithelium and in some sections appeared filled with spermatozoa but in others appeared empty (Fig. 1b, c). Connective tissue septae extended dividing the testis into compartments, seminifrous lobules or (Fig. 1b). Thickening in the spate between the seminifrous lobules was a vascular interstitial connective tissue containing oval or rounded vacuolated clustered steroid secreting Leydig cells (Fig. 1d). The testicular cranial and causal parts were similar and no secretory regions were found within the caudal portion indicating that the testes of catfish *C. gariepinus* were devoid of seminal vesicles. The seminiferous lobules enclosed spermatogenic cells and Sertoli cells (Fig. 1d, e). The seminiferous lobules of the catfish *C. gariepinus* were of cystic type; Spermatogenic cells of equal maturity grouped together within cysts made by the processes of the Sertoli cells. Spermatogenic cells included primary and secondary spermatogonia, primary and secondary spermatocytes, spermatids and spermatozoa. The primary spermatogonia were large ovoid cells with large vesicular central nucleus and distinct nucleolus and located at the periphery of the lobules. Secondary spermatogonia were slightly smaller and occurred in 2- or 4-cell cysts. Primary spermatocytes were even smaller than secondary spermatogonia and had much large rounded central nucleus and present in numerous-cell cysts. Secondary spermatocytes were smaller than primary spermatocytes and had nucleus with irregular-shaped deeply stained chromatin. Spermatids were the smallest and had elliptical condensed nucleus. Spermatozoa had rounded deeply stained nucleus and were observed free in the lumen (Fig. 1f). The spermatozoa were consisted of acrosome-less head, short midpiece and long single flagellum with scanty cytoplasm surrounded by the cell membrane. The head contained rounded electron dark nucleus underneath by an electron lucent depression, nuclear fossa (Fig. 1g). No sharp changes

were observed in each specific month during the spawning season; however, the testis exhibited one of three seminiferous lobule figures according to their maturation stage. Spermatogenic seminiferous lobules which were lined with peripherally situated primary spermatogonia and cystic secondary spermatogonia, primary and secondary spermatocytes and spermatids, and the lumen was lack of or had few spermatozoa (Fig. 2a, b). Pubertal seminiferous lobules were packed with spermatozoa and lined with very few spermatogenic cells (Fig. 2c, d). This type predominated during the peak of spawning in April and August. Spent seminiferous lobules contained remnants of spermatozoa and were lined with occasional spermatogonia and spermatogenic remnants (Fig. 2e, f). Histomorphometrical measurement revealed that the mean thickness of the tunica albuginea was 11.24±0.65 μm, diameter of the seminiferous lobules was 291.09±1.43 μm, volume percentage of seminiferous lobules was 95.38±0.42% and volume percentage of interstitial tissue was 4.61±0.42%.

DISCUSSION

The aim of the current study was to describe the morphological and histological structure of the catfish *C. gariepinus* testis from Egypt during the spawning season. The testes of the catfish C. *gariepinus* were paired elongated organs with fringed along their entire length and the GSI was about 1% of the body mass. The testis was surrounded by a connective tissue tunica albuginea and emitted septae dividing the testis into seminiferous lobules made by spermatogenic cells and separated by interstitial connective tissue which contained testosterone secreting Leydig cells. The catfish C. *gariepinus* shares the basic testicular structure to other fish (Meisner *et al*., 2000), avian (Friedlander *et al*., 1992) and mammalian species (Ahmed *et al*., 2012). Like in fish and unlike in mammals, the seminiferous lobules of the testes of the catfish *C. gariepinus* were of cystic type; cells similar in maturity stages grouped together in cysts (Loir *et al*., 1989; Chaves-Pozo *et al*., 2005; Suwanjarat *et al*., 2005; Van Dyk and Pieterse, 2008). The current study revealed that no seminal vesicle or accessory glandular structure was present within the caudal portion, thus spermatogenesis occurred along the entire cranial to caudal part of the testis. That is described as unrestricted testis type (Grier, 1981) and is similar to testis of the South American catfish *C. conirostris* (Chaves-Pozo *et al*., 2005). However, in some species the caudal region has no spermatogenic activity but instead seminal vesicle is present (Loir *et al*., 1989; Santos *et al*., 2001). It was shown that the testis of the catfish *C. gariepinus* observed with seminiferous lobules with a predominant type of three types. Spermatogenic testis with seminiferous lobules containing spermatogenic cells but no spermatozoa, pubertal testis with seminiferous lobules packed with spermatozoa with few spermatogenic cells and spent testis with seminiferous lobules having remnants of spermatogenic cells. Similar testicular cycles have been reported in different teleosts (Shrestha and Khanna, 1978; Suwanjarat *et al*., 2005; Cek and Yilmaz, 2007; Lawson, 2011). During spermiogenesis, the spermatids gradually differentiate into spermatozoa consisting of head with condensed nucleus and clear electron lucent penetration or nuclear fossa, short midpiece and long flagellum with scant cytoplasm and cell membrane. This characteristic features of the fish spermatozoa have been described in other teleosts (Lopes *et al*., 2004). Although, spermatozoa of catfish *C. gariepinus*, like most of other fish species, was of mono-flagellate type, spermatozoa of few fish species are biflagellate (Matos *et al*., 2002). The acrosome-less head of the sperm of the catfish *C. gariepinus* shown in this study *is* common in teleosts where external fertilization occurs (Lopes *et al*., 2004). The mature sperm are released into the spermatic duct which passes through the genital papillae. The current study showed that the spermatic ducts lined by cuboidal to columnar epithelium and surrounded by smooth muscle fibers. Contraction of muscular layer of the spermatic ducts may facilitate the transport of the spermatozoa.

CONCLUSION

In conclusion, the fringed testicular morphology without seminal vesicles of the Egyptian catfish *C. gariepinus* is similar to many other teleosts. The results presented in the current study should be considered as a baseline for future biological studies on the testis of the *C. gariepinus*.

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