Ultrastructure studies for Spermiogenesis of *Acanthostomum aswaninesis* (Digenea, Acanthostomatoidae), a helminth parasite of *Bagrus bayad* (Forsskal 1775) (Osteichthyes)

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**Abstract.** The present paper describes the characteristic ultrastructural features of spermiogenesis and the spermatozoon of *Acanthostomum aswaninesis* (Digenea, Acanthostomatoidae), a parasite of *Bagrus bayad* (Osteichthyes). It follows the general pattern of digeneans, with the initial formation of a differentiation zone, comprising striated rootlets associated with two centrioles and an intercentriolar body. The mature spermatozoon has features allowing the distinction of *A. aswaninesis* from other trematodes. It possesses an original anterior extremity. The two central elements of the axonemes appear prior to the peripheral doublets. The mitochondria assume a perinuclear arrangement, with the majority aligned parallel to the long axis of the spermatid. This work is the first description of early stages of spermatogenesis in adult *A. aswaninesis*.

**Keywords:** Fish; *Bagrus*; Digenea; *Acanthostomum aswaninesis*; Spermiogenesis; Nile River.

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**Introduction**

To date there have been numerous studies pertaining to the formation of sperm for the Phylum Platyhelminthes especially the trematodes. Both the Monogenea and Digenea have received attention by researchers for these groups. The advent of electron optics has greatly enhanced these studies. This is the first published article for spermiogenesis for *Acanthostomum aswaninesis*.

Representative of the early studies on spermatogenesis for the Digenea at the level of light microscopy include those of Anderson (1935). Later studies with the electron microscope were undertaken by Robinson and Halton (1982) and Erwin and Halton (1983)
who gave more detailed information on the complete process of spermatogenesis. Levron et al. (2009) added information at the electron level for representatives of the Phylum Platyhelminthes.

For the digenetic trematodes, the following articles are representative; Schistosoma margarebowiel (Awad and Probert, 1989), Paragonimus ohirai (Hirai and Tada, 1991), Fasciola hepatica (Gresson and Perry, 1961), Dicrocoelium dendriticum (Cifrian et al., 1993), and Dipherostomum brusinae (Levron et al., 2004).

The present study, using electorn microscopes, describes the spermatogenesis and spermiogenesis of Acanthostomum aswaninesis. This is the first published gametogenic sequence for this species adding to the published articles.

Materials and methods

Adult flukes were collected from B. bayad fish caught from the main stream of River Nile, Egypt. Flukes were fixed in 3% glutaraldehyde in a 0.1 M phosphate buffer (pH 7.2) and post fixed in 1% osmium tetroxide (O2O3) for 2 hours at 4°C then washed in the phosphate buffer. Specimens were dehydrated in an ascending series of ethanol then absolute acetone. Samples were then embedded in Spurr resin. Ultrathin sections, obtained with an ultra thin automated microtome using diamond knives, were stained with uranyl acetate and lead citrate and then examined with a JEOL transmission electron microscope (JEOL-JSTM 1200 EXII) at the Central Laboratory, Faculty of Science, Ain Shams University, Cairo, Egypt.

Results

Results of transmission electron microscopy study displayed that the testicular tissue rests on a basal muscle layer of the trematode (figure 1). Spermatogonia are represented by a peripheral layer of dense irregularly shaped cells characterized by a high nucleo-cytoplasmic ratio. Within the nucleus, clumps of heterochromatin are spread throughout the nucleoplasm and a prominent nucleolus is present. The cytoplasm is crowded with free ribosomes and contains many small mitochondria typically grouped at one pole of the cell (figure 1).

The cells close to the peripheral area of the reproductive tissue begin to enlarge and vary in shape. Following a phase of cellular growth and differentiation, mitosis of the tertiary spermatogonia result in an irregular order of 8 primary spermatocytes (figures 1 and 2). The first meiotic division of the primary spermatocytes generates secondary spermatocytes which are irregular in shape and exhibit a greatly increased cytoplasm to nuclear ratio. The nucleus is rounded to oval and contains dense chromatin granules distributed throughout the nucleoplasm and a number of small areas devoid of dark material. At least one nucleolus composed of fine chromatin granules, lying in a material of lesser density, is present in each spermatocyte (figures 2 and 3).

Mitochondria are numerous, dispersed throughout the cytoplasm in the primary developing cells but accumulate at the cell apex in secondary cells (figure 3).

Each secondary spermatocyte undergoes the second meiotic division. Thirty-two spermatids are formed connected to one another by thick convoluted stalks of cytoplasm. Each spermatid differentiates into a mature spermatozoon. The nucleus of the early spermatid is appreciably smaller in volume than that of a spermatocyte, with the chromatin evenly distributed throughout the developing cell (figure 4).

The developing cells and their nuclei undergo a process of elongation so that the distal ends of both become narrow. The nucleus becomes more elongate and migrates towards the cell apex and close to the plasma membrane. In this region, the membrane is supported by a row of closely spaced microtubules (figure 3). The mitochondria assume a perinuclear arrangement. The nuclear granules become more obvious and slowly become arranged in a alignment to form lamellae or filamentous structures (figure 4).
Transmission electron micrographs of the testis showed that an elongation of the spermatid and the formation of the zone of differentiation mark the beginning of spermiogenesis, distal to each of the spermatid nuclei. This region bulges out slightly and a row of microtubules appears below the plasma membrane. At the apex of each spermatid a conical projection develops and extends distally which is delimited from the rest of the cell by a circular groove called a collar (figures 5 and 6).

Development of the conical process is accompanied by marked changes in nuclear morphology. At first, it is oval in profile and contains evenly distributed chromatin granules and a dense nucleolus. The nucleus gradually elongates along the axis of the conical process and the chromatin condenses into twisted lamellae, giving it a longitudinally striated appearance (figure 5). This arrangement tends to be less ordered near the nuclear membrane.

Nucleoli persist for only a short time, appearing as small, dense bodies among the lamellae. The nucleus then migrates through the conical process with the two rootlets in the developing median cytoplasmic process (figures 5 and 6).

The median cytoplasmic process and the two lateral axonemes fuse along their lengths, to form the unipartite shaft of the spermatozoon then the nucleus passes through the zone of differentiation, between and slightly dorsal to the basal bodies, and into the median cytoplasmic process.

Numerous mitochondria also move into the zone of differentiation where they appear to fuse end-to-end to form a long, cylindrical body next to the nucleus. The mitochondria are considered to be positioned ventral to the nucleus. The arching membrane, close the cone shape of median process, separates its final structure from the common cytoplasmic mass. It becomes thickened and finally the spermatids appear with the peripheral microtubules (figure 7). The nucleus comes to rest in the proximal end of the median cytoplasmic process that will become the head region of the fully developed spermatozoon. The mitochondria lie distally to the nucleus in what will become the middle region of the spermatozoon.

Relatively few microtubules, numbering 8 to 17, extend into the median process and are in continuity with those supporting the dorsal and ventral plasma membranes of the conical process (figure 8). The number of cortical microtubules range from 7 to 15. Fewer, 4 to 5, microtubules occur towards the narrow distal end of the median process. The most distal part consists of one axoneme with cortical microtubules surrounded by the outer plasma membrane followed by the region having two axonemes and cortical microtubules without the nucleus and mitochondria. Then the mitochondria appear alone with the microtubules followed by the nuclear region which appears with the nucleus, mitochondria, two axonemes, and cortical microtubules. The last region of the sperm appears with one axoneme and the nucleus free from cortical microtubules (figure 12). The last part appears with a nucleus and one axoneme. By piecing together these profiles, it is possible to construct the mature spermatozoon of A. aswaninesis (figures 11, 12 and 13 from A to I). The anterior region, which is proximal in relation to its formation from the zone of differentiation, being with a single axoneme (A). This structure consists of one axoneme surrounded by a smooth plasma membrane with 4 to 5 cortical microtubules. Moving in an anterior to posterior direction, a second axoneme appears just below the anterior tip. The cortical microtubules form two groups, one above and one below the paired axonemes (B). The spermatozoon contains two elongate mitochondria with large amount of glycogen. The first mitochondrion is located close to the anterior tip, appearing narrow in diameter at first and is central in position, lying between the two axonemes (C). The first mitochondrion tapers off rapidly and it is separated from the second mitochondrion by a short intermediate region where only axonemes and microtubules can be seen (D). The second mitochondrion increases in diameter posteriorly, separating the two axonemes (E). There is an area of overlap between the mitochondrion and the nucleus (F). In this region, the cortical microtubules remain as two sets above and
below the other organelles (G). The nucleus appears to be relatively short in length in comparison with the mitochondria and judging by the relatively few sections containing the organelles in other micrographs.

**Figures 1-6.** TEM micrographs of the cross section of the testis of *A. aswanensis*: 1. Testicular tissue rest on the basal lamina (bl) and overlay muscle layer (m), early spermatid (est) with large number of mitochondria (mit), cytoplasm (C). 2. Irregular order of early spermatocytes (Esc), which are fusiform in shape with rounded nuclei (n). Mitochondria (mit) and sperm patches (Sppa). 3. Rosette of the late secondary spermatocyte (Sc) with oval nuclei (n), cytoplasm (C) which contain large number of mitochondria (mit), the cells are joined at the central cytophore (ccp). 4. Nuclear granules become more obvious and slowly become arranged in alignment to form lamellae of filamentous structures (fn) with early zone of differentiation (ZD), sperm patches (Spaa), rootlets (rol) and mitochondria (mit). 5. The migrating nucleus (n) and one of the two rootless (rot). 6. Critical separating stage of spermatozoa from the common cytoplasm, notice mitochondria (mit), nucleus (n), zone of differentiation (ZD), arching membrane (arc) and peripheral microtubules (pm).
The posterior region of the spermatozoon appears triangular in cross section (H). The nucleus enlarges and in the posterior region, the microtubules disappear. A central element is present in both axonemes and in the anterior tip, the two axonemes are staggered posteriorly and one eventually ends, leaving a single axoneme at the most posterior tip (I). The number of cortical microtubules increase in the actively moving region of the mature spermatozoa especially the anterior parts (figures 8, 9, 10).

Mature spermatozoa are long slender structures each consisting of two laterally running axonemes, two mitochondria, one large elongated nucleus and cortical microtubules. Spermatozoa usually grouped into batches each with 32 spermatozoa (figures 4 and 5).

A longitudinal section of a spermatozoan appears in figure 11 with a clear picture of axonemes consisting of the central core element (crt), limiting sheath, mitochondria (mit) and nucleus (n).

The axial structure is called the axoneme which possesses a 9 + “1” structure, 9 microtubule doublets and “1” central element called the central core element. This arrangement of microtubules differs from that of most cilia (9+2). Each microtubule doublet possesses two microtubules with two dyneine-side arms running in a clockwise direction. The 9 microtubule doublets (A and B) are connected to the central core element by 9 spokes (figures 8, 9, 10).

Discussion

The ultrastructural observations made in this study on the testes of adult A. aswaninesis displayed a sequence of events of spermatogenesis and spermiogenesis basically follows the common digenean and most monogenean sequences (Burton, 1972; Rees, 1979; Levron et al., 2009).

The described pattern in this study is similar to other observations with an exception that it may reach 128 in some patches (Xylander, 1989).

The formation of multicellular rosettes during spermatogenesis is typical of platyhelminthes, but there is controversy, not only about how this is achieved, but also as to the precise timing of the process. For A. aswaninesis, the rosette shape appear at the primary spermatocyte stages when the inner faces of the cells protrude towards the center of the group, and this would suggest that the rosette is formed partly by the fusion of the protuberances and partly by the subsequent incomplete cytokinesis of the cells.

Our results are similar to those described in most trematodes. The early spermatid develops a conical process but the nucleus still lies in the broad distal end of the cell. During the elongation of the spermatid, the nucleus also elongates and its chromatin material becomes filamentous.

For A. aswaninesis the formation of the zone of differentiation extends away from the cell to form the median process. The nucleus migrates, and becomes elongated parallel with the two rootlet structures, which form the axonemes. These events are similar to those occurring in F. hepatica.

The whole structure of the cell probably forms the basal bodies which are responsible for the formation of the axonemes. The central body may be responsible for the cortical microtubules that provide support for the developing median process.

The axonemes of the A. aswaninesis have a 9+“1” pattern of microtubules. The 9+“1” pattern is almost universal in parasitic platyhelminthes and was designated by Justine and Mattel (1981) as “1” because the central element is not a microtubule but a rod-like element.

The microtubule doublets possess two microtubules with two dyneine-side arms running in clockwise direction. This differs to the finding of Ashour et al. (2000; 2001), that the dyneine-side arms running counter-clockwise.
**Figures 7-11.** Micrographs (TEM) of the cross section of the testis of *A. aswanensis*. 7. Spermatozoon just after separating from the common cytoplasmic mass, notice mitochondria (mit), nucleus (n), two axonemes without the central core element and peripheral microtubules (pm). 8. Two migrating axonemes (ax) on the top of the photo, then after migration to the median process (mp) on the right of the photo, clearly one of differentiation (ZD), complete sperm (sp) with its mitochondria (mit). 9. Spermatozoa (sp), axonemes (ax), large nucleus (n), mitochondria (mit), peripheral microtubules (pm), and obvious characteristics of different levels of the spermatozoa. 10. The structure of axonemes (ax) with a single 9+1 microtubules; microtubule (A) (a), microtubule B (b) and dynein arms (dy) constitute 9 peripheral microtubule doublets. The central microtubules forming the central core element (crt), that attach to the peripheral microtubules by 9 spokes (sk), note also, mitochondria (mit), nucleus (n) and peripheral microtubules (pm). 11. Longitudinal section of mature spermatozoon one axoneme consisting of central core element (crt), limiting sheath ©, nucleus (n), mitochondria (mit) and spermatozoa (sp).

The present study is considered the first description of early stages of spermatogenesis in adult *A. aswanensis* and the Acanthostomatidae. A sequence of cross sections of the sperm helps in reconstruction of the fine structure of the spermatozoon.

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Figures 12-13. TEM of cross sections of the testis of A. aswanensis. 12. A series of transverse sections from the anterior to the posterior end of a mature spermatozoon. (A) Anterior tip with a single 9+2 axoneme (ax). (B) Section immediately posterior to the anterior tip showing two axonemes (ax). The cortical microtubules (pm) from a row above and below the axonemes. (C) Section showing the appearance of the first mitochondrion (mit). (D) Section showing the enlargement of the first mitochondrion. (E) Section through the intermediate region between the two mitochondria, in which no mitochondria are present. Two sets of cortical microtubules (pm) lie to either side of the two axonemes. (F) Section showing the appearance of the second mitochondrion (mit) between the two axonemes. (G) Section through the region of overlap between the second mitochondrion (mit) and the nucleus (n). (H) Section showing the region in which the second mitochondrion and the cortical tubes have disappeared. The nucleus (n) and the two axonemes (ax). (I) Section through the posterior tip of the spermatozoon showing a single axoneme (ax) are recognized. (I) Section through the posterior tip of the spermatozoon showing a single axoneme with large nucleus. No cortical microtubules are found. 13. Diagrammatic representation of the structure of the mature spermatozoon of A. aswanensis, depicted from successive transverse sections from A to I along its length. Sections A to L correspond to micrographs (A to I).
References


