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Electron-Microscopic Study on Spermatogenesis of Black Sailfin Molley, *Molliesia latipinna* LE SVERP

Kazuhiro Mizue

Electron microscopic observation was carried out on the structure of spermatozoa and spermatogenesis of black sailfin molley—*Molliesia latipinna* which is a fresh-water teleost fish and is generally called “tropical fish” in Japan. Since this fish is in mating period throughout the year and spermatogenesis is done continuously, the spermatozoa at all stages of progress are contained in the testis. The process of the spermatogenesis of this fish is almost the same as that of *Sebastiscus marmoratus* which is a marine teleost. But it is the remarkable characteristic that in this species the many sperms in a cyst form a perfect sperm-ball in the seminiferous tubule at the last stage of spermatogenesis. The spermatogenesis of this fish is classified into two periods, multiplying period and developing period. In the multiplying period, the nucleolus in the nucleus is clear but the chromosome is not clear. At the first stage of the developing period, the golgi complex and central body are found in the plotoplasm. The central body appearing at a distance from the nucleus is a short hollow tube surrounded by nine slender tubules each consisting of three fine tubules. The tail flagella appears from the central body and grows at right angles to the tube of the central body. Then the central body approaches the nucleus and the tail flagella grows up quickly in parallel direction to the nucleus. At the latter stage of the developing period, the chromosome in the nucleus of spermatozoa becomes many granules which are once gathered inside the nucleus. The outside of the nucleus where granules are not present shrinks quickly, and then outer granules in the nucleus become dense and grow in size. The nucleus wraps up the near part of the root of tail flagella towards inside, and at the same time complicated deep grooves enter into the nucleus from inside. Protoplasm no longer remains around the nucleus at this stage, and mitochondorias are gathered in the mitochondorial theath and surround the base of tail flagella without direct contact with the latter. The nucleus becomes the head of sperm cell. At the last stage of spermatogenesis, the granules are no longer found in the tapering slender sperm head. They migrate to the margin of a cyst and form a sperm-ball by arranging the heads at the outside and setting the tail flagellas at the center of the sperm ball. The structure of the tail flagella is the same as that of *Sebastiscus marmoratus* and there is no head cap at the sperm head of this fish either.
**Introduction**

There have been few electron microscopic studies on the spermatogenesis of marine animals. The structure and the growth of Crustacean spermatozoa have been studied to some extent but the study of this nature with the fish by electron microscope is very few. Harutsugu 11) clarified the structure of spermatozoa of Mozukugani, *Eriocheir japonicus*. Yasuzumi and Tsubo 12-13,14) described the pseudopodia that is derived from the nucleus of spermatozoa of Japanese crayfish, *Cambaroides japonicus*, the appearance and fate of the central body, and the continuous membranesystem by means of nuclear membranes and mitochondria. Moses 15,16) reported about the structure of spermatozoa of the crayfish, *Procambarus darkii*, and six stages of the growth of it. Yasuzumi 9-10) summarized the fine structure of spermatozoa of mammals, birds, insects and fish. Moreover other authors 1-2, 6-7-4-5) reported the structure of spermatozoa and the spermatogenesis of many kinds of mammals.

It is difficult to observe the spermatogenesis of the fish, because the spermatozoa in the fish testis grow simultaneously according to the annual sexual cycle, so we can observe only a certain stage of its spermatogenesis corresponding to the season the specimen is sampled and besides the sampling of the fish testis is more troublesome than that of other animals. For these reasons, reports on the fish spermatogenesis are very few in comparison with that of mammals and other animals. Fujimoto and Takaoka 8) clarified the fine structure of spermatozoa of lamprey, *Lampetra planeri*, by electron microscopic observation. Fujimura and others 8) observed by electron microscope testis sections of carp, *Cyprinus carpio*, and reported the fine structure of sperm head and tail axial fibrils of sperm containing proximal centriole. Mizue 18) studied by electron microscope on the spermatogenesis of Japanese rock-fish, *Sebastiscus marmoratus*, marine ovo-viviparious teleost fish, upon sampling in various seasons, and clarified the structure of spermatozoa and spermatogenesis. But the author made this study in order to learn whether or not there is any uy difference in spermatogenesis between the marine fish and fresh water fish.

The author wishes to express his appreciation to the Tomofuji Tropical Fish Company for offering the materials, Black sailfin molleys, and to Mr. T. Suematsu, School of Medicine, Nagasaki University, for his kind technical guidance in operation of electron microscope.

**Material and Method**

The material used in the present study is Black sailfin molly which is one of the tropical fresh water fish as usually called in Japan. This fish is shown in Fig.1. The body length is about 8 cm and body color is literally green black. The dorsal fin of male is very large and wide. As this species is kept in the water...
temperature between 26 °C and 29 °C, its testis is in reproductive condition all the time and it is easily imagined that the spermatozoa at all stages of progress are always present in that testis. It seems that this fish is a satisfactory material for observation of the fish spermatogenesis. One (7.1 cm) was dissected on 20 Dec. 1968 and its testis was fixed by Bouin's solution, made into paraffin section, precessed by normal stains and observed by optical microscope. Then, two additional individuals (7.6 cm and 8.1 cm) were dissected on 27 Dec. 1968 and two others (6.7 cm and 7.0 cm) on 10 Jan. 1969. These testis were immediately prefixed by glutaraldehyde for two hours and then fixed by osmic acid. These materials were dehydrated by acetone and embedded by epon 812. Phosphoric acid buffer was used at the pre- and fixation. The embedded materials were sliced into sections by LKB-4801 microtome, and after the staining by plumbum aceticum and uranyl acetate observed and photographed by the electron microscope (JEM-7A).

**Observation and Discussion**

**Optical microscopic observation of testis**

The optical microscopic macrofigure of the testis is shown in Plate I-1. By this figure it is clear that the spermatogenesis of this fish is almost the same as that of *Sebastiscus marmoratus* that is a marine teleost fish. In this fish also, the spermiogoniums appear on the basement membrane inside the seminiferous tubule and are divided and multiplied in a cyst. When the spermatozoa contained in the cyst are multiplied to be numerous, the multiplying period closes and each spermatozoon in the cyst begins to develop and finally becomes a sperm. This is the developing
period. And it is different from the general teleost fish as shown in Plate I-1,2 that in this fish the spermatozoa at all stages of progress are observed in one and the same section of testis. There can be observed spermatogonions which have just appeared on the basement membrane (Plate I-5) multiplying (Plate I-2) or developing spermatozoa in the cyst (Plate I-1, 3), sperm-ball (Plate I-1,4,5) that is the last stage of spermatogenesis, and empty seminiferous tubule that had discharged the sperm-ball (Plate I-5). This is one of the characteristics of this fish which is a tropical fresh water species.

Sperm-ball

There are a few fishes which form the sperm-ball in the seminiferous tubule at the last stage of spermatogenesis. For example, in Ditrema temmincki which is a marine viviparous teleost fish, many spermatozoa in a cyst of the seminiferous tubule get together and form a sperm-ball at the last stage of the spermatogenesis as reported by Mizue. But in Ditrema temmincki’s sperm-ball, the many sperms are merely crowded in a firm and irregular shape and the sperm-ball does not dissolve even after it is sent into the female’s ovary. In this species a group of sperms are crowded by a certain rule and forms a sperm-ball. As shown in Plate I-4, all the tail flagellas of sperms are situated at the middle portion of sperm-ball, and sperm heads are arranged at the outer margin of sperm-ball forming the external shell of sperm-ball. When sperm-balls are completed, they are pushed out of the seminiferous tubule and collected in the mesonephric duct (Plate I-6). In this species, a sperm-ball is formed in the seminiferous tubule by a group of sperms in a cyst like in case of Ditrema temmincki.

Seminiferous tubule after discharge of sperm-ball

In this fish, as soon as a sperm-ball is completed in the seminiferous tubule and begins to move out to the mesonephric duct, new spermiogoniums already appear on the basement membrane of seminiferous tubule and immediately begins to prepare for the next spermatogenesis (Plate I-5). In the other teleost fishes, the reproductive season is limited to a certain period occurring once a year, and the seminiferous tubule after the discharge of sperms remains in the resting condition for a long time. As the reproductive season of this fish is the whole year and reproduction is made throughout the year, so the seminiferous tubule is active continuously and the spermatogenesis is performed without ceasing.

Electron microscopic figure of spermatozoa in the multiplying Period

Plate II-7, 8 is the electron microscopic figure of spermatozoa in the multiplying period. The nucleus of this cell is comparatively large like in case of Sebastiscus marmoratus, but the protoplasm is small in comparison with that of the other kinds of cells. In the protoplasm, there are many mitochondrias and several mitochondrial rosettes surrounded by mitochondrias. This is the same with Sebastiscus marmoratus. Mitochondrias of these spermatozoa are various in shape
and the cristae therein are irregular in arrangement (Plates III, V). At the beginning of the multiplying period, the nucleolus of the nucleus is very clear (Plate II-7, 8), whereas the chromosomes in the nucleus are hardly visible, and the golgi complex and central body are not present yet in the protoplasm. But at the last stage of the multiplying period nucleoli are scattered in the nucleus. It can be seen that the nuclear membrane is twofold (Plate II-8, III-9).

**Small particulate components in protoplasm**

There are many small particulate components in the protoplasm. From the beginning of the multiplying period to the last stage of the developing period of spermatozoa, they are seen in protoplasm of the cell and they remain even in the mitochondorial sheath. It is believed that perhaps these dark small granules are PALADE'S particles. But these small particulate components have affinity to the membrane of endoplasmic reticulum, and they are distributed in uniformity in the protoplasm at all stages of the spermatozoa and crowded in small groups everywhere in the protoplasm.

**Beginning of developing period**

At the beginning of the developing period, the nucleus inclines extremely to one end of the cell, the greater part of protoplasm moves to the other end of the cell, the protoplasm contains many mitochondrias, golgi complex is seen in the protoplasm (Plate IV) and central body is formed nearby (Plate III, IV). The central body is a short hollow tube which is surrounded by nine slender tubules each consisting of three fine tubules (Plate III-10, 11). In the case of carp sperm [8], there is a little cavity at the lower pole of the spherical nucleus of spermatozoa and the central body is located in the cavity, but in this species the central body appears at a distance from the nucleus, and then the tail flagella begins to appear from the central body and grows at right angles to the short tubular central body (Plate IV-14, V-16, 17, 18, 19), and the nucleus begins to become granular.

**Last stage of developing period**

After the tail flagella of spermatozoa begin to grow, electron microscopic sections show many interesting figures. The tail flagella grows up quickly in parallel with the nucleus but not vertically (Plate IV-14, V-16, 17, 18, 19). This is different from the case of carp [8] and *Sebastiscus marmoratus* [18]. At the later stage of developing period the many granules in the nucleus gather at the inside of nucleus which is the same side as tail flagella (Plate V-16, 19, VI-20, 21) and the empty outer part of the nucleus where granules are not present shrinks quickly like in the case of *Sebastiscus marmoratus* [18]. And then the outside granules of nucleus become dense and grow in size (Plate VI-22, VII-23). At the same time the nucleus wraps up the near part of the root of tail flagella towards inside (Plate V-19, VII-23), and complicated deep grooves enter into the inside of the nucleus (Plate VI-20, 21, 22, VII-23). The fine structure of the root of tail
flagella is shown clearly in Plate V-16, 17, 18, 19, VII-23, 24. At this stage there is no protoplasm in front of the nucleus of spermatozoa, and protoplasm gather around the base of tail flagella and form the mitochondrial sheath. Every mitochondria in protoplasm gathers in the mitochondrial sheath surrounding the base of tail flagella at the distance of the sheath and cavity without any direct contact with the tail flagella (Plate VII-23, 24). In the meantime, the nucleus of spermatozoa already becomes the head of sperm cell. After this, every sperm cell in a cyst begins to move so as to form a sperm-ball with the sperm heads directing to the outside of the cyst and the tail flagellas in the central part of the cyst.

**Head of sperm cell**

Plate VIII-25, 26, 27 show the sperms which have accomplished spermatogenesis and formed a sperm-ball. The head part is perfectly repleted and granules are hardly noted in it. They are tapering slender. Like the sperm of *Sebastiscus marmoratus* there is no head cap at the tip of the sperm head which mammal sperms have at its head. Each sperm head of this species is covered with the two-fold nuclear membranes and a cell membrane, and these sperm heads are arranged around the outside of a cyst so as to form a sperm ball.

**Tail flagella of sperm**

The tail flagella of sperm is composed of a pair of central tail axial fibril and nine pairs of fibril surrounding it (Plate VI-20), and it is clear from Plate VII & V that the central tail axial fibril is twisted spirally. This tail flagella is very long in comparison with the sperm head and its vasic part gets into the sperm head deeply.

**Summary**

1. Fine structures of spermatozoa and spermatogenesis were observed by electron microscope about Black sailfin molley—*Mollienesia latipinna* which is a fresh water teleost fish. In the testis of this fish spermatogenesis is done throughout the whole year.
2. The spermatogenesis of this fish is almost the same as that of *Sebastiscus marmoratus*. But it is a remarkable characteristic that in this species the many sperms in a cyst form a sperm-ball in the seminiferous tubule.
3. The spermatogenesis of this fish is divided into two periods, multiplying period and developing period.
4. At the beginning of multiplying period, the nucleolus of nucleus is very clear, but at the last stage of that period nucleolus is scattered in the nucleus. The nuclear membrane is twofold.
5. At the beginning of developing period, the golgi complex and central body appear in the protoplasm of spermatozoa.
6. The central body is a short hollow tube surrounded by nine slender tubules, each slender tubule consisting of three fine tubules.
7. At first, the central body exists at a distance from the nucleus, and from the central body the tail flagella appears and grows up quickly in parallel direction with the
nucleus.
8. At the beginning of developing period, the nucleus begins to become granular, and these granules gather at the inside of nucleus and the empty outer part where granules are not present shrinks quickly.
9. And then outside granules of nucleus become dense and grow in size. The nucleus wraps up the base of tail flagella and complicated deep grooves enter into the inside of the nucleus.
10. At the last stage of spermatogenesis, every mitochondria of protoplasm gathers in the mitochonordial sheath surrounding the base of tail flagella.
11. The sperm of this species has a tapering slender head and the base of tail flagella gets into the sperm head deeply.
12. There is no head cap at the sperm head in this fish.
13. Tail flagella of this sperm is composed of a pair of central tail axial fibril and nine pairs of fibril surrounding it, and this tail flagella is very long in comparison with the head.
14. There are many small particulate components in the protoplasm at every stage.

References


**Explanation of Plates**

bm — Basement membrane, ca — Cavity, cb — Central body, c — Cyst, 
cf — Central fibril, ch — Chromosome, cm — Cell membrane, cr — Cristae, 
g — Golgi complex, sh — Sperm head, ig — Inside groove, m — Mitochondria, 
mr — Mitochondrial rosette, ms — Mitochondrial sheath, n — Nucleus, 
nm — Nuclear membrane, nu — Nucleolus, P — Protoplasm, s — Spermiogonium, 
sf — Surrounding fibril, st — Seminiferous tubule, sz — Spermatozoa, 
t — Tail flagella.

Plate I. All figures in Plate I are paraffin sections and were observed by optical microscope.

1. x100, There are many cysts which are different from each other in the developing stages.

2. x400, Spermatozoa in the cysts in the multiplying period.

3. x400, Spermatozoa at the last stage of developing period, every spermatozoon begins to move so as to form a sperm-ball with the sperm heads directed to the outside of a cyst and with the tail flagella set in the central part of a cyst.

4. x400, A perfectly completed sperm-ball.

5. x400, The sperm-ball which begins to move out to the mesonephric duct. New spermiogonions are seen on the basement membrane of seminiferous tubule preparing the next spermatogenesis.

6. x400, The many sperm-balls which gathered in the mesonephric duct.

Plate II

7. x5000, Spermatozoa in a cyst in multiplying period. Nucleus is large and nucleolus of nucleus is clear.

8. x12000, Spermatozoa in multiplying period. Nuclear membrane is twofold and nucleolus is clear.

Plate III

9. x14800, Spermatozoa at the beginning of developing period. The greater part of protoplasm moves to one part of the cell. The central body and golgi complex are present in the protoplasm and chromosomes are seen in the nucleus.

10. x20000, Central body. It is hollow and exists at a distance from the nucleus.

11. x49000, Central body. It is a short tube surrounded by nine slender tubules each consisting of three fine tubules.
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Plate IV
12. ×29200, Central body and golgi complex. Tail flagella begins to appear from the central body.
13. ×27000, Central body and golgi complex in the protoplasm.
14. ×32200, Tail flagella which begins to grow from the central body. The golgi complex is seen nearly.

Plate V
15. ×5600, Spermatozoa in developing period. Tail flagellas are seen in the protoplasm and the nucleus begins to become granular.
16. ×17400, Nucleus becomes granular and granules begin to gather at the inside of nucleus. The structure of the base of tail flagella which grows from the central body is clear. The tail flagella grows in parallel direction to the nucleus.
17. ×25800, Structure of the base of tail flagella and connection with central body.
18. ×21200, Structure of the base of tail flagella and connection with central body.
19. ×21000, Granules in nucleus have gathered at the inside of nucleus and outside empty part begins to shrink and nucleus begins to wrap up the base of tail flagella.

Plate VI
20. ×23600, Cross section of tail flagella. It is composed of a pair of central tail axial fibril and nine pairs of fibril surrounding it. A groove is seen in nucleus.
21. ×23600, Cross section of central body. This figure is the same stage as Fig. 20. A deep groove is seen in nucleus.
22. ×6800, Outside granules of nucleus have become dense and grown in size. Complicated deep grooves are seen in nucleus, and nucleus wraps up the base of tail flagella.

Plate VII
23. ×10800, Longitudinal section of spermatozoa at the same stage as Fig. 22. There is no protoplasm in front of nucleus of spermatozoa. The protoplasm have gathered around the base of tail flagella forming the mitochondrial sheath in which every mitochondria has gathered.
24. ×24600, Spermatozoa more developed than those in Fig. 23. The basic part of tail flagella gets deeply into the sperm head, and it is manifest from Fig. 19, 23, 24 that the central axial fibril is twisted spirally.

Plate VIII
25. ×5400, Sperm heads which form a part of shell of sperm-ball and mitochondria sheath which surround the base of tail flagella.
26. ×1600, A part of completed sperm-ball. There are many tail flagellas at the central part of sperm-ball.
27. ×30000, Sperm heads which are tapering slender and are covered with twofold nuclear membranes and a cell membrane but have no head cap.
Plate I
Plate II
Plate IV
Plate VI
Plate VII
Plate VIII