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Studies on Sharks—IV
Testes and Spermatogeneses in Selachians

Che-Tsung CHEN, Kazuyuki TESHIMA and Kazuhiro MIZUE

The male reproductive organs and the development of sperm cells in the selachians were studied. The reproductive organs of selachians are quite different from those of the teleosts; the testes and ovaries are formed at the anterior ends of epigonal organs which are developed in both sexes, and in male, the testis is connected to the epididymis. The spermiduct extending from the epididymis lies in the kidney, and the posterior end of spermiduct is expanded to form the sperm sac in some species. The spermatogenesis is also different from that of the teleosts, and the cyst is not formed. Moreover, the spermium shows a remarkably different shape in comparison with that of the teleosts; it has a slender and spiral head like a fiber, and many spermiums get together to form a sperm clump. In the seminiferous tubule, the spermiums in the sperm clumps are situated with their heads pointing towards the basement membrane, and with their tail towards the lumen.

Introduction

Of the teleosts, the histological studies on the development of testes and spermatogeneses in the fresh water fishes such as Guppy, Topminnow, Sword tail and Moon fish have been done to some extent, but not many in the marine fishes. Histological observations on testes of the marine fishes have been done in species such as Oryzias latipes, Culuepa pallasi, Liopsetta obscura, Cymatogaster aggregatus, Scomber japonicus, Sebastiscus marmoratus, Sebastes inermis, Ditrema temmincki and Girella punctata. Electron microscopic observations have also shown fine pictures of the spermatogeneses in Sebastiscus marmoratus and Mollinesia latipinna. Regarding this subject, an electron microscopic study is necessary to know the detail of spermatogenesis in the selachians.

KENDALL1) described the testes and spermiducts of Dog-fish. MATTHEWS2) studied in detail reproduction of Cetorhinus maximus, the basking shark, occurring in the cold waters, especially its testis and spermatogenesis. A series of studies on the testes of Scyliorhinus spp. in the Atlantic Ocean were done during the period from the end of the 19th century to the beginning of the 20th century. There are also some reports3) dealing with the length of claspers and the seasonal changes of testis weight.

It has been known that the structures of testis and spermatogenesis of fishes
are quite different from those of the mammals\textsuperscript{4,5}, Moreover, the spermiums of selachians show the special shape and the process of the spermatogenesis is also quite different from that of the teleosts. This report comprises some knowledges obtained through the observations on the testes and spermatogeneses in selachians.

**Materials and Methods**

The materials used in the present study are the male reproductive organs of nine species of sharks. Of these species, *Galeorhinus japonicus*, *Mustelus manazo*, *Squalus brevirostris*, *Heterodontus zebra*, *Heptranchias perlo* and *Cephaloscyllium umbratile* were collected at the Nagasaki fish market. These six species of sharks were caught in the East China Sea. The remaining three species, *Carcharhinus dussumieri*, *C. sp.* and *Rhinobatos schlegeli*, were caught during trawling operation in the South China Sea.

The collection at the Nagasaki fish market was done throughout the year, and very sound individuals were used to make the histological sections. The species caught in the South China Sea were preserved on board in formalin solution. The materials were prepared by paraffin embedding, cut as thin as possible, and stained with haematoxylin and eosin.

Although the histological sections of testes of *Galeorhinus japonicus* and *Mustelus manazo* were prepared throughout the year, the seasonal changes of mature testes of these two species will be described elsewhere.

According to Teshima and Mizue\textsuperscript{6}, *Carcharhinus dussumieri* and *C. sp.* in the South China Sea show no definite reproductive season. The testes of mature male are always mature throughout the year, and the testes of one individual show all the developmental stages in the spermatogenesis. It is, hence, possible to observe whole stages in the spermatogenesis by the materials collected during a short period (July to August).

**Observations and Discussion**

1. Reproductive organs

Although, in sex differentiation of the teleosts is usually made upon confirmation of the presence of ovary or testis by anatomy, the sex of the selachians is easily differentiated by the presence of claspers. Moreover, in viviparous species, the sex is differentiated by the external appearance at the relatively early stage of embryonic development, e.g., in *Galeorhinus japonicus*, the claspers are observed when the embryo is about 60–70 mm (parturition occurs when the embryo is approximately 250 mm). The sex differentiation by the gonads (testis and ovary) is difficult until the individual reaches the maturity.

The male reproductive organs are shown in Fig. 1. The epigonal organs lie
between digestive organs and kidney, and the posterior portion of the epigonal organ extends near the cloaca. The epigonal organs usually form a pair. In some mature males of *G. japonicus*, the posterior half portions of both epigonal organs are found to be combined, and divided by a very thin membrane at the center. These epigonal organs in both sexes show the same histology and the testes in male and the ovaries in female are formed at the anterior ends.

The anterior end of the testis is connected to the epididymis. The epididymis, a slender winding spermiduct, functions as a deferent duct. The spermiums formed in the testis are stored in the epididymis for a time, diluted by seminal fluid and are sent to the spermiduct in the kidney. A pair of slender kidneys attached to the dorsal side of the peritoneal cavity function as the urinary organs and the spermiduct lies in a zigzag line in the ventral side of the kidney. As the individual reaches the maturity, the spermiduct in the anterior portion of kidney develops greatly, and its histological section shows that greater part of kidney is occupied by the spermiduct. Although the spermiduct in the anterior half portion of kidney lies in a zigzag line, that in the posterior portion lies in a relatively straight line.

In *Galeorhinus japonicus*, the posterior end of spermiduct is expanded to form the sperm sac as shown in Fig. 1, and this sperm sac is also found in *Mustelus manazo*, but there are some species which form no sperm sac such as *Carcharhinus australis*. The seminal fluid stored in the sperm sac is discharged from the urinogenital papilla.

2. Spermatogenesis

The epigonal organs which form testes in male, or ovaries in female, at their anterior ends show the same histology in both sexes; they contain many capillaries and are filled with composed cells (Fig. 1, Plate I). In *Galeorhinus japonicus*, the testes lying at the anterior ends of the epigonal organs are already observed before the individual reaches the maturity. The testis is very small till it reaches the sexual maturity.

It has been known that the testes of fishes are divided into two types; seminiferous tubule type and lobule one, and these two types appear to be present in selachians. According to MATTHEWS, the testes of *Cetorhinus maximus* are of the lobule type, but those of *Galeorhinus japonicus, Carcharhinus australis* and *Mustelus manazo*, materials of the present study, belong to the seminiferous
tubule type (Fig. 2, Plate I).

Of the teleosts, all fishes except for tropical ones have a definite reproductive season when the testes work vigorously to increase their weight, i.e., when spermatogenesis is done. But, in other seasons, the testes are resting and the spermatogenesis is not done. The selachians described, here, however, shows that their spermatogeneses are done throughout the year. Although this observation is natural for *Carcharhinus dussumieri* and *C.* sp. which have no definite reproductive season, it is quite strange for the species such as *Galeorhinus japonicus* with a definite reproductive season. The testis of *C. dussumieri* is shown in Fig. 2, Plate I. This figure indicates that the testis consists of seminiferous tubules, and this figure also applies to the species such as *G. japonicus, Mustelus manazo, Heterodontus zebra, C.* sp. and *Rhinobatos schlegeli*.

Although the seminiferous tubules situating at the center of testis develop completely, those at the outer region are in early developmental stage. In the seminiferous tubules in the early developmental stage, the simple columnar epithelium covers the basement membrane of the seminiferous tubule (Fig. 3, Plate I), and spermatogonia developed from the basement membrane appear between the basement membrane and the seminiferous epithelium (Fig. 3, 4, Plate I). When many spermatogonia begin to appear, the seminiferous epithelium is pushed towards the lumen by them (Fig. 4, Plate I). These cells of the seminiferous epithelium, while being consumed, support the spermatogonia and give them nutrition; these cells are so-called nurse cells. In *Galeorhinus japonicus* and *Mustelus manazo*, these nurse cells appear again on the basement membrane as the spermatogenesis advances. Some of these cells move towards the center of the seminiferous tubule and support the sperm cells during the development. In *Carcharhinus dussumieri, C.* sp. and *Rhinobatos schlegeli*, however, these nurse cells do not appear during spermatogenesis.

a) **Multiplying period of sperm cells**

The spermatogenesis is divided into two periods; multiplying period and developing period. The spermatogonia which appear on the basement membrane inside the seminiferous tubule begin to be divided and eventually reduced in size (Fig. 5, Plate II). In the teleosts, one spermatogonium usually forms one cyst, and in that cyst, many sperm cells are divided. In selachians, however, the process of spermatogenesis is relatively similar to that of the mammals; the cyst is not formed and spermium is directly divided. After the multiplying period, the sperm cells develop into spermatocytes (Fig. 5, Plate II).

b) **Developing period of sperm cells**

The spermatocytes develop into spermatids by reduction division. After this, the spermatids transform their shape to develop into the spermiums, i.e., the tails are formed and develop gradually, and at the same time, the nuclei of cells gradually become slender and develop into the heads of the spermiums (Fig. 6, Plate
II). At this stage, approximately 30 sperm cells get together and begin to form a sperm clump. In mammals, the spermatids are divided as well as somatic cells, but in fishes including selachians, the spermatids transform their shape and develop into the spermiums gradually. Thus, spermatids are not divided to increase the sperm cells. The sperm clump is different from the sperm ball formed by the teleosts, and also different from the sperm mass formed by Dipirema temminckii.

The group of spermiums are situated with their heads pointing towards the basement membrane, and with their tails towards the center of lumen. About 20–30 sperm clumps are found in a transverse section of the seminiferous tubule (Fig. 9, Plate III). Each of the sperm cells in the sperm clump completes its shape gradually and its head becomes slender like fiber. Moreover, each of the heads becomes spiral and more slender and the sperm cells develop into spermiums. Each of the spermiums belonging to one sperm clump is regularly spiral. The sperm clump is formed not only in Mustelus manazo, and Galeorhinus japonicus, but also in Carcharhinus dussumieri, C. sp., Rhinobatos schlegeli, and Cetorhinus maximus. This sperm clump appears to be formed in all species of selachians.

The sperm clumps in the seminiferous tubule are taken out continuously to the epididymis. In Carcharhinus dussumieri, C. sp. and Rhinobatos schlegeli, preparation of the succeeding spermatogenesis is done when the sperm clumps begin to be formed in the seminiferous tubule, i.e. the spermatogonia begin to appear on the basement membrane inside the seminiferous tubule. In Galeorhinus japonicus and Mustelus manazo, the spermatogonia does not appear on the basement membrane, even if the sperm clumps are completely formed. The spermatogonia, however, appear and line up on the basement membrane by the time the sperm clumps have completed their arrangement in a line. The nuclei of cells of the seminiferous epithelium are larger than those of the spermatogonia, and these are not well stained with haematoxylin. It is, therefore, easy to separate the spermatogonia from the cells of seminiferous epithelium.

Galeorhinus japonicus and Mustelus manazo have a definite reproductive season, but Carcharhinus dussumieri, C. sp. and Rhinobatos schlegeli show that reproduction is done throughout the year; this difference in reproduction seems to indicate that the stage when the spermatogonia appear is different as described above.

3. Epididymis

In Galeorhinus japonicus and Mustelus manazo, the sperm clumps formed in the testis are continuously sent out to the epididymis through the seminiferous tubules. The epididymis is a complex deferent duct in which the spermiums move, and at the anterior portion of epididymis, the wall of duct consists of the glandular cells, i.e. the internal surface of duct is lined by the glandular epithelium (Fig. 10, Plate III). These glandular cells irregularly get together
and form a compound gland from which the fluids are secreted. These secreted fluids dissolve the sperm clumps sent from the testis, and spermiums are scattered over. The other part of epididymis is filled with parenchyma in which many capillaries develop. The glandular cells do not exist in the center and posterior portion of the epididymis, and the fluids, therefore, do not appear to be secreted; these portions consist of the parenchyma and complex winding deferent duct of spermiums. The duct of the epididymis is usually thick, and its epithelium consists of simple squamous cells, and its wall has no simple ciliated epithelium of columnar cells as found in the wall of spermiduct. The epididymis as shown in Fig. 1 was present not only in *G. japonicus* and *M. manazo* but also in all species studied by the present authors.

4. **Spermiduct**

The sperm clumps are dissolved by the fluids secreted in the epididymis, and sent to spermiduct as the seminal fluid. The spermiduct lies in the ventral part of kidney, and is situated along the slender kidney. The spermiduct in the anterior half portion of the kidney shows a very complicated way like a maze. The internal part of the spermiduct is divided into many branches, and moreover, the spermiduct itself is winding. The transverse section of the anterior half portion of kidney shows that the greater part of tissue is occupied by the spermiduct (Fig. 11, Plate III). In the anterior half portion of kidney, the spermiduct represents two big ducts running along the kidney, and is not very winding (Fig. 1). The spermiduct in this portion, however, contains many longitudinal folds. This suggests, therefore, that the spermiduct has many slender compartments, and the transverse section of this portion shows that the greater part of tissue is occupied by the kidney. Moreover, in this portion, urinary canals run along the ventral side of the spermiduct (Fig. 12, Plate IV). The internal surface of spermiduct is lined by the simple ciliated epithelium, and the epithelium consists of tall columnar cells. Each of the cells possesses long cilia extending towards the lumen (Fig. 13, Plate IV). These cilia aid in transport of the seminal fluid to posteriority.

5. **Sperm sac**

In *Galeorhinus japonicus* and *Mustelus manazo*, the posterior end of spermiduct running along the ventral side of kidney is greatly expanded to form the sperm sac, and this contains a large quantity of seminal fluid. This is shown in Fig. 1 and Fig. 14, Plate IV. The sperm sac is divided into many slender compartments by many longitudinal folds. The sperm sac and the urinary bladder open near the cloaca. A pair of urinary bladders are situated at the ventral side of the sperm sac, and the dorsal side of the sperm sac attaches to the flattened kidney. In *G. japonicus* and *M. manazo*, the sperm sac is remarkable, especially in the former. But the posterior end of the spermiduct in *Cetorhinus*
maximus, Carcharhinus dussumieri, Squalus brevirostris, Heptranchias perlo and Heterodontus zebra is not so expanded as in the previous species.

Summary

1. The male reproductive organs and spermatogeneses in Galeorhinus japonicus, Mustelus manazo, Carcharhinus dussumieri, C. sp., Rhinobatos schlegeli, Squalus brevirostris, Heterodontus zebra, Heptranchias perlo and Cephaloscyllium umbratile were studied.

2. In these species, a pair of epigonal organs are found, and at the anterior ends of these organs, testes in male or ovaries in female are formed.

3. The testes on selachians consist of seminiferous tubules. In early stage of development, the internal surface of the seminiferous tubule is lined by the seminiferous epithelium. The spermatogonia developed from the basement membrane appear between the seminiferous epithelium and the basement membrane, and the spermatogonia continue to be divided and reduced in size. This is the multiplying period of sperm cells.

4. After the multiplying period, the spermatocytes develop into spermatids by the reduction division, and the tails are formed. At the same time, cell nuclei become slender to form the heads. This is the developing period of the sperm cells.

5. The spermiums of selachians show a special shape and their shape is the same in every species. The head is slender as a fiber and spiral, and the tail is also slender. Approximately 30 spermiums get together to form a sperm clump, and the spermiums in the clump are arranged with their heads pointing towards the basement membrane and with their tails towards the center of lumen.

6. The sperm clumps are sent to the epididymis from the testis, and here, they are dissolved into the seminal fluid by the secreted fluid.

7. The spermiduct runs along the ventral side of kidney, and the internal part is divided into many branches. The spermiduct is quite winding, especially in the anterior half portion of kidney.

8. In Galeorhinus japonicus and Mustelus manazo, the posterior end of spermiduct is greatly modified to form a sperm sac. In other species, the spermiducts are modified to some extent.

References


### Explanation of Plates

c ..... capillary
s ..... spermium
bm ..... basement membrane
sec ..... seminiferous epithelium cell
sg ..... spermatogonia
sd ..... spermatid
sp ..... spermiduct
sf ..... seminal fluid
gc ..... glandular cell
k ..... kidney
ic ..... interstitial cell
fs ..... fold in spermiduct
e ..... epithelium
sc ..... sperm clump
st ..... seminiferous tubule
se ..... seminiferous epithelium
l ..... lumen
sc ..... spermatocyte
uc ..... urinary canal
dd ..... deferent duct
gt ..... glandular tissue
ss ..... sperm sac
cc ..... ciliated columnar epithelium
fss ..... fold in sperm sac
ub ..... urinary bladder

### Plate I

Fig. 1. 5 × 10. Section of the epigonal organ in *Mustelus manazo*, it is filled with composed cells and contains many capillaries.

Fig. 2. 5 × 4. Section of the testis in *Galeorhinus japonicus*, the testis contains many seminiferous tubules in which the sperm clumps are arranged in a line.

Fig. 3. 15 × 40. The originally seminiferous tubule in *Carharhinus dussumieri*, the seminiferous epithelium appears in the basement membrane while the spermatogonia do not appear.

Fig. 4. 10 × 40. The seminiferous tubules of *Carharhinus dussumieri* are more advanced than in Fig. 3, spermatogonia developed from the basement membrane appear between the basement membrane and the seminiferous epithelium which is pushed towards the lumen by them.
Plate II

Fig. 5. 10×10. The spermatocytes of *Mustelus manazo* formed by multiple divisions of the spermatogonia.

Fig. 6. 15×10. Section of the seminiferous tubules in *Galeorhinus japonicus*, showing the sperm clumps formed.

Fig. 7. 15×10×2. Section of the seminiferous tubules in *Galeorhinus japonicus*, the sperm clumps are arranged in a line and round the periphery. A new seminiferous epithelium appears again from the basement membrane.

Plate III

Fig. 8. 15×10. Section of the seminiferous tubules in *Carcharhinus dussumieri*, the sperm clumps are arranged in a line and round the periphery.

Fig. 9. 15×40. Sperm clumps in *Galeorhinus japonicus*, showing the heads of the clumps pointing towards the basement membrane. The whole of each head is slender like fibers and become spiral.

Fig. 10. 10×10. Deferent duct of the epididymis near the testis in *Galeorhinus japonicus*, showing the wall of duct consisting of the glandular cells.

Fig. 11. 5×1.2. Transverse section of the posterior portion of kidney in *Mustelus manazo*, the great part of tissue is occupied by kidney. The spermiduct is not winding.

Plate IV

Fig. 12. 5×1.2×2. Transverse section of the anterior portion of kidney in *Carcharhinus dussumieri*, the great part of the tissue is occupied by spermiduct which is winding.

Fig. 13. 10×10. The internal wall of spermiduct and the seminal fluid in *Carcharhinus dussumieri*, the internal wall is lined by simple ciliated epithelium. The sperm clumps are dissolved and separated from one another.

Fig. 14. 5×1.2. Transverse section of the sperm sac in *Galeorhinus japonicus*, the sperm sac is lined with many longitudinal folds.
Plate I

1

2

3

4

[caption and labels]
Plate II
Plate III