

## Fine Structural Observations on Spermatogenesis of the Goldeye Rockfish, *Sebastes thompsoni* (Teleostei: Scorpaenidae)

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Fine structural changes of the germ cells during spermatogenesis in the goldeye rockfish, *Sebastes thompsoni* were examined by means of the transmission and scanning electron microscopy. A spermatogonium has a large nucleus with a single nucleolus in the interphase. Primary spermatocytes are characterized by the formation of chromatin clumps and presence of the synaptonemal complex in the nucleus. The nucleoplasm of secondary spermatocytes is more condensed than that of primary spermatocytes, and the cytoplasm contains numerous mitochondria, endoplasmic reticulum and Golgi complex. The nuclei of spermatids in metamorphosis show sickle-like shape as the nucleoplasm becomes more condensed. In the cytoplasm of spermatids, the proacrosomal granules are not found at all. A spermatozoon consists of head, neck and tail. The acrosome is absent in the head. Four to five cytoplasmic collars are observed in the posterior portion of the head of spermatozoon. The well developed axonemal lateral fins are observed in the flagellum of spermatozoon.

**Key words :** *Sebastes thompsoni*, spermatogenesis, absence of acrosome, cytoplasmic collar, axonemal lateral fins

### Introduction

The mode of fertilization is generally divided into two types: the aquasperm and the introsperm by fertilized sites, and the acrosomal and anacrosomal sperm by presence or absence of the acrosome (Jamieson, 1991).

Several fine structural observations on teleost spermatozoa have been reported (Colak and Yamamoto, 1974; Chung and Lee, 1985; Jamieson, 1991; Chung and Chang, 1995; Lee, 1995, 1996). These studies showed that in many teleost spermatozoa do not bear the acrosome, and have some morphological characteristics.

Most species of the family Scorpaenidae are oviparous, but about 110 species of the genera *Sebastes*, *Sebastes*, *Heicolenus* and *Hozukius* are viviparous (Wourms, 1981). In Korea, little information is available on the reproductive biology on *Sebastes* which include several commercially important species.

The goldeye rockfish, *Sebastes thompsoni* are distributed in coastal waters of eastern and southern Korea (Chyung, 1977). Lee et al. (1998) reported that this species discharges the young fish in March.

In the present study, fine structural changes of the

germ cells during spermatogenesis in the goldeye rockfish, *Sebastes thompsoni* were examined by means of the transmission and scanning electron microscopy.

### Materials and Methods

Adult males of the goldeye rockfish with the total length over 22.0 cm collected in the southern coast of Korea in 1996 were used in this study.

#### 1. Transmission electron microscopic observations

The testes were removed and fixed in 2.5% glutaraldehyde solution (pH 7.2 buffered in 0.1M phosphate buffer) for 2~4 hours in 4°C. Then they were postfixed in 1% osmium tetroxide (OsO<sub>4</sub>) solution for 2 hours in 4°C. After fixation the specimens were washed by 0.1M phosphate buffer, then dehydrated by ethanol step by step, and finally embedded in Epon 812. Ultrathin sections (70 nm in thickness) were put on the copper grids (200 mesh) and double-stained with uranylacetate and lead citrate. Finally they were observed by the transmission electron microscope (JEM-1200EXII, JOEL).

## 2. Scanning electron microscopic observations

The testis specimens for scanning electron microscopic observations were treated in the same way as transmission electron microscopic observations; i.e. the fixation and dehydration processes were repeated. The dehydrated specimens were dried with the critical point dryer, and their outer surface was covered with gold ion particle (10 nm in thickness). And then the specimens were observed by the scanning electron microscope (DSM 940A, Carl Zeiss).

## Results

A testis consists of numerous seminiferous tubules and interstitial tissues. Each seminiferous tubule contains numerous testicular cysts (Fig. 1, A). In the testis of the early growing stage, the Sertoli cells of the testicular cyst epithelium are oblong-shaped and have elliptical nuclei, and mitochondrial rosette containing several mitochondria and core substance are present in the cytoplasm (Fig. 1, B). The spermatogenesis in the goldeye rockfish is divided into the following four stages: (1) spermatogonium, (2) spermatocyte, (3) spermatid, and (4) spermatozoon stage.

### 1. Spermatogonium stage

The spermatogonia in the interphase are ovoid (7.0  $\mu\text{m}$  in diameter). In this stage, the prominent nucleus reaches 5.0  $\mu\text{m}$  in size. The nucleus of a spermatogonium has a electron-dense nucleolus, and the cytoplasm is occupied with numerous vacuoles and mitochondria (Fig. 1, C). The spermatogonia in the multiplicative stage lack the nucleoli. The chromatin is condensed in the nucleoplasm, and a number of mitochondria begins to appear in the cytoplasm, but the cristae are poorly developed (Fig. 1, D).

### 2. Spermatocyte stage

The primary spermatocytes in the leptotene stage are approximately 4.5  $\mu\text{m}$  in diameter. The ratio of the volume of the nucleus to the cytoplasm becomes decreased. The electron-dense heterochromatin is easily

distinguished from the euchromatin. In the cytoplasm, relatively small number of mitochondria and endoplasmic reticulum are observed (Fig. 2, A). The primary spermatocytes in the pachytene stage are characterized by complex intra-nuclear structures such as formation of chromatin clumps (Fig. 2, B) and synaptonemal complex (Fig. 2, C). In the cytoplasm, a small number of vacuoles and a large number of mitochondria with enlarged cristae are observed (Fig. 2, C). The secondary spermatocytes have the nucleoplasm with more condensed chromatin and the cytoplasm increased with mitochondria and endoplasmic reticulum (Fig. 2, D).

### 3. Spermatid stage

The spermatids during spermiogenesis are divided into three steps (early, mid and late). After completion of the second meiotic division, the spermatids in the early stage are characterized by increment of the nucleoplasmic electron density and the Golgi complex with numerous cisternae and vacuoles (Fig. 3, A). The shape of the nucleus becomes deformed, and the centriole is observed below the nucleus (Fig. 3, B). The mitochondria of spermatids in the mid stage aggregate beneath the nucleus, and surround the axial filaments which are originated from the centriole (Fig. 3, C). In this stage, the nuclei are spherical (Fig. 4, A), and the axial filaments become elongate and protruded to form the tail. And the cytoplasm with mitochondria is pushed down around the centrioles beneath the nucleus (Fig. 3, D). In the late stage of spermiogenesis, the chromatin of spermatids is condensed near the nuclear membrane, and become more electron-dense, and form a large sickle-contour (Fig. 3, E). Then the nucleoplasm becomes more condensed, and form sickle-shape. The internal structures of mitochondria are clearly recognized as the tubular cristae swell (Fig. 3, F). However, the structural changes in the Golgi complex and the gradual formation of proacrosomal granules are not observed in all the stages during spermiogenesis. In the late stage of spermiogenesis, the general shape of the head of spermatids is elliptical, and the centriolar complex is observed beneath the head (Fig. 3, G; 4, B, C). But

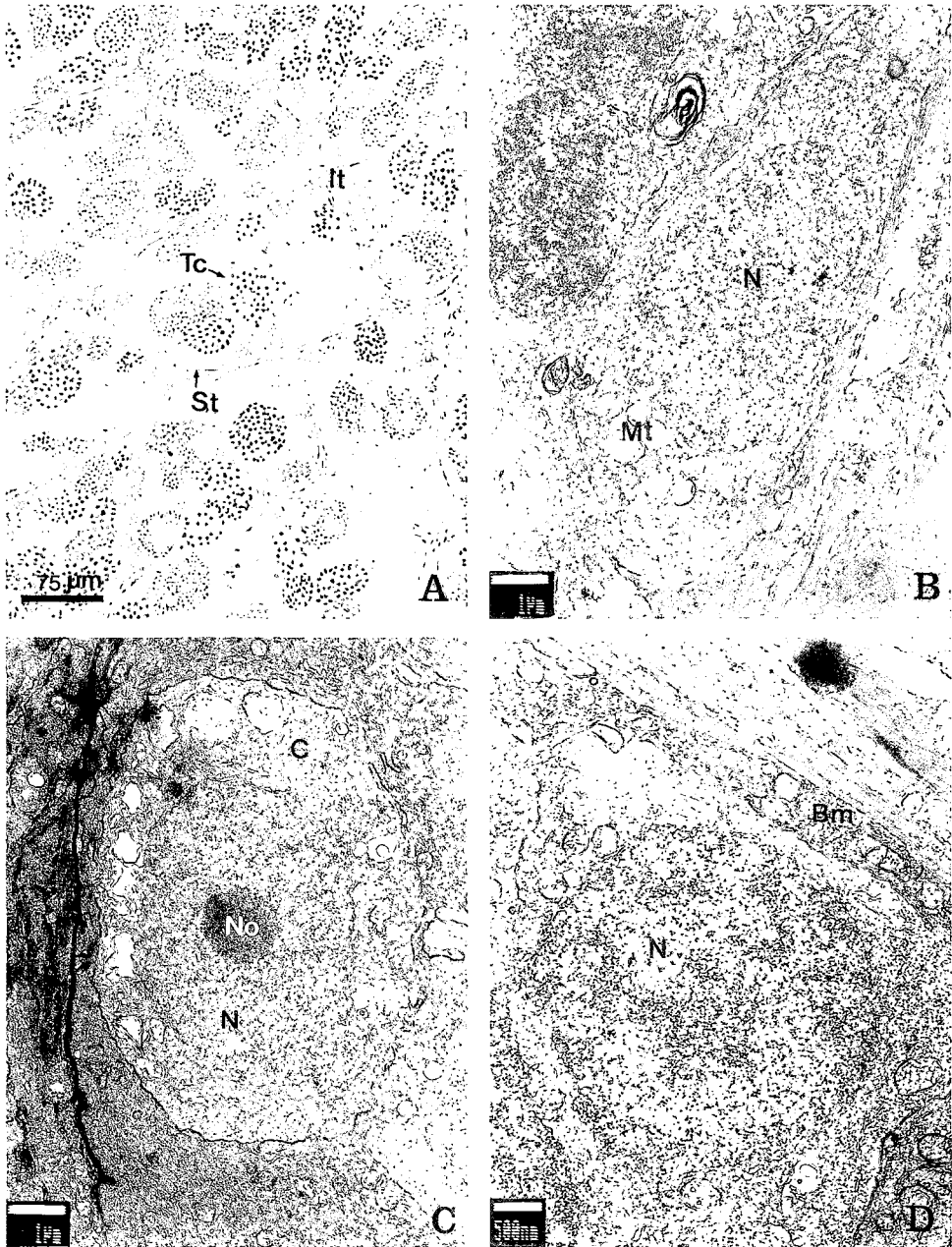


Fig. 1. Testicular structure and spermatogonia of the goldeye rockfish, *Sebastes thompsoni*. A. Section of a testis. The testis consists of seminiferous tubules (St) which contain many testicular cysts (Tc). It; interstitial tissue. B. Electron micrograph of a cyst cell. The cyst cell contains an elongate oval nucleus (N) and mitochondrial rosette (Mt) in the cytoplasm. C. A spermatogonium in the interphase. The spermatogonium contains a large nucleus (N) with a single nucleolus (No). C; cytoplasm. D. A spermatogonium attached to the basement membrane (Bm) in the multiplicative stage. N; nucleus.

these spermatids are transformed into the mature spermatozoa immediately after completion of spermiogenesis.

#### 4. Spermatozoon stage

From the scanning electron microscopic observations, it is found that a spermatozoon consists of three parts

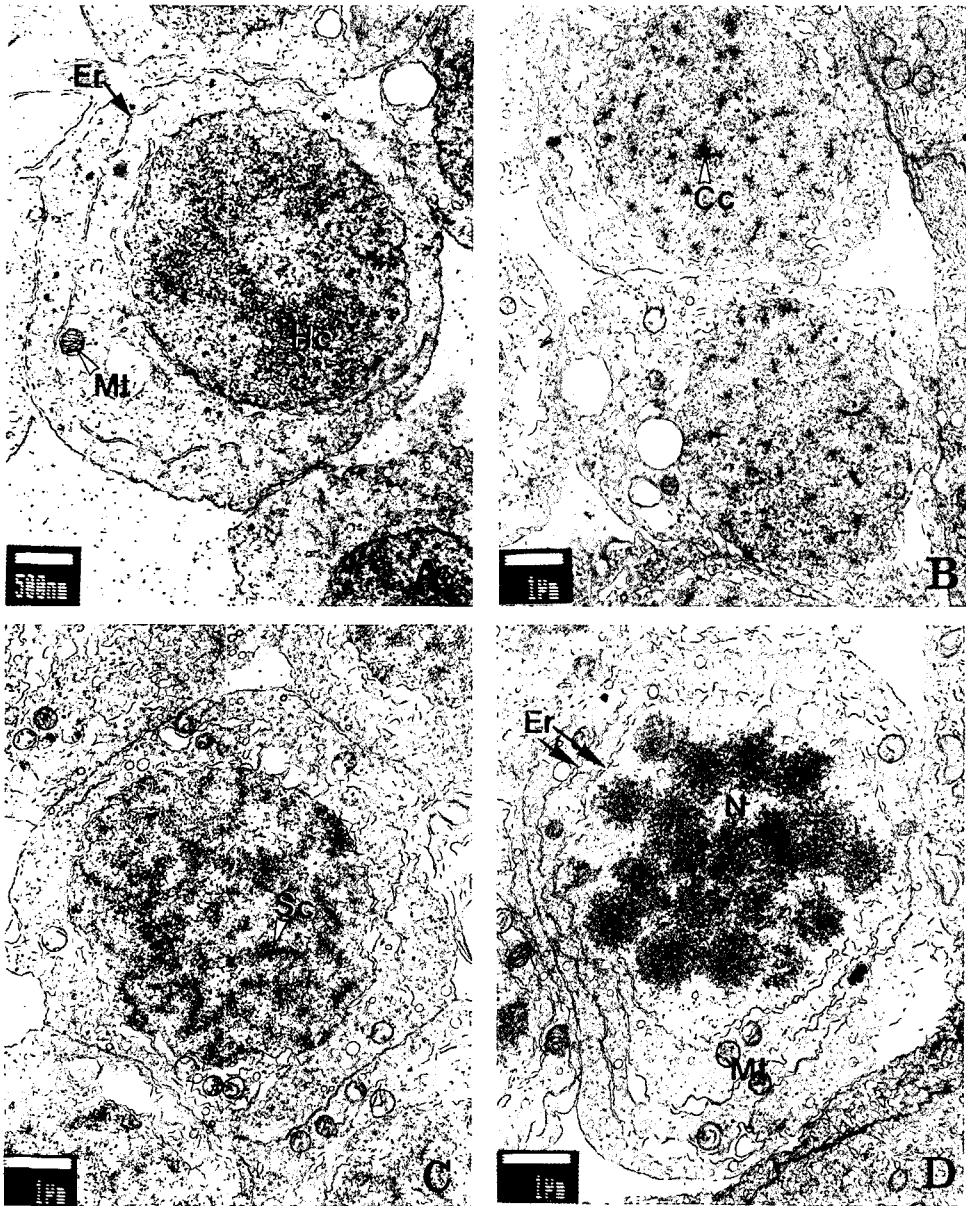


Fig. 2. Electron micrographs of spermatocytes of the goldeye rockfish, *Sebastes thompsoni*. A. Primary spermatocyte in the leptotene stage of the meiotic division. Note the heterochromatin (Hc) in the nucleus, and a few mitochondria (Mt) and endoplasmic reticulum (Er) in the cytoplasm. B. Primary spermatocyte in the pachytene stage of the meiotic division. Note the formation of chromatin clumps (Cc). C. Primary spermatocyte in the pachytene stage of the meiotic division. Note the synaptonemal complex (Sc). D. Secondary spermatocyte which contains the chromatin condensed in the nucleus (N) and numerous mitochondria (Mt) in the cytoplasm. Er; endoplasmic reticulum.

(head, neck and tail), and its head contours bear resemblance to a red pepper or an eggplant in shape (Fig. 4, D). In the transmission electron microscopic observations, the acrosome is not observed in the apical part

of the spermatozoon (Fig. 4, E). From the SEM observations, the following data on the spermatozoon are obtained: the head length ( $3.0\ \mu\text{m}$ ), the width of the head ( $1.8\ \mu\text{m}$ ), the tail length ( $17.0\ \mu\text{m}$ ), the width of

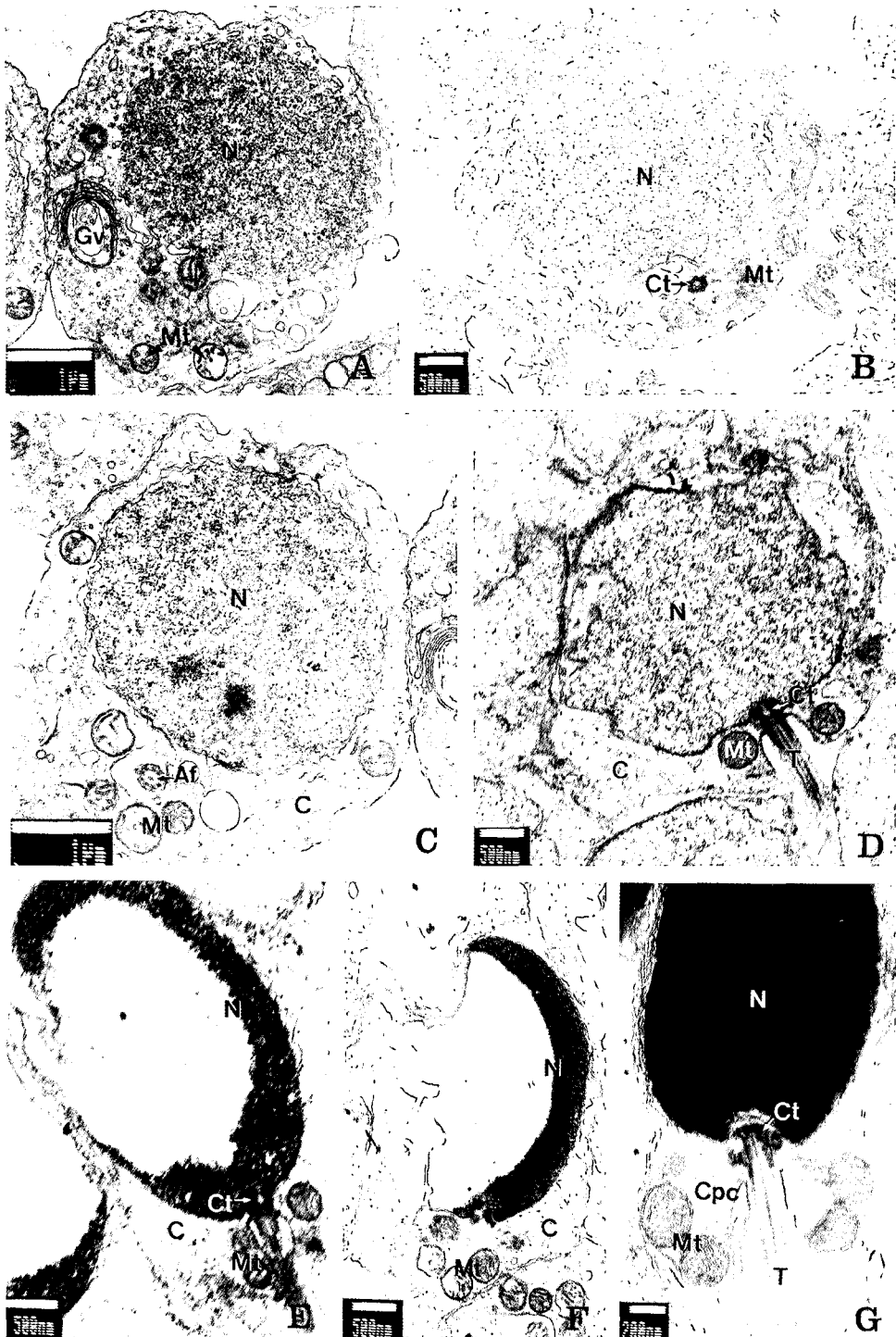


Fig. 3. Electron micrographs of spermiogenesis of the goldeye rockfish, *Sebastes thompsoni*. A and B. Spermatids during the early spermiogenesis. Note the well developed Golgi vacuoles (Gv), a centriole (Ct), and mitochondria (Mt) in the lower portion of the cytoplasm. N; nucleus. C and D. Spermatids during the middle spermiogenesis. Note the position of the axial filament (Af) and mitochondria (Mt). C; cytoplasm, Ct; centriolar complex, N; nucleus. E, F and G. Spermatids during the late spermiogenesis. Note the shape of the nucleus (N) and formation of cytoplasmic collar (Cpc). C; cytoplasm, Ct; centriolar complex, Mt; mitochondria, T; tail.

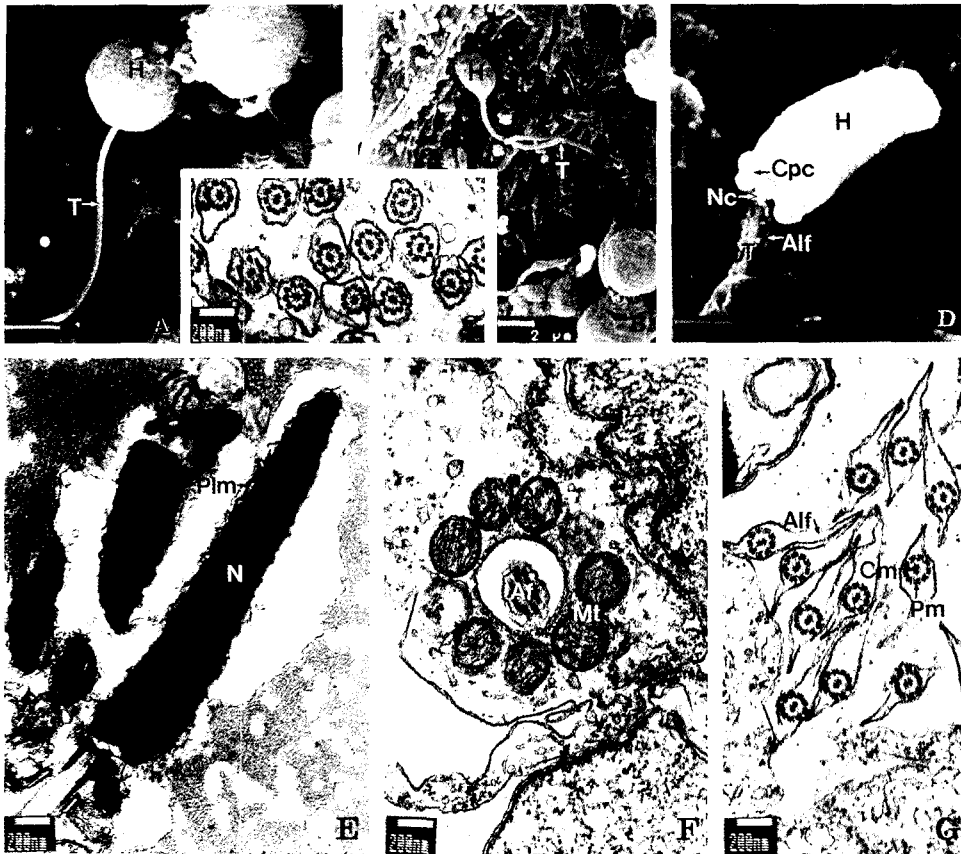


Fig. 4. Electron micrographs of spermatids and spermatozoa of the goldeye rockfish, *Sebastes thompsoni*. A. Spermatids during the middle spermiogenesis. The head (H) is round in form. T; tail. B. Spermatids during the late spermiogenesis. The head (H) is long and oval in form. T; tail. C. Cross section of spermatid tails. Note that the axonemal lateral fins are absent. D. External morphology of a sperm. The sperm consists of head (H), neck (Nc) and tail (T). Note the cytoplasmic collar (Cpc) of the head and the axonemal lateral fins (Alf) in the tail. E. Longitudinal section of the head of a spermatozoon. Note that the acrosome is absent. N; nucleus, Plm; plasma membrane. F. Cross section of the cytoplasmic collar part of a spermatozoon. Af; axial filament, Mt; mitochondria. G. Cross section of the sperm tails. Note the formation of the axonemal lateral fins (Alf) and axonemal structure in "9+2" system. Cm; central microtubules, Pm; peripheral microtubules.

the tail middle part ( $0.5 \mu\text{m}$ ) and the whole length ( $20.0 \mu\text{m}$ ). In the scanning electron microscopic observations, four to five cytoplasmic protuberances are found in the posterior part of the head (Fig. 4, D). These protuberances are confirmed to be cytoplasmic collars containing eight to ten mitochondria in a single layer (Fig. 4, F). In the cross section of the tail, the classical "9+2 system of axonemes" is clearly visible; that is, it is characterized by a pair of the central microtubules and nine pairs of the peripheral microtubules. The axial filaments are wrapped around by fibrous capsules which

manifest the bilaterally extended axonemal lateral fins in contour (Fig. 4, G). But the axonemal lateral fins are not found in the end part of tail.

## Discussion

It is well-known that external morphology and fine structures of the spermatozoa of fishes vary from one species to another and under the reproductive ecological conditions. The germ cells during spermatogenesis have been destined to undergo various cytological and

morphological changes. In the present study, we observed that the spermatogonia of the goldeye rockfish undergo remarkable changes in the nuclear size and chromatin density during spermatogenesis. Similar changes have also been reported from the previous observations on *Poecilia reticulata* (Billard, 1984), *Rudarius ercodes* (Lee and Hanyu, 1984), *Agrammus agrammus* (Chung and Lee, 1985), *Misgurnus mizolepis* (Kim, 1995), *Sebastes schlegeli* (Chung and Chang, 1995) and *Sebastes inermis* (Lee, 1996).

In this study, the formation of nuclear chromatin clumps and synaptonemal complex is one of the characteristics of primary spermatocytes of the goldeye rockfish. This characteristic appears only in prophase of the first meiotic division. Therefore it can be used as one of the criteria to distinguish the primary spermatocytes from the secondary spermatocytes.

During spermatogenesis, the Golgi complex developments are observed, but the proacrosomal granules are not found in any parts of the Golgi complex. The absence of proacrosomal granules results in the absence of the acrosome in the spermatozoon. In the late stage of spermiogenesis, the head shape has been transformed from round to red pepper-shaped gradually, and the cytoplasmic collars are formed in the posterior portion of the head, and the axonemal lateral fins are formed in the tail portion. These morphological changes of the head seem to be caused by condensation of the tubulins that are principal units of microtubules. On the other hand, based on the absence of lysosomes it can be suggested that the formation of the cytoplasmic collar and axonemal lateral fins is caused due to movement of the cytoplasm surrounding the nucleus backward to the posterior portion.

A mature spermatozoon of the goldeye rockfish consists of head, neck and tail. And it shows the characteristics of the anacrosomal introsperm type. The head is elongate and red pepper-shaped. The cross section of the flagellum shows "9+2 axonemal system", and the flagellum has bilateral axonemal fins like *Sebastes inermis* (Lee, 1996).

The spermatozoa of *Ditrema temmincki* (Lee, 1995) and *Neoditrema ransonneti* (Lee et al., 1995) among teleosts consist of head, middle piece with mitochondria, and tail.

However, the spermatozoa of *Sebastes thompsoni*, *Sebastes inermis* (Lee, 1996), *Limanda yokohamae* and *Eopsetta grigorjewi* (An et al., 1996) contain their mitochondria in a part of the head. Such part with mitochondria in the head is called the cytoplasmic collar. Therefore, it is not reasonable to designate the cytoplasmic collars as middle pieces in *Sebastes thompsoni* and the other three species.

The whole length (20  $\mu\text{m}$ ) of the spermatozoon of the goldeye rockfish is shorter than that of *Sebastes schlegeli* (45  $\mu\text{m}$ ) (Chung and Chang, 1995) and *Sebastes inermis* (27  $\mu\text{m}$ ) (Lee, 1996) belonging to the same genus.

It has been known that the following teleosts do not bear the acrosomes in their spermatozoa: *Agrammus agrammus* (Chung and Lee, 1995), *Anguilla japonica* (Colak and Yamamoto, 1974), *Cobitis striata* (Kim and Park, 1996), *Misgurnus mizolepis* (Kim, 1995), *Oryzias latipes* (Jamieson, 1991) and *Rhodeus ocellatus* (Ohta and Iwamatsu, 1983) in external fertilization fish, and *Amea splendens*, *Ataenobius toweri*, *Characodon lateralis*, *Xenotoca eiseni* (Grier et al., 1978), *Cymatogaster aggregata* (Gardiner, 1978), *Ditrema temmincki* (Lee, 1995), *Neoditrema ransonneti* (Lee et al., 1995), *Sebastes inermis* (Lee, 1996), *Sebastes schlegeli* (Chung and Chang, 1995) and *Sebastiscus marmoratus* (Mizue, 1968) in internal fertilization fish.

This fact suggests that the absence of the acrosome in spermatozoa and the morphological characteristics of the spermatozoan head are closely related with presence, shape, and structure of the micropyle of oocytes.

It has been reported that some species have the axonemal lateral fins in the flagellum: *Amea splendens* (Grier et al., 1978), *Aphyosemion gardneri*, *Craterocephalus marjoriae*, *C. stercusmuscarum*, *Gastrophysus hamiltoni*, *Hypseleotris galii*, *Iriatherina werneri*, *Lepisosteus osseus*, *Maccullochella macquariensis*, *Macquaria ambigua*, *Melanotaenia duboulayi*, *M. macullochi*, *Protopterus annectens*, *Pseudomugil mellis*, *P. signifer*, *P. tenellus* (Jamieson, 1991), *Eopsetta grigorjewi*, *Limanda yokohamae* (An et al., 1996), *Neoceratodus forsteri* (Jespersen, 1971), *Sebastes inermis* (Lee, 1996) and *Sebastiscus marmoratus*, (Mizue, 1968). This fact suggests that the number of

mitochondria and flagella, the morphology and length of flagella are related with the activity spermatozoa of each species.

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### References

- An, C.M., J.S. Lee and S.H. Huh. 1996. Ultrastructural study on spermatozoon of *Limanda yokohamae* and *Eopsetta grigorjewi*. Proceedings of Spring Joint Meeting of Korean Societies on Fisheries Science, pp. 340~343.
- Billard, R. 1984. Ultrastructural changes in the spermatogonia and spermatocytes of *Poecilia reticulata* during spermatogenesis. *Cell Tissue Res.*, 237, 219~226.
- Chung, E.Y. and T.Y. Lee. 1985. Studies on the reproductive cycle of greenling, *Agrammus agrammus*. *Bull. Nat'l Fish. Univ. Pusan*, 25, 26~42.
- Chung, E.Y. and Y.J. Chang. 1995. Ultrastructural changes of germ cell during the gametogenesis in Korean rockfish, *Sebastes schlegeli*. *J. Korean Fish. Soc.*, 28, 736~752.
- Chyung, M.K. 1977. *The Fishes of Korea*. Ilji-sa, Seoul, 727 pp (in Korean).
- Colak, A. and K. Yamamoto, 1974. An electron microscopic study of spermiogenesis in the Japanese eel, *Anguilla japonica*. *Bull. Fac. Fish. Hokkaido Univ.*, 25, 1~5.
- Gardiner, D.M. 1978. The origin and fate of spermatozoa in the viviparous teleost *Cymatogaster aggregata* (Perciformes: Embiotocidae). *J. Morphol.*, 155, 157~172.
- Grier, H.J., J.M. Fitzsimons, and J.R. Linton, 1978. Structure and ultrastructure of the testis and sperm formation in Goodeid teleosts. *J. Morphol.*, 156, 419~438.
- Jamieson, B.G.M. 1991. *Fish evolution and systematics: Evidence from spermatozoa*. Cambridge Univ. Press, New York, 319pp.
- Jespersen, A. 1971. Fine structure of the spermatozoon of the Australian lungfish *Neoceratodus forsteri* (Krefft). *J. Ultrastruct. Resear.*, 37, 178~185.
- Kim, B.S. 1995. Gonadal sex differentiation and development in diploid and induced triploidy mud loach, *Misgurnus mizolepis*. Ph.D. thesis, Nat'l Fish. Univ. Pusan, Korea, 108pp.
- Kim, I.S. and J.Y. Park. 1996. Structure of testis and spermatozoon of *Cobitis striata* (Pisces: Cobitidae) from Korea. *Korean J. Ichthyol.*, 8, 1~8.
- Lee, J.S. 1995. Studies on reproductive biology in viviparous teleost surfperch, *Ditrema temmincki*. Ph.D. thesis, Nat'l Fish. Univ. Pusan, Korea, 118pp.
- Lee, J.S. 1996. Ultrastructural study on spermatogenesis of rockfish, *Sebastes inermis* (Pisces: Scorpaenidae). *Korean J. Electron Microscopy*, 26, 267~275.
- Lee, J.S., C.M. An and S. H. Huh. 1998. Reproductive cycle of the goldeye rockfish, *Sebastes thompsoni* (Teleostei: Scorpaenidae). *J. Korean Fish. Soc.*, (in press).
- Lee, J.S., E.Y. Chung, C.M. An and P. Chin. 1995. Formation of spermatozoon and spermatozoa in the viviparous teleost, *Neoditrema ransonneti*. Proceedings of Autumn Meeting and Symposium of the Korean Fisheries Society, pp. 99~100.
- Lee, T. Y. and I. Hanyu. 1984. Reproductive cycle of small filefish, *Rudarius ercodes*. *Bull. Korean Fish. Soc.*, 17 (5), 423~435.
- Mizue, K. 1968. Studies on scorpaenous fish *Sebastiscus marmoratus* Cuvier et Valenciennes - VI. Electron microscopic study of spermatogenesis. *Bull. Fac. Fish. Nagasaki Univ.*, 25, 9~24.
- Ohta, T. and T. Iwamatsu, 1983. Electron microscopic observations on sperm entry into eggs of the rose bitterling, *Rhodeus ocellatus*. *J. Exp. Zool.*, 227, 109~119.
- Wourms, J.P. 1981. Viviparity: the material-fetal relationship in fishes. *Amer. Zool.*, 21, 473~515.

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