Ultrastructure of Germ Cells and the Functions of Leydig Cells and Sertoli Cells Associated with Spermatogenesis in *Pampus argenteus* (Teleostei: Perciformes: Stromateidae)

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Ee-Yung Chung, Young-Chul Yang, Hee-Woong Kang, Ki-Ho Choi, Je-Cheon Jun, and Ki-Young Lee (2010) Ultrastructure of germ cells and the functions of Leydig cells and Sertoli cells associated with spermatogenesis in *Pampus argenteus* (Teleostei: Perciformes: Stromateidae). *Zoological Studies* **49**(1): 39-50. The ultrastructure of germ cells and the functions of Leydig and Sertoli cells associated with spermatogenesis in male *Pampus argenteus* (Teleostei: Perciformes: Stromateidae) were investigated by transmission electron microscope observations. The intermitochondrial cement, which is involved in the propagation and grouping of mitochondria in the early developmental stages of germ cells, appeared in the cytoplasm near the nuclear envelope of spermatogonia. During the periods of active meiotic division and before spermiation, each well-developed Leydig cell contained an ovoid vesicular nucleus, and most of the cytoplasm was occupied by tubules containing a large number of smooth endoplasmic reticula, tubular or vesicular cristae of mitochondria, and several lipid droplets. Therefore, it was assumed that Leydig cells, as typical steroidogenic cells exhibiting several cytoplasmic characteristics, were involved in male steroidogenesis. Although a number of glycogen particles and a few lipid droplets were deposited in most Sertoli cells during the early and late stages of spermiogenesis, there was no clear evidence of steroidogenesis or a transfer of nutrients from Sertoli cells to spermatids. During the period of germ cell degeneration after spermiation, Sertoli cells had a lysosomal system that was associated with the performance of phagocytosis in seminiferous lobules, and it appeared that Sertoli cells of this species function in phagocytosis and resorption of products originating from degenerating spermatids and spermatozoa after spermiation: the occurrence of long slender cytoplasmic processes and various phagosomes (containing residual bodies, autophagosomes and autophagic vacuoles, myelin-like organelles, and granular lipid bodies) through phagocytosis by various lysosomes was observed within the cytoplasm of Sertoli cells. Spermatozoa of *P. argenteus* lack an acrosome, as seen in other teleosts. The structure of the spermatozoon in this species closely resembled that of the Perciformes (perciform-type teleosts). The flagellum or sperm tail of this species showed the typical 9+2 array of microtubules, interconnected by dynein and nexine arms.
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Key words: *Pampus argenteus*, Spermatogenesis, Germ cells, Leydig cells, Sertoli cells.

The silver pomfret, *Pampus argenteus*, is one of the commercially important edible fishes consumed in East Asian countries, including Korea, China, and Japan (Chyung 1977, Kim et al. 2005). In Korea, this fish is mainly found on its southern and western coasts. To study the reproductive mechanism associated with spermatogenesis, it is important to study the

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reproductive biology with reference to the functions of Leydig and Sertoli cells during germ cell development and degeneration. Several studies were previously conducted on fishes of the family Stromateidae (*Pampus* species), especially, *P. echinogaster*. Such studies investigated aspects of reproduction, including gonadal maturation (Jin 1990), growth and reproduction (Lee 1989), and reproductive ecology (Chung et al. 2008), as well as some additional ecological aspects (Abe and Kosaki 1964, Higashikawa et al. 1984). Several studies also investigated the reproduction of *P. argenteus*, including gonadal maturation (Jin 1990), reproductive ecology (Chung et al. 2008), and ecological aspects (Higashikawa and Mashumitsu 1974 1976, Higashikawa et al. 1981). Thus far, Leydig cells were found to be interstitial components of the testes of teleosts, in close association with blood vessels (Gresik et al. 1973, Grier 1981, Chung 2008). Sertoli cells were found to form the lining of the seminiferous lobules (Grier 1975 1992, Grier and Abraham 1983, Chung 2008). Despite this, there are still gaps in our knowledge regarding reproductive biology. Above all, studies on the development and degeneration of germ cells, Sertoli cells, and Leydig cells associated with spermatogenesis of *P. argenteus* are required to understand the reproductive biology of this fish. However, the functions and activities of Leydig and Sertoli cells associated with germ cell development during spermatogenesis are not yet clarified (Pudney 1993 1996, Chung 2008). Little information is available on the ultrastructure of spermatic germ cells and the functions of Sertoli and Leydig cells associated with spermatogenesis of this species, as determined by electron microscopic observations. Therefore, it is important to study germ cell development and the functions of Leydig and Sertoli cells associated with spermatogenesis using electron microscopic observations. In addition to spermatogenesis, the fate of undischarged germ cells after spermiation should be clarified in relation to the function of Sertoli cells. The purposes of the present study were to clarify the ultrastructure of germ cells and functions of Leydig and Sertoli cells during spermatogenesis and after spermiation.

MATERIALS AND METHODS

Sampling

In total, 133 specimens of *P. argenteus* were collected by trawl net from the coastal waters of Youngwang, Korea, for 1 yr from Jan. to Dec. 2006. After living fish were transported to the laboratory, specimens were used for electron microscopic observations.

Ultrastructural observations of germ cells, Leydig cells, and Sertoli cells

For production of tissue specimens for transmission electron microscopic (TEM) observations of the testicular structure of *P. argenteus*, excised pieces of testes were cut into small pieces and fixed in 2.5% glutaraldehyde- 2% paraformaldehyde (0.1 M cacodylate buffer, pH 7.5) for 2 h at 4°C. These were fully rinsed 3 times at around 30 min intervals with 0.1 M cacodylate buffer (pH 7.5), and fixed in 2% osmium tetroxide (0.2 M cacodylate buffer, pH 7.5) for 90 min at 4°C. Tissue fragments after fixation were dried using ethanol, transposed with propylene oxide, and embedded in an Epon-Araldite mixture. Ultrathin sections of Epon-embedded specimens were cut with glass knives on a Sorvall MT-2 ultramicrotome at a thickness of about 80-100 nm. Tissue sections were mounted on collodion-coated copper grids, doubly stained with uranyl acetate followed by lead citrate, and ultrathin sections were observed with a JEM 100 CX-2 (100 kV) electron microscope.

RESULTS

Ultrastructure of the testes

The general internal structure of the testes of *P. argenteus* was similar to those of other teleosts. The testes were located along the mid-dorsal side of the body cavity, occupying approximately 1/3 of the standard body length. A testis was composed of paired organs joined medially by connective tissue, and was asymmetrically shape. The testis was composed of a number of seminiferous lobules lined by Sertoli cells and germinal cysts that were filled with germ cells. The lobular lumen contained spermatogonia, spermatocytes, spermatids, and spermatozoa in the cysts. Lobules contained 2 cell types, somatic Sertoli cells and germ cells.

Spermatogenesis of this species entirely occurred within spermatocysts, which contained isogenic germ cells, the borders of which were formed by multiple Sertoli cells. Germ cells in various developmental stages were found in the cysts. In the testicular structure, Leydig and Sertoli cells, which appeared near germ cells in germinal cysts, were involved in spermatogenesis. Clusters of Leydig cells in the interlobular space were easily distinguishable from the connective tissue composing the walls of the seminiferous lobules. Leydig cells were located in the interstitium, which was surrounded by seminiferous lobules. The ultrastructure and activity of Leydig cells, which varied with the different stages of germ cell development, were observed within the interstitium. In particular, well-developed Leydig cells were found during the period of active meiotic division and before spermiation. Morphological changes and activities of Sertoli cells showed different characteristics with germ cell developmental stages. After spermiation, Sertoli cells were involved in the formation of several phagosomes that originated from degenerating spermatids and whole sperm cells by phagocytosis.

Electron microscopic observations of germ cell development, and Leydig and Sertoli cells during spermatogenesis

Based on morphological characteristics and the development of germ cells in the seminiferous lobules of the testis, spermatogenesis were classified into 4 successive stages: (1) spermatogonium, (2) spermatocyte, (3) spermatid, and (4) spermatozoon stages.
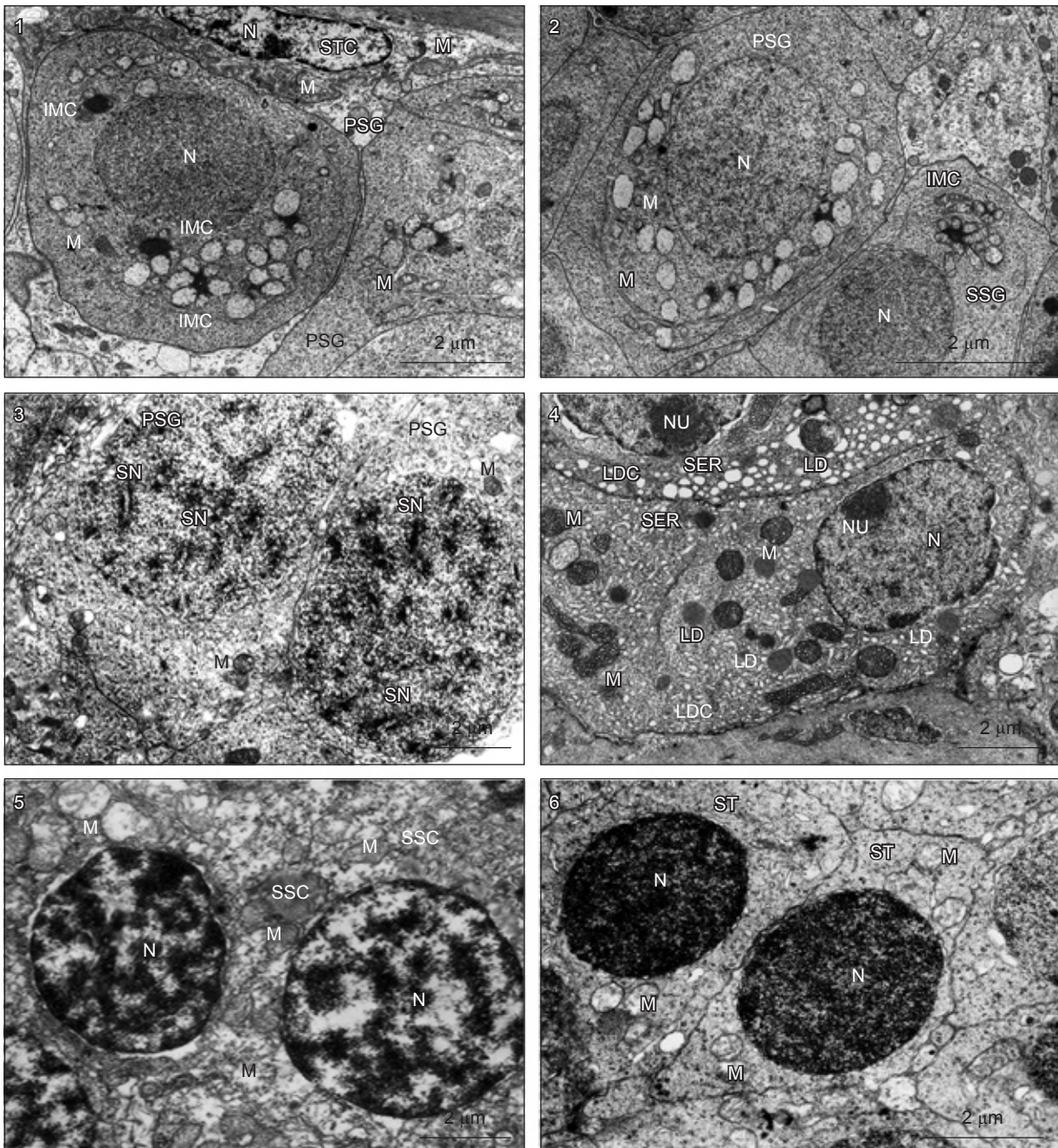
Spermatogonium stage: Spermatogenesis occurred in cysts of the seminiferous lobules. In this stage, primary spermatogonia were individually surrounded by a Sertoli cell and displayed a very low electron density and regular outlines on electron micrographs. Sertoli cell contained an elongated nucleus, while the development of the cytoplasm was weak. Primary spermatogonia (approximately 9-10 μm in diameter) were the largest germ cells in the testis, and were present throughout the year. These cells were slightly oval-shaped or spherical, and a single prominent nucleus (about 3 μm in diameter) contained a nucleolus. Chromatin materials in the nucleus were frequently observed in different degrees of condensation according to the mitotic stage.

At this stage, in particular, some intermitochondrial cement and several mitochondria

appeared in the cytoplasm near the nuclear envelope of the primary spermatogonium (Fig. 1). The primary spermatogonium divided mitotically to produce secondary spermatogonia. Secondary spermatogonia (approximately 8.5-9.5 μm in diameter) were characterized by a decreased cell size compared to primary spermatogonia, and were grouped within spermatocysts, which were bordered by Sertoli's cell processes. Secondary spermatogonia also contained intermitochondrial cement and several mitochondria in the cytoplasm (Fig. 2).

Spermatocyte stage: Primary spermatocytes arose from the mitotic division of secondary spermatogonia. Primary spermatocytes (approximately 7.0-8.0 μm in diameter) were oval-shaped, and it was sometimes hard to distinguish the cellular limits. The nucleus of the primary spermatocyte contained chromatin which was slightly denser than that of the secondary spermatogonium. At this stage, the nucleolus was not visible, and chromosomic masses (referred to as synaptonemal complexes) were frequently found. In primary spermatocytes, several synaptonemal complexes in the nucleus appeared in the prophase during the 1st meiotic division. During pachytene, synaptonemal complexes were particularly evident (Fig. 3). During the period of meiotic division, clusters of developing Leydig cells in the interlobular space of the interstitium appeared near the connective tissue composing the wall of the seminiferous lobule, and the cells were round to oval-shaped. Each of the developing Leydig cells (approximately 10.5-11.5 μm in diameter) contained 4 main morphological, structural characteristics: a vesicular nucleus, ovoid and elongated mitochondria with tubular cristae, a number of smooth endoplasmic reticula, and lipid droplets in the cytoplasm (Fig. 4). Secondary spermatocytes, which arose from the meiotic division of primary spermatocytes, were present in large numbers within the cysts. Compared to primary spermatocytes, secondary spermatocytes (approximately 6.0-7.0 μm in diameter) showed decreases in cellular and nuclear sizes. They possessed spherical nuclei with condensed chromatin that was irregularly distributed. Their cytoplasm was reduced, and contained several mitochondria and lipid droplets (Fig. 5).

Spermatid stage: Secondary spermatocytes gave rise to spermatids through the 2nd meiotic division. Spermatids were seen in different stages of spermiogenesis, as indicated by the

Figs. 1-6. **1.** *Pampus argenteus*. An electron micrograph of Sertoli cells (STCs) and primary spermatogonium (PSG). An STC contains an elongated nucleus (N) and several mitochondria (M) in the cytoplasm, while the PSG contains an oval nucleus (N) and intermitochondrial cement (IMC) in the cytoplasm. **2.** Primary spermatogonium (PSG) and secondary spermatogonium (SSG). The PSG contains oval nuclei (N) and several mitochondria (M) in the cytoplasm, while the SSG contains intermitochondrial cement (IMC) in the cytoplasm. **3.** Primary spermatocytes (PSCs) in the pachytene stage of the prophase. Many synaptonemal complexes (SNs) are present in the nuclei, and several mitochondria (M) appear in the cytoplasm of PSCs. **4.** Developing Leydig cells (LDCs) in the interstitium during meiotic division and spermatogenesis. An LDC contains a large vesicular nucleus (N) with a nucleolus (NU), numerous smooth endoplasmic reticula (SER), several mitochondria (M) with tubular or vesicular cristae, and a few lipid droplets (LDs) in the cytoplasm. **5.** Secondary spermatocyte (SSC). A few SSCs contain electron-dense chromatin in the nucleus (N) and several mitochondria (M) in the cytoplasm. **6.** Spermatids (STs) in the cyst after meiotic division. STs contain round or oval nuclei (N) with electron-dense heterochromatin and several mitochondria (M) in the cytoplasm.

degree of chromatin condensation. Spermatids underwent a shape remodeling and a size reduction during spermiogenesis. In the early part of the spermatid stage (approximately 4.0-4.5 μm in diameter), nuclei were oval or rounded, and became smaller as the chromatin condensed. At this stage, in particular, condensation of electron-dense heterochromatin masses appeared in the nucleus (Fig. 6). In an early stage of spermiogenesis, well-developed Sertoli cells, which had ovoid nuclei, appeared near several spermatids. Mitochondria, a number of glycogen particles, and a few lipid droplets were present in the cytoplasm of Sertoli cells. At this time, the activity of Sertoli cells was very high, although no clear evidence of steroidogenesis was found (Fig. 7). During the period of spermiogenesis, well-developed Leydig cells were also observed near blood vessels within the interstitium. Normally, well-developed Leydig cells (about 10.7-12.0 μm in diameter), which were located near the cysts containing secondary spermatocytes and spermatids, were ovoid or cuboidal, and each cell contained an ovoid vesicular nucleus possessing slightly condensed chromatin around the nuclear envelope. Most of the cytoplasm was occupied by tubules of smooth endoplasmic reticula, tubular or vesicular cristae of mitochondria, several lipid droplets, and dense osmiophilic bodies. Thus, Leydig cells showed several structural characteristics seen in typical steroidogenic cells (Fig. 8). During spermiogenesis, the morphology of the spermatid nucleus gradually changed, and several mitochondria and a centrosome moved to a position just behind the nucleus of the spermatid. At this time, well-developed Sertoli cells, which had elongated or slightly triangular nuclei, appeared near the spermatids during spermiogenesis, and cytoplasm of Sertoli cells contained Golgi complexes, mitochondria, a large number of glycogen particles, and several lipid droplets. Although the activities of these cells were very high, there was still no clear evidence of steroidogenesis in Sertoli cells (Fig. 9). In the late stage of spermiogenesis, the final spermatids were still in the cysts. At this time, 2 centrioles were initially located nearly the nuclear envelope; the proximal centriole appeared near the nuclear envelope, and the distal centriole formed the basal body of the flagellum. The basal body gave rise to a flagellum. A flagellum formed from each centriole prior to the mitochondrial arrangement in the region that would become the midpiece. The final spermatids, which still contained a large

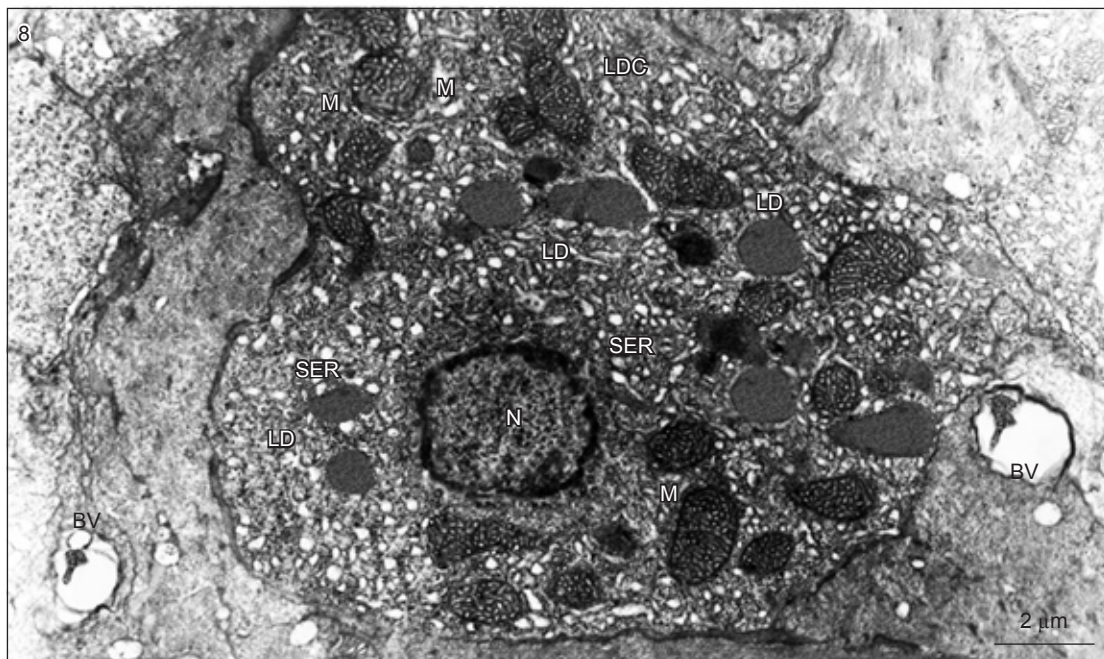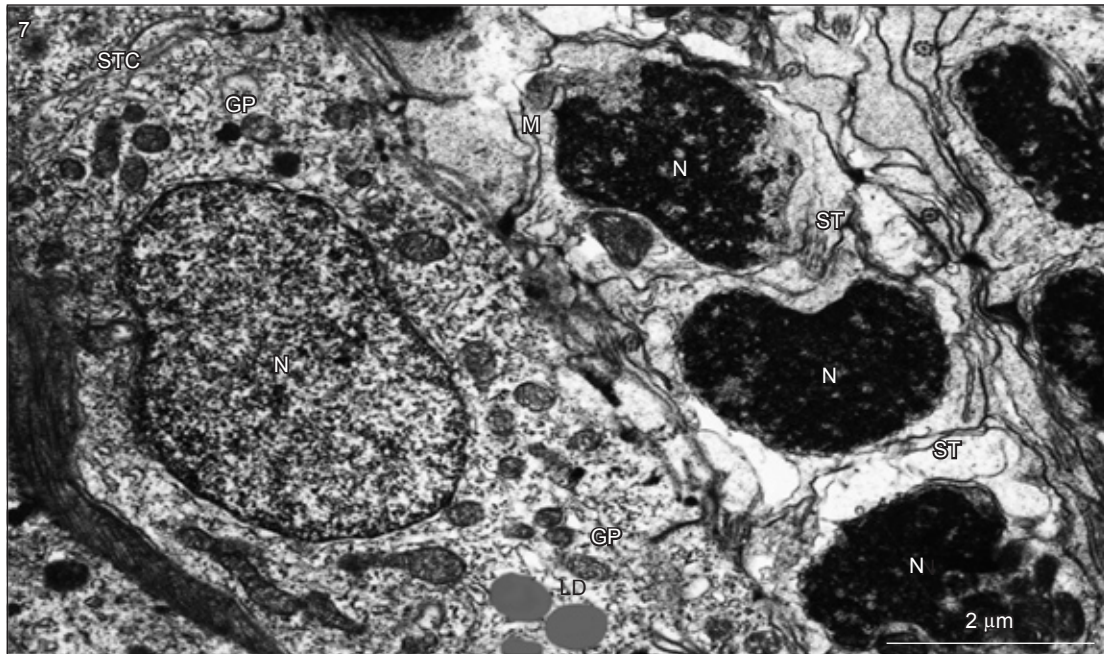amount of cytoplasm, eliminated the excess (Figs. 10, 11).

Spermatozoon stage: In this stage, the differentiation of spermatozoa was completed, and the heads of these spermatozoa were created at the periphery of the cysts toward the lumen of seminiferous lobules, with their tails oriented in the opposite direction. The spermatozoon of *P. argenteus* had a head, a small midpiece, and a single flagellum. The proximal centriole was anterior and perpendicular to the distal centriole. Therefore, the sperm structure of this species showed a primitive type, as found in species that perform external fertilization, and closely resembled that of the order Perciformes. The oval or round sperm nucleus of this species was densely packed with chromatin material. The spermatozoa of *P. argenteus* lacked an acrosome, as seen in other teleost fish. The sperm head was approximately 3 μm long. In the midpiece of the sperm, the proximal centriole and distal centriole appeared beneath the nucleus, and the sperm tail (flagellum) was about 40 μm long (Fig. 12). The sperm flagellum contained an axoneme with 9 doublets of peripheric microtubules and 2 central singlet microtubules; the flagellum showed the typical 9+2 array of microtubules, interconnected by dynein and nexine arms. Two flagellum lateral fins were shown in the cross-section of the flagellum of the sperm, as found in species that perform external fertilization (Fig. 13).

Germ cell degeneration: Just before spermiation, Sertoli cells had an obvious nucleolus in the ovoid or triangular nuclei, and some lysosomes began to appear near the interdigitation of the cells (Fig. 14). After spermiation, clusters of degenerating Leydig cells were also present in the interstitium between seminiferous lobules, and nuclei of these cells were irregularly shaped. Notably, degenerating smooth endoplasmic reticula and mitochondria also appeared in the cytoplasm of Leydig cells near degenerating spermatids in the cysts (Fig. 15). After numerous spermatozoa had been discharged, Sertoli cells had irregular pycnotic nuclei. At this time, Sertoli cells underwent active phagocytosis by various lysosomes, and produced slender cytoplasmic processes into the cyst lumen. During the period of germ cell degeneration, the cyst walls and a part of the basement membrane of Sertoli cells were broken around the periphery of the seminiferous lobules by various lysosomes. In particular, residual bodies and autophagosomes (or autophagic vacuoles) were observed in the
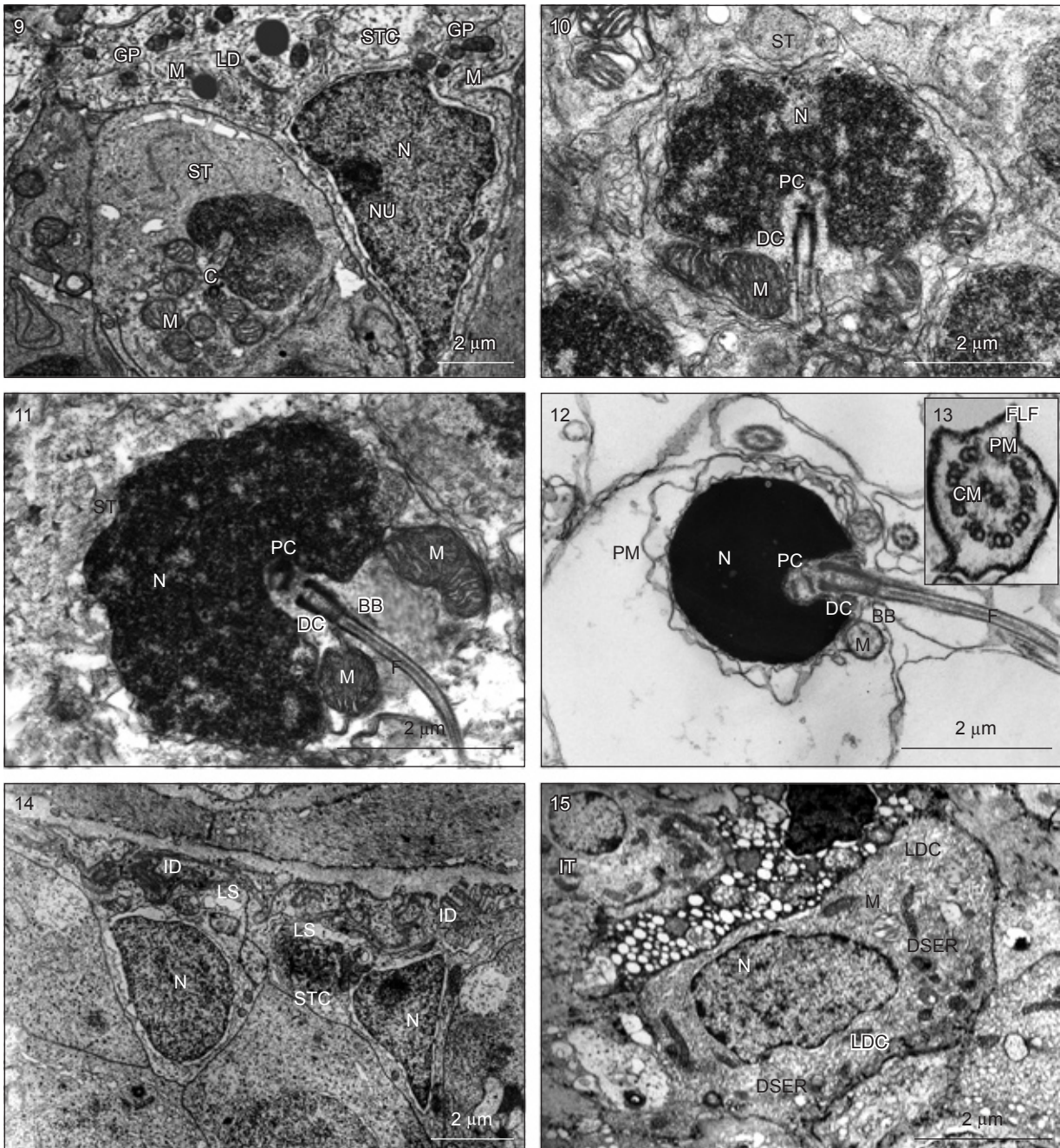
cytoplasm near pycnotic nuclei of these cells. At this time, slender cytoplasmic processes encircled a residual body in a phagocytic vacuole that was shed by degenerating spermatids and whole sperm cells in the cyst lumen, and then the residual body

was removed from the lumen of the seminiferous lobules (Fig. 16).

Phagocytosis of degenerating spermatids and whole sperm cells was only observed in seminiferous lobules. Thereafter, various

Figs. 7-8. 7. *Pampus argenteus*. An electron micrograph of a Sertoli cell (STC) and spermatids (STs) in the early stage of spermiogenesis. The STC contains an oval nucleus (N) filled with heterochromatin and several mitochondria (M), a number of glycogen particles (GPs), and several lipid droplets (LDs) in the cytoplasm, while nuclei (N) of STs undergo morphological changes during spermiogenesis. **8.** A Leydig cell (LDC) near the blood vessels (BVs) in the connective tissue in the late stage of spermiogenesis. A well-developed LDC contains a vesicular nucleus (N), a large quantity of smooth endoplasmic reticula (SER), oval or spherical mitochondria (M) with tubular or vesicular cristae, and several lipid droplets (LDs) in the cytoplasm.
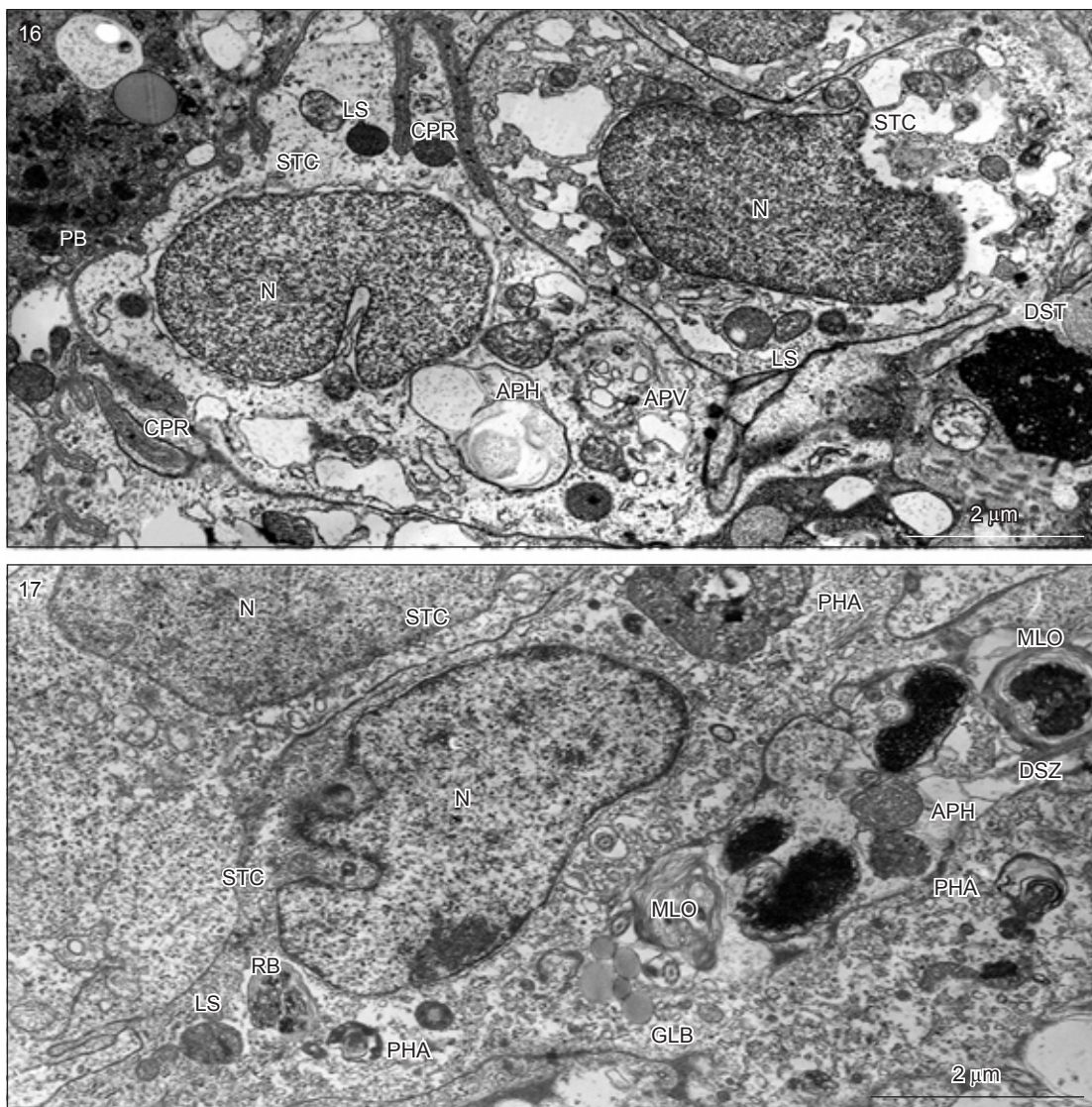
Figs. 9-15. 9. *Pampus argenteus*. An electron micrograph of a spermatid (ST) near a Sertoli's cell (STC) during spermiogenesis. The centrosome (C) and several mitochondria (M) move to beneath the nucleus (N) of the ST, and the STC contains a triangular N with a prominent nucleolus (NU), a number of glycogen particles (GPs), mitochondria (M), a Golgi complex (G), and several lipid droplets (LDs) in the cytoplasm. **10.** Spermatid (ST) during spermiogenesis. Several mitochondria (M), a proximal centriole (PC), and a distal centriole (DC) appear in the cytoplasm near the nucleus (N). **11.** Spermatid (ST) in the late stage of spermiogenesis. Several mitochondria (M), a proximal centriole (PC), a distal centriole (DC), and a basal body (BB) appear in the midpiece under the nucleus (N). **12.** Completed spermatozoon in the mature stage. The spermatozoon has an oval sperm nucleus (N) with no acrosome, but with condensed chromatin in the plasma membrane (PM), a proximal centriole (PC), distal centriole (DC), basal body (BB), and mass mitochondria (M) in the midpiece, and a flagellum (F) characteristic of sperm of the order Perciformes. **13.** Cross-sectioned sperm tail. The sperm tail has a flagellum axoneme showing the 9+2 array of microtubules (9 peripheral doublet microtubules (PMs) and 2 central singlet microtubules (CMs)) and 2 flagellar lateral fins (FLFs). **14.** Sertoli cells (STCs) just before spermiation. Each STC has an obvious nucleolus (NU) in the ovoid or triangular nuclei (N), and some lysosomes (LSs) near the interdigitation (ID) of the STC cytoplasm. **15.** Degenerated Leydig cells (LDCs) in the interstitium (IT) after spermiation. Each degraded LDC has an irregularly shaped nucleus (N), and contains degenerated smooth endoplasmic reticula (DSER) and a few mitochondria (M) in the cytoplasm.

phagosomes were formed by the phagocytosis of degenerating spermatids and sperm by various lysosomes in the cytoplasm of Sertoli cells. Specifically, myelin-like organelles were observed within phagosomes, which originated from degenerating spermatids and spermatozoa by various lysosomes. Occasionally, large phagosomes containing granular lipid bodies, residual bodies, autophagic vacuoles, and myelin-like organelles were observed within the cytoplasm of Sertoli cell (Fig. 17).

DISCUSSION

Cystic spermatogenesis, which is characteristic of most teleosts, occurs in cysts. In this study, *P. argenteus* was found to undergo cystic spermatogenesis. Germ cells in cysts were found to be at various stages of development. However, germ cells in the same developmental stage were found in each cyst in the lobular lumen, as seen in *Boleophthalmus pectinirostris* (Chung 2008).

Recently a few studies reported on the

Figs. 16-17. 16. Degenerating Sertoli cells (STCs) after spermiation. Lysosomes (LSs), cytoplasmic processes (CPRs), residual bodies (RBs), autophagosomes (APHs), and autophagic vacuoles (APVs) appear in the cytoplasm of degenerating STCs near degenerating spermatids (DSTs). **17.** Degenerating Sertoli cells (STCs) after spermiation. Irregular nuclei (N), several phagosomes (PHAs), residual bodies (RBs), autophagosomes (APHs), myelin-like organelles (MLOs), and granular lipid bodies (GLBs), which are from degenerating spermatozoa (DSZ) and spermatids by way of phagocytosis by lysosomes (LSs), appear in degenerating STCs.

function of the intermitochondrial cement which appears in germ cells in the early developmental stages of germ cells (Toury et al. 1977, Billard 1984, Muñoz et al. 2002, Jun et al. 2006, Chung 2008). In this study, particularly in the early developmental stages of germ cells, intermitochondrial cement appeared in the cytoplasm of primary and secondary spermatogonia during early spermatogenesis of *P. argenteus*. In terms of the occurrence of intermitochondrial cement, Billard (1984) stated that mitochondrial groupings were associated with this cement. Toury et al. (1977) described how the major constituents of intermitochondrial cement are RNA and protein, and the majority of the proteins present in the cement are destined to be incorporated into the mitochondria that are formed in the clusters. Therefore, it is thought that intermitochondrial cement is involved in the propagation and grouping of mitochondria in early developmental stages of germ cells.

In most teleost fishes, after the secondary spermatogonium develops into primary spermatocytes, the nuclei of primary spermatocytes undergo complex morphological changes during the early meiotic prophase. At this stage, the nucleolus is not visible, while chromosomic masses, which are referred to as synaptonemal complexes, were frequently found. In this study, several synaptonemal complexes in the nucleus in the primary spermatocyte appeared in the prophase during the 1st meiotic division. During the period of meiotic division, in most teleosts, clusters of Leydig cells in the interstitium are easily distinguishable from the connective tissue composing the walls of the seminiferous lobules.

According to Chung (2008), well-developed Leydig cells during the period of active meiotic division have 3 major morphological characteristics: (1) a vesicular nucleus, (2) mitochondria with tubular cristae, and (3) smooth endoplasmic reticula. Of these 3 characteristics, it is well known that vesicular nuclei are found in steroid-secreting cells of teleosts (Follenius 1964, Asahina et al. 1985, Jun et al. 2006). Colombo and Burighel (1974) stated that lipid droplets and dense osmiophilic bodies were also occasionally found in the cytoplasm of Leydig cells, and that this combination of organelles is typical of steroidogenic cells.

In this study, well-developed Leydig cells of *P. argenteus* were found to have the same 3 major morphological characteristics as reported by the authors above. Therefore, it appears that Leydig cells, as typical steroidogenic cells, are involved in male sex steroidogenesis. Regarding the presence or absence of lipids in Leydig cells of most teleost species, many authors (Stanley et al. 1965, Oota and Yamamoto 1966, Wiebe 1969, Nicholis and Graham 1972, Gresik et al. 1973, Cauty and Loir 1995, Chung 2008) reported that lipid droplets are present in these cells in many teleost fish testes except those of *Thalassoma duperrey* (Hourigan et al. 1989), *Esox lucius* (Grier et al. 1989), *E. niger* (Grier et al. 1989), and *Kareius bicoloratus* (Jun et al. 2006). Exceptionally, a few authors (Follenius and Porte 1960, Follenius 1968, Gresik et al. 1973) reported that lipid droplets are not found in healthy Leydig cells of teleost species. In this study, the accumulation of lipid droplets in the cytoplasm of Leydig's cells of *P. argenteus* appeared during the periods of gonadal development and maturation, indicative of active steroidogenesis, as seen in *Oncorhynchus mykiss* (Cauty and Loir 1995) and *B. pectinirostris* (Chung 2008). Thus, Leydig cells in the testes of this species showed morphological characteristics of typical steroidogenic cells. Regarding the function of Sertoli cells in seminiferous lobules of teleosts, Gresik et al. (1973) reported that these cells have 3 functions: (1) nutrition, (2) phagocytosis, and (3) steroidogenesis. Chung (2008) reported that mitochondria, endoplasmic reticula, a large number of glycogen particles, and a few lipid droplets were present in the cytoplasm of mature Sertoli cells of *B. pectinirostris*. He supposed that in particular, glycogen particles in these cells appeared to be involved in spermatid nutrition during spermiogenesis. In this study, most Sertoli cells of *P. argenteus* contained a large number of glycogen particles and a few lipid droplets during the period of multiplication of spermatogonia and spermiogenesis. Thereafter, the numbers of glycogen particles and lipid droplets subsequently dropped during spermiation. However, there was no indication of a transfer of nutrients to spermatids during spermatogenesis, and the fine structure of Sertoli cells did not provide clear evidence of steroidogenesis or steroidal activity.

Van Vuren and Soley (1990) reported that after sp ermiation, cytoplasmic processes, particularly the long slender variety that encircle patches of whole sperm cells, are an indication of the phagocytic properties of Sertoli cells of *Tilapia rendalli*. Lo Nostro et al. (2003) reported that the lysosomal systems of Sertoli cells phagocytose residual bodies and undischarged germ cells, and

they supposed that the uptake of residual bodies may be facilitated by the activity of Sertoli cells in many teleosts.

In this study, phagosomes containing degenerating spermatids and spermatozoa, lipid materials (lipid droplets and globular lipid bodies), and residual bodies were observed within Sertoli cell cytoplasm. Large phagosomes and myelin-like organelles (or myelin figures) were observed within the phagosomes. Long, slender cytoplasmic processes encircling patches of whole sperm cells appeared in Sertoli cell cytoplasm. After residual bodies were phagocytosed, several autophagosomes and autophagic vacuoles appeared in Sertoli cell cytoplasm. Following spermiation, Sertoli cells in the testes of many teleost species undergo fatty degeneration by various lysosomes and are presumed to be resorbed. Thus, the characteristics of phagocytosis in Sertoli cells in *P. argenteus* were similar to the results reported by Van Vuren and Soley (1990) and Lo Nostro et al. (2003).

With reference to phagocytosis of residual bodies in mammals, Morales et al. (1985) and Sylvester et al. (1989) reported that after spermiation, residual bodies, which are composed of large vacuoles, multivesicular bodies, condensed mitochondria, and lipid droplets, appeared in the cytoplasm of Sertoli cells of rats. At that time, residual bodies are initially covered by thin cytoplasmic processes of Sertoli cells. Similarly, Chung (2008) reported that in teleosts such as *B. pectinirostris*, various lysosomes in Sertoli cells phagocytose and lyse the residual bodies that detach from spermatids after spermiation, and lipid materials (a few lipid droplets and granular lipid bodies) in the cytoplasm of Sertoli cells may then be produced by phagocytosis of the residual bodies. In this study, similar phenomena as reported by Chung (2008) were also found in Sertoli cell cytoplasm of *P. argenteus*.

Poirier and Nicholson (1982) reported that in the case of species that perform external fertilization, the spermatozoa of teleost fishes are considered to be the primitive type, with a round nucleus and short midpiece containing a few mitochondria, and a tail consisting of a long flagellum. In the present study, the spermatozoon of *P. argenteus* showed a typical primitive type of externally fertilizing sperm.

The spermatozoa lack an acrosome, as in all teleost fish spermatozoa (Romagosa et al. 1999, Jun et al. 2006, Chung 2008). This fact is generally related to the presence of an egg micropyle (Romagosa et al. 1999, Chung 2008). *Pampus argenteus* belongs to the order Perciformes. As reported by Mattei (1991) and Jamieson (1991), the sperm type of this species was observed in species of the order Perciformes. In the sperm of *P. argenteus* (family Stromateidae), 2 masses of mitochondrial materials lie on 2 sides, and the nucleus of the spermatozoon is asymmetrical. The type and structure of the sperm of this species are of the primitive type, and consist of a spermatozoon with a flagellum possessing the 9+2 pattern. According to reports of Mattei (1988) and Lo Nostro et al. (2003), the spermatozoon of this species belongs to the uniflagellate anacrosomal aquasperm group (type 1 sperm, reported by Mattei), and this is an externally fertilizing species.

Jamison (1989) reported that flagellum lateral fins on the flagellum of the spermatozoon are present in most externally fertilizing sperm. In this study, *P. argenteus* was found to be an externally fertilizing species, and the spermatozoa have 2 flagellum lateral fins. Therefore, the results of this study are in agreement with those reported by Jamieson (1989).

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