

Structural and Histochemical Changes in the Cyst cell and the Interstitial Cell in the Testis of a Teleost *Hexagrammos agrammus* associated with the Reproductive Cycle

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In order to understand fine structural and histochemical changes in the cyst cell and the interstitial cell in the testis of the spottybelly greenling *Hexagrammos agrammus* associated with the reproductive cycle from January to December, 1992, these cells were studied by electron microscopy and light microscopy.

The cyst cells in the mature testis show a weak affinity to haematoxylin, while they become larger in size. At this time, these cells appear to be more functional than those in the growing stage because several mitochondria, endoplasmic reticulum, glycogen particles and a few lipid droplets appear in the cytoplasm of the cyst cell. It appears, therefore, that the cyst cell of this species has vital functions for nutrition, secretion and steroidogenesis.

Well-developed interstitial cells contain large rod-shape or spherical mitochondria with tubular cristae and the large quantities of smooth endoplasmic reticulum and electron-dense materials in the vesicle at the mature and spawning stage. The interstitial cells of this species show characteristics of steroid interstitial cells having a vesicular nucleus, mitochondria with tubular cristae, and smooth endoplasmic reticulum. However, these interstitial cells of teleost give negative histochemical reactions for Sudan black B.

Introduction

The cyst epithelial cells that line the spermatogenetic cyst of the testes in lower vertebrates, such as Pisces and Amphibia are believed to be the homolog of the Sertoli cell in higher vertebrates (Loft, 1968; Hoar, 1969). In higher vertebrates Sertoli cells may have a nutritive, contractile, or steroidogenic function (Leblond and Clermont, 1952; Roosen-Runge, 1955; Marsall and Woolf, 1957 and Sapsford et al., 1969; Nicholls and Graham, 1972).

In the spottybelly greenling *Hexagrammos agrammus*, the testicular lobules contain the cyst cells (Sertoli cell) which extend from the basement membrane of the tubule to its lumen and exhibit a close association with the developing spermatids.

An endocrine function of the interstitial cell(Leydig cell) of the testis of teleosts has been inferred from seasonal proliferative changes (Courrier, 1921). Subsequent histochemical investigations of teleost testes for lipids have provided evidence for steroidogenesis by the interstitium(Marshall and Lofts, 1956; Loft and Marshall, 1957; Hoar, 1969).

Since Follenius and Porte(1960) described active interstitial cells present in *Lebistes* testis, electron microscopic examinations of interstitial cells(Leydig cells) have been reported briefly for immature trout(Oota and Yamamoto, 1966), seasonal changes in the stickleback, *Gasterosteus aculeatus*(Follenius, 1968), histochemical and ultrastructure of the testis in *Oryzias latipes*(Gresik *et al.*, 1973), and for the greenling *Agrammus agrammus*(Chung and Lee, 1985).

In teleostean testes, however, structural changes in the cyst cell and interstitial cell in accordance with the reproductive cycle have not yet been satisfactorily clarified. Therefore, the present work aimed to conduct a study on the structures of the cyst cells (so-called sertoli cell) and the interstitial cells in the testis of a spottybelly greenling in an attempt to compare their ultrastructure with other teleosts and mammalian homologs.

Materials and methods

Specimens of *Hexagrammos agrammus* were collected monthly at the intertidal zone of Tongbaeksum, Pusan, Korea for one year, from January to December, 1992. A total of 160 adult males were used for histochemical and electron microscope study.

Analysis of gonadal phase in the spottybelly greenling *Hexagrammos agrammus* was made by light microscopical examination of histological preparation. The tissues were subjected to standard histological procedures(testes were fixed in Bouin's fixative, dehydrated in alcohol and embedded in paraffin). Embedded tissues were thin sectioned(5-7 μm) on a rotary microtome. Sections were mounted on glass slides, stained with Hansen's hematoxylin-0.5% eosin, Mallory's triple stain and PAS stain, and examined using the light microscopy.

For histochemical study, all the following reactions were performed on fresh frozen sections of testes prepared in a cryostat at -27°C . Sudan Black B staining for neutral fats were used for detecting lipids.

For electron microscopical observations the excised pieces of the gonads were cut into small pieces and prefixed immediately in 2.5% paraformaldehyde-glutaraldehyde in 0.1M phosphate buffer solution(pH 7.4) for 2 hours at 4°C . Subsequently fixed specimens were dehydrated in increasing concentrations of ethanol, cleared in propylene oxide and embedded in Epon-Araldite mixture. Ultrathin section of Epon-embedded specimens were cut with glass knives on a Sorvall MT-2 microtome and LKB ultramicrotome at a thickness of about 800-1,000 \AA . Tissue sections were mounted on collodion-coated copper grids, doubly stained with uranyl acetate followed by lead citrate, and observed

JEM 100 CX- II (80KV) electron microscope.

Results

1. General morphology and structure of the testis

The testes of the spottybelly greenling are white and elongate, they are located in both sides in the posterior half of the abdominal cavity(Fig. 1). The testis consisting of a pair of lobular structures in the right and left is united in the posterior seminal vesicle.

Interlobular spaces are generally narrow, but here and there they become somewhat broad and angular, and are full of compact aggregates of small poorly defined cells. The cells appear to be round or oval and have a comparatively large roundish nucleus stained deeply with haematoxylin.

The interstitial region between the lobules contains blood vessels, clearly discernible islets of interstitial tissue can be detected between the developing testicular lobules.

The islets vary in thickness from one to a few cells or many consist of pockets of up to 20–30 interstitial cells, these islets of small interstitial cells appear to communicate with each other through narrow, interlobular connective tissue corridors and efferent ducts.

Just inside each testicular lobule lie the cyst cells. Within any lobule the enclosed spermatogenic tissues are all in approximately the same stage of development, i. e., all spermatogonia, spermatocytes or developing spermatids. The cyst cells appear somewhat similar to the sertoli cells of Mammalia.

2. Histological changes in the cyst cells and the interstitial cells in the testis.

In the early growing testes collected in July, the lobules are filled with the spermatogonia, spermatocytes and cyst cells.

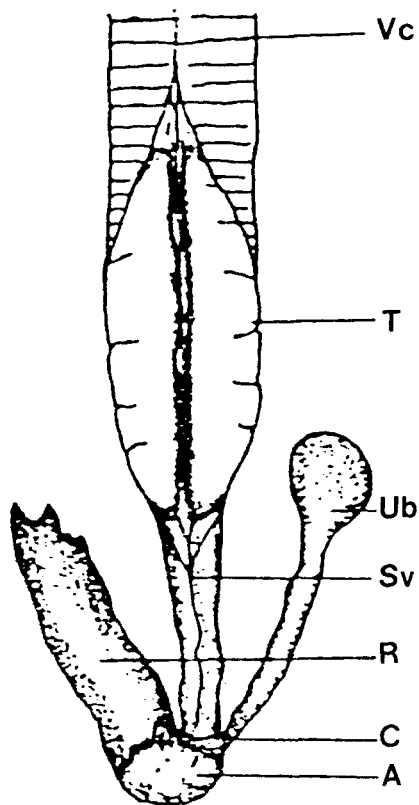


Fig. 1. Reproductive organ of *Hexagrammos agrammus*.

A: Anus C: Cloaca R: Rectum Sv: Seminal vesicle T: Testis Ub: Urinary bladder Vc: Vertebral column

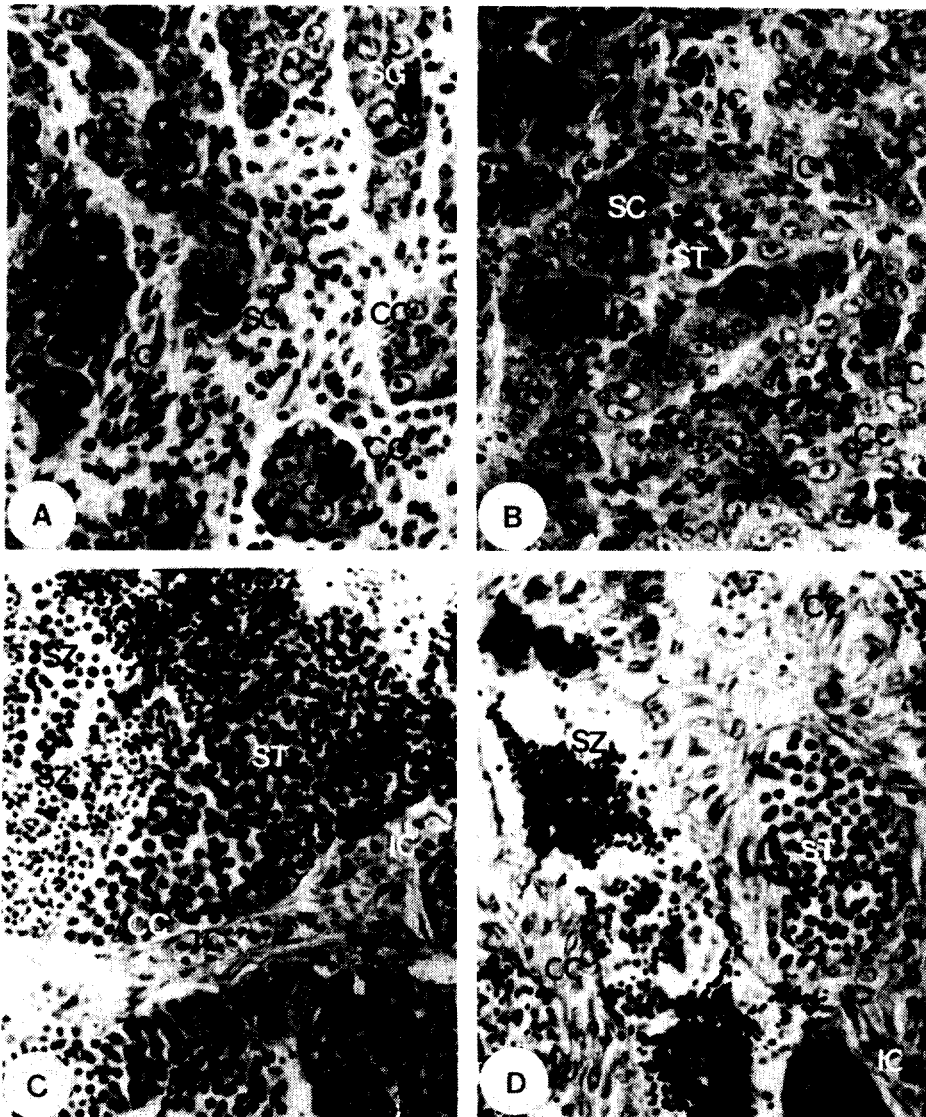


Fig. 2. Light micrographs showing the gonadal phases of *Hexagrammos agrammus*.

A. transverse section of the testis in the early growing stage. Note the testicular lobules filled with the spermatogonia (SG), spermatocytes(SC), cyst cells(CC) and the islets of interstitial cells(IC) located in interlobular spaces: B. transverse section of the testis in the late growing stage. Note the spermatogonia(SG), spermatocytes(SC), spermatids(ST) and cyst cells(CC) in the testicular lobules, and interstitial cells(IC) in the interstitial tissue: C. section of the testicular lobules in the mature and spent stage. Note a number of spermatozoa(SZ), spermatids(ST) and cyst cells(CC) in the lobules, and the islets of interstitial cells(IC): D. section of the testicular lobules in the recovery stage. Note a number of remaining spermatozoa(RSZ), spermatids(ST) and cyst cells in the lobules and a few interstitial cells(IC) in the interstitial tissue. A,B,C,D. $\times 400$.

and the islets of interstitial cells are located in interlobular spaces. The cyst cells are spherical, oval, spindle or oblong in shape. Their nuclei are deeply stained with haematoxylin, while the cytoplasm is faintly stained.

At this time, the interstitial cells appear to communicate with each other, the mean interstitial cell size is $9.0 \mu\text{m} \pm 0.04 \mu\text{m}$ and the mean nuclear size is $3.60 \mu\text{m} \pm 0.02 \mu\text{m}$. These cells and fibroblasts of the connective tissue stain similarly with haematoxylin-eosin. But there are no lipid deposits in the interstitial cells in this stage. These interstitial Leydig cells give negative histochemical reactions for sudan black B. Therefore, the volume of cytoplasm is very small and there is no evidence of the interstitial cells having secretory function by histochemical observations (Fig. 2A).

In the late growing testes of fishes collected between August and September, most of the germ cells in the lobules are spermatocytes and spermatids. With increasing growth, numerous cyst cells are clear, most of these cells located along the lobule wall have each a nucleus of triangular or elliptical shape. The interstitial cell is much better developed than the previous stage. Most of the cells are compactly arranged forming large aggregation. And the cell size is $9.5 \mu\text{m} \pm 0.02 \mu\text{m}$, the mean diameter of a nucleus is $4.01 \mu\text{m} \pm 0.05 \mu\text{m}$ in diameter. Their cell boundaries are not clear in the aggregation (Fig. 2B).

The interstitial cells show negative histochemical reactions for Sudan black B as in the previous stage.

In the testes of mature and spent fishes collected between September and October, with an increase in amount of mature spermatozoa, testicular lobules become larger in size. Most of the cyst cells are present along the lobule wall, and they showed a weak affinity to haematoxylin and become larger in size than those in the previous stage. Thus, these cells appear to be much functional. At this time the cytoplasm of the interstitial cells continue to increase in size and reach the maximum size ($16.70 \mu\text{m} \pm 0.04 \mu\text{m}$), while their nuclear sizes are not changed remarkably in comparison with the previous stage (Fig. 2C). The interstitial cells also give negative histochemical reactions for Sudan black B.

After spawning, in the recovering testes of fishes collected through November to February, a number of remaining spermatozoa are scattered in the testicular lobules. And then the intralobular cyst cell on the germinal epithelium were newly formed and activated again, and they show somewhat strong affinity to haematoxylin, while decrease markedly in size.

At this time, the interstitial cells are hard to find in the interlobular interstitium, most of cell areas and its size ($7.16 \mu\text{m} \pm 0.12 \mu\text{m}$) are markedly reduced in comparison with the previous stage except for their nuclei size. therefore, the activities of the their cells are rapidly decreased (Fig. 2D).

3. Electron microscopic observation

1) Cyst cell (Sertoli cell)

The outermost cyst cells form a continuous layer around the periphery of the testicular

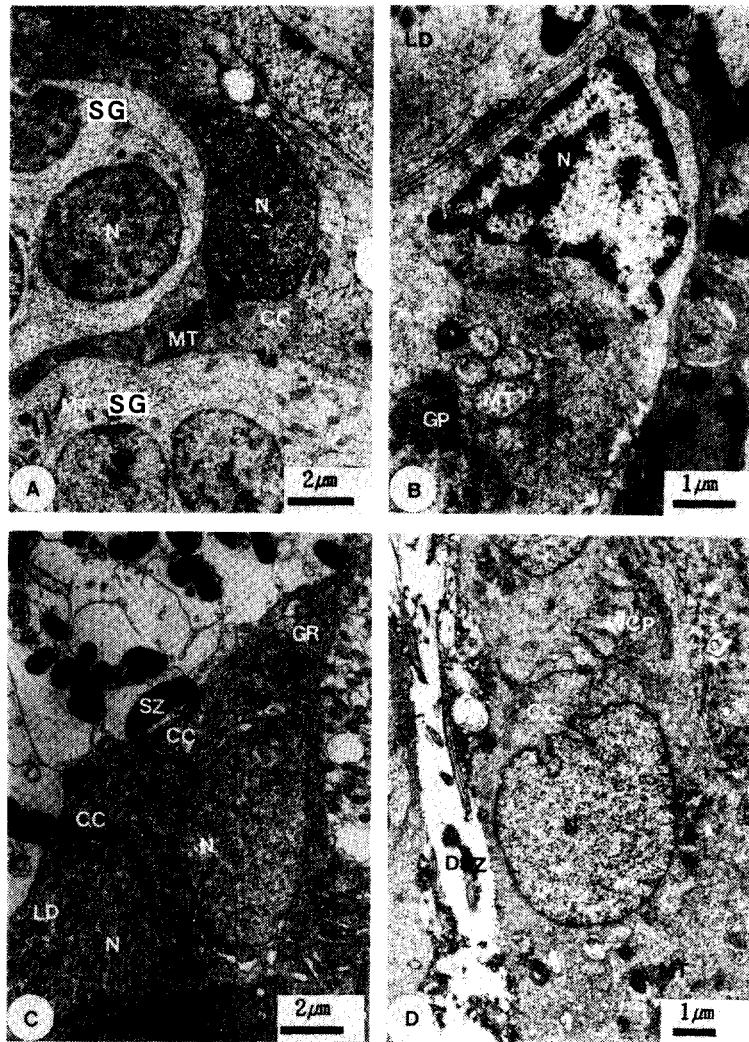


Fig. 3. Electron micrographs of the cyst cells in *Hexagrammos agrammus*.

A. cyst cell in the testicular lobule in the early growing stage. Several spermatogonia(SG) are surrounded by the cyst cell(CC) containing an elongated oval nucleus(N), and a few mitochondria(MT) and small quantities of glycogen particles in the cytoplasm of the cyst cell. $\times 6,000$; B. cyst cells in the testicular lobules in the late growing stage. The cyst cells(CC) contain a triangular nucleus with heterochromatin, and they possess the smooth endoplasmic reticulum, mitochondria, a few lipid droplets(LD), large quantities of glycogen particles(GP) in the cytoplasm, and desmosome(DM) between adjacent cells is found. $\times 11000$; C. cyst cells in the testicular lobules in the mature and spent testes. Several mitochondria(MT), endoplasmic reticulum(ER), glycogen particles(GP), a few lipid droplets(LD) and granules appear in the cytoplasm. $\times 6,000$; D. cyst cell in the recovery stage. a number of remaining or degenerating spermatozoa(DSZ) are attached to the cyst cells(CC) with a degenerating wrinkled oval or irregular nucleus, several degenerating wrinkled cytoplasmic projections(WCP) are found in cyst cells. $\times 8,000$.

lobule and are separated from the interstitial capillaries and interstitial cells by a layer of connective tissue elements and thin bands of intercellular material.

In the early growing testes, several spermatogonia are found in the cyst. The cytology of the peripheral cyst cells appear variable, the cyst cell contains an elongated oval nucleus. Small number of spherical mitochondria, and glycogen depositions are existed in the cytoplasm(Fig. 3A).

As the spermatocytes grow, the cyst cells contain a remarkable triangular nucleus with heterochromatin near the nuclear envelope, and they possess granular endoplasmic reticulum, several electron dense granules, spherical mitochondria in the cytoplasm in the late growing stage. At this time, each peripheral cyst cell joins tightly with those of the adjacent cell. Especially, desmosome between adjacent cells is found, and glycogen particles are scattered throughout the cytoplasm. Therefore, these cells are appear to be much functional for secretion or metabolism(Fig. 3B).

In the mature and spent testes, the testicular lobules containing maturing spermatozoa appear large in volume than those containing spermatogonia or spermatocytes in the late growing stage. The cyst cells with a triangular nucleus increase in number and length, and the area of contacting plasma membrane between the cyst cells is increased.

Especially, several mitochondria, endoplasmic reticulum, glycogen particles, a few lipid droplets and granules appear in the cytoplasm of these cells in the mature stage Fig. 3C). Therefore, these cyst cells show to be more much functional for secretion than the previous stage.

In the recovering testes, a number of remaining or degenerating spermatozoa are existed in the lobules, and the nucleus of the cyst cell shows degenerating wrinkled oval or irregular forms in shape during the degenerating period. At this time, several mitochondria, glycogen particles, especially, several degenerating wrinkled cytoplasmic projections are found in the cytoplasm (Fig. 3D).

2) Intersitial cell (Leydig cell)

The aggregated interstitial cells in the interlobular space are easily distinguishable from connective tissue cells composing the wall of lobules due to the difference in shape of the nucleus, internal organization of mitochondria and endoplasmic reticulum.

In the early growing stage testes, the clusters of the interstitial cells found in the interlobular spaces are surrounded with the fibroblasts in intercellular spaces of various widths. The cells are generally round to oval in shape and somewhat irregular in outline. Frequently, the areas of their cells show a complicated membraneous structure, the nucleus is large in size and highly variable in shape. The nucleoplasm exhibits a fine granularity with an almost homogeneous density, distinct nucleoli are recognizable in the nucleus. The cytoplasm is scanty, but if contains characteristic organelles. The mitochondria are several in number but large in size, the large ones measuring more than $2\mu\text{m}$. Ovoid or spherical mitochondria are common. The Golgi complex is found on rare occasions.

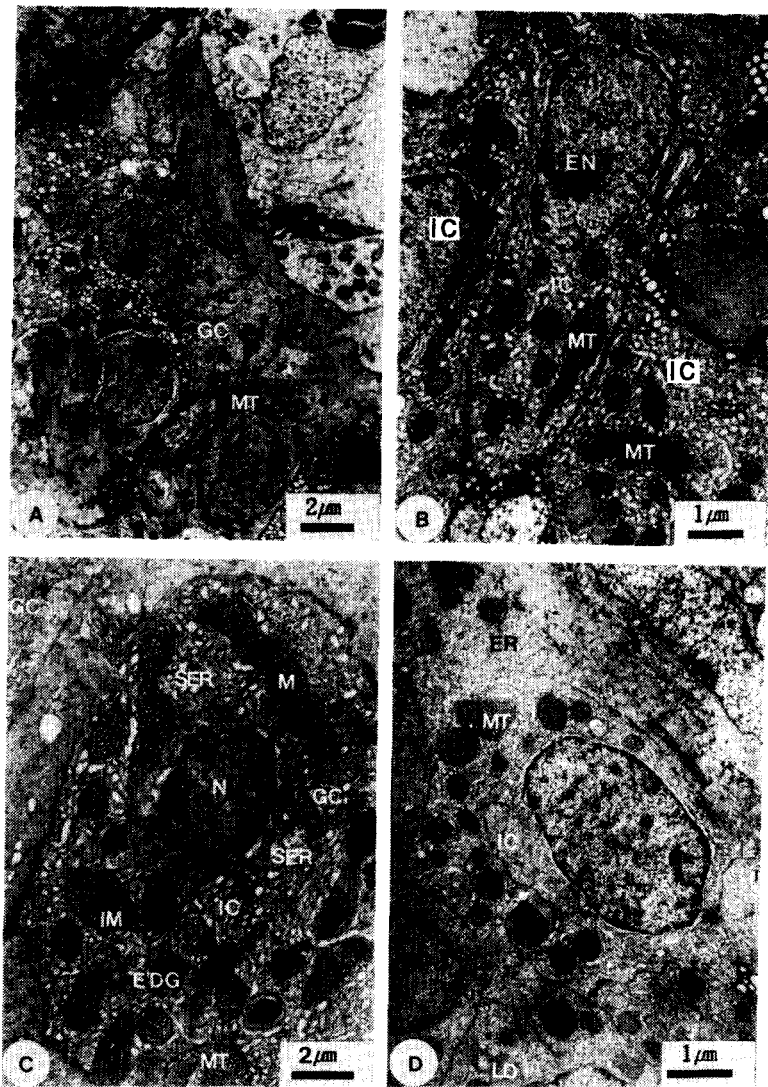


Fig. 4. Electron micrographs of the interstitial cells in *Hexagrammos agrammus*.

A. Cluster of the interstitial cells(IC) in interstitial space. The cells are generally round to oval in shape, contain ovoid or spherical mitochondria, Golgi complex(GC), a number of smooth endoplasmic reticulum(SER). $\times 5,000$.; B. Clusters of the interstitial cells(IC) in interlobular spaces. The cells are oblong in shape, each with a relatively large euchromatic nucleus, contain several large mitochondria(MT) with tubular cristae, a number of smooth endoplasmic reticulum. $\times 10,000$; C. Well-developed interstitial cell in the mature and spent stage. The interstitial cells are ovoid or cuboidal in shape, and they contain a number of mitochondria with well developed tubular cristae and intramitochondrial granule(IM) electron dense granules(EDG) in the vesicles. $\times 6,000$; D. the interstitial cells in the recovering stage. Number of interstitial cells decreased, a few lipid droplets (LD) and several mitochondria(MT) appear during degeneration. $\times 12,000$.

Free ribosomes are common, but not so abundant in the cytoplasmic matrix.

In the late growing testes, interstitial cells fill the entire central regions of the interstitium, they tend to group around, and come into close association with the numerous capillaries. Growing Leydig cells are elongated oval cell, each with a relatively large euchromatic nucleus. Most of the cytoplasm is occupied by the smooth endoplasmic reticulum. The mitochondria with tubular cristae are relatively large (up to 2.7 μm in length and 0.7 μm in diameter), they appear to vary in shape from almost elongate to oval; this may be simply indicate sectioning in different planes (Fig. 4B).

In the mature and spent testes, typical Leydig cells are ovoid or cuboidal cells. The mitochondria with well-developed tubular cristae are the largest in size (up to 6.7 μm in length and 0.7 μm in diameter), they are rod-shape or spherical type. At this time, well-developed interstitial cells may contain increased quantities of smooth membrane and a few Golgi apparatus in the cytoplasm. And the electron dense granules are homogeneous and either completely fills the vesicle or is surrounded by an electron-lucent halo of varying size (Fig. 4C).

In the recovering testes, the interstitial cell does not appear to collapse after spermiation, although the evidence indicates that gonads have reduced interstitial cell in size and number. A little lipid droplets and a few mitochondria appear during degeneration (Fig. 4D).

Discussion

1. Cyst cell (Sertoli cell)

Gresik *et al.* (1973) suggested that the fine structure of the cyst epithelial cells have capacities for several functions: 1) nutrition 2) phagocytosis and 3) steroidogenesis.

In the present study, there are a few organelles (mitochondria, endoplasmic reticulum, etc.) and small amount of glycogen particles in the cytoplasm of the cyst cells in the early growing stage, but cell organelles, a little lipid droplets and the large amount of glycogen particles are increased in the cells in the mature stage.

Therefore, glycogen particles seem to be implicated in the nutrition of spermatids during spermiogenesis.

Active phagocytosis is indicated by the occurrence of vacuoles containing degenerating spermatids, residual cytoplasm of spermatids, residual bodies in the cyst lumen of *Oryzias latipes* (Gresik *et al.*, 1973).

Reddy and Svoboda (1967) described that abnormalities of spermatogenic elements lead to their phagocytosis by Sertoli cells in rats. Vaupel (1929) considered the cyst epithelial cells of guppy to be phagocytic. Thus, similar phenomenon can be seen in the cyst lumen in the testis of the spottybelly greenling.

The smooth endoplasmic reticulum of cyst epithelial cells could be involved in steroid biosynthesis. Such a function has been postulated for cyst epithelial cell of sharks (Collenot and Ozon, 1964, Simpson and Wardle, 1967) and of salmon (Wiebe, 1969).

In the present study, the amount of glycogen particles and smooth endoplasmic reticulum, glycogen particles in the cyst cells are increased during the spermiogenesis remarkably. Therefore, it can be assumed that the cyst cell of this species is functioning actively for nutrition, phagocytosis and steroidogenesis.

2. Interstitial cell (Leydig cell)

The interstitial cells situated in the interlobular space in the testis of this species are quite similar to interstitial cells in the adult mammalian testes (Fawcett and Burgos, 1960; Christensen and Fawcett, 1961; Hyder, 1969; Gresik *et al.*, 1973).

The most interesting finding in this investigation are those which relate to the comparative development of the spermatogenic cyst cell (Sertoli cell) and the interstitial tissue elements of the spottybelly greenling testis.

In the early stage interstitial tissue does not appear by cytological and histochemical appearance to be secretory. The tissue is sparse, and cells are small and bear no lipid concentration. However, rapid interstitial cell developments occur during mature and spermiation. These phenomena are coincide with the other studies (Hyder, 1969; Gresik *et al.*, 1973).

From the evidence of their location in the testis, from histochemical and ultrastructural observation, the interstitial Leydig cells of teleosts would seem to be homologous with those of birds (Nicholls and Graham, 1972) and mammals (Christensen and Mason, 1965). It remains to be proved that they are the principle source of androgens in fish, though this would seem likely.

In reference to the evidence for the synthesis of steroids in the Leydig cells of teleosts, and indeed in those of other vertebrates, there is little visual indication of their secretion by the cell. Follenius (1968) has described that although the Golgi complex is prominent in the Leydig cells of *Gasterosteus*, no evidence of its involvement in steroid secretion can be found by electron microscopy.

However, in the present study, the electron dense material was homogeneous and filled the vesicle completely or surrounded by an electron-lucent halo of varying size. Vesicles of similar size and morphology were sparsely dispersed in the cytoplasm of the interstitial cell. Some were in association with the plasma membrane with cytological features suggestive of secretion into the intercellular space.

Cytologically, the interstitial Leydig cells of *Hexagrammos agrammus* show three features characteristic of steroid interstitial cells of other species : (1) a vesicular nucleus, (2) mitochondria with tubular cristae, (3) smooth endoplasmic reticulum.

This type of vesicular nucleus is seen in the steroid secreting cells of most mammals

(Christensen and Gillim, 1969), of other teleosts (Follenius, 1964; Follenius and Porte, 1960). And the rod-shaped mitochondria are very common in steroidogenic cells (Belt and Pease, 1956; Christensen and Gillim, 1969; Rhodin, 1971). The large intramitochondrial inclusions have been observed in the Leydig cells of other vertebrates (Christensen and Fawcett, 1961; Oota and Yamamoto, 1966). It has been suggested that these inclusions may represent lipids within the mitochondrial matrix (Oota and Yamamoto, 1966).

The interstitial cells are filled with the smooth endoplasmic reticulum. Smooth endoplasmic reticulum, especially when present in a tubular form, is characteristic of cells which metabolize steroids (Christensen and Gillim, 1969; Fawcett *et al.*, 1969).

The absence of positive Sudan B staining in the Leydig cells of the spottybelly greenling is easily understood from the findings of this study. Electron microscopy reveals an absence of lipid concentrations.

No lipid droplets were observed. Similarly, fine structural studies of testicular steroidogenic cells of the guppy (Follenius and Porte, 1960) and of the trout (Oota and Yamamoto, 1966) have revealed a lack of lipid droplets. Follenius (1968) saw lipid droplets only in degenerating Leydig cells of *Gasterosteus aculeatus*. This phenomenon coincides with the result in the present study.

Acknowledgement

The authors are grateful to Mr. Ye-Gyu Lee of the Electron Microscope Laboratory, Korea University, for his assistance with the transmission electron microscopy. Thanks are due to Mr. Sung-Yeon Kim of Department of Marine Biology, National Fisheries University of Pusan, for assistance in preparation of the manuscript.

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The Cyst cell and the Interstitial Cell in the Testis of a Teleost *Hexagrammos agrammus*

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生殖周期에 따른 노래미, *Hexagrammos agrammus* 精巢內的 cyst 細胞
및 間質細胞의 構造的, 組織化學的 變化

鄭 義 泳 · 李 基 永

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1992년 1월 부터 12월 까지 노래미, *Hexagrammos agrammus*의 生殖周期에 따른 精巢內的 cyst 細胞와 間質細胞의 微細構造的 變化를 알기위해 전자현미경 및 광학현미경적 조사를 하였다.

成熟한 精巢內的 cyst 細胞들은 haematoxylin에 미약하게 염색되나 세포의 크기는 커진다. 이때 이들 세포는 여러개의 미토콘드리아와 소포체, 글리코겐 입자들 및 소수의 脂肪滴들이 cyst 세포의 세포질내에 나타나고 있어, 成長期의 cyst 세포들 보다 기능이 더 활발하게 보여 本種의 cyst 세포는 영양공급, 분비 및 스테로이드 合成機能이 큰것으로 추정된다.

成熟 및 産卵期에 잘 발달된 間質細胞들은 관상의 크리스테를 갖는 杆狀 또는 球狀의 미토콘드리아들과 다수의 활면소포체들, 그리고 小胞內的 미확인된 전자밀도 물질을 함유하고 있다. 따라서 本種의 間質細胞들에는 胞狀의 핵과 관상의 크리스테를 갖는 미토콘드리아와 활면소포체가 나타나고 있어 steroid 間質細胞의 특징을 보이고 있으나, 間質細胞들의 Sudan black B에 대한 조직화학적 반응은 음성반응을 나타낸다.