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The Ultrastructure of Testis and Spermatogenesis in Bluespotted Mud Hopper(*Boleophthalmus pectinirostris*)

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짱뚱어, *Boleophthalmus pectinirostris* 정소의 미세구조 및 정자형성

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ABSTRACT : The present study observed the ultrastructure of testis of bluespotted mud hopper(*Boleophthalmus pectinirostris*), and spermatogenesis was discussed also. The testis was surrounded by a thin adventitia, inside which spermatocyst composed the parenchyma of testis. Each lobule was enwrapped by many spermatocysts, which were filled with different kinds of spermatogenic cell clusters at the same developmental stage. In the lobule lumen there are large numbers of spermatozoa. The thin adventitia(outer wall) of testis was composed of outer epithelium, and the underlying layers, such as collagen fiber layer, and myoid tissue. The myoid tissue elongated into the inside of testis, became the main componentof interstitium between spermatocyst, where spermatogenesis occurred. In addition, interstitial cells containing dense homogeneous nucleus and abundant mitochondria were observed. Spermatogonia contained round nucleus with diffuse chromatin and nucleolus, and dense nuclear bodies surround by mitochondria in cytoplasm. The synaptonemal complex was observed in primary spermatocytes clearly. Early spermatid presented larger round nucleus composed of granular chromatin, which was located in the center of cytoplasm. The nucleus of mid-spermatid composed of finely granular chromatin lied on one side of spermatid, and abundant mitochondria had migrated another side. A nuclear fossa appeared in the site near mitochondria in late-spermatid, and the centriole was formed in nuclear fossa.

Key words : *Boleophthalmus pectinirostris*, Spermatogenesis, Testis, Ultrastructure.

요 약 : 짱뚱어 정자의 미세구조 및 정자형성과정에 관한 연구를 하였다. 정소는 정자낭으로 구성되어 있으며 얇은 막으로 둘러싸여 있었다. 정자낭은 여러 발생단계의 정자들을 포함하고 있으며, 정자낭의 내강에는 다수의 정자들이 위치하고 있었다. 정소의 외막은 상피층, 콜라겐층, 근양체(myoid tissue)등으로 구성되어 있었다. 근양체는 정소 안쪽까지 연결되어서 정자낭 사이의 간질조직의 주요 구성체였다. 게다가 핵과 다수의 미코톤드리아를 포함한 간세포(interstitial)도 관찰되었다. Synaptonemal complex가 1차 정모세포에서 확인되었다. 초기 정세포에서 과립상의 염색질로 구성된 구형의 핵이 관찰되었다. 치밀한 과립상의 염색질로 구성된 중기 정세포의 핵이 정세포의 한쪽에 자리잡고 있었고, 미코톤드리아가 다른 한쪽에 자리잡고 있었다. nuclear fossa가 후기 정세포의 미토콘드리아의 근처에서 관찰되었다.

INTRODUCTION

Bluespotted mud hopper(*Boleophthalmus pectinirostris*) is subjected to *Perciformes*, *Gobiidae*, an intertidal and amphibious air-breather that actively shuttles back and forth between tide-pools and air(Martin and Bridges, 1999), and mainly distributes in China, Korean Peninsula, Japan etc. It creeps around and browses benthic diatom on mud flats at low tide, breathing through skin, tail fin and gill cavity, and stays in a burrow in the mud at high tide(Masuda et al., 1984). The flesh of this fish has the premium quality, contains sixteen amino acids, eight of which are essential amino acid(Wang and Su, 1994), and can be used in Chinese medicine(Tang, 1987). In addition, bluespotted mud hopper has strong adaptability to changes in temperature and salinity, short food chain and low trophic level, and is easy to culture and long-distance transportation, which all make this

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fish an ideal species for aquaculture(Zhang and Hong, 2003).

The aquaculture of bluespotted mud hopper has been carried out since 1960's in Taiwan, and many authors have investigated the basic and reproductive biology of bluespotted mud hopper. The annual histological changes of ovaries, and microscopic structure and ultra-structure of oocyte development were observed by Cai(1994) and Ni et al.(1995). Chung et al.(1991) also investigated the sexual maturation histologically on the gonadal development, and by gonadosomatic index(GSI) and egg diameter composition. Cai(1996) studied the population structure and reproductive characteristics, and Ryu et al.(1995) investigated its ecology and life history. Regarding to the artificial production of bluespotted mud hopper seedling, the studies on the artificial reproduction, embryonicdevelopment, and larval feeding and growth, have been investigated by Zhang et al.(1987 and 1989). Hong et al.(2000, 2001) optimized the hatching techniques of fertilized eggs, and successfully induced the ovulation. Zhao et al.(2002) investigated the effect of pheromones on maturation and spawning. However, the ultrastructure of testis and spermatogenesis has not been reported. The present study observed the ultrastructure of testis of bluespotted mud hopper, and spermatogenesis was discussed also.

MATERIALS AND METHODS

1. Fish

Adult bluespotted mud hoppers were collected from a fish market in Yosu city during reproductive season.

2. Histology and cytology of testis

The testes were obtained through dissection and fixed in Bouin's solution for histology and in 2.5% glutaraldehyde for cytology, respectively. After 24h the samples for histology were processed for routine histological examination using paraffin-embedded procedures. The microstructure of testes was observed and light micrographs were made using the Olympus CX41 microscope. The samples for cytology were post-fixed in 1% OsO4 for 1h, embedded in Spurr resin. Ultrathin sections were contrasted with alcoholic uranyl acetate and lead citrate, and examined with a JEM 1200 EX-?transmission electron microscope.

RESULTS

1. Histology

The testis of bluespotted mud hopper was surrounded by a thin out of wall, inside of which spermatocysts composed the parenchyma of testis(Fig. 1A). The interstitial cells with acidophilic cytoplasm were observed between lobules(Fig. 1B). Each lobule was enwrapped by many spermatocysts, which were filled with difference kinds of spermatogenic cell clusters at the same developmental stage. The different spermatogenic cells including spermatogonium, spermatocyte, and spermatid, could be distinguished in terms of the cell size, cytoplasm to nuclear volumetric ratio and the basophilicdegree under a light microscope. In the lobule lumen there are large numbers of spermatozoa(Fig. 1B-D).

2. Cytology

The thin outer wall of testis was composed of outer epithelium, and the underlying layers, such as collagen fiber layer, and myoid tissue(Fig. 2A). The erose epithelia surrounded the thick collagen fiber lays. The underlying myoid tissue consisted of many myoid cells, which were filled with abundant myoid

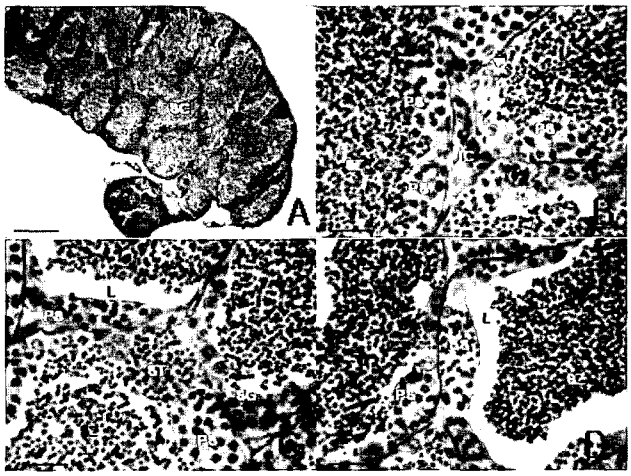

Fig. 1. Light micrographs of H&E stained *B. pectinirostris* tissue sections of the testis. A: The parenchyma of testis composed of spermatocysts. B-D: Higher modification of Fig. 1A. IC; interstitial cell, L; lumen of spermatocyst, PS; primary spermatocyte, SC; spermatocyst, SG; spermatogonium, SS; secondary spermatocyte, ST; spermatid, SZ; spermatozoa, An arrow indicates a spermatocyte undergoing meiosis. Bar=50 μm(A), 5 μm(B-D).

fibers(Fig. 2A, 3A). The nucleus of myoid cell was homogeneous, but there was difference in the electronic density between myoid cells, while density of nucleus was accordant with cytoplasm in the same cell(Fig. 3A). The myoid tissue elongated into the inside of testis, became the main component of interstitium between spermatocyst, where spermatogenesis occurred(Fig. 2A). The term "interstitium"was used in this study to designate structures and substances that were in the spaces between spermatocysts and separated from them by the basal membrane of interstitial cells. In addition, interstitial cells containing dense homogeneous nucleus and abundant mitochondria were observed in interstitium(Fig. 2B, Fig. 3A, B).

Spermatocysts were filled with difference kinds of spermatogenic cell clusters at the same developmental stage along the basal membrane of interstitial cells. The basal membrane was dense, and often extended into the spermatocyst and wrapped spermatogonia(Fig. 2B, C). Spermatogonia contained round nucleus with diffuse chromatin and nucleolus, and dense nuclear bodies surround by mitochondria in cytoplasm(Fig. 2B-D). Spermatocytes were obviously smaller than spermatogonia, and the synaptonemal complex was clearly observed in primary spermatocytes(Fig. 3C). Spermatid could be divided into three stages obviously. Early spermatid presented larger round nucleus composed of granular chromatin, which was located in the center of cytoplasm(Fig. 3D). The nucleus of mid-spermatid composed of finely granular chromatin lied on one side of spermatid, and abundant mitochondria had migrated another side(Fig. 4A). A nuclear fossa appeared in the site near mitochondria in late-spermatid, and the centriole was formed in nuclear fossa(Fig. 4A).

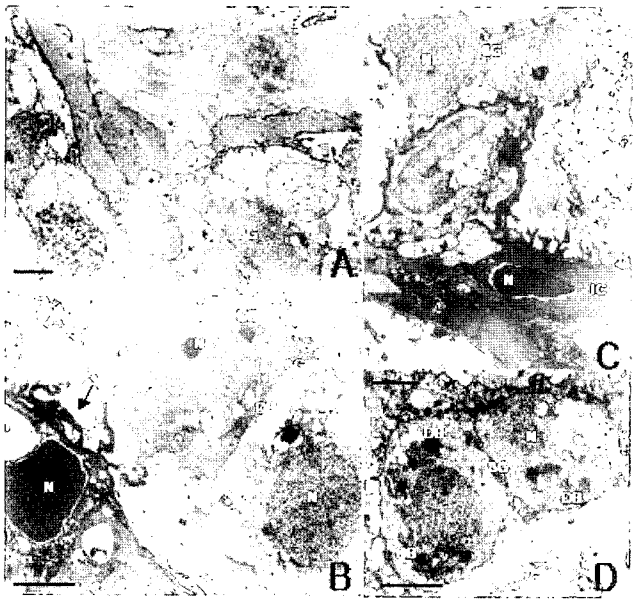

Fig. 2. The ultrastructure of testis of *B. pectinirostris*. A: The outer wall of testis composed of outer epithelium, and the underlying layers, myoid tissue, and collagen fibers. Two spermatocysts containing spermatogonia and spermatocytes were separated by the extended myoid tissue. B: Spermatogonia containing round nucleus with diffuse chromatin and nucleolus, and dense nuclear bodies surround by mitochondria. An interstitial cell containing dense nucleus and mitochondria and its basal membrane(arrow) was closed to it. C: Basal membrane(arrow) extended into the spermatocyst and wrapped spermatogonia. D: Two spermatogonia containing many dense nuclear bodies. DB; dense nuclear body, E; epithelium, F; collagen fibers, I; interstitial cell, M; mitochondria, MT; myoid tissue, N; nucleus, NS; nucleolus, S; spermatocyte, SC; spermatogonium. Bar=2 μm.

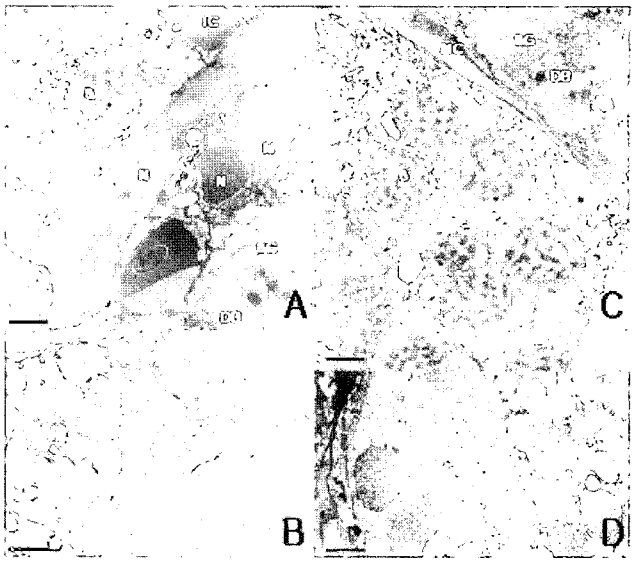

Fig. 3. Ultrastructure of testis of *B. pectinirostris*. A: Interstitium was composed of myoid cells, and interstitial cell containing dense nucleus and abundant mitochondria. There was difference in the electronic density between myoid cells. Two spermatocysts containing spermatogonia with dense nuclear bodies and spermatocytes were separated by interstitium. Bar=2 μm. B: Magnification of two interstitial cells showed abundant mitochondria. An arrow indicates the plasmatic membrane. Bar=0.5 μm. C: Primary spermatocytes containing synaptonemal complex. Bar=2 μm. D: Early spermatid containing granular chromatin. Bar=2 μm. DB; dense nuclear body, IC; interstitial cell, M; mitochondria, MC; myoid cell, N; nucleus, S; spermatocyte, SG; spermatogonium.

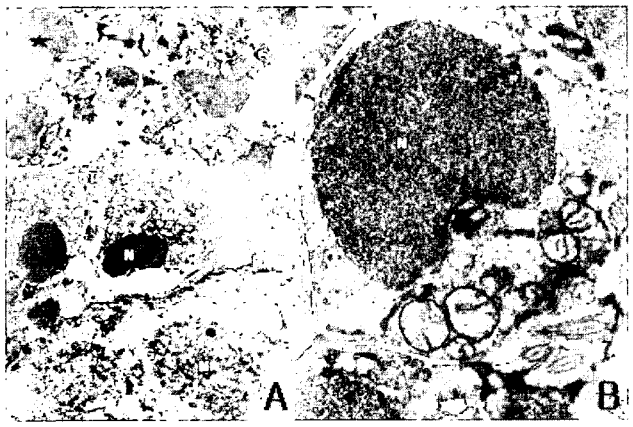

Fig. 4. The ultrastructure of testis of *B. pectinirostris*. A: Mid-spermatid containing finely granular chromatin and abundant mitochondria, late-spermatid with a nuclear fossa housing centriole(asterisk), and spermatogonia in the same spermatocyst. Bar=2 μm. B: Magnification of late-spermatid. The mitochondria had migrated to underside of nuclear fossa. Bar=0.5 μm. C; centriole, M; mitochondria, N; nucleus, SG; spermatogonium, ST; spermatid.

DISCUSSION

The gonad organization and gametogenesis in teleosts have been extensively studied and summarized in several work(Grier, 1993, 2000; Colombo and Grandi, 1996; Grandi and Columbo, 1997; Nakamura et al., 1998; Grier and Lo Nostro, 2000; Koulish et al., 2003). Comparisons have shown that despite general agreement on cell form certain details of sperm andtheir organelles are specific to various taxe, and thus have been used as evolutionary markers(Mattei, 1988). Efforts have also been made to establish the correlations between type and form of fertilization, whether external or internal, and the form and dimensions of the sperm head and sperm tail. With these in mind, it was of interest to study the testis structure and spermato-genesis of *B. pectinirostris*, an intertidal and amphibious air-breather.

The outer wall consisted of epithelium, and subjacent layers that include collagen fiber layer, and myoid tissue. The subjacent layers enclosing the gonad appear to be analogous with the tunica albuginea, a compact collagenous connective tissue layer enclosing the testes in mammals(Greep and Weiss, 1973). The myoid layer not only surrounded the testis under collagen fiber, but also elongated into the inside of testis, became the main component of interstitium between spermatocyst. The wide distribution of myoid cells suggests that a contractile mechanism extends throughout the testis, which probably aids the sperm in moving from cysts to collecting ducts(Koulish et al., 2003).

The organization of spermatogenic cells suggested that the testis of bluespotted mud hopper belonged to lobular type. Prior to spermatocyst formation the spermatogonia are closely associated with Sertoli cells in a tissue that rests on a basement membrane that delineates the lobule. The lobule opens into a lumen. This kind of organization has been termed as the germinal epithelium and fulfills the textbook definition of an epithelium(Grier, 2000; Grier and LoNostro, 2000). In *B. pectinirostris*, however, there was no enough morphological evidence to indicate the pre- sence of Sertoli cells. Spermatogonia often were wrapped with the basal membrane of interstitial cells and myoid cells.

The interstitial cell of *B. pectinirostris* was characterized by dense homogeneousnucleus and abundant mitochondria. There was some difference between these cells and Leydig cells described in other teleost fishes, i.e., rich in anastomizing SER and have numerous vesicles, except the presence of abundant mitochondria. More researchshould be performed to illustrate the function of interstitial cell, for example, whether they can produce steroid.

In spermatogonium, dense nuclear bodies surrounded by mitochondria were observed. It was found that these bodies appeared almost concomitantly as precursors of the nucleolus and crossed the nuclear membrane pores in the filamentous form of small, 18S rRNA subunits(Fishelson, 2003). They would condense and become surrounded by mitochondria that might presumably serve as energy depots for RNA activities. Beginning with spermatid formation, they were no longer visible.

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