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Comparative Ultrastructure on Spermatogenesis of Diploidand and Triploid in Mud Loach, *Misgurnus mizolepis*

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2배체와 3배체 미꾸라지(*Misgurnus mizolepis*) 수컷의 정자형성과정에 따른 미세구조 비교

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ABSTRACT

Ultrahistology of spermatogenic cells on spermatogenesis were analyzed from triploid males of the mud loach, *Misgurnus mizolepis*. All the testis of triploid males were smaller in thickness and shorter in length than those of diploid males, but the testes developmental stages in triploid males were very similar to those of diploid males. And cytological characteristics were also almost identical to each other. Also Sertoli cells with high activity were recognized at intralobuli of the testis in triploid males during the period of spermiogenesis. And then a few matured spermatozoa were observed in testis of triploid, and interstitial cells also appeared high active in interlobuli. But nucleus sizes of spermatogenic cells of triploid male according to developmental stages were larger than those of diploid overall. Especially, spermatozoa of triploid showed abnormal morphology such as two or more tail flagella, significantly larger head sizes, nucleus size, and diameter of axial filaments etc. than those from diploid.

Keywords : Mud loach, Triploid, Spermatogenesis, Ultrastructure, Sterility

INTRODUCTION

With recent development of transgenic technique, this technique was using for enhanced agriculture productivity in crops (Clive, 2003). Also, this technique was also beginning to using for enhanced aquaculture productivity in fishes and shellfishes (Rasmussen & Morrissey, 2007). Especially, Food Drug Administration of America has been reviewed for the past 10 years, whether to use food as living modified (LM) atlantic salmon with fast growing. Most of experts expect that this fish will firstly serve at table in near future. On the other hand, GM fluorescence aquarium fish has been already sold some countries such as America, Taiwan, Singapore etc. (Gong et al., 2003).

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Transgenic fish has properties such as increased growth rates, feed conversion efficiency, disease resistance, cold tolerance, and improved metabolism of land-based plants. However, use of transgenic organisms in aquaculture is a very controversial topic due to a number of environmental and human health concerns such as escapement and introduction of LM fishes into the food chain (Rasmussen & Morrissey, 2007). Accordingly, a controversy on biodiversity and food safety concern of LM fishes has being continued (Isaeva & Morosov-Leonov, 2006). In response, some transgenic research has also been focused on including sterility to reduce the risk of transgenic organisms breeding with wild species. A method of chromosome manipulation, referred to as polyploidy, provides the option of creating sterile organisms (Rasmussen & Morrissey, 2007). One of promising technique to induce sterile in fish and shellfish is induction of triploid. Triploid males were almost sterile. Accordingly, this technique can be potentially used as a mean for biological confinement of transgenic fish in recent years.

Actually, studies on artificial triploid induction in fish have been started to improve generally their productivity with turning reproductive energy to body growth energy through sterilization (Isaeva & Morosov-Leonov, 2006). Gonadal characteristics of triploid male has been reported for a number of fishes such as three spined stickleback (Swarup, 1959), hybrid triploid of olive flounder × halibut (Lincoln, 1981), Rainbow trout (Lincoln & Scott, 1984), Grass carp (Allen & Wattendorf, 1987), and Mud loach (Kim et al., 1994, 1995), loach (Arias-Rodriguez et al., 2010) etc. Although a lot of studies on spermatogenesis of the male triploid were reported, a few reports on sperm production of triploid male fish and shellfish (Gui et al., 1991, 1992; Komaru et al., 1994; Rosalio & Ibarra, 2002; Arias-Rodriguez et al., 2010). To control mating between LM fish and wild fish in the aquatic environment, studies on gonadal characteristics of triploid is more needed. Therefore, we reports here the testicular development and ultrahistological characteristics according to spermatogenesis of triploid male mud loach for providing basic information with related to environmental risk assessment on the transgenic fishes releasing to environment.

MATERIALS AND METHODS

Triploid mud loach *Misgurnus mizolepis* was produced according to the procedures described by Kim et al. (1995). Triploid mud loach have been cultured in indoor circulated culture system up to the nine months of age (11 cm in total length) and mature triploid males were kept about 4 years old.

To analysis ultrahistology of triploid male, ten specimens were collected at an interval of five days from 10 days after induced triploid to nine months after hatching (March to December, 1996) in triploid and diploid *M. mizolepis* respectively. Gonad samples were pre-fixed with a 2.5% glutaraldehyde solution in 0.1 M phosphate buffer, pH 7.2, for two to four hours at 4˚C. The pieces were then washed in phosphate buffer solution three times for 10 minutes, and post-fixed in 1% osmium tetroxide (O_sO_4) in the same buffer for two hours at 4˚C. Samples were then dehydrated in an ethanol series for 20 minutes in each concentration, and twice in 100% propylene oxide for 20 minutes. They were embedded in Epon 812 mixture according to the protocol. Section of 60 ~ 70 nm were obtained with an ultra microtome (UKB, USA), mounted on a 200 mesh copper grid and stained 2% uranyl acetate for 20 minutes and lead citrate for five minutes. Photo microscopy was done using a JEOL JEM 1200Ex II TEM.

Diameters of germ cells and nuclei according to spermatogenic stages were measured by photographic prints of TEM and high power LM. The data were analyzed using student t-test. All data obtained from triploid males were compared with data from diploid mud loach.

RESULTS

1. Diagrammatic sketch of the Gonad

Testis structures of the triploid mud loach, *Misgurnus mizolepis* at nine-month-old were presented in Fig. 1. Mature testis of diploid showed a pair of rod shape in external shape (Fig. 1A), On the other hand, testis of triploid was rather thinner and smaller to that from diploid (Fig. 1B).

2. Cytological characteristics of germ cell in triploid male

Cytological characteristics of germ cell were investigated in triploid male according to testicular developmental stage using Transmission Electron Microscopy (Fig. 2). All their characteristics were compared to those of diploid mud loach.

1) Spermatogonial cell

Diploid : The testes were firstly recognizable from mud loach of 24.3 mm in total length (25 days after hatching). At that time, the testes showed as a pair of cyst filled mesenchymal tissue, each cyst was composed of primordial germ cells and two to

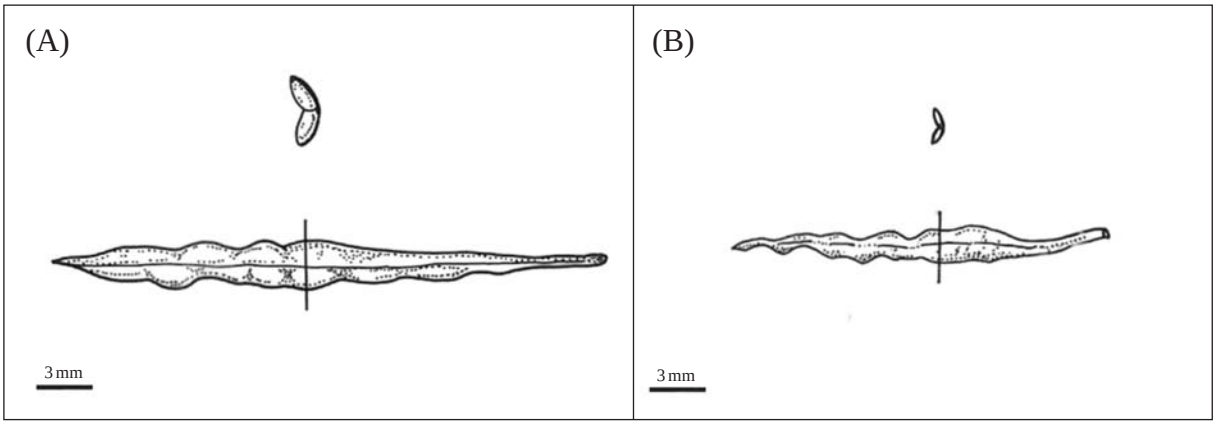


Fig. 1. Diagrammatic sketch of external feature of mature testis in diploid (A) and triploid (B) males mud loach, *Misgurnus mizolepis*.

three spermatogonium. As primitive gonad was distinguished to testis, spermatogonium was arranged condense each other in the cyst (named to cluster by LM) located among the testicular lobule. The nucleus of spermatogonium was spherical, it has high electronic density, and size of nucleus was about 4.40 μm in diameter. Heterochromatin with high density clearly appeared in nucleus. Although cytoplasmic content was less quantity than that of their nucleus, core substance composed of many mitochondria with cristea was formatted to mitochondrial rosset (Mr) in cytoplasm. Endoplasmic reticulum, golgi bodies, and the other cell organelles also appeared in the cytoplasm (Fig. 2A).

Triploid : The testes were firstly recognizable from mud loaches of 31.1 mm in total length (30 days after hatching). All testes of triploids were recognized later in age than in diploid. Relatively larger spermatogonium compared to diploid was multiplicated in the cyst. As seen in diploid, mitichondrial rossets in spermatogonium were located in cytoplasm near the nuclear envelope, and there were much and bigger than that from diploid. At that time, size of nucleus was about 5.48 μm in diameter. Nevertheless, histological characteristics were very similar to that of diploid (Fig. 2B).

2) The primary spermatocytes stage

Diploid : As testis was gradually increased in volume, a pair of cyst consist of several spermatogonium were developed to the testicular lobuli. At that time, age of male fry was about 30 days after hatching. Many primary spermatocytes appeared in the cyst of the testicular lobules. As the testis advanced, primary spermatocytes on the germinal epithelium become relatively smaller in size and more condenser than spermatogonia. Meanwhile, heterochromatin with high density clearly appeared in nucleus. Also many of mitochondrial rossets were recognized in the cytoplasm of spermatocyte as seen in the spermatogonia. Size of nucleus of primary spermatocyte was about 3.88 μm in diameter (Fig. 2C).

Triploid : The spermatogonia in cyst developed into the spermatocytes in testis of fry. At that time, age of male fry was about 50 days after hatching. As early cyst turned to lobule, lobule began to be filled with connective tissues and somatic cells. Size of primary spermatocyte was larger than spermatogonium of the triploid and spermatocyte of the diploid. So a cytoplasm of primary spermatocyte had some mitochondrial rossets, but their cell membrane is not clear and irregular compared to that of diploid. A nucleus of primary spermatocyte with a plentiful heterochromatin also had a larger than that of diploid as about 5.35 μm in diameter. Nevertheless, histological characteristics were very similar to that of diploid (Fig. 2D).

3) The secondary spermatocytes

Diploid : When primary spermatocytes developed into secondary spermatocytes throughout the second maturation division in the cyst, cell size of secondary spermatocytes was rather smaller than that of primary spermatocytes. Size of nucleus of the secondary spermatocyte was about 3.58 μm in diameter. A chromatin with electronic high density in nuclear was firstly recognized to show mitotic characteristics as appears in somatic cell division. The secondary spermatocytes had many vacuoles and several mitochondria in the cytoplasm (Fig. 2E).

Triploid : Size of the secondary spermatocytes was larger than that of diploid, but it was smaller in diameter of cells than those of the primary spermatocytes in triploid. Size of nucleus of the secondary spermatocyte was about 4.30 μm in diameter. However, spermatogenic stage in triploid were very similar to that

of diploid, and its cytological characteristics was almost identical to that of diploid (Fig. 2F).

4) Spermatid stage

Diploid : As the further development of testis advanced, spermatogonia and spermatocytes in the testis of immature mud loach were developed into a variety stage of the germ cells, including spermatogonia, spermatocytes, and spermatids. At that time, age of immature male was about 50 days after hatching. Nuclear of the spermatid which followed the secondary spermatocyte by secondary maturation division, appeared to be spherical shape with high electronic density. Size of nucleus of spermatid was about 1.71 μm in diameter. At that time, several mitochondria and all vacuoles in cytoplasm moved to posterior portion of nuclear. Also, axial filament was firstly appeared at centriole throughout cytoplasmic canal (Fig. 2G). During spermatids progressed spermiogenesis, Sertoli cell in the cyst has showed very highly activities: low electron dense chromatin in the nucleus, and several mitochondria rossets, endoplasmic reticular, the large amount of glycogen particles in the cytoplasm (Fig. 2I).

Triploid : Gonadal development process of the triploid was rather retarded than those of diploid. But some spermatids could be appeared in frontier portion of testis from 70 days after hatching. At that time, testis of diploid mostly developed into the spermatids. Spermatids in triploid male had much quantity cytoplasm than those of diploid, but it were similar to that of diploid with condensed nuclearplasm throughout spermiogenesis. Size of nucleus was about 3.50 μm in diameter (Fig. 2H). The spermatids of triploid attached to active Sertoli cells as seen in the cyst of diploid Sertoli cell within cyst of triploid showed almost identical characteristics as seen in diploid with relativity larger nuclear, several mitochondria rossets, endoplasmic reticular, lipid droplet, and the large amount of glycogen particles in the cytoplasm (Fig. 2J).

5) Spermatozoon structure

Diploid : At eighth days after hatching, spermatids in testis of diploid mostly developed into spermatozoa. At that time, spermatozoa were very sharply modified by spermiogenesis. Spermatids were also distinguished from spermatocytes. But, mainly spermatozoa stage was started from 120 days after hatching of diploid male. When spermatids developed into mature spermatozoa which followed spermiogenesis, the matured spermatozoa were filled up within the cyst. The spermatozoa which consisted of head, midpiece, and tail flagellum was seen in Fig. 2K and Fig. 3. A head of the spermatozoon was rounded

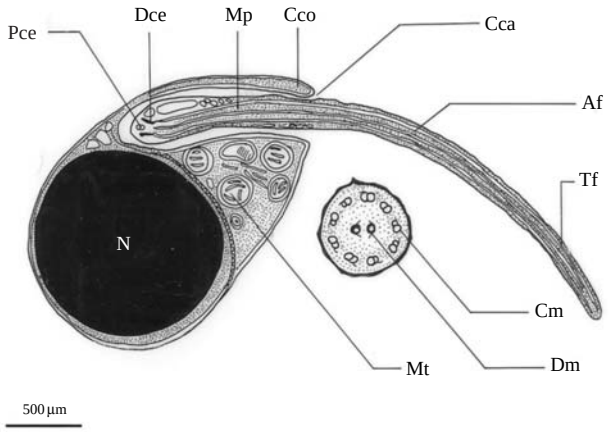


Fig. 3. Diagrammatic sketch of a mature sperm in mud loach, *Misgurnus mizolepis*. Abbrevations: Af, axonemal flagellum; Cca, cytoplasmic cannal; Cco, cytoplasmic collar; Cm, central microtubule; Dce, distal centriole; Dm, doublet microtubule; Mp, midpiece; Mt, mitochondria; N, nucleus; Pce, proximal centriole; Tf, tail flagellum.

in shape with about 1.51 μm in diameter. Also, a head of the spermatozoon had condensed nucleoplasm, and contain dense electronic density. No acrosome could be found, a number of mitochondria were located in posterior cytoplasm of the head, and then it was formed paranucleus. Also proximal centriole appeared in this area; axial filament was derived from distal centriole located near proximal centriole throughout cytoplasmic collar. Midpiece of spermatozoon was covered with axoneme surrounded by seven outer densefiber (Fig. 2K), tail flagellum consisted of nine pairs of central microtubules at the periphery and one pair of central microtubule at the center, and each doublet had two dynein arms connected with each doublet. And then, axoneme of spermatozoon has showed a simple 9+2 arrangement and had not any lateral axonemal fins (Fig. 2M). Therefore, spermatozoa of mud loach were aquasperm of uniflagellum anacrosomal type.

Triploid : When triploid male growing up to 150 days after hatching, although a few spermatozoa were observed after spermiogenesis, spermatozoa reached the mature stage. Interstitial cells and cyst cells in intralobuli also appeared to be active. But, mainly spermatozoa stage was started from 210 days after hatching. Spermatozoa through spermiogenesis were observed in lobule of testis as seen in diploid (Fig. 2L). A head of the spermatozoon has condensed nucleoplasm as seen in diploid. As a size of head was about 2.35 μm in diameter, a head of triploid spermatozoa larger than that of diploid counterpart, its distribution was relatively various. But, axoneme of spermatozoon has showed a simple 9+2 arrangement and had not any lateral axonemal fins as seen in diploid. Nevertheless, sometimes we

Table 1. Comparisons of each elements values of the spermatozoon between diploid and triploid of mud loach, *M. mizolepis*

Ploidy	Cytoplasmic collar length (μm)	Cytoplasmic cannal width (μm)	Tail flagellum Length (μm)	Tail flagellum Diameter (μm)
Diploid				
Min.	1.50	0.48	10.70	0.13
Max	2.00	0.50	11.20	0.15
Mean±S.D.	1.71±0.17	0.49±0.01	11.06±0.20	0.14±0.01
Triploid				
Min.	3.45	0.85	10.9	0.19
Max	3.54	0.93	11.4	0.21
Mean±S.D.	3.50±0.04*	0.89±0.03*	11.10±0.18	0.20±0.01*

*$P < 0.01$

can find out a few abnormal spermatozoons with two or more tail flagella (Fig. 2N).

3. Size of elements consist of spermatozoon

Sizes of each elements of spermatozoon were showed in Table 1.

Diploid : Cytoplasmic collar was about 1.71±0.17 μm in length and cytoplasmic canal was about 0.49±0.01 μm in width. Tail flagellum was about 11.06±0.20 μm, 0.14±0.01 μm in length and diameter respectively.

Triploid : Cytoplasmic collar was about 3.50±0.04 μm in length and cytoplasmic cannal was about 0.89±0.03 μm in width. Tail flagellum was about 11.06±0.18 μm, 0.20±0.01 μm in length and diameter respectively. As seen this results, cytoplasmic collar's length and width and tail flagellum's diameter of triploid spermatozoon were almost two times larger or thicker than those of diploid. But tail flagellum's length of triploid was same that of the diploid.

DISCUSSION

A promising biotechnological tool for increased production of food from aquaculture and creation of sterile organisms is polyploidy. Polyploidy refers to a genetic state that can be produced artificially in fish and shellfish through manipulation of embryos. Polyploid individuals have extra sets of chromosomes beyond the normal 2, with triploids having 3 and tetraploids having 4. There were reports on gonadal development of triploid males for a number of fishes such as three spined stickleback (Swarup, 1959), hybrid triploid of olive flounder × halibut (Lincoln, 1981), Rainbow trout (Lincoln & Scott, 1984), Grass carp (Allen & Wattendorf, 1987), and Mud loach (Kim et al., 1994, 1995) etc. We could find out spermatogenesis of triploid males in mud loach, *Misgurnus mizolepis*.

Triploid fish and shellfish are viable and tend to be sterile due to a lack of gonadal development (Rasmussen & Morrissey, 2007). So, testis of triploid males was smaller than that of diploid counterpart in size, but external shape of testis had very similarity between diploid and triploid (Gui et al., 1991). In our study, all the testes of triploid male were smaller in thickness and shorter in length than those from diploid males.

From the point of view of gonadal development, the testis of diploid males in mud loach was firstly recognized at twenty-five days after hatching. On the other hand, testis of triploid was firstly recognized at about thirty days after hatching later which was rather than that of diploid. While the spermatid stage of testis in diploid males reached at about fiftieth days after hatching, the spermatid stage of triploid was recognized about seventh days after hatching, which had much quantity cytoplasm. While the spermatozoa of diploid males showed at about eightieth days after hatching, spermatozoa of triploid males were showed at 150 days after hatching in their testis at last. So the testicular developmental stage of triploid male was delayed to compare to diploid. I think that is because replication, recombination, and segregation would not be effective on spermatogenesis. To the successful spermatogenesis, there were three problems in a cell; replication, recombination, and segregation (Rosalio & Ibarra, 2002). The delay in the onset of gametogenesis known to occur in triploid males can be explained by the replication mechanism as first checkpoint in a cell (Guo & Allen, 1994; Komaru et al., 1994; Ruiz-Verdugo et al., 2000).

Differences in sperm cell size between triploid and diploid fish and shellfish have been reported some papers (Komaru et al., 1994; Rosalio & Ibarra, 2002). Rosalio & Ibarra (2002) suggested that increase of cell size in triploid catarina scallop might be caused by the fact that organelles (mitochondria, endoplasmic reticulum, ribosomes) were much than in diploid. In the present

study, germ cell size of all spermatogenic stages of the triploid was shown to be larger in diameter than that of diploid.

From the cytological of view of triploid males of mud loach, mitochondrial ressetts located at the cytoplasm near to nuclear envelope of the germ cells of showed very high activity during spermatogenesis and syneptonemal complex found in secondary spermatocyte also was prominent as seen in diploid counterpart. During spermatids did progressive spermiogenesis, Sertoli cell in the cyst of testis of male triploid showed very high activities. Sertoli cells had chromatin with low electron dense in the nucleus, and several mitochondria rossets, endoplasmic reticular, and the large amount of glycogen particles in the cytoplasm as seen in diploid counterpart. Gresik et al. (1973) suggested that Sertoli cells had several functions; nutrition, phagocytosis, and steroidogenesis. In our study, Sertoli cells in triploid male seem to be implicated in the nutrition supply to spermatids and to spermiogenesis as seen in diploid counterpart.

Generally, the structure of spermatozoon was very similar in fishes of same family and genus (Jamison, 1991). The spermatozoa of triploid mud loach were morphologically similar to that of diploid, although it showed a larger head. Head of the spermatozoa was rounded in shape, and had condensed nucleoplasm with dense electronic density. No acrosome could be found, a number of mitochondria was located in posterior cytoplasm of the head. In spite of the size increase, the spermatozoa of triploid mud loach had the same number of cross-sectioned mitochondria in diploid. Proximal centriole appeared in posterior region of the head, and axial filament was derived from distal centriole located near proximal centriole through cytoplasmic collar. The tail flagellum consisted of nine pairs of central microtubules at the periphery and one pair of central microtubule at the center, and each doublet had two dynein arms connected with each doublet. Tail structure (axoneme) of spermatozoon in diploid mud loach has showed a simple 9+2 arrangement and had not any lateral axonemal fins. But spermatozoa of triploid males showed abnormal morphology such as two or more tail flagella, significantly larger head nucleus size, and diameter of axial filaments etc. than those from diploid. These characteristics also appeared in male triploid of grass carp (Allen et al., 1986). Allen et al. (1986) suggested that maybe they have been different in DNA content. A DNA content of spermatozoa in diploid grass carp measured by flow cytometry was all haploid. But a DNA content of spermatozoa in triploid grass carp was classified of three groups; haploid, diploid, and triploid. Benfey & Sutterlin (1986) reported that the spermatozoa in triploid rainbow trout have contained 1.5n by randomly segregation of the extra set of chromosomes. Cherfas (1986) suggested that meiosis of chromosome in triploid occurred by three spindle fiber set at each centriole in triploid gynogenesis of silver crucian carp. Yamashita (1993) reported that triploid gynogenesis of crucian carp Ginbuna had doubled to 6N from 3N after this specimen skipped the firstly maturation division. And then this specimen has matured to 3N after segregate from 6N when this specimen carries out the secondary maturation division. In our study, size of sperm head was two times larger than that of diploid counterpart, although its variation was change the width of the larger. Komaru et al. (1994) reported that there was due to increase genome size. Generally, Gui et al. (1992) also suggested that the sperm production of triploid males in fishes would be effected by the extra set of chromosomes during the maturation division. If mostly male fishes failed the maturation division, the species could produce the allosperm or might be sterile. The use of sterile triploids in aquaculture can help protect the genetic diversity of native populations and prevent establishment of populations of escapee organisms (Rasmussen & Morrissey, 2007). In our study, although a few spermatozoa were observed from male triploid mud loach at the level of ultrahistology, further research on activity and fertility of the spermatozoa of triploid males mud loach will be need more.

REFERENCES

Arias-Rodriguez L, Yasui GS, Kusuda S, Arai K: Reproductive and genetic capacity of spermatozoa of inter-populational hybrid males in the loach, *Misgurnus anguillicaudatus*. J Appl Ichthyol 26 : 653-658, 2010.

Allen SK Jr, Thiery RG, Hagstrom NT: Cytological evaluation of the likelihood that triploid grass carp will reproduce. Trans Am Fish Soc 115 : 841-848, 1986.

Allen SK Jr, Wattendorf RJ: Triploid grass carp status and management implication. Fisheries 12 : 20-24, 1987.

Benfey TJ, Sutterlin AM: Growth and gonadal development in triploid landlocked Atlantic salmon (*Salmo salar*). Can J Fish Aquat Sci 41 : 1387-1392, 1986.

Cherfas NB, Abramenko MI, Emelyanova OV, Ilyina ID, Truveller KA: Geneticpeculiarities of induced gynogenesis in the hybrids between crucian carp and common carp. Genetika (moscow) 22 : 134-139, 1986.

Clive J: Global review of commercialized transgenic crops. Current Science 84 : 303-309, 2003.

Gong Z, Wan H, Tay TL, Wang H, Chen M, Yan T: Development of transgenic fish for ornamental and bioreactor by strong expression of fluorescent proteins in the skeletal muscle. Bioch Biophys

Res Comm 308 : 58-63, 2003.

Gresik EW, Quirk JG, Hamilton JB: A fine structural and -study of the Leydig cell in the testis of the teleost. *Oryzias latipes* (Cyprinidontiformes). Gen Comp Endocrinol 20 : 86-98, 1973.

Gui JF, Xiao WH, Chen L, Liang SC, Jiang YG: Gonadal development in artificial triploid transparent colored crucian carp. Acta Zoologica Sinica 37 : 297-304, 1991.

Gui JF, Jia J, Liang SC, Jiang YG: Meiotic chromosome behavior in male triploid transparent colored crucian carp *Carassius auratus* L. J Fish Biol 41 : 317-326, 1992.

Guo X, Allen Jr SK: Reproductive potential and genetics of triploid Pacific oysters *Crassostrea gigas* (Thunberg) Biol Bull 187 : 309-318, 1994.

Isaeva NM, Morosov-Leonov SY: Transgenic fishes : modern state of the problem. Cyto Genetic 41 : 256-261, 2006.

Jamieson BGM. Fish evolution and systematic Evidence from spermatozoa: New Yolk Cambridge Univ Press. 319 pp, 1991.

Kim DS, JY Jo, Lee TY: Induction of triploidy in mud loach (*Misgurnus mizolepis*) and its effect on gonad development and growth. Aquaculture 120 : 263-270, 1994.

Kim BS, An CH, Kim DS: Histological studies on gonad and germ cell development of diploid and triploid mud loach, *Misgurnus mizolepis*. J Aquacult 8 : 327-341, 1995.

Komaru AK, Konishi K, Wada KT: Ultrastructure of spermatozoa from induced triploid Pacific oyster *Crassostrea gigas*. Aquaculture 123 : 217-222, 1994.

Lincoln RF: Sexual maturation in triploid male plaice (*Pleuronectes platessa*) and plaice x flounder (*Platichthys flesus*) hybrids. J Fish Biol 19 : 415-426, 1981.

Lincoln RF, Scott AP: Sexual maturation in triploid rainbow trout, *Salmo gairdneri* (Richardson). J Fish Biol 25 : 385-392, 1984.

Rasmussen RS, Morrissey MT: Biotechnology in aquaculture: transgenics and polyploidy. Comprehensive Reviews in Food Science and Food Safety 6 : 2-16, 2007.

Rosalio MA, Ibarra AM: Ultrastructural characteristics of spermatogenesis in diploid and triploid catarina scallop (*Argopecten ventricosus* Sowerby II, 1842). J Shellfish Research 21 : 93-102, 2002.

Ruiz-Verdugo CJ, Ramirez JL, Allen Jr SK, Ibarra AM: Triploid catarina scallop (*Argopecten ventricosus* Sowerby 2, 1842): growth gametogenesis, and suppression of functional hermaphroditism. Aquaculture 186 : 13-32, 2000.

Swarup H: Effect of triploidy on the body size, general organization and cellular structure in *Gasterosteus aculeatus* (L.). J Genet 56 : 143-155, 1959.

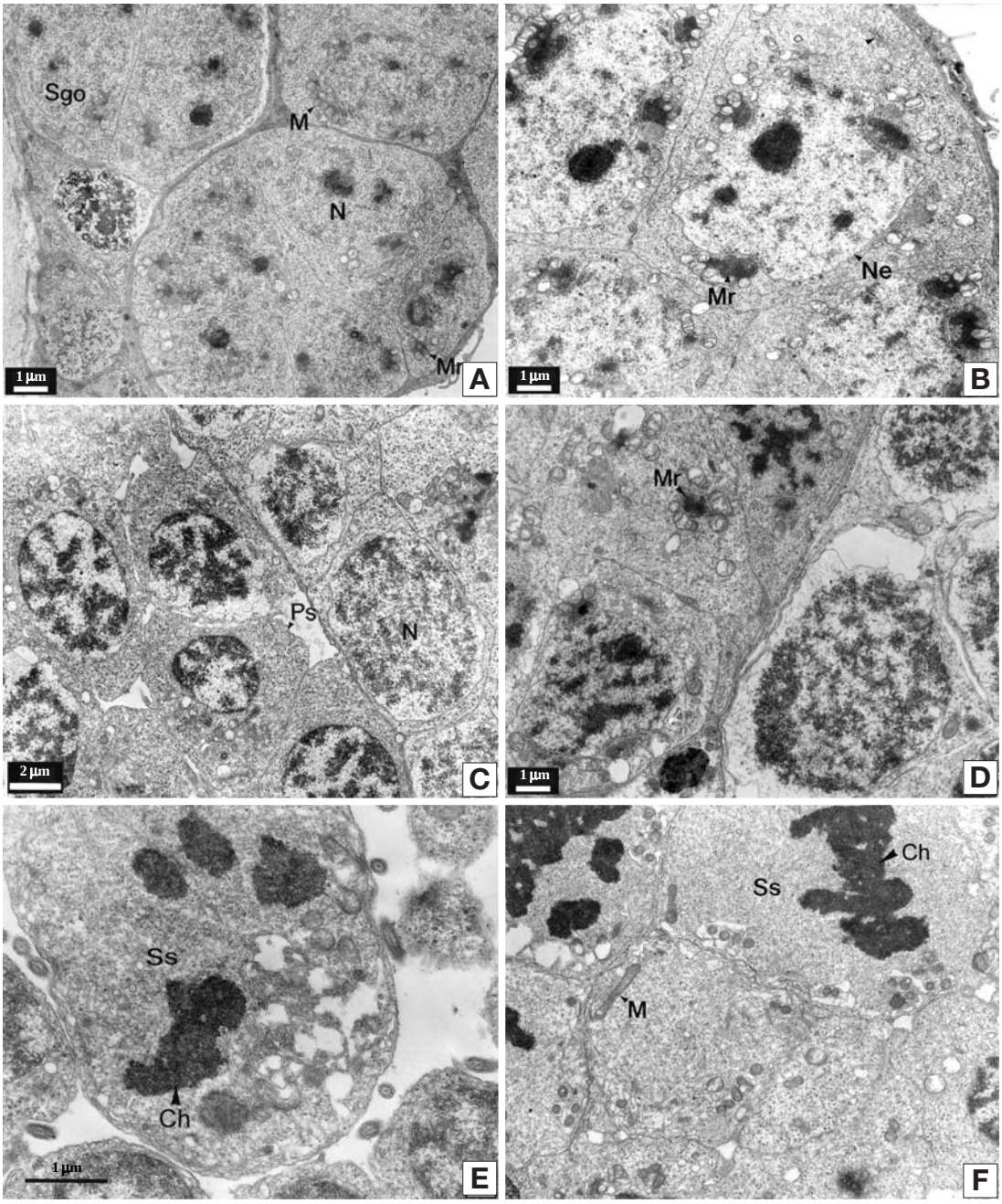
Yamashita M, Jiang J, Onozato H, Nakanishi, Nagahama Y: A triploid spindle formed at meiosis assures the retention of the original ploidy in the gynogenetic triploid crucian carp Ginbuna *Carassius auratus langsdorfii*. Devel Growth Differ 35 : 631-636, 1993.

＜국문초록＞

성숙한 2년산 미꾸라지 (*Misgurnus mizolepis*) 친어로부터 3배체를 유도하여 이들 3배체의 정자형성과정에 따른 생식세포의 미세구조를 조사하여 2배체 숫컷과 비교하였다. 3배체 숫컷의 정자형성과정과 세포학적 미세구조는 2배체와 유사한 특징을 나타내었다. 정자변태과정이 일어나는 시기에 3배체의 정소 소엽 내 Sertoli cell은 세포질 내 다수의 미토콘드리아 로제트, 소포체, 그리고 소량의 지방적 등을 함유하는 등 높은 활성을 나타내었다. 3배체 숫컷의 정소에서 일부 성숙한 정자들이 식별되었다. 변태를 완료한 3배체의 정자는 두부, 중편 그리고 미부축사로 구성되어 2배체 정자와 같은 anacrosomal aquasperm으로 나타났으나 생식세포 발달단계별 핵의 크기가 2배체보다 크고, 정자를 구성하는 cytoplasmic cannal의 직경이 크며 cytoplasmic collar의 길이가 길고 미부축사의 직경이 굵고 두 개 이상의 미부축사를 가지는 등 비정상적인 구조를 나타내었다.

FIGURE LEGENDS

Fig. 2. Electron micrographs of spermatogenesis in male diploid and triploid mud loach, *Misgurnus mizolepis*. A, Spermatogonia were present in the cyst, with a large nucleus with heterochromatin and the mitochondrial rossets, and endoplasmic reticulum in the cytoplasm of spermatogonia in diploid testis. B, Relatively larger spermatogonia in triploid testis than those of diploid are shown in the cyst, several mitochondrial rossets near the nuclear envelop; C, Many primary spermatocytes are existed in the cyst of the testicular lobules in diploid, with a large nucleus with heterochromatin and somewhat mitochondrial rossets in the cytoplasm; D, The primary spermatocytes are presented in the testicular lobules in triploid testis, with relativily larger primary spermatocyte than those as seen in diploid, and a large nucleus with heterochromatin and somewhat mitochondrial rossets in the cytoplasm; E, The secondary spermatocytes during the second maturation division in the cyst of diploid testis, with electron dense chromatin in the nucleus vacuoles and mitochodria in the cytoplasm; F, The secondary spermatocytes during the second maturation division in the cyst of triploid testis, with electron dense chromatin in the nucleus and sperical or rod spaped mitochodria with tubular cristae. Abbrevations: Ch, chromosome; M, mitochondria; Mr, mitochondrial rossets; N, nucleus; Ne, nuclear envelop; Ps, primary spermatocyte; Sco, synaptonemal complex; Sgo, spermatogonium; Ss, secondary spermatocyte; G, The spermatids after secondary maturation division in the cyst of diploid testis, with condesed chromatin in the nucleus and several mitochodria, vacuoles in the cytoplasm; H, the spermatids ager secondary maturation division in the cyst of triploid testis, with relatively larger nuclei than those of diploid; I, Sertoli cell during spermiogenesis in diploid, with low electron dense chromatin in the nucleus and several mitochondrial rossets, endoplasmic reticulum, and the large amount of glycogen particles in the cytoplasm; J, The Sertoli cells during spermiogenesis in triploid, with many spermatids during spermiogenesis attached to active Sertoli cells as seen in cell organelle in diploid; K, longitudinal section of a mature spermatozoon in diploid, with sperm head including condensed chromatin, many mitochodria in the middle piece and the tail flagellum. L, Longitudinal section of mature spermatozoa in triploid, with relatively larger sperm heads than those of diploid; M, Transverse sections of axonemes of the tail flagella of the completed spermatozoa in diploid, with axonemes represented 9+2 type; N, Transverse section of axonemes of the tail flagella of the completed spermatozoa in triploid, with relatively larger tail flagella than those as seen in diploid. Abbreviations; A, axoneme; Af, axonemal flagellum; Cco, cytoplasmic collar; Cm, central microtubule; Dm, doublet microtubule; Er, endoplasmic reticulum; Gp, glycogen particles; Mp, glycogen particles; Mr, mitochondrial rosset; Mt, mitochondria; N, nucleus; Pce, proximal centriole; Ser, Sertoli cell; Stid, spermatid; Tf, tail flagellum; V, vacuole.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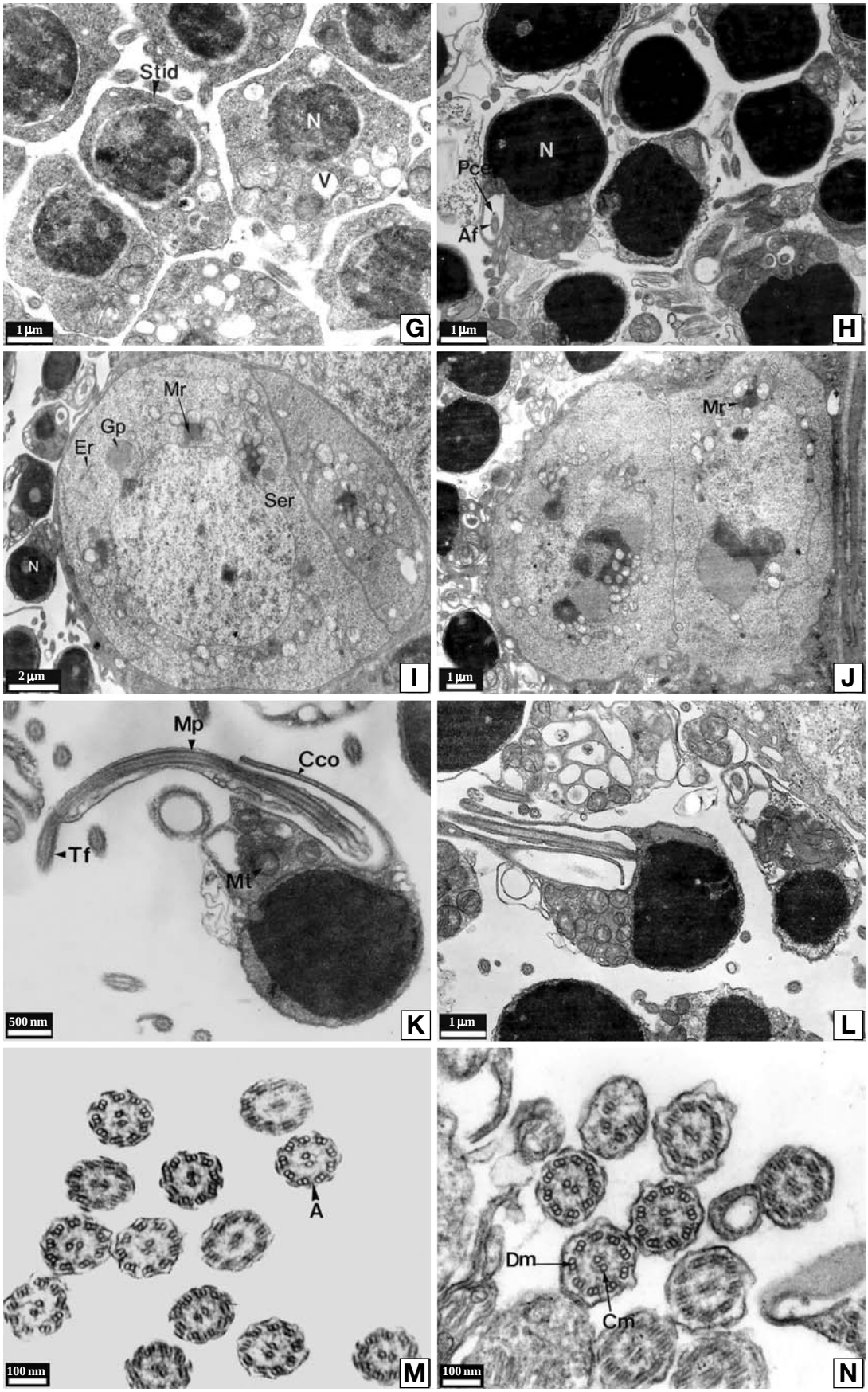
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