Korean J. Ichthyol. 2(1), 95~105, 1990. 韓魚誌, 2(1), 95~105, 1990.

Gonadal Sex Differentiation in *Misgurnus mizolepis*

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Gonadal sex differentiation of *Misgurnus mizolepis* was studied during growth from the size of 3.5 mm (2-day-old) larvae to about 80 mm in total length (100-day-old) adult fish.

One pair of primordial germ cells were found 2 days after hatching, and the development of prominent genital ridge was noticed at both stages of 4 days and 5 days after hatching. The gonadal sexes of this species were clearly distinguishable in 8 days, when the fry reached 15. 0 mm in total length. Spermatids first appaered at the stage of 20 days after hatching and 100 -day-old female fish had well-developed ovaries.

Above results indicate that *M.mizolepis* belongs to the differentiated type of gonochoristic teleosts.

Introduction

The loaches have a Eurasian distribution with greatest diversity in Southeast Asia and there are close to 150 species (in 3 subfamily) which range in size from 3 to 30 cm (Berra, 1981).

The cyprinid loach, *Misgurnus mizolepis*, a popular and an important food fish in Korea, is considered to be a good candidate for culture because of their market potential and high tolerance to varying environmental conditions. Although many studies have been carried out on rearing and stocking of this fish, little research has been directed toward genetic improvements that would increase its value as a cultured species. One genetic improvement that may provide benefits to fish culture is the production of mono-sex population through the manipulation of physiological or chromosomal sex (Yamazaki, 1983).

M. mizolepis exhibit sexually related dimorphic growth in which females grow faster and reach larger ultimate size than males. Therefore, artificial control of sex may be an important and interesting subject for genetic improvement of this species. Manipulation of sex by hormonal treatment requires an understanding of the timing of various stages of sexual differentiation (Malison *et al.*, 1986). In this respect, we examined normal histological development of *M. mizolepis* gonads.

This work was supported in part by the grant from the Korea Science and Engineering Foundation, 1989

Materials and Methods

Fish - All fish used were the offsprings of *M. mizolepis* (captured from the Nakdong-River, Kimhae, Kyeongsangnam-do) that had been artificially spawned and reared in the laboratory at 25 ± 0.2℃.

Sampling - To observe the process of early gonadal sex differentiation of the larvae, fish were sampled at 1day intervals until 20 days after hatching and examination of the gonads from juveniles and adult fish was made at intervals of 10 - 30 day during the succeeding period.

Histological analysis - Larvae were starved for about 6 hours prior to being sacrificed and fixed in Bouin's fluid. Every fixed sample was sectioned with 4 μm thickness from mouth to anus. Gonads from juveniles and adult fish were excised and fixed in Bouin's fluid. Paraffin sections of specimens were cut serially at 4μm in thickness. Hematoxylin and eosin-phroxine stained sections were prepared for examination.

Results

At the age of 2 days after hatching, when the fry averaged 3.5 mm in total length, one pair of primordial germ cell were found, which were located between just belowed mesonephric duct and gut. The primordial germ cells were quite large (11~15μm in diameter) and slightly oval in shape with a large nucleolus (Fig. 1).

At the stage of 3 days, the germ cells began to intrude into the peritoneal cavity with a few somatic ones enclosing them in anterior region of the presumtive gonadal area. No mitotic germ cells were detectable at this stage (Fig. 2a). The development of prominent genital ridge was noticed at both stages of 4 days and 5 days after hatching and signs of mitotic activity were observed in some of the larger germ cells within the gonad, but the majority were seen in a typical resting condition (Fig. 2b & Fig. 3).

The gonads of the half of the fish examined 6 days after hatching possessed not only a few single and clusters gonial cells but also many cysts of germ cells in meiotic prophase (Fig. 4).

At the age of 8 days after hatching, when the fry reached 15.0 mm in total length, two types of gonads were observed. One type of gonad did not show any sign of the meiotic activity but Sertoli-like cells and lobule were observed (Fig. 5a). The gonads of the rest of the fish were observed primary growth of oocytes (Fig. 5b).

At the stage of 14 days, when the fry attained to 22.5 mm in total length, some germ cells in meiosis were observable in the testis (Fig. 6a). Spermatogenesis proceeded rapidly after the initiation of meiosis, and the spermatids were first appearance at the stage of 20 days after hatching (Fig. 6b). Active spermatogenesis of spermatogonia seemed to take place about 70-100 days after hatching (Fig. 7a and 7b).

Some oocytes of peri-nucleolus stages were observed in ovaries 20 days after hatching, (Fig.

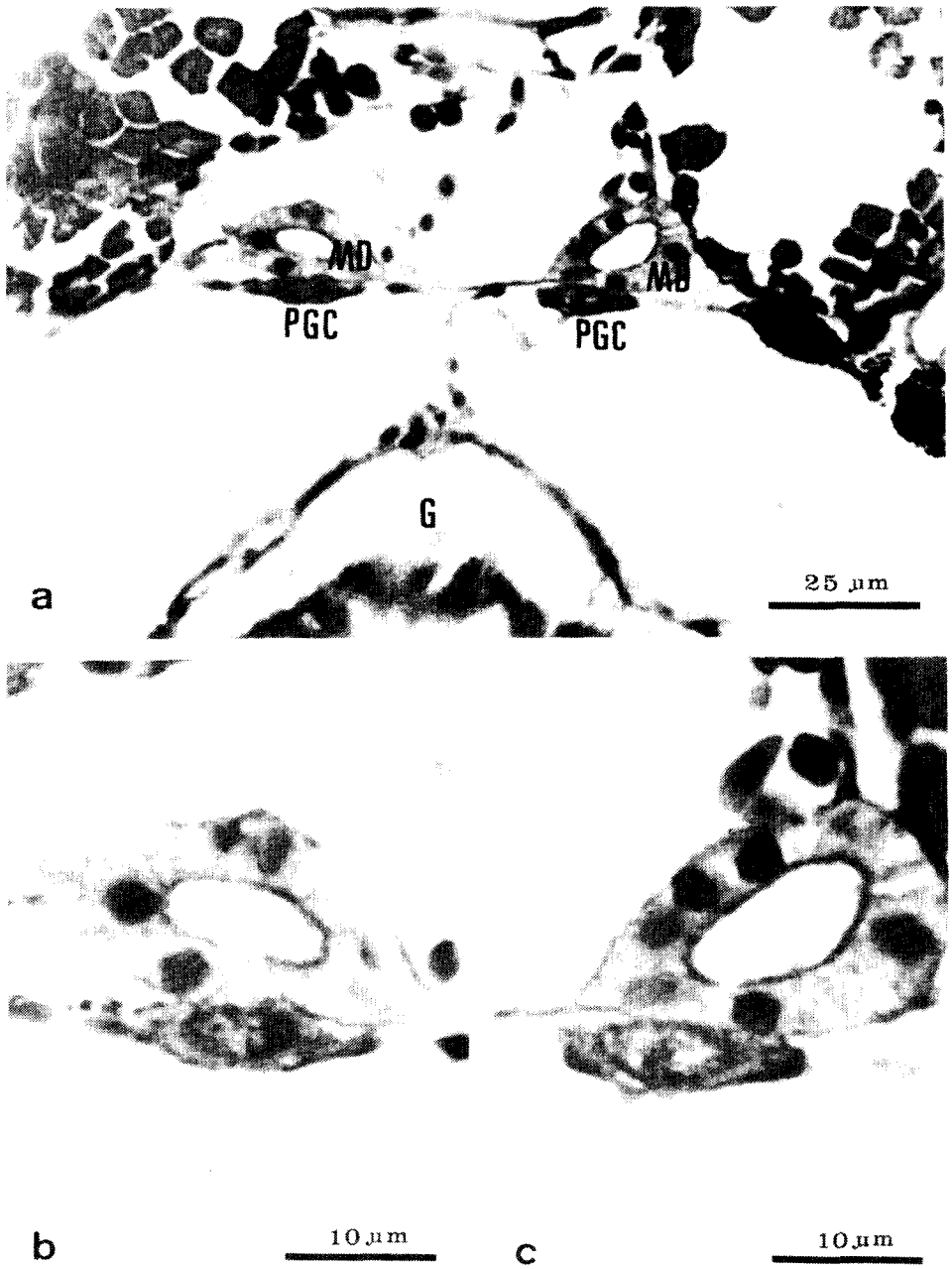

Fig. 1. Primordial germ cell in a 2-day-old larva.
a ; Primodial germ cells (PGC) were located between the mesonephric duct(MD) and the gut (G). b & c ; High power view of primordial germ cells.

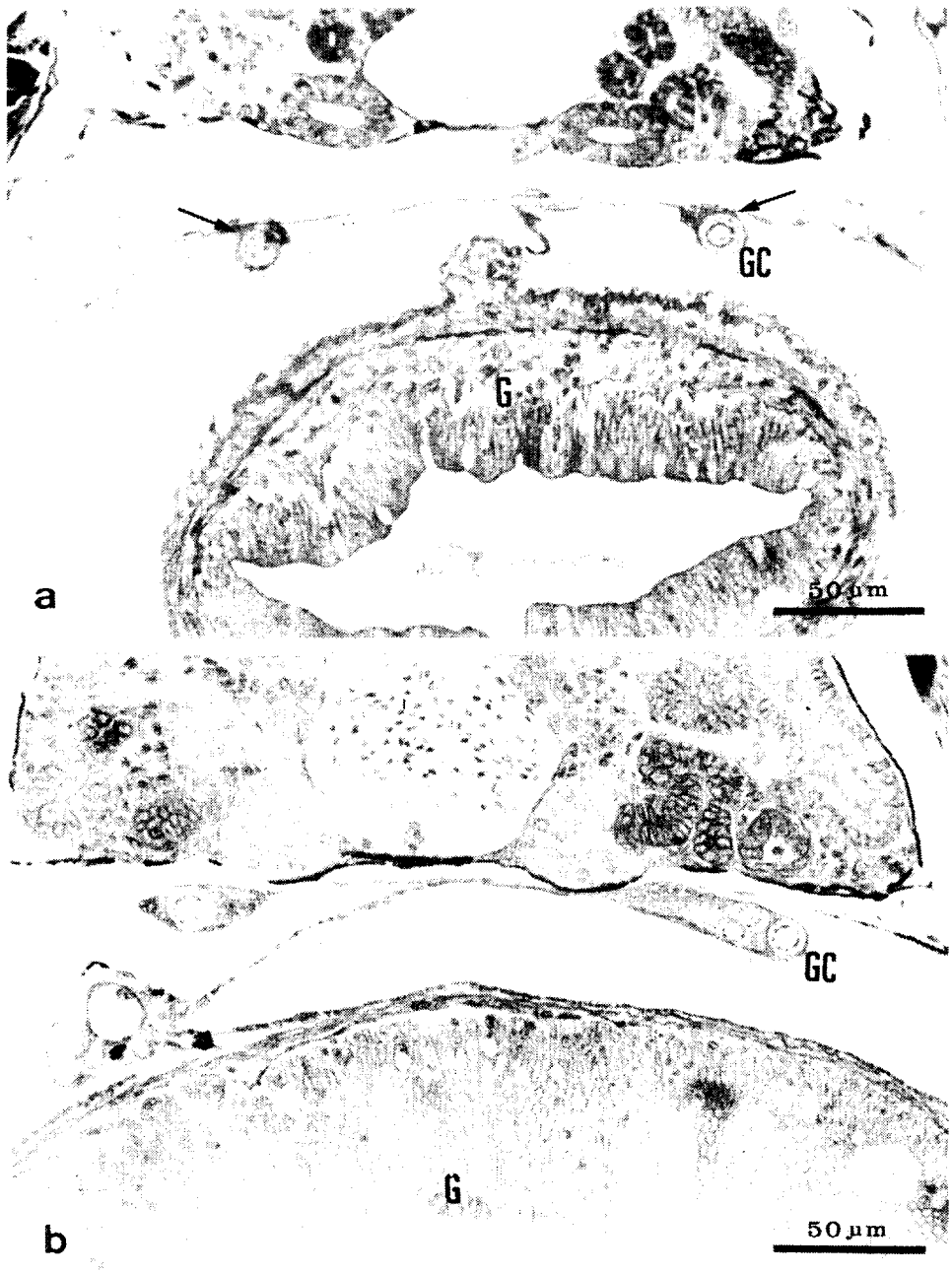

Fig. 2. Transverse sections of a larva, 3 days after hatching (a) and 4 days after hatching (b). Arrows show somatic cells enclosing germ cell (GC). G ; gut

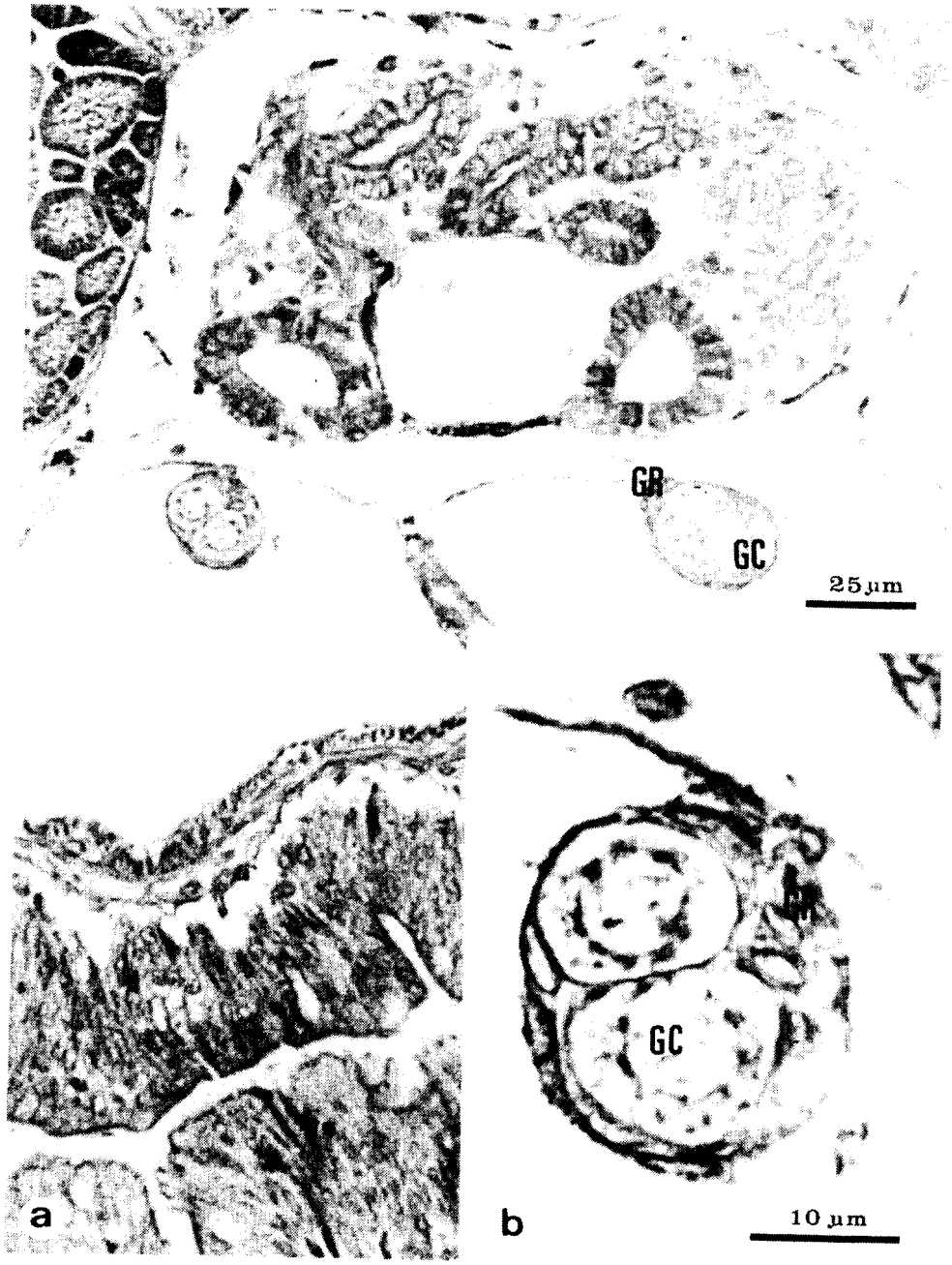

Fig. 3. a ; Cluster of germ cells (GC) in the genital ridge (GR) of a 5-days-old larva. G ; gut
b ; High power view of cluster of germ cells(GC).

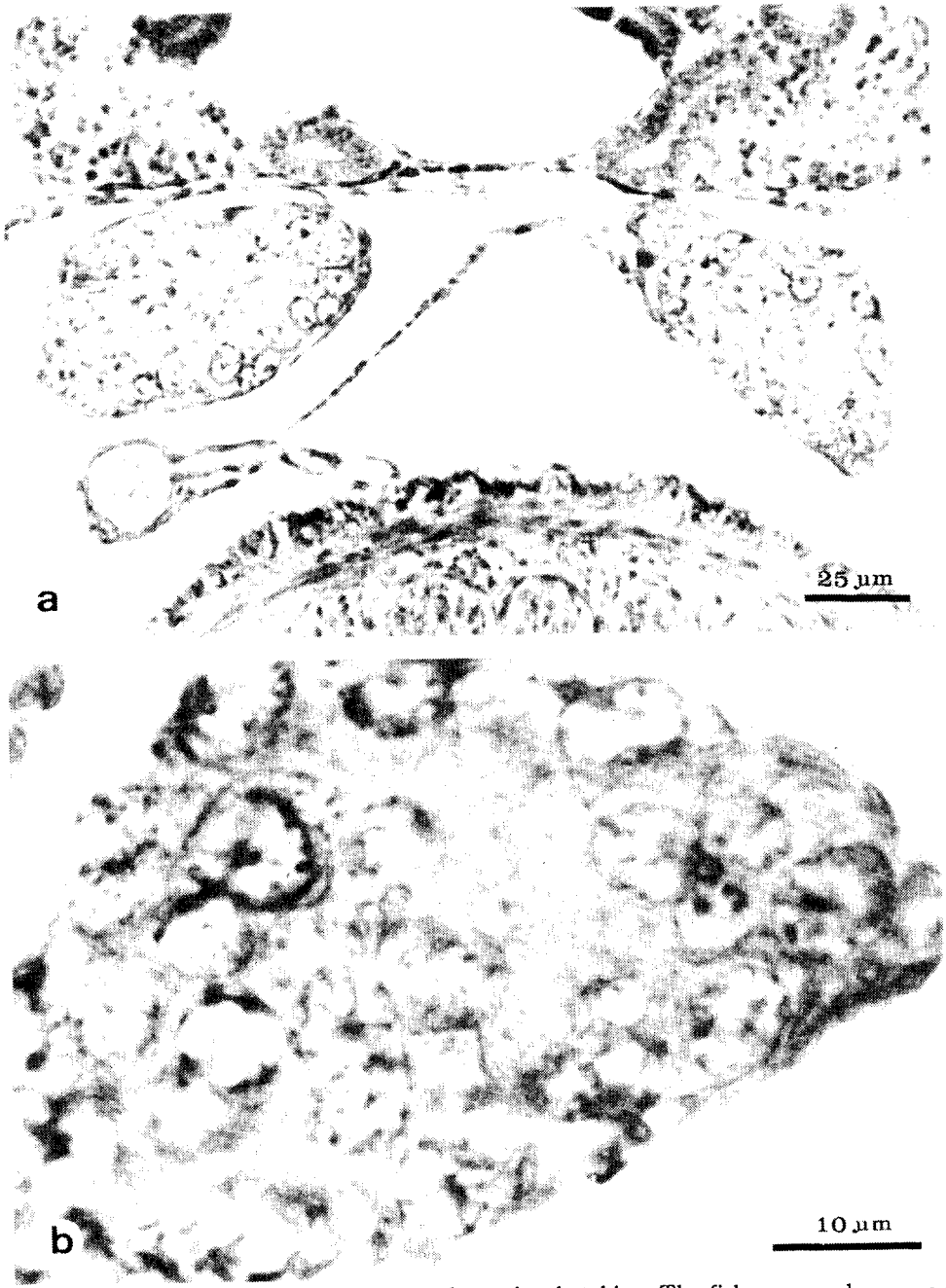

Fig. 4. a ; Transverse section of a larva, 6 days after hatching. The fish possessed many cysts of germ cells in meiotic prophase. b ; High power view of a primordial gonad.

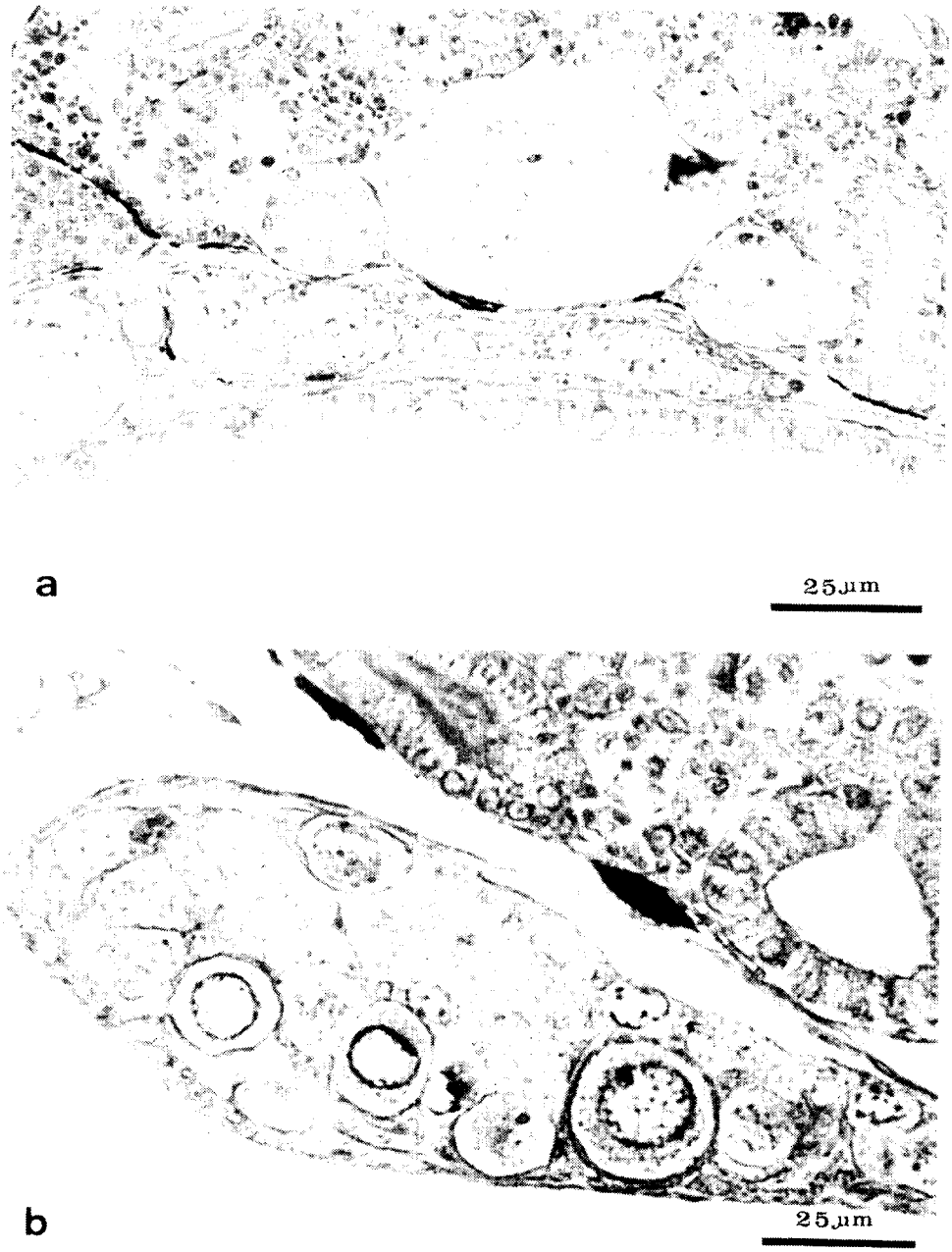

Fig. 5. Transverse sections of the larvae, 8 days after hatching. Two types of gonads were ovserved. a ; Testicular tissue b ; Overian tissue

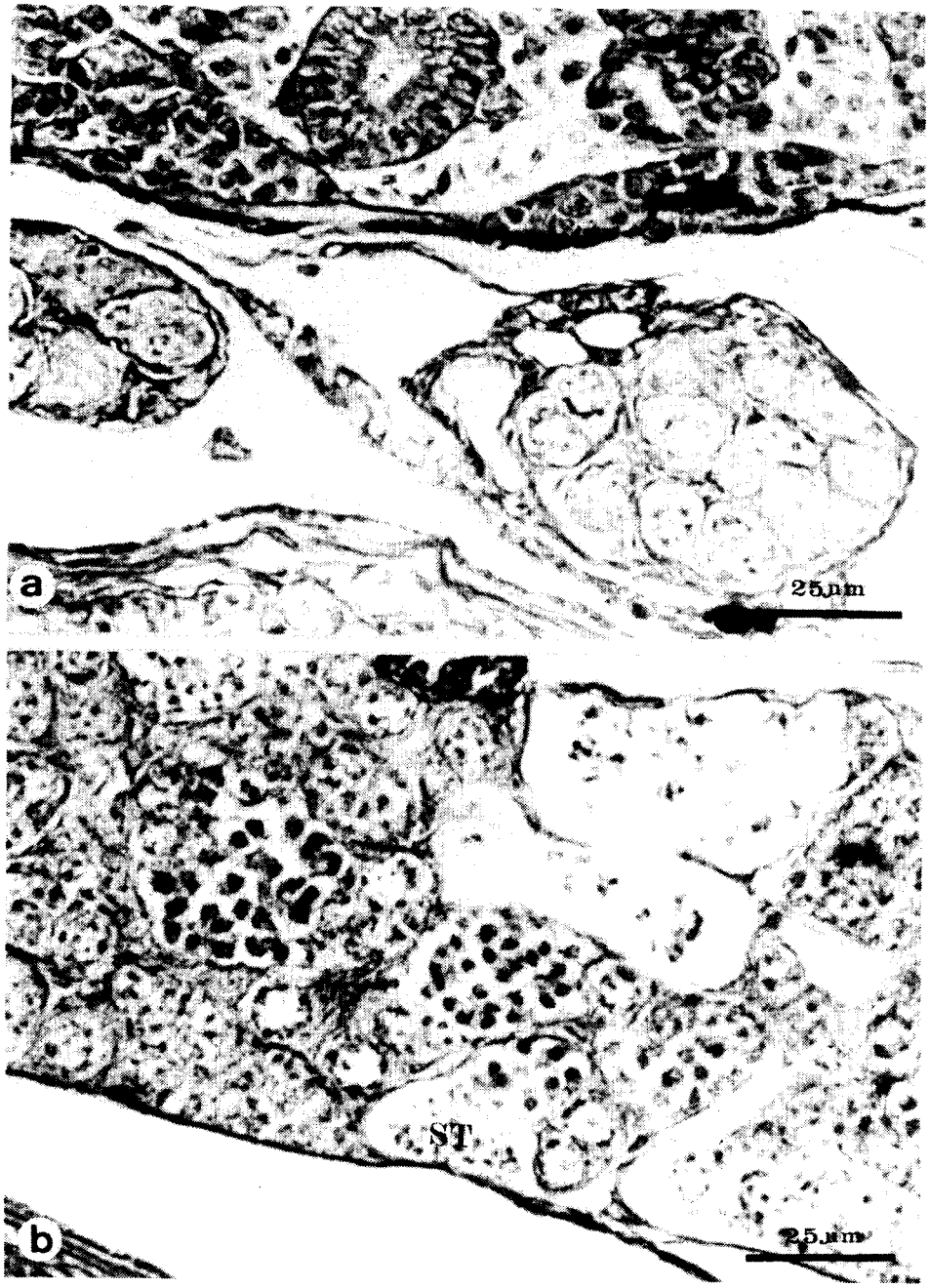

Fig. 6. a ; Testicular tissues from 14-day-old fish.
b ; Testicular tissues from 20-day-old fish.

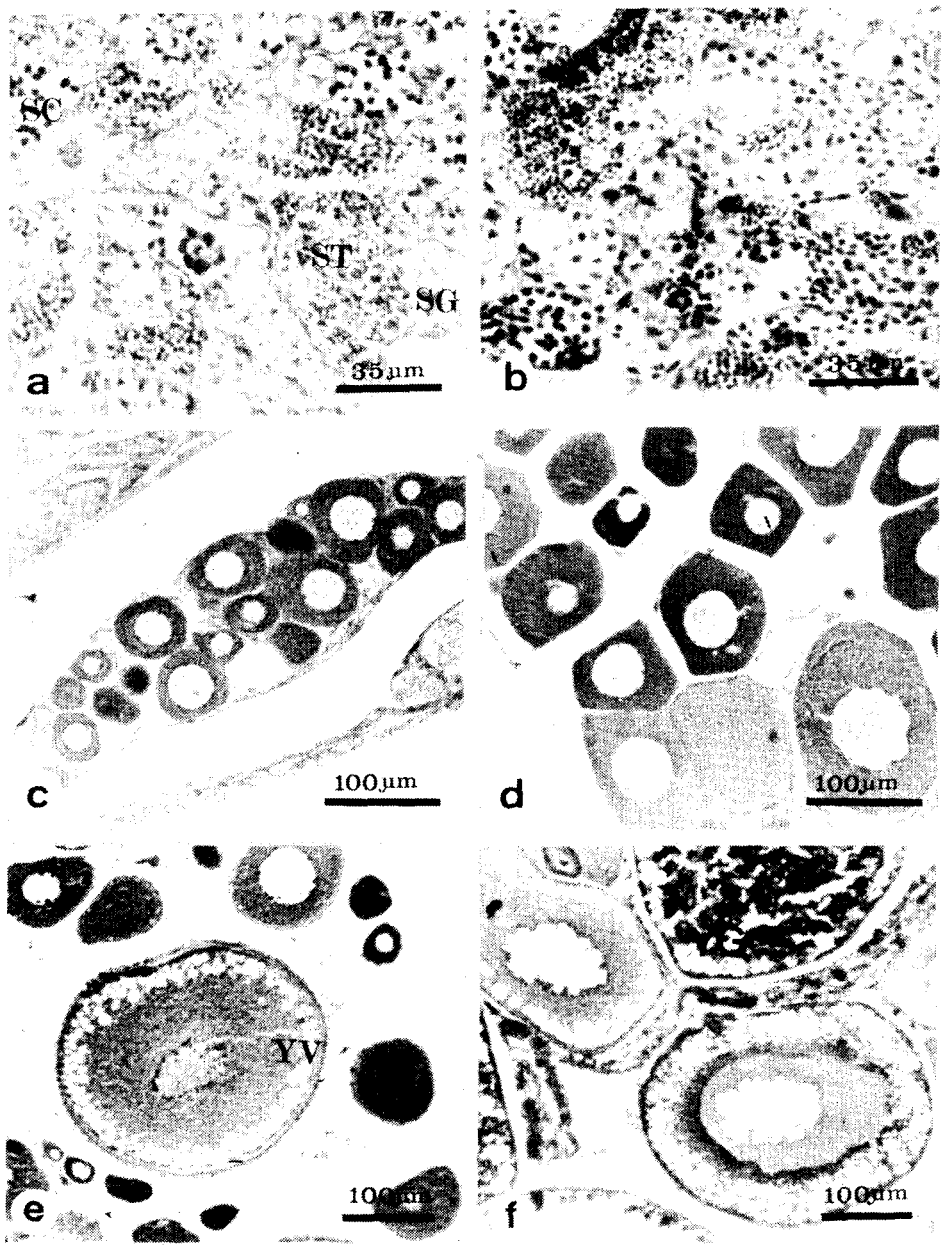

Fig. 7. a & b; Portion of testis from a fish belonging to the mature groups. Some lobules contain a small amount of spermatozoa (a; 70-days-old fish). Lobules packed with mature spermatozoa (b; 100-day-old fish). SG: spermatogonia, SC: spermatocyte, ST: spermatid c-f; Portion of ovary from a fish belonging to the immature and mature groups (c; 20-day-old fish, d; 30-day-old fish, e; 70-day-old fish, f; 100-day-old fish).
YV: yolk vesicle, YG: yolk granule

7c), and vitellogenesis in oocytes began 30 days after hatching (Fig. 7d). About 70 days after hatching, late growing oocytes with yolk vesicle were found in premature ovaries of the female fish (Fig. 7e) and 100-day-old female fish had well-developed ovaries with oocytes at various stages of maturation and well-formed ovarian cavities (Fig. 7f).

Discussion

Generally, fishes have one pair of bilateral gonads, and at the beginning of ontogenesis the gonads rise as a pair to form the genital ridge composed of the primodial germ cells and the connective tissue surrounding them (Hibiya, 1982).

Most of the studies on the identification of germ cells during the early phases of gonadal development have been based on cell size and staining affinity using the light microscope (Satoh, 1974). In *M. mizolepis* used in the present study, primordial germ cells could be distinguishable from the somatic cells by the histological features such as oval in shape an relatively large in size.

Gonochorism in fish is having either testis or ovaries in one individual and the great majority of fish are either undifferentiated or differentiated gonochorists (Yamamoto, 1969). Many teleosts have been considered to be undifferentiated. However, several species of cultured fish have been reported to be differentiated gonochorism (Davis and Takashima, 1980; Kim *et al.*, 1988; Nakamura, 1981; Nakamura and Takahashi, 1973; Robertson, 1953; Takahashi *et al.*, 1980; Yamamoto, 1969; Yoshikawa & Oguri, 1977). Yamazaki(1983) suggested that the artificial control of gonochoristic sex of differentiated species is normally successeful only when treatment with sex steroid is carried out during a particular stage of gonadal development. In the present study, the gonadal sexes of *M. mizolepis* were indiscernible at least histologically at 6 days after hatching but became clearly distinguishable by 8 days of age. This results indicate that *M. mizolepis* belongs to the differentiated type of gonochoristic teleosts.

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미꾸라지(*Misgurnus mizolepis*)의 성 분화에 관한 연구

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암컷의 성장이 수컷에 비해 빠른 미꾸라지의 유전육종을 위한 연구의 일환으로 성전환을 시키기 위하여 우선 본 종의 성장에 다른 생식소 형성 및 그의 분화과정을 조사하였다. 그 결과, 부화후 2일이 경과 하여 전장이 3.5mm 정도에 이르면 원생식세포가 중신관과 창자 사이에서 관찰되었다. Genital ridge의 형성은 4 및 5 일자 자어에서 이루어졌으며, 전장이 15.0mm 되는 8 일자 자어에서는 정소와 난소가 뚜렷이 구별되었다. 숫컷의 경우 20 일자 치어에서 최초로 정세포가 정소조직내에 존재하였고, 암수 모두 100 일자에 이르면 생식소내에 완전 성숙된 정 난자가 관찰되었다. 이상의 결과로 미루어 본종의 성분화는 differentiated형의 gonochorism인 것으로 확인되었다.