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Hormonal Induction of Sex Reversal in Serranid Fish, *Epinephelus septemfasciatus*

Young-Don Lee, Hyung-Bae Kim*, Choon-Bok Song**, Sum Rho** and Jung-Jae Lee**

Marine Research Institute, Cheju National University, 3288 Hamdŏk-ri, Chochŏn-eup, Pukjeju-gun, Cheju-do 695-810, Korea
**Marine Biotechnology Laboratory, KORDI, Ansan P.O. Box 29, Seoul 425-600, Korea*
***Department of Aquaculture, College of Ocean Science, Cheju National University, Cheju-do 690-756, Korea*

호르몬처리에 의한 능성어(*Epinephelus septemfasciatus*)의 성전환

이영돈 · 김형배* · 송춘복** · 노 섬** · 이정재**

제주대학교 해양연구소 · *한국해양연구소 해양생물공학연구그룹 · **제주대학교 증식학과

Hormonal induction of sex reversal was examined by using sex steroid hormones in serranid fish, *Epinephelus septemfasciatus*. Young fish were collected from the coastal area of Cheju Island, and reared for 2 years before fish were used for the experiments. Without any hormonal treatment, gonads of fish (1,000~2,800 g in body weight) were occupied by oocytes of the perinucleolus stage and bundles of protogonial cells in the area of germinal epithelium. When the induction of sex reversal was attempted by daily oral administration of 17α-methyltestosterone (0.5 mg/kg fish) for 90 days, active spermatogenesis was induced, and spermatogonia and spermatocytes and spermatids were appeared in all gonads we examined. However, after daily, oral treatment of 17β-estradiol (0.5 mg/kg fish) for 50 days with the following injection of human chorionic gonadotrophin (1,000~1,500 IU/kg fish) mature oocytes were not induced in fish gonad.

Key words : Sex reversal, Sex steroid, Spermatogenesis, *Epinephelus septemfasciatus*

Introduction

In Korea, groupers inhabit around the rocky reef, usually 5 to 60 m under the water surface, around the coastal area and Southern Sea of Cheju Island. Kelp grouper (*Epinephelus bruneus,*) sevenband grouper (*E. septemfasciatus,*) red grouper (*E. akaara*), blue spotted grouper (*E. fario*), and black tipped grouper (*E. fasciatus*) are known to inhabit naturally near Cheju Island (Kim and Lee, 1994). Because of their high growth rate, daintiness and market value, these groupers are known recognized to be one of the most important future mariculture fish in Korea, Japan, and the other South-

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east Asian cantries. Therefore, many researches have been focused on their reproductive biological characteristics.

Induction of sex reversal and gonadal development in grouper have been previously reported for *E. tauvina* (Tan and Tan, 1974), *E. akaara* (Chan and Yeung, 1983 ; Hwang, 1993) and *E. fario* (Kuo et al., 1988). Studies on egg development and early growth of groupers have been conducted by using *E. tauvina* (Chen et al., 1977), *E. salmonoides* (Huang et al., 1986), *E. suillus* (Doi et al., 1991), and *E. fuscoguttatus* (Kohno et al., 1993). On the other hand, with *E, septemfasciatus,* Kitajima et al. (1991) described the development of eggs, larvae and juveniles mainly based on its morphological changes. However, no studies on sex reversal in this fish have been attempted so far.

Therefore, this study were performed to promote the better understanding of the effects of sex steroid hormones on sex reversal with regard to the proper concentration and duration of hormone treatment.

Materials and methods

Young fish of *E. septemfasciatus* were collected at the coastal area of Cheju Island. Reared for 2 years at Halla Aquaculture Farm located in Cheju Island and reached from 1.0 to 2.8 kg in body weight.

Fish were divided into three experimental groups of 20 fish per group ; 17α-methyltestosterone (MT) treatment group (A) for the induction of sex reversal, 17β-estradiol (E_2) treatment group (B) for induction of gonadal development, and one control group (C). MT (0.5 mg/kg fish) was administered to A group for 90 days, while E_2 (0.5 mg/kg fish) was provided for 50 days and then human chorionic gonadotrophin (1,000~1, 500 IU/kg fish) was injected for B group. The concentration and duration of hormone treatment generally followed the method by Kuo et al. (1988). For the histological analysis of gonads, preserved samples treated with Bouin's fixative solution were embedded by the following routine procedure ; dehydrated in alcohol and embedded in paraffin. Sections from the embedded samples were attached on glass slides, stained with Delafield haematoxylin and eosin, and examined by using a microscopy.

Results

Protogonial cells of 6~8 μm and the perinucleolus oocytes of 25~30 μm in size, were scattered in the ovarian sac (Pl. IA). Each ovarian sac appeared to be contracted, while undifferentiated mesenchymal tissue was observed along the germinal epithelium. Such observations were made in fish collected from February to April. On the other hand, the ovarian lamellae of fish sampled from June to August were compacted by a large number of the perinucleolus oocytes and each ovarian lamellae was expanded and the germinal epithelium became very thin (Pl. IB).

The total amount of MT administered to group A was 45.0 mg per kg-fish of the daily dosage of 0.5 mg per kg-fish for 90 days. As a result of MT treatment, both male and female germinal cells were intermingled in the gonad. The remnants of the perinucleolus oocytes were scattered through the testicular tissue, and each of the testicular lobules contained bundles of protogonial cells, spermatocytes, and spermatids (Pl. IC, ID).

When E_2 was administered to B group at the daily dosage of 0.5 mg per kg-fish for 50 days and ended up to be 25.0 mg per kg-fish, the remnants of perinucleolus oocytes were distributed in the ovarian lamellae and a number of mesenchymal tissue and interstitial cells were appeared in the germinal epithelium (Pl. IE). Finally after E_2 treatment for 50 days an experiment with HCG injection of 1,500 IU per kg-fish was conducted, a small number of the perinucleolus oocytes were scattered and a large number of interstitial cells occupied the ovarian lamellae (Pl. IF).

Discussion

The size and age of serranid fish showing sexual maturity and sex reversal were varied depending on their species. *E. tauvina* reached sexual maturity at the age of three around 2.5 kg in body weight, and 40 to 50 cm in body length, and their sex reversal began at the age of seven (Tan and Tan, 1974). Induction of sex reversal was reported in *E. fario* which were nine years old and 11 kg in body weight (Moe, 1969). It was also observed in the five-year-old *E. salmonoides* (*E. amblycephalus*), 60~70 cm in body length and 6 kg in body weight (Tang et al., 1979). Hwang (1993) reported that *E. akaara* reached sexual maturity between two to three years old, as their body weight reached to 0.22~0.63 kg and total length from 19.5 to 35.2 cm, and then sex reversal began at the age of three, while their gonads were ovotestis. In this study, *E. septemfasciatus*, that is 41~55 cm in total length and 1.1~2.8 kg in body weight, was still immature and their gonad contained a large number of perinucleolus oocytes scattered in the ovarian lamellae.

Kuo et al. (1988) reported that mature males of the two-year-old *E. fario* were obtained by the oral administration of MT at daily dosage of 0.5 mg or 1.0 mg MT/kg body weight for five months, and spawning was successfully induced by doing multiple injections of HCG (at dose of 2,000~3,000 IU/kg fish) using the four-year-old fish. In the present study, when 17β-estradiol were administered at daily dosage of 0.5 mg/kg body weight for 50 days, and then HCG at the dosage of 1,000~1,500 IU/kg fish to *E. septemfasciatus*, it was observed that only the immature oocytes were scattered, and a large number of the somatic cells occupied in the gonadal lamallae of *E. septemfasciatus*. This implied that *E. septemfasciatus* used for this study were still immature. Therefore, the appropriate dosage and concentration of sex hormone should be administrated. In addition the age and size of experimental fish turned out to be very important for successful spawning and sexual maturity. On the other hand, the perinucleolus oocytes and sexually undifferentiated protogonial cells were intermingled in the gonad of serranid fish at the age from serranid fish two to three-year-old (*E. septemfasciatus*). Of these two kinds of cells, protogonial cells seemed to be more easily affected by the action of sex steroid hormone because of their sexual bipotentiality. The specific effects of sex steroid hormones on protogonial cells and its functional significance are remained to be clarified.

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References

Chan, S. T. H. and W. S. B. Yeung, 1983. Sex control and sex reversal in fish under natural conditions. In : W. S. Hoar, D. J. Randall, and E. M. Donaldson eds.), Fish Physiology. Vol. IX, Part B. Academic Press, New York, pp. 171−222.

Chen, F. Y., M. Chow, T. M. Chao and R. Lim, 1977. Artificial spawning and larval rearing of the grouper, *Epinephelus tauvina*, in Singapore. Singapore J. Prim. Ind., 5 : 1 −21.

Doi, H., M. M. Nawi, N. R. Lah and Z. Tailb, 1991. Artificial propagation of the grouper, *Epinephelus suillus* at the marine finfish hatchery in Tanjong Demong, Terengganu, Malaysia. Department of Fisheries, Ministry of Aqriculture, Malaysia. pp. 1−42.

Huang, T. S., K. J. Lin, C. L. Yen, C. Y. Lin and C. L. Chen, 1986. Experiments on the artificial propagation of black spotted grouper, *Epinephelus salmonoides* (Lacepede). I. Hormone treatment, ovulation of spawners and embryonic development. Bull. Taiwan Fish. Res. Inst., 40 : 241−58.

Hwang. S. I., 1993. Gonadal development and sex reversal by 17-methyltestosterone treatment of *Epinephelus akaara* (Temmink et Schlegel). M. S. thesis, Cheju National

Univ., Graduate school. pp. 1−35.

Kim, I. S. and W. O. Lee, 1994. Fish fauna from Cheju Island, Korea. The study group of Korean Fish Fauna Dept. Biol., Chonbuk Nat. Univ., pp. 1−51.

Kitajima, C., M. Takaya, Y. Tsukashima and T. Arakawa, 1991. Development of egg, larvae and juveniles of the grouper, *Epinephelus septemfasciatus,* reared in the laboratory. Japan. J. Ichthyol., 38 : 47−55.

Kohno, H., S. Diani and A. Supriatna, 1993. Morphological development of laval and juvenile grouper, *Epinephelus fuscoguttatus.* Japan J. Ichthyol., 40 : 307−316.

Kuo, C. M., Y. Y. Ting and S. L. Yeh, 1988. Induced sex reversal and spawning blue spotted grouper, Aquaculture, 74 : 113−126.

Moe, M. A. J., 1969. Biology of the red grouper, *Epinephelus mario* (Valenciennes), from the Eastern Gulf of Mexico. Florida Dept. Res. Prof. Pap., 2207 : 1−20.

Tan, S. M. and K. S. Tan, 1974. Biology of tropical grouper, *Epinephelus taivina.* I. A preliminary study on hermaphroditism in *E. tauvina.* Singapore J. Prim. Ind., 2 : 123−133.

Tang, H. W., J. Y. Twu and W. C. Su, 1979. Preliminary report on artificial propagation of black spotted grouper, *Epinephelus amblycephalus* (Bleeker). Bull. Taiwan Fish. Res. Inst., 31 : 511−517.

Plate I

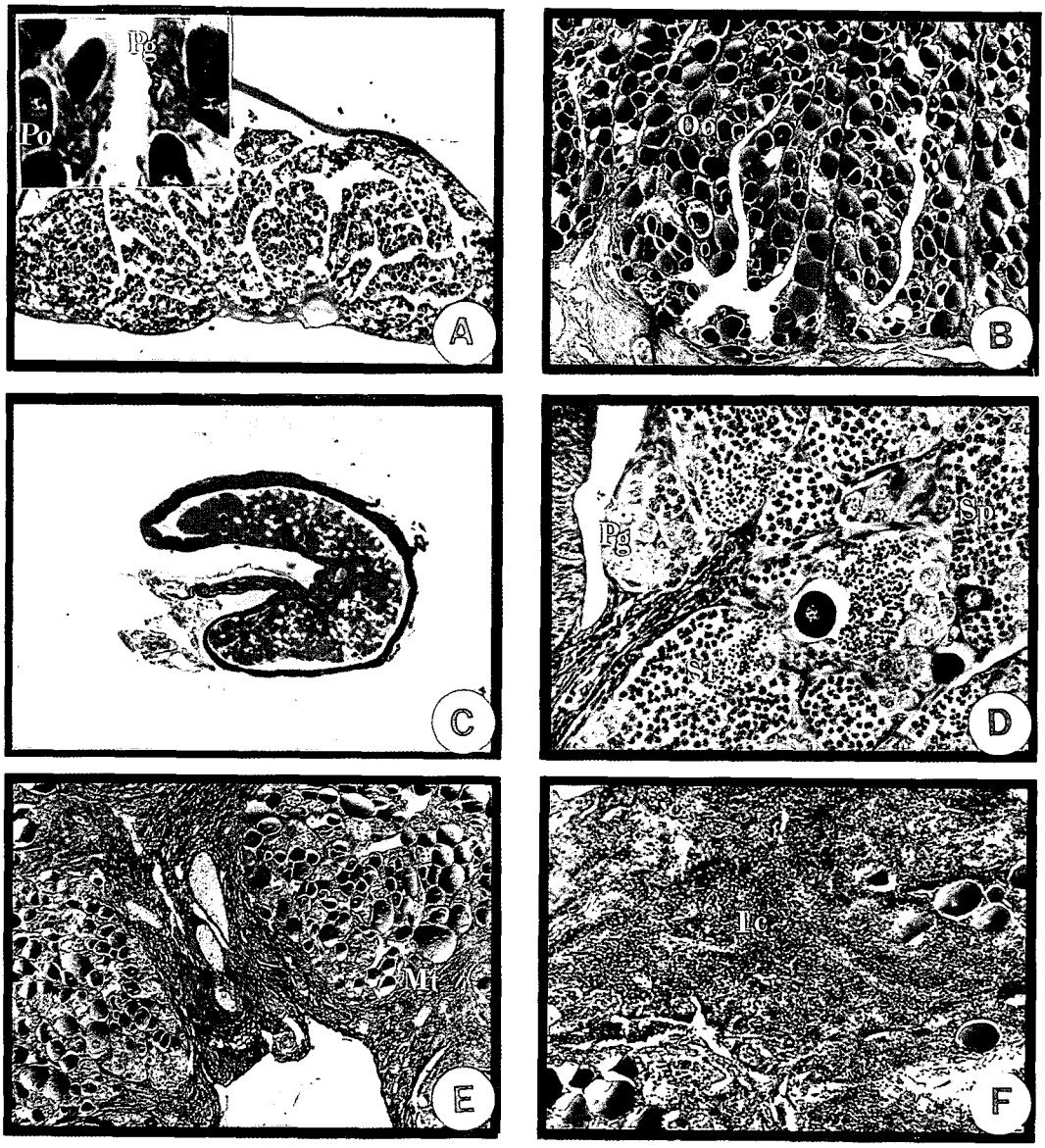

Plate I. Various sexual stages in the gonads of *E. septemfasciatus*. 1 : Ovary of the resting and multiplicative stage. The ovarian sac is composed of protogonial cells (Pg) and perinucleolus oocytes (Po), ×50. 2 : Ovary of the early growing stage. The ovarian lamellaes contain a large number of the oocytes (Oo), ×100. 3 : Induced ovotestis by 17α-methyltestosterone. The remnant of the oocytes are scattered through the testicular tissue, ×50. 4 : Partial magnification of the ovotestis (Pl. IC). The testicular lobe is composed of protogonial cells, spermatocytes (Sp) and spermatids (St), ×200. 5 : Ovary treated with 17β-estradiol showing the extensive development of the mesenchymal tissue (Mt) with the degenerative of the oocytes, ×100. 6 : Effected ovary by the injection of HCG showing the incresement of the intestitial cells (Ic) with the degenerative of the oocytes, ×100.