

## Induction of Primary Male in Juvenile Red Spotted Grouper *Epinephelus akaara* by Immersion of 17 $\alpha$ -Methyltestosterone

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**ABSTRACT** : We investigated the androgenic effects of 17 $\alpha$ -methyltestosterone (MT) on gonadal sex reversal in juvenile red spotted grouper *Epinephelus akaara*. The fish were immersed in 17 $\alpha$ -MT at 1 and 5 mg/L. Treatment method of 17 $\alpha$ -MT was once weekly for 4 and 8 weeks. Fish were sampled at 12 months after end of the treatment period in order to histological analysis. At the initiation of an experiment (70 day after hatching), juvenile red spotted grouper have the paired primordial gonads with somatic cells below kidney in the posterior portion of the body cavity. Formation of ovarian cavity indicates that the ovarian differentiation beginning at 70 DAH in red spotted grouper. At 12 months after end of the treatment period, control group, 17 $\alpha$ -MT 1 mg/L treatment group for 4 and 8 weeks, and 17 $\alpha$ -MT 5 mg/L treatment group for 4 weeks were all female. However, sex-changed males without ovarian cavity were observed in the 17 $\alpha$ -MT 5 mg/L treatment group for 8 weeks. In grouper, we firstly reported that the red spotted grouper be able to induce the primary males by hormone treatment prior to gonadal sex differentiation.

**Key words** : Red spotted grouper, *Epinephelus akaara*, Gonadal sex differentiation, Sex change, Primary males

### INTRODUCTION

The red spotted grouper, *Epinephelus akaara* is one of the most important grouper species for aquaculture in Korea, China, Japan and Southeast Asian countries. In particular, groupers are ideal candidate species for intensive aquaculture in Asia Pacific region because of high consumer demand,

desirable taste, efficient feed conversion, and rapid growth (Kohno et al., 1993; Chen & Tsai, 1994; Millamena, 2002). Despite its advantages as commercial importance culture species is still in infancy with adult management, natural spawning and seed production because most groupers require culture periods of several years until first maturation and spawning is achieved (Song et al., 2005). The problems of

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grouper aquaculture development has been hindered by the difficulty of mature male grouper security, which is due to the lack of a standardized method of sex change and the unavailability of mature male broodstock.

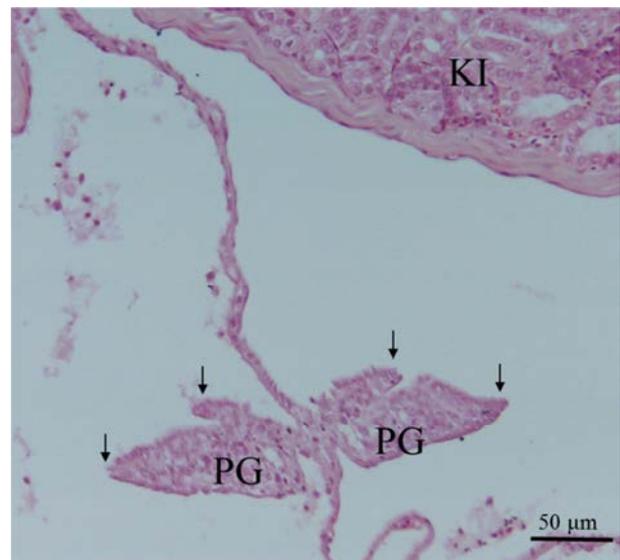
Most groupers including the red spotted grouper are protogynous hermaphrodites, first become sexually mature as females and later change sex to become males. Natural sex change in grouper species occurs between 3 and 11 years of age depending on the species (Tan & Tan, 1974; Chauvet, 1988). The steroid hormones play important role of sex change in the grouper species (Li et al., 2006; Zhou & Gui, 2010). Many study have been reported on different grouper species to develop standardized methods for sex reversal by treatment with androgens or aromatase inhibitors (Bhandari et al., 2005). But sex-changed male grouper had testis with ovarian cavity and sperm production is very low. Thus, in order to have a stable supply of sperm, it is necessary for male groupers without ovarian cavity using hormone treatment prior to ovarian cavity formation. In this study, we examined that the effect of  $17\alpha$ -methyl-testosterone (MT) to induce the primary male of juvenile red spotted grouper *E. akaara*.

## MATERIALS AND METHODS

The experiments were carried out in Marine Science Institute, Jeju National University. At each experimental group, 200 fish were reared in 300 L circle tank with flow-through sea water, under natural water temperature and photoperiod. At 70 days after hatching (Total length,  $3.33 \pm 0.38$  cm; Body weight,  $0.47 \pm 0.14$  g), fish were immersed in  $17\alpha$ -MT at 1 and 5 mg/L. Treatment method of  $17\alpha$ -MT was once weekly for 4 and 8 weeks. Fish were sampled at 12 months after end of the treatment period in order to histological analysis. The gonads were fixed in Bouin's solution, embedded in paraffin, cross-sectioned, and stained with haematoxylin and eosin using the standard methods for light microscopy.

## RESULTS AND DISCUSSION

At the initiation of an experiment (70 day after hatching), juvenile red spotted grouper have the paired primordial gonads with somatic cells bellow kidney in the posterior portion of the body cavity. Each lobe of the primordial gonads began elongation to form an ovarian cavity (Fig. 1). In grouper species, gonad development during sexual differentiation is a little studied but similar patterns as early characteristic of female differentiation were reported (Murata et al., 2009; Liu & Sadovy, 2009; Tsai et al., 2011; Sao et al., 2012). In *E. malabaricus*, the initial ovarian cavity was observed at 47 day after hatching by elongations of somatic cells in the gonads on the sides facing the gonad lateral walls, an early event of initial ovarian cavity formation (Murata et al., 2009). In *E. coioides* and *E. bruneus*, the initial ovarian phase was observed at 4 month after hatching and 60 day after hatching, respectively (Tsai et al., 2011; Sao et al., 2012). In this study, early morphological change to form the initial ovarian cavity in red



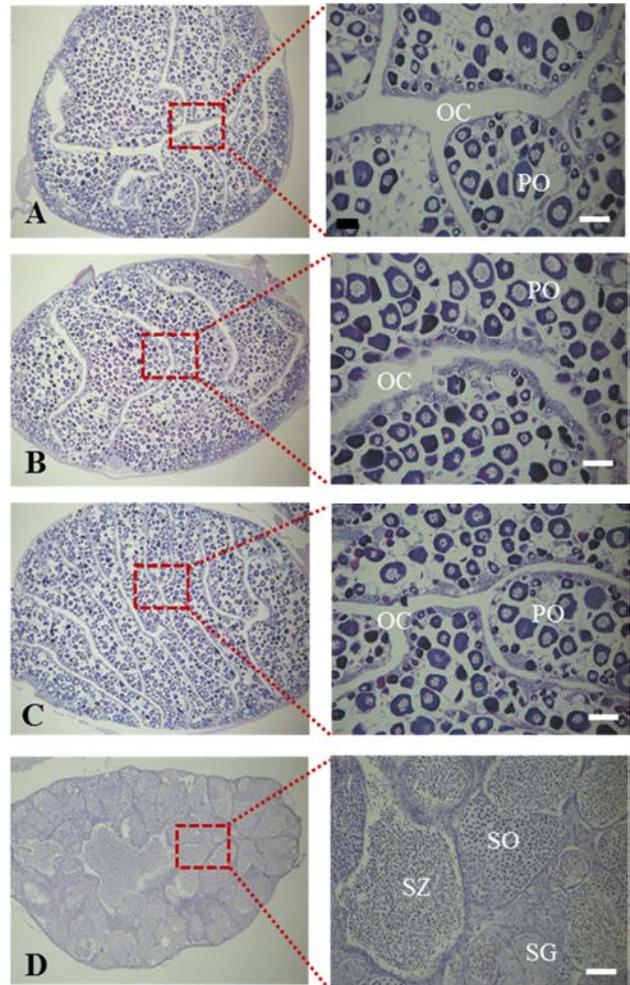
**Fig. 1. Cross section gonad of juvenile red spotted grouper at 70 day after hatching.** Two elongations of somatic tissue (arrow), indicating ovarian cavity formation, can be seen. KI, kidney; PG, primordial gonad.

spotted grouper were observed by around 70 day after hatching.

Sex pattern of protogynous hermaphroditic fish can be divided into two types, monandry and diandry, according to the male development pathway (Reinboth, 1967). Monandric species follow a single male developmental pathway; all males in a population are secondary males derived exclusively from functional females via sex change. Diandric species follow two male developmental pathways, i.e., primary males develop from juveniles through sexual differentiation and secondary males develop via sex change. In *Halichoeres poecilopterus*, *H. tenuispinis*, and *Ps. sieboldi* were diandric type with primary and secondary male. Most grouper is monandric type with secondary male via sex change from functional female (Lee et al., 2006).

At 12 months after end of the treatment period, control group, 17 $\alpha$ -MT 1 mg/L treatment group for 4 and 8 weeks, and 17 $\alpha$ -MT 5 mg/L treatment group for 4 weeks were all female, which is contained oogonia and perinucleolus oocytes in ovary. However, sex-changed males were observed in the 17 $\alpha$ -MT 5 mg/L treatment group for 8 weeks. The gonad of sex changing individuals was consisted of numerous testicular lobules and filled with sperm, but cannot observed the ovarian cavity (Fig. 2).

In many grouper species, successful induction of artificial sex change has been reported mostly in small sized females (Tukashima & Kitajima, 1983; Chao & Chow, 1990; Tsuchihashi et al., 2003). Androgen administration including 11-ketotestosterone (11-KT) and the synthetic 17 $\alpha$ -methyltestosterone (MT) widely used for sex reversal induction in grouper, but various dose concentrations and methods of hormone administration (Chao & Lim, 1991; Tsuchihashi et al., 2003; Lee et al., 2010). However, these methods of hormone administration have problem such as incomplete sex inversion and reverted back to being females during the next reproductive period (Marino et al., 2000; Sarter et al., 2006; Oh et al., 2013). Therefore, the most effective treatment time for artificial sex change induction by



**Fig. 2. Cross section gonad of juvenile red spotted grouper at 12 month after hatching.** A, control group; B, treatment group after immersion with 17 $\alpha$ -MT 1 mg/L for 4 and 8 weeks; C, treatment group after immersion with 17 $\alpha$ -MT 5 mg/L for 4 weeks; D, treatment group after immersion with 17 $\alpha$ -MT 5 mg/L for 8 weeks. In A to C group, fish were all female with ovarian cavity and consisted of numerous ovarian lamellae with perinucleolus oocyte. In D group, fish were induced primary male without ovarian cavity and consisted of numerous testicular lobules with spermatogonia, spermatocyte and spermatozoa. OC, ovarian cavity; PO, perinucleolus oocytes; SG, spermatogonia; SO, spermatocyte; SZ, spermatozoa. Scale bars indicate 50  $\mu$ m (A to C) and 100  $\mu$ m (D).

steroid hormone is during gonadal sex differentiation. In this study, sex-changed males without ovarian cavity were observed when juvenile red spotted grouper (70 DAH) were immersed in 17 $\alpha$ -MT 5 mg/L for 8 weeks. These results suggested that the red spotted grouper be able to induce the primary males by hormone treatment prior to gonadal sex differentiation.

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