Morphological and physiological studies on sex change in tropical fish: Sexual plasticity of the ovaries of hermaphroditic and gonochoristic fish

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Abstract  To clarify the role of estrogens in the onset of sex change in fish, estrogen levels were artificially reduced in hermaphroditic and gonochoristic female fish via the treatment with aromatase inhibitors (AIs). AI treatments caused depletion in blood estrogen levels and induced complete sex change from a female to a male in protogynous three-spotted wrasse and the honeycomb grouper. Opposite-direction sex change of the protandrous yellowtail anemonefish was also induced by AI treatments. Not only in hermaphrodites, AI treatments induced testicular differentiation and, in certain circumstances, a complete sex change in the developing ovaries of the gonochoristic fish: tilapia, medaka, zebrafish, carp and golden rabbitfish. We demonstrated that estrogen depletion induces the female to male sex change in both hermaphroditic and gonochoristic fish. Results suggested that some germ cells in the ovaries of both hermaphroditic and gonochoristic fish maintain sexual bipotentiality, which is the ability to differentiate into both female and male germ cells, throughout the life and that the fate of these germ cells’ differentiation depends on the level of endogenous estrogen. Higher levels of circulating estrogen maintain femaleness, while lower levels force their differentiation into males. These studies contribute to the progress of aquaculture.

Keywords  Aromatase inhibitor, Sex change, Estrogen, Plasticity, Ovary, Testis

Introduction  For over 50 years, the lead author of this review, Masaru Nakamura, studied the morphological and physiological mechanisms of sex differentiation in teleost fish. His focus remained on the role of endogenous sex hormones in sex differentiation and on the establishment of techniques applicable to aquaculture, such as sex control of tilapia and salmonid fish by the treatments of exogenous sex hormones. Through collaboration with other scientists, he demonstrated that endogenous estrogen func-
tions as an ovarian inducer and that the lack of estrogen induces testicular differentiation (Nakamura et al. 1998, 2003; Nakamura 2000, 2013; Develin and Nagahama 2002; Strüssmann and Nakamura 2002). Researchers found that developmental window of morphological sex differentiation is very sensitive to exogenous estrogen and androgen and that treatments of developing gonads with exogenous estrogens and androgens around this window can cause a reversal of their phenotypic sex. On the other hand, dogma of reproductive biology suggested that it is impossible to induce a complete sex reversal after sexual differentiation of gonads (Nakamura and Takahashi 1973; Nakamura 2000), suggesting that the testes and ovaries of fish lose sexual plasticity after sex differentiation. While still an undergraduate, Masaru Nakamura learned from his university lectures that fish can change sex in adulthood (Atz 1964). His interests unfolded to understand mechanisms underlying regulation of sexual plasticity in naturally sex changing fish. Beginning in 1984, his studies initially focused on the physiological analysis of sex change in fish, using the protogynous saddleback wrasse, Thalassoma duperrey (Quoy & Gaimard, 1824), in collaboration with various scientists, namely Prof. Gordon E. Grau, at the University of Hawai’i, Dr. Y. Nagahama, Dr. S. Adachi, and Dr. T. Kobayashi at National Institute for Basic Biology, and Dr. K. Yamauchi at Hokkaido University. In 2000, he moved to Tropical Biosphere Research Center, Sesoko Station, of the University of the Ryukyus. Although the facilities for the culture of fish and corals were available, the supply of seawater was initially inadequate. In three years, a building was constructed and facilities for the state-of-the-art research were established, mainly a constant supply of seawater, tools for marine biological research, and fish holding system (10- and 50-ton tanks). He started a highly functional laboratory to study further mechanisms underlying sex change in hermaphroditic fish. In the meantime, many undergraduate students, graduate students, and postdoctoral researchers participated in his research project. Many researchers from across Japan and the world collaborated with him. This review paper summarizes the research of Masaru Nakamura and his collaborators at Sesoko Station on the sexual plasticity of the fish gonads and the role of estrogens in the maintenance of ovaries in the hermaphroditic and gonochoristic fish.

The sexuality of fish is usually divided into two types; hermaphroditism (herm) and gonochorism (Kuwamura et al. 2020). The herm is subdivided into synchronous herm and sequential herm, which is further subdivided into three types; protogynous herm, protandrous herm, and bi-directional sex change. Protogynous fish change their sex from female to male, and protandrous fish change it from male to female. Bi-directional sex change can change sex in both directions repeatedly. To analyze the physiological mechanisms regulating each type of sex change, we used the protogynous three-spotted wrasse, Halichoeres trimaculatus (Quoy & Gaimard, 1834), the Marber grouper, Epinepherus malabaricus Bloch & Schneider, 1801, the honeycomb grouper, E. merra Bloch, 1793. The yellowtail clownfishes, Amphiprion clarkii (Bennett, 1830) and Trimma okinawae (Aoyagi, 1949), were used to study protandrous and bi-directional sex change, respectively. To compare with hermaphrodites, we used the gonochoristic tilapias, Oreochromis niloticus (Linnaeus, 1758) and O. mossambicus (Peters, 1852), and the golden rabbitfish, Siganus guttatus (Bloch, 1787). Gonadal structures change dramatically during sex change. It is difficult to recognize in detail the gonadal changes from external attributes, such as nuptial colorations, sexual behavior, and urinogenital papillae. Taking histological and ultrastructural analysis approaches, we studied morphological changes in gonadal germ cells and somatic cells of the fish undergoing sex change. To understand the role of sex hormones in sex change, we measured their serum levels. In addition, the expression of steroidogenic enzymes essential for sex hormone production was immunohistochemically examined, using specific antibodies, in gonads during sex change.

These studies of the physiology of sex change in fish were performed at Sesoko Station and have been published in English and Japanese (Nakamura et al. 2003, 2005; Paul-Prasanth et al. 2011; Kobayashi et al. 2013, 2018; Kobayashi and Nakamura 2016; Nakamura and Kobayashi 2019; Murata et al. 2020; Nagahama et al. 2020; Nozu et al. 2021).

We demonstrated that estradiol-17β (E2, the primary estrogen in fish) depletion, rather than the increase of 11-ketotestosterone (11-KT, the primary androgen in
fish), correlates tightly with the beginning of sex change in the saddleback wrasse, *T. duperrey* (Nakamura et al. 1989). From this finding, we hypothesized that estrogen depletion might trigger sex change. However, it is difficult to analyze the loss of function of estrogen in the sex change of fish because estrogen production in the ovaries usually occurs in the follicle cells, which have close contact with germ cells (Kagawa et al. 1982). Thus, it is technically challenging to separate germ cells from the effects of estrogen in the ovaries during sex change. To test this hypothesis, we used aromatase inhibitor (AI) to induce estrogen depletion in live fish. AIs act by inhibiting cytochrome P450 enzyme aromatase, which catalyzes the conversion of androgen to estrogen (Steele et al. 1987). AIs have been used to treat breast and ovarian cancer in postmenopausal women (Smith and Dowsett 2003; Howell et al. 2005). It is also known that AI treatments around sex differentiation in fish can induce sex change (Piferrer et al. 1994; Nakamura et al. 1999; Kroon et al. 2000; Rukusana et al. 2010) reported that the administration of AI (Fadrozole) induces sex change in protogynous fish. We also examined the effects of estrogen depletion on the ovaries of hermaphroditic fish treating them with AIs. We also examined the effects of AI on the developing ovaries of gonochoristic fish which do not change sex naturally.

A. The effects of AI on the ovaries of hermaphroditic fish

The protogynous three-spotted wrasse, *H. trimaculatus*

Females of the three-spotted wrasse usually change sex to male when the largest male disappears from the group consisting of a larger male and some smaller females. Ovaries of females consist of ovarian tissue only. By feeding females with the diet containing AI (Fadrozole. 500 μg/g diet) for three (AI-3), five (AI-5), and 10 days (AI-10) (Nozu et al. 2009, 2013, 2015), we examined whether depletion of estrogen results in their sex reversal to males. The gonads of the fish in the treated and control groups were examined histologically at the end of the AI treatment and at 30 days after the start of the experiment. At the end of AI treatment, all individuals in the AI-3-treated group had gonads with degenerating yolky oocytes, indicating the onset of sex change. Most individuals in the AI-5 treated group had ovaries with atretic yolky-oocytes, similar to those of the AI-3 treated group. One of the fish had male germ cells, presumably spermatogonia, on the outer periphery of the ovigerous lamellae together with degenerate yolky oocytes (Fig. 1A). The time point at which the ovary had just committed to transformation into a testis was not detected histologically at the end of AI treatment. However, by 30 days after the AI treatment had started, the gonads of the AI-5 treated group (testes) were different from those of the AI-3 treated group (ovaries). This finding suggested the presence of molecular or physiological differences in the ovaries of the AI-3- and AI-5-treated fish by the end of AI treatment. We were able to identify the site of testicular differentiation in the ovary during sex change in these fish.

All individuals in the initial control and control groups were females with mature ovaries (Fig. 1B). At 30 days

![Fig. 1](image-url) Histological sections of the gonads of three-spotted wrasse. (A) Gonad in AI-5 treated fish at the end of AI treatment. (B) Mature ovary of fish in the initial control. (C) Mature testis with an ovarian cavity from a fish in the AI-5 treated group at 30 days after the start of experiment. Abbreviations: OC, Ovarian cavity; PSG, spermatogonia; SZ, spermatozoa; VO, vitellogenic oocytes. Scale bar = 100 μm
after the onset of the experiment, approximately 70% of the individuals in the AI-3 group had mature ovaries. The remaining fish had a mature testis with active spermatogonic germ cells and an ovarian cavity, indicating that the sex change had occurred. All individuals in the AI-5, AI-10 group had mature testes (Fig. 1C). At the end of the AI treatment, serum E2 levels of fish in the AI-3, AI-5, and AI-10 groups were significantly lower than those in control fish (Fig. 2). The results indicate that a short-time AI treatment (for five days) resulted in a complete sex change. In other words, the initiation of physiological sex change in females of the three-spotted wrasse, meaning the point of no return, took only five days under rapid estrogen depletion.

Fig. 2 The serum E2 levels at the end of AI treatment. The asterisks indicate significant differences from controls (P < 0.05).

The protogynous honeycomb grouper, E. merra

Groupers exhibit protogynous sex change. The undifferentiated gonads of all individuals differentiate into ovaries during the fry stage, and the gonads mature in adulthood (Murata et al. 2020). After sexual maturation, an ovary changes into a functional testis (Nakamura et al. 2005). To investigate artificial sex change in the breeding season, we implanted females honeycomb groupers (76.08±24.02 g in body weight; 21.6–15.8 cm in total length) with AI (Fadrozole; 10 mg/kg BW), either alone or in combination with E2 (10 mg/Kg BW) (Bhandari et al. 2005). After 75 days of treatment, all the control fish remained as females (Fig. 3A), whereas all the AI-treated fish had undergone complete sex reversal from the female to the male (Fig. 3B). In contrast, cotreatment of AI with E2 blocked ovaries from sex change (Fig. 3C). AI treatment significantly reduced circulating levels of E2 (Fig. 4A). The plasma 11-ketotestosterone (11-KT) levels were increased in the AI-treated fish, while the levels in the E2–supplemented fish were low compared to controls (Fig. 4B).

The protandrous yellowtail anemonefish, A. clarkii

Individual groups of yellowtail anemonefishes, which maintain a monogamous mating system, are symbiotic with a sea anemone. A male changes sex to female when the largest female disappears from a group (Moyer and Nakazono 1978; Hattori 1991; Buston 2003). The gonad of fish during the male phase consists of both immature ovarian tissue and mature testicular tissue (Godwin 1994). In contrast, during the female phase, only ovarian tissues, which carry many mature oocytes (Fig. 5A), are present. Thus, testicular tissue in the bisexual gonads of the male phase disappears completely during sex change. To elucidate the mechanisms underlying the sexual plasticity in the ovary of female anemonefish, we administered functional female with AI (Fadrozole, 500 μg/g diet) for 80 days (Nakamura et al. 2015). Three out of 5
Fig. 4  The plasma levels of E2 (A), and 11-KT (B) in the honeycomb grouper treated with AI. Bars with different letters (a and b) differ significantly from another (P<0.05).
Abbreviations: AI, AI-treated female grouper; AI/E2, AI and E2 cotreated female grouper.

Fig. 5  Histological sections from the gonads of yellowtail anemonefish. (A) The ovary of fish in the control group. (B) The ambisexual gonads of fish in the AT-treated group. Abbreviations: S, spermatozoa; Oc, ovarian cavity; Y, yolky oocyte. Scale bar = 200 µm

Fig. 6  The plasma levels of E2, and 11-KT in AI and control groups. Bars with different letters (a and b) differ significantly from another (P<0.05). Abbreviations: IC, initial control group; C, control group; AI, AI treated group.
fish in the AI-treated group had ambisexual gonads displaying both testicular tissue (Fig. 5B), including many cysts of spermatogenic germ cells and ovarian tissue which contained many meiotic germ cells and oocytes at the peri-nucleolus stage, a situation similar to the bisexual gonads of male phase fish. Blood E2 levels initially were high in females but dropped significantly when treated with AI (Fig. 6A). The mean plasma 11-KT levels in the AI-treated group was significantly higher than at the beginning of treatment and in the control group (Fig. 6B). From these results, we concluded that AI treatment induced estrogen depletion and that depletion of estrogen can cause an opposite-directional sex change, from functional female to male in the protandrous anemonefish. Protandrous black porgy Acanthopagrus schlegelii (Bleeker, 1854) under 2 years old has the bisexual gonads both functional testicular tissue and immature ovarian tissue (Wu et al. 2010). It is known that ovarian tissue develops, whereas testicular tissue regresses during natural sex change from male to female. Lee et al. (2002) demonstrated that AI treatment blocked natural sex change of the black porgy.

The effects of AI on the ovaries of gonochoristic fish.

Nile tilapia, O. niloticus

Gonochoristic fish do not undergo natural sex change and their sexual plasticity was believed to be lost after phenotypic sex differentiation of gonads. To examine their ability to change artificial sex change, we treated females with the AI for 180 days (Paul-Prasanth et al. 2013). All gonads of adult females treated with AI (Fadrozole 200 μg/g diet) for 180 days had sex-reversed gonads with spermatogenic germ cells occupying either the entire or at least one-half of the gonad (Fig. 7A). On the other hand, female tilapia receiving co-treatment of AI and E2 (200 μg/g diet each) had ovaries with follicles at different stages of development comparable to those in the control ovaries (Fig. 7B). In female fish treated with AI alone, plasma levels of E2 were significantly lower than those of the control group at 90 and 180 days of treatment (dot) (Fig. 8A and C). In contrast, no discernible changes were seen in the levels of 11-KT in fish at 60 (data not shown) and 90 days of treatment (dot) (Fig. 8B), whereas the plasma E2 and 11-KT levels in fish with co-treatment of E2 were comparable to those of the control (Fig. 8A, B, C and D). Significant increases in plasma levels of 11-KT were observed in female tilapia treated

![Histological sections from the gonads of Nile tilapia.](image)

**Fig. 7** Histological sections from the gonads of Nile tilapia. (A) Ovary of AI-treated female tilapia having transformed into testes with spermatozoa and sperm (white arrows). (B) An intact mature follicle in the ovary of an AI/E2 treated female tilapia. Scale bars are 100 μm and 1 μm.

![Plasma levels of E2 and 11-KT in AI-treated female tilapia](image)

**Fig. 8** Plasma levels of E2 (A) and 11-KT (B) in AI-treated female tilapia at 90 days of treatment (dot), and E2 (C) and 11-KT (D) at 180 dot. Abbreviations: Co, control; AI, AI-treated female tilapia; AIE, AI+E2 co-treated female tilapia. P < 0.05.
with AI at 180 dot (Fig. 8D).

To identify the site of testicular differentiation in the immature ovary of tilapia, females at 60–80 days after hatching (dah) were given a diet containing AI (Fadrozole 200 μg/g) for over one month (unpublished data). Ovaries of females in the control group at 60–80 dah had many young oocytes at the peri-nucleolus stage and many oogonia, which usually were distributed in the area surrounding the ovarian cavity (OC) on the lateral side of the body wall (Fig. 9A). Somatic tissue facing the bottom of the OC (Fig. 9B) changed irregularly in females treated with AI for more than one month. In the next stage, presumptive spermatogonial germ cells increased in number in this area (Fig. 9C). Subsequently, testicular tissue containing active spermatogenic germ cells spread along with the OC (Fig. 9D). Some sperm fluid appeared in the newly differentiated and developed efferent ducts. These results demonstrated the site of testicular differentiation in the developing ovaries of tilapia.

Other gonochoristic fish

We were also able to induce testicular differentiation in the developing ovaries of gonochoristic fish (Ogawa et al. 2008; Paul-Prasanth et al. 2013; Takatsu et al. 2013; Rahaman et al. 2020). One-year-old genetically female (XX) carp, *Cyprinus carpio* Linnaeus, 1758, were fed a diet containing AI (Fadrozole 200 μg/g diet) for 24 weeks (Ogawa et al. 2008). Testicular lobule-like structures and cysts of spermatocytes began to appear after eight weeks of treatment, and spermatozoa were observed first at week 16 and then spread to the whole gonad in some individuals by week 18 (Fig. 10A). A fish treated with AI

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**Fig. 9** Histological sections from the ovaries of genetic female Nile tilapia. (A) Normal immature ovary of a fish at about 60 days of hatching. (B) The ovary of a fish treated with AI for more than one month. Note the tissue structure (arrow) changes near the edge of the ovarian cavity (OC). (C) Initial testicular differentiation in the ovary; testicular tissue, including spermatogonia and spermatocyte (arrow), appear near the OC. (D) Developed spermatogenic tissues in the ovary (arrow) spreads along with the wall of the OC. Bar indicates 50 μm.

**Fig. 10** Histological sections from the gonads of genetic female carp after 22 weeks, AI-treated and control groups. (A and B) Testicular tissue (arrow) develops in the ovary of fish treated with AI. (C) Females in control group have immature ovaries with young oocytes. The asterisk indicates atretic oocytes. Bars indicate: 100 μm in (A), 600 μm in (B) and 50 μm in (C).
had the bisexual gonads both matured testicular tissue and ovarian tissue (Fig. 10B). Females in the control group had immature ovaries (Fig. 10C). Mature female medaka, *Oryzias latipes*, were exposed to AI (Exemestane 100 μg/L water) for 60 days (Paul-Prasanth et al. 2013). Females in control had matured ovaries (Fig. 11A and B). Functional testes were developed in the gonads of the female by inhibition of aromatase for 60 days (Fig. 11C). Immuno-positive cells against P450scc antibody appeared in the testicular tissue (Fig. 11D). Co-treatment of E2 inhibited AI-induced sex reversal. Spermatogenic cysts originated from single germ cells or clusters of germ cells that exhibited characteristics of germline stem cells in the germinal epithelium on the side of the ovary adjacent to the ovarian cavity. Control fish treated with the vehicle (99% ethanol) showed more amount of E2 in the ovaries, while AI-treated ovaries had an amount equal to that in the testis of the normal males (Fig. 12A) at 10 dot. 11-KT was significantly increased in the exposed ovaries at 10 dot when compared with that of the control ovaries, but was lesser than that in the testis of the normal males (Fig. 12B). Sexually mature zebrafish females, *Danio rerio*, were fed a diet containing AI (Fadrozole 200 μg/g diet) for five months (Takatsu et al. 2013). Eventually, the ovaries of females (Fig. 13A) changed to functional testes containing a large number of sperm (Fig. 13B). Artificial fertilization using sperm from the sex-changed females was successful. Recently, Rahaman et al. (2020) succeeded to induce rapid sex change of female by the AI-treatment for two months. In a one-year-old golden rabbitfish (*S. guttatus*) fed a diet containing AI (Exemestane 1 mg/g diet) for 4 months (unpublished data), testicular tissues containing cysts of spermatogonia, spermatocytes, and spermatids were found among degenerating oocytes in one ovary of the three females.

![Fig. 11](image1.png)

**Fig. 11**  
Histological sections from the ovaries of XX medaka. (A) Ovary of control XX medaka showing vitellogenic and post-vitellogenic follicles together with post-ovulatory follicle (black arrow). (B) Magnified image of the post-ovulatory follicle shown in the dotted rectangle area in (A). (C) Testis formed in an adult breeding XX-female by AI for 60 days. (D) P450scc-positive cells appear in the testicular tissue. Scale bars indicate 500 μm (A), 250 μm (B), 80 μm (C) and 50 μm (D).

![Fig. 12](image2.png)

**Fig. 12**  
(A) Tissue levels of E2 in the ovary of AI-treated XX medaka. (B) Tissue levels of 11-KT in the ovary of AI-treated medaka. Abbreviations: Co, control; Al, AI-treated female tilapia; AIE, AI/E2 co-treated female tilapia. The asterisks indicate significant differences from controls (P<0.05).
treated with AI (Fig. 14). A small amount of sperm was also found in the newly developed efferent ducts.

From the series of AI experiments using the females of various gonochoristic fish, we demonstrated that estrogen depletion causes testicular differentiation in the developing ovaries of gonochoristic fish and that some germ cells in the ovaries of gonochoristic fish retain plasticity.

B. Estrogen depletion and the germ cells’ differentiation in the ovary of teleost fish

In the present study, treatment with AI induced estrogen depletion and sex change from female to male in the hermaphroditic three-spotted wrasse, the honeycomb grouper, and the yellowtail anemonefish. On the other hand, co-treatment of AI and E2 prevented sex change in the ovary of the wrasse and the grouper (Higa et al. 2003; Bhandari et al. 2005). Zang et al. (2013) evidenced that methylation of the promoter region of cyp19a1a acts as the sex change of protogynous rice field eel Monoperus albus (Zuiw, 1793). Wu et al. (2016) also demonstrated that a high level of methylation at the cyp19a1a promoter in the ovary of the black porty was the primary predator of whether the testis of the digonic gonad is developed or degenerated. These results strongly suggest that estrogen depletion in females mediates sex change in hermaphroditic fish. We also demonstrated that AI treatment induces low estrogen serum levels in females of the gonochoristic fish tilapia (serum) and medaka (gonad) and induced testicular differentiation or complete sex reversal in the developing ovaries of females of five species of gonochoristic fish. Conversely, co-treatment with AI and E2 also inhibits AI-induced sex change in medaka and tilapia (Paul-Prasanth et al. 2013). These findings suggest that the loss of function of E2 results in testicular differentiation or complete sex change to males not only in females of hermaphrodites but also in females of gonochoristic fish. In other words, higher estrogen levels in the ovary play a role not only in the differentiation of female germ cells and the maintenance and maturation of the ovaries but also in preventing differentiation of bipotential germ cells into male germ cells.

Ohta et al. (2007) reported that androgen, but not estrogen, plays a principal role in the changes in both gonadal morphology and body color in the transformation from female to male in the wrasse, Pseudolabrus sieboldin Mabuchi & Nakabo, 1997.

However, in the saddleback wrasse, we did not detect substantial increases in androgen levels in the fish just after the onset of natural sex change (Nakamura et al. 1989). We confirmed increases in serum or plasma levels
of 11-KT, instead of decreases in E2, during sex change in the grouper, the yellowtail anemonefish, tilapia, and medaka (gonad) during sex change artificially induced by the AI treatment. We also demonstrated that androgen treatments can induce sex change in the three-spotted wrasse and the honeycomb grouper (Higa et al. 2003; Bhandari et al. 2006). We believe that treatment with androgen or AI may have caused changes in the ratio of estrogen (lower) to androgen (higher) in the body. We, therefore, hypothesize that low estrogen levels, relative to androgen levels, are important for the initiation of sex change from female to male.

Ovaries of some protogynous fish contain some traces of spermatogenic germ cells (Robertson 1972; Moyer and Nakazono 1978; Reinboth 1980; Shpigel and Fishelson 1986). However, we did not find spermatogonia in the ovary of the saddleback wrasse and the yellowtail anemonefish, similar to ovaries of gonochoristic fish. Thus, it is interesting to know the origin of spermatogenic tissues in developing ovaries of hermaphroditic and gonochoristic fish that lack apparent testicular tissue. We found that spermatogenic tissues arose from some gonial germ cells and germline stem cells in the ovaries of the protogynous three-spotted wrasse and the gonochoristic fish, tilapia, and medaka (Nozu et al. 2009; Paul-Prasanth et al. 2013). Some gonial germ cells in the developing ovaries of hermaphroditic and gonochoristic fish seem to retain bipotentiality, the ability to differentiate into spermatogonia or oocytes. We, therefore, assume that some gonial germ cells, so-called oogonia, in the ovaries of most gonochoristic fish might have bipotentiality. These cells usually differentiate into oogenetic germ cells in presence of higher estrogen levels, whereas they become spermatogenic germ cells when experience depleting levels of estrogen in the ovary.

C. The application to aquaculture

With AI treatment, we successfully induce precocious male development in the honeycomb grouper. These males mated with females and successfully fertilized eggs (Bhandari et al. 2004a, 2004b; 2005; Alam et al. 2006). Later, it was found that AI treatments effectively induce precocious and functional sex change in females of various species of groupers (Li et al. 2006; Hur et al. 2012; Garcia et al. 2013; Wu et al. 2015; Evliyaoğlu et al. 2019). This new technique for the masculinization of smaller females by AI makes a significant contribution to seed production in the aquaculture of groupers, obviating the need to obtain large males from the wild for brood stock (Nakamura and Kobayashi 2019; Murata et al. 2020; Nozu et al. 2021).

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Compliance

All animal handling and experiments were conducted in accordance with our Guide for the Care and Use of Laboratory Animals (Doubutu-jikken-kisoku, 19.6.26)
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