Gonadal maturation of Indonesian leaffish (Pristolepis grootii) using pregnan mare serum gonadotropin and luteinizing hormone-releasing hormone analog

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Gonadal maturation of Indonesian leaffish (*Pristolepis grootii*) using pregnan mare serum gonadotropin and luteinizing hormone-releasing hormone analog

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ABSTRACT

Indonesian leaffish (*Pristolepis grootii*) is one of Indonesia's endemic fish species that has the potential to be cultivated. The unavailability of quality seeds is a main obstacle in *P. grooti* farming. This study aimed to induce gonadal maturation of *P. grootii*. A total of 95 females (100.81 ± 0.78 g), reared in 15 aquaria (n=9), fed with a commercial pellet, and injected twice on the 7th and 14th day.with PMSG 10 IU.kg^{-1} of BW (T1), PMSG 10 + LHRHa 50 g.kg^{-1} of BW (T2), PMSG 20 (T3), PMSG 20 + LHRHa 50 (T4), and control (T5). Blood samples were collected from the caudal vein at 0, 30, and 60 days post-injection. The results showed that *P grootii* injected with a combination PMSG 20 + LHRHa 50 had the highest estradiol 17β (905.46±83.09 ρg.ml^{-1}), total cholesterol (383.78±40.57 mg.dL^{-1}), GSI (1.68±0.12 %), fecundity (2,946±174.72 egg.fish^{-1}), and oocyte diameter (922.64±11.54 μm). The combination of PMSG 20 IU.kg^{-1} of BW + LHRHa 50 g. kg^{-1} of BW was the best treatment for gonadal maturation of *P. grootii*.

1. INTRODUCTION

Gonadal maturation in vertebrates (including the fishes) is controlled by the hypothalamus-pituitary-gonad (HPG) axis and gonadotropin-releasing hormones (GnRH) regulate the differentiation of the gonad via the HPG axis. GnRH plays an important role in the regulation of the reproductive system. Its pulsatile secretion determines the pattern of secretion of the follicle-stimulating hormone (FSH) and luteinizing hormone (LH) which then regulate both the endocrine function and gamete maturation in the gonads [1]. FSH and LH are secreted into the bloodstream, regulating the synthesis of sex steroids (androgens and estrogens). FSH predominantly regulates early gonadal development and induces maturation, and LH dominates gonadal maturation. Thus, the activation of the HPG axis is commonly regarded as the initiation of maturation and its advancement can therefore be attended by measuring androgens and or estrogens in the blood [2].

Wild fish was adapted to the captive rearing tend to experience a reproductive system dysfunction. These disturbances probably result from the combination of captivity-constrained stress and the lack of a suitable "natural" spawning environment [3], [4]. Therefore, hormonal induction is needed [5]. The direct stimulation of gonadotropin used a type of hormonal therapy, which replaced the insufficient production of endogenous with exogenous hormone. This is done by injection of synthetic gonadotropin analog, both for maturation and spawning. Currently, a variety of highly potent synthetic GnRHa agonists are available, as well as advanced-release delivery systems for their controlled administration. These methods have contributed significantly to the development of more reliable and less species-specific methods for controlling the reproduction of captive broodstock.

Hormonal manipulations can accelerate gonadal maturation and ovulation [6], [7]. Hormones that can be used for maturation include pregnant mare serum gonadotropin (PMSG) and luteinizing hormone-releasing hormone (LHRH). The success of gonadal maturation induction using PMSG, among others, in eel, *Anguilla bicolor bicolor* [8]; silver pompano, *Tracinotus blochii* [9]. The use of LHRH for gonad maturation of fish was also successful in the Benni fish, *Barbus sharpeyi* [10]; waigieu seaperch, *Psammoperca waigiensis* [11]; kuntum, *Rutilus frisii kutum* [12]; channel catfish, *Ictalurus punctatus* [13], and spotted sand bass, *Paralabrax maculatofasciatus* [1].

The introduction of new species into aquaculture is often hampered by the unreliable supply and quality of larvae and juveniles. In most species, sophisticated protocols are required to induce maturation, and synchronize breeding in captivity, via hormonal manipulation [5]. The Indonesian leaffish (*Pristolepis grootii*) is one of Indonesia's endemic fish species that has the potential to be cultivated [14]. It is locally known as 'sepatung'. “patong” or 'katong', and nationally “sepatung”. These fish are found in rivers, canals, swamps, floodplains in South Sumatra [15]. This species has been traded globally as ornamental fish. Local people use it as side dishes and aquarium fish [16]. Anthropogenic activities such as overexploitation by overfishing, swamp reclamation, and degradation of spawning habitats have led to a decline in the natural population of *P. grootii*. Considering consumers' preferences and market value and to preserve the biodiversity, this species should be protected from being extinct, via aquaculture. This study was conducted to evaluate the effectiveness of the administration of LHRHa and PMSG for gonadal maturation of *P. grootii*.

2. MATERIALS AND METHODS

2.1 Fish rearing

The immature females of *P grootii* were from the Kelekar river, Ogan Ilir regency, South Sumatra, Indonesia. Fish were acclimatized for one month in one fiber tank (2000 liters). During rearing, fish were fed with commercial pellets (protein 30-33%, fat 4-6%, crude fiber 2.7%), three times a day at satiation. Then the fishes were selected (n=95), weighed and the initial weight obtained was 100,81±0,78 g.fish^{-1}, with the condition of gonad maturation previtellogenic phase. Ninety fish were randomly transferred into 15 aquaria (65x50x50 cm^3, n=6), a water volume of 90 liters. The water of the aquaria was renewed every seven days, 10% volume. Water temperature, dissolved oxygen, and pH were monitored in-situ daily. Water temperature ranged from 25.9 to 30 °C during the experiment, pH, and dissolved oxygen ranged from 5.3 to 6.8; 5.88-7.91 mg.L^{-1}, respectively. Fish from all groups were kept under a natural photoperiod regime for local geographic location.

2.2 Hormone treatment

Two synthetic gonadotropin hormones used were pregnant mare serum gonadotropin (PMSG) (Folligon®, Intervet International B.V., Netherlands) and luteinizing hormone-releasing hormone analog (LHRHa) (Quintrol®, Dong Bang. Co.Ltd, Taiwan). The doses of hormones used were PMSG 10 $IU.kg^{-1}$ of BW (Code T1), PMSG 10 $IU.kg^{-1}$ of BW + LHRHa 50 $g.kg^{-1}$ of BW (T2), PMSG 20 $IU.kg^{-1}$ of BW (T3), PMSG 20 $IU.kg^{-1}$ of BW +LHRHa 50 $g.kg^{-1}$ of BW (T4), and one group was injected with a physiological solution (NaCl 0.9%) (T5). All fish in all experimental groups were injected intramuscularly, twice on the 7th and 14th days. Prior to injection, the fish were anesthetized using tricaine methane-sulfonate (MS-222) at a dose of 1 mL per 3 liters of water.

2.3 Blood sampling

Blood samples were taken three times, namely on days 0, 30, and 60 post-injection. Previously, the fish were anesthetized (MS-222, 60 ppm). Blood were taken from the caudal vein using a 1 mL syringe with anticoagulant (EDTA, ethylene diamine tetraacetate), as much as 0.5 to 1 mL (pulling from 3 fish). The blood samples were put into a microtube and immediately put into an icebox, then the sample was centrifuged at 3000 rpm for 15 minutes. The separated blood plasma was put into a new microtube, labeled according to treatment, stored in a freezer at -20 °C for further analysis. The measurement of estradiol-17β (E2) concentration was done by enzyme-linked immunosorbent assay (ELISA) method using a commercial kit (DRG International Inc). Total cholesterol were determined by the cholesterol oxidase-phenol amino phenazone (CHOD-PAP) method using a commercial kit (®Glory Diagnostic, Spain). The analysis procedure following manufacture instructions.

2.4 Sampling of gonad and liver

Sampling of gonad and liver was done on day 0 and day 60 for analysis of gonadosomatic index (GSI) = [weight of gonad (g) / weight of fish (g) x 100], hepatosomatic index (HSI) = [weight of liver (g) / weight of fish (g) x 100], and histology of gonad. Fish were sacrificed and dissected. Gonads and livers were weighed with digital scales (nearest 0.01 g).

The gonads were cut into small slices, fixed in Bouin's solution, dehydrated and infiltrated, then embedded in paraffin, cut at 5 μm, and stained with hematoxylin and eosin [17]. Histological examination with a binocular microscope (Olympus, Tokyo) at 40x10 magnification. The oocytes were observed under a microscope, then an image was taken. The images were analyzed using ImageJ software which measured the diameter of one hundred oocytes.

2.5 Statistical analysis

The data were tabulated and analyzed by one-way analysis of variance (ANOVA), and the significant difference between the means was evaluated using the Duncan test. Statistical analysis was performed using Statistical Package for the Social Sciences software (version 25, SPSS Chicago, IL, USA), and differences were considered to be statistically significant at $P<0.05$. Data are presented as mean ± standard of deviation (SD).

3. RESULTS AND DISCUSSION

3.1 Estradiol-17β

The blood estradiol-17β level of fish injected with the combination of PMSG and LHRHa (T2 and T4) was higher than that of fish injected without combination (T1 and T3) and control (T5) ($P<0.05$). The highest estradiol-17β levels appeared at T2 and T4, observed on D30 ($P <0.05$). Treatment T1, T3, and T5 increased during the observation period. Treatment T2 and T4 decreased in the observation of D60 (Figure 1).

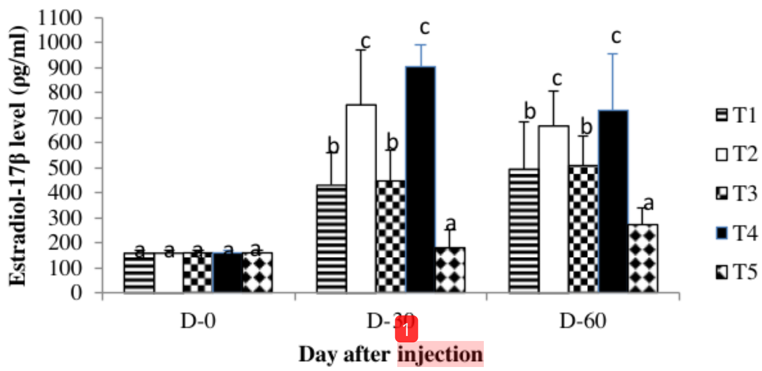

Figure 1. Indonesian leaffish (*Pristolepis grootii*) estradiol-17β levels after injection of PMSG 10 IU.kg^{-1} of BW (T1), PMSG 10 IU/ kg^{-1} of BW + LHRHa 50 g.kg^{-1} of BW (T2), PMSG 20 IU. kg^{-1} of BW (T3), PMSG 20 IU/ kg^{-1} of BW + LHRHa 50 g. kg^{-1} of BW (T4), control (T5). Different letters indicate a significant difference between treatments at the same time point (P<0.05). Data presented as mean ± SD (n=3).

3.2 Total cholesterol

The total cholesterol level of the injected fish was higher than that of the uninjected fish. In the D30 observation, the cholesterol level of fish injected with a combination of PMSG and LHRHa hormones (T2 and T4) was higher than that of fish injected without combined (T1 and T3). The highest total cholesterol levels appeared at T2 and T4, observed on D30 (P <0.05). Treatment T1, T3, and T5 increased during the observation period. Treatment T2 and T4 decreased in the observation of D60 (Figure 2).

Figure 2. Indonesian leaffish (*Pristolepis grootii*) total cholesterol levels after injection of PMSG 10 IU.kg^{-1} of BW (T1), PMSG 10 IU/ kg^{-1} of BW + LHRHa 50 g.kg^{-1} of BW (T2), PMSG 20 IU.kg^{-1} of BW (T3), PMSG 20 IU/ kg^{-1} of BW + LHRHa 50 g. kg^{-1} of BW (T4), control (T5). Different letters indicate a significant difference between treatments at the same time point (P<0.05). Data presented as mean ± SD (n=3).

3.3 GSI, HSI, fecundity, and oocyte diameter

The results showed that *P. grootii* was induced by a combination of PMSG 20 IU/ kg^{-1} of BW + LHRHa 50 g. kg^{-1} of BW had the highest GSI, fecundity, and oocyte diameter with values of 1.68±0.1%, 2.946±174.72 egg, and 922.64±11.54 μm, respectively. The GSI, HSI, fecundity, and oocyte diameter values of *P. grootii* are presented in Table 1.

Table 1 The gonadosomatic index (GSI), hepatosomatic index (HIS), fecundity, and oocyte diameter of

Indonesian leaffish (*Pristolepis grootii*) injected with pregnant mare serum gonadotropin (PMSG) and luteinizing hormone-releasing hormone analog (LHRHa)

Treatment Code	Parameters (mean±SD)			
	GSI (%)	HSI (%)	Fecundity (egg)	Oocyte diameter (μm)
T1	1.05±0.04^{a}	1.03±0.07^{a}	2.558±183.59^{a}	889.92±37.03^{b}
T2	1.41±0.15^{c}	1.05±0.05^{a}	2.813±205.55^{b}	905.44±22.68^{c}
T3	1.14±0.10^{b}	1.02±0.04^{a}	2.619±186.51^{a}	899.52±48.99^{b}
T4	1.68±0.12^{d}	1.01±0.03^{a}	2.946±174.72^{c}	922.64±11.54^{c}
T5	0.98±0.05^{a}	1.07±1.05^{a}	2.306±152.96^{a}	820.16±45.95^{a}

T1=PMSG 10 IU.kg^{-1} of BW, T2=PMSG 10 IU/ kg^{-1} of BW + LHRHa 50 g.kg^{-1} of BW, T3=PMSG 20 IU.kg^{-1} of BW, T4=PMSG 20 IU/ kg^{-1} of BW + LHRHa 50 g. kg^{-1} of BW, T5=control. Different letters in the same column indicate a significant differences between treatments (P<0.05).

3.4 Gonadal histology

At the end of rearing (D60) the gonad maturity level of the fish was injected with a combination of PMSG and LHRHa hormones (T2, T4) in the final maturity phase (Figure 3-A). The fish injected with PMSG (T1) and LHRHa (T3) alone (Figure 3-BC) at the maturity phase, and the control group (T5) were in the end phase of vitellogenesis (Figure 3). 3-D). An overview of the gonadal structure of female *P grootii* is shown in Figure 3.

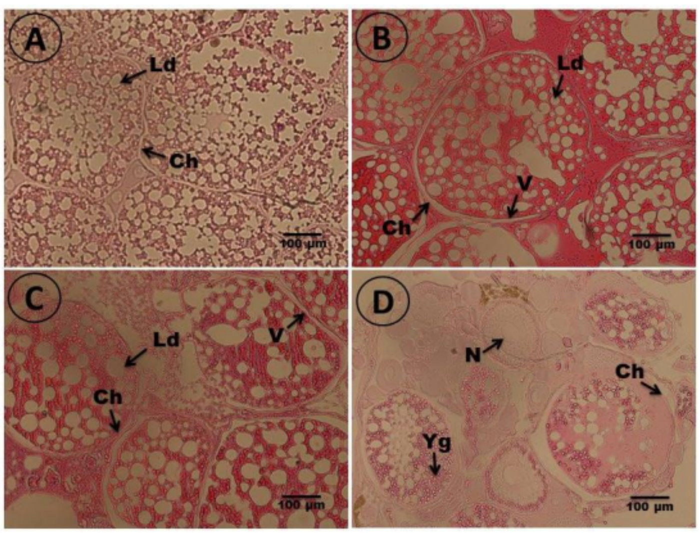

Figure 3. Histology of the ovaries of the female Indonesian leaffish (*Pristolepis grootii*) after injection of PMSG 10 IU.kg^{-1} of BW (A), PMSG 10 IU/ kg^{-1} of BW + LHRHa 50 g.kg^{-1} of BW (C), PMSG 20 IU.kg^{-1} of BW (B), PMSG 20 IU/ kg^{-1} of BW + LHRHa 50 g. kg^{-1} of BW (D), N= nucleus, Ld= lipid droplets, Yg= yolk granule, V= vitelline, Ch= chorion.

In this study, the highest levels of estradiol-17β and cholesterol were in fish injected with a combination of PMSG 20 IU.kg^{-1} of BW + LHRHa 50 g.kg^{-1} of BW, observed D30. Elevated cholesterol levels, associated with increased estradiol-17β levels. In fish, steroidogenesis occurs in the gonads, where cholesterol is processed into pregnenolone and subsequently into estradiol [18]. The conversion of cholesterol to pregnenolone involves enzyme action [19]. The sequential action of several steroidogenic enzymes results in the conversion of pregnenolone to active steroids such as estradiol-17β [20]. Increased levels of E2 indicate that the gonads are in the process of development. At the observation of D60, the level of estradiol-

17β decreased. This condition indicates that the gonads have entered the mature phase.

GSI is not only used to predict spawning season but also an indication of gonadal maturity. The highest GSI value was in the T4 treatment. The increase in GSI values correlates with HSI values because vitellogenin produced from the liver will be carried by the bloodstream to the gonads, then absorbed and stored in oocytes. Continuous absorption causes an increase in oocyte size and yolk accumulation, which causes the GSI value to increase [21]. The increase in GSI values also correlated with the resulting fecundity. The accumulation of increased vitellogenin in the gonads increases the resulting fecundity. The highest fecundity produced was 2,946±174.72 in T4 treatment. Fecundity along with other indices such as GSI and HSI are used to access fish reproductive conditions [22]. Vitellogenin absorption in oocytes affects oocyte diameter. The increase in oocyte diameter is accompanied by an increase in the level of gonadal maturity and stops at late maturity. The highest oocyte diameter (922.64±11.54) in T4 treatment. The increase in oocyte diameter is accompanied by an increase in GSI.

4. CONCLUSIONS

Hormonal induction for gonadal maturation of *P. grootii* has been successfully carried out. Hormone injection using a combination of PMSG 20 IU.kg^{-1} of BW + LHRHa 50 g. kg^{-1} of BW was the best treatment for gonadal maturation of *P. grootii*.

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