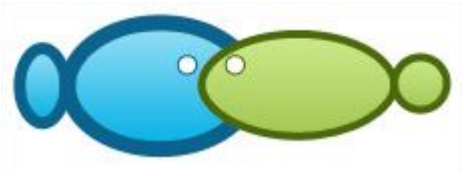


Induction of ovarian rematuration in striped catfish (*Pangasianodon hypophthalmus*) using pregnant mare serum gonadotropin hormone in out-of spawning season

[1,2]Wahyu Pamungkas, [2]Dedi Jusadi, [2]Muhammad Zairin Jr., [2]Mia Setiawati, [2]Eddy Supriyono, [1]Imron Imron

[1] Research Institute for Fish Breeding, Ministry of Marine Affairs and Fisheries, Subang, West Java, Indonesia; [2] Department of Aquaculture, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Dramaga Campus, Bogor 16680, West Java, Indonesia. Corresponding author: D. Jusadi, siflounder@gmail.com

Abstract. An experiment was conducted to study the effect of pregnant mare serum gonadotropin hormone (PMSG) on the induction ovarian rematuration of striped catfish (*Pangasianodon hypophthalmus*) in out-of spawning season. The experimental catfish were assigned to a completely randomized design consisting of four doses of PMSG (0, 5, 10 and 20 IU kg^{-1} BW) with three replication. Sixty females (weights ranging from 2 to 4 kg) and sixty males (weights ranging from 2 to 3 kg) were used in this study. Blood samples of striped catfish were collected at week 0, 2, 4, 6 and 8 to measure the concentrations of plasma estradiol-17ß and at week 0, 4 and 8 to measure vitellogenin. The reproduction parameters of the catfish were measured at week 8. The results showed that induction PMSG at a dose of 20 IU kg^{-1} BW resulted higher reproduction performance of the catfish, including higher concentration of estradiol and vitellogenin in blood plasma during the vitellogenesis process, accelerated oocyte development and ovarian rematuration, higher gonadal maturity level (100 percent), ovi somatic index, fecundity, larval production and survival rate than other treatment. Overall data suggested that induction of PMSG at a dose 20 IU kg^{-1} could be used to improve the reproductive performance of the striped catfish in out-of spawning season.
Key Words: striped catfish, pregnant mare serum gonadotrophin hormone, ovarian, hormone injection.

Introduction. The striped catfish, *Pangasianodon hypophthalmus*, is one of the superior aquaculture species, as a major aquaculture product on the world market (McGee 2014). Striped catfish have a great economic importance in India (Paniyar et al 2014) and are an important freshwater species in South and Southeast Asia, including Vietnam (Bui et al 2010), Malaysia (Asdari et al 2011), Bangladesh (Ahmed & Hasan 2007) and Indonesia (Griffith et al 2010). According to Ministry of Marine Affairs and Fisheries, Republic of Indonesia (2013), the increase of striped catfish production in Indonesia from 2010 to 2013 reached 95.57%. Also, the Ministry (2018) reported that the price of this particular species ranged between Rp. 20,000 and 28,000 per kilogram.

The striped catfish have been widely cultivated in most of region in Indonesia nearly 15 years. However, several problems such as the periodical seed availability due to low frequency of spawning, the quality and quantity of mature broodstock, etc., altogether result in unsustainable seed production. This happens because naturally striped catfish is a type of fish that spawns in certain seasons. The main spawning of catfish generally occurs in the rainy season, whereas during the dry season it is difficult to find gonadal maturation in females and took about six months for gonadal maturation (Zairin 2000; Manosroi et al 2004; Moses et al 2016). The duration of gonadal maturation is a problem in the continuation of yearly production of catfish, especially in the dry season.

The process of fish gonad maturation is naturally influenced by environmental signals received by the central nervous system and then the message forwarded to the hypothalamic gland (Bernier et al 2009), where the hypothalamic gland release the gonadotropin-releasing hormone (GnRH) into the pituitary gland; further the pituitary will release follicle stimulating hormone (FSH) (Chen & Fernald 2008). The increase of FSH concentration stimulates the aromatase enzyme to synthesize testosterone to estradiol-17β, thereby stimulating the synthesis of vitellogenin in the liver. Furthermore, vitellogenin is carried through the bloodstream to the gonad and its absorption occurs in the gonad by the follicular layer of the oocyte (Mylonas et al 2010).

Based on the role of hormonal factors on the gonadal maturation process, the acceleration of gonadal rematuration can be done by appropriate hormone manipulation. Research to accelerate maturation and rematuration have been conducted using several hormones, including pregnant mare gonadotropin serum (PMSG), gonadotropin salmon releasing analogous hormone (sGnRH) combined with antidopamine (AD), human chorionic gonadotropin (hCG) and estradiol-17β (Nagahama et al 1991; Legendre et al 2000; Sinjal et al 2014). The PMSG hormone is a type of gonadotropin hormone that is very important for the reproductive process. PMSG is one of the steroid hormones that comes from horse serum which contains gonadotropin as follicle stimulating hormone (FSH) and a least amount of luteinizing hormone (LH). PMSG containing FSH activate gonads to synthesize estradiol-17β which in turn stimulates the liver to produce vitellogenin (Nagahama 1983; Swanson et al 2003).

Based on the foregoing previous studies, a study on the induction of ovarian rematuration on the striped catfish broodstock was carried out using PMSG hormone to accelerate the gonad maturation of striped catfish. The aim of this study was to obtain an optimal dose that can accelerate the ovarian rematuration of the striped catfish broodstock in out of spawning season, especially on dry season.

Material and Method. The experiment was conducted in the experimental ponds of the Research Institute for Fish Breeding (RIFB), Subang, West Java, Indonesia on March - July 2018. Sixty female catfish (*Pangasionodon hypopthalmus*) from RIFB collection, weighing 2.5-4 kg were used as experimental models; the catfish was chosen due to its high fecundity. Sixty males weighing 2-3 kg were also used in this study. The sperm of males was used in the spawning process.

Striped catfish rearing. The selected catfish based on the gonad maturity stage (no eggs) were acclimated to experimental conditions for 2 weeks before hormonal treatment. The catfish broodstock were kept into 12 net cages, (3 m x 5 m x 1.5 m) located in 6000 m^2 of earthen pond at a stocking density of 10 fish per cage, consist of 5 females and 5 males for 8 weeks. During the experiment, the fish were fed two times a day using commercial feed (38% of crude protein) at 3% of the body weight (BW). Fish broodstock were tagged using a microchip to make it easier on observation of gonadal development, estradiol and vitellogenin profile of each individual. Blood samples were collected from two fish from each treatment at 0, 2, 4, 6 and 8 week to measure the plasma estradiol and at 0, 4 and 8 week to measure vitellogenin concentration. The gonad maturity stage of the experimental catfish were observed every two weeks. The ovi somatic index, fecundities, fertilization rate, hatching rate, larvae production and survival rate of larvae were measured on the end of this experiment.

Hormonal treatment. The study used a complete randomized design with three replications. The treatment consists of 4 doses of PMSG, there were A) 0; B) 5; C) 10; and D) 20 IU kg^{-1}. Hormone induction was carried out every two weeks for eight weeks. Observation of gonad maturity level of females were done every two weeks by inserting a catheter into the genital hole to obtain an egg sample. The female broodstock which have reached gonad maturity (stage 4) were intramuscularly injected with 500 IU HCG kg^{-1}. Ten hours after inducing HCG, female and male were induced by ovaprim with a dose of 0.5 mL kg^{-1}. Afterward, the induced female and male were stripped for fertilization.

Incubation and hatching of eggs were carried out in conical glass fiber. Thirty larvae were kept in a 10 liter container to observe the larval vitality for 4 days.

Plasma estradiol-17b and vitellogenin concentrations. Blood samples were collected every 2 weeks, i.e., at weeks 0, 2, 4, 6 and 8 for measurements of plasma estradiol-17b concentration and at weeks 0, 4 and 8 for measurements of vitellogenin concentrations. Blood was collected from the caudal artery using a syringe internally coated with heparin. Plasma was separated by centrifuging at 3000 g for 15 min and stored at -20°C until further use. The estradiol-17b concentrations were measured to determine the variations of estradiol-17b concentrations during gonadal maturation process. The concentrations of estradiol-17b in the plasma were measured by ELISA (EIA1561 DRG International Inc., Marburg, Germany). The plasma vitellogenin concentrations were measured to determine the variations of plasma vitellogenin concentrations during gonadal maturation process.

Vitellogenin concentrations in the plasma were measured in two steps. The first step was the isolation of the vitellogenin from the plasma of experimental catfish by using SDS polyacrylamide gel electrophoresis (SDS-PAGE) (Bio-Rad, Hercules, California, USA) (Walker 2002). The second step was the quantification of the isolated vitellogenin using the Bradford method (Kruger 2002).

Histology of eggs. Egg histology analysis was carried out based on the Hurvitz et al (2007) method. The eggs were fixed using Bouin's solution for 24 hours and then stored in 70% ethanol solution before the histology preparation process. Egg histology observation was carried out using a light microscope (Olympus BX 21).

Water and environmental quality parameters. Water quality parameters were measured every two weeks, while humidity and rainfall were based on the data from Indonesia's Agency for Meteorology, Climatology and Geophysics (BMKG) during the study. Water parameter consisting of temperature, pH and disolved oxygen were measured by water quality analyzer.

Data analysis. The data of the estradiol, vitellogenin level, egg histology, water and environment quality were presented descriptively to describe the changes of E2 and vitellogenin level development. The data of reproductive performance was analyzed statistically using Microsoft excell 2016 and SPSS program (ver. 25). One-way analysis of variance (ANOVA) was chosen to measure the significance of different treatment. If the treatment effect was significant, the differences between the means were examined further using Duncan's test.

Results. The concentration of estradiol-17β in blood plasma in all treatments increased during the gonadal maturation process. The value of estradiol concentration in the striped catfish female induced by PMSG at a dose of 20 IU kg^{-1} increased rapidly from the second week to the sixth week and decreased at the eighth week (Figure 1).

The SDS-Page profile of vitellogenin in the striped catfish female induced by PMSG hormone is presented in Figure 2. The results of blood plasma electrophoresis of the striped catfish female showed that the vitellogenin protein bands were in molecular weight between 140-180 kDa. The estimated weight of the vitellogenin molecule is about 153 kDa as measured by the marker molecular weight.

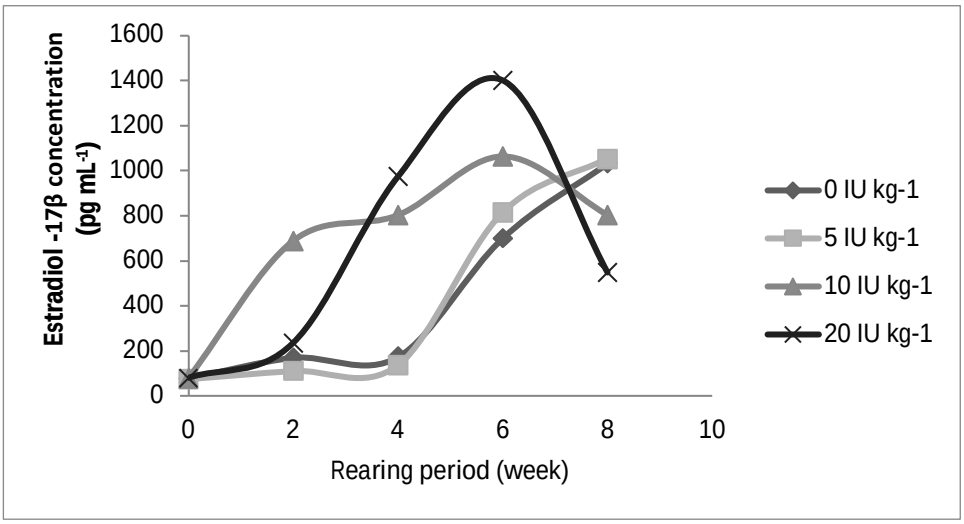
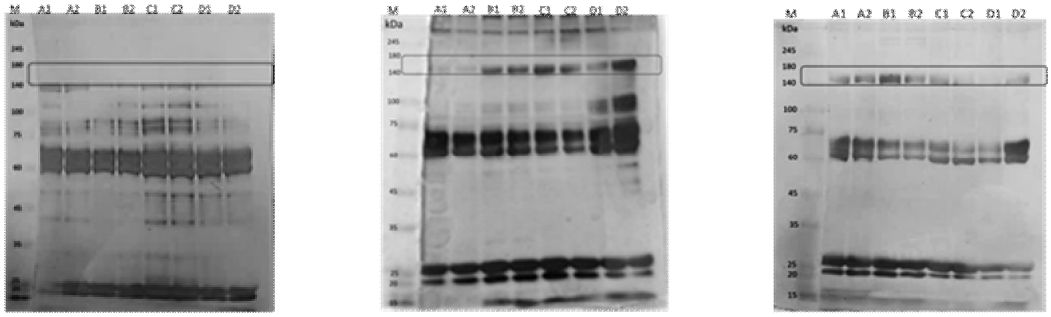


Figure 1. The concentration of estradiol in the blood plasma of the striped catfish broodstock.



At week 0 At week 4 At week 8

Figure 2. SDS-page profile of vitellogenin in blood plasma of the striped catfish injected with PMSG hormone at week 0 (initial), 4 and 8. M: molecular weight markers (kDa), lines A1 and A2: treatment of 0 IU kg^{-1}, B1 and B2: 5 IU kg^{-1}, C1 and C2: 10 IU kg^{-1}, D1 and D2: 20 IU kg^{-1}.

The vitellogenin protein band in the plasma of striped catfish female at week 0 (initial study) has not been seen in all treatments. The vitellogenin protein band at week 4 looked thin in treatment A (control) and thick in treatments B, C and D. The vtg protein band at week 8 looked very thin in treatments C and D, thinning at treatment B and starting thickened in treatment A.

The concentration of vitellogenin in the blood plasma of the catfish female induced by PMSG increased at week 4 and decreased at week 8. The highest vitellogenin concentration was detected in the striped catfish female injected with 20 IU kg^{-1} PMSG at week 4 (63.041 mg mL^{-1}), followed by striped catfish female induced by PMSG 10 IU kg^{-1} (58.860 mg mL^{-1}) and 5 IU kg^{-1} (45.717 mg mL^{-1}). In the control treatment, the highest vitellogenin concentration occurred at week 8 (47.522 mg mL^{-1}), at the time the other treatments had decreased. Increased vitellogenin concentration is in line with the increased of estradiol concentrations in blood plasma (Figure 3).

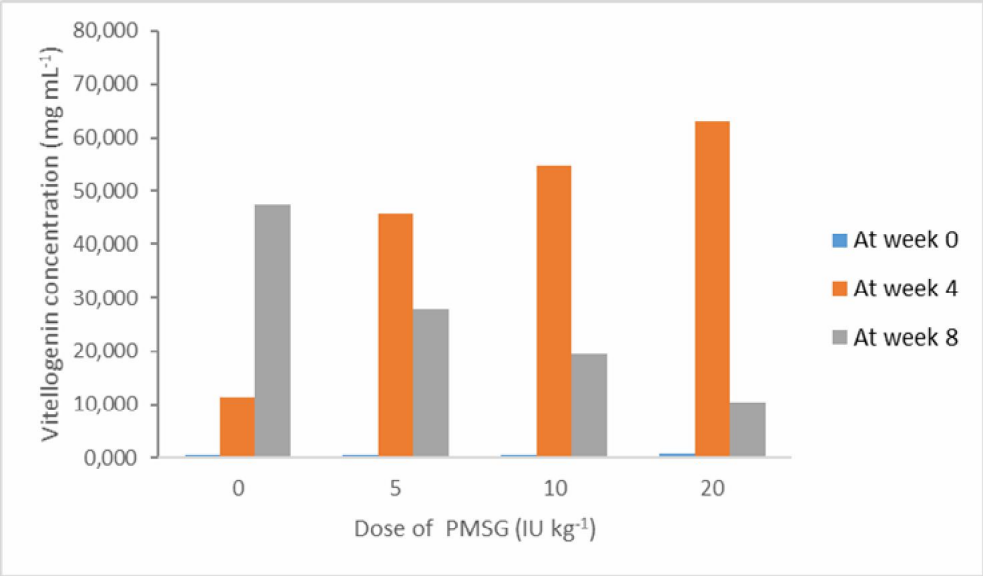


Figure 3. Vitellogenin concentration in the blood plasma of the striped catfish broodstock induced by PMSG hormones at different doses

The oocytes development observed microscopically showed differences between treatments and control (Figure 4). The striped catfish female induced by PMSG began to show the development of oocyte size at week 4. The process of vitellogenesis in the female injected with PMSG takes place from the fourth week and ends when the egg was mature. The striped catfish broodstock induced by PMSG 10 IU kg^{-1} and 20 IU kg^{-1} showed the development of oocytes that have reached the final stage of vitellogenesis and mature. Most of the female induced by PMSG 0 IU kg^{-1} were still many undeveloped eggs and some females catfish induced by PMSG at dose 5 IU kg^{-1} still showed the development of stage II gonad maturity, and several other females have reached the mature stage of gonads.

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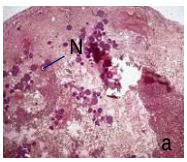
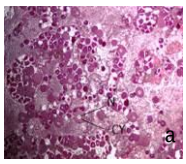
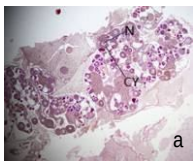
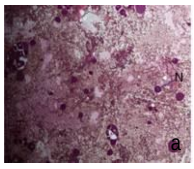
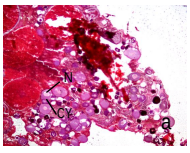
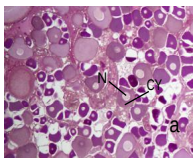
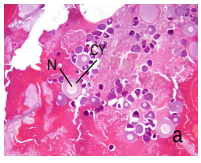
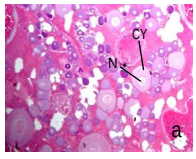
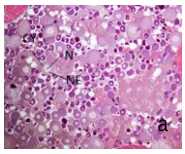
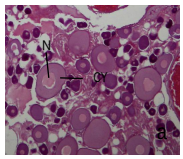
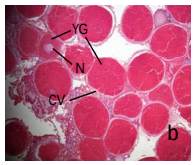
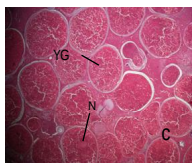
0				
4				
8				

Figure 4. Development of striped catfish oocytes induced by PMSG at different doses during maintenance (n = nucleus, cy = cytoplasma, cv = corticol vesicle, yg = yolk globule, a = previtellogenesis, b = vitellogenesis, c = mature).

Observations on gonadal maturity, ovi somatic index (OSI), fecundity, fertility rate (FR), hatching rate (HR), larval production and larval survival rate on day 4 are presented in Table 1. Gonadal maturity level of striped catfish broodstock during the study showed that 100 percent of the female broodstock induced by 20 IU kg^{-1} of PMSG experienced mature gonads and were ready to be spawned in the 8th week. Statistical analysis showed significant differences between treatments. The highest gonadal maturity level were observed in the fish on 20 IU kg^{-1} of PMSG induction, then fish on 10 IU kg^{-1} of PMSG induction. The lowest percentage of gonadal maturity occurred in fish which were induced by 0 IU kg^{-1} of PMSG.

The pond water quality was maintained in a controlled condition throughout the study. The average water temperature ranged from 30 to 34°C, pH ranged from 7.39 to 7.70 and dissolved oxygen ranged from 3.80 to 4.90. Standard water temperature, pH and dissolved oxygen are in the range 28-30°C, 6.85-7.50 and 3-6, respectively (Minggawati 2012). Air humidity and rainfall during the study ranged from 63.30 to 94.20 and from 0.00 to 20.80 mm respectively.

Table 1

Gonadal maturity rate (GMR), ovi somatic index (OSI), fecundity, fertilization rate (FR), hatching rate (HR), larval production, larval survival rate (SR) on day 4

Parameter	*0 IU kg^{-1}*	*5 IU kg^{-1}*	*10 IU kg^{-1}*	*20 IU kg^{-1}*
GMR (%)	26.67 ± 11.55^{a}	53.33 ± 23.09^{b}	66.67 ± 11.55^{b}	100 ± 0.00^{c}
OSI (%)	8.68 ± 1.17^{a}	9.49 ± 0.12^{a}	10.56 ± 0.95^{a}	12.92 ± 1.54^{b}
Fecundity (egg kg^{-1})	123407 ± 10876^{a}	132014 ± 18287^{ab}	163812 ± 25677^{bc}	190734 ± 12982^{c}
FR (%)	83.68 ± 2.53^{a}	93.13 ± 4.20^{b}	93.52 ± 4.20^{b}	96.57 ± 1.28^{b}
HR (%)	83.66 ± 0.77^{a}	83.82 ± 1.91^{a}	86.50 ± 3.34^{a}	93.81 ± 2.70^{b}
Larval production (larva/female)	299307 ± 10485^{a}	371209 ± 45342^{a}	483874 ± 66231^{b}	659890 ± 33922^{c}
SR (%)	11.11 ± 5.85^{a}	15.37 ± 2.63^{a}	16.85 ± 5.94^{a}	34.44 ± 15.12^{b}

Mean values in same row with different superscripts vary significantly ($p < 0.05$).

Discussion. The results of the study described that the induction of the PMSG hormone can accelerate the rematuration of the striped catfish female. Gonadal maturation process of the striped catfish broodstock induced by the PMSG hormone was characterized by increased levels of estradiol-17β and vitellogenin concentrations in blood plasma of the striped catfish and oocyte development during the maturation process.

Estradiol-17β is the main estrogen in female fish (Wang et al 2008; Cabrita et al 2008) which plays a role in the gonadal maturation process (Klinge 2000; Nilsson et al 2001). The concentration of estradiol in plasma increases and the concentration level remains high during the process of vitellogenesis (Lubzens et al 2010). This happened due to the process of vitellogenesis, the production of estradiol-17β in the body will increase and then enter in the vascular system and stimulate the liver to synthesize vitellogenin (Ng & Idler 1983). Vitellogenin produced by the liver is secreted into the bloodstream to the gonad so that oocyte growth and development occurs. Nagahama et al (1995) stated that the development of oocytes from previtellogenesis to vitellogenesis is caused by an increased production of estradiol-17β.

Vitellogenin is an egg yolk precursor protein that is synthesized in the liver under the control of the estrogen hormone and secreted into the bloodstream (Brion et al 2000; Ding 2005; Prakash et al 2007). Vitellogenin as raw material for egg yolk has a high molecular weight (MW). In some fish species, vitellogenin has MW between 140-200 kDa (Tyler 1991; Komatsu & Hayashi 1997). Meanwhile, Ding (2005) reported that vitellogenin has MW between 200-700 kDa, as in killifish, *Fundulus heteroclitus*, which has a range of 200 kDa vitellogenin molecules. The MW of vitellogenin in some Cyprinidae is around 150 kDa in goldfish, 156 and 190 kDa in *Cyprinus carpio* (Fukada et al 2003) and 167 kDa in *Puntius conchonius* (Shi 2004).

The vitellogenin protein band in the plasma of striped catfish female at week 0 (initial study) has not been seen in all treatments. At week 0, the striped catfish has not occured the process of gonadal maturation. The vitellogenin protein band at week 4 looked thin in treatment A (control) while thick in treatment B, C and D. During the 4th week, the striped catfish broodstock induced by PMSG began to occur gonadal maturation process (stage II). Vitellogenin protein bands looked very thin in treatments C and D, thinning at treatment B and starting to thicken in treatment A at week 8. At the 8th week, the striped catfish female treated C and D had reached the gonadal maturity stage IV, whereas in treatment B reached the level of gonad maturity stage III to IV. In control (A) most of the females start the process of gonad maturation stage II and III. During the process of vitellogenesis, the concentration of vitellogenin in the plasma will increase and is indicated by thickening of protein bands in the electrophoresis results. The concentration of vitellogenin in the plasma begins to decrease when the process of vitellogenesis ends and the female fish has reached the stage IV of gonadal maturity. It is indicated by thinning of the vitellogenin protein band in the electrophoresis results. Matsubara et al (1994) stated that the level of vitellogenin in fish showed the maturation stage of the female parent gonads.

In this study, the striped catfish induced by PMSG began to occur the process of vitelogenesis at week 4, which was indicated by increased concentrations of estradiol and vitelogenin in blood plasma. Increased estradiol concentration was in line with increased vitellogenin concentrations in blood plasma (Figures 1 and 3). Vitellogenin is synthesized in the liver under the stimulation of the hormone estradiol, then vitellogenin secretion is distributed through the bloodstream in the form of compounds with Ca_2^+ (Yaron & Silvan 2006). Stimulation of the hormone estradiol causes an increase in plasma vitellogenin concentration. Tang & Affandi (2000) suggested that vitellogenin is a major component of oocytes that have grown, in the form of glycophosphoproteins and contain about 20% fat, especially phospholipids, triglycerides and cholesterol. The vitellogenesis phase is an important stage because in the process it supports oocyte growth up to 90% nearing the egg size (Sun & Pankhurst 2004). When vitellogenesis takes place, the yolk granule increase in number and size, so the oocyte volume increases (Yaron & Silvan 2006). The development of eggs at the stage of absorption of vitellogenin stops when the oocytes have reached the maximum size.

The results of OSI and fecundity values were not significantly different between treatments ($p > 0.05$), while FR values showed differences between treatment and controls. The striped catfish induced by PMSG at a dose of 20 IU kg^{-1} showed the highest HR values, larval production and SR. It is significantly different from other treatments.

Injection of PMSG accelerated the gonadal rematuration process of the striped catfish broodstock during the dry season. PMSG contains more FSH which plays a role in the process of vitellogenesis (Gallego et al 2012). Furthermore, Hafez et al (2000) confirmed that the effect of FSH in PMSG was more dominant than LH. FSH plays a role in inducing the process of gonadal maturation and oocyte development (vitellogenesis), while LH triggers late stage oocyte maturity. FSH stimulates theca cells through the cAMP system to produce testosterone. The aromatase enzyme converts testosterone to estradiol-17β causing oocyte development.

Conclusions. The results obtained in the present study showed that the PMSG can induce the acceleration of the striped catfish broodstock rematuration in out of spawning season. Induction of PMSG at a dose of 20 IU kg^{-1} is the best dose to accelerate ovarian rematuration of the striped catfish broodstock female with the percentage of gonad maturity reaching 100 percent.

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Authors:
Wahyu Pamungkas, Research Institute for Fish Breeding, Ministry of Marine Affairs and Fisheries, Jl. Raya 2 Sukamandi, Subang 41256, West Java, Indonesia, e-mail: yhoe_pamungkas@yahoo.co.id
Dedi Jusadi, Department of Aquaculture, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Dramaga Campus, Bogor 16680, West Java, Indonesia, e-mail: siflounder@gmail.com; dedidj@apps.ipb.ac.id
Muhammad Zairin Junior, Department of Aquaculture, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Dramaga Campus, Bogor 16680, West Java, Indonesia, e-mail: zairinmz@live.com
Mia Setiawati, Department of Aquaculture, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Dramaga Campus, Bogor 16680, West Java, Indonesia, e-mail: miasetiawati25@yahoo.com; miasetia@apps.ipb.ac.id
Eddy Supriyono, Department of Aquaculture, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Dramaga Campus, Bogor 16680, West Java, Indonesia, e-mail: eddy_supriyono@yahoo.com
Imron Imron, Research Institute for Fish Breeding, Ministry of Marine Affairs and Fisheries, Jl. Raya 2 Sukamandi, Subang 41256, West Java, Indonesia, e-mail: imronnawawi@gmail.com

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