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Induced spawning of seurukan fish, *Osteochilus vittatus* (Pisces: Cyprinidae) using ovaprim, oxytocin and chicken pituitary gland extracts

[1]Zainal A. Muchlisin, [1]Gusnar Arfandi, [2]Muhammad Adlim, [3]Nur Fadli, [4]Sugianto Sugianto

[1] Department of Aquaculture, Faculty of Marine and Fisheries, Syiah Kuala University, Indonesia; [2] Department of Chemistry, Faculty of Teaching Training and Education, Syiah Kuala University, Indonesia; [3] Department of Marine Sciences, Faculty of Marine and Fisheries, Syiah Kuala University, Indonesia; [4] Department of Soil Science, Faculty of Agriculture, Syiah Kuala University, Indonesia. Corresponding author: Z. A. Muchlisin, muchlisinza@unsyiah.ac.id

Abstract. The objective of the present study was to examine the effectiveness of ovaprim, oxytocin and chicken-pituitary-gland extracts on induced spawning of seurukan fish (*Osteochilus vittatus*). The non factorial completely random design was utilized in this study. The only female broodfishes were administered with tested hormones at recommended doses, while the males were not. The ovulated eggs were mixed with diluted sperm at ratio of 1:4 (sperm:eggs), then about 100 eggs were taken randomly and incubated in a plastic basin at three replications. The study showed that the fishes administered with ovaprim gave 424 minutes (7 hours) of latency period with 93.33% of fertilization, 82.33% hatching and 80.66% survival rates. Inducing with chicken pituitary gland extracts gave 776 minutes (13 hours) of latency period with 82.33% of fertilization, 66.66% of hatching and 45.66% of survival rates; no hatching and survival rates observed for the oxytocin treatment. These data show that ovaprim and chicken pituitary gland extracts were successfully induced for spawning of seurukan fish and ovaprim gave the better effect.
Key Words: Aceh Province, artificial spawning, freshwater fish, latency period, gonadotropin.

Introduction. Seurukan fish (*Osteochilus vittatus*) is one of the indigenous tropical freshwater fish which occurre in Indonesia waters, this species being commonly found in Nagan Raya and Aceh Selatan Districts of Aceh Province (Muchlisin & Siti-Azizah 2009). *O. vittatus* is a potential target for aquaculture (Muchlisin 2013) and currently the cultures of species have been initiated in Aceh Province, Indonesia. The larvae were supplied from outside of Aceh, for example North Sumatra and Java, because the hatchery of freshwater fish industry has not been well developed in this region. Due to high price, some fish farmers use low quality larvae collected from the wild. Therefore, the backyard hatchery facilitated with induced breeding technique is crucial to be introduced especially in rural areas to mitigate the current problem.

In general, the induced breeding of fish may be approached in two ways, hormonal treatment or environmental stimulations. These approaches have become of practical importance in the fish farming industry (Kahkesh et al 2010). The environmental spawning approach is the common technique in the backyard hatcheries; but this method is inefficient because it much depends on the condition of the maturity of the broodfish. In addition, the influence of specific environmental factors that stimulate ovulation and spawning has not been well known in most of tropical cultured fishes (Marte 1989). Thereby, the artificial spawning using induced breeding technique could be a good choice for the farmers; but the administration of reproductive hormones becomes a challenge. The popular commercial hormones used for artificial spawning of freshwater fishes were ovaprim and human clorionic gonadotropin (HCG), but the availability and the high price of these hormones are a serious problem for traditional hatcheries at remote

areas in Indonesia. Thereby, it is crucial to find some alternative materials for inducing the ovulation in artificial spawning especially for *O. vittatus*. The potential inducing materials are from pituitary-gland extract of mammal, poultry or fish.

Andalusia (2008) used chicken-pituitary-gland extracts for induced breeding in goldfish *Carassius auratus* and African catfish, *Clarias gariepinus* and Oka (2005) has reported the application of cattle's pituitary-gland extracts for induce breeding in common carp, *Cyprinus carpio;* however in general, the information on alternative hormones for induce breeding in fish was still scarce. Besides pituitary gland extracts, the oxytocin hormone has also been studied previously for alternative inducing ovulation for fish. This hormone is known as neuromodulator on the central nervous system (Lee et al 2009), but the role of oxytocin in fish reproduction is unclear and still interesting to explore (Viveiros et al 2003).

Induce breeding is affected by the types and doses of utilized hormones and strongly depends on the quality of the broodstock (Muchlisin et al 2006). Therefore, broodstock management should be well conducted prior to breeding program. The objective of the present study was to examine the effectiveness of a popular commercial hormone (ovaprim) and two alternative hormones (chicken-pituitary-gland extracts and oxytocin) for induced breeding of *O. vittatus*.

Material and Method

Study design. The complete random experimental design was utilized in this study. Three types of hormone i.e. ovaprim (Syndel, Canada), oxytocin (Novart, China) and chicken-pituitary-gland (hypophisis) extract were tested at three replications. The chicken pituitaries were collected from local market in Nagan Raya District. The ovaprim and oxytocin doses were 0.5 mL kg^{-1} and 1.0 mL kg^{-1} of body weight, respectively (the recommendation doses from manufactory), while the chicken-pituitary-gland extracts was 500 mg kg^{-1} body weight used, it is a best dose for comet fish *Carassius auratus* (Andalusia et al 2008), the similar family taxonomically with *O. vittatus* (Cyprinidae).

Broodstock. A total of 20 males (90-160 g body weight) and 15 females (120-210 g body weight) of prospective broodfish were collected from the wild in Nagan Raya District, Indonesia and reared in the groudpond (10 m x 15 m x 1 m). The broodfish were fed with commercial pelleted diet (30% proteins) at ratio of 3% body weight per day for eight weeks. The broodfish was regularly monitored for their gonad maturation and the breeding program was conducted when the fish reached the level four of gonad maturation levels. The selected broodfish (4 females and 8 males) were reared separately in two different hapas according to their sexes, the broodfish were fasted for one day prior induced with hormones.

Hormones preparation and injection. The ovaprim and oxytocin hormones were packaged in sealed bottles by manufactories and no special preparation is needed. The hormones were taken directly from the bottle using a syringe at appropriate doses. The pituitary glands were taken from freshly slaughtered chicken heads at local market. The glands were washed by 45% alcohol and gently ruined in a muller. Approximately 5mL of physiological solution (0.9% saline water) were added into the muller then centrifuge for 5 minutes at 10,000 rpm, then hypofisar extracts were taken using a syringe (Streit Júnior et al 2005).

A single dose of tested hormones were applied at dorso-lateral section of female broodfish on 09.00 pm then injected broodfish were returned to the hapa. No hormone application was done for male broodfish. The successful of ovulation was monitored for two hours after hormone application with one hour interval thereafter.

Sperm and eggs collection. Sperm was collected from mature males with gentle finger pressure to the abdomen. The genital pore was cleaned with tissues to avoid urine and water contamination. Sperm was collected with a syringe and then it was diluted with physiological solution extender (0.9% saline water); ratio of sperm and extender was

1:20 (v/v) (Muchlisin et al 2004) and the diluted sperm was kept in the syringe in an ice box (4^{o}C) prior to use for fertilization.

The eggs were collected with gentle pressure to the abdomen as applied for the male previously. This was done when the females have been ovulated. The collected eggs were put in a plastic basin and kept at 4^{o}C in cruches ice box. Then the eggs and the sperm were mixed with ratio of 1:4 v/v (sperm:eggs) then two drops of tap water were added and mixed using a feather and left in contact for 5 minutes (Muchlisin et al 2010; 2014). A total of 100 eggs from the basin were taken randomly then incubated in a plastic jar at three replications. The successful fertilization was calculated after 2 hours incubation and the hatching eggs were monitored in one hour interval for 48 hours. The larvae were fed with chicken egg yolk solution starting with the third day after they hatched and the survival rates were recorded after 7 days.

Parameters. Four main parameters were recorded namely: (a) latency time, the duration time between hormone applications until ovulation, (b) fertilization rate = (total fertilized eggs/total incubation eggs) x 100%, (c) hatching rate = (total of hatched eggs/total of fertilized eggs) x 100%, and (d) survival rate= (total of larvae at 7 days after hatched/total hatched larvae) x 100%.

The main water quality parameters i.e. temperature of incubation water (hatching medium), dissolved oxygen (DO) and pH were also recorded during the study.

Statistical analysis. All data were subjected to analysis of variance (ANOVA), followed by comparison of means using Duncan's multiple range test. All statistical analyses were performed using SPSS ver.17.0

Results and Discussion. The results showed that the shorter latency period was found at ovaprim application (423 minutes) followed by chicken pituitary gland extracts (776 minutes) and the longer latency period was occurred at oxytocin application (1,800 minutes, Figure 1). The higher fertilization, hatching and survival rates were found at fish injected with ovaprim and followed by chicken pituitary gland extracts and no hatching rate was observed for fish injected with oxytocin (Table 1).

The Anova test showed that application of hormones gave a significant effect on the latency period, fertilization, hatching and survival rates of seurukan fish larvae ($p < 0.05$). The best results were found on fish injected with ovaprim, however the fertilization rate was not significantly different from the treatment with chicken-pituitary-gland extracts.

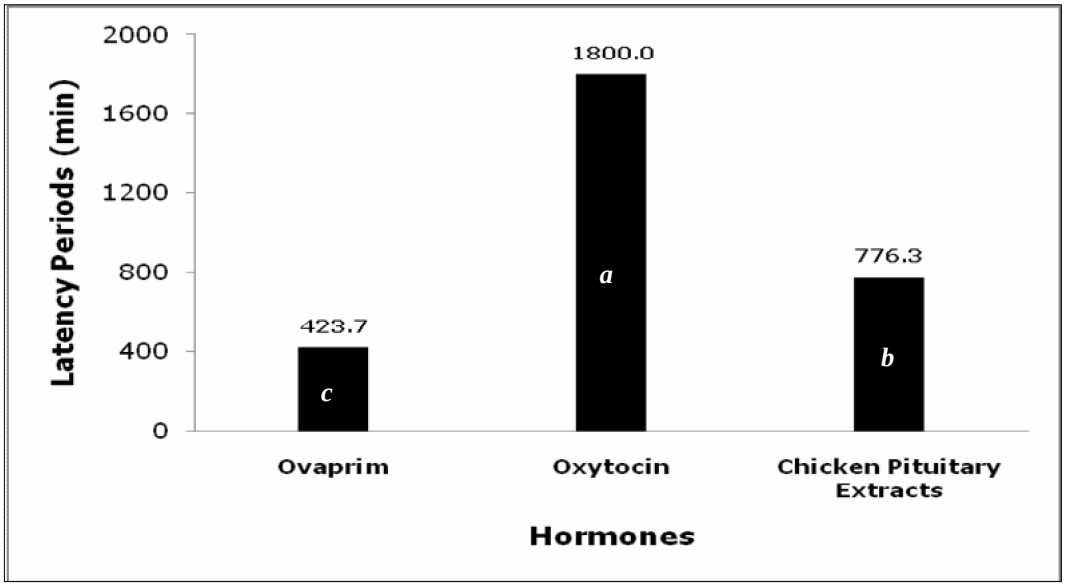

Figure 1. The latency periods of *Osteochilus vittatus* eggs according to tested hormones. Bars with the different letter were significantly different ($p < 0.05$).

The study revealed that ovarprim and chicken pituitary gland extracts have successfully induced spawning for *O. vittatus*, while the oxytocin application was not effective for this fish due to no eggs had hatched during the study. The effectiveness of ovaprim probably was caused by the composition and amount of reproductive hormones contained in this solution. They were sufficient to trigger the activity of reproductive hormones in the body of fish, then they induced gonad maturation and eggs ovulation faster if is compared with chicken pituitary gland extracts and oxytocin treatments. According to Rokade et al (2006), the ovaprim contains a salmon gonadotropin releasing hormone analog (sGnRHa; D-Arg^6-Pro^9-NEt) at a concentration of 20 µg mL^{-1} and domperidone, a dopamine antagonist, at 10 mg mL^{-1} (Hill et al 2009). The ovaprim activates and stimulates the GnRH in the body and/or inhibits the gonadotropin delivery. This releases inhibiting factor (GnRIF) causing the pituitary to secrete gonadotropins (GtH) and when GtH reached a certain level, vitellogenic oocytes undergo the process of final oocyte maturation (Peter et al 1986; Lin & Peter 1986; Marte 1989). Ovaprim has been applied on a wide variety of fishes for induced breeding program. The successful application of ovaprim has been reported on some fish species, for example common carp *Cyprinus carpio* (Reny 2008), catfish *Clarias batrachus* (Sahoo et al 2007), gouramy *Osphronemus goramy* (Arfah et al 2006) and mrigal carp *Cirrhinus mrigala* (Rokade et al 2006).

Table 1

The average values of latency period, fertilization, incubation time, hatching and survival rates of *Osteochilus vittatus* (± SD). The values with the same superscript (a/b) at same row were significantly different ($p<0.05$)

Parameter	*Hormone application*		
	Ovaprim	*Oxytocin*	*Chicken pituitary extracts*
Fertilization rate (%)	93.33±3.055^a	45.07±14.30^b	82.33±3.512^a
Incubation time (minute)	660±6.04^b	-	780±5.20^a
Hatching rate (%)	82.33±3.51^a	-	69.33±3.51^b
Survival rate (%)	80.66±2.52^a	-	66.66±3.79^b

As mentioned early, the chicken pituitary gland extract has successfully induced the ovulation of *O. vittatus*, however the results were lower compared to ovaprim, but better than oxytocin. This indicates that chicken pituitary gland extract can be used as alternative hormone to stimulate the ovulation of *O. vittatus*). This is probably because pituitary gland contains the similar hormones as ovaprim, but the composition and concentration migt be lower than ovaprim. According to Bowen (2004) the pituitary gland produces gonadotropin that has an important role in gonad maturation of animals. This hormone is composed by Follicle-stimulating Hormone (FSH) and Luteinizing hormone (LH), and the role has been described previously. Previously, chicken pituitary gland extracts have been applied and succesfully stimulated the ovulation of African catfish *Clarias gariepinus* (Taufek et al 2009), common carp (Kruger et al 1984) and goldcarp *Carassius auratus* (Andalusia et al 2008), but the appropriate doses are crucial where too high doses lead to decrease of hatching and survival rates of larvae, but if it is too low, it caused the fish failed to spawn, as the consequence of low amount of LH and FSH hormones in the blood of the fish, then they cannot stimulate the gonadal maturation process and ovulation.

The study revealed that the oxytocin was not suitable to use for induced breeding of *O. vittatus*, because no eggs had hatched during the study. Probably the oxytocin has only stimulated the contraction of muscles within gonad causing ovulation, but has not stimulated the gonad maturation and therefore the fish produces low quality eggs, which then failed to hatch as recorded in this study. Based on its physiological effect, the oxytocin function is to stimulate birth delivery on human by inducing muscle contraction of uterus. Ramad (2013) also stated that oxytocin in the blood lead to increases the late stage of pregnancy where uterus is the traditional target of oxytocin. In generally most of the bony fishes have no uterus (Braungart 1951) and therefore the oxytocin was not suitable for *O. vittatus* as recorded in this study, moreover the role of oxytocin in the fish

birth process for example sharks is still unclear. The application of oxytocin has been reported on the male of *C. gariepinus* that successfully increased sperm density (Viveiros et al 2003), but the administration of this hormone on the female fish of *O. vittatus* has not gave a satisfy achievement.

The direct observation showed that the eggs batch produced by broodfish injected with oxytocin has yellowish color, which indicates immature eggs while the matured eggs have dark greenish color as appeared on those injected with ovaprim and chicken pituitary gland extracts in this study. The incubation time of each treatment was significantly different, for example the eggs which have been produced by broodfish injected with ovaprim started to hatch about in 11 hours, in 13 hours when they were treated with chicken pituitary gland extract and no hatched eggs with oxytocin treatment. This implied that the incubation time of *O. vittatus* eggs were affected by hormone application. It was presumed that the hormone application has increased the eggs quality and affected the hatching time as revealed in this study. We recorded that the water temperature of incubation medium has ranged between 28 and 29^{o}C, pH 6.9 to 7.1 and dissolved oxygen has ranged between 4.9 and 5.2 ppm. Also according to Wijayanti et al (2010) the optimum range of water temperature for incubation of *O. vittatus* eggs was 26 to 30^{o}C. Also Wijayanti et al (2010) reported that optimum level of dissolved oxygen and pH for *O. vittatus* eggs ranged between 4.0 and 7.7 ppm and from 6 to 9, respectively; it indicates that the water quality parameters were in the optimum level for hatching of seurukan eggs.

Conclusions. The ovaprim and chicken pituitary-gland-extracts have succesfully induced the spawning activity of seurukan fish. The application of ovaprim gave higher of latency time, fertilization, hatching and survival rates. Chicken-pituitary-gland extracts exhibit similar effect and it was much better than oxytocin but not as effective as ovaprim. It is concluded that the chicken-pituitary-gland extract which is cheap, as waste of poultry industry and available everywhere can be used as alternative hormone in induced breeding program of *O. vittatus*.

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References

Andalusia R., Mubarak A. S., Dhamayanti Y., 2008 [Application of chicken pituitary gland extracts on the latency time, fertilization and hatching rates of comet fish *Carassius auratus auratus*]. Berkala Ilmiah Perikanan 3(1):21-27 [in Indonesian].

Arfah H., Mafyucha L., Carman O., 2006 [Artificial spawning of gouramy O*spronemus gouramy* using ovaprim]. Jurnal Akuakultur Indonesia 5(2):103-112 [in Indonesian].

Bowen R., 2004 Pathophysiology of the endocrine system. Colorado State University, Colorado. Accessed on August 20, 2014. Available at: http://www.vivo.colostate.edu/hbooks/pathphys/endocrine/basics/index.html.

Braungart D. C., 1951 A comparative study of the reproductive systems of several teleost fishes. Copeia 1951(3):203–204.

Hill J. E., Kilgore K. H., Pouder D. B., Powell J. F. F., Watson C. A., Yanong R. P. E., 2009 Survey of ovaprim use as a spawning aid in ornamental fishes in the United States as administered through the University of Florida tropical aquaculture laboratory. North American Journal of Aquaculture 71:206–209.

Kahkesh F. B., Feshalami M. Y., Amiri F., Nickpey M., 2010 Effect of ovaprim, ovatide, HCG, LHRH-A2, LHRHA2+CPE and carp pituitary in Benni (*Barbus sharpeyi*) artificial breeding. Global Veterinaria 5(4):209-214.

Kruger J. C. De W., Smit G. L., Van Vuren J. H. J., Ferreira J. T., 1984 Some chemical and physical characteristics of the semen of *Cyprinus carpio* L. and *Oreocromis mossambicus* (Peters). Journal of Fish Biology 24:263-272.

Lee H. J., Macbeth A. H., Pagani J. H., Young W. S. 3rd, 2009 Oxytocin: the great facilitator of life. Progress in Neurobiology 88(2):127-151.

Lin H. R., Peter R. E., 1986 Induction of gonadotropin secretion and ovulation in teleosts using LHRH analogous and catecholaminergic drugs: a review. In: The First Asian Fisheries Forum. Maclean J. L., Dizon L. B., Hosillos L. V. (eds), Asian Fisheries Society, Manila, Philippines, pp. 667-670.

Marte C. L., 1989 Hormone-induced spawning of cultured tropical finfishes. Advances in Tropical Aquaculture, AQUACOP IFREMER, Actes de Colloque 9:519-539.

Muchlisin Z. A., 2013 Potency of freshwater fishes in Aceh waters as a basis for aquaculture development program. Jurnal Iktiologi Indonesia 13(1):91-96.

Muchlisin Z. A., Siti Azizah M. N., 2009 Diversity and distribution of freshwater fishes in Aceh Water, Northern-Sumatra, Indonesia. International Journal of Zoological Research 5(2):62-79.

Muchlisin Z. A., Hashim R., Chong A. S. C., 2004 Preliminary study on the cryopreservation of bagrid catfish (*Mystus nemurus*) spermatozoa; the effect of extender and cryoprotectant on the motility after short-term storage. Theriogenology 62:25-34.

Muchlisin Z. A., Hashim R., Chong A. S. C., 2006 Influence of dietary protein levels on growth and egg quality in broodstock female bagrid catfish (*Mystus nemurus* Cuv. & Val.). Aquaculture Research 37:416-418.

Muchlisin Z. A., Nadiya N., Nadiah W. N., Musman M., Siti-Azizah M. N., 2010 Preliminary study on the natural extenders for artificial breeding of African catfish *Clarias gariepinus* (Burchell, 1822). AACL Bioflux 3(2):119-124.

Muchlisin Z. A., Mastura S., Asraf A., Fadli N., Hendri A., Siti-Azizah M. N., 2014 A preliminary study to evaluate the effects of powder milk solution on the eggs adhesiveness and fertilization rates of African catfish, *Clarias gariepinus*. AACL Bioflux 7(1):15-19.

Oka A. A., 2005 [Application of livestock pituitary extracts to induce spermation of common carp *Cyprinus carpio* L.]. Majalah Ilmiah Peternakan 8(5):1-8 [in Indonesian].

Peter R. E., Chang J. P., Nahorniak C. S., Omeljaniuk R. J., Sokolowska M., Shih S. H., Billard R., 1986 Interactions of catecholamines and GnRH in regulation of gonadotropin secretion in teleost fish. Recent Prog Horm Res 42:513-548.

Ramad F. T., 2013 [Application of oxytocin and ovaprim, and its combination on induced spawning of *Synodontis eupterus*]. Master Thesis, Institut Pertanian Bogor, Bogor. [in Indonesian].

Reny I., 2008 [Effect of various doses of GnRHa+domperidon on ovulation of common carp *Cyprinus carpio* punten strain]. Karya Ilmiah Perikanan 3(2):9-16 [in Indonesian].

Rokade P., Ganeshwade R. M., Somwane S. R., 2006 A comparative account on the induced breeding of major carp *Cirrhina mrigala* by pituitary extract and ovaprim. Journal of Environmental Biology 27(2):309-310.

Sahoo S. K., Giri S. S., Chandra S., Sahu A. K., 2007 Effect of ovaprim doses and latency periods on induced spawning of *Clarias batrachus*: observation on larval deformity. Indian Journal of Experimental Biology 45:920-922.

Streit Júnior D. P., de Moraes G. V., Ribeiro R. P., Sakaguti E. S., Povh J. A., Moreira H. L. M., 2005 Effects of three different sources of pituitary extract on gonadal inducer in male and female pacu (*Piaractus mesopotamicus*). Journal Acta Scientiarum - Animal Sciences 27(4):439-447

Taufek N., Harmin S. A., Christianus A., 2009 Effect of chicken gonadotropin-releasing hormone (cGnRH-II) on plasma steroid hormone, maturation and ovulation in african catfish *Clarias gariepinus* (Burchell). African Journal of Biotechnology 8(23):6700-6709.

Viveiros A. T. M., Jatzkowski A., Komen J., 2003 Effect of oxytocin on semen release response in african catfish *Clarias gariepinus.* Theriogenology 59:1905-1917.
Wijayanti G. E., Sugiarto, Susatyo P., Nuryanto A., 2010 [Embryogenesis of *Osteochilus hasselti* at different levels of water temperature of incubation]. Prosiding Semnas Basic Science 7(3):180-187 [in Indonesian].

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Authors:
Zainal A. Muchlisin, Department of Aquaculture, Faculty of Marine and Fisheries, Syiah Kuala University, Banda Aceh 23111, Indonesia, e-mail: muchlisinza@unsyiah.ac.id.
Gusnar Arfandi, Department of Aquaculture, Faculty of Marine and Fisheries, Syiah Kuala University, Banda Aceh 23111, Indonesia, e-mail: gusnar_fd@yahoo.com
Muhammad Adlim, Department of Chemistry, Faculty of Teaching Training and Education, Syiah Kuala University, Banda Aceh 23111, Indonesia, e-mail: adlimbandang@yahoo.com
Nur Fadli, Department of Marine Sciences, Faculty of Marine and Fisheries, Syiah Kuala University, Banda Aceh 23111, Indonesia, e-mail: ivad29@yahoo.com
Sugianto Sugianto, Department of Soil Science, Faculty of Agriculture, Syiah Kuala University, Banda Aceh 23111, Indonesia, e-mail: s.sugianto@unsyiah.ac.id

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