

Original Article

Induced breeding and seed production of seurukan fish (*Osteochilus jeruk*) using synthetic reproductive hormones

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Abstract: The commercial aquaculture of seurukan fish (*Osteochilus jeruk*) is common in Indonesia; however, seed availability from the wild is limited. Therefore, induced breeding technology in seurukan fish using synthetic reproductive hormones is crucial. This study evaluated the efficacy of five different types of synthetic reproductive hormones and their corresponding doses on the reproductive performance of seurukan fish in captivity. The methodology consisted of two experiments; first, four types of synthetic reproductive hormones were tested: ovaprim, ovaspec, human chorionic gonadotropin (HCG), and ovalumon. Second, several doses of ovaprim hormone were tested with doses of 0.25, 0.50, 0.75, and 1.0 ml kg⁻¹ body weight. The results showed that ovaprim, ovaspec, and HCG successfully induced ovulation and spawning in seurukan fish. The hormones significantly affected the latency period, number of eggs released, fertilization, and survival rates ($P < 0.05$), but did not significantly affect the hatching rate and survival of larvae ($P > 0.05$). Ovaprim produced better egg quality compared to ovaspec and HCG. In the second experiment, the results showed a significant difference in the latency period and egg size ($P < 0.05$) of fish treated with varying doses of ovaprim hormone. The hormone doses did not significantly affect the number of eggs, hatching rate, fertilization rate, survival rate, or larval survival ($P > 0.05$). The dose of 0.5 ml kg⁻¹ BW of ovaprim produced the best results. Therefore, a dose of 0.5 ml kg⁻¹ BW was recommended as an effective dose for induced spawning and seed production of seurukan fish.

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Introduction

The seurukan fish, *Osteochilus jeruk*, is a native freshwater fish species found in Sumatra, Indonesia. This species has high economic value and potential for aquaculture. The distribution spans across the inland waters of Nagan Raya to South Aceh, Indonesia, primarily in the rivers that originate in the rainforest of the Leuser ecosystem (Hadiaty and Siebert, 1998). The biology, food, and feeding habits, as well as the reproductive biology of seurukan fish, have been reported by Hadiaty (2000). The fish is an omnivore and spawns multiple times in wild conditions. Currently, the market supply comes from wild catch,

and fishermen frequently use unfavorable and destructive fishing practices, such as poisoning and electric shocks, to catch fish. This has caused the wild population to decrease sharply in recent years (Personal communication with the local fishermen in Nagan Raya district). Therefore, it is essential to develop viable aquaculture and captive breeding techniques for seurukan fish farming to meet the market demand and alleviate fishing pressure on wild populations.

The culture of seurukan fish was initiated by fish farmers in Nagan Raya District, Aceh Province. However, the larvae collected from the wild were of

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low quality and showed poor growth performance. The availability of high-quality seed is a key factor for success in fish farming (Eriani et al., 2017), and a reliable supply of high-quality larvae must be secured. The high-quality larvae are mostly produced in hatcheries that use high-quality broodstock (Nagahama, 1994; Abidin et al., 2006; Dewantoro et al., 2017). Currently, the technology for breeding seurukan fish is not available. Therefore, breeding technology for this fish is crucial to produce high-quality fish larvae that support commercial aquaculture.

In hatcheries, breeding is typically performed using reproductive stimulating hormones to accelerate the maturation process and induce ovulation in fish. Currently, several brands of commercial synthetic reproductive hormones are available on the market, such as ovaprim, ovaspec, human chorionic gonadotropin (HCG), and ovalumon. Ovaprim and ovaspec contain a combination of salmon gonadotropin-releasing hormone analogs (sGnRH-a) and anti-dopamine (Zadmajid et al., 2017; El Mohajer et al., 2022). This hormone stimulates the pituitary gland to produce and release gonadotropin hormone (GTH), which triggers the maturation and ovulation processes in fish, thereby reducing the latency period (Rosyida et al., 2021; Zahra et al., 2021). HCG is a hormone that mimics the luteinizing hormone (LH) in fish, triggering ovulation and final maturation of eggs in the breeding of fish. Ovalumon contains ethinylestradiol, and its use in fish triggers the estrogenic hormone content of ethinylestradiol in fish blood, thereby accelerating the process of vitellogenesis and gonad maturity in female fish (Hafeez-ur-Rehman et al., 2015). Therefore, these four synthetic reproductive hormones have shown promising results in inducing maturation and ovulation in different fish species.

Studies on applying synthetic reproductive hormones such as ovaprim, ovaspec, HCG, and ovalumon to induce ovulation in broodfish have been widely conducted and proven effective. For instance, ovaprim has been used in the induced breeding of African catfish, *Clarias gariepinus* (Sinjal, 2014),

Silver barb, *Barbonymus schwanenfeldii* (Dewantoro et al., 2017), common carp *Cyprinus carpio* (Sinaga and Telaumbanua, 2020), Hai catfish, *Pangasius hypophthalmus* (Leonita et al., 2021), and Goldfish, *Carrasius auratus* (Mustari, 2021). Ovaspec has been used on the Peres fish, *Osteochilus kappeni* (Lijana et al., 2021), the Nilem, *O. vittatus* (Madihah et al., 2023), Siamese catfish, *Pangasianodon hypophthalmus* (Agustinus et al., 2023), and climbing perch *Anabas testudineus* (Maharani et al., 2024). HCG successfully induced ovulation in the Mekong catfish, *Pangasius bocourti* (Cacot et al., 2002), and ovalumon stimulated ovulation in the African catfish, *C. gariepinus* (Abdel-Latif et al., 2021; Tama et al., 2022). Studies related to using these synthetic reproductive hormones in breeding seurukan fish have not been reported. Therefore, this work aimed to determine the efficacy of different reproductive stimulating hormones, namely ovaprim, ovaspec, HCG, and ovalumon, and their dosage to accelerate the ovulation process in seurukan fish.

Materials and Methods

Experimental design: This study was conducted in May 2024 in Gampong Meunasah Krueng, Beutong District, Nagan Raya Regency, Aceh Province, Indonesia. The methodology consisted of two experiments. The first experiment evaluated the different synthetic reproductive stimulating hormones to determine the most effective reproductive hormone. The second experiment aimed to determine the optimal dose of the hormone that elicited the best spawning and reproductive performance. The first experiment used a completely randomized design (CRD), with each hormone treatment having four replications. Four types of commercial reproductive hormones were used: ovaprim (Syndel Laboratory, Canada), ovaspec (Spectrum Asia, Germany), HCG (Chorulon, United States), and ovalumon (Estradiol, Philippines) at a dose of 0.5 ml kg^{-1} body weight (BW) female broodstock. In the second experiment, the most effective hormone determined from the first experiment was evaluated using different hormone doses, 0.25, 0.50, 0.75, and 1.0 ml kg^{-1} BW, with four

replications for each dose.

The first experiment

Broodstock selection and hormone injection: A total of 40 males (18-20 cm total length and 120-150 g body weight) and 20 females (25-30 cm total length and 250-300 g body weight) broodstocks were collected from local fishermen in the Nagan Raya district. The selected broodfish have reached the late-gonad maturity level and were distributed into 20 happas nets (1x1x1 m), which were placed in a flowing water pond. Each happa was stocked with one female and two males. Subsequently, fish were injected with synthetic reproductive hormones at a dose of 0.5 ml kg⁻¹ and 0.25 ml kg⁻¹ for female and male broodfish, respectively. Broodstock was released back into the happa and monitored until spawning occurred. After spawning, broodfish were separated and moved to the pond. All eggs in the happas were collected, and the total number was counted.

Eggs incubation and larval rearing: Approximately 200 eggs were taken randomly and then incubated in a 5L plastic jar equipped with aeration. The percentage of fertilization was observed after 2 hours of incubation. The fertilized eggs appeared transparent, while the unfertilized ones looked milky white. Unfertilized eggs were removed from the hatching container, while fertilized eggs were left in the incubation container. The hatching rate was then calculated for each hormone. After hatching, the larvae were maintained in the same container for 14 days. Feeding was performed with Artemia cysts for 5 days, followed by Tubifex three times a day at satiation.

The second experiment

Broodstock selection and hormone injection: A total of 16 females and 32 males matured broodstock were used in this study. The selected broodstock was weighed for body weight, measured for total length, and distributed into 16 happas (1x1x1 m) with a ratio of one female and two males. Subsequently, female broodstock was injected intramuscularly with ovaprim at a tested dose of 0.25, 0.50, 0.75, and 1.0 ml kg⁻¹ BW with one injection, while male broodstock was injected with ovaprim at a dose of 0.25 ml kg⁻¹ BW.

After being injected, the broodstock was released back to the happa and monitored every 2-hour intervals for spawning success. The pair successfully spawned was moved to the broodstock pond, and eggs collected in the happa were counted.

Eggs incubation and larval rearing: A total of 200 eggs were taken randomly from the happa, and then incubated in a 5L plastic jar equipped with aeration. The fertilization rate was calculated 2 hours after incubation. Unfertilized eggs were removed from the incubation container, while fertilized eggs were kept until hatched. After hatching, the larvae were kept in the same container for 14 days. During this period, they were fed on artemia cysts for 5 days, followed by tubifex three times a day at satiation.

Measured parameters

Latency period, total number of released eggs, and egg size: The latency period refers to the time elapsed between injection and the fish's ovulation (or spawning). It was calculated based on Donaldson (1983), while egg size was calculated following Harianti (2013): Egg size = $\sqrt{Dh} \times Dv$, where Dh is the horizontal axis of the egg (mm), and Dv is the vertical axis of the egg (mm).

Fertilization, hatching, and survival rates: The percentage of fertilization success was calculated as follows: Fertilization rate (%) = (total fertilized eggs/total incubated eggs) x 100. The percentage of the hatching rate was calculated using the following formula: Hatching rate (%) = (total of hatched eggs/total of fertilized eggs) x 100. Meanwhile, the survival rate was calculated as follows: Survival rate of larvae (%) = (total number of larvae – total number of dead larvae)/total number of larvae x 100.

Data analysis: The data were subjected to a one-way analysis of variance (one-way ANOVA) followed by a Duncan multiple range test using SPSS version 20.0 software. A *P*-value < 0.05 was considered statistically significant.

Results

Effect of different synthetic reproductive hormones: The results showed the impact of different synthetic reproductive hormones on spawning success

and egg quality in seurukan fish larvae (Table 1). Among the four tested hormones, ovaprim, ovaspec, and HCG successfully stimulated ovulation and spawning in Seurukan fish. However, fish injected with ovalumon did not show any spawning performance. The results showed that different hormones had a significant effect on the latency period and the number of released eggs ($P < 0.05$) but did not affect egg diameter, fertilization, hatching, survival rate, and normality of larvae ($P > 0.05$). The fastest latency period and the largest egg size were recorded in ovaprim treatment, i.e., 380 min and 3,970 eggs, respectively. The latency period in ovaprim treatment was significantly different from ovaspec and HCG treatments, while the number of eggs was not significantly different. The best egg size, fertilization rate, and survival rate of seurukan fish were also obtained by ovaprim, namely 1.78 mm, 82.40%, and 100%, respectively; however, these values were not significantly different from ovaspec and HCG treatments. The hatching rate of fish eggs successfully ovulated reached 100% in all treatments, and larvae were observed under normal conditions.

Effect of ovaprim dose: The data presented in Table 2 show the impact of varying ovaprim doses on the spawning response of fish related to egg quality and larval survival. The results showed that ovaprim hormone dose produced a significant effect on the latency period and egg size ($P < 0.05$) but did not significantly affect the number of eggs, hatching rate, fertilization rate, survival rate, and normality of larvae ($P > 0.05$). The shortest latency period and the largest egg size were obtained in ovaprim treatment at a dose of 0.50 ml/kg BW with values of 367 minutes and 2.50 mm, respectively. These values were significantly different from 0.25, 0.75, and 1.0 ml kg⁻¹ BW doses.

The total number of eggs released showed no significant differences among the various doses, ranging from 1783.3±368.55 to 2475.0±106.0 eggs. However, the highest number was observed at the 0.50 ml kg⁻¹ dose (2475.0±106.0), indicating that this dose may be optimal for maximizing egg release. The fertilization rate remained high across all tested doses, ranging from 79.28±2.61 to 88.05±8.06%. All doses

demonstrated a hatching success rate of 100%, reflecting the high quality of fertilized eggs produced at all levels of Ovaprim. Survival rates varied significantly among the different doses. The 0.5 ml kg⁻¹ BW dose produced the highest survival rate (100.00±0.00%), while 0.25 and 0.75 ml kg⁻¹ doses showed slightly lower survival rates (99.33±1.55% and 99.00±1.32%), respectively. The 1.00 ml kg⁻¹ BW dose had a lower survival rate (97.17±1.04%).

Discussions

This study showed that among the four commercial synthetic reproductive hormones, only three were successful in triggering ovulation and spawning in seurukan fish, namely ovaprim, ovaspec, and HCG, while ovalumon did not induce spawning. Ovaprim and ovaspec contain an analog of salmon GnRH (sGnRH_a) and a brain neurotransmitter (dopamine) inhibitor (Leonita et al., 2021; Syarif et al., 2021). These combinations are effective in inducing ovulation and improving the quality of eggs and larvae of fish (Lin and Peter, 1996; Brzuska, 2010). HCG contains Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) (Leonita et al., 2021). Although ovaprim and ovaspec contain GnRH and dopamine inhibitors, the doses are slightly different. For example, ovaprim contains 20 µg ml⁻¹ of sGnRH_a and 10 mg ml⁻¹ of dopamine inhibitors (Leonita et al., 2021), while Ovaspec contains 18 µg ml⁻¹ of GnRH-analog and 10 mg ml⁻¹ of anti-dopamine (Syarif et al., 2021). Therefore, ovaprim resulted in the best results compared to ovaspec.

sGnRH_a analogs and anti-dopamine play a crucial role in triggering ovulation and spawning in fish. Generally, GnRH stimulates the pituitary gland to release gonadotropins, which in turn induce the production of luteinizing hormone (LH) that triggers ovulation and spawning. Under natural conditions, gonadotropin secretion is inhibited by dopamine; hence, when the antagonist blocks dopamine, the function is inhibited, leading to increased gonadotropin secretion (Rosyida et al., 2021). GnRH hormone also stimulates the adenohypophysis to produce FSH, which enhances gonadotropin secretion

Table 1. Egg quality, survival, and normality of seurukan fish (*Osteochilus jeruk*) larvae based on the type of hormone used. Mean±SD values in the same column with different superscripts are significantly different.

Treatment	Latency period (min.)	Eggs size (mm)	Total released eggs	Fertility (%)	Hatching (%)	Survival (%)	Larvae normality (%)
Ovaprim	380.20±9.83 ^a	1.78±0.40 ^a	3970.0±668.5 ^c	82.40±6.56 ^a	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a
Ovaspec	556.60±19.50 ^b	1.74±0.31 ^a	3483.4±649.3 ^{ab}	80.57±6.32 ^a	100.00±0.00 ^a	99.00±1.06 ^a	100.00±0.00 ^a
HCG	736.00±11.31 ^c	1.44±0.08 ^a	2886.0±351.7 ^a	78.83±3.90 ^a	100.00±0.00 ^a	98.50 ±1.54 ^a	100.00±0.00 ^a
Ovalumon	-	-	-	-	-	-	-

Table 2. Egg quality, survival, and normality of seurukan fish (*Osteochilus jeruk*) larvae based on ovaprim dosage. Mean±SD values in the same column with different superscripts are significantly different.

Ovaprim dosage (ml kg ⁻¹)	Latency period (min.)	Eggs size (mm)	Total released eggs	Fertility (%)	Hatching (%)	Survival (%)	Larvae normality (%)
0.25	392.67±11.24 ^b	1.47±0.11 ^a	2083.3±340.34 ^a	79.28±2.61 ^a	100.00±0.00 ^a	99.33±1.55 ^{ab}	100.00±0.00 ^a
0.50	367.50±10.60 ^a	2.50±0.00 ^b	2475.0±106.0 ^a	88.05±8.06 ^a	100.00±0.00 ^a	100.00±0.00 ^b	100.00±0.00 ^a
0.75	406.00±2.00 ^b	1.53±0.11 ^a	2016.6±425.24 ^a	85.38±8.55 ^a	100.00±0.00 ^a	99.00±1.32 ^{ab}	100.00±0.00 ^a
1.00	405.00±5.56 ^b	1.53±0.11 ^a	1783.3±368.55 ^a	83.08±1.15 ^a	100.00±0.00 ^a	97.17±1.04 ^a	100.00±0.00 ^a

(Maggi, 2016). These hormones work synergistically to ensure the smooth running of the entire reproductive system (Tsutsui and Ubuka, 2021). Meanwhile, ovalumon contains ethinylestradiol (Rehman et al., 2015), and this hormone fails to trigger ovulation and spawning in seurukan fish. Intramuscular administration of ethinylestradiol to fish can increase the production of the hormone in fish blood, thereby accelerating the process of vitellogenesis and gonad maturity (Tama et al., 2022). In seurukan fish, this hormone is ineffective and even causes negative effects, including hardening of the stomach, production of excessive feces, and failure to spawn.

The results showed that ovaprim produced better results than ovaspec and HCG. In the second experiment, ovaprim administration at a dose of 0.5 ml kg⁻¹ BW yielded the best results. Several studies reported that ovaprim at the same dose produced the best spawning performance in fish, including eel-tailed catfish, *Tandanus tandanus* (Cheah and Lee, 2000), African catfish, *Clarias gariepinus* (Namadina, 2019), seabass, *Dicentrarchus labrax* (Zohar and Mylonas, 2001), and Asian stinging catfish, *Heteropneustes fossilis* (Chaube et al., 2014).

The administration of reproductive hormones not only induces broodfish to ovulate and spawn but also improves egg quality and fertilization rates, as shown in this study, where the use of ovaprim resulted in better egg quality and a higher fertilization rate compared to ovaspec and HCG. Egg quality affects the fertilization rate of fish (Abidin et al., 2006). Therefore, using ovaprim facilitates increased fertilization and hatching rates in seurukan fish. Stimulating hormones at optimal doses can create ideal conditions in fish, enhancing the reproductive process and increasing fertilization efficiency (Sinjal, 2014; Kumar et al., 2021). Lower or higher doses of ovaprim than optimal requirements can cause a decrease in egg quality. This is because hormones function optimally at certain doses, and lower or higher than optimal limits reduce effectiveness (Yuatiati et al., 2015; Fatemi et al., 2021).

The injection of GnRH hormone into fish bodies

increases the dose in the blood, which then induces the pituitary gland to release gonadotropins, such as LH and FSH. LH and FSH go through the bloodstream to the gonads, where LH stimulates the production of enzymes that break down follicle walls, causing ovulation. At the same time, FSH plays a crucial role in the development of eggs within the follicle. Anti-dopamine enhances the effects of GnRH by inhibiting dopamine, a neurotransmitter that generally blocks the release of GnRH, leading to high GnRH production and increased release of LH and FSH (Hill et al., 2009).

Conclusions

In conclusion, ovaprim, ovaspec, and HCG successfully stimulated ovulation and spawning of seurukan fish, while fish injected with ovalumon failed to spawn. Ovaprim produced better egg quality, fertilization, and survival rates. The second experiment showed that Ovaprim at a dose of 0.5 ml kg⁻¹ BW produced better results compared to other tested doses. Therefore, Ovaprim 0.5 ml kg⁻¹ BW was recommended as the optimal dose for induced breeding of seurukan fish.

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