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INDUCED BREEDING AND SEED PRODUCTION OF NALEH FISH Barbonymus sp. USING SYNTHETIC HORMONES

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ABSTRACT

The Naleh fish, *Barbonymus* sp., is a native freshwater fish in Aceh province, Indonesia, and is considered one of the most popular fish species for food and ornamental purposes. Induced breeding technology is crucial for the success of any fish farming practice, but currently, the effective hormone treatments and induced breeding and seed production methods for Naleh fish are unknown. Therefore, the present study aims to determine the suitable hormone for effective induced breeding of Naleh fish. The study was conducted in January 2025 at the Community Fish Breeding Center of Meunasah Krueng Village, Nagan Raya District, Aceh Province, Indonesia. The Completely Randomized Design method with 3 treatments and 5 replications for each hormone was tested. Three types of commercial available hormones, namely Ovaprim, Ovaspec, and HCG, were evaluated. The results show that the types of hormones tested significantly affected latency time, fertilization rate, and survival rate ($p<0.05$) but did not significantly affect hatching rate and larval malformation rate ($p>0.05$). The highest reproductive performance parameters were found in the Ovaprim induced fish groups. Therefore, it was concluded that Ovaprim hormone is the suitable hormone for the effective induction of spawning and seed production of Naleh fish.

INTRODUCTION

Naleh fish *Barbonymus* sp. is a native freshwater fish species with significant economic value and considerable potential for commercial aquaculture. It is primarily distributed in the Southwestern region of Aceh, Indonesia, especially in the Nagan Raya area (Muchlisin *et al.*, 2015; Batubara *et al.*, 2018). Intensive fishing practices by local communities have led to overexploitation and decline of wild populations (Batubara *et al.*, 2019). Reports from fishermen in Nagan Raya and adjacent regions indicate that a consistent decline in Naleh fish catches, with large individuals over 20 cm now rarely observed in recent decades. Concurrently, the demand for Naleh fish is projected to rise due to the increased demand and nutritional importance of fishderived animal protein for the burgeoning population. To address these challenges, the development of an integrated aquaculture system, and captive breeding and seed production are essential to satisfy market demand and alleviate fishing pressure on wild stocks. Further, the production of high-quality larvae is fundamental to successful aquaculture business (Muchlisin *et al.*, 2025). Such larvae are typically produced through induced spawning using high-quality certified and captive reared broodstock (Dewantoro, 2015; Muchlisin *et al.*, 2025).

Several synthetic hormonal preparations have been commonly employed in fish for gonadal maturation, and induced spawning and ovulation in different fish species such as Ovaprim, Ovaspec, and Human chorionic gonadotropin (HCG) (Marimuthu *et al.*, 2007; Marimuthu *et al.*, 2009; Marimuthu and Haniffa, 2010; Rahman *et al.*, 2014; Muchlisin *et al.*, 2025). Ovaprim and Ovaspec contain salmon gonadotropin-releasing hormone analogs (sGnRHa) and dopamine antagonist (Syarif *et al.*, 2021; Nargesi *et al.*, 2025). These hormones play a crucial role in enhancing the efficiency of mass induced fish breeding and seed production in hatchery conditions and ultimately leading to improved fish production in aquaculture. The main function of the sGnRHa is to stimulate the production of endogenous gonadotropin hormones, while dopamine antagonist inhibit the dopamine receptors in the pituitary, thereby increasing pituitary activity to release GnRH, then accelerating the ovulation process (Quiniou and Bosworth, 2020; Abdel-Latif *et al.*, 2021; Rosyida *et al.*, 2021). HCG consists of approximately 90% luteinizing hormone (LH) and 10% follicle-stimulating hormone (FSH). FSH stimulates follicle development and maturation, and enhances vitellogenesis. LH promotes the production of testosterone, estrogen, and progesterone, which are essential for the final maturation of oocytes (Betz *et al.*, 2008; Longo *et al.*, 2025).

The effectiveness of the hormone and dosages is species-dependent (Nazir *et al.*, 2023; Siddique *et al.*, 2024), and among the commercial spawning hormones, Ovaprim, Ovaspec, and HCG are the most widely used. For instance, Ovaprim has effectively induced ovulation and spawning in spotted snakehead fish *Channa punctatus* (Marimuthu and Haniffa, 2010), seurukan fish *Osteochilus vittatus* and *O. jeruk* (Muchlisin *et al.*, 2014; Muchlisin *et al.*, 2025), freshwater catfish *Abramis brama* (Nargesi *et al.*, 2025), and African catfish *Clarias gariepinus* (Urom, 2024). Ovaspec has demonstrated efficacy in Seluang fish *Rasbora einthovenii* (Syarif *et al.*, 2021) and climbing perch *Anabas testudineus* (Maharani *et al.*, 2024). HCG has successfully induced ovulation in pikeperch *Sander lucioperca* (Křišt'an *et al.*, 2013), Royal danio *Barilius bakeri* (Antony and Natarajan, 2016), and silver carp *Hypophthalmichthys molitrix* (Gupta *et al.*, 2024). Therefore, individual fish species-specific studies are needed to ensure the correct hormone and optimum dose for successful induction of breeding.

Breeding technology for high-value native fish, particularly Naleh fish, needs to be developed to produce high-quality fish larvae and fingerlings for the aquaculture of this species. The Naleh fish is one of the popular fish species in high demand and a potential candidate for farming due to its huge potential as an ornamental fish and a food fish in Indonesia. Several basic studies related to the Naleh fish have been reported by several researchers, including studies on the bioecology and genetics of the Naleh fish as a basis for their sustainable fisheries management (Batubara, 2019; Batubara *et al.*, 2021). Efizon *et al.* (2021) have studied the reproductive biology of the Naleh fish in the Nagan River. Abdan and Sulistiono (2023) studied the growth and reproductive patterns of the Naleh fish, and Yulvizar *et al.* (2025) screened lactic acid bacteria from the intestines of Naleh fish as a natural probiotic to improve fish health and support sustainable cultivation of freshwater fish. However, studies on induced breeding and seed production using ovulation-stimulating hormones, specifically Ovaprim, Ovaspec, and HCG, have never been reported. Therefore, this study aims to determine the suitable hormone to accelerate gonad maturity and ovulation in Naleh fish using these three different hormones.

MATERIALS AND METHODS

Experimental design

This study was conducted in January 2025 at Meunasah Krueng Community Breeding Center, Nagan Raya District, Indonesia. Three commercial reproductive stimulating hormones, namely: Ovaprim (Syndel Laboratories, Canada), Ovaspec (Spectrum Asia, Germany), and HCG (Chorulon, United States) were tested in this experiment. These hormones were selected due to their widespread market availability and relatively low cost. The study complies with Research Ethics in animal used in research of Universitas Syiah Kuala Code No. 958/ 2015.

Broodstock preparation

A total of 50 male broodstock with a weight ranges from 30-70 g and a total length ranges from 17-20 cm, and 30 females with a weight ranges from 40-70 g and a length ranges from 18-28 cm were collected from the Nagan River, Nagan Raya Regency, Aceh Province, Indonesia, and its tributaries. The captured broodstock fishes were stocked in hapa nets placed in earthen ponds with a flowing water system. During conditioning, the broodstock were given commercial feed with a crude protein content of 30% and crude lipid of 18% and the fish were fed at the rate of 5% of body weight twice daily for two two-week acclimatation period.

After two weeks, broodfish were evaluated for gonadal development, and only individuals at the late gonad maturity stage were selected for the induced spawning experiments. Mature males were identified by a reddish genital papilla and the easy release of sperm upon gentle abdominal pressure. Mature females exhibited an enlarged, soft abdomen, a swollen and reddish genital opening, and released oocytes with minimal blood loss when the abdomen was pressed. The mature female Naleh fish is showed in Figure 1.

Figure 1. Matured female Naleh fish *Barbonymus* sp.

Breeding process

Thirty males and fifteen females were selected for the study. The broodfish were allocated to fifteen hapa nets, each measuring 1 x 1 x 1 x 1m, and placed in flowing-water ponds with water temperatures ranging from 26 to 28 oC, pH 6.6 - 7.2, and dissolved oxygen levels of 5.8 - 8.2 ppm. Each net stocked with one female and two males. Each hormone was intramuscularly administered at doses of 0.5 ml/kg for female and 0.25 ml/kg for male broodstock, respectively, with a single injection given at 5:00 PM. For each hormone, 5 replicate treatments were evaluated. Following the injection, the broodfish were returned to the hapas and monitored until spawning. After spawning, broodfish were transferred to separate ponds. Eggs from each hapa were collected and the total number of eggs from each breeding set was counted.

Incubation of eggs and larvae rearing

Following the enumeration of total released eggs, a total of 200 eggs from each treatment group were randomly selected and placed in a 5-liter plastic jar containing aerated water for incubation. Fertilization success was assessed after 2 hours, and embryo development was monitored at 2-hour intervals for 24 hours by randomly sampling eggs for microscopic observation. Upon hatching, larvae were reared for 15 days. From day 3 to day 8 larvae received boiled egg yolk dissolved in water and the larvae were fed twice daily at *ad libitum*. Feeding commenced using solid food on the third day, coinciding with the complete absorption of the yolk sac. From day 8 to 15, larvae were fed with zooplankton (*Daphnia* sp.).

Measured parameters

- The latency period was calculated using the following formula:

Latency period (hours) = Ovulation time (minutes) – Last hormone injection time (minutes).

- Egg diameter was measured using a Zeiss microscope by calculating the long axis and short axis, then averaging.
- Fertility rate (FR) was calculated based on the following formula:

FR (%) = (number of fertilized eggs) / (number of incubated eggs) x 100.

- Hatching rate (HR) was calculated using the following formula:

  HR (%) = (number of hatched eggs) / (number of incubated eggs) x 100.

- The rate of normal larvae (RNL) (was calculated as the proportion of larvae without any morphological defects (in the head or tail, eyes, and fins) to the total number of larvae observed using the following formula:

  RNL (%) = (Number of fish larvae without any morphological defects) / (Total number of larvae observed) x 100.

- Survival rate (SR) of larvae was calculated using the following formula:

  SR (%) = (Number of fish larvae at the end of the study) / (Total number of larvae observed) x 100.

In addition, microscopic embryonic and larval development was also observed. The embryogenesis was observed from eggs after fertilization until the eggs hatching, at certain phases such as cell division, morula, blastula, gastrula, organogenesis, and larvae. Observations were carried out every 2-hour interval using a microscope for 24 hours or until the eggs hatched. The embryonic development was recorded in this study only for the Ovaprim treatment, because there was no substantial difference in the embryogenesis observed among the other hormone treatments. The main water quality parameters such as temperature, pH, and dissolved oxygen (DO) were also measured at the beginning and end of the study in all the incubation tanks using a YSI water checker (SKU – 626870-1).

Data analysis

The experimental data were analysed using SPSS ver. 25.0. The data were initially tested for the normality assumption and when the data were not normal, they were arcsine transformed. The transformed data were analysed using one-way ANOVA, followed by Duncan's multiple range test to identify which pairs of group means are statistically significant.

Results and Discussion

The results show that there was a significant difference in reproductive performance among the hormone treatments especially for the latency period, egg diameter, total eggs released, fertility, and survival rates ($p<0.05$), however there was no significant difference noticed among the hormones with regard to hatchability and larvae abnormality ($p>0.05$). The average latency period ranged from 380 to 472 minutes. Significantly low latency period (380.0±7.9 minutes) was found in the Ovaprim hormone administered fishes; followed by Ovaspec (410.0±22.6 minutes) and HCG hormone treatments (472.6 ± 17.5 min). The egg diameter ranged from 1.9 to 2.9 mm. The fish administered with Ovaprim showed larger eggs (2.9 ± 0.18 mm) than those treated with Ovaspec (2.3 ± 0.11 mm) and HCG (1.9 ± 0.20 mm). The total number of eggs released ranged from 2573 to 3853 eggs. The higher number of eggs was recorded in the Ovaprim treatment (3853.6 ± 492.3), but there was no significant difference noticed from the Ovaprim and Ovaspec hormone treatment groups. The lowest number of eggs were observed in the HCG (2573.4 ± 570.7) administered groups. (Table 1).

The fertilization rates ranged from 74.28 to 94.49%, among the three hormones. the highest fertility was found in the Ovaprim treatment, but it was not significantly different from the Ovaspec treatment. The hatching rate ranged from 97.5 to 100%, The highest hatching rate (94.49 ± 3.46 %), was found in the Ovaprim treatment, but was not significantly different from the Ovaspec (88.86 ± 3.97 %) and HCG hormones (74.28 ± 10.80 %). Significantly, the highest percentage of survival (100%) and normal larvae (100%) was also obtained in the Ovaprim hormone treatment. The percentage of morphologically normal larvae did not differ significantly among the hormone treatments ($P>0.05$) Ovaprim and Ovaspec had very high rates of normal larvae (100.0% and 99.2 ± 0.83%, respectively), while HCG was slightly lower (93.2 ± 6.22%).

The observations of the embryonic development phases in Naleh fish generally include the fertilization, morula, blastula, gastrula, and organogenesis phases. The morula phase occurs 3 hours after the egg is fertilized; this phase lasts for 2 hours. At the 5th hour, the embryogenesis process enters the mitotic division phase and forms many cells called a morula. After this phase ends, at the 7th hour, the embryogenesis process enters the blastula phase. In this phase, division continues, and the formation of body cavities occurs. Furthermore, at the 8th hour, the embryogenesis process enters the gastrula phase, where the body shape becomes more clearly visible; this phase lasts for approximately 2 hours. After 2 hours, or at the 9th hour after fertilization, the embryogenesis process enters the blastula phase. This phase lasts for approximately 3 hours, after which the embryonic development process enters the organogenesis phase, which is 12 hours after fertilization. This organogenesis process lasts for approximately 5 hours, and the eggs begin to hatch to produce larvae approximately 15 hours after fertilization (Figure 2). In addition, the incubation water temperature, pH, and dissolved oxygen were also measured in situ, with the respective value ranges from temperature 25 – 27°C, pH 7 – 8.9, and DO 6 – 7.9 mg/L, indicating that, in general, the water quality was still within the tolerance of fish larvae.

The present study evaluated the efficiency of three hormonal preparations such as Ovaprim, Ovaspec and HCG on the different reproductive performance and larval parameters of Naleh fish. The study demonstrated that all three commercially available breeding hormones effectively induced ovulation in Naleh fish under captive conditions. Although Ovaprim yielded outcomes and best reproductive performance, followed by Ovaspec. This similarity in reproductive efficacy is likely attributable to both hormones containing the Salmon Gonadotropin Releasing

Hormone analog (sGnRHa) and a dopamine antagonist compound (Syarif *et al.*, 2021). Despite both products containing sGnRH-a, their molecular structures differ. Ovaprim includes the salmon analog D-Arg6, Pro9NEt, which has been shown to induce ovulation, enhance sperm production, and improve the quality of eggs and larvae (Lin and Peter, 1996; Brzuska, 2021). In contrast, Ovaspec contains the D-Ala6 analog, Pro9-NEt, which is reported to be more effective in increasing fertilization rates (Brzuska, 2021). The sGnRHa and dopamine antagonist function to stimulate gonadal maturation and ovulation in fish. Gonadotropin-releasing hormone (GnRH) prompts the pituitary gland to secrete gonadotropins, which subsequently induce the production of Luteinizing Hormone (LH). LH initiates ovulation, allowing fish to release eggs. Under natural conditions, dopamine inhibits gonadotropin secretion; thus, blocking dopamine with an antagonist that increases gonadotropin release (Rosyida *et al.*, 2021). GnRH also stimulates the adenohypophysis to produce Follicle-Stimulating Hormone (FSH), which regulates the concentration and timing of gonadotropin secretion, thereby affecting the ovulation cycle and egg production (Kasiyati, 2018). LH and FSH act synergistically to promote reproductive success (Oduwole *et al.*, 2021; Li *et al.*, 2024).

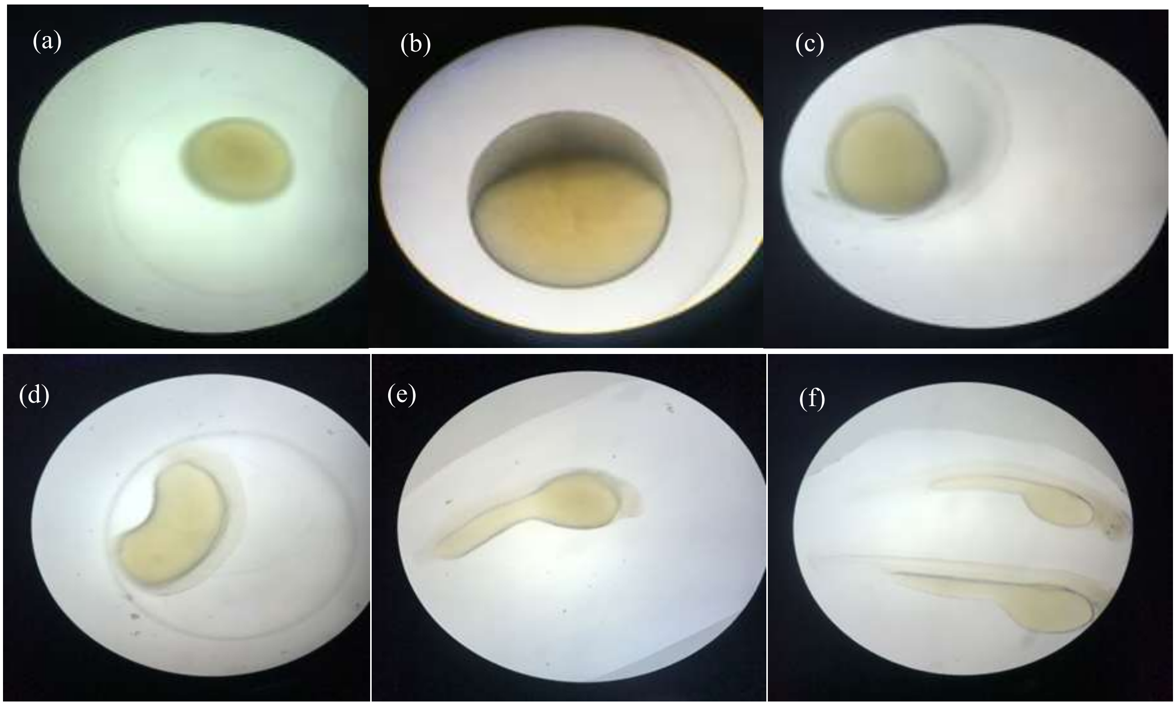

Figure 2. Embryogenesis of Naleh fish *Barbonymus* sp. induced breeding with Ovaprim hormone; (a) clavage phase, (b) morulla phase, (c) blastula phase, (d) gastrula phase, (e) organogenesis phase, (f) larvae phase

Table 1. The reproductive performance of Naleh fish (*Barbonymus* sp.) fish induced with three types of hormones

Hormone treatment	Latency period (minutes)	Egg diameter (mm)	Total number eggs released	Fertilization Rate (%)	Hatching Rate (%)	Survival rate of larvae (%)	Normal fish larvae (%)
Ovaprim	380.0±7.9[c]	2.9±0.18[a]	3853.6±492.26[a]	94.49±3.46[a]	100.0±0.00[a]	100.0±0.00[a]	100.0±0.00[a]
Ovaspec	410.0±22.6[b]	2.3±0.11[a]	3544.6±184.21[a]	88.86±3.97[a]	99.4±0.54[a]	98.3±1.35[a]	99.2±0.83[a]
HCG	472.6±17.5[a]	1.9±0.2[b]	2573.4±570.69[b]	74.28±10.80[b]	97.5±4.61[a]	89.6±3.18[b]	93.2±6.22[a]

Each value is the mean and standard deviation of five replicate treatments. The mean values with different superscripts are significantly different ($p<0.05$)

In addition to being effective in induced breeding of Naleh fish as shown in this study, the Ovaprim hormone has also been reported to successfully induce ovulation and improve reproductive performance in stinging catfish *Heteropneustes fossilis* (Hossain *et al.*, 2013; Yasmin *et al.*, 2024), common carp *Cyprinus carpio* (Sinaga and Telaumbanua, 2020), Siamese catfish *Pangasius hypophthalmus* (Ihwan *et al.*, 2021), serukan fish *Osteochilus vittatus* and *O. jeruk* (Muchlisin *et al.*, 2014; Muchlisin *et al.*, 2025), Indian carp *Labeo mrigala* (Kumar, 2023), and blackfin sea bream *Acanthopagrus berda* (Abbas *et al.*, 2019). In addition to successfully inducing ovulation in female fish, Ovaprim has also been reported to be effective in inducing spermiation in male fish, for example, in silver rasbora *Rasbora argyrotaenia* (Al Adawiyah *et al.*, 2019), common bream *Abramis brama* (Nargesi *et al.*, 2025), and African catfish *Clarias gariepinus* (Gbemisola and Adebayo, 2014). Although HCG successfully triggered ovulation in naleh fish, its results were less satisfactory than those achieved with Ovaprim and Ovaspec. Nevertheless, HCG remains suitable for the induced breeding of several fish species for example, catfishes, *Clarias macrocephalus* (Mollah and Tan, 1983) and *Clarias lazera* (Ahmed and Manofal, 2017), silver pompano *Trachinotus blochii* (Putra and Mulah, 2019), freshwater fish golden perch, *Macquaria ambigua* (Rowland, 1983), and Nile tilapia *Oreochromis niloticus* (Rbbani *et al.*, 2025). Therefore, this study confirms that the effectiveness of hormone type is species-dependent.

Observations of fertilized eggs incubated at 26 to 27°C demonstrate that Naleh fish embryogenesis proceeds through distinct phases: cleavage, morula, blastula, gastrula, and organogenesis. These phases occur at 3, 5, 7, and 9 hours post-fertilization, respectively. The organogenesis phase, marked by the development of the head, tail, and somites, lasts for 3 hours. Hatching begins 15 hours after fertilization. The embryonic development rate of naleh fish is faster than that of other freshwater fish species. For instance, the climbing perch (*Anabas testudineus*) exhibits an incubation period of 24 hours at 29°C (Suriansyah, 2021), while the Siamese catfish (*Pangasius hypophtalmus*) requires 25 hours at 28°C (Sitinjak *et al.*, 2019). However, the duration of embryogenesis and organogenesis in fish is strongly influenced by incubation temperature (Bondarenko *et al.*, 2015). In the Black saddled coral grouper (*Plectropoma laevis*), eggs incubated at 32°C hatch after 14 hours, whereas at 26°C, hatching occurs after 18 hours (Andriyanto *et al.*, 2013). Similarly, in the Tinfoil barb (*Barbonymus schwanenfeldii*), eggs incubated at 24°C hatch after 80 hours, compared to 94 hours at 28°C (Suhada *et al.*, 2022).

Conclusion

This study observed that reproductive performance parameters varies among the hormones administered. The hormone significantly influenced latency time, egg diameter, number of eggs released, fertilization rate, and survival rate. However, it did not have a significant effect on hatching rate or on normality of larval morphology. Among the hormones tested, Ovaprim showed the most favorable results in induced breeding of Naleh fish (*Barbonymus* sp.), with an average latency time of 380 minutes, egg diameter of 2.9 mm, approximately 3853 eggs released, a fertilization rate of 94.4%, and both hatching and survival rates at 100%. Based on these results, Ovaprim is recommended for the effective induced breeding and seed production of Naleh fish under captive conditions. Further research could explore the underlying mechanisms of how Ovaprim enhances these reproductive performances. Additionally, investigating the long-term effects of using Ovaprim on fish health and population dynamics would be beneficial for sustainable aquaculture practices. Further study is needed on how to increase larval survival and growth, for instance, by providing enhanced natural feed and good water quality management.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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