



# Increasing of Spawning Performance of Snakehead Fish (*Channa striata*) with the Use of Ovaprim Hormone Dosage Variations in Male and Female Broodstock

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**Abstract.** Snakehead fish (*Channa striata*) is an economically important fish. In order to meet market needs, the current supply is more dependent on catches in public waters than on cultivation. One reason is the lack of seed production from hatcheries. The purpose of this study was to analyze the effect of using various doses of the ovaprim hormone on male and female snakehead fish to improve the spawning performance of snakehead fish. The study was an experiment using a 2 x 2 factorial completely randomized design (CRD) with 3 replications. The first factor was the dose of the male broodstock ovaprim hormone, with two dose levels of 0.5 mg/kg and 1.0 mg/kg, and the second factor was the dose of the female broodstock ovaprim hormone, 1.5 mg/kg and 3.0 mg/kg. The results showed that the latency time of snakehead fish in this study ranged from 22.6 to 31.2 hours. Hormone dose significantly affected the latency time of male and female broodstock, but no significant interaction. Fertilization rate 85.58 – 88.86% and egg hatchability 93.3 – 96.7%. These parameters of fertilization rate for males are significant, but females and the interaction show no significant differences. Then, there was no difference in egg hatchability between all treatments. They concluded that variations in ovaprim hormone doses only affected latency time and fertilization rate, and egg hatchability had no effect.

**Keywords:** Ovaprim Hormone, Performance, Spawning, Snakehead Fish.

## 1 Introduction

The snakehead fish (*Channa striata*) is an economically important freshwater fish in several Asian countries. In Indonesia, it is mostly found on the main islands of Java, Sumatra, and Kalimantan [1, 2], and the population's consumption of snakehead fish is very high, especially in South Kalimantan [3]. Snakehead fish is a commercial freshwater fish species that supports the fisheries sector and significantly improves the welfare of local communities [4, 5]. The snakehead fish is a type of fish native to swamp waters and is a carnivorous fish that can be cultivated [6]. Market demand for snakehead fish continues to increase, while production (exploitation) of snakehead fish in South Kalimantan tends to increase annually [7]. In fact, according to the Deputy of Bank

Indonesia, snakehead fish significantly influences the inflation rate in South Kalimantan [8].

The development of snakehead fish farming in controlled environments remains limited. In addition to the limited dissemination of cultivation technology, the availability of seeds is a limiting factor. Identification and inventory of snakehead fish farmers in South Kalimantan revealed that these are only small and side businesses, with no large-scale operations. More than 95% of the snakehead fish seed used is still sourced from wild catches [3]. Efforts to increase snakehead fish seed productivity can be achieved by mastering seed production technology, followed by growth. Fish farmers are willing to successfully cultivate snakehead fish if they have adequate seed sources, readily available fish feed, and a market ready to accommodate the harvest. Generally, demand for snakehead fish for consumption is quite high, at 2–5 tons per month, while only 60% of the demand can be met. Therefore, it is necessary to increase the production of snakehead fish both naturally, semi-naturally, and intensively, which is carried out proportionally and sustainably [7].

Several studies on snakehead fish include domestication and cultivation [9, 10], research on the reproductive aspects of snakehead fish in their habitat [7], and research on the first gonad maturity of snakehead fish, the results were 190 mm long and 50.3 g in weight [11]. Furthermore, research on the technical cultivation of snakehead fish using the biofloc system to be more efficient in terms of feed and water quality can be maintained, especially from nitrates and ammonia, which are poisons for fish [12], and maintenance carried out in two phases with increasing fish size. In the first phase, fingerling size (weight  $1.94 \pm 0.71$  g, length  $6.29 \pm 0.81$  cm) was stocked @ 200 m<sup>3</sup> in a biofloc tank, which was reduced to 15 m<sup>3</sup> in the second phase using the same stock [13]. Research on the use of probiotic technology in snakehead fish cultivation [1], followed by research on snakehead fish cultivation using an aquaponic system [14], and research on snakehead fish cultivation using a recirculation system to maintain stable water quality parameters [15].

*Channa striata* seed and breeding programs must be consistently promoted to increase production [16]. Research results have not yet demonstrated proven technology for snakehead fish seed production. Mass production of snakehead fish fry, which is necessary for its cultivation, is very difficult. The development of snakehead fish cultivation is very slow, with seed availability being one of the obstacles [17]. Studies have shown that snakehead fish are very difficult to spawn or mate naturally. Therefore, to accelerate spawning, hormonal intervention is needed to stimulate gonad maturity, a process known as semi-artificial spawning. According to [18], fish spawning is influenced by several factors, namely hormone induction, the environment, and broodstock quality. Although hormonal induction is known, the optimal dosing strategy for male and female snakehead broodstock has not been explored. This study aims to determine the effect of various combinations of ovaprim hormone doses induced in male and female snakehead broodstock on spawning performance, namely latency time, degree of fertilization, and egg hatchability. We will get a stable snakehead fish breeding technology that can be applied to aquaculturists.

## **2 Materials and Methods**

### **2.1 Location and Time**

This research was conducted in tarpaulin ponds specifically constructed for research purposes and located in Samhurang Village, North Labuan Amas District, Central Hulu Sungai Regency, South Kalimantan. The research period was from September to December 2022.

### **2.2 Materials and Equipment**

The tools and materials used included broodstock snakehead fish, ovaprim hormone, 10% formalin, distilled water, 12 tarpaulin ponds measuring 2.5 m x 1.5 m x 1 m, a water pump, a Styrofoam box, a basin, a bucket, a scoop, a digital scale, a fish length gauge, a measuring cup, a Petri dish, a syringe, a thermometer, a pH meter, a DO meter, ammonia test kits, a plankton net, sample bottles, and a microscope. The test fish used in this study were male and female broodstock snakehead fish, each weighing 300-400 g, kept in tarpaulin ponds and fed pellets. The number of broodstock required for spawning was 12 males and 12 females. The gonadotropin hormone administered to spawning snakehead fish was ovaprim.

### **2.3 Research Procedures**

The broodstock of snakehead fish to be spawned were first kept in a 4 m x 2.5 m x 1.2 m tarpaulin pond with a water level of 1 m. Male and female broodstock were kept separately, each with a stocking density of 10 broodstock per tarpaulin pond and fed trash fish at a dose of 7% of their body weight. Every day, the gonad maturity of both males and females was monitored with the hope that the broodstock would develop gonad maturity at Gonad Maturity Level (GML) IV. The characteristics of female broodstock with GML IV include a larger, softer abdomen due to the presence of eggs. When massaged, the female releases orange eggs. Meanwhile, males with GML IV have milky white testicles, larger and firmer than those at previous maturity levels. The surface of the testicles feels slightly rough, and they are ready to be released during the spawning process.

Water temperature measurements in the tarpaulin pond for the maintenance of snakehead broodstock are carried out every morning and evening, the results of which range from 26-28°C °C, which meets the standard of 25- 32 °C. Furthermore, water pH, dissolved oxygen, and ammonia levels are measured once a week. The results of pH measurements are in the range of 6.5-6.6, and this meets the standard of 4.5-7.0. Dissolved oxygen measurements are in the range of 4.9-5.4 mg/L, the standard of 3.7-5.7 mg/L, and ammonia levels are in the range of 0.04-0.05 mg/L, meeting the standard of below 0.1 mg/L.

Male and female broodstock that have reached GML IV maturity are taken from the broodstock pond and then injected with the hormone ovaprim. The weight and body length of the broodstock snakehead fish are measured to calculate the hormone requirements according to the treatment dosage. The injection is performed

intramuscularly in the back, with the needle positioned at a 30-45° angle and inserted to a depth of 1 cm. The broodstock snakehead fish are placed in tarpaulin ponds with a ratio of 1 male to 1 female. Each spawning pond contains a pair of gonad-mature broodstock snakehead fish that have been stimulated with gonadotropin hormone (ovaprim).

Observations were made on the length of time between the first male and female parent being injected with the hormone ovaprim until the time of spawning, calculated as the latency time. Furthermore, when the eggs were removed from the spawning female parent, observations were made on the degree of fertilization, namely the ratio of the number of fertilized eggs to the total number of eggs, and observations on the number of hatched eggs compared to the total number of eggs, calculated as a percentage of egg hatchability.

#### 2.4 Research Design, Hypothesis, and Data Analysis

The statistical research design used a Completely Randomized Design (CRD) Factorial 2 x 2 (two factors, two levels), and three replications. The treatments were as follows: Factor M, the ovaprim dose for male broodstock, with two levels: M<sub>1</sub> = dose 0.5 mL/kg; M<sub>2</sub> = dose 1 mL/kg  
Factor F, the ovaprim dose for female broodstock, with two levels: F<sub>1</sub> = dose 1.5 mL/kg; F<sub>2</sub> = dose 3 mL/kg

The hypotheses in this study were: (1) differences in ovaprim doses for male broodstock have a significant effect; (2) differences in ovaprim doses for female broodstock have a significant effect; and (3) there is an interaction between the ovaprim doses given to male and female broodstock. Hypothesis testing uses Analysis of Variance (ANOVA), and if the results are significantly different, then it is continued with Duncan Multiple Range Test (DMRT) to obtain the differences between the different treatments.

#### 2.5 Research Parameters

The parameters observed from the results of this experiment were reproductive performance, including:

**Latency Time.** Which is the time from the ovaprim hormone injection to the ovulation of the female fish. The equation is:

$$TL = t_1 - t_0 \quad (1)$$

Where: TL: Latent time (hours)

t<sub>1</sub>: Spawning time

t<sub>0</sub>: Injection time

**Fertilization Rate.** The number of fertilized and unfertilized eggs is determined through a complete census by directly observing the egg color and its equation:

$$\text{Degree of Fertilization} = \frac{\text{Number of fertilized eggs}}{\text{Total number of eggs}} \times 100\% \quad (2)$$

**Egg Hatchability.** The number of eggs that hatch is determined through a complete census, followed by calculating the hatchability using the following formula:

$$\text{Egg hatchability} = \frac{\text{Number of eggs hatched}}{\text{Number of eggs fertilized}} \times 100\% \quad (3)$$

### 3 Research Methods

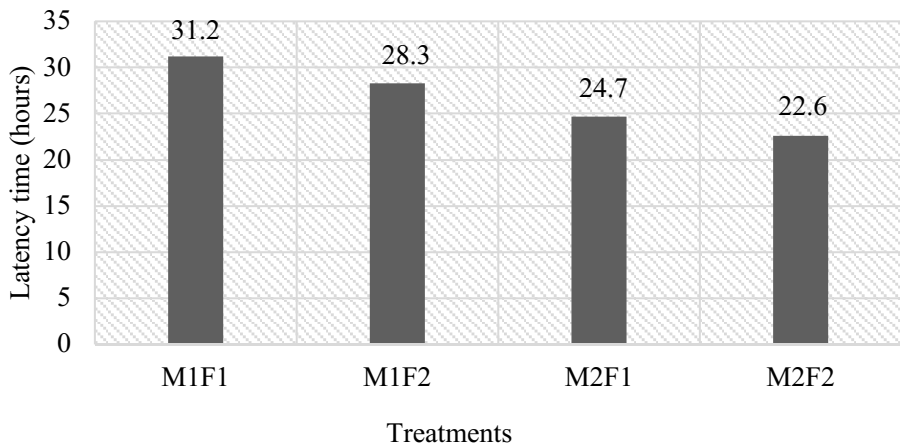
#### 3.1 Latency Time

Latency time is the period from fish spawning after the ovaprim hormone injection until the parent fish ovulates (releases eggs). The following data is obtained and presented in Table 1.

**Table 1.** Latency time data for snakehead broodstock after ovaprim hormone injection.

Number	Treatment	Latency time (hours)			Average and Standard Deviation
		1	2	3	
1	M <sub>1</sub> F <sub>1</sub>	33.2	29.3	31,1	31.2 ± 1.95
2	M <sub>1</sub> F <sub>2</sub>	26.7	30.3	28,0	28.3 ± 1.82
3	M <sub>2</sub> F <sub>1</sub>	24.8	23.6	25,8	24.7 ± 0.90
4	M <sub>2</sub> F <sub>2</sub>	22.2	23.2	22,4	22.6 ± 0.53

From the data in Table 1, the graph presented in Fig.1 below is shown:



**Fig. 1.** Latent time graph (hours) of snakehead fish for each treatment.

The graph above explains that the latency time after ovaprim hormone injection in male and female broodstock was longest in the M<sub>1</sub>F<sub>1</sub> treatment (ovaprim dose of 0.5 mL/kg male and 1.5 mL/kg female), which was 31.2 hours, and the fastest in the M<sub>2</sub>F<sub>2</sub> treatment (1 mL/kg male and 3 mL/kg female) which was 22.6 hours. This latency time is faster than the research of [19], which obtained a latency time for snakehead fish

spawning of 30-37 hours, after injecting ovaprim hormone only in female broodstock with a dose of 0.3-0.5 ml/kg. However, the latency time of this study was slower than the research of [20], which only required a latency time of approximately 24 hours. Then, the application of Ovaprim 0.4 ml/kg to male broodstock and 0.9 ml/kg to female broodstock successfully stimulated the reproduction of snakehead fish [21].

To determine whether there were significant differences between treatments and which treatments were significantly different, an Analysis of Variance (ANOVA) was performed. If significant differences were found, a Duncan Multiple Range Test (DMRT) was used. The results of the analysis of variance for the latency time data for the snakehead fish broodstock in this study are presented in Table 2.

**Table 2.** Analysis of Variance (ANOVA) Data for Latency Time of the Snakehead Fish Broodstock

Source	Sum of Squares (SS)	Degree of Freedom (df)	Mean Square	F value	F table		p Value
					5 %	1%	
M (Male)	111.63	1	111.63	51.76**	5.32	11.26	< 0.001
F (Female)	18.75	1	18.75	8.69*	5.32	11.26	= 0.018
M x F	0.40	1	0.05	0.19	5.32	11.26	= 0.680
Error	17.25	8					
Total	148.04	11					

\*) significant, \*\*) very significant

Based on the analysis of variance above (Table 2), it was found that M (Male hormone dose) has a highly significant effect on latency ( $p < 0.001$ ), while F (Female hormone dose) also has a significant effect ( $p \approx 0.018$ ). However, the interaction between M and F is not significant ( $p \approx 0.680$ ), meaning the effects of M and F on latency act independently here. Furthermore, Duncan's Multiple Range Test (DMRT) showed that the  $M_1 F_1$  treatment did not significantly differ in latency time from the  $M_2 F_1$  treatment, and the  $M_1 F_2$  treatment with  $M_2 F_2$  was also not significantly different. However, the  $M_1 F_1$  treatment was significantly different from  $M_1 F_2$ , and the  $M_2 F_1$  treatment was significantly different in latency time from  $M_2 F_2$ . Further analysis showed no interaction between the ovaprim dose factors in male and female broodstock. These results illustrate those variations in the ovaprim hormone dose in male broodstock did not affect latency time, but variations in the dose injected into female broodstock did and resulted in significant differences between treatments. This is according to the study by [22] that administering different ovaprim doses will result in different latency times. However, research [16] found that the use of synthetic gonadotropin hormones with different doses had no different effects on latency time.

Duncan's Multiple Range Test (DMRT) showed that all treatments were significantly different ( $p < 0.01$ ), but there was no interaction between hormone doses in male and female parents (M x F). These results illustrate those variations in the ovaprim hormone dose in male parents affect latency time, as well as in female parents, but the male and female parents stand alone, because there is no relationship (interaction) between the hormone doses of male and female parents. This is in accordance with research by [22] that giving different doses of ovaprim will result in

different latency times. However, research [16] found that the use of synthetic gonadotropin hormones with different doses did not have different effects on latency time.

According to [16], the fast latency period is due to the optimal hormone dose, which causes the female parent to release pheromones more quickly for ovulation. The pheromone response causes an increase in neurophyseal hormones, so that when the levels have reached a certain level, the female parent releases eggs more quickly [23]. The latency period or ovulation time limit is influenced by several factors, namely hormonal factors in the form of stimulation from gonadotropin hormone injections on the spermiation process and environmental factors in the form of water quantity and quality [24]. According to broodfish injected with the hormone ovaprim, the concentration of gonadotropin hormones in the blood is increased, thereby inducing egg development and spawning. This proves that injecting a dose of gonadotropin hormone (including ovaprim) intramuscularly (into the muscle) into mature gonad snakehead fish can stimulate ovulation [25].

The use of different doses of ovaprim hormone causes the latency period for snakehead fish to also vary. This is the opinion of [26], who stated that semi-artificial spawning treatment using hormones causes an acceleration of the latency period for spawning. The influence of GnRH and anti-dopamine hormones, the more they are given, the more GtH is secreted by the pituitary gland. Too much GtH hormone can cause its presence in the blood to be longer, which can maximize gonadal maturity and accelerate ovulation. This is also explained by [27], who stated that the combination of LHRH-a and anti-dopamine can cause high GtH secretion and its presence in the blood plasma for a longer period.

### 3.2 Fertilization Rate

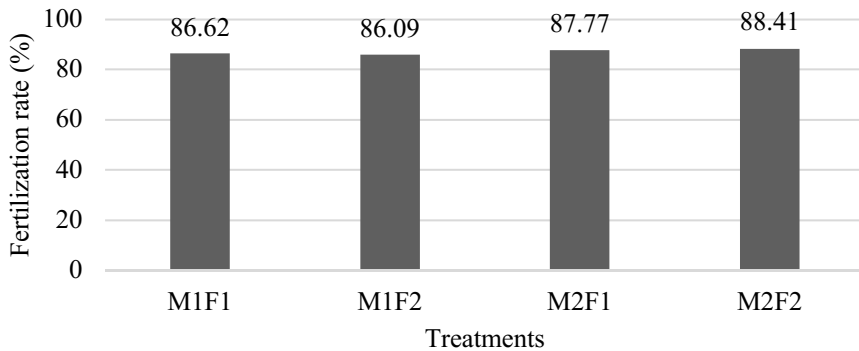
Fertilization is the fusion of male and female gametes, culminating in the union of the nuclei of the two gametes to form a zygote [28]. According to, fertilization can be divided into two types: internal and external. Fertilization, which generally occurs in fish, is external because it occurs outside the parent's body. The success of the fertilization process is influenced by the sperm's ability to fertilize the egg. Unstored sperm (fresh sperm) has a higher fertilization capacity than stored sperm.

Fertilization rate can be calculated from the percentage of fertilized eggs after ovulation by directly observing the egg color. The results of the snakehead fish egg fertilization calculation in this study are presented in the following Table 3.

**Table 3.** Fertilization rate in snakehead fish spawning with various combinations of ovaprim hormone

Number	Treatment	Fertilization rate (%)			Average and Standard Deviation
		1	2	3	
1	M <sub>1</sub> F <sub>1</sub>	87.11	85.98	86.67	86.62 ± 0.52
2	M <sub>1</sub> F <sub>2</sub>	85.58	85.81	86.88	86.09 ± 0.69
3	M <sub>2</sub> F <sub>1</sub>	87.97	88.43	86.91	87.77 ± 0.78
4	M <sub>2</sub> F <sub>2</sub>	88.43	88.86	87.94	88.41 ± 0.46

From the data in Table 3, the graph presented in Fig. 2 below is shown.



**Fig. 2.** Degree of Fertilization in snakehead fish spawning for each treatment.

The graph above explains that the highest fertilization rate after ovaprim hormone injection in male and female broodstock was in the M<sub>2</sub>F<sub>2</sub> treatment (ovaprim dose of 1.0 mL/kg male and 3.0 mL/kg female) which was 88.41%, and the lowest was in the M<sub>1</sub>F<sub>2</sub> treatment (0.5 mL/kg male and 3 mL/kg female) which was 86.09%. Fertilization from this study was lower than the study conducted by [29], which was (96.2 ± 2.4%), but higher than the study of Hossain et al. (2013), which was only 80.0%. Meanwhile, the study by [27] found that the reported fertilization rate of *Channa striata* eggs was around 58.83%, but lower than the fertilization rate of *Channa punctatus*, which was around 78.3%. However, previous research, namely by [16], found that fertilization of snakehead fish or fertilized eggs reached 99.75%.

To determine whether there were significant differences between treatments and which treatments were significantly different, an analysis of variance (ANOVA) was performed. If significant differences were found, a duncan multiple range test (DMRT) was used. The results of the analysis of variance for the fertilization rate data for the snakehead fish broodstock in this study are presented in Table 4.

**Table 4.** Analysis of Variance (ANOVA) Data for Fertilization Rate of the Snakehead Fish Broodstock

Source	Sum of Squares (SS)	Degree of Freedom (df)	Mean Square	F value	F table		p Value
					5 %	1 %	
M (Male)	9.2050	1	9.2050	22.66**	5.32	11.26	< 0.001
F (Female)	0.0154	1	0.0154	0.038	5.32	11.26	= 0.018
M x F	0.9690	1	0.9690	2.38	5.32	11.26	= 0.680
Error	3.2505	8	0.4063				
Total	13.4399	11	-				

\*\* ) very significant

There is a very significant difference between treatments in the given of ovaprim hormone doses given to male parents on the degree of fertilization, where the calculated

F value = 22.66 > F table (0.01) = 11.26, while there is no difference between treatments in given to female parents, calculated  $F = 0.038 < F \text{ table } (0.05) = 5.32$ . Likewise, with the interaction source (M x F), there is no significant difference, where calculated  $F = 2.38 < F \text{ table } (0.05) = 5.32$ . This means that there is an increase in the percentage of fertilization if the male parent is given a higher dose of hormone, while in the female parent, it has no effect, and there is also no interaction between the hormone doses given to the male and female parents. In other words, the increase in hormone doses is only related to the level of fertilization played by the male parent; it has nothing to do with the female parent. Duncan's multiple range test (DMRT) showed that the  $M_1F_1$  treatment was significantly different in its fertilization capacity from the  $M_1F_2$  treatment, and the  $M_2F_1$  treatment with  $M_2F_2$  was also significantly different. Further analysis showed no interaction between the ovaprim dose factors in male and female broodstock. These results illustrate that the dose variation in male broodstock affects fertilization capacity, but the dose variation in female broodstock does not. Thus, to increase the degree of fertilization, it is necessary to optimize the ovaprim hormone dose in male broodstock of snakehead fish.

According to [31], in general, several factors influence the spawning process, namely internal factors that influence reproduction, namely gonadotropin drivers and inhibitors, pre-ovulation, and ovarian response to GtH, gonadal maturity level, and fish health conditions during reproduction. External factors that influence spawning are photoperiod, temperature, and substrate. Furthermore, it is said that synthetic gonadotropin hormones, such as ovaprim at a dose of 0.4 ml/kg BW, can be used as an appropriate spawning agent for successful breeding and production of snakehead fish seeds in conditions in cultivation containers [32]. Research by [33] on supplementing broodstock diets with 10.8% lipids can increase the success of snakehead fish spawning. The impact of this semi-artificial spawning practice can improve the quality of offspring and the sustainability of the species [23]. The strategy that must be implemented is to carry out semi-artificial spawning to accelerate the reproduction of snakehead fish [34].

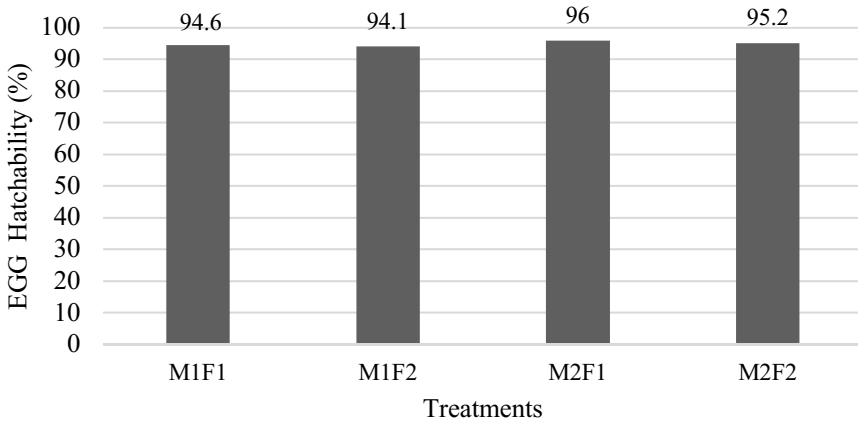
According to [22] stated that hormone injection can cause an increase in the concentration of 17,20-dihydroxypren-4-en-3-one (17,20-P) in the blood plasma of fish, and further, [35] said that the ovaprim hormone can provide higher spawning stimulation, higher fertility values, faster egg diameter enlargement, and shorter latency time. The quality of the male fish sperm is very important in determining the success or failure of fertilization. In some cases of artificial fish breeding with hormone injection, there is often a time lag between the availability of sperm and the presence of eggs (asynchronous gonadal maturity of male and female fish) or a difference in the distance between the presence of sperm and the presence of eggs [36]. Sperm's normal viability after leaving the testicles is only about 1-2 minutes, resulting in many eggs not being fully fertilized [37]. Reduced sperm motility makes it difficult for sperm to find or penetrate the egg micropyle, resulting in low egg fertilization, requiring hormonal stimulation [38]. The results of the snakehead fish's fertilization ability in this study were quite good, ranging from 86.09 to 88.41%, indicating that the sperm's ability to fertilize was quite high and in sync with the maturity of the fish's eggs. This is due to the injection of the hormonal ovaprim, but the doses applied in this study were not significantly different, meaning that the lowest dose for males, 1.0 mg/kg, and the lowest dose for females, 1.5 mg/kg, were sufficient for optimal fertilization.

### 3.3 Egg Hatchability

Hatchability is the percentage of eggs that hatch from a given number of fertilized eggs. High egg hatchability can be influenced by several factors, including egg quality, water quality, and handling during hatching [39]. The egg hatchability (%) of snakehead fish from various treatments in this study is presented in Table 5 and Fig. 3.

**Table 5.** Egg hatchability in snakehead fish spawning with various combinations of ovaprim hormone

Number	Treatment	Egg Hatchability (%)			Average and Standard Deviation
		1	2	3	
1	M <sub>1</sub> F <sub>1</sub>	95.2	93.8	94.8	94.6 ± 0.72
2	M <sub>1</sub> F <sub>2</sub>	93.8	93.3	95.2	94.1 ± 0.99
3	M <sub>2</sub> F <sub>1</sub>	96.7	95.2	96.7	96.0 ± 0.87
4	M <sub>2</sub> F <sub>2</sub>	96.0	94.5	95.1	95.2 ± 0.62



**Fig. 3.** Hatchability of snakehead fish eggs for each treatment.

The graph above shows that the highest hatchability was found in the M<sub>2</sub>F<sub>1</sub> treatment (ovaprim dose of 1 mL/kg for males and 1.5 mL/kg for females) and the lowest in the M<sub>1</sub>F<sub>2</sub> treatment (ovaprim dose of 0.5 mL/kg for males and 3 mL/kg for females). To determine whether there were significant differences between treatments and which treatments were significantly different, an Analysis of Variance (ANOVA) was performed. If significant differences were found, a Duncan Multiple Range Test (DMRT) was used. The results of the analysis of variance for the egg hatchability rate data for the snakehead fish broodstock in this study are presented in Table 5.

**Table 6.** Analysis of Variance (ANOVA) Data for Egg Hatchability of the Snakehead Fish Broodstock

Source	Sum of Squares (SS)	Degree of Freedom (df)	Mean Square	F value	F table		p Value
					5 %	1%	
M (Male)	3.5509	1	3.5509	4.44	5.32	11.26	< 0.001
F (Female)	1.7706	1	1.7706	3.14	5.32	11.26	= 0.018
M x F	0.2706	1	0.2706	0.48	5.32	11.26	= 0.680
Error	4.5044	8					
Total	10.0965	11	-				

Based on Table 6, there were no significant differences between the treatments, so it can be concluded that the combination of ovaprim hormone doses given to male and female broodstock had no significant effect on egg hatchability parameters. This means that egg hatchability parameters are not related to hormone dosage. In various studies on egg hatchability, water temperature, dissolved oxygen, light intensity, and the optimal conditions of other water quality parameters be the most influential. However, research [16] showed that the use of synthetic gonadotropin hormones with different doses had a significantly different effect on the percentage of fish egg hatching, and the highest hatchability was 78.47%. According to [40] stated that the quality of the broodstock, egg quality, and egg diameter greatly affect the hatchability of fish eggs. Hatching occurs due to mechanical work, where the embryo often changes position and finally comes out of its shell. Furthermore, it is said that the factors that influence the hatchability of fish eggs are internal factors (egg quality and hormones) and external factors (temperature, alkalinity, salinity, ammonia, lighting, and pH) [41].

According to [42], hatching of fish eggs is an enzymatic process, namely the endodermal glands in the embryonic pharynx area, and the enzyme is called chorionase. Embryo activity and chorionase formation are influenced by internal factors, such as yolk volume, while external factors, namely temperature, oxygen, light intensity, salinity, pH, and other factors [43]. According to research by [44], the fastest hatching of snakehead fish eggs was obtained with a time of 1,540 minutes, with a percentage of 85.33% and the slowest hatching with an average time of 1,782 minutes, with a hatching percentage of 79.33% at a temperature of 29-30°C. The results of the study by [27] showed that the hatching rate of snakehead fish eggs in this study was lower than *C. striatus* and *C. punctatus*, which were reported at around 62.33% and 90.6%, respectively. Furthermore, the total number of fertilized eggs from the artificial spawning process was 12,860 eggs, with a mortality rate of 11% and the final result after hatching was around 11,527 fish larvae [34]. The results of this experiment indicate that the use of synthetic gonadotropin hormones with different doses has a significantly different effect on the hatching percentage of snakehead fish eggs.

The hatching process generally occurs faster at higher temperatures because at higher temperatures the metabolic process runs faster, resulting in faster embryo development, which further results in more intensive embryo movement within the shell. However, temperatures that are too high or change suddenly can inhibit the hatching process and cause death. The temperature found during the hatching study was 27–30 °C. This is supported by [45], who stated that high temperatures can cause premature larvae because the pro-larvae are not yet ready to accept their environmental

conditions. According to [46], temperatures that are too low or too high from the optimal range can cause death in fish. Sufficient dissolved oxygen of 4.6–7.0 mg/L is very important in hatching because eggs and fry have a high metabolic rate. Lack of oxygen not only slows embryo development but can also cause embryo death. The dissolved oxygen concentration is not less than 4–5 mg/L at all times during hatching [47].

## 4 Conclusion

The latency time of snakehead broodstock after being given various combinations of ovaprim hormones was 22.2 – 33.2 hours. This latency time parameter indicates the treatment. M (Male hormone dose) has a highly significant effect on latency, while F (Female hormone dose) also has a significant effect. However, the interaction between M and F is not significant, meaning the effects of M and F on latency act independently here. The fertilization rate ranged between 85.58 – 88.86%, where the combination of ovaprim hormones given only affected the male parent, while giving different doses to the female parent had no effect, and there was no interaction between the male and female parents in terms of this degree of fertilization. Egg hatchability ranged between 93.3 – 96.7%, which is a fairly high hatchability, and the administration of the ovaprim hormone combination provided no significant effect, whether given to male and female parents, and there was no interaction between factors. To accelerate latency time, a dose of 1.0 ml/kg for males and 3.0 ml/kg for females can be used. However, to increase fertilization rates, a dose of 1.0 ml/kg for males and 1.5 ml/kg for females is used. To increase hatchability, the minimum dose is 0.5 ml/kg for males and 1.5 ml/kg for females. Overall, it is recommended to use the minimum dose for greater efficiency, namely 0.5 ml/kg for males and 1.5 ml/kg for females.

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