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EFFECTIVENESS OF OVAPRIM HORMONE IN INDUCING OVULATION, FERTILIZATION AND HATCHING NILEM FISH (OSTEOCHILUS HASSELTI) EGGS

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ABSTRACT

This study aims to determine the effectiveness of using the hormone ovaprim on ovulation time, fertilization degree and hatching degree of nilem fish (*Osteochilus hasselti*) eggs. The number of parents used was four males and two females (2:1). The dose of the ovaprim hormone used was 0.3 mL/kg body weight for the male parent and 0.5 mL/kg body weight for the female parent. Ovulation time is done every hour by observing his behavior. After the fish shows signs of impending ovulation, they are removed and stripped. Eggs and sperm are collected and then fertilized. Analysis of the degree of fertilization and hatching was done descriptively. The results showed that giving ovaprim was effective and inducing ovulation, fertilization and hatching of *Osteochilus hasselti* fish. Ovulation occurred at 8 hours after being injected with the hormone ovaprim, the degree of fertilization and egg hatching was quite high, respectively 98% and 97%.

Keywords: Fertilization, hatching, *Osteochilus hasselti*, ovaprim.

1. INTRODUCTION

Nilem fish (*Osteochilus hasselti*) is one of the freshwater fish commodities belonging to the Cyprinidae family, which is widely cultured by farmer in Java, especially in West Java. This fish is generally used as a byproduct of the aquaculture of *Cyprinus carpio, Oreochromis nilotica, O. mossambicus* and *Osphronemus goramy* [1].

GSJ: Volume 10, Issue 12, December 2022
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Besides being concentrated in West Java, this fish is found in many rivers and swamps on the island of Sumatra [2]. In the fishing industry, *Osteochilus hasselti* besides having the potential to be developed in aquaculture, also has the potential to be processed into various processed products, including caviar, boiled, baby fish, and beef jerky [3]. The potential possessed by the *Osteochilus hasselti* commodity must be maximized so that it becomes a profitable economic opportunity for fish cultured.

In an effort to develop the potential of *Osteochilus hasselti* fish, the first step that can be done is to increase seed production to reduce fish exploitation in nature. The application of artificial spawning techniques to broodstock induced by the use of the hormone ovaprim is one of the efforts to increase nilem seed production. Artificial spawning by providing hormone stimulation to the broodstock with the aim of accelerating gonadal maturation and spawning [4]. According to Wulandari et al. (2017) [5] the benefits of artificial spawning are to increase fertilization and hatching rates and have a higher survival rate. The purpose of this study was to analyze the effectiveness of using the hormone ovaprim in artificial spawning of *Osteochilus hasselti* fish for fry products produced based on several observational parameters including egg fecundity, fertilization and hatching rates and survival of the larvae and fry produced.

2. MATERIALS AND METHODS

The broodstock Maintenance

A total of four selected male (average weight 82 g) and two female (200 g average weight) reared separately in brood tanks. The broodstock were given pellet feed which had been mixed with Ovagrow at a dose of 0.1 g/kg pellet. Feeding 3% of the biomass, with a frequency of giving twice a day, namely morning and evening.

Administering Ovaprim Hormones to the broodstock

The purpose of giving the hormone ovaprim to the parent is to induce the process of spawning the parent, both male and female parent. The hormone ovaprim is given by injection intramuscularly (under the dorsal fin) with a 45^{o} inclination of the syringe. The dose of the ovaprim hormone given was 0.3 mL/kg body weight for the male parent and 0.5 mL/kg body weight for the female parent. The injection was carried out at night around 20.00 WIB [6]. At the time of injection, the fish are wrapped in a wet cloth to maintain moisture, calm and avoid excess stress from the parents.

Ovulation Process

The broodstock which have been injected with the hormone ovaprim, are then returned to the brood rearing pond, waited for 8-12 hours until there are signs that the fish are about to spawn, then spawning is carried out artificially. Artificial spawning is carried out by means of male and female parents each striped on the abdomen downwards (urogenitalia) to assist in the process of releasing sperm and eggs (ovulation). Sperm and eggs from striping are placed in different containers (basins). Sperm that are already in the basin are added to a physiological solution of NaCl twice the volume of sperm released, with the aim that the sperm can survive in the external environment.

Fertilization Process

GSJ: Volume 10, Issue 12, December 2022
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The available sperm and eggs are then put together in a basin for the fertilization process to be carried out by slowly mixing the sperm and eggs using a chicken feather. Left for two minutes to ensure the fertilization process has occurred. Then wash and rinse the eggs that have been fertilized by sperm with water that flows slowly before being put into the hatching tub. The characteristics of a fertilized egg are that the egg is clear and green at the core, while an egg that is not fertilized will be pale white. Next, the calculation of the fertilized egg or the degree of fertilization (Fertilitation Rate / FR) is carried out. The calculation is done by sampling three times. Sampling using a petri dish and then counting the number of fertilized eggs. The calculation results of each sampling are entered into the formula:

$$FR = \frac{\text{Jumlah Telur Terbuahi}}{\text{Jumlah Telur}} \text{ x } 100\%$$

Egg hatching

After the fertilization process, the eggs are put into the hatching tub, which is a fiber tub filled with 800 L of water and equipped with an aeration installation for oxygen supply. Furthermore, observations were made of fertilized eggs and eggs that hatched.

Hatching eggs is a continuation of the fertilization process. In this stage the process of embryo formation occurs, the final stage of embryo formation is the hatching process. Not all eggs containing embryos reach the hatching stage, therefore it is necessary to calculate the number of eggs that can hatch by calculating the hatching rate using the formula:

$$HR = \frac{\text{Jumlah Telur Menetas}}{\text{Jumlah TelurTerbuahi}} \text{ x } 100\%$$

The calculation of the degree of hatching was carried out by sampling three times. The presence of eggs that hatch is indicated by the presence of larvae swimming in the hatchery. Feeding the larvae is carried out after the food reserves in the form of egg yolk (yolk sac) are used up, that is, on the 4th to 7th day. The feed given is boiled chicken egg yolk which is spread evenly over the entire surface of the tub.

3. RESULTS AND DISCUSSION

Ovulation time

Based on the results of observations, the sign that a female fish is ready for ovulation is characterized by a bulging belly and when rubbed or pressed, the urogenital part throbs, while in male fish, when rubbed or pressed, it will secrete white liquid on the urogenital part. Ovulation time begins to occur at the 8th hour after the injection with the hormone ovaprim. While the results of the study by Sinjal (2014) [7], showed that brood catfish were given the hormone ovaprim at a dose of 0.3 ml, the average spawning time was slower, which was around 9.2 hours from the time of injection. This shows that the dose of the hormone ovaprim given is sufficient to induce gonadotropins in the fish's body for ovulation to occur. As according to Fujaya (1999) [8], fish that are given hormone stimulation are able to spawn because the gonadotropin content in the fish's body increases.

GSJ: Volume 10, Issue 12, December 2022
ISSN 2320-9186

Degree of egg fertilization (Fertilization)

Calculation of the degree of egg fertilization can be done after the ovulation process occurs, which is about 8 hours after the ovulation process. The results of calculations on the degree of fertilization of nilem fish for each time of sampling did not show any significant differences and resulted in high values (Table 1).

Table 1. Fertilization Degree of *Osteochilus hasselti* Fish

Sampling to -	Average Fertilization	Degree
1	$\frac{132}{133}$ x 100% = 99%	
2	$\frac{129}{130}$ x 100% = 99%	98%
3	$\frac{117}{121}$ x 100% = 96%	

Table 1 shows the average degree of fertilization of *Osteochilus hasselti* fish eggs induced with the hormone ovaprim which produces a fairly high value, namely 98%. This shows that the dose of the hormone ovaprim given to the broodfish, namely 0.3 mL/kg and 0.5 mL/kg body weight of the broodstock, had a good effect on increasing the degree of fertilization. The degree of fertilization that occurs in fish is a fertilization process that is monospermic, that is, only one spermatozoa can enter the egg cell through the microfil hole [9, 10]. The process of fertilization occurs because the egg cell secretes a compound called 'fertilizing' which can stimulate spermatozoa to swim and enter the egg.

In this study the degree of fertilization is quite high, which is equal to 98%. According to Woynarovich and Horvath [9] the degree of fertilization in fish is largely determined by the quality of eggs, spermatozoa, media and human handling. Egg quality determines the degree of fertilization. Good quality eggs are eggs that reach maximum egg maturity, so that the fertilization process will occur properly and will produce good larvae and seeds. As the results of research conducted by Leonita *et al*., (2021) [11] show that fecundity, fertilization and hatching are very dependent on the mechanism of action and the dose of the hormone given. The ovaprim hormone gave the best results on the reproductive parameters of Siamese catfish such as fecundity, fertilization and hatching compared to HCG and spawnprim hormones. The high average percentage of fertilization is due to the presence of the GnRH+ domperidone hormone found in the broodstock fish's body. The use of hormones (sGnRH+dopamine) not only induces the mother to ovulate, but affects the fertilization process. The mechanism of action of exogenous hormones given by injection, namely the hormone ovaprim by increasing the action of LHRA-a in the blood so that it can encourage ovulation. Naturally the work of the LHRH-a hormone in the fish's blood is very slow, and has decreased so it needs hormone induction from outside. According to Leonita et al. (2021) [11] ovaprim contains sGnRH-a, which is able to stimulate the pituitary gland to produce GtH I and GtH II in fish. As according to Joshua *et al*. (2021) [12] administration of hormones containing GnRHa has been shown to be very effective for inducing oocyte maturation and ovulation, increasing sperm production and spawning in many fish. The same opinion was expressed by Dewantoro et al. (2017) [13], that administration of the hormone ovaprim through injection can increase egg maturity. This is because the hormone given is able to increase the content of gonadotropin-releasing hormone (GnRH) which is induced by the hypothalamus. In

GSJ: Volume 10, Issue 12, December 2022
ISSN 2320-9186

addition, the presence of antidopamine will inhibit dopamine activity, thereby increasing the performance of the hypothalamus gland in inducing GnRH. Aziz (2018) [14] states that giving hormones can affect egg quality and the percentage of fertilization. Some hormones that affect the level of fertilization in an individual include FSH (Folicle stimulating hormone), LH (Luteinizing hormone), estrogen, progesterone, and thyroid. These hormones can be secreted by administering synthetic hormones containing GnRH. The mechanism of action of GnRH is to stimulate the pituitary gland to produce these hormones. So according to Fujaya (2004) [15], brood fish injected with pituitary hormones can directly increase the concentration of gonadotropin hormones in the blood so that they can induce egg development and spawning. However, according to Nandeesha *et al*. (1990) [16] the use of ovaprim gave better results compared to the use of the pituitary gland in the process of spawning and fertilization. The use of ovaprim provides spawning stimulation and a higher degree of fertilization and results in larger egg diameters, shorter latency times and lower mortality rates.

The dose of the ovaprim hormone used affects the degree of fertilization, as the results of a study conducted by Semidang *et al*. (2018) [3], showed that the use of the hormone ovaprim at a dose of 0.2 mL/kg body weight of *Osteochilus hasselti* resulted in a lower fertilization rate, which was 74.7%. According to Sugistia (2016) [17] the difference in the degree of fertilization in artificial spawning can be influenced by the dose of the hormone ovaprim. At a lower dose will produce a lower degree of fertilization as well. This is presumably because the mechanism of action of the hormone will run normally (optimally) at certain levels, decreasing or increasing it is thought to reduce the biological potential of the hormone towards the target so that it influences the stimulation of egg development.

According to Murtidjo (2001)[18], failure or low degree of fertilization, because the release of sperm and egg cells does not occur simultaneously and is relatively short, resulting in failure in the fertilization process. Another factor is because the sperm are not actively moving in the plasma fluid, so they cannot immediately enter the microphile hole which is located on the surface of the egg. Sperm that are not actively moving, because plasma fluid has a higher concentration than sperm fluid. According to Effendi (1997) [19], spawning eggs that are fertilized then develop into embryos and eventually hatch into larvae, while eggs that are not fertilized will die and rot. The percentage of egg maturity affects the number of eggs that hatch.

Egg Hatching Degrees

Based on observations, nilem eggs that have been fertilized hatch at ±24 hours after fertilization. The occurrence of hatching is indicated by the presence of larvae swimming around spread throughout the hatchery tub, but more are found congregating in the corners of the tub. In principle, the hatching process occurs due to mechanical and enzymatic work. Mechanical work occurs because the embryo that has been formed in the egg often moves to change its position to get a wider space, because the size of the embryo in the egg is larger than the environment in the egg shell. With these movements, the soft part of the egg shell will break so that the embryo comes out of the shell [10]. Apart from mechanical work, egg hatching is also triggered by enzymatic work, chemical elements of enzymes secreted by endodermal glands located in the pharynx of the embryo. The newly hatched larvae still have food reserves in the form of egg yolk (yolk sac), and on the 4th day the yolk begins to disappear so that the larvae begin to be fed in the form of boiled egg yolk which has been dissolved in water.

GSJ: Volume 10, Issue 12, December 2022
ISSN 2320-9186

The results of the calculation of the hatching degree of nilem eggs induced by the hormone ovaprim did not show a significant difference for each sampling and produced high values (Table 2).

Tabel 2. Degree of hatching of *Osteochilus hasselti* fish eggs

Sampling to -	Average Fertilization	Degree
1	$\frac{128}{132}$ x 100% = 96%	97%
2	$\frac{128}{129}$ x 100% = 99%	
3	$\frac{115}{117}$ x 100% = 98%	

Table 2 shows the average hatching degree of nilem fish eggs induced with the hormone ovaprim which is quite high, namely 97%. According to Manickam and Joy (1989) [20] the increase in hatchability of fish eggs fed with ovaprim solution was caused by an increase in the Follicle Stimulating Hormone (FSH) content so that the follicles developed and egg hatchability also increased. The results of research conducted by Subagja *et al.* (2015) [21], showed that nilem fish whose ovulation process was induced with the hormone ovaprim at a dose of 0.3 mL/kg body weight for male brooders and 0.4 mL/kg body weight for female fish resulted in a lower hatching rate, which was 87 .88%. In his research, the female broodstock was used with an average weight of 255 g, while the male broodstock had an average weight of 54.4 g. The difference in the degree of hatching was probably influenced by the readiness of the broodstock to spawn, especially in the male broodstock. According to Sumantadinata (1981) [22], parent nilem which is good for spawning has reached a size of 120 – 180 g/head and is 6 – 12 months old. So the lower hatching degree values that have been carried out by Subagja *et al.* (2015) [21], it is possible that the sperm produced by the male parent has not reached optimal and uniform maturity, because the male parent weight used has only reached a weight of 54.4 g.

From the description above, it shows that the ovulation process with the help of the hormone ovaprim or known as artificial spawning can be used as an effort to produce nilem fish seeds. As according to (Satyani 2008) [6] injection of stimulating hormone in nilem fish can help speed up the ovulation process. Artificial spawning can reduce the failure rate in hatchery activities. According to Sinjal (2014) [7], the results of artificial spawning can produce egg hatchability and larval survival values of more than 80%.

4. CONCLUSION

Based on the research results, it can be concluded:

Administration of the hormone ovaprim by injection at a dose of 0.3 mL/kg body weight of the male parent and 0.5 mL/kg body weight of the female parent is effective enough to induce ovulation, fertilization and hatching of *Osteochilus hasselti* fish. Ovulation occurred at the 8th hour after being injected with the hormone ovaprim, the degree of fertilization and egg hatching was quite high, respectively 98% and 97%.

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ISSN 2320-9186

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