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# Use of ovaprim and Chorulon hormones for spawning induction of G4 transgenic Mutiara catfish Brood stock in rearing indoor Hatchery

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**Abstract**
This research was conducted at Hatchery Building 4, the Fisheries Biotechnology and Marine Sciences Laboratory of Padjadjaran University from October to November 2022, aims to obtain the optimal combination dosage of ovaprim and HCG hormones on the reproductive performance of mutiara catfish. The study used a completely randomized design (CRD) with four treatments (pair A: 0.4 ml ovaprim + 0.6 ml HCG, B: 0.5 ml ovaprim + 0.5 ml HCG, C: 0.6 ml ovaprim + 0.4 ml HCG for transgenic fish pairs, and D: 0.4 ml ovaprim + 0.6 ml HCG for non-transgenic fish pairs) with three pairs of broodstock as replicates. The research results showed that the best dosage to induce spawning in transgenic G4 mutiara catfish was found in treatment B (0.5 ml ovaprim/kg female and male broodstock weight and 0.5 ml HCG/kg male broodstock weight). After spawning, the broodstock pairs in treatment B showed the best results with an average relative fecundity of 85,103 eggs/kg broodstock, fertilization rate (FR) reaching 88.150%, hatching rate (HR) reaching 90.056%, and survival rate (SR) reaching 88.034%.

**Keywords:** Fecundity, hatching rate, HCG, ovaprim, spawning

## 1. Introduction
The third-generation production of mutiara catfish was successfully achieved by the Balai Penelitian Pemuliaan Ikan (BPPI) Sukamandi in 2014 through genetic improvement. This fish is the result of the crossbreeding of four strains of catfish, namely Paiton, Sangkuriang, local Dumbo, and Mesir, through individual selection and artificial breeding using composite sperm and egg mixing techniques. Mutiara catfish exhibit accelerated growth up to 70%, maintain uniform sizing, achieve a feed conversion rate up to 0.5, display robust endurance, and show stress tolerance toward environmental conditions [1]. Transgenic technology, such as the insertion of estrogen hormone and exogenous GH genes into mutiara catfish, has improved reproductive performance and the quantity of mutiara catfish fry. Additionally, transgenic mutiara catfish can be bred at an age of less than one year [2]. Furthermore, to accelerate gonad growth in transgenic mutiara catfish broodstock G4 (MTG4), a 1.5-month maturation process is conducted using hormonal stimulation through injection. Hormones like Human Chorionic Gonadotropin (HCG) and ovaprim are capable to optimizing broodstock spawning [3].
The success of spawning induction using a combination dosage of ovaprim and HCG hormones can be measured by relative fecundity, fertilization rate (FR), hatching rate (HR), and survival rate (SR). Assessment of these parameters indicates that the reproductive performance of MTG4 broodstock is capable of achieving mass production of MTG5. Based on the description above, the spawning performance of MTG4 can be induced using a combination of ovaprim and HCG hormones.

## 2. Materials and Methods
### 2.1 Time and Place
The research was conducted at Hatchery Building 4, Fisheries Biotechnology Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Padjadjaran. The research was carried out from August 2022-October 2022.

The production of G4 transgenic mutiara catfish is carried out by spawning the male and female parent pairs of G4 transgenic mutiara catfish, which is a continuation of the breeding scheme as shown in (Figure 1).

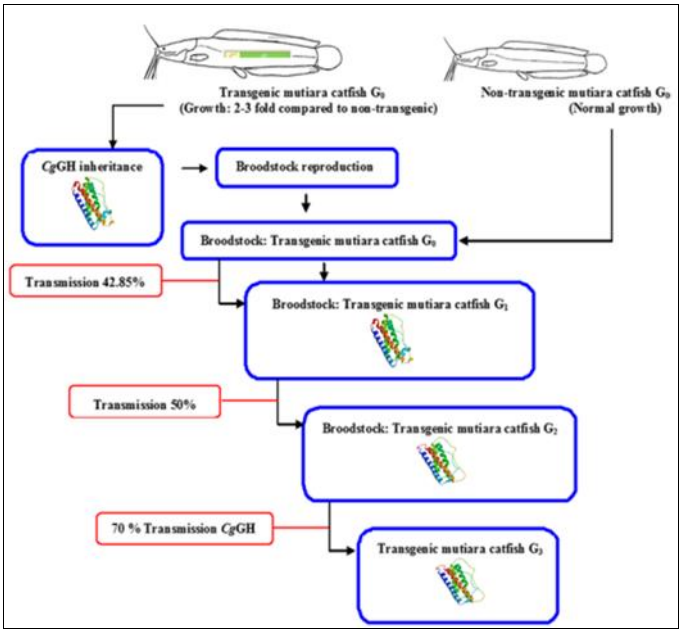


**Fig 1:** Breeding Scheme of G4 Transgenic Mutiara Catfish [4].

This research procedure comprises several stages. The first stage involves maturation in Treatment A, Treatment B, Treatment C (transgenic), and Treatment D (non-transgenic) for 1-1.5 months. The maturation takes place within a fiber tank with a diameter of 1.3 meters and a depth of 1 meter, filled with water to a height of 50 centimeters. Within the tank, a heater is installed to maintain the temperature 28±1 ºC, along with aeration for oxygen supply. The broodstock are provided with HI-PRO-VITE 781 feed. The next stage involves selecting broodstock to choose a mature male and female catfish that are ready for spawning. Spawning is conducted using a semi-artificial method. Induction of spawning in transgenic mutiara catfish is performed by injecting ovaprim hormone into both male and female broodstock. Additionally, the injection of HCG hormone is administered only to male broodstock two hours after injecting ovaprim into the female broodstock. After injection, the paired broodstock are placed into a 1000 L breeding tank with controlled water temperature and oxygen levels. The spawning process occurs within 8-12 hours after hormone injection. A hatching grid is used within the breeding tank to collect the fertilized eggs. The eggs at the bottom of the tank are sampled to determine the fertilization rate. Finally, the fertilized eggs are moved to a glass aquarium measuring 40x25x25 cm, filled with approximately 30 L of water at a temperature of 26 °C±1 °C, and provided with aeration. Over a period of 14 days post-hatching, the catfish larvae are fed with Artemia, and the survival rate (SR) is calculated during this period.

### 2.3 Parameter and Data Analysis

**Relative fecundity (Rf)**

The calculation of spawned eggs for each treatment is conducted using the following formula [5] as follows:

$$Rf = Wg/We \times \sum \text{egg sample}$$

**Information**

Fr = Relative fecundity
Wg = Gonad weight (g)
We = Mean weight of egg samples (g)

**Fertilization Rate (FR)**

The calculation of fertilization rate utilizes the formula [6] as follows:

$$FR = \frac{\sum \text{fertilized eggs}}{\sum \text{total Egg}} \times 100\%$$

**Hatching Rate (HR)**

The calculation of hatcing rate is performed 18-24 hours after the fertilization process. he hatching rate is calculated using the formula [7] as follows:

$$FR = \frac{\sum \text{fertilized eggs}}{\sum \text{total Egg}} \times 100\%$$

**Survival Rate (SR)**

Survival rate is the percentage of larvae that are alive at the final stage of rearing. The calculation of survival rate is based on the formula [6] as follows:

$$SR = \frac{\sum \text{live larvae}}{\sum \text{total larvae}} \times 100\%$$

**Data Analysis**

The collected data from each test parameter are analyzed using One Way Anova with Duncan's Multiple Range Test (Sigma plot 12.2) for determining the best treatment.

## 3. Results and Discussion

### 3.1 Mutiara Catfish G4 Broodstock

Three pairs of MTG4 broodstock successfully spawned through hormonal induction using a combination of ovaprim and HCG, while six pairs of other transgenic broodstock and three pairs of non-transgenic broodstock did not spawn. In the case of transgenic mutiara catfish, the spawning failure is caused by the eggs remaining unfertilized due to incomplete gonad maturation, while non-transgenic mutiara catfish experienced spawning failure due to immature gonad development and secondary sexual characteristics that do not meet the spawning requirements.

### 3.2 Relative fecundity

The relative fecundity from three pairs of successfully spawning transgenic mutiara catfish broodstock is presented in (Table 1).

**Table 1:** Relative Fecundity

| Perlakuan | Fekunditas Relatif (butir/kg induk) |
|---|---|
| (A) ♂MTG G4 >< ♀MTG G4 | 81.546 |
| (B) ♂MTG G4 >< ♀MTG G4 | 85.103 |
| (C) ♂MTG G4 ><♀MTG G4 | 83.306 |

The results of relative fecundity indicate that Treatment B achieved higher values (♂MTG_G4 B2.2 X ♀MTG_G4 A1.1), reaching 85,103 eggs/kg of broodstock using a dosage of ovaprim 0.5 ml/kg + HCG 0.5 ml/kg. In comparison, Treatment C (♂MTG_G4 C2.1 X ♀MTG_G4 B1.1) had a relative fecundity value of 83,306 eggs/kg of broodstock with

a dosage of ovaprim (0.6 ml/kg + HCG 0.4 ml/kg), and when compared to treatment A (♂MTG_G4 A3.2 X ♀MTG_G4 C2.1), the relative fecundity reached 81,546 eggs/kg of broodstock using a dosage of ovaprim (0.4 ml/kg + HCG 0.5 ml/kg). The presence of the *Cg*GH gene in transgenic mutiara catfish enhances the cells in the gonads, such as oogonia. The greater number of oogonia leads to higher fecundity [9].

### 3.3 Fertilization Rate (FR) and Hatching Rate (HR)

Fertilized eggs will undergo mitosis rapidly. Fertilized eggs can be identified by their transparent color, while unfertilized eggs can be identified by their cloudy white color, as shown in (Figure 2).

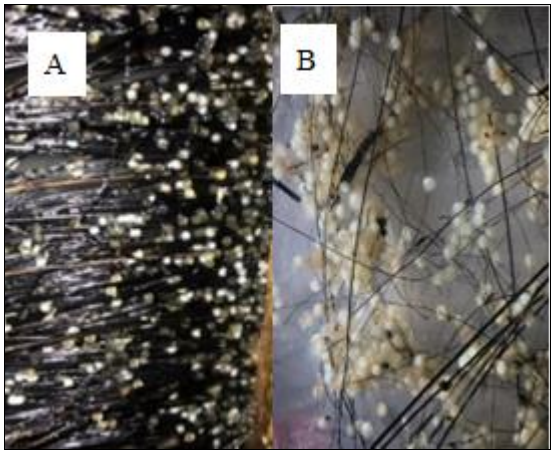


**Fig 2:** Eggs of G4 Transgenic Mutiara Catfish (A = Fertilized, B = Unfertilized)

The fertilization rate values of G4 transgenic mutiara catfish during the study can be seen in Figure 3

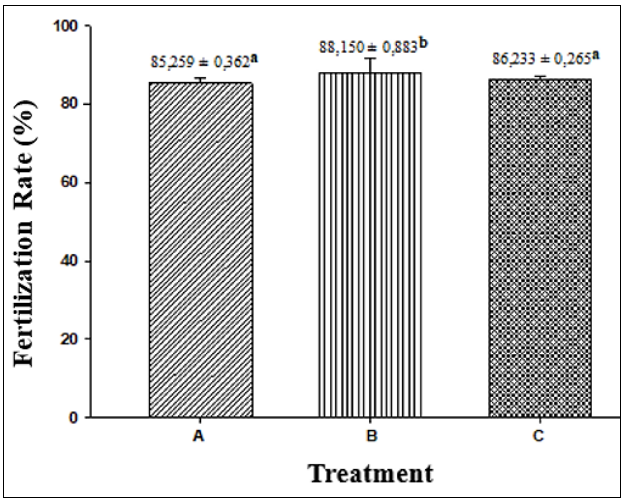


**Fig 3:** Fertilization Rate Graph

Based on the results of the conducted research, it is shown that the injection of sGnRH-a and anti-dopamine at different dosages on G4 transgenic mutiara catfish (MTG) significantly influences the fertilization rate. The highest value was obtained in treatment B, reaching 88.150% (Figure 4). Treatment C followed with 86.233%, while treatment A had 85.259%. The use of sGnRH and anti-dopamine hormones not only stimulates the fish to spawn but is also suspected to be related to the success of fertilization. According to [10], the injection of sGnRH-a and anti-dopamine hormones into the fish's body induces an increase in gonadotropin levels, which, in turn, elevates the GtH-II or LH (Luteinizing Hormone) hormone during the final stages of vitellogenesis. Consequently, the egg cell nucleus withdraws and undergoes a momentary fusion just before ovulation, a process referred to as GVBD (germinal vesicle break down).

The percentage of fertilization rate with higher injection dosages of sGnRH-a and anti-dopamine, at a dose of 0.6 ml/kg of broodstock weight, showed a decrease in the fertilization rate. This is suspected to be because the dosage is too high for the transgenic mutiara catfish. As a result, transgenic mutiaracatfish that have matured gonads with injections of excessively high dosages will experience a decline in egg quality.

Meanwhile, at a dosage of 0.4, a low fertilization rate is obtained, suspected to be due to the lack of gonadotropin release stimulation, resulting in incomplete maturation or germinal vesicle break down (GVBD). Consequently, fertilization cannot proceed effectively. This is supported by [11], where at low doses (suboptimal), the injected hormones cannot stimulate the optimal release of gonadotropins, leading to incomplete egg maturation. Eggs that have not matured completely result in an ineffective fertilization process. On the other hand, at higher dosages, early ovulation occurs, and the ovulated eggs remain in the ovarian lumen under hypoxic conditions for a longer period. This leads to a decline in egg quality.

The hatching rate (HR) values of transgenic mutiara catfish during the research can be seen in (Figure 4).

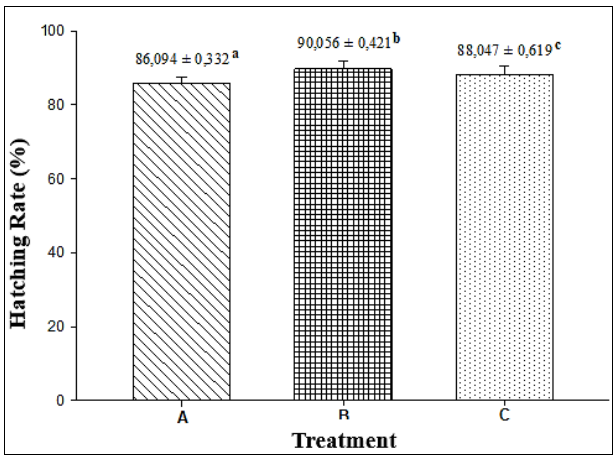


**Fig 4:** Hatching Rate Graph

Based on the results of the conducted research, it is shown that the injection of sGnRH-a and anti-dopamine at different dosages on transgenic mutiara catfish significantly influences the hatching rate [13]. The highest value was obtained in treatment B, which reached 90.056%, followed by treatment C with 88.047%. Meanwhile, treatment A had a hatching rate of 86.094%. Female broodstock of transgenic mutiara catfish injected with ovaprim (sGnRH and anti-dopamine content) at a dosage of 0.5 ml/kg body weight showed high results in the hatching process. This is suspected to be because this dosage is optimal and can influence the hatching rate. The significant effect also indicates an improvement in the egg hatching ability of catfish treated with ovaprim solution due to the increased *Follicle Stimulating Hormone* (FSH) content, which leads to follicle development and an increased hatching rate [14].

According to [14], sGnRH+domperidone or ovaprim not only stimulates the fish to ovulate but also has a correlation with

the fertilization rate, hatching, and resulting larvae. The optimal dosage can enhance the biological performance towards its target.

The administration of sGnRH and anti-dopamine at a dosage of 0.6 ml/kg of fish weight resulted in a decreased hatching rate. This is supported by [16], which states that the hormone's mechanism of action will function normally (optimally) at a certain level.

### 3.4 Survival Rate (SR)

The results of the survival rate of MTG4 catfish larvae showed that the highest survival rate was found in treatment B (88.034%) and treatment C (86.989%). Meanwhile, treatment A had a larval survival rate of 85.983% (Figure 5).

The high survival rate is one of the advantages of transgenic female broodstock. Female broodstock has the ability to pass on mitochondrial DNA (mtDNA) to their offspring, so only the egg cells contribute mtDNA. Transgenic catfish containing GH insertion (*Cg*GH) can increase in the number of mitochondria in the cells, increase in glycogen content, increase in muscle fibers, but a decrease in lipid droplets in their cells [15]. Transgenic catfish show a higher survival rate compared to non-transgenic catfish. The better survival rate in transgenic catfish is suspected to be due to a stronger immune system and better body resistance compared to non-transgenic catfish.

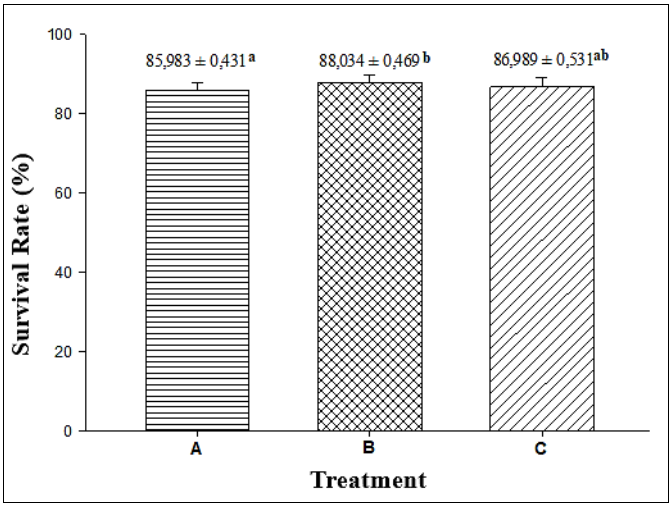


**Fig 5:** Survival Rate Graph

## 4. Conclusions

The best dosage to induce spawning in G4 transgenic mutiara catfish was found in treatment B, using ovaprim at a dose of 0.5 ml/kg of female and male broodstock weight, and HCG at a dose of 0.5 ml/kg of male broodstock weight. After spawning, the performance of the broodstock pairs in treatment B showed the best results with a relative fecundity of 85,103 eggs/kg of broodstock, fertilization rate (FR) of 88.150%, hatching rate (HR) of 90.056%, and survival rate (SR) of 88.034%.

## 5. References

1. Ikan BPP. Academic Manuscript on Fast-Growing Catfish Resulting from Individual Selection. Fish Breeding Research Center, Center for Fisheries Research and Development; c2014.
2. Buwono I, Iskandar D, Grandiosa R. Dissemination of Mutiara Catfish Farming at Padjadjaran University in the Lele Cileunyi Farmers Group. Journal of Applied Science and Technology for Society. 2021;10(4):273–278. Https://Doi.Org/10.24198/Dharmakarya.V10i4.35331
3. Mulah A, Raza'I TS, dan Putra WKA. Effectiveness of Human Chorionic Gonadotropin (HCG) and Ovaprim Hormones on Latency Time and Fecundity in Spawning Star Pompano (*Trachinotus Blochii*). Intek Aquaculture Journal. 2017;1(2):1–6. https://doi.org/10.31629/intek.v1i2.260
4. Buwono ID, Iskandar dan Grandiosa R. *CgGH* and *IGF-1* expression level and growth response of G4 transgenic mutiara strain catfish (*Clarias gariepinus*) reared at different stocking densities. Aquaculture International. 2022;31:827-846 https://doi.org/10.1007/s10499-022-01002-6
5. Witthames R, Peter Thorsen A, Murua H, Saborido-Rey F, Greenwood NL, Dominguez R, *et al*. Advances In Methods For Determining Fecundity: Application Of The New Methods To Some Marine Fishes. Fishery Bulletin. 2009;107(2):148–164.
6. Effendie MI. Fisheries Biology. Yayasan Pustaka Nusantara; c1997.
7. Effendie MI. Fisheries Biology. Yayasan Pustaka Nusantara; c2002.
8. Buwono ID, Iskandar Agung MUK, dan Subhan U. Perakitan Ikan Lele (*Clarias* Sp.) Construction of transgenic catfish (*Clarias* sp.) using sperm electrophoration technique. Biology Journal. 2016;20(1):17–28.
9. Haryono. Biological Aspects of Exotic and Rare Tambra Fish (Tor Tambroides Blkr) as the Basis for Domestication. Biodiversitas. 2006;7(2):195–198.
10. Rosyida A, Basuki F, Nugroho RA, Yuniarti T, Hastuti S. Reproductive Performance of Parent Nilem Fish (*Osteochilus hasselti*) Injected with Synthetic Hormone sGnRH-a and Anti Dopamine with Different Doses. Journal of Tropical Aquaculture Science. 2021;2:97-106.
11. Dewantoro E, dan Yudhiswara R. The Effect of Ovaprim Hormone Injection on the Spawning Performance of Sharks (*Barbonymus Schwanenfeldii*). Ruaya Journal: Journal of Research and Studies in Fisheries and Marine Sciences. 2017;5(2):1–9.
12. Nuraini H, Alawi Nurasiah, Aryani N. Effect of sGnRH + Domperidone and Different Latency Time on Ovulation and Hatching of Jamun Fish Eggs. Worst Fishery Periodic. 2013;41(2):1-8.
13. Mehdi Y, dan Mousavi SE. A Review of The Control Of Reproduction And Hormonal Manipulations In Finfish Species. African Journal of Agricultural Research. 2011;6(7):1643–1650.
14. Manickam P, Joy KP. Induction of Maturation and Ovulation by Pimozide LHRH Analogue Treatment and Resulting High Quality Egg Production in the Asian Catfish, *Clarias batrachus* L. Aquaculture. 1989;83:193-199.
15. I'tishom R. Pengaruh sGnRHa + Domperidon dengan Dosis Pemberian yang Berbeda terhadap Ovulasi Ikan Mas (*Cyprinus carpio* L.) Strain Punten. J Berkala Ilmiah Perikanan. 2008;3(1):9-16.
16. Dunham RA, Liu Z. Aquatic Genomics: Steps Toward a Great Future. In Gene Mapping, Isolation and Genetic Improvement in Catfish. Springer-Verlag. c2002. p. 45–60.