Research in
AGRICULTURE, LIVESTOCK and FISHERIES
ISSN : P-2409-0603, E-2409-9325
An Open Access Peer-Reviewed International Journal

**Article Code: 0336/2021/RALF**
**Article Type: Research Article**

**Res. Agric. Livest. Fish.**
**Vol. 8, No. 1, April 2021: 171-179.**

# DOSE OPTIMIZATION OF OVATIDE HORMONE FOR INDUCED BREEDING OF FRESHWATER GANG MAGUR, *Hemibagrus menoda* (Hamilton, 1822)

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**ARTICLE INFO**

**Received**
21 March, 2021

**Revised**
20 April, 2021

**Accepted**
26 April, 2021

**Online**
May, 2021

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**Key words:**
Dose optimization
Gang magur
*Hemibagrus menoda*
Induced breeding
Ovatide

**A B S T R A C T**

An experiment on induced breeding of gang magur, *Hemibagrus menoda* (Hamilton, 1822) using Ovatide hormone was carried out at in the Field Laboratory Complex of Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh. This study consists of three treatments each with three replications. The objective of the experiment was to find out the effective dose of Ovatide hormone for induced breeding. A total number of 54 brood fish were used for the experiment among which 36 were male and 18 were female. Brood fish were kept in the ratio of 2♂:1♀ for breeding purpose. Female brood fish were injected at the rate of 7, 5, 3 ml Ovatide/kg body weight while the males were injected with 3, 2.5 and 1.5 ml Ovatide/kg body weight respectively in $T_1$, $T_2$, and $T_3$ at the same time. The brood fish were injected with single dose of Ovatide in all treatments. Ovulation rates were 0%, 100%, 63%; fertilization rates were 0%, 97%, and 90%, and hatching rates were 0%, 95% and 76% in treatments $T_1$, $T_2$, and $T_3$ respectively. Fertilized eggs were incubated for 21-22 h in all the treatments. Higher ovulation rate (100 %), fertilization rate (97%), hatching rate (95%) and survival rate (85%) were found in treatment $T_2$. Therefore, the optimum dose of Ovatide hormone for induced breeding of *H. menoda* is 2.5 and 5 ml Ovatide/kg body weight of male and female brood fish, respectively.

**To cite this article:** Hasan M. Z., M. F. Islam, S. A. Haque, M. S. Islam, M. M. Rahman and M. I. Miah, 2021. Dose Optimization of Ovatide Hormone for Induced Breeding of Freshwater Gang Magur, *Hemibagrus menoda* (Hamilton, 1822). Res. Agric. Livest. Fish., 8 (1): 171-179.

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## INTRODUCTION

Among the 293 freshwater fish species of Bangladesh, the biodiversity status of 253 freshwater fishes was assessed by International Union for the Conservation of Nature (IUCN, 2015). Sixty-four (64) are under threat, among them 9 Critically Endangered, 30 Endangered and 25 Vulnerable. This has been followed by 26 species as Near Threatened. *Hemibagrus menoda* (Hamilton, 1822) is an important freshwater catfish that is fast disappearing from the rivers, haors and beels of Bangladesh, hence, categorized as Near Threatened (Rahman, 2005; IUCN, 2015). In order to preserve this fish for future generations, this study attempts to develop conservation strategies through domestication and conservation aquaculture. *H. menoda*, a catfish commonly named Menoda catfish and locally called Gang tengra, Golsa-tengra, Arwari, Gang magur, koune magur in Bangladesh. *H. menoda* has been utilized as experimental animal and is a valuable food fish because of its large size (450 mm or 17.7" SL, but can attain up to 800 mm), tasty flesh and high market value, but it is less frequently encountered in markets compared to other genera of large Bagrid catfishes such as Rita and Sperata (Hoque *et al*., 1998; Ng and Ferraris, 2000). Although the total production of these catfishes has been increased in recent years, but the availability of many species of catfishes is declining day by day from natural waters (Hoque *et al.,* 1998). The induced breeding technique has been made possible to produce fish seed all year round (Ayinla, 1988). There are various types of commercial hormones are used for artificial propagation of different catfishes. Among them, Pitutary Gland (PG), Deoxycorticosterone Acetate (DOCA), Ovaprim, Ovulin, Human Chorionic Gonadotropin (HCG), Ovopel, Dagin and Aquaspawn etc. are most familiar in the markets. (Cheah & Lee, 1980; Brzuska & Adamek, 1999; Zohar & Mylonas, 2001; Adebayo & Popoola, 2008).There are several studies have been documented about the effectiveness of using different doses of synthetic hormones for artificial propagation of different catfishes (Olubiyi *et al.,* 2005; Sahoo *et al.,* 2005; Achionye-Nzeh & Obaroh, 2012; Shinkafi & Ilesanmi, 2014; Marimuthu *et al.,* 2015), but very little is known about the doses of Ovatide in catfish breeding. Ovatide, an injectable inducing hormone consisting of Gonadotropin Releasing Hormone (GnRH) analogue in combination with dopamine antagonist is efficient in induced spawning of tropical fishes such as *Labeo rohita, Clarias batrachus* (Gupta *et al.,* 2002 and Sahoo *et al*., 2004). Moreover, probably it is the first study of induced breeding in menoda catfish (*Hemibagrus menoda*) by using Ovatide hormone.

There has been an alarming trend of the conservation status of *H. menoda*. Captive breeding is an alternative strategy for conservation of a species in order to prevent it from extinction. A successful captive breeding of fish requires establishing a dependable induced breeding and fry rearing technique. Successful spawning of any species is the first and foremost step in its stability to the aquaculture industry by providing a consistent and trustworthy supply of eggs and larvae. Moreover, the capability to control spawning ensures the availability of seed supply to be year round rather than solely during peak spawning season (Torano *et al*., 2000). The seeds of *H. menoda* are unavailable primarily because this fish, as is the case with other catfishes, do not spawn naturally in captivity which is a huge barrier to its successful culture. Therefore, our investigation has been designed to evaluate effects of different dosages of Ovatide hormone on breeding performance of *H. menoda.*

## MATERIALS AND METHODS

### Site selection and duration of the study

This experiment was carried out at Field Laboratory Complex, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh during March 2017 to July 2018. The aim of the experiment was to evaluate effects of different dosage of Ovatide hormone for induced breeding of *H. menoda*. The brood fish were collected from Jaria-Jhanjail point, Kangshariver, under Netrokona district, Bangladesh.

### Brood fish collection and rearing

A total of 54 live H. menoda were collected during March 2017 and February 2018 from Kangsha river located at Jaria-Jhanjail, Netrokona district, Bangladesh. During the rearing period, the fish were fed with SIS (small indigenous species of fish), and CPC feed, a special feed enriched with protein and vitamin-E was fed at a rate of 4-5% body weight which enhances the gonadal maturation in fishes. Regular manuring, fertilization, and liming were performed whenever necessary.

**Brood selection**

To carry out the induced breeding trial, male and female broods were caught from the brood rearing ponds using a cast net. The average weight of the male and female were 371±50 g and 778±80 g, respectively. The gravid male and female were selected based on the following criteria. Males with relatively smaller body, elongated and slender in shape having swollen and reddish urogenital papillae were selected. Contrariwise, the female was identified by having rounded and protruding abdomen which is soft when touched with fingers and the swollen genital opening sometimes reddish in color.

**Conditioning**

Selected broods were transferred to the cemented circular tank in the hatchery with continuous water showering for about five hours prior to administering hormones. Male and female were kept off-feed during conditioning.

**Collection and preparation of Ovatide**

Ovatide hormones were used as inducing agents. The proper doses of Ovatide hormone were calculated based on the recommended dose and body weight of the brood fish using this formula:

$$\text{Amount of ovatide (mL)} = \frac{\text{Wt.} \times \text{Pt.}}{1000}$$

Where, Wt. = the total body weight of the fishes injected (g)

Pt. = the rate in mL ovatide injected·kg $^{-1}$ body weight under a particular treatment.

For Ovatide preparation, the powder form synthetic hormone was diluted with distilled water to dissolve it in the contained vial and shaked for a few minutes. The prepared solution was then taken out in a 5 ml disposable syringe for injection.

**Hormone injection**

The hormone solution was administered intramuscularly to the female and male between the dorsal fin and lateral line maintaining about 45° angular position with the body. The injected fish were then released into breeding hapa placed in tanks for synchronized spawning.

**Observation of reproductive behavior**

After injection, both male and female broods were kept together in the spawning tank (temperature 25.5 °C; pH 7.2; dissolved oxygen 6.5 ppm) and their reproductive behavior was closely monitored.

**Experimental design**

This experiment was conducted using commercial Ovatide hormone consisting of three treatments $T_1$, $T_2$, and $T_3$ with three replications each in a Completely Randomized Design (CRD). The doses of Ovatide are presented in Table1. A total of 54 brood fish which were taken and kept them at a ratio of 2 male: 1 female in the tanks. Semi artificial (or induced natural) propagation method was adopted which involved synchronized spawning in breeding hapas whereby the injected brood fish (male and female spawners) were placed into a breeding hapa fixed in circular and rectangular tanks (dimensions: 1.22×2.74×0.37 m). Double hapa (upper and lower) were used with the mesh size of the upper hapa (dimensions: 1.35×1.12×0.36 m) being larger than the lower hapa (1.24×1.02×0.36 m). After ovulation of 21-22 h and fertilization, the upper hapa was then removed along with the spent spawners while the fertilized eggs settled in the lower hapa which served as a shelter during the incubation period. Larvae were also hatched in the hapa.

**Table1. D**oses of Ovatide (ml Ovatide/kg body weight) hormone for induced breeding of *Hemibagrus menoda* at 1♀:2♂ ratio

| Sex | Treatment | | |
|---|---|---|---|
| | $T_1$ | $T_2$ | $T_3$ |
| Male | 3 ml/kg | 2.5 ml/kg | 1.5 ml/kg |
| Female | 7 ml/kg | 5 ml/kg | 3 ml/kg |

**Spawning, determination of fertilization rate and incubation of eggs**

Ovulation and fertilization occurred in the spawning hapa. After ovulation, the upper hapa was removed along with the spent spawners leaving the fertilized eggs. Fertilization rate was determined by taking 100 eggs from each treatment and then incubated in 1.5 L bowls each having sufficient water flow via a PVC pipe with inlet and outlet. Then the eggs were observed under a magnifying glass and fertilized eggs were counted. The fertilized eggs are not transparent as the hatching egg. The fertilized egg was easily identified by the presence of transparent shell with gray spot within the eggshell, while the unfertilized egg was whitish and opaque. The fertilization rate was determined (Islam and Biswas, 2017) by using the following formula:

$$\text{Fertilization rate (\%)} = \frac{\text{No. of fertilized eggs}}{\text{Total no. of eggs (fertilized+unfertilized)}} \times 100$$

**Care of hatchlings and determination of hatching rate**

Hatching started after 125-130 h of fertilization. Care of hatchlings started from the moment the eggs began to hatch. The separation of larvae from unhatched eggs was achieved by siphoning with a 1.5 mm rubber hose. The larvae were closely monitored so as to observe the time of yolk sac absorption for first feeding of larvae. Aeration was provided using aerators and flow through systems. After completion of hatching, about 6 h post hatching; larvae/hatchlings number in each bowl was counted by visual observation using a magnifying glass and recorded. Hatching rate was determined (Mollah *et al.,* 2008) by using the following formula:

$$\text{Hatching rate (\%)} = \frac{\text{No. of eggs hatched}}{\text{Total no. of fertilized eggs}} \times 100$$

**Determination of survival rate**

Survival rate was determined by randomly collecting 300 hatchlings from each replication under a particular treatment and stocked in the trays containing water for 10 days (without feeding). All other conditions during experimentation were maintained same. After completion of the experiment at 10th day, the number of total live larvae in the tray was counted separately for the calculation of survival rate.

**Physico-chemical parameters analyses**

The physico-chemical conditions of water during the experiment are shown in the Table 2. Temperature, pH and dissolved oxygen of water during treatments ranged from 26-28 °C, 6.7-7.5 and 5.5-6.5 ppm respectively. It indicates that the water quality was within the suitable range.

**Table 2.** Physico-chemical parameters of different doses of Ovatide hormone for induced breeding of *Hemibagrus menoda*

| Parameters | $T_1$ | $T_2$ | $T_3$ |
|---|---|---|---|
| Incubation Temperature (°C) | 26- 27 | 26-27 | 26-28 |
| Dissolve $O_2$ (ppm) | 5.5 | 7 | 6.5 |
| pH | 6.8 | 7.2 | 7.5 |

**Statistical analyses**

For statistical analysis of data, one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) at a significance level of $P<0.05$ was employed. The statistical data analysis was carried out with the aid of the computer software SPSS version 20 and necessary graphs were drawn by MS Excel 2013.

## RESULTS

### Latency period and incubation temperature

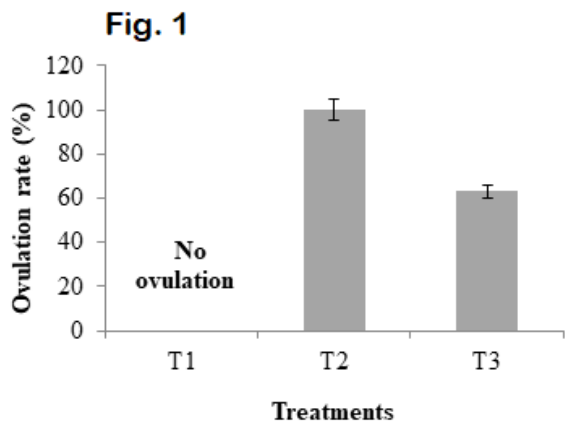
The time interval between the injection of Ovatide hormone and ovulation varied between 21 to 22 h in the treatments whereas the incubation temperature varied from 26 to 27°C.

### Spawning rate

No spawning occurred in treatment $T_1$ i.e. in females injected 7 ml Ovatide/kg body weight (Figure 1). The percent ovulation rate was lower in $T_3$ than other treatments of $T_2$ in which highest ovulation occurred.

### Fertilization rate

The average fertilization rates were recorded as 97 % and 90 % in $T_2$ and $T_3$, respectively (Figure 2). The highest fertilization rate (97 %) was recorded in 5 ml Ovatide/kg body weight in treatment $T_2$.



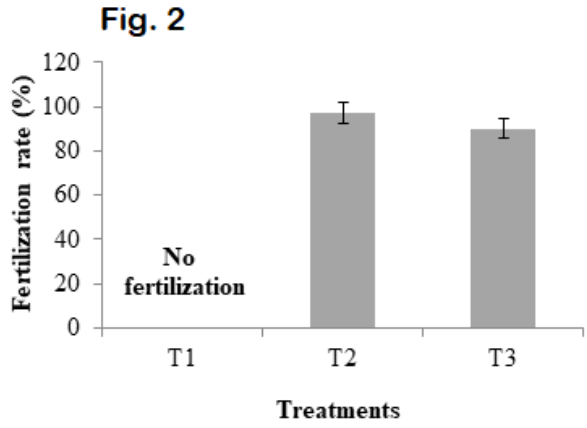



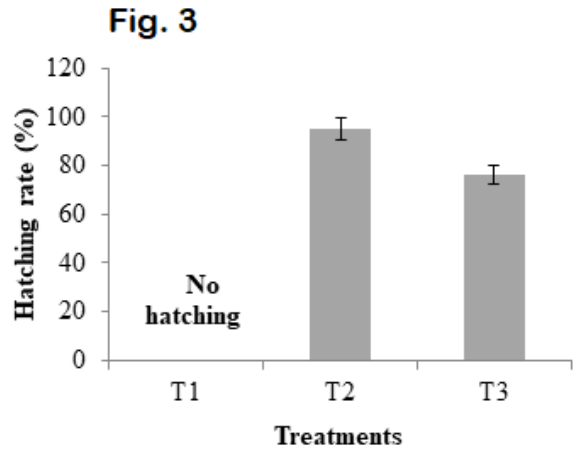

**Figure 1.**Comparison of ovulation rate (%) with different treatments
**Figure 2.** Comparison of fertilization rate (%) with different treatments

### Hatching rate

During the dose optimization of Ovatide, hatching rates were found to be 95 %, 76 % in $T_2$ and $T_3$ respectively (Figure 3). Hatching rate showed that $T_2$ was higher than all other treatment ($T_1$ and $T_3$).

### Survival rate

The survival rates of *H.menoda* with different treatment were 85 %, 80 % in $T_2$ and $T_3$ respectively (Figure 4) after 7 days of experiment also revealed that $T_2$ higher than the rest of the treatment.



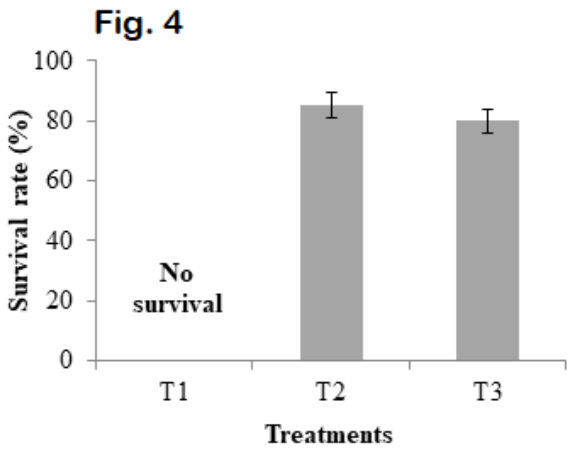




**Figure 3.**Comparison of hatching rate (%) with different treatments
**Figure 4.**Comparison of survival rate (%) with different treatments

## DISCUSSION

In order to conserve the *H. menoda* species, brood fishes were collected from the wild in the Kangsha River, Netrakona and reared up to the attainment of gonadal maturation under captive condition in the pond. Pillay (1993) pointed out that the administration of the appropriate dose of hormone is the basic task to the success of induced breeding, coupled with the brood condition and environmental conditions. In this study, induced breeding trials performed using different doses of Ovatide had resulted in the successful spawning of *H. menoda* under captivity.

Irrespective of the dosage of Ovatide applied in different treatments, the time interval between the first hormonal injection of Ovatide extract and ovulation (latency period or response time) varied between 21 and 22 h and occurred within a temperature range of 26 – 28 °C. The longtime of latency was attributed to lack of synchronization in achievement of readiness of spawning by the fish (Rahdari *et al*., 2014). Tan-Fermin and Emata (1993) recorded a latency period of 12–16 h when *Clarias gariepinus* and *Clarias macrocephalus* were induced to breed using pituitary and ovaprim which is lower than the present findings.

The hormone doses of 7 ml ovatide/kg body weights of fish did not yield any ovulation. Female bellies hard and vent blocked. No ovulation occurred after 21 hours of the hormone injection. However, successful ovulation occurred in the females of *H. menoda* injected 3, 5 ml ovatide/kg body weight. This suggests that *H. menoda* can be induced to ovulate using the Ovatide dosage from 3 to 5 ml/kg body weight. Higher doses from 7 ml Ovatide/kg body weight failed to induce ovulation.

Induced breeding trial of *H. menoda* using three different doses of Ovatide hormone in three treatments gave the highest fertilization rate of 97 % at 5 ml Ovatide/kg body weight of female. The fertilization rate recorded in this study is lower than the 98 % fertilization rate found in *Heteropneustes fossilis* injected with Pituitary Gland (PG) extract at 75 mg/kg body weight (Ali *et al.*, 2014). Lower fertilization rate (75 %) was recorded for *Cirrhina reba* (Verghese, 1969). Differences in the results from other studies could be due to age and physiological state of brood fish and environmental factors. The present findings agree with Haniffa and Sridhar (2002) who observed that irrespective of hormones and fish species, fertilization in *Channa punctatus* was 70 % and above. Since fertilization rate was above 70 % for both $T_2$ and $T_3$, they could be recommended for the induced spawning of *H. menoda.* The higher fertilization rate from the hormones could be the result of appropriate timing and the appropriate dosage administration.

Hatching rate was highest (95 %) in a dose of 5 ml Ovatide/kg body weight of female and lowest (76 %) at a dose of 3 ml Ovatide/kg body weight of female. The hatching rates recorded in this study are quite higher than many other fish species. Islam (2002) recorded a hatching rate of (76.21 %) for *Ompok pabda* in a PG dose of 18 mg/kg and lowest (36.59 %) at a dose of 20 mg PG/kg body weight of fish. Begum *et al*. (2001) reported 38 % hatching rate as the highest for Shing, *Heteropneustes fossilis* injected with PG dose of 75 mg/kg body weight. These differences may be attributed to age, maturity of donor fish or seasonal variation rather than the hormone dosage.

The highest (85 %) larval survival rate at 72 h post hatching was recorded in *H. menoda* treated with ovatide dose of 5 ml/kg body weight of female is indication of the appropriateness of the dose for the induced spawning of the fish. The lower survival rate (42.96±3.56) recorded in dose of 6500 IU Human Chorionic Gonadotropin (HCG)/kg body weight could be due to the higher dose given or low egg quality from the brood female. The survival rate of 5 day old hatchling of *Mystus vittatus* varied from 55.5 – 68 % (Sarker *et al*., 2002; Alam *et al*., 2006; Islam *et al*., 2011), which is lower than the present finding. Our finding further extends the assertion from Okere *et al*. (2015) that, type of hormonal agent determines fry survival rate. Survival rate recorded in this study is remarkably higher than that obtained by Haniffa and Sridhar (2002) who reported survival rate of 55 % in *Channa punctatus* injected 3000 IU HCG/kg body weight. Single dose of 5 ml Ovatide/kg body weight of female could be considered the optimum dose for the induced spawning of *H. menoda* in a 2♂:1♀ ratio.

## CONCLUSION

This study investigated the optimum dose of Ovatide hormone for induced breeding of this species. Induced breeding of *H. menoda* has been successful using single doses each of Ovatide hormone 5, 3 ml/kg body weight of female. Comparison of the spawning successes of Ovatide hormones revealed that 5 ml/kg body weight of female and 2.5ml/kg body weight of male could be considered the optimal dose for the induced spawning of *H. menoda* in a 2♂:1♀ ratio.
This study provides the first data on the induced breeding technique of *H. menoda*. The success achieved in this study over the control of this fish under captivity makes its domestication possible and a promising candidate as a culture fish. The present findings on the optimum doses of Ovatide may serve as basis for the subsequent induced breeding and fry nursing of *H. menoda* in Bangladesh. The seed production technology of the species is the most prerequisite of development of culture package technology. The applicability of the induced breeding program for *H. menoda* should be demonstrated in hatcheries for the benefit of farmers throughout Bangladesh.

## CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

## ACKNOWLEDGMENTS

Authors are thankful to the Field Laboratory Complex of Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh for assistance in conducting research successfully as well as express their gratefulness to the Ministry of Science and Technology, Bangladesh for funding under National Agricultural Technology Transfer Programme-Phase II Project (NATP-2).

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