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PROOF
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# Artificial Propragation of Oranda Fancy Goldfish (Carassius auratus auratus) by Using Novel Synthetic Ovulin Hormone

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## Abstract

The present study aimed to evaluate the artificial propagation of Oranda fancy goldfish (*Carassius auratus auratus*) through intra-peritoneal administration of synthetic ovulin hormone.  A total of 45 broodfish (30 males and 15 females) were used in this study and ovulin was injected with double doses for females and single doses for males, using different levels of dosage: 0 (control group), 0.20, 0.40, 0.60, and 0.80 mL/kg of body weight. A 2:1 male-to-female ratio was maintained in the breeding tank. Ovulation occurred within 10−12 hours, yielding yellowish, spherical eggs measuring 0.6−0.9 mm. Fertilized eggs hatched within 40−48 h of fertilization. The brood stocks injected at a dose of 0.40 mL/kg exhibited significantly higher results ($P<0.05$) in all aspects of breeding performance compared to all other dosages. This dosage resulted in a relative fecundity of 21.74%, fertilization rate of 73%, hatching rate of 71%, and larval survival rate of 69%. This study clearly demonstrated that varying hormone doses lead to differences in the breeding performance of oranda goldfish. The positive response to the synthetic ovulin hormone, evidenced by notable fecundity, fertilization rates, and hatching rates, suggests that this method could facilitate commercial breeding and mass seed production for oranda goldfish.

## Introduction

In recent years, the passion for aquarium fishkeeping has become a popular hobby among millions and is a significant commercial activity supporting rural development in many developing countries (FAO, 2010). As ornamental fish are in high demand, their culturing and trading offer a viable livelihood (Siddiky and Mondal, 2016). The knowledge of artificial breeding is a key attribute because it allows intensive seed production of a given species under controlled conditions. Only a reliably induced breeding and larva-rearing technique can supply quality fish seeds (Mollah et al., 2008).  In Bangladesh, the ornamental fish trade has historically been limited to its own territory, often necessitating costly imports to meet domestic demand. Established breeding techniques for many ornamental species remain scarce. Therefore, developing effective local breeding methods, such as through induced breeding, can significantly reduce import dependency and boost the domestic aquaculture sector.

Goldfish are becoming increasingly important in fisheries science, not only as an ornamental fish but also as an experimental animal due to their adaptability to fluctuating environmental conditions (Blanco et al., 2018). Centuries of selective breeding have produced a wide variety of goldfish with different body types, colors, fin and eye configurations, and sizes (Smartt, 2001). According to Mohanta et al. (2008), more than 100 varieties of goldfish appeal to a wide range of aquarium fish lovers. The oranda goldfish (*C. auratus auratus*) is one of the oldest captive-bred fancy goldfish varieties and belongs to the Cyprinidae family, as there are no wild populations of this fish (Mohanta et al., 2008). It is one of the most popular goldfish in the world and is valued for its egg-shaped body and hood and fleshy bubble-like growth on the top of its head called the wen (Geoff and Nick, 2004).

Hormones used widely in the induced breeding of fish include pituitary extract, deoxycorticosterone acetate (DOCA), ovaprim, ovulin, ovatide, human chorionic gonadotropin (HCG), ovopel, dagin, and aquaspawn (Brzuska 2004, 2005; Zohar & Mylonas, 2001). Among these, ovaprim, ovopel, and aquaspawn are increasingly popular and considered efficient for successful spawning due to their GnRH and dopamine blocker receptors (Hassan et al., 2018). Ovulin is a ready-made synthetic inducing agent manufactured by Ningbo Sansheng Pharmaceutical Company Ltd., China, with each 10 ml vial containing 100 mg domperidone and 0.2 mg GnRH analogue. It serves as a peptide supplement to extend the spawning season. While various studies have explored aspects of *Carassius* species worldwide (Salas et al., 2006; Tsoumani et al., 2006), and ovulin has been successfully applied in the propagation of African catfish (Mamndeyati et al., 2018; Maradun, 2018; Ataguba et al., 2023; Jega et al., 2024) and common carp (*Cyprinus carpio*) (Mohammadnejad et al., 2022). To date, there have been no documented reports on the effectiveness of ovulin hormone in the breeding performance of captive oranda goldfish. This study represents the first scientific investigation into optimizing ovulin for this specific species. Selecting the appropriate inducing agent and its effective dose is crucial for improving overall reproductive success. Therefore, this study was conducted to assess the breeding performance and to establish an induced breeding technique for oranda goldfish (*C. auratus auratus*) using synthetic ovulin hormone.

## Materials and Methods

### Collection and Acclimatization of Broods

This study was conducted at the Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh. Prior to the breeding program, all necessary preparations for the induced breeding of oranda goldfish (*Carassius auratus auratus*) were made in the laboratory, including ensuring the availability of brood conditioning tanks, breeding bowl setups, water supply facilities, and working space. Oranda broods (30–38 g) were purchased from local breeders from Chattogram. This experiment was conducted in compliance with the guidelines of the Animal Care and Use Committee of Chattogram Veterinary and Animal Sciences University.

Before hormone administration, we acclimatized procured broodfishes (n=45) for one month in three glass aquaria (60×30×45 cm) with 70 liters of water holding capacity and each tank housed 15 oranda broods. Water quality parameters, including temperatures (25−27ºC), dissolved oxygen concentration (4−6mg/L) and pH (7.0−8.0) were regulated to maintain suitable environment for the broods. During acclimatization, fish were fed twice daily with dried tubifex at 3% of their body weight. We measured the length and weight of the broods after the acclimation period. The length and weight of the collected fish are listed in Table 1.

### Experimental Design

To evaluate the breeding efficiency of the experimental fish, we administered four distinct doses of ovulin (Table 1), with a control group that received no hormonal dose. Each treatment was replicated three times to enhance the reliability of the findings. The study utilized a total of fifteen breeding tanks, each with a capacity of 20 liters. In each tank, two males and one female (2:1 male-to-female ratio) were maintained to optimize breeding conditions. Prior to hormone administration, the fish were acclimated to their environment for 24 h. Throughout this acclimation phase, we rigorously monitored water quality parameters to ensure an optimal habitat. The water temperature was consistently maintained at 26.2±0.17ºC, while dissolved oxygen levels were recorded at 4.69±0.65 mg/L, thus ensuring adequate respiratory conditions. Additionally, the pH was carefully regulated to 7.6±0.48, thereby creating a balanced aquatic environment conducive to successful breeding.

**Table 1.** Standard length (SL), total length (TL), and body weight (BW) of collected oranda goldfish males (n=30) and females (n=15) used in this study

| Treatments (ml ovulin/kg) | Male TL (cm) | Male BW (g) | Female TL (cm) | Female BW (g) |
|---|---|---|---|---|
| $T_0$ | 12.86±0.38 | 30.80±0.96 | 11.21±0.69 | 32.91±2.60 |
| $T_1$ (0.2) | 13.07±0.83 | 31.47±1.31 | 11.17±0.63 | 36.53±3.83 |
| $T_2$ (0.4) | 13.06±0.55 | 32.14±1.14 | 11.16±0.72 | 33.26±2.88 |
| $T_3$ (0.6) | 12.70±0.92 | 31.08±0.86 | 10.26±1.08 | 34.41±3.39 |
| $T_4$ (0.8) | 13.10±0.44 | 32.33±1.54 | 12.52±0.14 | 35.38±7.05 |

### Administration of Hormone

We purchased synthetic ovulin hormone from local trader which was preserved in 10 ml vial and stored at 4ºC. Synthetic ovulin hormone doses of 0.2, 0.4, 0.6, and 0.8 ml/kg body weight were administered via intraperitoneal injection in the brood fish using a standard 1 ml intramuscular syringe, held at a 45-degree angle to the fish's body (Shinkafi and Ilesanmi, 2014), with no dilution required as raw doses were administered. Females received two doses, whereas males received a single dose of the hormone (Viveen et al., 1985). The male fish were injected simultaneously with the second dose administered to the females. The hormone doses administered to the broods are listed in Table 2.

### Collection of Egg/Sperm and Fertilization

At the end of the ovulation period, we performed stripping by applying slight pressure to the posterior ventral side of the females in de-chlorinated water to minimize fungal attacks. Subsequently, the respective males were stripped in the same breeding bowl and gently mixed with female eggs. We counted the eggs from each treatment by taking three subsamples from the total egg volume and averaging the count to estimate the total number of eggs. Fertilization was assessed approximately 1−2 hours post-mixing. Translucent eggs with visible embryonic eye spots were identified as fertilized (Sahoo et al., 2005) and counted through direct observation by taking subsamples in a slide from each replication. Dead eggs that appeared opaque were carefully removed by siphoning, allowing only the viable eggs to be incubated at room temperature (25−27ºC).

### Reproductive Performance Analysis

The relative fecundity, percentage fertilization, percentage hatchability, and survival rate were recorded for each treatment to determine the breeding performance of ovulin at various dose levels as follows:

Relative fecundity was calculated using the formula described by Kahkesh et al. (2010) as follows:

Relative fecundity (%)= (Number of stripped eggs / Body weight of female) x 100

Percentage fertilization: The mean fertilized eggs in all replications were expressed as percentage fertilization per female (Adebayo and Popoola, 2008) as follows:

Fertilization (%)= (Number of fertilized eggs / Number of eggs counted) x 100

Percentage hatchability: This was calculated using the formula described by Lambert (2008) as follows:

Hatchability (%)= (Number of eggs hatched / Number of fertilized eggs) x 100

Survival rate: Data of survived larvae was collected for one week, and the survival rate of oranda goldfish larvae was determined using the following formula used by Ayinla and Akande (1988):

Survival rate (%)= (Number of larvae at the end of experiment / Number of larvae hatched) x 100

### Statistical Analysis

The data obtained for the breeding performance study were subjected to one-way analysis of variance (ANOVA) to determine significant differences among the treatments and to describe significant differences within and between treatments. Tukey's-b multiple range tests (P<0.05) were used. Data are expressed as means ± standard deviation (SD). Statistical analysis was performed using IBM SPSS Statistics (Version 22.0).

## Results

### Ovulation and Hatching Period

The results of induced breeding of *C. auratus auratus* are presented in Table 3. Oranda goldfish eggs ovulated within 10−12 hours and hatched within 40−48 hours of fertilization at the temperature of 25−27ºC among all the injected groups. Ovulation periods did not differ significantly (P>0.05) across different hormone administrations (Table 3). While mean hatching periods also showed no significant difference overall (P>0.05), the highest dose (T4) showed a longer hatching duration.

**Table 2.** Hormonal administration protocol of the study for the breeding trial of the oranda gold fish

| Treatment | Sex | Ovulin (ml/kg body weight) | | The time interval between the doses |
|---|---|---|---|---|
| | | $1^{st}$ dose | $2^{nd}$ dose | |
| $T_1$ | Female | 0.2 | 0.2 | 6 hours |
| | Male | - | 0.2 | |
| $T_2$ | Female | 0.4 | 0.4 | |
| | Male | - | 0.4 | |
| $T_3$ | Female | 0.6 | 0.6 | |
| | Male | - | 0.6 | |
| $T_4$ | Female | 0.8 | 0.8 | |
| | Male | - | 0.8 | |

**Assessment of Breeding Performance**

The breeding performance indices for the samples administered at the four doses are given in Table 4. The highest number of eggs was found in $T_2$ treated females, which was significantly higher than in other treatment groups (Table 4). The parameter "Eggs/Females" represents the mean total eggs stripped per female across replicates. The diameter of eggs was found to vary between 0.73 and 0.77mm, and no significant variation was observed among hormonal treatments. Female of $T_2$ had the highest percentage of relative fecundity (21.74±3.68%), while the lowest relative fecundity (10.08±5.99%) was recorded in the $T_4$ sample (Table 4).

The percentage of fertilization varied among the different doses of hormone administration. Egg samples of $T_2$ and $T_3$ had significantly ($P<0.05$) highest percentage fertilization of 73.00±4.58 and 56.33±9.07, respectively, while the lowest fertilization (30.67±2.08) was found in a sample of $T_4$ (Figure 1). $T_2$ females significantly ($P<0.05$) yielded the highest percentage of hatchability (71.07±4.02), while the lowest hatchability (31.50±4.22) occurred in $T_4$ (Figure 2). The highest and most significant ($P< 0.05$) survival rates were obtained for $T_2$ (69.64±6.65) and $T_3$ (51.74±6.65) (Figure 3).

## Discussion

This study represents the first documented scientific investigation into the efficacy of synthetic ovulin hormone for the induced breeding of oranda goldfish (*Carassius auratus auratus*). Our primary objective was to establish an effective breeding

**Table 3** Ovulation and hatching period of ornate goldfish after hormone administration and time were measured as hours

| Treatment | Ovulation period (hour) | Hatching period (hour) |
|---|---|---|
| $T_1$ | 10.00±2.00 | 40.33±3.84 |
| $T_2$ | 10.33±1.52 | 43.66±2.96 |
| $T_3$ | 11.67±0.57 | 40.00±2.64 |
| $T_4$ | 11.33±0.28 | 48.66±1.15 |

**Table 4**. Breeding performance index of *C. auratus auratus* at different dose levels of ovulin

| Parameter | Treatment (mL $kg^{-1}$ b.wt.) | | | |
|---|---|---|---|---|
| | $T_1$ | $T_2$ | $T_3$ | $T_4$ |
| Eggs/ Females | 360.67±47.04[a] | 717.67±87.12[b] | 427.67±105[ab] | 380.00±288[a] |
| Egg diameter (mm) | 0.77±0.12 | 0.73±0.14 | 0.77±0.13 | 0.75±0.09 |
| Relative fecundity | 11.01±2.42[a] | 21.74±3.68[b] | 12.33±2.18[ab] | 10.08±5.99[a] |

*Mean values with different superscripts are statistically significant at ($P<0.05$).

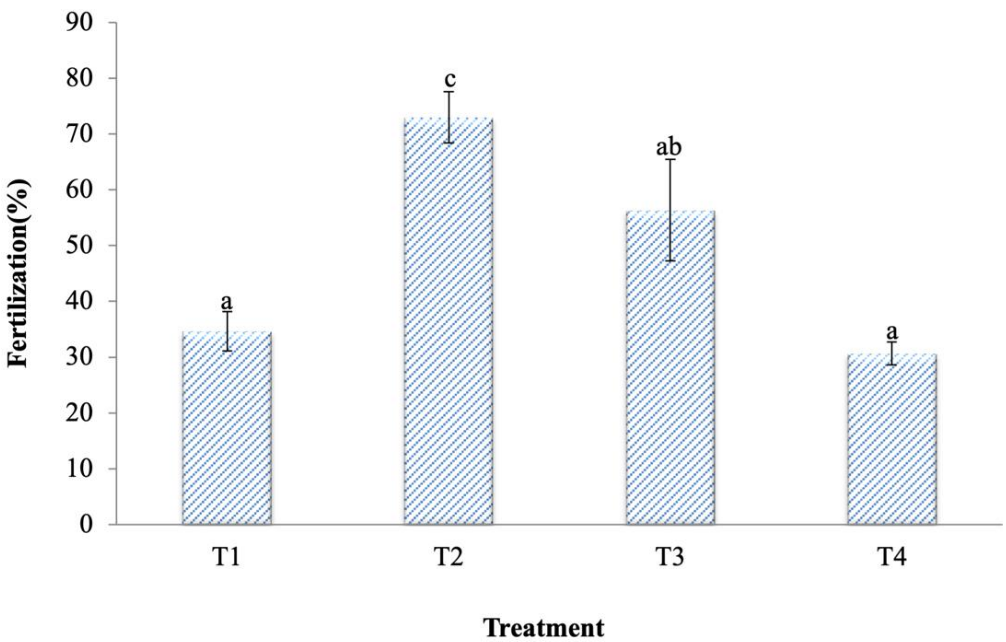


**Figure 1.** Comparison of fertilization rate (%) of *C. auratus auratus* during induced breeding with the administration of different doses (T1, T2, T3 and T4: 0.2 ml/kg body weight, 0.4 ml/kg body weight, 0.6 ml/kg body weight and 0.8ml/kg body weight) of synthetic ovulin hormone. Values are presented as mean±SEM. Mean values with the same superscript did not show any significant difference ($P>0.05$).

technique and identify the optimal ovulin dosage for this popular ornamental species.

The effectiveness of ovulin across various fish species (Mamndeyati et al., 2018; Maradun, 2018; Mohammadnejad et al., 2022) suggested its promising potential for oranda goldfish, despite no prior applications. The positive breeding outcomes indicate that *C. auratus auratus* is highly responsive to ovulin, irrespective of broodstock weight (Mahmud et al., 2012), aligning with findings that synthetic hormones are typically administered undiluted for efficacy (Olumuji and Mustapha, 2002). This approach addressed a gap in the literature concerning diluted hormone versions for induced breeding (Maradun et al., 2019). Our selected ovulin dosages were reported in successful applications for other species (Ibrahim et al., 2019; Uruku et al., 2018; Ukwe and Abu, 2016; Ayoola et al., 2012) and also for synthetic hormones in goldfish

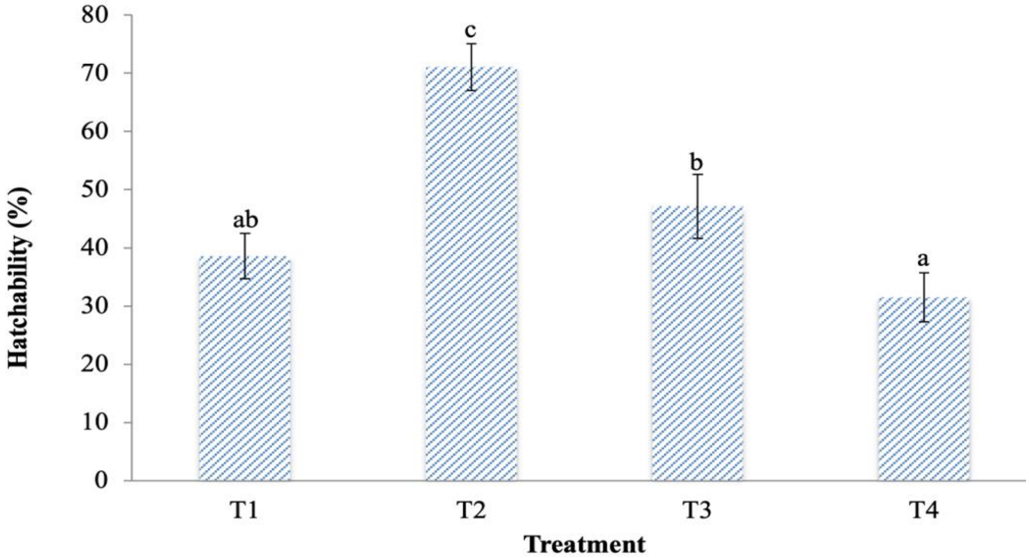


**Figure 2.** Comparison of hatchability (%) of *C. auratus auratus* during induced breeding with the administration of different doses (T1, T2, T3 and T4: 0.2 ml/kg body weight, 0.4 ml/kg body weight, 0.6 ml/kg body weight and 0.8ml/kg body weight) of synthetic ovulin hormone. Values are presented as mean±SEM. Mean values with the same superscript did not show any significant difference (P>0.05).

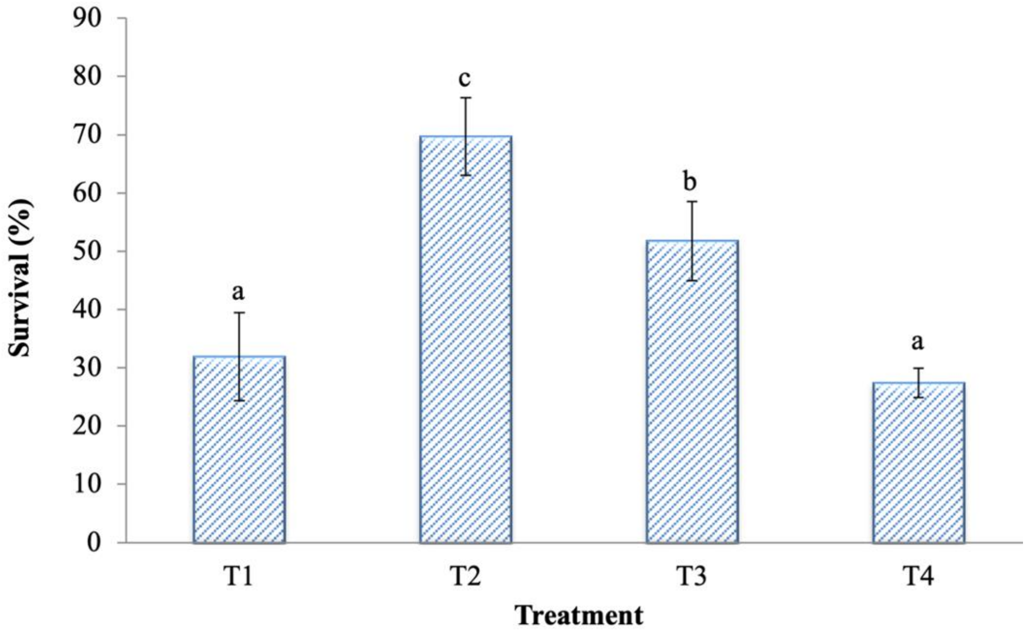


**Figure 3.** Comparison of survival (%) of *C. auratus auratus* during induced breeding with the administration of different doses (T1, T2, T3 and T4: 0.2 ml/kg body weight, 0.4 ml/kg body weight, 0.6 ml/kg body weight and 0.8ml/kg body weight) of synthetic ovulin hormone. Values are presented as mean±SEM. Mean values with the same superscript did not show any significant difference (P>0.05).

breeding (Nirmal et al., 2016; Cejko and Kucharczyk, 2015; Paulo and Antonio, 2005). All ovulin-administered broodfish successfully ovulated, a higher rate than previously reported in other study (Cejko and Kucharczyk, 2015). The consistent ovulation period of 10−12 hours at 25−27ºC suggests ovulin's reliable efficacy. This latency period is comparable to common goldfish induced with ovaprim and cloprostenol at similar temperatures (Jagtap, 2011). While some studies noted that increased hormone doses could shorten ovulation periods (Mahadevi et al., 2018), our study focused on optimizing the overall breeding performance. Ovulation periods in other species, such as *Clarias gariepinus*, also exhibit similar ranges with ovulin (Uruku et al., 2018; Ukwe and Abu, 2016; Ayoola et al., 2012). Previous reports also demonstrate application of different other synthetic hormone in goldfish breeding which requires larger volumes in inducing the goldfish. For example, 0.7ml/Kg and 1.4ml/Kg WOVA-FH was required for inducing *C. auratus auratus*, while 0.5−1.2ml/Kg ovaprim required in comet goldfish indicates the efficacy of ovulin in goldfish breeding.

Regarding reproductive output, the highest number of stripped eggs (717.67±87.12 eggs) was observed at 0.4 mL/kg body weight of ovulin, a figure exceeding fecundity in crucian carp but lower than the 1189 eggs reported for telescopic-eyed goldfish treated with WOVA-FH (Mahadevi et al., 2018). This highlights variations in reproductive potential among different strains and hormonal treatments. The relative fecundity of oranda goldfish at 0.4 mL/kg (21.74±3.68%) was, however, lower than some reports for common goldfish (Vahid et al., 2012), yet comparable to crucian carp administered natural carp pituitary hormone (13-17% at 0.5 mL/kg) (Targon´ska et al., 2012). This suggests that while ovulin effectively enhances reproductive output in oranda goldfish, its efficacy for fecundity might differ when compared to other species or hormones. Consistent with previous observations in *Carassius* species (Jagtap, 2011; Mamun and Basudev, 2016), our results provide corroborating evidence for hormonal influence on fecundity. While the egg diameter in this study (0.73−0.77 mm) was notably differed from (1.55mm by Ruhul et al., 2013; 0.8−1.00 mm by Mondal et al., 2018; 1.32mm by Vahid et al., 2012; and 0.9−1.0mm by Mahmud et al., 2012) studies possibly reflecting variation in the strain, rearing conditions, hormonal or genetic influences. This discrepancy warrants further investigation into influencing factors like hormonal dosage, environmental conditions, or genetic strain.

Fertilization rates varied widely depending on the hormone and species, underscoring species-specific responses. Our highest recorded fertilization rate for oranda goldfish (73.00%) was slightly lower than some reports for *Clarias gariepinus* with ovulin (81% by Ukwe and Abu, 2019; 88.12% by Maradun et al., 2018), but significantly higher than others (15.27% by Uruku et al., 2018; 67% by Ayoola et al., 2012). Compared to other goldfish species, ovulin in oranda goldfish yielded superior fertilization to ovaprim in comet goldfish (Mahmud et al., 2012) and was comparable to cloprostenol and transpose applications (Jagtap, 2011). Our study also observed higher hatching rates than those reported for comet goldfish (Mahmud et al., 2012), though lower than telescopic-eyed goldfish (Mahadevi et al., 2018), suggesting promising avenues for improving breeding efficiency.

The larval survival rate in this study was notable, with 69.64% at 0.4 mL/kg body weight and 51.74% at 0.6 mL/kg body weight. These rates surpassed other groups in this study and align with higher survival rates in *Clarias gariepinus* using ovulin (Maradun et al., 2018). However, it's important to note that even higher survival rates have been documented for goldfish (Paulo and Antonio, 2005; Mondal et al., 2018) and crucian carp (Targon´ska et al., 2012) in other contexts, indicating room for further optimization. Our findings emphasize that optimal breeding performance in oranda goldfish requires precise dose calibration, as dose lower than 0.40 mL/kg may not be optimal, while at higher dose at T4, this species demonstrates poor performance. This aligns with observations that excessive inducing agents can diminish fertilization, while insufficient use may delay male inducement (Pronob et al., 2016; Routray et al., 2007). At T4, overdose of ovulin may provide negative feedback to the HPG axis in reproductive output. Moreover, the hormone dosage is also dependent on the “readiness" of female in terms of age, size, sensitivity, and a variety of other criteria. In tropical and subtropical environments, fish metabolism is much higher (because to higher temperatures), and the likelihood of hormone waste is consequently higher than in temperate zones. In this study, a control group (receiving no hormonal dose or saline water) was included; however, these fish showed no pairing and subsequently no spawning process. This observation is crucial, as it clearly demonstrates that induced breeding with ovulin was necessary to achieve successful propagation under our experimental conditions. While our results highlight the effectiveness of ovulin across various doses, the lack of natural spawning in the control group further underscores the hormone's direct and absolute impact in initiating the breeding process. The positive responses observed across all injected groups strongly support ovulin's utility as an effective inducing agent for Oranda goldfish. However, future studies with a greater number of samples, year-round breeding trials with different temperature regimes and measuring different hormonal level will further elucidate breeding performance of this species.

## Conclusion

This study successfully established an effective induced breeding technique for oranda goldfish (*Carassius auratus auratus*) using synthetic ovulin hormone and identified 0.4 mL/kg body weight as the optimal dosage for commercial breeding, balancing fecundity (21.74%), fertilization (73%), and larval survival (69.64%). The consistent ovulation observed across all treated groups, contrasted by the lack of spawning in the control group strongly supports ovulin's critical role in the artificial propagation of this species under the given conditions. Our findings offer valuable insights for commercial breeding programs, demonstrating ovulin's potential for mass seed production of oranda goldfish. This method could contribute significantly to reducing import dependency and fostering the domestic aquaculture sector in Bangladesh. This study paves the way for further research aimed at improving artificial breeding of goldfish species. Future investigations could focus on optimizing hormone application, environmental parameters, and other biological factors, such as testing ovulin in hybrid goldfish strains, evaluating its efficacy under varying temperature thresholds, and conducting cost-benefit analyses against alternative inducing agents like ovaprim to assess its overall commercial viability.

## Ethical Statement

The study was approved by The CVASU Ethics Committee.

## Funding Information

This research received no external funding.

## Author Contribution

Md. Main Uddin Mamun: Conceptualization, Methodology, Project administration, funding acquisition, formal analysis, Writing, Review & Editing; Md. Moudud Islam: Supervision, Investigation, Data Curation, Methodology, Data curation, Review & Editing; Sk. Ahmed Al Nahid: Review & Editing, Supervision; Md. Mahiuddin Zahnagir: Conceptualization, Validation, Resources, Writing, Review & Editing. Fatema Akhter and Md Nayeem Hossain: Writing, Review & Editing.

## Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this study.

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