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Study on effects of synthetic hormone for breeding of koi carp (*Cyprinus carpio*)

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Abstract
The brooders of size varied from 375 to 540 g were taken for the present investigation. The Female Koi carp received WOVA-FH at 0.2 ml/kg (T_1), 0.3 ml/kg (T_2), and 0.4 ml/kg (T_3) in triplicate treatments. Similarly male brooders were injected with 0.1 ml/kg (T_1), 0.2 ml/kg (T_2), and 0.3 ml/kg (T_3) WOVA-FH, also in triplicates. Brooders were introduced into breeding hapas at a 1:1 male to female ratio. Brooders treated with 0.3 ml/kg body weight (T_2) showed 100% spawning success, releasing 11814.0±1220.7 no of eggs. In comparison, brooders given 0.2 ml/kg (T_1) released 7870.0±1464.9 eggs, while those treated with 0.4 ml/kg (T_3) laid 9645.00±1019.47 no of eggs. When brooders received a lower dose of 0.2 ml/kg (T_1), only partial spawning occurred. Egg numbers ranged from 6890 to 9554, with fertilization percentages of 65.0±0.7 and hatching rate of 59.29±0.3. Brooders treated with WOVA-FH at 0.3 ml/kg (T_2) showed fertilization and hatching rates of 71.5±0.5% and 64.8±0.1%, respectively. In T_3, the total number of eggs laid ranged from 8741 to 10750, with fertilization rates of 68.31±0.71. Corresponding hatching rates was 62.2±0.4. The findings indicate that WOVA-FH is an effective inducing agent for achieving successful breeding in Koi carp. Administering WOVA-FH at a dosage of 0.3 ml/kg body weight yielded complete breeding success, including the highest egg output along with improved fertilization and hatching rates.

Keywords: *Cyprinus carpio*, WOVA-FH, fertilization, hatching

Introduction
One of the most popular and a growing industry worldwide of these days is the demand of ornamental fish. More than 2500 fish species that are used in the ornamental fish market worldwide, 60% are freshwater species. Despite being an important component of the global fish trade, the ornamental fish industry is modest. In many developing nations, it has a positive impact on rural development.

With a variety of technologies and increased farming intensity, carp culture in India has expanded geographically during the past 50 years. In India, carp production is considered the foundation of freshwater aquaculture and its proportion has increased from 46% in the 1980s to over 85% in recent years. The three largest carps of India-*Labeo rohita*, *Cirrhinus mrigala* and *Catla catla* have been the primary contributors to the nation's fish supply. The three domesticated exotic carp i.e *Cyprinus carpio*, *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix* are thought to be the second most significant group in Indian fish farming (Laxmappa, 2014) [7]. Common carp (*Cyprinus carpio*), in addition to Indian major carps, has become an important fish species for culture, particularly in seasonal tanks. Additionally, consumer's preference for small-sized common carp has contributed to the common carp's rise as a significant and sought-after fish species for culture, as opposed to Indian major carps, which only breed during the monsoon season.

Induced breeding, sometimes referred to as the hypophysation technique allows fish that are unable to reproduce in a stagnant body of water to do so by injecting artificial hormones (Bhuiyan *et al*., 2007) [4]. WOVA-FH emerges as one of the effective synthetic hormones for the induced breeding practices of several varieties of fish species. It is readily available commercially. In aquaculture, WOVA-FH, a synthetic hormone formulation based on GnRH, is frequently used to trigger gonadal maturation and spawning in crustaceans and finfish. Improved rates of fertilization and hatching, better growing conditions, and enhanced

protection of larvae from predators and adverse environmental conditions are all advantages of induced breeding, which entails hormone treatment and artificial incubation of fertilized eggs. Therefore, studies on hormonal dosage may help determine the optimal hormone concentration that could increase larval production.

Materials and Methods

Experimental Site: This investigation was conducted during the period from December, 2024 to June, 2025 in the cement tanks at the Instructional Fish Farm, College of Fisheries, Berhampur, Odisha, India. The breeding experiments involved the use of both the hapa system and the stripping method to induce spawning in Koi carp (*Cyprinus carpio*).

Collection and Maintenance: To perform the breeding experiments, healthy, disease-free advanced fingerlings and broodstock of Koi carp were sourced from the State Fish Farm Kaushalyaganga, Odisha during the first week of December 2024. The fish were then transported to the cement tanks at the Instructional Fish Farm and maintained under standard broodstock management protocols. Before introducing the fish, important water quality parameters of the tanks were assessed, and the fish were acclimatized accordingly. Disinfection measures were strictly followed prior to stocking.

Initially, fish were fed twice daily with commercial ornamental feed. Later, their diet was supplemented with a 1:1 mixture of rice bran and groundnut oil cake enriched with vitamins and minerals. During the first four months, fish were fed at 4% of total biomass per day, divided into two feedings. Thereafter, the feeding rate was lowered to 2% to avoid fat accumulation around the abdominal region near the vent. Periodic application of manure was practiced to encourage the growth of phytoplankton and zooplankton, providing a natural food source. Feeding was completely halted in May 2025, ahead of the breeding trials. The breeding assessment using WOVA-FH hormone was conducted in June, 2025 to evaluate its influence on induced breeding outcomes in Koi carp.

Identification of Male and Female Brood Fish: Healthy and mature brooders, particularly those older than one year, were selected. Female brooders were identified by their distended, soft, and elastic abdomen, especially noticeable in the lower region where the outline of the ovaries was visible on both sides. A pinkish vent also indicated readiness for spawning. Additionally, females had pointed and soft pectoral fins, distinguishing them from males. Male brooders were selected based on traits such as a flattened abdomen and compressed body, with milt released upon gentle abdominal pressure. Males also displayed larger, rougher pectoral fins compared to females, which further aided in sex differentiation for induced breeding.

Formation of Breeding Sets: Breeding trials were initiated in June, 2025 to evaluate hormone effects. Once the sexes were determined, breeding sets consisting of mature male and female fish of roughly equal size and weight were prepared and placed in breeding hapas, which were installed in cement tanks at the Instructional Fish Farm. The breeding hapas, measuring 2 x 1 x 1 meters, were constructed from muslin cloth with a cover and were used for spawning activities. Multiple hapas were arranged in cement tanks according to specific treatments and replicates. Prior to breeding, all relevant water quality parameters were carefully measured and monitored.

Inducing Hormone: WOVA-FH was the hormone used for induction in this study. This cost-effective, newly developed hormone is designed for the artificial breeding of fish and consists of a combination of salmon gonadotropin-releasing hormone analogue (GnRH) and a dopamine antagonist, typically domperidone. It stimulates the hypothalamic-pituitary-gonadal (HPG) axis effectively through a single intramuscular injection. Each ml of WOVA-FH contains 20 µg of salmon GnRH analogue and 10 mg of domperidone, produced by M/s. USV Limited, Mumbai.

Experimental Design: Breeding sets were arranged with three treatment groups and one control group, each having three replications to ensure accurate result computation. The trials were conducted in cement cisterns with a volume of 21 m^3 (dimensions: 7.0 × 3.0 × 1.0 m). For each set, the synthetic hormone WOVA-FH was administered in calculated doses. The control group was not given any hormone to allow for natural or spontaneous spawning.

Control/ Treatments	Female	Male
T_0	No hormone	No hormone
T_1	0.2 ml/kg of body weight	0.1 ml/kg of body weight
T_2	0.3 ml/kg of body weight	0.2 ml/kg of body weight
T_3	0.4 ml/kg of body weight	0.3 ml/kg of body weight

Collection of Broodstock for Hormone Administration: For the induced breeding trials, mature brood fish were collected from cement tanks and separated by sex, with males and females placed in separate hapas. Each brooder was accurately weighed to determine individual body weight, which was essential for calculating the correct hormone dosage based on sex.

Hormone Administration: Prior to hormone application, WOVA-FH was stored under proper conditions. Brooders were segregated by sex and weighed to obtain their initial body weights. The breeding sets were prepared in the early morning. The hormone was administered intramuscularly using an insulin syringe to ensure precise dosing. Female brooders received WOVA-FH in doses varied from 0.2 to 0.4 ml/kg body weight, while males received 0.1 to 0.3 ml/kg. The goal was to identify the optimal dose for maximum breeding efficiency. After injection, brooders were transferred to the breeding hapa for spawning.

Response Time: The Response time or latency period refers to the interval between hormone administration and the initial signs of spawning. This period was recorded in hours.

Egg Count Estimation: To estimate the total number of eggs released, a 3 ml sample of water-hardened eggs was collected and counted. This process was repeated three times with different sub-samples. Excess water was removed using a strainer. The average number of eggs per sample was calculated, scaled up to one litre, and multiplied by the total volume of water-hardened eggs to estimate the total egg count.

Fertilization Rate: Fertilization percentage was determined for each treatment group by examining water-hardened eggs. Three sub-samples were collected per breeding set and the number of fertilized eggs (n) out of the total eggs (N) in each sample was counted to calculate the fertilization rate. The fertilization percentage was then calculated by using the standard method.

Hatching Percentage: Hatching percentage is defined as the proportion of fertilized eggs that successfully hatched into spawn. To determine this, the number of hatchlings in 1 ml of sample was counted and then extrapolated to calculate the total number of hatchlings from the entire volume collected. This value was used to estimate the overall hatching rate.

Analysis of Water Quality Parameters: Water quality was regularly assessed during the stocking, rearing, and breeding phases. To ensure optimal conditions, only filtered water, free from contaminants, was used throughout the experiment. The important water quality parameters measured included water temperature, pH, dissolved oxygen, alkalinity, and hardness. Overall, the water quality parameters remained within acceptable limits, showing minimal fluctuations during the course of the investigation (Table-1).

Statistical Analysis: In the present experiment, the treatments are not homogeneous so the statistical tool "Randomized Complete Block Design" was used to know the significant difference between the treatments.

Results and Discussion

In the present study, details of breeding response of *Cyprinus carpio* to WOVA-FH are given in Table-1. Female Koi carp (*Cyprinus carpio*) treated with different hormonal doses exhibited variations in their spawning response time. When female brooders were injected with WOVA-FH at 0.2 ml/kg body weight (T_1), spawning occurred after approximately 9.5 hours. However, when higher doses of 0.3 ml/kg (T_2) and 0.4 ml/kg (T_3) were used, the spawning response time decreased, occurring within 8 to 9 hours. Ghosh *et al*. (2012) [6] observed spawning behaviour 1-8 hours after hormone treatment. Mahadevi *et al*. (2018) [8] found a 5-9 hours latency period for Koi carp treated with 0.7 ml/kg and 1.4 ml/kg doses for males and females respectively. Basudha *et al*. (2017) [2] reported a latency period of 7-10 hours in *Bangana dero* at 26 °C using WOVA-FH. Sit *et al*. (2023) [12] recorded 15-17 hours latency with 1.5 ml/kg Ovatide in *Puntius chola*. Raze *et al*. (2017) [10] reported latency of 8-12 hours in Vietnamese Koi (*Anabas testudineus*) treated with 0.20 ml/kg Flash for females and 0.13 ml/kg for males. Similarly, Roy *et al*. (2011) [11] reported 8-12 hours latency in Thai Koi treated with pituitary gland hormone.

The number of eggs laid by female Koi carp under different doses of WOVA-FH is presented in Table-2. The study revealed significant differences (p<0.05) in fecundity among treatments. Brooders treated with 0.3 ml/kg body weight (T_2) showed 100% spawning success and released 11814.0±1220.7 eggs. In comparison, brooders given 0.2 ml/kg (T_1) released 7870.0±1464.9eggs, while those treated with 0.4 ml/kg (T_3) laid 9645.0±1019.4 eggs. Other studies have reported similar findings. Ghosh *et al*. (2012) [6] found fecundity ranging from 15000±2000 to 70000±10000 eggs/kg body weight in summer, and 1500±200 to 7000±1000 eggs/kg in winter using 0.7 ml/kg Ovaprim. Amin *et al*. (2015) [1] achieved 100% ovulation in Vietnamese Koi with 2 mg/kg for males and 7 mg/kg for females using pituitary extract alone or with Ovaprim. Begum *et al*. (2022) [3] recorded 17000-18000 eggs in Koi carp and 4500-5000 eggs in goldfish using Ovaprim C (0.5 ml/kg for females and 0.1 ml/kg for males). Mahadevi *et al*. (2018) [8] recorded 1185-1205 eggs per female in telescopic eye goldfish using 0.7 ml/kg for males and 1.4 ml/kg for females WOVA-FH. Basudha *et al*. (2017) [2] reported 17790 eggs in *Bangana dero* using 0.5 ml/kg WOVA-FH, while Dey *et al*. (2016) observed 1440-7050 eggs in *Barilius barila* using 0.5 ml/kg WOVA-FH. Mahmud *et al*. (2012) observed 70% fecundity in comet goldfish using 0.7 ml/kg Ovaprim. Sit *et al*. (2023) [12] reported 106308±3075 eggs in *Puntius chola* with 1.5 ml/kg Ovatide. Roy *et al*. (2011) [11] recorded 28762 eggs per 100 g female in Vietnamese Koi using varying 1.1-1.6 mg PG/kg doses for females and 0.6 mg/kg for males.

In the present investigation, 100% spawning success was recorded when WOVA-FH was administered at 0.3 ml/kg body weight (T_2) to Koi carp (*Cyprinus carpio*). The fertilization and hatching percentages were highest at this dose. These results confirm that the optimal spawning response in Koi carp was achieved at 0.3 ml/kg WOVA-FH. The required hormone dosage for successful spawning is influenced by several factors such as broodstock condition, maturity stage, season, and climate. Variations in these parameters necessitate adjustments in hormone dosage, highlighting the need for further dose standardization under diverse agro-climatic conditions to optimize fertilization and hatching rates for efficient seed production. Ghosh *et al*. (2012) [6] observed maximum fertilization and hatching rates using 1ml/kg Ovaprim, achieving 61.82% and 55% in summer, and 39.23% and 31.67% in winter. Amin *et al*. (2015) [1] reported 70.22±3.40% fertilization and 51.22±3.96% hatching in Vietnamese Koi with 2 mg PG/kg (female) and 7 mg PG/kg (male). Begum *et al*. (2022) [3] documented 74% fertilization, 57% hatching, and 42.5% survival in Koi carp and goldfish using Ovaprim C (0.5 ml/kg females, 0.1 ml/kg males). Mahadevi *et al*. (2018) [8] observed 74% fertilization and 82% hatching in telescopic eye goldfish with WOVA-FH doses of 0.7 ml/kg (male) and 1.4 ml/kg (female). Basudha *et al*. (2017) [2] found 87.33% fertilization and 87.73% hatching in *Bangana dero* treated with 0.5 ml/kg WOVA-FH. Sit *et al*. (2023) [12] induced *Puntius chola* using 1.5 ml/kg Ovatide, achieving 79.28±0.59% fertilization and 78.03±0.49% hatching with 106308±3075 eggs. Raze *et al*. (2017) [10] recorded 79.89% fertilization and 66.22% hatching in Vietnamese Koi with 0.2 ml/kg Flash. Roy *et al*. (2011) [11] noted 50-59% fertilization and 58.67% hatching in Thai Koi using 1.0-1.6 mg PG/100 g (female) and 0.6 mg PG/100 g (male).

Table 1: Water quality parameters (Mean ± SD) of cement tanks during the experimental period

Water quality parameters	Tank-1	Tank-2	Tank-3
Water temperature (°C)	26.60±0.26	26.47±0.40	26.43±0.50
pH	8.1±0.35	7.91±0.21	7.75±0.31
Dissolved oxygen(ppm)	7.10±0.20	7.40±0.26	7.20±0.26
Alkalinity(ppm)	184.00±4.00	185.33±2.52	185.33±3.79
Hardness(ppm)	164.67±5.03	167.00±4.36	168.67±5.69

Table 2: Details of breeding response (Mean ± SD) of *Cyprinus carpio* to WOVA-FH

Treatments	Size of female(g)	Size of male (g)	Latency period(h)	No. of egg laid by female	Fertilisation (%)	Hatching (%)
T_0	420.0±36.0	400.0±30.00	0	0	0	0
T_1	428.3±79.7	407.0±50.2	9.5	7870.0±1464.9	65.0±0.7	59.2±0.3
T_2	496.6±51.3	451.6±57.5	8.0	11814.0±1220.7	71.5±0.5	64.8±0.1
T_3	480.0±50.7	448.3±53.4	9.0	9645.0±1019.4	68.3±0.7	62.2±0.4

Conclusion

Administering WOVA-FH at a dosage of 0.3 ml/kg body weight yielded complete breeding success, including the highest egg output along with improved fertilization and hatching rates. Therefore, it is suggested that induced breeding of Koi carp be carried out in the month of June with WOVA-FH administered at 0.3 ml/kg body weight.

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