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Comparative Efficacy of Synthetic Hormones, Wova-FH and Ovatide on Breeding Performance of a *Cypirinus carpio* Var. communis in the Captive Conditions of Kashmir

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Abstract

This study assessed the efficacy of two GnRH-based synthetic hormones, Ovatide and Wova-FH, in the induced breeding of common carp (*Cyprinus carpio*) under the environmental conditions of Kashmir. A Completely Randomized Design (CRD) with three treatments for each hormone and a control group, performed in triplicates, was employed. The latency period varied significantly, with Ovatide showing superior performance. The shortest latency period (38±0.38 hours) was recorded at the highest dosage of Ovatide (0.7 mL/kg), compared to 44±0.57 hours for Wova-FH (0.5 mL/kg) and 55.5±0.5 hours for the control. Ovatide also achieved the highest ovulation rate (88.8%), significantly outperforming Wova-FH (55.5%) and the control (22.2%). Fertilization rates were significantly higher in Ovatide-treated groups, ranging from 85.25%±0.98 to 86.20%±1.12, compared to 83.42%±0.69 to 84.04%±1.15 in Wova-FH groups. However, hatching rates ranged from 67.80%±0.66 to 71.08%±1.37 across all treatments with no significant differences ($p>0.05$) between hormone-treated and the control groups, indicating that hatching success is independent of hormone type and dosage.

This study provides evidence supporting the use of Ovatide as a more effective inducing agent for carp breeding in Kashmir for sustainable aquaculture development in the region.

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Introduction

With the global population projected to reach 9.7 billion by 2050, (United Nations, 2019), the demand for nutrient-rich food will rise by 25-70% (Hunter, Smith, Schipanski, Atwood, & Mortensen, 2017). Increased fish production is crucial to meet this need due to growing living standards and shifting diets (Bene et al., 2015; Hua et al., 2019). To meet the nutritional needs of growing population, aquaculture must scale production rapidly (FAO, 2018). Induced breeding techniques offer an effective way for consistent supply of seeds from captive stocks without affecting the capture fishery. The common carp (*Cyprinus carpio*) is commercially important carp which account for about 8% of global finfish aquaculture (FAO, 2018). Introduced in 1956, the common carp have adapted well to Kashmir's water bodies, notably contributing 69.13% of Dal Lake's fish catch by weight, with *C. c.* communis making up 59.2% of the total, highlighting its commercial importance. To boost carp production locally, an ample supply of seed is required and primary inducing agents like Ovatide™ and Wova-FH™ are widely used in the region; however, lack of data on their comparative efficacy of these inducing agents in Kashmir presents a research gap. Studies from other regions, such as Jhajhria (2002); Saud, Hazarika, Verma, and Goswami (2013), have shown Ovatide to be effective, requiring lower dosages and resulting in high spawn production. However, there are no comparative studies from Kashmir, that have suggested the most suitable dose of these inducing agents.

Therefore, this study aims to assess the efficacy of Ovatide and Wova-FH in induced spawning of common carp in Kashmir, and evaluating the dosages proven effective in prior research to ensure high fertilization rates and minimal stress. The primary reason for selecting Ovatide and Wova-FH from the range of commercially available synthetic hormones was their widespread use in the aquaculture industry in Kashmir. Ovatide primarily contained a synthetic analog of Gonadotropin-Releasing Hormone (GnRH) combined with a dopamine antagonist, pimozide. Wova-FH contained a synthetic analog of GnRH and a dopamine antagonist, domperidone. By documenting the performance of these agents in local conditions, this research will enable breeders to make informed choices, optimizing breeding practices for increased production and help reduce cost involved in induced breeding of the fish in Kashmir.

Materials and Methods

Mature fish (n=126) were collected from Dal Lake, Srinagar district, Kashmir, and stocked in brood ponds. For the experiments, fish were randomly selected from brood ponds based on their external appearance. Male fish were identified by their flat abdomen and rough pectoral fins, while females were distinguished by their swollen abdomen and soft pectoral fins (Table 1). Males with freely oozing milt and ripe females were paired in a 1:1 ratio for spawning. The fish were segregated by sex using external morphological characteristics and then transferred to a conditioning tank for acclimatization for 6–7 days.

For hormone administration, mature fish (females: 364.6±10.5 g; males: 320.7±16.0 g), approximately 2 years old, were carefully removed from the conditioning tank using a scoop net and placed on a sponge platform. The fish were wrapped in soft, moist cloth, and a hormone injection was administered near the base of the pectoral fin using a graduated syringe with a 2 ml capacity. The fish were then monitored at 6-hour intervals to record their latency response and accurately determine the timing of spawning. Two synthetic GnRH-based hormones, Ovatide (Suyog Pharmaceuticals Pvt. Ltd., Tarapur, Thane) and Wova-FH (Biostadt India Ltd., Worli, Mumbai), were used in the experiment.

The study followed a Completely Randomized Design (CRD) with three treatments of each hormone injection, along with a control, performed in triplicates. Each treatment group consisted of 18 fish (9 males and 9 females) randomly assigned to groups, ensuring unbiased distribution. This randomization minimized the influence of pre-existing differences among the fish, and to ensure that the variations between groups are solely as a result of the different treatments. The doses of Ovatide (OT) and Wova-FH (WFH) used for males and females in each treatment are summarized in Table 3.

Table 1. Selection criteria for the mature brood fish

Male	Female
Abdomen normal, not bulky like female	Abdomen bulging, elastic and soft
Pectoral fins rough	Pectoral fins slimy
Usually have tubercles on the head and gill plates during breeding season	Tubercles absent on head and gill plates
Gently pressing on abdomen, male releases milt.	Gently pressing on abdomen, eggs ooze out.

Latency period- It is the period between final injection of inducing hormone i.e. 0.00 hr and the time of spawning in experimental fishes, expressed in hours.

Spawning rate was calculated by using the formula

$$\text{Spawning rate} = \frac{\text{Number of ovulated females}}{\text{Number of injected females}} \times 100$$

Estimation of fecundity

a) Spawning fecundity: The total number of eggs stripped (spawned) was estimated by counting the egg in 1 g of stripped eggs and then multiplied with total weight of eggs (Sahoo, Giri, & Sahu, 2005).

b) Relative fecundity: This was calculated as per the formula given below by (Kahkesh, Feshalami, Amiri, & Nickpey, 2010)

$$\text{Relative fecundity} = \frac{\text{Absolute fecundity}}{\text{Weight of fish}}$$

A petri dish containing acetone was used to hold the eggs for examination under a microscope. A soft, thin brush was carefully used to count the fertilized eggs one hour after fertilization. Fertilized and unfertilized eggs were distinguished based on the

colour of the eggshell. Unfertilized eggs were characterized by their opaque white appearance, while fertilized eggs appeared transparent. The fertilization rate was calculated using the formula provided by Adebayo and Popoola (2008):

$$\text{Fertilization rate} = \frac{\text{No. of fertilized eggs in the sample}}{\text{Total number of eggs in the sample}} \times 100$$

After hatching was completed, the hatchlings were collected in a pot or dish and counted visually using a magnifying glass. The number of eggs hatched was recorded. The hatching rate was determined using the formula described by Haniffa and Sridhar (2002):

$$\text{Hatching rate (\%)} = \frac{\text{Number of eggs hatched}}{\text{Total number of fertilized eggs}} \times 100$$

Water quality parameters such as temperature, pH, and DO were recorded during the experiment. DO was measured based on the standard methods given in APHA (2004), and pH was determined using a digital pH meter (pH ep® Hanna Instruments, Italy).

The results are presented as mean ± standard error (SE). The Kruskal-Wallis one-way ANOVA ($p<0.05$) was used to compare differences in medians between treatments. Dunn's post hoc test with Bonferroni correction was applied to identify significant pairwise differences.

Results and Discussion

The experimental fish were bred in captivity during May 2020, which coincided with the peak spawning season. Water quality parameters were regularly monitored throughout the experiment to maintain optimal conditions for induced spawning (Table 2). The water temperature ranged between 10-15°C, with an average of 12.7±1.85°C. Dissolved oxygen levels varied from 7.8 to 8.4 ppm, with a mean value of 8.05±0.26 mg/L, and the pH remained stable between 7.3 and 7.8, with a mean of 7.5±0.11.

The latency period, defined as the time between hormone injection and ovulation, showed significant differences between the Wova-FH and Ovatide treatment groups (Table 5, Fig. 1). In the Wova-FH-treated groups, latency ranged from 44±0.57 hours (T3) to 48±0.57 hours (T1). For Ovatide-treated fish, the latency period ranged from 38±0.38 hours (T6) to 42±0.25 hours (T4). The control group recorded the longest latency period of 55.5±0.5 hours. The results indicated that higher doses of both hormones significantly reduced the latency period, with Ovatide demonstrating superior efficacy. The shortest latency period, 38 hours, was observed in the group administered the highest Ovatide dosage (0.7 mL/kg) (Table 4).

Table 2. Water quality parameters in the breeding tanks during the experimental period

Parameter	Range	Mean±SD
Water temperature	10-15 ºC	12.7±1.85
Ph	7.3-7.8	7.5±0.17
Dissolved oxygen(mg/l)	7.8-8.4	8.05±0.26

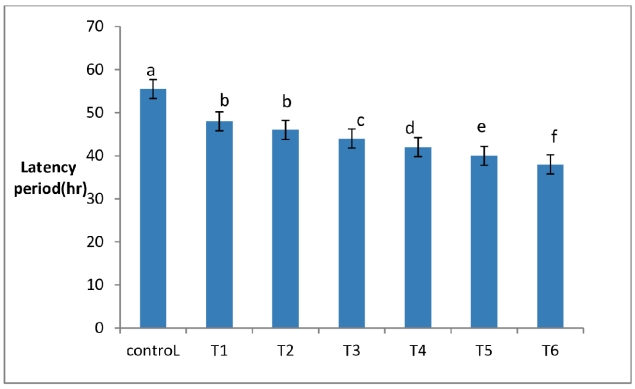

Fig. 1. Effect of different dosages of Wova-FH and Ovatide on the latency period of *C. carpio* var. communis. The means with same character are not statistically significant ($p>0.05$)

These findings agree with the results of Zadmajid and Butts (2018), who reported faster ovulatory responses at higher hormone doses. However, the latency periods recorded in this study were longer than those reported for Common Carp by Yaron (1995); Dorafshan, Mostafavi, & Amiri, (2003), likely due to the lower water temperatures (10–15°C) noted in this experiment, as suggested by Heyrati, Mostafavi, Toloee, and Dorafshan (2007). The prolonged latency period observed in the control group further highlights the effectiveness of hormone administration in accelerating the spawning process.

The ovulation rate followed a similar trend, with Ovatide outperforming Wova-FH. The highest ovulation rate (88.8%) was recorded in the T5 group (Ovatide at 0.5 mL/kg), while Wova-FH showed a peak ovulation rate of 55.5% in the T2 group (Table 4). The control group exhibited the lowest ovulation rate at 22.22%. These results highlight the effectiveness of Ovatide in inducing ovulation at both low and high doses. The increased ovulation rate in Ovatide-treated groups is consistent with findings by Rahman et al. (2013), who reported higher ovulation rates in fish treated with Ovaprim compared to traditional hormonal agents like PGE. The spawning rate was lower in fish administered 0.3 and 0.7 mL/kg body weight of Ovatide and Wova-FH, compared to those given 0.5 mL/kg (T2) of both hormones. Ghanemi and Khodadadi (2017) also observed that using Ovaprim in both low and high doses did not induce reproduction in *Shirbot*. At low doses, reproductive hormones may not support all maturation events, such as germinal vesicle migration, breakdown, and subsequent ovulation (Rottmann, Shireman, & Chapman, 1991).

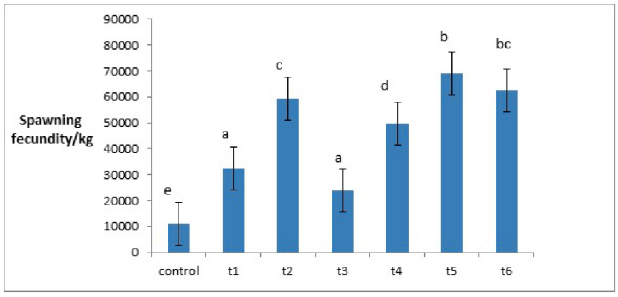

Fig. 2. Effect of different dosages of Wova-FH and Ovatide on the spawning fecundity of *C. carpio* var. communis. The means with same character are not statistically significant (p>0.05).

Fecundity, expressed as the total number of eggs released per kg body weight, significantly varied among the treated groups (Table 5, Fig 2). In Wova-FH-treated fish, fecundity ranged from 23,968±1,097 (T3) to 59,220.33±2,316 (T2), while in Ovatide-treated groups, it ranged from 49,577±2,019 (T4) to 68,931±3,064 (T5) (Table 4). The control group showed the lowest fecundity at 10,993±467. Relative fecundity followed a similar pattern, with Wova-FH treatments showing a range from 23.96±1.09 to 59.21±2.316, and Ovatide treatments ranging from 49.576±2.018 to 68.92±3.06 eggs per kg body weight. Both hormones induced significantly higher fecundity than the control ($p<0.01$). These findings are consistent with studies by Das et al. (2016); Behera, Das, Singh, and Sahu, (2007), who reported higher fecundity in hormone-treated fish compared to controls. The highest fecundity was observed in the Ovatide group T5, indicating that Ovatide not only shortened latency periods but also improved fecundity. Similar results were reported by Behera et al. (2007); Audu and Ofojekwu (2010); Fernandez-Palacios et al. (2014) in other species.

Fertilization rates varied significantly across the treatments, with the Wova-FH groups showing rates between 83.42%±0.69 (T2) and 84.04%±1.15 (T1), while the Ovatide groups exhibited slightly higher rates, ranging from 85.25%±0.98 (T6) to 86.20%±1.12 (T5), both showing significant differences (Table 5, Fig. 3). In contrast, the control group recorded a fertilization rate of 82.05%±1.43.

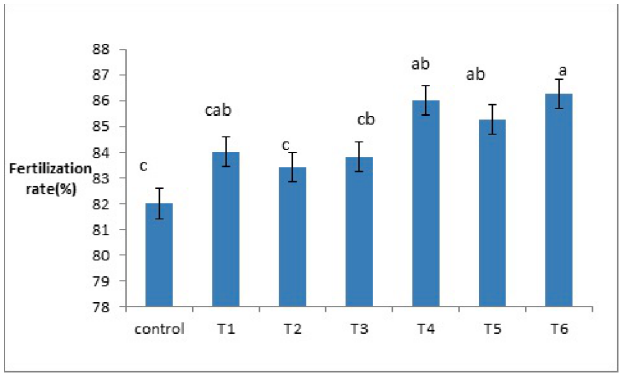

Fig. 3. Effect of different dosages of Wova-FH and Ovatide on the fertilization rate (+/- SE o) of *C. carpio* var. communis. The means with same character are not statistically significant (p>0.05).

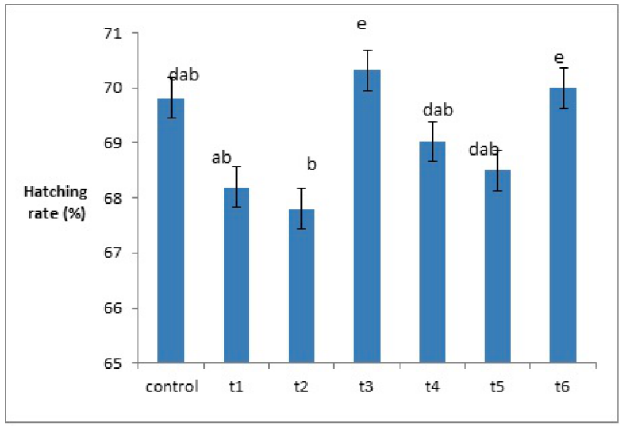

Fig. 4. Effect of different dosages of Wova-FH and Ovatide on the hatching rate (SE) of *C. carpio* var. communis. The means with same character are not statistically significant (p>0.05).

Table 3. Treatments based on type of hormone and dose applied

Treatments	Dosage ♀	Dosage ♂
Control	No inducement	No inducement
T_1	0.3ml kg^{-1} WFH	0.1 ml kg^{-1} WFH
T_2	0.5 ml kg^{-1} WFH	0.2 ml kg^{-1} WFH
T_3	0.7 ml kg^{-1} WFH	0.3 ml kg^{-1} WFH
T_4	0.3 ml kg^{-1} OT	0.1 ml kg^{-1}OT
T_5	0.5 ml kg^{-1} OT	0.2 ml kg^{-1}OT
T_6	0.7 ml kg^{-1} OT	0.3 ml kg^{-1}OT

OT= Ovatide; WFH = Wova- FH.

Hatching rates across all treatments ranged from 67.80%±0.66 to 71.08%±1.37. There was no significant difference observed in hatching rates between the hormone-treated groups and the control ($p>0.05$) (Table 5, Fig. 4). These findings suggest that while hormone treatments can enhance ovulation and fertilization rates, they do not necessarily improve hatching success.

The results are consistent with studies by Yeasmin Rahman, Haq, Hossain, and Rahman, (2013); Dash, Pradhan, and Gupta (2018), which concluded that egg viability post-ovulation is not influenced by hormone dosage or type. Additionally, no significant effect of hormone type on hatching rate was observed, further supporting the notion that egg viability is independent of hormone type and dosage. These finding contrasts with Behera et al. (2007) and other studies, which reported dose-dependent hatching rates. However, the current results align with those of Ghanemi and Khodadadi (2017), who found no significant differences in hatching rates across various Ovaprim doses in *Shirbot*.

This study evaluated the comparative efficacy of Ovatide and Wova-FH in the induced breeding of common carp under the unique environmental conditions of Kashmir. The findings highlight the

Table 4. Results of the breeding performance of *C. carpio* var. *communis* with different treatment groups of WOVA-FH and Ovatide

Breeding parameters	Control	T_1 (0.3 ml/kg) Wova FH	T_2 (0.5 ml/kg) Wova FH	T_3 (0.7 ml/kg Wova FH	T4 (0.3 ml/kg) Ovatide	T5 (0.5 ml/kg) Ovatide	T6 (0.7 ml/kg) Ovatide	P- value
No.of females (N)	9	9	9	9	9	9	9	
No.of males (N)	9	9	9	9	9	9	9	
Body weight of female	364.58± 10.51	375.64± 13.04	345.12± 12.04	374.66± 11.69	340.68± 12.94	345.611± 10.64	335.611± 11.47	
Body weight of males	320.72± 16.89	325.66± 17.78	303.41± 22.36	303.49± 25.37	308.57± 18.57	314.296± 23.1	338.38± 13.41	
No. of females strippable	2	3	5	3	6	8	6	
Ovulation rate (%)	22.22%	33.33%	55.55%	33.33%	66.66%	88.88%	66.66%	
Latency period (hr)	55.5± 0.5	48± 0.57	46± 0.54	44± 0.57	42± 0.25	40± 0.32	38± 0.38	<0.01
Spawning fecundity/kg	10993± 467	32301± 1926	59220± 2316	23968± 1097	49577± 2019	68931± 0.7	56747± 2937	<0.01
Relative fecundity	10.9± 0.467	32.3± 1.9	59.2± 2.3	23.96± 1.0	49.5± 2.01	68.9± 3.06	56.7± 2.9	<0.01
Fertilization rate (%)	82.0± 1.43	84± 1.15	83.424± 0.69	83.86± 1.01	86.14± 1.35	85.25± 0.9	86.25± 1.28	<0.05
Hatching rate (%)	69.82± 0.66	68.2± 1.28	67.8± 0.663	70.32± 0.59	69.025± 1.3	68.56± 0.52	70.0± 1.37	>0.05

superior performance of Ovatide over Wova-FH, as evidenced by significantly shorter latency periods, higher ovulation rates, and greater spawning fecundity across all dosage levels. The highest efficacy was observed with Ovatide at 0.7 mL/kg, which resulted in the shortest latency period (38 hours), the highest ovulation rate (88.8%), and the greatest fecundity. These results emphasize the effectiveness of Ovatide in ensuring efficient induced spawning in common carp, making it a suitable choice for aquaculture in the region.

While hormone treatments significantly improved fertilization rates, hatching success remained unaffected; indicating that egg viability post-ovulation is independent of hormone type and dosage. These findings suggests that while hormones enhance spawning and fertilization processes, they may not influence subsequent hatching success. However, the results also contrast with studies reporting dose-dependent hatching rates, highlighting the need for further research to explore factors affecting egg viability and hatching.

The study also underscores the importance of optimizing hormone dosages and understanding regional environmental factors, such as lower water temperatures, which may influence spawning outcomes. By documenting the performance of Ovatide and Wova-FH in local conditions, this research provides a foundation for informed decision-making by breeders and policymakers to enhance carp production in Kashmir.

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