



## Single-Dose Ovaprim-C Induced Breeding in Selective Carp Species at Ratta Kulachi Fish Hatchery, Dera Ismail Khan

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### Abstract

The current study examined the effects of intramuscular ovaprim-C injection on the number of eggs/kg, fertilization rate, and hatching percentage of economically significant and rapidly growing food fishes, specifically grass carp (*Ctenopharyngodon Idella*), rohu (*Labeo rohita*), and mohri (*Cirrhinus Mrigala*), at Fish Hatchery, Ratta Kulachi, Dera Ismail Khan, Pakistan, from May to June 2024. For this study, which took place from May 18 to June 28, 2024, 66 fish in total—24 grass carps (16 males and 8 females in a 2:1 ratio), 18 Rohu (12 males and 6 females in a 2:1 ratio), and 24 mohri (16 males and 8 females in a 2:1 ratio)—were used. According to calculations, the combined weight of the female grass carps, Rohu and Mohri, was 15.8, 10.1, and 16.8 kg, respectively. After



receiving a single injection of ovaprim-C (luteinizing hormone releasing hormone analogue) at a rate of 0.5 ml/kg for females and 0.25 ml/kg for males, fish were effectively spawned. The fish were taken straight to the Chinese Hatchery's breeding ponds after being dosed. After nine to eleven hours of dosage, spawning occurred. The twitching movement began ten to twelve hours after spawning. After 20–24 hours of fertilisation at 28–30°C, hatching took place. The average number of eggs per kilogramme of body weight of grass carp, Rohu, and Mohri was found to be 91866, 82370, and 88154, respectively. Rohu, Mohri, and grass carp fertilization rates were found to be 78.4%, 73, and 70%, respectively. Grass carp, Rohu, and Mohri hatching percentages were 75.3%, 76.2, and 67.2%, respectively.

**Keywords:** Ovaprim-c, Induce breeding, *Ctenopharyngodon Idella*, *Labeo rohita*, *Cirrhinus Mrigala*

## **I. INTRODUCTION**

Carp cultivation is growing quickly in Pakistan, however one of the main obstacles to this industry's growth is the lack of high-quality fish seed. The rapid progress of carp culture has been substantially aided by the significant advancement made by Chaudhry and Alikunki [1] in the induced breeding of Indian big carps using pituitary extract. Even though the hypophysation technique is successfully used, there are some issues that have kept fish producers from using it broadly. In 1964, Pakistan began importing grass carp (*Ctenopharyngodon Idella*) from China. Temperatures between 20 and 30 °C (68 and 86 °F) are ideal for grass carp to enter reproductive state and spawn [2]. The grass carp (*C. idella*) reaches maturity at the age of two years and above; among cultivable carps, males often reach maturity a little earlier than females [3]. It has been noted that a certain proportion of *Labeo rohita* males and females reach sexual maturity in just one year, at the end of their second year of life in ponds [4]. According to reports, *Mrigala* reached its first maturity at the end of the year [5] and at the age of two [6, 4]. Fish

reproduction occurs on a periodic basis and is governed by both internal (endogenous) and external (environmental) regulatory mechanisms. There are two primary methods of induced reproduction in fish. The first involves altering the culture environment to replicate key elements of the fish's natural spawning habitat. The second tactic involves giving brood fish injections or food that contains one or more naturally occurring reproductive hormones or their synthetic analogues. However, two lines of hormone-induced spawning appear to be the most effective for successful breeding at the lowest cost, according to recent studies and commercial aquaculture methods. i) GnRH analogue (LHRH analogue) can be injected or administered orally with a dopamine antagonist; ii) pure gonadotropin (such as human chorionic gonadotropin, or HCG) can be injected either by itself or in combination with common carp pituitary extract to increase its potency [7]. The product Ovaprim contains 20 ug/ml of salmon gonadotropin-releasing hormone analogue (sGnRH;D-Arg6,Pro9,Net) and 10 mg/ml of domperidone, a dopamine antagonist. Ovaprim is mostly administered intramuscularly or intraperitoneally to induce ovulation and spermiation in fish. Bruising, redness, sores, ulceration, darkened body coloration (hyperpigmentation), and loss of body color (hypopigmentation) at the injection site are among the reported adverse effects. Overall, post-spawning death occurred (about 1.3%).[8]. When ovaprim was supplied to all six Government hatcheries in Punjab in 1991, it was employed for the first time in Pakistan. It was used experimentally in 1991. In 1992, Ovaprim was used to raise carps in all of the Punjab Government's hatcheries [9]. This study sought to determine how the Grass carp (*C. idella*), Rohu (*L. rohita*), and Mohri (*C. mrigala*) were induced to spawn, fertilize, and hatch under culture conditions at Fish Hatchery, Ratta Kulachi, Dera Ismail Khan

## **II. Materials and Methods**

### **II.I Study Area**

To determine the relative fecundity—that is, the number of eggs per kilogram of body weight and the rate of fertilization—by artificial spawning in grass carp (*C. idella*), rohu (*L. rohita*), and mohri (*C. mrigala*), the experiment was carried out at Fish Hatchery, Ratta kulachi, D I Khan. This study, which took place from May 18 to June 28, 2024, involved 66 fish in total: 24 grass carps (16 males and 8 females in a 2:1 ratio), 18 Rohu (12 males and 6 females in a 2:1 ratio), and 24 mohri (16 males and 8 females in a 2:1 ratio). According to calculations, the combined weight of the female grass carps, Rohu and Mohri, was 15.8, 10.1, and 16.8 kg, respectively. Hypodermic A 2 ml syringe with 0.1 ml increments and a number 22 needle was utilized. Using the best water quality parameters and a maximum water capacity of 5000 liters, tests were carried out in a circular spawning tank that was 9 feet in diameter and 3 feet deep. A day before the trial started, the fish were acclimated. Because studies by [10] have shown that a single, simultaneous injection of ovaprim-C is efficacious, it was administered as a single dose.

## **II.II Choosing**

## **Brooders**

For artificial propagation to be successful, male and female brood fish must be of high quality and mature [11]. Fish hatchery Ratta Kulachi D I Khan and a few private fish farms provided the brooders. After being moved into the fish hatchery's cement holding tanks, the fish were put to sleep using a 50 L fibreglass tank that was partly filled with tap water and a 100–120 ppm 2-phenoxyethanol solution. Fish between the ages of 4 and 5 that were healthy, disease-free, and ripe (2–5 kg wt.) were chosen. Rohu and Mohri of both sexes were chosen for their good distinctive qualities, as well as the grass. Secondary traits of Rohu and Mohri grass carp, both sexes, that demonstrated these fishes' full maturity during selection.

**Figure 1: Collection of Brooders**



**Figure 2: Prepare Circular Tank**



### **II.III Care of Brooders**

Before breeding was induced, Rohu and Mohri, grass brooders, were raised in composite culture. All the males and females were separated and placed in separate hatchery holding tanks made of cement prior to the day of breeding. To prevent fish injuries, feeding was halted during this period, and extra caution was used when handling brooders for induced breeding and when deploying drag netting. To reach full maturity, grass carp brooders were given a lot of green fodder in the form of aquatic weeds like Vallisneria, Ceratophyllum, and Hydrilla at a rate that doubled their body weight

**Figure 3: Collected Brooders in Holding Tank**



**Figure 4: Fish Ready for Injection**



### **II.IV Method of injection**

The dosage of ovaprim-C solution was determined by weighing the brood fishes in accordance with [12]. After cleaning the area with an alcohol-soaked cotton swab, male brooders received a single intramuscular injection of 0.25 ml/kg and females 0.5 ml/kg into the dorsolateral region of the fish [13]. A woman's round, soft abdomen and swollen, projecting, pinkish-red vaginal opening, as well as her swollen and reddish anus, are examples of markers of

ripeness. Secondary sexual traits were visible in males. By holding the needle upright and removing the air from the syringe, the necessary amount of ovaprim-C was extracted from the bottle. Brood fish were injected while lying laterally in a cotton bag in water with their upper halves kept above the water's surface. A needle was carefully entered towards the head at a 45° angle to the body's longitudinal axis to a depth of roughly 1.5 cm, and the injection was made slowly at the inner side of the scale-less pectoral fin.

$$\text{Weight of Brood Fish (kg)} \times \text{Ovaprim-C Dosage (mL/kg)} = \text{Injection Volume (mL)}$$

**Figure 5: Dose of Ovaprim Ready to Inject**

**Figure 4: Fish Ready For Injection**



## II.V Handling and Transfer of Brooders

Immediately after administering the hormones, the breeding sets (two males and one female) were released into cemented tanks (capacity 5000 L) containing dechlorinated tap water, flowing at the rate of 12 L/min [13] Acclimatization was maintained to avoid fish mortality.

**Figure 8: Male and Female Brooders in Breeding Tank after Injection**



## **II.VI Breeding and Spawning**

After single dose of injection to both males and females, they were put in the ratio of 2:1 (male: female) in the cemented circular tank especially in the morning time. Then after about 4-5 hours, showering and water jets started to create circular water motion, soon after about 7-8 hours of showering they got excited and showed sexual play. Males started to chase females and forced them to lay eggs. Spawning took place after 9-11hours of injection at temperature 28-30°C which heavily depended on temperature

**Figure 9: Eggs Spawned After Injection**

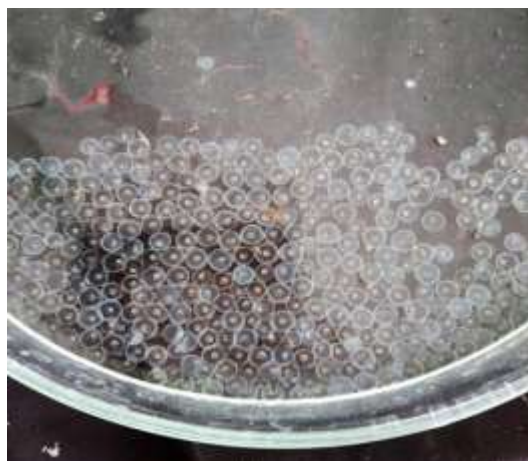


## **II.VII Counting of Eggs**

The volumetric approach was used to determine the quantity of eggs. To determine the number of eggs in each beaker, the number of eggs was counted in triplicate in a beaker and the average of the three estimates was calculated. The total number of eggs was determined by multiplying this by the number of beakers that were measured.

$$\text{Number of Eggs per kg} = \frac{\text{Total Weight of Fish (kg)}}{\text{Total Number of Eggs}}$$

**Figure 10: Calculation of Fertilization Rate by Dry Method**



**Figure 12:**

**Calculation of Number of Hatchling**



## **II.VIII Hatching and Incubation**

Temperature is the primary determinant of hatching and incubation; due to the high temperatures in D I Khan, the hatching duration was observed as 20–24 hours following fertilization at 30-32°C. For four days, the freshly born larvae were kept in the circular incubation pool until the yolk was absorbed. Baby milk powder (lactogen) was introduced in the incubation pool three days later, and on the fifth day, they were moved to nursery ponds for additional rearing.

## **II.XI Calculation of Fertilization Rate, Hatching Rate**

About 100 fish eggs were randomly selected using a small net once they reached the middle gastrula stage, which occurs 16–20 hours after fertilization. The eggs were then placed on a white dish, and any that were visible to the naked eye—such as turbid, white, empty, or rotting eggs—were discarded. By only dipping the eggs in a diluted KMnO<sub>4</sub> in a petri plate, a different technique was used for more precision to separate the fertilized and

unfertilized eggs. While the fertilized eggs stayed the same, the unfertilized eggs developed color. % calculation of the fertilized eggs.

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

When hatching was completed, the hatchlings were collected in a pot (dish) and counted by visual observation using magnifying glass and recorded. The hatching rate was determined by the following formula.

$$\text{Percentage of Hatchlings} = (\text{Total Number of Hatchlings} \div \text{Total Number of Fertilized Eggs}) \times 100$$

**Figure 11: Hatchling Produced**



For **statistical analysis** of data, we were calculating the following:

**Mean( $\mu$ )**

$$\mu = \Sigma X / N$$

where: X = individual values, N = total number of values

**Standard Deviation ( $\sigma$ )**

$$\sigma = \text{sqrt} (\Sigma (X - \mu)^2 / N)$$

**Pearson Correlation Coefficient (r)**

$$r = \Sigma (X - \bar{X}) (Y - \bar{Y}) / \text{sqrt} (\Sigma (X - \bar{X})^2 * \Sigma (Y - \bar{Y})^2)$$

where:

- X, Y are variables (e.g., weight and eggs, temperature and fertilized eggs)
- $\bar{X}$ ,  $\bar{Y}$  are the means of X and Y respectively

### III. RESULTS

We utilized 24 grass carps (16 males and 8 females in a 2:1 ratio), 18 Rohu (12 males and 6 females in a 2:1 ratio), and 24 mohri (16 males and 8 females in a 2:1 ratio) in this investigation, which was conducted between May 18 and June 28, 2024. The female grass carps, Rohu and Mohri, were found to weigh 15 kg, 10.8 kg, and 16.8 kg combined.

**Table 1: Number and Weight of Sample**

Groups	No.of fishes	Males	Females	Weight of males	Weight of females
Grass carp	24	16	8	15.2	15
Rohu	18	12	6	17.9	10.8
Mohri	24	16	8	25.1	16.8

**Table 2: Presenting the mean values and ranges of various water quality parameters for Grass Carp, Rohu, and Mori Carp**

Parameters	Grass Carp (Mean ± SD, Range)	Rohu (Mean ± SD, Range)	Mori Carp (Mean ± SD, Range)
Water Temperature (°C)	27.90±0.55 (27.2-28.6)	27.75±0.60 (27.0-28.5)	27.80±0.58 (27.1-28.5)
pH	8.30±0.37 (8.0-8.6)	8.40±0.35 (8.1-8.7)	8.35±0.38 (8.1-8.6)
D.O (mg/L)	7.40±0.34 (7.1-7.9)	7.35±0.36 (7.0-7.8)	7.38±0.35 (7.0-7.8)
Carbonate (mg/L)	24.75±2.60 (21.5-27.5)	24.40±2.65 (21.0-27.0)	24.50±2.64 (21.0-27.0)

Bicarbonate (mg/L)	122.50±10.50 (112.0-137.0)	121.00±10.40 (110.0-135.0)	121.75±10.43 (111.0-136.0)
Total Carbonate (mg/L)	149.00±11.60 (138.5-163.0)	148.50±11.65 (138.0-161.0)	148.75±11.64 (138.0-162.0)
Calcium (mg/L)	169.00±10.20 (156.0-181.0)	167.50±10.30 (154.0-179.0)	168.25±10.275 (155.0-180.0)
Hardness (mg/L)	194.00±15.00 (176.0-210.0)	193.00±15.20 (174.0-208.0)	193.50±15.11 (175.0-209.0)
Chloride (mg/L)	24.50±1.88 (23.2-27.2)	24.10±1.90 (22.8-26.8)	24.25±1.89 (23.0-27.0)
T.D.S (mg/L)	662.00±18.00 (640.0-681.0)	660.50±18.20 (638.0-679.0)	661.25±18.08 (639.0-680.0)

### III.I Results of Fecundity, Fertilization Rate and Hatchling Percentage

#### III.I.I Grass Carp

In the present study total 24 grass carp were treated (16 males and 8 females). The total weight of females was calculated as 15 kg. In May and June of 2022, fish that received a single injection of ovaprim-C were successfully induced to spawn. Eight females received ovaprim-C injections; fish in these treatments ovulate 100% of the time, yielding 91866 kg-1 of eggs overall. The overall fertilization and hatching rates were 78.4 and 75.3%, respectively, as indicated in Table 2.

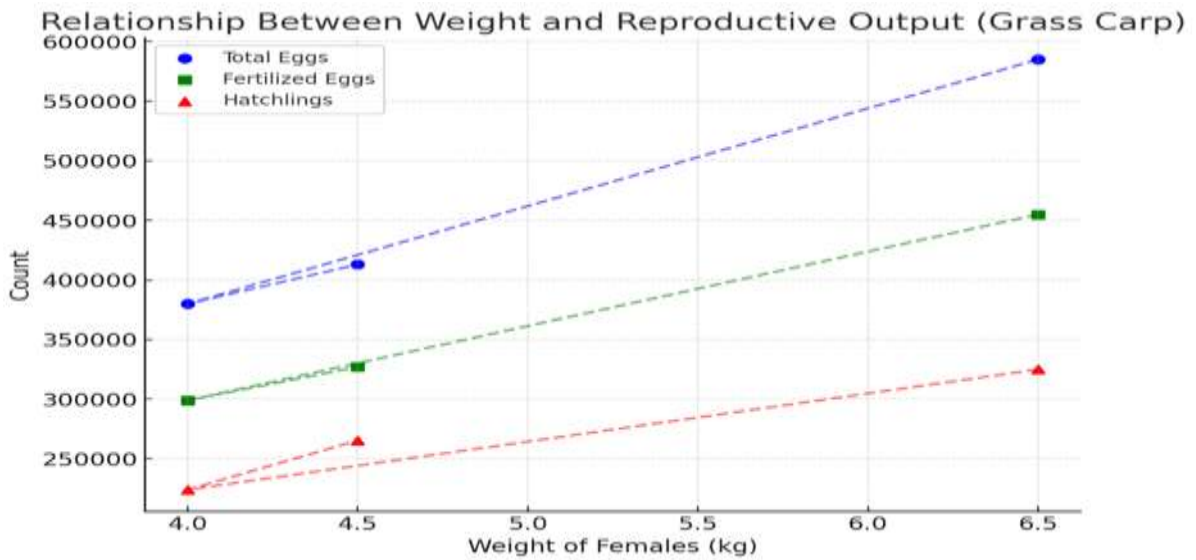
**Table: 3 Date Wise treatment Of Ovaprim in Grass Carp**

Date	Temperature (°C)	Number of Females	Weight of Females (kg)	Dose (ml/kg)	Number of Eggs	Fertilized Eggs	Number of Hatchlings	Mean ± SD (Fertilized Eggs)	Correlation (Weight vs Eggs)
18/05/2024	24-26	4	6.5	0.5ml	585000	455000	325000	360,333 ± 83,170	r = 0.999 (p = 0.012)
22/06/2024	25-27	2	4	0.5ml	380000	299000	224000	360,333 ±	r = 0.999

								83,170	(p = 0.012)
28/06/2024	28-30	2	4.5	0.5ml	413000	327000	265400	360,333 ± 83,170	r = 0.999 (p = 0.012)

\*It will be Significant (if  $p < 0.001$ ) and the correlation value ( $r = 0.999$ ,  $p = 0.096$ ) is a single statistical result calculated from all the data points together

**Figure 12:** Grass Carp, showing the relationship between female weight and the number of eggs, fertilized eggs, and hatchlings



**Table 4: Shows The Percentage of Hatchlings, Fertilization Rate, And Overall Fecundity In Grass Carp**

Number of females treated	8
Total weight of females	15kg
Average Temperature	25-30°C
Spawning period	9-11 h
Total number of eggs spawn	1378000
Total number of eggs fertilized	1081000
Hatchling period	20-24h
Total number of hatchings	814800
Overall Fertilization percentage	78.4%

Overall Hatchling percentage	75.3%
Average number of eggs/kg	91866
Fertilized egg count per kilogram on average	72066
Average hatchling number per kilogram	54320

### III.I.II Rohu

A total of 18 *Labeo rohita* (12 males and 6 females) were treated in this study. The females' total weight was determined to be 10.8 kg. In May and June of 2022, fish that received a single injection of ovaprim-C were successfully induced to spawn. Six females received ovaprim-C injections; fish in these treatments ovulate 100% of the time, yielding 82370 kg-1 of eggs overall. The overall fertilization and hatching rates were 73 and 76.2%, respectively, as indicated in Table 4.

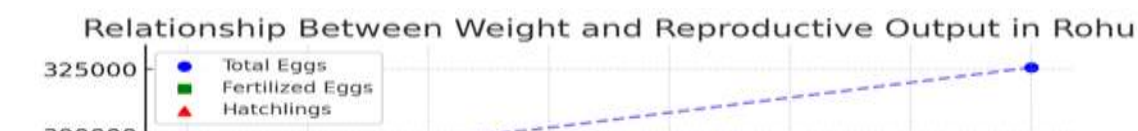
**Table: 5 Date Wise Treatment Ovaprim in Rohu**

\*It will be Significant (if  $p < 0.001$ ) and the correlation value ( $r = 0.989$ ,  $p = 0.096$ ) is a

Date	Temperature (°C)	Number of Females	Weight of Females (kg)	Dose (ml/kg)	Number of Eggs	Fertilized Eggs	Number of Hatchlings	Mean $\pm$ SD (Fertilized Eggs)	Correlation (Weight vs Eggs)
24/05/2024	24-27	2	4	0.5ml	325600	231000	187000	218,800 $\pm$ 13,686	$r = 0.989$ ( $p = 0.096$ )
7/6/2024	25-27	2	3.5	0.5ml	297000	221400	156200	218,800 $\pm$ 13,686	$r = 0.989$ ( $p = 0.096$ )
28/06/2024	26-30	2	3.3	0.5ml	267000	204000	157000	218,800 $\pm$ 13,686	$r = 0.989$ ( $p = 0.096$ )

single statistical result calculated from all the data points together.

**Figure 13: Rohu, showing the relationship between female weight and the number of eggs, fertilized eggs, and hatchlings**



**Table: 6 Overall fecundity, fertilization rate and hatchling percentage in Rohu**

Number of females treated	6
Total weight of females	10.8 kg
Average Temperature	25-30°C
Spawning period	8-10h
Total number of eggs spawn	889600
Total number of fertilized eggs	656000
Hatchling period	20-24h
Total number of hatchlings	500200
Percentage of total fertilization	73%
Total percentage of hatchlings	76.2%
Average quantity of eggs per kilogram	82370
Fertilized egg count per kilogram on average	60740
Average hatchlings count per kilogram	46314

**III.I.III Mohri**

A total of 24 Mohri (*C.mrigala*) were treated in this study (16 males and 8 females). The females' total weight was determined to be 16.8 kg. In May and June of 2022, fish that received a single injection of ovaprim-C were successfully induced to spawn. Eight females received ovaprim-C injections; fish in these treatments ovulate 100% of the time, yielding

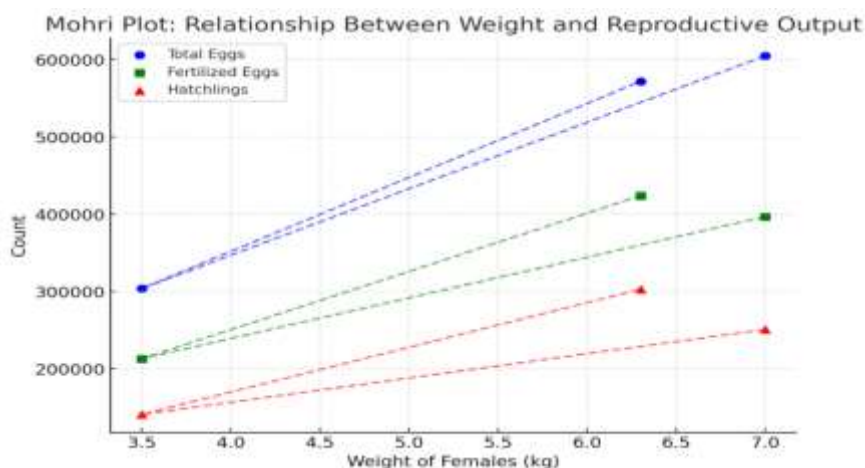
88154 kg-1 of eggs overall. Table 6 shows that the overall fertilization and hatching rates were 76.2 and 67.2%, respectively.

**Table: 7 Date wise treatment of Mohri**

Date	Temperature (°C)	Number of Females	Weight of Females (kg)	Dose (ml/kg)	Number of Eggs	Fertilized Eggs	Number of Hatchlings	Mean ± SD (Fertilized Eggs)	Correlation (Weight vs Eggs)
24/05/2024	24-27	3	7	0.5ml	605000	397000	251000	344,667 ± 114,823	r = 0.996 (p = 0.057)
7/6/2024	25-27	2	3.5	0.5ml	304000	213000	141000	344,667 ± 114,823	r = 0.996 (p = 0.057)
22/06/2024	26-28	3	6.3	0.5ml	572000	424000	303000	344,667 ± 114,823	r = 0.996 (p = 0.057)

\*Significant ( $p < 0.001$ ) and the correlation value ( $r = 0.989$ ,  $p = 0.096$ ) is a single statistical result calculated from all the data points together

**Figure 14: Mohri-style plot showing the relationship between female weight and the number of eggs, fertilized eggs, and hatchlings**



**Table: 8 Overall Fecundity, Fertilization Rate and Hatchling Percentage in Mohri**

Number of females treated	8
Total weight of females	16.8kg

Average Temperature	25-30°C
Spawning period	9-11h
Total number of eggs spawn	1481000
Total number of fertilized eggs	1034000
Hatchling period	20-24
Total number of hatchings	695000
Percentage of total fertilization	70%
Total percentage of hatchlings	67.2%
Average quantity of eggs per kilogram	88154
Fertilized egg count per kilogram on average	61547
Average hatchlings count per kilogram	41369

**III.II Overall fecundity, fertilization rate and hatchling percentage in Grass, Rohu and Mohri**

The current study examined the effects of intramuscular ovaprim-C injection on the number of eggs/kg, fertilization rate, and hatching percentage of economically significant and rapidly growing food fishes, namely grass carp (*Ctenopharyngodon Idella*), rohu (*Labeo rohita*), and mohri (*C. mrigla*), at Fish Hatchery, Ratta kulachi, Dera Ismail Khan, Pakistan, from May to June 2024. For this study, which took place from May 18 to June 28, 2024, 66 fish in total—24 grass carps (16 males and 8 females in a 2:1 ratio), 18 Rohu (12 males and 6 females in a 2:1 ratio), and 24 mohri (16 males and 8 females in a 2:1 ratio)—were used.

According to calculations, the combined weight of the female grass carps, Rohu and Mohri, was 15.8, 10.1, and 16.8 kg, respectively. After receiving a single injection of ovaprim-C (luteinizing hormone releasing hormone analogue) at a dose of 0.5 ml/kg for females and 0.25 ml/kg for males, fish were effectively spawned. The fish were taken straight to the Chinese Hatchery's breeding ponds after being dosed. After nine to eleven hours of dosage, spawning occurred. The

twitching movement began ten to twelve hours after spawning. After 20–24 hours of fertilization at 28–30°C, hatching took place. The average number of eggs per kilogram of body weight of grass carp, Rohu, and Mohri was found to be 91866, 82370, and 88154, respectively. Rohu, Mohri, and grass carp fertilization rates were found to be 78.4%, 73, and 76.2%, respectively. Grass carp, Rohu, and Mohri hatching percentages were 75.3%, 76.2, and 67.2%, respectively.

**Table: 9 Overall fecundity, fertilization rate and hatchling percentage in Grass, Rohu and Mohri**

Parameters	Grass	Rohu	Mohri
Fecundity/kg	91866	82370	88154
Number of females treated	8	6	8
Total weight of females	15	10.8kg	16.8
Temperature	25-30c	25-30c	25-30c
Spawning period	9-11	8-10	9-11
Toil Number. of eggs	1378000	889600	1481000
Total Number of fertilized eggs	1081000	656000	1034000
Hatchling period	20-24	20-24	20-24
Total Number of hatchling	814800	500200	695000
Fertilization percentage	78.4 %	73%	70%
Hatchling percentage	75.3%	76.2%	67.2%

#### IV. DISCUSSION

In the present study, single intramuscular injection of synthetic hormone, ovaprim-C resulted in successful spawning of Grass carp (*C. idella*), Rohu (*Labeo rohita*) and Mohri (*C.mrigala*). The results of the hormonal stimulation in the current work are like the effectiveness and usefulness by using ovaprim-C. But speed and gentleness during fish capture and handling are of utmost importance [14].

Certain hormones are used individually like different analogues of LH-RH, without pituitary gland, which results in failure of spawning and clearly indicates that dopamine blocks the action of LH-RH on the secretion of gonadotropin [15]. Then Canada introduced the ovaprim- C containing the analogue of salmon gonadotropin releasing hormone (D-Arg6, Pro9, Net) and dopamine antagonist, and studies conducted in India [10] and Pakistan [16,17,18] revealed the superiority of ovaprim-C in induced spawning. Dose of ovaprim-C used in Grass carp (*C. idella*), Rohu (*Labeo rohita*) and Mohri (*C. mrigala*) in the present experiment is 0.5 ml/kg, while experiments conducted by Nandeesh et al. (1990) and Peter et al. (1986) reported the dose rate of 0.7 ml/kg. So, the present study reveals that ovaprim-C use is more economical in commercial carp seed production, as it saves a considerable amount of time and avoids the excessive handling of brood fish. In India trials with Ovaprim have given very encouraging results [10,12] Kaula and Rishi (1986) reported the successful spawning of Mrigala with Ovaprim. [16] reported very satisfactory results in trials with Ovaprim. reported successful spawning of Rohu and Mrigala with Ovaprim at Fish seed hatchery Rawal Town, Islamabad. However, a major handicap in the use of Ovaprim is its high viscosity, which causes difficulty in injection. Its high cost is also a prohibitive factor.

Dose of Ovaprim-C used in *L. rohita* in the present experiment is 0.5 ml/kg, while experiments conducted by [16] reported the dose rate of 0.3 to 0.5, 0.4 ml/kg, respectively. So, the present study reveals that Ovaprim-C use is more economical in commercial carp seed production, as it saves a considerable amount of time and avoids the excessive handling of brood fish. The positive response of Rohu to ovaprim indicated the higher potency of this drug inducing the spawning. *L. rohita* have been reported to spawn with 10 mg of Pimozide (Kaul and Rishi, 1986) and the results obtained from Ovaprim is supported by the results of the trials in 1988 and 1989 [16,18]. Moreover, reliable estimates for absolute and relative fecundity can be attained by using

the predictive equations with a fair amount of accuracy. In the present experiment, the highest fertilization rate of Rohu was 73%. After 20 hours of fertilization the hatching started. Hatching was carried out in circular tank and the highest hatching rate of Rohu was 76.2%. However, the fertilization and hatching percentage was high because the number of males was sufficient. It was also found that fertilization and hatching rate of natural sources was lower than this study. Other responsible factors that might affect fertilization and hatching percentage are temperature, photo period, dissolved oxygen, salinity and water flow. Temperature plays a vital role in gonadal reappearance and timing of ovulation. If the optimum temperature cannot be maintained the maturation may delay or the eggs may release spasmodically, and the total amount of eggs may also be reduced [20]. These may be the reasons for lower fertilization and hatching rate in natural sources. The result of this study on Rohu is almost like the Saud et.al. (2013) which have a fertilization rate 71-78%. Grass carp (*C. Idella*) preferably spawn during mid-April to June. Fertilization rate is 78.4% and hatching percentage is 75.3% (Table 8) at the favorable temperature of 26 to 30°C as shown in Table 2. Generally, the number of eggs spawned by Grass carp (*C. Idella*) in the present study was almost like previous report [21,22,23]. In the present study the fecundity in Mohri is good but fertilization and hatching percentage is slightly lower than grass carp and rohu. There may be various factors like temperature, photoperiod, dissolved oxygen, salinity and water flow. Temperature plays a vital role in gonadal reappearance and timing of ovulation. During the experiment with Mohri two main factors Temperature and dissolved oxygen in water effect the fertilization and hatching production. In the present study fecundity, fertilization rate and hatching rate in Mohri is greater than Ali .M. A et al (2015) but less than more et al (2010) [22].

## **V. CONCLUSION AND RECOMMENDATION**

In conclusion it may be suggested that more emphasis should be given to develop the technique of induced breeding of fish and popularize it all over the

country. It is a known fact that a guaranteed supply of high-quality fish seed is the primary requirement for the growth of inland fisheries and successful intensive fish farming. The important economic estuarine fish varieties should be produced using this method profitably to breed and obtain seed for brackish water fish farming. The experiments that have been carried out thus far have been very positive and have indicated a lot of potential for future study. To meet the need for fish seed in Pakistan, concerted efforts are being made to refine the procedures. It is anticipated that sincere efforts and cooperation from all sides would result in the commercial production of fish seeds of acceptable types. To standardize dosage and collect more data on the size, growth rate, survival, and other characteristics of the eggs and hatchlings produced by Ovaprim therapy, more experiments are now necessary. A higher demand for high-quality fish seeds might be met with additional advancements in breeding and hatching methods as well as dose standardization.

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