DOI: 10.5281/zenodo.10682989

CHAPTER 20

Efficacy of various doses of Ovaprim on breeding performance of *Labeo rohita* (Rohu)

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Received: 02 February, 2024; Accepted: 07 February, 2024

Abstract

Fishes are easily renewable but they are not infinite so they have to be managed properly to sustain the world population. To satisfy ever growing human consumption demands large scale cultivation of organisms is necessary. Here comes the need for pure fish seed. A single dose of 0.4 ml/Kg Ovaprim was effective over to 0.3 & 0.5 ml/Kg in induce breeding in Labeo at hatchery.

Keywords: Induced breeding, Ovaprim, Labeo Rohitha, Rohu, Hypophysation, Linpe method

Introduction

Carp culture India is the polyculture of three to six species of major carps . Of these three are Indian major carps namely Catla (*Catla catla*), Rohu (*Labeo rohita*), Mrigal (*Cirrhinus mrigala*), and three exotic carps viz. Silver carp (*Hypophthalmichthys molitrix*), Grass carp (*Ctenopharyngodon idell*a) and Common carp (*Cyprinus carpio*).In India major carp fishes like *Catla catla, Labeo rohita, and Cirrhina mrigala* are chosen for pond culture because they can grow quickly in confined waters , non-predatory food habits and general preference as food by the public. Carp fishes constitute the bulk of the world aquaculture production, with silver carp, grass carp and common carp occupying third, fourth and fifth places, respectively Crespi (2004). The widely cultivated Indian major carps, namely, *Labeo rohita, Catla catla and Cirrhinus mrigala,* contribute to over 90 % of the total Indian aquaculture production and 70-75 % of total fresh water production . The exotic carps, namely, silver carp (*Hypophthalmichthys molitrix*); grass carp (*Ctenopharyngodon idella*) and common carp (*Cyprinus carpio*) form a second 11 important group contributing to 25-30 % of this production along with catfishes FAO(2015).

With the ever increasing population, necessity of augmenting the production of fish through the development of more effective techniques for fish culture for supply of animal protein is the need of the hour. A

ISBN: 978-93-95369-85-5

major factor that constrains the speed and extent of development of fish farming is the inadequate supply of quality fish seed Nwokoye et al., (2007). Non-availability of quality fish seed is the major problem in the carp culture Naeem et al.,(2005a & b) and timely seed supply is one of the major constraint Radheyshyam (2010). To satisfy ever growing human consumption demands large scale cultivation of organisms is needed. This is successful only when the resource is renewable Harvey and Hoar (1979). Fishes are easily renewable but they are not infinite so they have to be managed properly to sustain the world population.

The technique of hypophysation of the Indian major carps in the late 1950s Chaudhuri and Alikunhi (1957) , grass carp and silver carp by Alikunhi et al. (1963 a,b) has changed the fishery sectors scenario completely .It has brought a big revolution in breeding carps in standing waters and to get quality seed. Yet there were inherent difficulties with the method Later with development of Ovaprim, an easy to spawn agent only one dose of hormone did the job with higher success rate. Easily available synthetic hormone have increased the seed production immensely

Review of literature

Technique of hypophysation was first developed in Brazil Von Ihering et.al., (1935),Ihering (1937). Induced ovulation in fish started in the late 1930s where ground pituitaries and pituitary extracts was used to achieve success. Since, then the pisciculturists are using this method as a part of routine fish culture method. During 1955 and 1956 the technique of induced spawning for the first time by using pituitary gland extract of Indian major carps in India was done by Chaudhari, Alikunhi (1957) with a thrust on mass production of quality spawn in controlled environment, thereby reducing dependence on natural seed collection. Carp species viz. *Labeo rohita, Cirrhinus mrigala, Cirrhinus reba, Labeo bata and Puntius sarana* were successfully induced bred by injecting pituitary extract from carp fishes. Fish pituitary gland extract was effective as ovulating agent all over the world but, it had some inherent problems involved in it Nandeesha et.al (1990b). As a result there is a shift from using pituitary gland extracts towards using synthetic hormonal formulations. Luteinizing hormone, Follicle stimulating hormone, or luteinizing hormone + Follicle stimulating hormone, pregnant mare serum (PMSG) and Human chorionic gonadotropin (HCG) in crude or purified forms have taken the place of pituitary gland extracts. Dopamine antagonist,pimozide when administered along with LH-RH analogue increased ovulation in fish Chang and Peter(1983).Effects of LHRH-A and sGnRH-A alone or combination with the pimozide and domperidone on chinese loach and common carp was studied by Lin et al.(1988). This study proved that sGnRH-A proved to be more effective in inducing ovulation in common carp when it was combined with domperidone. Linpe method is commercialized by a Canada -based company, M/S Syndel laboratories, Inc under the trade name Ovaprim Ovaprim is supplied in liquid form it contains 20μg/ml salmon gonadotropin-releasing hormone analog (sGnRHa) and 10mg/ml domperidone. It is in the form of a ready-to-inject solution and is used the whole world over today.

Materials and methods

- **Description of the study area**

Fish seed hatchery selected for studying the breeding performances of Indian major carps with Ovaprim is located 5 km away from Jagtial district of Telangana state, India.

- **Overview of the Fish Seed Hatchery**

Breeding of carps in this hatchery is done utilizing continuous flow of water by gravity. This technique was developed in China. The advantages of this system are viz. a continuous flow of water can be maintained which simulates riverine environment, hatching is more effective because oxygenated water gets distributed evenly, eggs can be protected from being washed out with water by placing a plastic tube in the middle and a screen at the water outlet, water flow rate and water quality parameters can be easily accessed and maintained.

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The components of this hatchery are: 1) Overhead tank 2) Spawning pool 3) Egg collection chamber 4) Hatching pool 5) Spawn collection chamber

- ***Labeo rohita* breeder**

During the breeding season the pectoral fins of mature males are rough on the dorsal surface, while those of females are smooth. The species breeds naturally in rivers, reservoirs, and in large lakes in which fluvial conditions are simulated. The breeding season generally coincides with the south-west monsoon i.e. June-September. Male and Female breeders of 2 + age and weighing 2 kg are selected Bishtet.al (2013)

- **Collection and maintenance of brood fish**

The brooders of *Labeo rohita* were collected from local rivers during March – May in The brooders were fed with supplementary feed mixture of rice bran and mustard oil cake daily in 1 : 1 ratio at the rate of 3-4 % of their body weight during March and April , later on it was reduced to 2 % till they are used for breeding. This is done to ensure proper growth and gonadal maturity Charula (2008)

- **Identification of male and female brooders & their maintenance**

On the basis of their secondary sexual characters male and female breeders are identified Jhingran and Pullin (1985). The ripe males were identified by roughness of the dorsal surface on the pectoral fin, which on the contrary was very smooth in the female. The roughness in pectoral fins was felt by touching the surface of fin close to the body. The mature male and female fishes were also distinguished from the shape of their body, condition of the vent and secretion of milt in males.

Protocol of the experiment

Male and female breeders were netted out with drag net and are put into the breeding pool for acclimatization. Water jets are released to simulate riverine conditions and fountains are also opened to give the rainy effect. Breeders are starved for 6-8 hours before beginning of breeding programme, to clean up their guts so that no release of fecal matter occurs while breeding .In the evening breeders are separated sex wise. Ratio of female to male selected for breeding is in the ratio of 1:1 by weight More et.al (2010)

Three doses of Ovaprim i.e., 0.3, 0.4 & 0.5 ml/kg body weight were injected to female breeders in replicates of four. Male fishes are administered with only 0.2 ml/kg body weight Pandey and Singh (1997)

The schedule of hormonal dose administered is given in Table No: 1

Table 1: Dosage of hormone administered to Labeo rohita

Name of the fish	Dosage of Ovaprim administered to female breeder (ml/kg)			Dosage of Ovaprim administered to male breeder (ml/kg)
	Treatments			
	T1	T2	T3	T1 , T2 and T3
Labeo rohita	0.3	0.4	0.5	0.2

- **Administration of Ovaprim**

Glass syringes and non-disposable needles are boiled to make them sterile. Brooders are netted out one by one and held in a cloth hapa to avoid wriggling movement and then injected with ovaprim. There are several ways of administering ovaprim to carps viz. intracranial, intraperitoneal, and intramuscular. In this study only intra muscular injection is given in the dorsolateral region of both and female fish in a single dose Haniffa and Sridhar (2002) at the base of the caudal above the lateral line. Later on injected brooders are released into the breeding pool.

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- **Dosage of Ovaprim to be administered**

The brood fish are weighed and quantity of Ovaprim to be injected to male and female breeders is calculated as follows Nandeesha et. al. (1991)

Quantity to be injected (ml) = Dosage of Ovaprim (ml) x Weight of brood fish (Kg)

Methods for assessing results

- **Latency**

Latency is the time between injection of hormone and ovulation Montchowui et.al (2011) it is expressed in hours. When ovulation was observed females were hand stripped after drying the genital papilla with a paper towel and eggs were collected in a container, later stripping of male was done for collection of milt Chaudhuri et.al (1966), Naeem et.al (2005a). Eggs were gently mixed with milt with a quill. Fertilized eggs were rinsed three or four times with water and then transferred to a circular hatching pool.

- **Counting of Eggs and Average Number of eggs Kg^{-1}**

Number of eggs laid by fishes was measured using volumetric method More (2010). A beaker of known volume is taken and numbers of eggs were counted in triplicate and the average number of eggs per beaker was calculated. Total number of eggs per beaker was calculated using the following formula:

Total number of eggs laid = Average number of eggs in sample beaker x Number of beakers measured

The average number of eggs Kg^{-1} was obtained by dividing total number of eggs obtained by total weight of female breeders

- **Fertilization percentage**

After 3 to 4 h of fertilization, the fertilized and unfertilized eggs can be distinguished. Fertilized eggs of Indian major carps are transparent, non-adhesive, round in shape while unfertilized eggs are opaque. To estimate fertilisation percentage 2-3 samples of water hardened eggs were taken from the breeding pool in random and number of fertilised eggs in each sample was counted and average value was determined More *et.al.,* (2010)

$$\text{Fertilisation percentage} = \frac{\text{Average number of fertilized eggs}}{\text{Average number of eggs in a sample}} \text{ x } 100$$

Fertilisation percentage is calculated using the following formula

- **Hatching percentage**

It is was calculated by taking sub sample of a uniform volume (5ml) from fertilised eggs and incubating them in a plastic tray filled with water which is used for egg incubation. Eggs were kept rotating by putting an aeration stone in one corner of the tray Charula (2008). Hatching percentage was estimated by the following formula Naeem (2013).

$$\text{Hatching percentage} = \frac{\text{Total number of hatchlings}}{\text{Total number of fertilized eggs}} \text{ x } 100$$

Results

Breeding performance of *Labeo rohita* with different doses of Ovaprim

Labeo rohita were tested with different doses of Ovaprim i.e 0.3, 0.4, 0.5 ml/kg under (T1), (T2), (T3) treatments respectively. Every experimental dose was tested in four replicates. In each experiment exactly four numbers of male and female breeders with individual weight of 2 kg are selected .in 1: 1 ratio by weight.

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Latency under (T1) and (T2) ranged from 9.00 - 11 hrs and 8.15 - 9 hrs respectively where and it was between 9.45 - 11.15 in (T3). Highest latency of 11.15 was seen with (T3) and lowest i.e., 8.15 hr. seen in (T2.)

Number of eggs spawned were in the range of 10.03 -10.54 x 10^5 (T1), 10.96– 11.64 x10^5 in (T2) and 10.01 – 11.08 x10^5 in (T3). Lowest number of eggs 10.01 x 10^5 were observed in (T3) and highest 11.64 x10^5 in (T2)

Average number of eggs /kg in (T1) were in which the range of 1.25 – 1.32 x 10^5. Whereas it was 1.37 – 1.46 in (T2) and 1.25 – 1.38 x10^5 in (T3) .Lowest average number of eggs /kg 1.25 x 10^5 were found in (T1) and (T3) and highest 1.46 x10^5 in (T2)

Number of fertilized eggs obtained were 8.1 – 8.71 x10^5 in (T1), 9.95 – 10.78 x x10^5 in (T2) and 8.61 – 9.8 x10^5in (T3). Lowest number of fertilized eggs 8.1 x 10^5 were found in (T1) and highest 10.78x10^5 in (T2).

Fertilization percentage under (T1), (T2), (T3) was in the range of 79.41-85.73, 87.45-96.14, 85.74-88.45 respectively. Lowest and highest fertilization percentages were seen in T1 and T2 with 79.41 and 96.14 respectively.

Number of hatchlings obtained were 6.9-7.7 x 10^5 in (T1), 9.25 – 9.91 x 10^5 in (T2) and 7.35 – 8.9 x 10^5 in (T3). Lowest number of hatchlings 6.9 x 10^5 in (T1) and highest 9.91 x10^5 in (T2) were obtained.

Hatching percentage under (T1), (T2), (T3) was in the range of 85.19-89.02, 86.45-96.48, 80.77 – 91.46 respectively. Lowest and highest fertilization percentages were seen in (T3) and (T2) with 80.77 and 96.48 respectively (All results are tabulated in Table: 2)

Pooled results (Mean ± SD) of breeding performance of *Labeo rohita* with different doses of Ovaprim

The pooled results of breeding performance of *Labeo rohita* with different doses of Ovaprim i.e. 0.3, 0.4, 0.5 ml/kg under (T1), (T2), (T3) treatments respectively are depicted as Mean ± SD values.

Latency under (T1) and (T2) was 10.1 ± 0.84 and 8.45 ± 0.38 hrs respectively where and it was 10.42 ± 0.78 in (T3). Highest latency was seen in T3 and lowest in T2

Total Number of eggs laid were 10.23 ± 0.21 lakhs (T1), 11.32 ± 0.33 lakhs in (T2) and 10.42 ± 0.46 lakhs in (T3). Highest total number of eggs were seen in (T2) and lowest in (T1.)

Average number of eggs /kg in (T1) were 1.279 ± 0.027 lakhs. Whereas it was 1.415 ± 0.04 (T2) and 1.303 ±0.057 lakhs. Lowest average number of eggs was found in (T1) and highest in (T2)

Total Number of fertilized eggs obtained were 8.49 ± 0.27 lakhs in (T1), 10.38±0.42 lakhs in(T2) and 9.07 ± 0.52 lakhs in (T3). Lowest number of fertilized eggs were found in (T1) and highest in (T2)

Overall fertilization percentage under (T1), (T2), (T3) was in the range of 83.01± 2.86 l 91.74 ± 3.62, 86.96 ± 1.30 respectively. Lowest and highest fertilization percentages were seen in (T1) and (T2) respectively.

Total Number of hatchlings obtained were 7.43 ± 0.36 lakhs in (T1), 9.58 ±0.26 lakhs in(T2)and 7.94 ± 0.7 lakhs in(T3). Lowest number of hatchlings in (T1) and highest in (T2) were obtained.

Overall hatching percentage under (T1), (T2), (T3) was in the range of 87.4 ± 1.63, 92.45 ± 4.4, 87.54 ± 4.9 respectively. Lowest and highest fertilization percentages were seen in (T1) and (T2) respectively (All results are tabulated in Table: 3 and Graphs: 1, 2, 3)

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Table 2: Breeding performance of Labeo rohita with different doses of Ovaprim

Treatments	Dosage of Ovaprim (ml/kg)		No. of fishes		Weight of each breeder (kg)		Total weight of breeders (Kg)		Latency (Hrs)	Number of eggs (x10 ^{5})	Average number ofeggs /Kg (x10 ^{5})	Number of fertilised eggs (x10 ^{5})	Fertilisation %	Number of hatchlings (x10 ^{5})	Hatching %
	♂	♀	♂	♀	♂	♀	♂	♀							
T1	0.2	0.3	4	4	2	2	8	8	9	10.2	1.27	8.1	79.41	6.9	85.19
			4	4	2	2	8	8	10	10.16	1.27	8.71	85.73	7.6	87.26
			4	4	2	2	8	8	10.45	10.03	1.25	8.51	84.85	7.5	88.13
			4	4	2	2	8	8	11	10.54	1.32	8.65	82.07	7.7	89.02
T2	0.2	0.4	4	4	2	2	8	8	8.15	11.13	1.39	10.7	96.14	9.25	86.45
			4	4	2	2	8	8	8.25	10.96	1.37	9.95	90.78	9.6	96.48
			4	4	2	2	8	8	8.30	11.64	1.46	10.78	92.61	9.91	91.93
			4	4	2	2	8	8	9	11.55	1.44	10.1	87.45	9.59	94.95
T3	0.2	0.5	4	4	2	2	8	8	11	10.01	1.25	8.61	86.01	7.5	87.11
			4	4	2	2	8	8	10.20	10.38	1.29	9.1	87.67	7.35	80.77
			4	4	2	2	8	8	9.45	10.24	1.28	8.78	85.74	8.03	91.46
			4	4	2	2	8	8	11.15	11.08	1.38	9.8	88.45	8.9	90.82

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Table 3: Pooled results (Mean ± SD) of breeding performance of *Labeo rohita* with different doses of Ovaprim

Parameter	Treatments		
	T1	T2	T3
No.of males treated	16	16	16
Total weight of males (Kg)	32	32	32
No.of females treated	16	16	16
Total weight of females (Kg)	32	32	32
Latency (Hrs)*	10.1 ± 0.84	8.45 ± 0.38	10.45 ± 0.78
Total number of eggs obtained (x10 ^{5})*	10.23 ± 0.21	11.32 ± 0.33	10.42 ± 0.46
Average number of eggs/kg (x10 ^{5})*	1.279 ± 0.027	1.415 ± 0.04	1.303 ± 0.057
Total number of fertilized eggs obtained (x10 ^{5})*	8.49 ± 0.27	10.38 ± 0.42	9.07 ± 0.52
Overall fertilization percentage*	83.01 ± 2.86	91.74 ± 3.62	86.96 ± 1.30
Total number of hatchlings obtained (x10 ^{5})*	7.43 ± 0.36	9.58 ± 0.26	7.94 ± 0.7
Overall hatching percentage*	87.4 ± 1.63	92.45 ± 4.4	87.54 ±4.9
*Values are indicated as Mean ± SD			

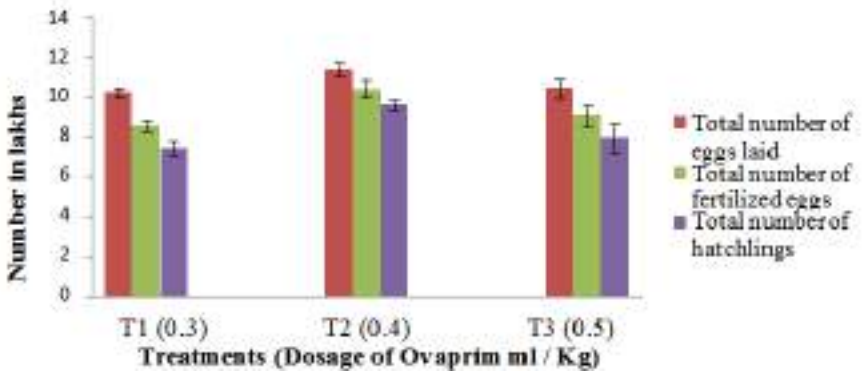

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Fig. 1: Total number of eggs, fertilised eggs and hatchlings obtained (Mean±SD) from *Labeo rohita* using different doses of Ovaprim

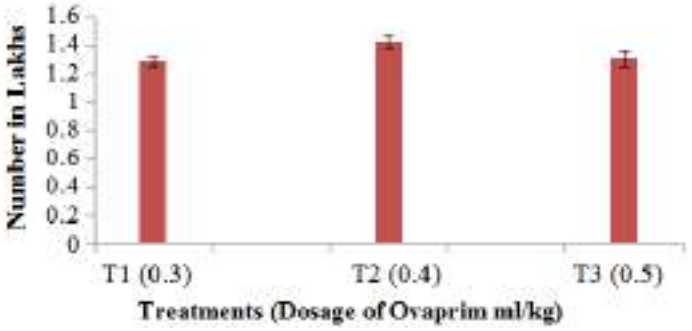

Fig. 2: Average number of eggs Kg^{-1} (Mean ±SD) of *Labeo rohita* with different doses of Ovaprim

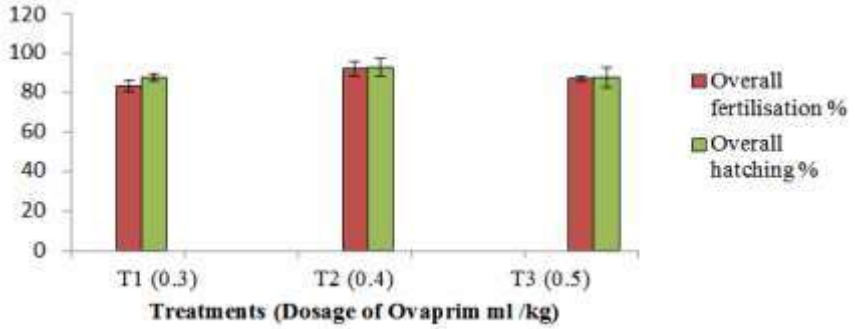

Fig. 3: Overall fertilisation and hatching percentage (Mean ±SD) of *Labeo rohita* with different doses of Ovaprim

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Fertilized eggs of rohu

Hatchlings of rohu

Discussion

Labeo rohita in (T2) showed a lowest latency period of 8.45±0.38 hr. in (T2) compared with that of (T1) and (T3). Result obtained is very similar to the latency of 8-9 hrs. achieved by Basavaraja (2007) who used sGnRH-a +DOM. Pandey and Singh(1997) got 6-8 hrs. of latency with 0.4 ml/kg.

In (T2) Total number of eggs obtained (11.32±0.33 lakhs), Average number of eggs/kg (1.415±0.04 lakhs) overall fertilization percentage (91.74 ± 3.62), and hatching percentage (92.45 ± 4.4) were highest when compared with (T1) and (T3). Gurpreet and Raman (2012) reported fecundity of 0.38 lakh eggs/kg body weight, fertilization rate 61.3 % and hatching rate of 72.2 % with 0.4 ml/kg Ovaprim on breeders weighing 1.5 kg on an average. Osman et.al., (2012) compared three doses of Ovaprim i.e., 0.3, 0.4, 0.5 ml/kg to female but a uniform dose of 0.2 ml/kg to male and reported a highest fertilization and hatching percentages of 86 and 85 respectively with 0.4 ml/kg dose.

Nandeesha et.al (1990a) used 0.4 ml/kg dose to female but 0.15 ml /kg to male and got 76-94 % of fertilization. Even higher doses of 0.6 and 1.2 ml/kg dose to female did not give better results than with 0.4 ml/kg. Basavaraja (2007) used a similar dose to female but for the male he used 0.1-0.2 ml/kg Ovaprim and reported fertilization percentage of 90±3.60. Naeem et.al (2013) used 1-3 Kg weight female breeder and injected 0.4 ml/kg for female got 63,574 average number of eggs / kg body weight of female with fertilization and hatching percentage of 77.50 and 81.39 respectively. By using 0.5 ml/kg to female and 0.2 ml/kg Ovaprim to male rohu Khan et.al., (2013) obtained fertilization percentage of 86 and hatching percentage of 75 whereas, Chakrabarthi et.al (2016) got spawning fecundity of 0.88-1,00,000 lakh eggs/kg bodyweight, fertilization and hatching percentage of 90-95 and 85.5- 92.5 % respectively using females weighing 0.9-1.7kg on an average. Indira et.al (2012) also used 0.5ml/kg Ovaprim to female and got less fertlisation and hatching percentage i.e. 75-85% and 70-85 % respectively. Jhajhria (2011) reported fertilization percentage as 50% and hatching percentage as 89% with 0.5 ml/kg dose of Ovarpim. Dhawan and Kaur (2004) used 0.5 mg/ kg Ovaprim to female and 0.25 mg/kg to male and reported a mean fertilization percentage of 80.55 %. Khan et.al (2006) administered a still higher dose of 0.6 ml/kg but obtained 58,000 average number of eggs/kg body weight, fertilization percentage of 54% and hatching percentage of 42%. More (2010) used doses of Ovaprim to female and male in the range of 0.4-0.6 and 0.2-0.3 ml/kg respectively and reported overall fertilization percentage as 94.06 and overall hatchling percentage of 91.36. Sharma and Singh (2002) used doses of 0.3-0.5 ml/kg Ovaprim to female and uniform dosage of 0.2 ml/kg and reported fertilization percentage in the range of 72.55±3.84 with a minimum of 55.94 % and maximum of 93.24 %. In rohu, latency was less (8.45±0.38) with 0.4ml/kg Ovaprim, followed by (10.1±0.84) with 0.3 ml/kg and (10.45±0.78) with 0.5 ml/kg dose. Total number of eggs laid were (11.32 ±0.33 lakhs) with 0.4ml/kg Ovaprim, followed by (10.42±0.46 lakhs) with 0.5 ml/kg and (10.23±0.21lakhs) with 0.3 ml/kg dose. Average number of eggs/kg laid were more (1.415 ±0.04 lakhs) with 0.4ml/kg Ovaprim, followed by (1.303±0.057 lakhs) with 0.5 ml/kg and (1.27±0.027 lakhs) with 0.3 ml/kg dose. Overall fertilisation percentage and Total number of fertilised eggs laid were more (91.74±3.62) and (10.38±0.42 lakhs) with 0.4ml/kg Ovaprim,

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followed by (86.96 ±1.30) and (9.07 ±0.52 lakhs) with 0.5 ml/kg and (83.01 ±2.86) and (8.49±0.27lakhs) with 0.3ml/kg dose Overall hatching percentage and Total number of hatchlings obtained were more in rohu it was (92.45±4.4) and (9.58±0.26 lakhs) with 0.4ml/kg Ovaprim ,followed by (87.54 ±4.9) and (7.94±0.7 lakhs) with 0.5 ml/kg, (87.4±1.63) and (7.43 ±0.36 lakhs) with 0.3 ml/kg dose. *Labeo rohita's* breeding performance was found to be very effective with a single dose of 0.4 ml/Kg of Ovaprim.

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