

EFFECTS OF OVAPRIM ON REPRODUCTIVE PERFORMANCE OF FRESH WATER CARP, *CIRRHINA MRIGALA* (F. HAMILTON, 1822)

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ABSTRACT

Eighteen female and nine male individuals were selected to induce breeding trials using ovaprim. Three doses of ovaprim were administered i.e. T1 (0.3 mL/kg body weight), T2 (0.4 mL/kg) and T3 (0.5 mL/kg). Six females were kept in a group and a group was treated with T1, T2 or T3. Two male individuals per group (2:1) received 0.2 mL/kg of ovaprim. The females treated with T3 exhibited better reproduction with maximum number of eggs (303227 ± 15389), maximum number of hatched eggs (210460 ± 10597), maximum number of unhatched eggs (63381 ± 4011), maximum number of hatchlings (210460 ± 10597) and maximum working fecundity (83000). The proportion of unhatched eggs to total number of eggs or hatched eggs to total of eggs remained proportionally same in the treatment. The proportion of fertilized eggs increased from 58.34% in T1 to 90.31% in T3. The proportion of unfertilized eggs likely declined from T1 to T3. It was concluded that a level of ovaprim, 0.5 mL/kg, was probably the most suitable which can fulfill the demand of fish seed for successful propagation in case of *Cirrhina mrigala*.

KEYWORDS: Induced breeding, Ovaprim, Reproductive response, Ovulation, Carp Biology.

INTRODUCTION

Fish farming is expanding in Pakistan but still passing through its neonatal stage. There is a need to further strengthen the production potential of fresh water fish species. Conventionally, aquaculture activities are based on members of cultivable fish species (monoculture or polyculture) and sources of nutrients supplied by manure trials or by supplemental feeding strategies. Irrespective to culturing practices the most important aspect is the selection of fish species e.g., stocking of indigenous carps [*Labeo rohita* (Rohu), *Cirrhina mrigala* (Mori) and *Catla catla* (Thaila)] (Jhingram and Pulin, 1985). Besides, exotic fish species have also been introduced from different countries.

The need for perpetual supply of carps is important which can be accomplished through natural resources. Unfortunately over fishing, use of banned nets for fishing and most important the environmental degradation have caused great decline in fish population. These are serious issues in the field of seed production of fish. There are several methodologies employed for artificial propagation. Induced reproduction is one of them which allow farmers to obtain more spawn from breeders that do not naturally reproduce in controlled environment and to manipulate the breeding seasons to favour production cycles. Dorafshan *et al.* (2003) tested GNRHa (gonadotropin releasing hormone analogue alone or in combination with Domperidone in common carp, *Cyprinus carpio*). They found that spawning was higher in treated fishes. It requires basic information regarding the brood stock collection, management, artificial breeding, hatcheries and nursery management. In the present studies, we have investigated the effects of ovaprim on reproductive performance of fresh water carp.

MATERIALS AND METHODS

Experimental design: To find out the induced effects of synthetic hormone (Ovaprim) on fresh water carp (*Cirrhina mrigala*) with special emphasis on reproductive response, following experiment was conducted.

Weight of Fish: Female of T1 treatment varied in size from 2.5 to 3.86 kg, 2.24 to 4.05 kg in T2 and 3.07 to 4.25 kg in T3. While male fish also varied in weight from 2.75 to 3.80 kg in group I, 2.83 TO 4.10kg in group II and 2.97 to 3.82 kg in group III.

Identification of male and female: Male and female fishes were identified on the basis of secondary sexual characters. As described by Chondar (1994) morphological characters such as roughness of pectoral fins, scales and operculum are developed on the advent of breeding season, protruded body shape with reddish vent are common in both male and female carp but males secrete milt on gentle press and females produce ova.

Selection of breeders: For the selection of productive males and females the criteria set by Chondar (1994) was adopted. The fully ripped males were selected based on the out coming of milt freely. When a gentle press was applied on their belly, where as females were selected on the basis of reddish ventral region with more rounded and bulging abdomen. It was considered during the selection procedure that all selected breeders should be of moderate size because of little or no deposition of fat contents in the body cavity they were fed with formulated diets.

Method for administration of ovaprim: In common practice there are two modes of administration of ovaprim i.e. peritoneal (within the body cavity) at the ventral part of the fish body and intramuscular (within the musculature) at the dorsal part of the fish body. The method used by Jhingran and Pullin (1985) was adopted to inject ovaprim in the present experiment. The scales of experimental breeders were lifted for the insertion of injection needle. The angle to the base of pectoral fin was between 45° - 90°. A moist towel was also used to wrap the examined fish for further steps.

Calculation of dosage of ovaprim: Ovaprim (Syndel Laboratories Ltd, Canada) was procured from local scientific store in liquid form (10 mL) contains 20 µg of Salmon GnRHa and 10 mg of doxeridon (Brzuska and Bialowas, 2002; Afzal *et al.*, 2008). These active ingredients then dissolved in propylene glycol as suggested by Peter *et al.* (1998). Eighteen females of *Cirrhina mrigala* (mean weight T1 = 3.307 ± 0.208, T2 = 3.588 ± 0.166, T3 = 3.653 ± 0.185) were divided into three groups as they were tested against three serially arranged doses of Ovaprim i.e. 0.3 mL/kg, earlier tried by Nandeeshia *et al.* (1990), 0.4 mL/kg by Das (2006) and 0.5 mL/kg by Nandeeshia *et al.* (2009). Side by side six males were also injected at the rate of 0.2 mL/kg of body weight and the ratio of females and males were 2:1 (Naeem *et al.*, 2005).

Ovulation and spawning: The ovulation process in fish is certainly a stress full phenomenon. It is followed by excitement, restless movement, active swimming or jumping behavior. The tested individuals exhibited these signs just one hour after the administration of Ovaprim. The spawning started after eight hours of injection by intermittent splashing in circular tank when male chased the females. This actively chasing was prolonged for approximately 30 - 60 minutes. All the stocked fish were matted out for hand stripping. Every male was held gently and wrapped in a moist towel. Slightly press was applied on the abdominal region which resulted as some eggs oozed out from the urinogenital pore. Subsequently all injected females were stripped and their eggs were collected in the dry plastic container already tagged with respect to their numbers. Side by side induced males were stripped for obtaining milt as done by Chaudhuri *et al.* (1966) and Naeem *et al.* (2005). A large sized quill was used to mix milt and eggs vigorously for 15-30 second before washing with normal saline and tannin solution. Washing lasted for about one hour during which eggs increased in size due to absorption of water via vitelline membrane. Three fertilized egg samples (one gram) were separated out from the plastic container for counting and weighing. The total number of eggs, number of fertilized eggs and number of hatchlings (hatched eggs) for each fish were determined following Mollah *et al.* (1990):

- Number of eggs spawned = Average number of eggs in one gram of sample × Total weight of eggs
- Fertilization = Number of fertilized eggs / Total number of eggs (fertilized + unfertilized) × 100
- Hatching = Number of eggs hatched / Total number of eggs × 100
- Fecundity per kg B.W. = Number. of eggs stripped / Body weight of fish (Dorafshan *et al.*, 2003).

RESULT AND DISCUSSION

Figure 1 presents the weight of males and females fishes grouped into three groups of males with three individuals in each group and three groups of females with six individuals in each group. One group of females was treated with 0.3 mL/kg, and other groups with 0.4 mL/kg and 0.5 mL/kg ovaprim. The males were treated with 0.2 mL/kg B.W. dose of Ovaprim. At the time of latency period, spawning activities were started by both sexes. Some excitement, restless movements and active swimming or jumping behavior were observed at this time. The same indications were also reported by Haniffa *et al.* (2002). They noted the courtship behavior took place at the bottom. All males showed their participatory action and touched females frequently. Males made a circular movement around the females during mating, male aligned on either side and rubbed the body against the female and released milt. The adhesive eggs were discharged to accomplish spawning. The same observations were put forward by Naeem *et al.* (2005), during the hand stripping of silver carp against a single dose of Ovaprim-c. The reproductive performance of *Cirrhina mrigala* against the single dose of Ovaprim is categorized as weight of eggs from each female and their counting.

The mean weight of eggs in T1 treated females was 227.8 ± 14.4g in females treated with 0.3mL/kg B.W. ovaprim, 272.2 ± 12.7 g in treatment T2 (0.4mL/kg B.W. ovaprim) and 326 ± 16.6g in fishes treated with 0.5 mL/kg B.W. (T3). These quantity of eggs under the influence of Ovaprim are in the line with the study of Bhuiyan *et al.* (2006), an artificial breeding of *Puntius gonionotus* 100 % release of eggs were noted.

The mean number of eggs released in T1 was 211627 ± 13333, in T2 254772 ± 11800 and in T3 303227 ± 15389. Here it is an increasing trend with respect to body weight of females. The same pattern of releasing number of eggs against the administration of ovaprim in major carps and it was claimed by Panday and Singh (1997). 100 % spawning was happened along with 100 million number of eggs were obtained. Likewise present spawning rate Kumara and Seneviratne (1988) also obtained 100,000 eggs from grass carp and 120,000 eggs from big head carp by using LHRH-a, HCG and PG. Exactly the same attributions were suggested by Mirza *et al.* (1994) from 60 females of *Catla catla*, *Labeo rohita* and *Cirrhina mrigala*. The mean body weight was 3.75, 1.93 and 1.58 kg respectively. All females were treated by CPH and HCG as a single dose. The Rohu females release 96,000 eggs and Mrigal and Catla released 92,000 eggs.

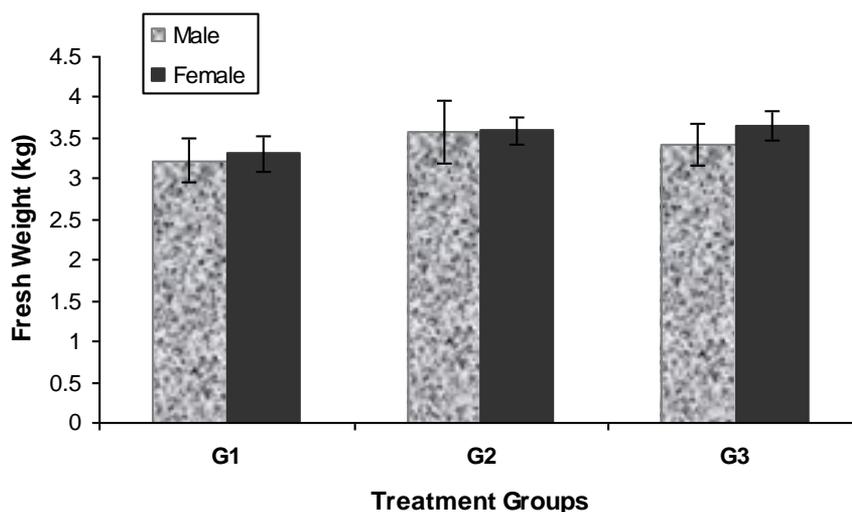


Fig. 1. Mean fresh body weight of male (N=3) and Female (N = 6) fishes of three treatment groups (G1, G2, and G3). All males were treated with 0.2 mL Ovaprim / kg body weight and the females of the three groups were treated as follows: G1 = 0.3 ML Ovaprim per kg body weight, G2 = 0.4 mL Ovaprim per kg body weight and G3 = 0.5 mL per kg body weight. Average similarity on body weight amongst three groups of males was $91.64 \pm 1.91\%$ and amongst three groups of females was $88.70 \pm 2.10\%$ on the basis of Czekanowski's (1913) index.

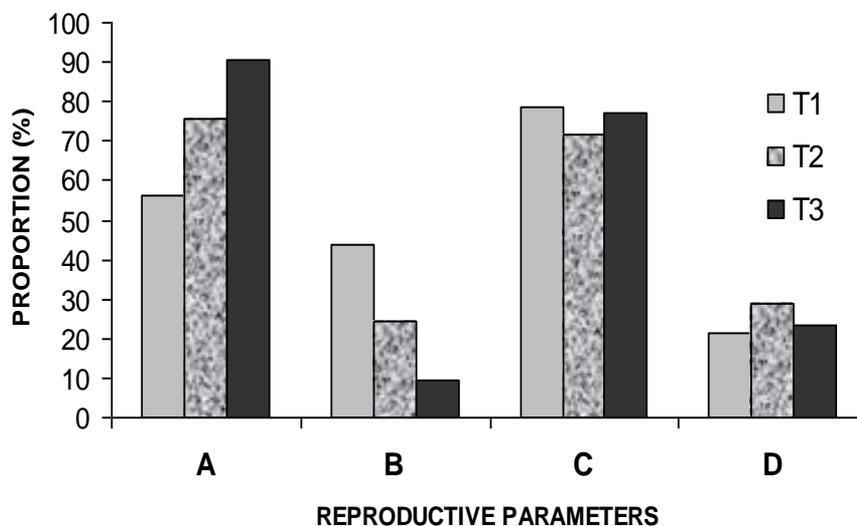


Fig. 2. The proportion of various reproductive parameters under three doses of ovaprim (T1-T3). A, Proportion of fertilized eggs to the total number of eggs; B, proportion of unfertilized eggs to the total number of eggs; C, Proportion of hatched eggs to the total number of fertilized eggs and D, Proportion of unhatched eggs to the total number of fertilized eggs.

All the under examined females in T3 were capable to producing maximum number and proportion of fertilized eggs i.e., 273841 ± 14101 and over 90 % respectively as the average values (Fig. 2). The females of T2 ranked next to T3 showing 193205 ± 9438 number of egg and 75.83 % of fertilization. The much less efficiency in terms of these parameters was noted by females of T1 i.e., 119233 ± 23309 and $58.34 \pm 1.20\%$. These factors are supposed to be decisive that T3 (0.5 mL/kg) is a well reporting dose of ovaprim (Table 1). These findings supported the study of Panday and Singh (1997). They tried 0.2 mL/kg of ovaprim to *Catla catla*, *Labeo rohita* and *Cirrhina mrigala*. The fertilization was obtained between 65-98% with an average of 80%. Moreover, the findings of More *et al.* (2010) was found to be same i.e. the 88.11% to 97.11% of fertilization noted when ten breeding trials were carried out by administration of ovaprim to Indian major carps including *Cirrhina mrigala*. Naem *et al.* (2011) claimed 73.92% of fertilization when Silver carp were tested and suggested that ovaprim-c is a well suited alternative to other inducing hormones. The result put forward by Rokade *et al.* (2006) was in line of present findings. They attributed the satisfactory performance with significant increase in rate of fertilization as maximum as 91% and suggested that ovaprim is clearly best spawn of major carps. Haniffa *et al.* (2000) tried both natural and synthetic hormone on other warm blooded fishes i.e., *Channa striatus*. PE and HCG tried as natural hormone while LHRH and ovaprim as synthetic hormone. PE took long time to respond and exhibited the lowest fertilization rate. Among rest of HCG, LHRH and ovaprim, eggs diameter and high fertilization rate revealed that ovaprim was the superior one compared to other tested hormones.

Table 1. Effects of Ovaprim on spawning of *Cirrhina mrigala*.

Parameters	T1 (Mean ± SE)	T2 (Mean ± SE)	T3 (Mean ± SE)
Weight of eggs (gm)	227.8 ± 14.4	272.2 ± 12.7	326.2 ± 16.6
Number of eggs	211627 ± 13333	254772 ± 11800	303227 ± 15389
Number of fertilized eggs	119233 ± 23309	193205 ± 9438	273841 ± 14101
Number of unfertilized eggs	92394 ± 6018	61567 ± 2598	29386 ± 2230
Number of hatched eggs	93685 ± 7984	137541 ± 7449	210460 ± 10597
Number of unhatched eggs	25548 ± 1353	55664 ± 2981	63381 ± 4011
Number of hatchlings	93685 ± 7984	137541 ± 7449	210460 ± 10597
Working fecundity / kg B.W.	64000	76283	83000

T1, 0.3 mL/kg; T2, 0.4 mL/kg and T3, 0.5 mL/kg

The number of unfertilized eggs was counted as mean values against each treatment. Much high quantity of unfertilized eggs were counted i.e., 92394 ± 6018 in T1 followed by T2 (61567 ± 2598) and T3 (29386 ± 2230). When these figures were analyzed as their percentage from total number of eggs produced. Only a small figure came out i.e. 9.6 ± 0.521 % in T3 indicating much less degree of spoilage or deterioration compared to 23.18 ± 0.57 (T2) and 33.85 ± 1.2 (T1). These high rates of unfertilized eggs were probably due to bacterial activities and mishandling. The findings of Lin *et al.* (2000) reflect these kinds of lapses during the study of induced breeding of grass carp. They developed an automated system to handle fertilized eggs to reduce the risk of spoilage as well as to reduce the economics and improve water quality as well. Khan *et al.* (2006) compared efficacy of ovatidae and ovaprim and inject to *Labeo rohita*. They found comparatively less rate of fertilized and high numbers of opaque and translucent eggs, ovatidae was much effective than ovaprim. According to Mortezaavizadeh *et al.* (2009) likewise efficacy of ovatidae, carp pituitary extract (CPE) may be proven as good as ovatidae regarding the production of maximum number of viable eggs. These observations are contradicted to present observations.

The hatched eggs were successfully fertilized and ready to go for their embryonic development. From all the six females that were treated with T1 released 93685 ± 7984 numbers of hatched eggs, in T2, 137541 ± 7449 and T3, 210460 ± 10597 (the maximum number of eggs). The presented contributions of proportion hatching of eggs were in T1, 78.57 ± 1.26, in T2 71.89 ± 0.83 and in T3, 76.89 ± 0.67. Afzal *et al.* (2008) tested ovaprim and ovatidae to obtain high percentage of hatched eggs and numbers of fry to fingerlings. Those females who were treated with ovatidae showed significantly lower values. The similar efforts were also taken by Naeem *et al.* (2011) who elucidate the high rate of hatching (73.92 %) with 18-24 hours of latency period. However, Bhattacharyya and Homeochuduri (2009) showed some reservations regarding the use of ovaprim to non-carp species although they obtained better spawn with significantly higher rate of fertilization, but the brooders experienced high mortality rates. The ready product for embryonic development can be maximizing by keeping all a biotic preservatives measures as done by Dwivedi and Reddy (1986) in CIEF-D81 type of carp hatchery. In contrast to the ovaprim usage Bhatti *et al.* (1994) tried 220 IU of HCG / kg of body weight to grass carp females initially and then 1600 IU as secondary dose. The 76 - 90 % of hatching was noticed consist of 96,000 and 89,000 eggs.

There were some failures noted in case of hatched eggs and counted unhatched eggs. These eggs were fertilized successfully but not proceeded to next steps of embryonic development. It was concluded from the facts mentioned in table 2 that females treated with T3 over all produced large quality of eggs out of them maximum were preceded to developmental steps and only 63647 ± 4031 eggs were spoiled. The percentage of spoilage was much less i.e. 23.20 ± 0.67. As far as the rates and numbers of spoilage were concern in T2 and T1, 56508 ± 3061 and 46714 ± 2807 spoiled eggs were counted, respectively. The percentage compositions were 28.88 ± 0.833 and 33.57 ± 1.26 for T2 and T1. These results are in line with the findings of Naeem *et al.* (2011) who reported 26.08 % of deterioration of eggs against the administration of ovaprim-c in silver carps.

The hatchlings resulted after passing embryonic developmental stages. The hatchlings produced by six tested females, in T3 were in large numbers (210460 ± 10597) compared to T2 (137541 ± 7449) and T1 (93685 ± 7984). There was a marked difference noted at this stage which reflect light on the efficacy of T3 and proven that all females respond brightly in every manner right from the beginning to the production of hatchlings. As the T3 results were appreciable, the findings of Afzal *et al.* (2000) are also confirming the effectiveness of ovaprim in major carps. The female exhibit substantially greater number of viable eggs converted to fry after proceeding through metamorphosis. In contrast to it, a finding was recorded by Kahkesh *et al.* (2010) who worked on *Barbus*, a member of family cyprinidae. The reproductive response consists of spawning rate, latency period, fecundity, fertilization and hatching rates. The drug was used by them were ovaprim, ovatidae, HCG, LHRH-A2, CPE and LHRH-A2 + CPE. Best drug was proven by accessing rate of hatchings produced and fecundity i.e., the mixture of LHRHA + CPE. In the continuation of contradiction to ovaprim Lee *et al.* (1988) concluded that HCG can be a better replacement of ovaprim. However, the cost and response are not up to the mark. Das *et al.* (2006) argued the rate of development of fertilized eggs toward hatching stage. They clearly indicated that temperature significantly influenced the hatching rate not the quantity of synthetic hormone. Mirza *et al.* (1994) tried mixture of carp pituitary homogenetic (CPH) and HCG in major carps. They noticed that 75 - 90 % of fertilization along with the hatching power of 80000, 67000 and 74000 eggs out of 96000 from Rohu and 92000 from

Catla and Mrigal, respectively. Bhatti *et al.* (1994) obtained 110,000 and 97,000 eggs of grass carp by injecting HCG @ 220 IU / kg as first dose and 1600 IU / kg as second dose. Out of which 76 – 90 % of hatchlings were produced equally to prove that HCG can be another drug to produce substantial results.

Working fecundity is, in fact, the actual numbers of viable eggs that can be converted eggs to hatchling stage. In T1 it was 64000, in T2, 76283 and into T3, 83000 eggs. The difference among all treatments was correlated with the body weight of females.

Multiple correlation and regression analysis for egg weight and number of eggs as influenced by ovaprim doses and body weights of female fish is presented in Table 2. It is evident from the correlation studies that both body weight of fish and ovaprim dose had significant effect on egg weight and number of eggs.

Table2. Regression equations for egg weight and number of eggs as influenced by ovaprim dose and body weight of fish.

Loge Egg Weight (g) = -81.240 + 392.781 ovaprim + 57.876 Body weight ± 15.282			
	t=-3.06	t=8.58	t=8.22
	p < 0.008	p < 0.0001	p < 0.0001
Zero correlation =		0.905	0.757
Partial correlation=		0.911	0.776
R= 0.963, R ² = 0.928 Adj. R ² =0.918, N=18, F=96.22 (p < 0.0001)			
Loge Numbers of Egg =11.103 + 1.439ovaprim + 0.219 Body weight ± 0.5984			
	t=106.83	t=8.03	t=7.94
	p < 0.001	p < 0.0001	p < 0.0001
Zero correlation =		0.768	0.768
Partial correlation=		0.901	0.899
R= 0.959, R ² = 0.921 Adj. R ² =0.910, N=18, F=96.22 (p < 0.0001)			

The present results are in accordance with the findings of Naeem *et al.* (2005) who reported that total body weight influences the weight of eggs released. However, Brzuska and Bialowas (2002) reported that statistically the weight of eggs have no significant effect on weight of eggs. However, better fecundity rates along with percentage survival and hatching can be achieved against mixture of LHRHA + CPE in a member of cyprinidae family i.e. Barbus. Tyler and Sumpter (1996) noted that fecundity rate was depended over genetics and pattern of gametogenesis. The same suggestions were also put forward by Naeem *et al.* (2005), that body weight have influence on total number of eggs and number of eggs/kg which means significant influence on absolute fecundity.

The out comes from above mentioned results may be concluded that if a particular female is heavy means, its body weight is enough than it will produce more mass of eggs, more number of eggs and ultimately after fertilization more number of hatchlings can be produced with considerable working fecundity but rate of fertilization and rate of hatching have not any effect on the seed production. The reason is that the whole experiment is based on artificial mixing by hand and there is a great chance to improper mixing of eggs and milt. The eggs which were transferred by sperm got fertilized status and those who failed to treat as unfertilized status. The same attribution for number of eggs verses contributing variables. If more number of eggs are available then more number of hatchlings produced along with maximum working fecundity.

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