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Fecundity and Induced Spawning of Silver Carp, *Hypophthalmichthys molitrix* by Using a Single Intramuscular Injection of Ovaprim-C at Fish Hatchery Islamabad, Pakistan

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Abstract: In the present study effect of intramuscular injection of Ovaprim-C was studied on the number of eggs kg^{-1}, fertilization rate and hatching percentage during May, 2002 in fish hatchery Islamabad on silver carp, *Hypophthalmichthys molitrix*. Fish spawned successfully following a single dose of injection of ovaprim (LH-RH analogue) with 0.6 mL kg^{-1} for female and 0.2 mL kg^{-1} for male. Ova and milt were stripped simultaneously and mixture was stirred for 15-30 sec during which fertilization occurred. Hatching occurred within 18-32 h after fertilization. Regression analysis was applied to assess the body weight dependence of absolute and relative fecundity. It was observed that body weight has positive influence on absolute ($r = 0.990$) and relative fecundity ($r = 0.708$). Equations were developed to describe these relationships. If it is impossible to determine the absolute and relative fecundity directly, then these parameters can be determined from the body weight. The equations of these parameters are highly significant ($p<0.001$) and can be used to estimate the absolute and relative fecundity with a fair amount of accuracy

Key words: Induced spawning, fecundity, Ovaprim-C, LHRH, gonadotropin, fishes, silver carp, *Hypophthalmichthys molitrix*

INTRODUCTION

In Pakistan, the carp culture is rapidly expanding but the non-availability of quality fish seed is one of the major constraints in the development of this industry. Major breakthrough achieved by Chaudhry and Alikunhi[1] in induced breeding of Indian major carps using pituitary extract has greatly contributed to the rapid development of carp culture. Although technique of hypophysation is practiced successfully but there are certain problems, which have prevented it being taken up widely by fish farmers.

In recent years, Human Chorionic Gonadotropin (HCG) has received some attention as a substitute for pituitary but has met with little success, except in the breeding of silver carp[2].

Lutinising releasing hormone (LH-RH), a mammalian hypothalamic peptide, has the capacity to release gonadotropin from pituitary gland[3]. The Chinese report on successful use of mammalian based LH-RH analogue (D-Ala_6, Pro_9, Net) for induced breeding of carps created world wide interest on the use of LH-RH for breeding various species of fish[4]. A major break through in fish breeding research was the finding that dopamine, acts as an inhibitory factor for synthesis of gonadotropin[5]. When LH-RH was used alone, without Pituitary Gland, spawning failure clearly indicates that dopamine blocks the action of LH-RH on the secretion of gonadotropin. Thus blocking of dopamine action with some antagonists like pimozide, potentiate the action of LH-RH resulting in successful spawning[6].

Among the various analogues of salmon releasing hormones D-Arg_6, Trp_7, Leu_8, Pro_9 and Net has been found to be highly effective and this particular analogue is used in Ovaprim. This high effectiveness of salmon releasing hormone is due to its higher affinity for binding sites in the pituitary[7]. Syndel Laboratories Ltd, Canada developed Ovaprim, that contains an analogue of salmon gonadotropin releasing hormone (D-Arg_6, Pro_9 and Net) and dopamine antagonist[8].

There has been considerable research in India on spawning of carps with ovaprim. Kaul and Rishi[9] reported the successful spawning of mrigal. There are interesting observations reported by other workers in review of Nandeesha *et al.*[8] During 1989, trials were conducted in nine states of India to understand the efficacy of this drug in different agro climatic-regions and reported the effect of ovaprim in comparison with pituitary. Khan *et al.*[10] reported the successful spawning of rohu and mrigal with ovaprim (LH-RH analogue) at Fish Hatchery Islamabad,

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Pakistan. The present study is the outcome of breeding trials carried out at Fish Hatchery and Research Center (FHRC) Islamabad on silver carp, *Hypophthalmichthys molitrix*.

MATERIALS AND METHODS

A total of 30 silver carp, *Hypophthalmichthys molitrix* brooders weighing from 2.0-4.2 kg were used for this study conducted in May 2002. Fish were selected from stocks reared in 2.5 ha reservoir at Islamabad Hatchery.

Selection and handling: Females with soft, distended belly and pink-red genital papilla and males, which released milt when subjected to gentle pressure on the abdomen, were selected. Fish were transferred into cemented holding tanks of fish hatchery and anesthetized with 100-120 ppm 2-phenoxyethanol solution in a 50 L fiberglass tank half, filled with tap water.

Hormone injection: Sex ratios of one female to two males were used per spawning trial. Fishes were injected ovaprim intramuscular in a single dose to both sexes following protocol of Nandeesha *et al.*[8] Males were injected 0.1 mL kg^{-1} ovaprim, for female the dose was 0.6 mL kg^{-1}.

Ovulation and spawning: Spawning behavior occurred at about 9-10 h after the single dose of Ovaprim-C. This was indicated by the intermittent splashing on the water surface as males chased the females. The activity lasted for about 30-60 min after that fish were netted out for stripping. A ripe female, which upon slight pressure on the abdomen, released some eggs from the urinogenital pore when fish was held ventrally. Females were stripped and eggs were collected in dry plastic bowl. In case of partial spawning, fish were returned to the spawning tank to ovulate further. One or two males were stripped simultaneously with a female to fertilize eggs following the semi-dry fertilization method[11]. Milt was mixed with the egg using a bird feather and stirred for 15-30 sec prior to washing; three one gram egg samples were weighed out and counted. The total number of eggs spawned was calculated by multiplying the average number of eggs from the three one gram samples with the total weight of eggs sampled.

Incubation: Breeding test were conducted in circular spawning tanks of 2 m diameter. Underground water with total hardness of not less than 174 ppm was used as hatching medium[12]. Percent fertilization rate expressed as the ratio of fertilized eggs to total eggs counted (n = 100) from each of three sampling of known volume (eggs+water) was determined 6-7 h post incubation period (early blastula stage). Hatching occurred after 18-24 h at water temperature of 24.8-25.8°C, hatching rate was then determined. Newly hatched larvae remained in the circular spawning tank for three days until yolk was absorbed. Water quality parameters during experiments were given in Table 7.

RESULTS AND DISCUSSION

Fish given single injection of Ovaprim-C were successfully induced to spawn (Table 2). Thirty females were injected with Ovaprim-C; ovulation of fish in these treatments was 100%. Total number of obtained eggs was 91778 kg^{-1}, fertilization and hatching percentage was 72.56 and 71.09, respectively (Table 1).

Absolute fecundity and relative fecundity were found to be related to body weight in silver carp. Regression analysis was applied to assess the body weight dependence of these variables.

Body weight has a positive influence on absolute fecundity (Table 3). Relative fecundity increases with increasing body weight (Table 4). Each of these relationships are statistically significant ($p<0.001$) and well described by a linear equation.

$$Y = a + bX$$

Where, a and b are constant, X is the body weight and Y is dependent variable.

Table 1: Effect of Ovaprim-C on spawning of silver carp, *Hypophthalmichthys molitrix*

Parameters	Ovaprim treatments
No. of females treated	30.00
Total weight of females (kg)	86.30
Total No. of eggs	7998500.00
Total No. of fertilized eggs	5785000.00
Total No. of hatchling	4112800.00
Overall fertilization percentage	72.56
Overall hatching percentage	71.09
Average No. of eggs/kg	91778.00
Average No. of fertilized eggs kg^{-1}	66559.00
Average No. of hatching kg^{-1}	48108.00

Table 2: Spawning response of female silver carp, *Hypophthalmichthys molitrix*

Month	Temperature (°C)	No. of females	Total weight of females (kg)	Dose of ovaprim (mL kg^{-1})	No. of eggs	Fertilization rate	No. of hatching
May, 2002	25.0	10	27.6	0.6	2530000	1705000	1157000
May, 2002	25.6	8	24.4	0.6	2300000	1640000	1170000
May, 2002	24.8	5	14.2	0.6	1340000	990000	735800
May, 2002	25.8	7	20.1	0.6	1828500	1450000	1050000

Table 3: Statistical parameters of various relationships involving body weight versus total number of eggs of silver carp, *Hypophthalmicthys molitrex*

Relationships	r	a	b	SE (b)
Wet body weight (x) Total No. of eggs (y)	0.990***	-5037	1101	2907

Table 4: Statistical parameters of various relationships involving body weight versus number of egg kg^{-1} of silver carp, *Hypophthalmichthys molitrix*

Relationships	r	a	b	SE (b)
Wet body weight (x) No. of egg kg^{-1} (y)	0.708***	7474	5920	1115

Table 5: Statistical parameters of various relationships involving log wet body weight versus log total number of eggs of silver carp, *Hypophthalmichthys molitrix*

Relationships	r	a	b	SE (b)
Log wet body weight (x) Log total No. of eggs (y)	0.987***	4.8753	1.1933	0.0361
Log wet body weight (x) Log No, of eggs kg^{-1} (y)	0.710***	4.8753	0.1933	0.3614

***p<0.001, Correlation coefficient (r), intercept (a), regression coefficient (b), standard error of b (SE) and probabilities (p), n = 30 in each

Table 6: Dosage of Ovaprim-C for carps at different locations

Fish species	Dose of Ovaprim-C for (♀)	References
Catla catla	0.4-0.5	[8]
Labeo rohita	0.3-0.4	[8]
Labeo rohita	0.4	[10]
Cirrhina mrigala	0.25-0.3	[8]
Cirrhina mrigala	0.4	[10]
Hypophthalmichthys molitrix	0.4-0.7	[8]
Ctenopharyngodon idella	0.4-0.8	[8]
Aristichthys nobilis	0.4-0.5	[8]
Hypophthalmichthys molitrix	0.6	Present study

Table 7: Mean values and ranges of various water quality parameters during the induced spawning experiments of silver carp, *Hypophthalmichthys molitrix*

Parameters	Mean±SD	Range
Water temperature (°C)	25.30±00.47	24.8-25.8
pH	8.27±00.17	8.1-8.5
DO (mg L^{-1})	7.60±00.45	7.0-8.0
Carbonate (mg L^{-1})	28.25±02.87	26.0-32
Bicarbonate (mg L^{-1})	157.00±18.49	137.0-176
Total carbonate (mg L^{-1})	185.25±19.65	166.0-208
Calcium (mg L^{-1})	161.50±13.62	145.0-175
Hardness (mg L^{-1})	183.25±07.63	174.0-190
Chloride (mg L^{-1})	23.75±02.50	21.0-27
TDS (mg L^{-1})	587.00±41.23	548.0-644

When total values of absolute fecundity and relative fecundity of silver carp, *Hypophthalmichthys molitrix* were transformed into log-log scale, a linear relationship of the form:

$$\text{Log } Y = a + b \log X$$

was obtained showing a high degree of correlation (Table 5).

Statistical analysis, including regression analysis and calculation of correlation was carried out by using a computer package EXCEL following Zar[13].

Results of this study showed that successful induction of spawning in silver carp is achieved by using a single dose of Ovaprim-C. Certain drugs and different analogues of LH-RH are being tested for breeding fishes with varying degree of success[14]. However, it was only when the dopamine inhibitory activity in the synthesis of gonadotropin was demonstrated[6] that the reason behind the spawning failures became clear. Investigations have now clearly shown the potentiated actions of analogues when they are combined with dopamine antagonists like pimozide or doperidon[15-19]. Based on the extensive research on Chinese carp, Peter *et al.*[5] defined a new method of breeding called the Linpe in which LH-RH analogue is combined with a dopamine antagonist. It is major break through in the history of aquaculture. Ovaprim is a new drug developed essentially on this new method combining releasing hormone with dopamine antagonist. Earlier studies conducted in India, Nandeesha *et al.*[8] and Khan *et al.*[10] has clearly demonstrated superiority of ovaprim in induced spawning of major carps. Dosage of Ovaprim used for carps at different locations are given in Table 6.

Silver carp does not appear to be a difficult fish to spawn under local conditions. Dose of Ovaprim-C used was 0.6 mg kg^{-1} for female. This was based on report of Nandeesha *et al.*[8] and Peter *et al.*[5] who reviewed dopamine activity in various fish species and indicated that it may vary considerably between species. The positive response of both male and female bighead carp to a single simultaneous injection of Ovaprim-C is very significant from the point of view of commercial carp seed production, as it saves a considerable amount of time and avoids excessive handling of brood fish[8]. Present experiments showed that silver carp mature within three years and preferably spawn during mid May to June (Table 2). Fertilization rate was 72.56% and while hatching percentage was 71.09%, favorable temperature for incubation was 24.8 -25.8°C (Table 2).

Generally, the number of eggs spawned by silver carp in the present study was lower than previously reported[20-22]. This may be due to the low nutritional status of the brood fish as brood stocks depended completely on the natural productivity of the reservoir and were not given artificial feed throughout the rearing period. Ovaprim-C being ready to use form will simplify the breeding operation and hence can be easily adopted in carp seed production. Further trials are needed to standardize the dosage for adoption on a commercial scale.

Although the several investigator[8,10] have published on fecundity and successful induction of spawning

ovaprim but no study on silver carp have yet attempted to correlate the absolute and relative fecundity with weight. Absolute and relative fecundity increases with increasing body weight of the fish. In many species fecundity is shown correlate with body weight, total length and standard length and is used to measure the relationship between number of eggs and weight of fish[23,24]. It is concluded that if it is impracticable to estimate the absolute and relative fecundity of silver carp the body weight will provide satisfactory estimation of these variables using predictive regression models developed in this study a reasonable amount of accuracy.

Based on our experiments with Ovaprim, it is therefore concluded that ovaprim usage may have the following advantages over that of commercial pituitary:

- Reduced handlings of brood fish due to the single injection given to both the sexes simultaneously. This not only well decrease/avoid post spawning mortality of fish but also increase spawning response.
- Additional studies may provide valuable information on the growth of hatchlings.
- Additional experiments are essential to confirm the impact of Ovaprim on the growth and survival of hatchling.

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