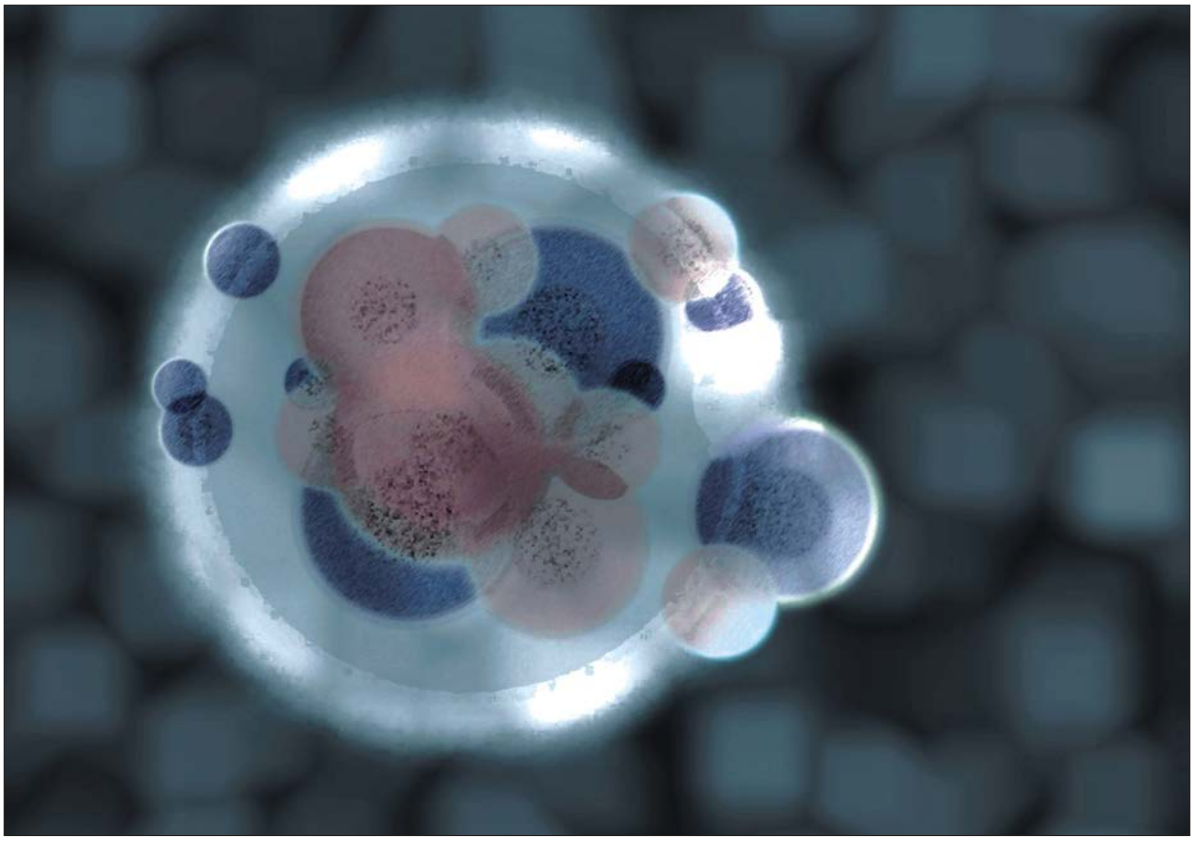

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Induced Spawning of Major Carp *Catla catla* by a Single Intramuscular Injection of Ovaprim-C and Fecundity at Fish Hatchery Islamabad, Pakistan

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Abstract: In the present study 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 Thaila, *Catla catla*. Fishes were spawned successfully following a single dose of injection of ovaprim (LH-RH analogue) with 0.7 mL kg^{-1} for female and 0.2 mL kg^{-1} for male, ova and milt were stripped simultaneous and mixture was stirred for 15-30 sec during which fertilization occurred. Hatching occurred within 18-32 h after fertilization. Experiment was conducted in circular spawning tank with 2m diameter. Regression analysis was applied to assess the body weight dependence of absolute fecundity (total number of eggs) and relative fecundity (number of eggs kg^{-1}). It was observed that body weight has positive influence on absolute fecundity ($r = 0.983$) while relative fecundity remains fairly constant with increasing body weight ($r = 0.453$). Equations were developed to describe these relationships. If it is impossible to determine the relative fecundity then it 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 relative fecundity with a fair amount of accuracy.

Key words: Induced spawning, fecundity, Ovaprim-C, LHRH, gonadotropin, fishes, thaila, *Catla catla*

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 Alikunki[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 Hormone Releasing Hormone (LH-RH), a mammalian hypothalamic peptide, has the capacity to release gonadotropin from pituitary gland[3]. 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, 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, 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 Nandeeshas *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[9]. Khan *et al.*[10] reported the successful spawning of rohu and mrigal with Ovaprim (LH-RH analogue) at Fish Hatchery Islamabad, Pakistan. The present study reported the outcome of breeding trials

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carried out at Fish Hatchery and Research Center (FHRC) Islamabad on Thaila, *Catla catla*.

MATERIALS AND METHODS

A total of 22 Thaila, (*Catla catla*) brooders weighing from 2.2-6.5 kg were used for this study conducted in June 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 and for female the dose was 0.7 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 egg samples 1 g were weighed out and counted. The total number of eggs spawned was calculated by multiplying the average number of eggs from the three 1 g sub 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 209 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 27.1-28.5°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 noted and recorded as shown in Table 1.

Table 1: Mean values and ranges of various water quality parameters during the induced spawning experiments of Thaila, *Catla catla*

Parameters	Mean±S D	Range
Water temperature (°C)	27.85±0.580	27.1-28.5
pH	8.35±0.38	8.1-8.6
D.O (mg l^{-1})	7.370±0.35	7.0-7.8
Carbonate (mg l^{-1})	24.50±2.64	21.0-27
Bicarbonate (mg l^{-1})	121.75±10.43	111.0-136
Total carbonate (mg l^{-1})	148.75±11.64	138.0-162
Calcium (mg l^{-1})	168.25±10.275	155.0-180
Hardness (mg l^{-1})	193.50±15.11	175.0-209
Chloride (mg l^{-1})	24.25±1.89	23.0-27
T.D.S (mg l^{-1})	661.25±18.08	639.0-680

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 67670 kg^{-1}, fertilization and hatching percentage was 91.01 and 67.50, respectively (Table 3).

Absolute fecundity was found to be related to body weight while relative fecundity remains constant in *Catla catla*. Regression analysis was applied to assess the body weight dependence of these variables.

Body weight had a positive influence on absolute fecundity (Table 4). Relative fecundity remained constant with increasing body weight (Table 5). Each of these relationships were 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.

When total values of Absolute fecundity and relative fecundity of Thaila, *Catla catla* were transformed into log-log scale, a linear relationship of following form was obtained showing a high degree of correlation (Table 6):

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

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 Thaila, *Catla catla* was achieved by using a single dose of Ovaprim-C. Certain

Table 2: Spawning response of female Thaila, *Catla catla*

Months	Temperature (°C)	No. of females	Total weight of) females (kg	Dose of Ovaprim (mL kg^{-1})	No. of eggs (Million)	Fertilization rate (Million)	No of hatching (in Million)
June, 2002	26.0	5	26.5	0.7	1.835	1.641	1.201
June, 2002	26.0	9	32	0.7	2.225	2.045	1.39
June, 2002	26.5	4	15.8	0.7	1.0755	0.981	0.63
June, 2002	26.0	4	10.9	0.7	0.6805	0.625	0.387

Table 3: Effect of Ovaprim-C on spawning of Thaila, *Catla catla*

Parameters	Ovaprim treatments
No. of females treated	22
Total weight of females (kg)	85.2
Total No. of eggs	5816000
Total No. of fertilized eggs.	5292000
Total No. of hatchling	3608000
Overall fertilization percentage	91.01%
Overall hatching percentage	67.50%
Average No. of eggs kg^{-1}	67670
Average No. of fertilized eggs kg^{-1}	61620
Average No. of hatching kg^{-1}	41584

Table 4: Statistical parameters of various relationships involving body weight versus total number of eggs of Thaila, *Catla catla*

Relationships	r	a	b	SE (b)
Wet body weight (x)				
Total No. of eggs (y)	0.983***	-33928.5	77027.81	3193.21

***p< 0.001

correlation coefficient (r), intercept (a), regression coefficient (b), standard error of b (S.E.) and probabilities (p), n = 22 in each case

Table 5: Statistical parameters of various relationships involving body weight versus No. of egg kg^{-1} of Thaila, *Catla catla*

Relationships	r	a	b	SE (b)
Wet body weight (x)				
No of egg kg^{-1} (y)	0.453 $^{n.s}$	59472.4	2116.75	929.409

***p < 0.001, correlation coefficient (r), intercept (a), regression coefficient (b), Standard Error of b (SE) and probabilities (p), Non-Significant (NS), n = 22 in each case

Table 6: Statistical parameters of various relationships involving body weight versus total number of eggs of Thaila, *Catla catla*

Relationships	r	a	b	SE (b)
Log wet body weight (x)				
Log total No. eggs (y)	0.976***	4.7520	1.1346	0.0563
Log wet body weight (x)				
Log No. of eggs kg^{-1} (y)	0.471 $^{n.s}$	4.7520	0.1346	0.0563

***p < 0.001, correlation coefficient (r), intercept (a), regression coefficient (b), Standard Error of b (SE) and probabilities (p), Non-Significant (NS), n = 22 in each

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

Table 7: 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]
Catla catla	0.7	Present study

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] had clearly demonstrated superiority of ovaprim in induced spawning of major carps. Dosage of Ovaprim used for carps at different locations are given in Table 7.

Thaila, *Catla catla* did not appear to be a difficult fish to spawn under local conditions. Dose of Ovaprim-C used was 0.7 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 thaila mature within three years and preferably spawn during mid May to June (Table 2). Fertilization rate was 91.01% and while hatching percentage was 67.50%, favorable temperature for incubation was 27.1-28.5°C (Table 2).

Generally, the number of eggs spawned by thaila 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 have published on fecundity and successful induction of spawning

Ovaprim[8,10] but no study on *Catla catla* have yet attempted to correlate the absolute and relative fecundity with weight. Absolute and relative fecundity increased with increasing body weight of the fish. In many species fecundity was shown to 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 was concluded that if it is impracticable to estimate the absolute and relative fecundity of *Catla catla*, the body weight will provide satisfactory estimation of these variables using predictive regression models developed in this study with a reasonable amount of accuracy.

Based on present 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 will decrease/avoid post spawning mortality of fish but also will 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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