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Induced Spawning, Fecundity, Fertilization Rate and Hatchling Rate of Indian Major Carp *Catla catla* by Using Syntheting Hormone and Carp Pituitary Extract

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Abstract: In the present study during the period June –August 2009 applying the appropriate doses of the hormones ovaprim and carp pituitary extract was studied on the number of eggs/Kg body weight, fertilization rate and hatchling rate at Fish breeding center at Jaikwadi, Paithan Dist. Aurangabad. Fishes were spawned successfully by using appropriate doses of synthetic Ovaprim and carp pituitary extract. The percentage of fertilization ranged (92.00% -95.00%) was found with ovaprim treatment and (69.58% - 85.29%) with pituitary extract treatment. The percentage of hatchling ranged (89.31 – 94.21%) with Ovaprim treatment and (58.82% - 78.82%) with pituitary extract treatment.

Key words: Synthetic hormone ovaprim · Carp pituitary extract · Fish breeding center · *Catla catla*

INTRODUCTION

As the population of world continuously increasing, demand of fish products has been increased beyond total supply, resulting in a reduction of the global per capita capture fisheries. To fill the gap, aquaculture boomed. Currently, aquaculture is the fastest growing animal production sector in the world expanding at an average of 9.2% per year since 1970 compared to only 1.4% for captured fisheries and 2.8% for terrestrial animal meat products [1].

India is the third largest producer of fish in the world and second in inland fish production which contributes 45 per cent of total production in the country. The country's fish production was 28.34 and 17.89 lakhs tones respectively marine and inland fisheries sector in 2000. It has been projected that the marine resources potential is about 39 lakhs tones and inland 45 lakhs tones. The production pattern of inland fisheries indicated a contribution of 54.7 per cent by major carps followed by common carp (8.1 per cent), other carps (6.1 per cent), murrels (3.6 per cent), hilsa (2.2 per cent) and other fishes (25.3 per cent). Thus carps emerge as prime species in the inland sector [2].

With the progress in agriculture, human being simultaneous moved towards water recourses as an alternative for its exploitation where aquatic animals have become the matter of interest and curiosity. Scientist community has been conducting experiment on this line since long ago for the better yield of fish.

Induced breeding techniques are important in fishery development. The brooders are injected with spawning agent. The basic principle comes from biological mechanism propagation, because the extent ecological condition in ponds cannot satisfy the reproductive requirement of the brood fish.

Hypophysation technique consists of gonadotropin hormones secreted by the pituitary gland play an important role in maturation of the gonads and spawning in fishes. Hypophysation techniques consist of intramuscular injection of a suspension or extract of the fish pituitary gland.

This technique is based on the fact that the Gonadotropic Hormones (FSH and LH) secreted by the pituitary gland which, play an important role in maturation and spawning of fishes is called "Hypophysation" the Brazilian were the first to develop technique in 1930,

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followed by Russians who successfully applied this method to berrd sturgeons. This method is now popular in Europe, America and Asia.

Various induced breeding agents were reported for Indian major carps such as pituitary gland extract, human chorionic gonadotropin (HCG), sex steroid hormones, thyroid hormones, clomiphene citrate, sexovid, tamoxifen, ovaprim (sGnRHA), ovatide and pheromones etc.

MATERIALS AND METHODS

The experiments were carried during June 2009-August 2009 (breeding season) at fish breeding center Jaikwadi, Paithan, Dist. Aurangabad in Maharashtra state 55 km away from Aurangabad.

Brooders were collected from the stocking pond of fish breeding center at Jaikwadi. Healthy male and female were selected by external morphological characteristics for said experiments. Pituitary extract was injected intramuscularly in the dorsolateral region at the second installments to female. The first dose 0.2 - 0.4 ml/kg body weight was administrated to the female for promoting maturation and Second dose 0.6 - 0.8 ml/kg body weight was administrated to the female for inducing spawning after 6 - 8 hrs. Where as a single dose of ovaprim was administered to the both male and female to induce spawning.

Injected brooders were released in a breeding pool. Experimental brooders were observed for 36 hrs after injection; at interval of 4 to 6 hrs the fishes gave response to behavioral changes, ovulation and spawning.

To carry out the induced breeding experiments, facilities of Chinese circular hatchery, stocking pond, breeding pool and incubation pool were availed to us by Govt. of Maharastra, Fishery Development Officer with their kind co-operation during the course of research work.

Chemicals: In the present experiment synthetic hormone Ovaprim was used to induce final maturation. Spawniong, fecundity, fertilization rate and hatchling rate is compared with pituitary extract.

Ovaprim (sGnRHA): Ovaprim is a synthetic drug (spawning hormone for fish) manufactured by M/s Syndel Laboratories Ltd., Canada, containing 20 µg sGnRH (salmon gonadotropin releasing hormone) and 10 mg domperidone in 1 ml solution.

Preparation of Pituitary Extract: The pituitary glands were collected from Indian major carp *Catla Catla* in the month of June to August. To gain access to the pituitary, the top of the skull was removed with a knife. Pituitary gland was left behind on the base of the skull. Collected pituitaries were homogenized in 0.6% salt solution or distilled water. The solution was centrifuged and the clear supernatants were used for injection. Sometimes preserved pituitary gland may also be used for extract preparations. Pituitary glands were preserved in absolute alcohol immediately after collection. Each gland was kept in a separate phial with fresh absolute and stored in a cool shady place at room temperature or under refrigeration until needed.

Selection of Brooders: Healthy and disease free brooders of *Catla catla* were selected for every trial in the (2:1) ratio of male and female (For the collection of brooders drag net was used for netting (to avoid gill injury). Brooder fishes were identified and selected for the experiment on the basis of following morphological characteristics, such as, the bulging abdomen, soft ventral abdominal region, comparatively larger size, felt pectoral spine, smooth pectoral fin and swelling anal fin with reddish colour of females. But in comparison to males the normal abdomen, milt comes out with gentle pressure on the abdomen, smaller size of similar age, serrated pectoral spine, rough pectoral fin and concave anus from exterior had been observed at the time of selection of brooders.

Counting of Egg: The eggs of Indian Major Carp *Catla catla* are non-floating, non-adhesive and round in shape, average diameter of eggs 4.6 mm and color is light red. The eggs were collected in a bucket they put in a mosquito net held in water. The eggs were measured in beaker of known volume. Total number of eggs laid (approx), percentage of fertilization and percentage hatchling can be calculated by using following formula.

Total no. of eggs Laid (approx) = Average no. of eggs each sample beaker X number of beakers of eggs

Percentage of Fertilization: Fertilized eggs of Indian major carp were transparent, non–adhesive, round in shape while unfertilized eggs were opaque. The fertilization rate was calculated through random sampling by examining 2-3 samples from each breeding tank by using following formula.

$$\text{Fertilization rate (\%)} = \frac{\text{Eggs in a sample}}{\text{Average no. of eggs in a sample}} \text{ X } 100$$

Percentage of Hatchling: Percentage of hatchling was calculated by following formula

$$\text{Hatchling rate\%} = \frac{\text{Total no. of spawn}}{\text{Total no of fertilized eggs}} \text{ X } 100$$

RESULTS

In the present study from June to August 2009 (Breeding season). The results of the experiment carried out on Indian major carp *Catla catla* treated with Ovaprim and Pituitary extract has been presented in Table no1 &2 and pituitary extract has been presented in Table no. 3&4.

Aggressiveness in the brooders was noticed after 4-6 hrs, of the second dose (0.6 - 0.8 ml/kg body weight) of pituitary extract to female and single dose (0.2 - 0.4 ml/kg body weight) of pituitary extract to male. Whereas the single dose (0.2 - 0.4 ml/kg body weight) of Ovaprim were administred to male and female *Catla catla*.

The ratio of the male: female were selected (2:1) for each trial. In the present study, fourty females and eighty healthy disease free males were selected for the experiment. For a years 2009 and average result has been tabulated during breeding season.

Spawning Response Due to Injection of Pituitary Extract: In the month June 2009 minimum response was found such the average number of eggs obtained 980000, average number of fertilized eggs were 680000, average number of hatchlings were 400000, average number of eggs per kg, was 81666, average number of fertilized eggs per kg was 56666, average number of hatchling per kg was 33333, fertilization rate (%) was 69.38% and average hatchlings rate (%) was 58.82% of *Catla catla* presented (Table No. 1).

While in the month August 2009 the maximum response was found such the average number of eggs obtained 1700000, average number of fertilized eggs were 1450000, average number of hatchlings were 1135000, average number of eggs per kg, was 81666, average number of fertilized eggs per kg was 67441, average number of hatchling per kg was 52790, fertilization rate (%) was 85.29% and average hatchlings rate (%) was 78.82% of *Catla catla* presented (Table No. 1).

Spawning Response Due to Injection of Ovaprim: In the month June 2009 minimum response was found such the average number of eggs obtained 980000, average number of fertilized eggs were 680000, average number of hatchlings were 400000, average number of eggs per kg, was 81666, average number of fertilized eggs per kg was 56666, average number of hatchling per kg was 33333, fertilization rate (%) was 69.38% and average hatchlings rate (%) was 58.82% of *Catla catla* presented (Table No. 2).

While in the month August 2009 the maximum response was found such the average number of eggs obtained 1700000, average number of fertilized eggs were 1450000, average number of hatchlings were 1135000, average number of eggs per kg, was 81666, average number of fertilized eggs per kg was 67441, average

Table 1: Spawning response of female *Catla catla* with Pituitary extract. (Year - 2009)

Months	No. of female treated	Total wt of female (kg)	Average no. of eggs obtained	Dose of Pitutary extract I st	Dose of Pitutary extract II nd	Average no. of fertilized eggs	Total no. of hatchling	Average no. eggs Kg^{-1}	Average no. fertilized eggs Kg^{-1}	Average no. Hatchling eggs Kg^{-1}	Average fertilization rate (%)	Average Hatchling rate (%)
June 09	4	12.0	980000	0.2-0.4	0.6-0.8	970000	680000	400000	79069	56666	69.38	58.82
July 09	4	16.5	1300000	0.2-0.4	0.6-0.8	1000000	690000	78787	60606	41818	76.92	69
July 09	4	16.5	1200000	0.2-0.4	0.6-0.8	930000	635000	72727	56363	38484	77.5	68.27
Aug.09	4	17.5	1300000	0.2-0.4	0.6-0.8	970000	6300000	74285	55428	36000	74.61	64.94
Aug.09	4	21.5	1700000	0.2-0.4	0.6-0.8	1450000	1135000	81666	67441	52790	85.29	78.82

Table 2: Spawning response of female *Catla catla* with Ovaprim (Year - 2009)

Months	No. of female treated	Total wt of female (kg)	Average no. of eggs obtained	Dose of ovaprim	Average no. of fertilized eggs	Total no. of hatchling	Average no. eggs Kg^{-1}	Average no. fertilized eggs Kg^{-1}	Average no. Hatchling eggs Kg^{-1}	Average fertilization rate (%)	Average Hatchling rate (%)
June 09	4	19.0	2000000	0. 4-0.6	1900000	1790000	121052	92682	87317	92.00	89.31
July 09	4	12.0	1450000	0.4 - 0.6	1370000	1230000	95294	114166	102500	94.48	89.78
July 09	4	16.0	1760000	0.4 - 0.6	1690000	1560000	120833	105625	97500	96.02	92.30
Aug.09	4	19.0	2300000	0.4 - 0.6	2116000	1890000	110000	111368	99473	92.00	89.31
Aug.09	4	20.5	2300000	0.4 - 0.6	2116000	1890000	110000	111368	99473	95.00	94.21

Table 3: Overall effect Pituitary Extract and Ovaprim on spawning *Catla catla* with Ovaprim (Year - 2009)

Pituitary Extract		Ovaprim	
Parameters	Results	Parameters	Results
86.5No. Of females Treated	20	No. Of females Treated	20
Total weight of females	84	Total weight of females	86.5
Total no. of eggs obtained	6480000	Total no. of eggs obtained	9810000
Total no. of fertilized eggs	5320000	Total no. of fertilized eggs	9192000
Total no. of hatchling	9440000	Total no. of hatchling	8360000
Average no. eggs per Kg.	141493	Average no. eggs per Kg.	111435.8
Average no. fertilized eggs per Kg.	63781.4	Average no. fertilized eggs per Kg.	107041.8
Average no. hatchling per Kg.	45151.6	Average no. hatchling per Kg.	97252.6
Overall fertilization %	76.74	Overall fertilization%	93.9
Overall hatchling %	67.97	Overall hatchling%	90.982

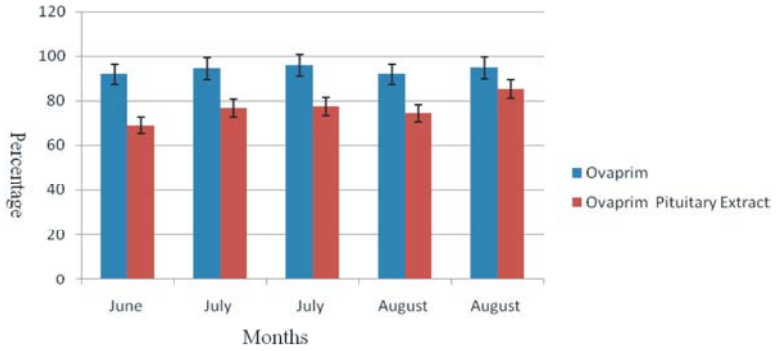

Fig. 1: Fertilization rate (%) in Catla catla due to compared Ovaprim with Pituitary Extract (2009)

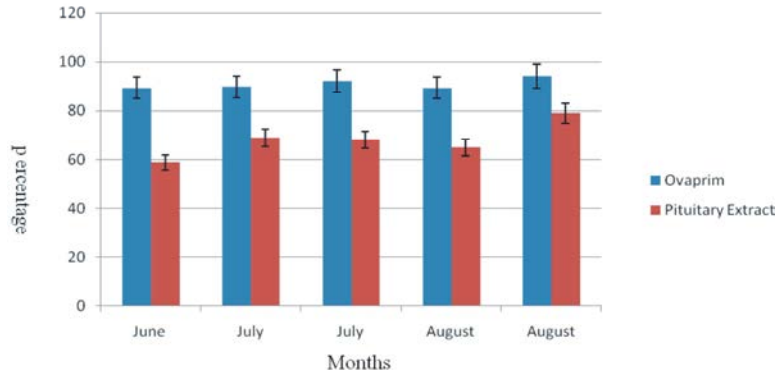

Fig. 2: Hatchling rate (%) in *Catla catla* due to compared Ovaprim with Pituitary Extract (2009)

number of hatchling per kg was 52790, fertilization rate (%) was 85.29% and average hatchlings rate (%) was 78.82% of *Catla catla* presented (Table No. 2).

The overall effects of pituitary extract and ovaprim on spawning presented (Table No. 3). The result of all trials were summarized as fertilization (%) rates, hatchlings (%) rates and fecundity due to pituitary extract and Ovaprim effects have been recorded (Table No. 1, 2 &3).

Fecundity: During the present study, 20 numbers of female of *Catla catla* were treated with ovaprim and 20 numbers of female of *Catla catla* were treated with pituitary extract. In every trial ratio was 2:1 of male and female was kept constant. As shown in the Table No. 1 & 2 the effective dose of ovaprim was found to be 0.2 - 0.3 ml/ Kg and 0.4 – 0.6 ml/ Kg body weight of male and female respectively which induced spawning after 6- 8 hours of dose. However, Pituitary extract was injected twice in two different doses to female. First does was given 0.3 ml/kg body weight followed by second injection 0.6ml/kg body weight and the time interval between two doses was 6 hrs. While a single dose at 0.2- 0.3 ml/kg body weight was given at the time of second dose to female. Spawning taken place after 6 hrs. of the second dose.

During the present study it has been observed that fecundity rate was better due to the ovaprim administration as compared to the pituitary extract. Pituitary extract produced 69230 – 81666 eggs/Kg of body weight on average basis. While, ovaprim produced 94444 -140000 eggs/Kg of body weight on average basis. Fecundity found high in ovaprim treated fish as compared with pituitary extract treated fish.

DISCUSSION

In the present study, a single intramuscular dose of Carp pituitary extract and synthetic hormone ovaprim were resulted in successful spawning of Indian Major Carp *Catla catla*. The results of the hormonal stimulation in the present study are similar to the effectiveness and usefulness by using ovaprim-C [3].

Certain hormones are used individually like different analogues of LH-RH, without pituitary gland, which results in failure of spawning clearly indicates that dopamine blocks the action of LH-RH on the secretion of gonadotropin [4]. However, use of dopamine antagonists like pimozide or doperidon, potentiate the action of LH-RH, resulting in successful spawning [5]. Major breakthrough in the history of aquaculture happened when extensive research on Chinese carp [6] and a new Linpe method was introduced in which LH-RH analogue is combined with a dopamine antagonist. Then Canada introduced the ovaprim-C containing the analogue of salmon gonadotropin releasing hormone (D-Arg6, Pro9 and Net) and dopamine antagonist. Earlier studies conducted in India and Pakistan [7, 8] had clearly demonstrated superiority of ovaprim-C in induced spawning of Major Carps.

Various workers were reported that the dosage of ovaprim required for female brood fish Indian Major Carps as 0.40-0.50 ml/kg to *Catla catla,* 0.30-0.40 ml/kg to *Labeo rohita,* 0.25-0.30 ml/kg to *Cirrhinus Mrigala* [9]. In the spawning of *Catla catla* 0.7 ml/kg dose of ovaprim was reported and the recorded overall fertilization percentage (91.01%), overall hatchling percentage (67.50%), average number of egg Kg^{-1} (67670), average number of fertilized egg Kg^{-1} (61620), average number of hatchling Kg^{-1} (41584) in *Catla catla* [10].

The rate of fertilization and hatchling percentage are higher with ovaprim as compared with pituitary extract [11, 12]. The number of eggs obtained 2,40,000 with fertilization rate 90% and hatchling rate 90% in *Labeo rohita* and 1,40,000 number of eggs obtained with fertilization rate 95% hatchling rate 80% in *Cirrhinus mrigala* [13]. Present study showed that, the result of fertilization and hatchling percentages were higher in Ovaprim treatment as compared with pituitary extract treatment.

[14] has reported 28 -100% fertilization in *C. striatus* with regard to pituitary extract and [15] has reported 45% fertilization in *H. fossilus* [16] noticed percentage of fertilization 60-68% with regard to pituitary extract and percentage of fertilization (95-98%) with regard to Ovaprim.

In the present study similar spawning responses were obtained as average number of egg per kg 75636, overall fertilization rate 77.12% and overall hatchling rate 68.25% with regard to Pituitary extract and average number of egg per kg 112964, overall fertilization rate 94.20% and overall hatchling rate 92.05% with regard to Ovaprim.

In the present study only a single dose of ovaprim induced while the two dose of pituitary extract were given to the female fish, still their spawning was delayed and fertilization and hatchling rates were less than those of the ovaprim dose. One of these reasons for this difference is the poor quality of pituitary glands used in various farms. Besides this aspect, the difference in the mode of action between pituitary and other hormones also appear to be responsible for comparatively better results with pituitary extract and ovaprim. The hormone ovaprim are known to act at pituitary level leading to the secretion of endogenous gonadotropins are introduced into the body [17].

[18] had reported that in India, most of the breeders have been preparing ovaprim, as a survey showed that only10-15% of fish breeders use extract due to its complexity of technique ovaprim is effective in induced spawning because it contains salmon GnRh, native peptide found in the most teleports also contain a dopamine inhibitor (brain neurotransmitter). Present result indicated that ovaprim might be considered the best suitable effects in induced breeding compared with pituitary extract.

When single intramuscular injection of Ovaprim-C was applied, ovulation, fertilization and hatchling values were found 100%, 50.99% and 73.92%, respectively. However, in a previous study conducted at fish hatchery Islamabad, Pakistan [19], they reported corresponding values as 100%, 72.56% and 71.09% for *Hypophthalmichthys molitrix*, respectively. These variations in results were due to change in topographical condition. This variation may also be due to change in climatic condition and low nutritional status of brood fish [20].

The trials conducted on the Ovaprim-C under the present study it was indicated that this hormone had no adverse effect on brood fish or spawns. The several investigators have published their work on fecundity and successful induction of spawning of carp by Ovaprim on different species; Labeo rohita and Cirrhinus mirigala [21].

During the present study in the year 2009 and 2010, in every trial showed that number of eggs per Kg were higher in ovaprim treated fish as compared with the carp pituitary extract (Table No.1 & 2). [22] had been repeated that Ovaprim treatment as compared to the Ovatide and carp pituitary extract. Ovaprim perform 0.38-lac eggs/kg, carp Pituitary extract 0.35 lac eggs/kg and ovatide as 0.37-lac egg/kg on average basis. Fecundity remains high in Ovaprim treated fishes as compared with ovatide and carp pituitary extract treated fish.

In the present study fecundity rate was observed high due to the Ovaprim treatment as compared with the carp pituitary extract and showed that Fecundity was higher in Ovaprim treated fish as compared with carp pituitary extract.

Many workers have proved that ovaprim has better result induced spawning, fertilization and hatchling rate as compared to pituitary extract and some other hormones of these some are [23-28].

CONCLUSIONS

Based on present study it is consequently concluded that the rate of fertilization and hatchling were generally higher in ovaprim when compared to pituitary extract. Reduced handlings of brood fish due to the single dose administrated to both the sexes at the same time due to this decrease post spawning mortality of fish and increase spawning response in ovaprim treatment when compared to pituitary extract treatment.

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