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Original Article

Evaluation of spawning efficacy of ovatide for carp breeding in Pakistan

Abdul Majid Khan*, Ayesha Iqbal, Hafiz Abdullah Shakir and Muhammad Ayub

Department of Zoology, University of the Punjab, Quaid-e-Azam Campus Lahore (AMK, AI, HAS), Punjab Fisheries Department, 2-Sanda Road, Lahore (MA), Pakistan

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Abstract
"Ovaprim" manufactured by a Canada-based company, M/s Syndel laboratories, is in use all over the world today for induced spawning of fishes. Ovaprim contains 20μg of Salmon Gonadotropin Releasing Hormone (GnRH) and 10mg of domperidone /ml. "Ovatide" is a highly potent and ready-to-use injectable formulation consisting of a synthetic GnRH analogue and a dopamine antagonist dissolved in a mixture of aqueous and organic solvents. The viscosity of the solution has been specifically maintained at a level that makes its administration in the fish particularly easy. The rapidly increasing cost and viscosity of the ovaprim proved to be the prohibitive factor. However, ovatide is low-priced and less viscous as compared to ovaprim. The data presented in this paper are the outcome of trials planned to find out the comparative efficacy and usefulness of ovatide using native major carps and Chinese carps. A comparison was carried out for both the drugs for fecundity, fertilization, and hatching rate during the induced spawning of carps. The ovatide induced complete spawning comparable to the ovaprim in all the species tested under the study.
Key words: Induced spawning, Ovatide, Carps, GnRH analogue.

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INTRODUCTION

Most of the fishes for aquaculture such as Asiatic carps, mullet, and milkfish are open water breeders and breed under the influence of environmental stimuli. They become refractory when subjected to confinements of ponds. They either do not mature or become incapable to reproduce in confined environment. Efforts were made to find out ways and means to induce these fishes to breed under controlled conditions and facilitate easy availability of seed for aquaculture.

For many pond breeding carps, a suitable hormone is necessary for synchronizing large-scale spawning. Till 1960s, the major part of carp seed required for culture was collected from riverine sources. Various spawning inducing agents are reported in fishes namely, fish pituitary extract (Khan, 1938; Alikunhi *et al.,* 1962), sex steroid hormones (Jalabert, 1976), antiestrogens (non-Steroidal compounds): clomiphene citrate, sexovid and tamoxifen as ovulating agents (Pandey and Hoar, 1972; Donaldson *et al.,* 1981), domperidone or pimozide, ovaprim (sGnRHA) (Peter *et al.,* 1986, 1988) and Ovatide (Pandey *et al.,* 2001; Thakur and Reddy, 1998). In India experiments with ovaprim have given very encouraging results (Nandeesha et al., 1991). Kaula and Rishi (1986) reported the successful spawning of Cirrhinus mrigala with ovaprim. Nandeesha et al. (1991) reported very satisfactory results in trials with ovaprim. Khan et al. (1992) reported successful spawning of Labeo rohita and Cirrhinus mrigala with ovaprim in Pakistan. However, a major hindrance in the use of ovaprim is its high cost and viscosity, which causes difficulty in serving the dose and cause damage to scales around the injected point at fish body which in time lead to infections and cause mortality of brood fish after spawning. High cost of ovaprim is also a prohibitive factor.

The aforesaid constraining factors of ovaprim promoted a Mumbai-based pharmaceutical company, M/s Hemmo Pharma, to undertake synthesis of GnRH analogues and develop an indigenous hormonal formulation, which can be made available to the fish hatchery operators at an affordable price. This ultimately led to the formulation of ovatide. The Central Institute of Fisheries Education (CIFE) conducted extensive field trails in Madhya Pradesh, Andhra Pradesh, Haryana and

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*Corresponding author: memorablefish123@gmail.com

Maharashtra on induced breeding of fishes including carps using ovatide and reported encouraging results (Thakur and Reddy, 1998). In Pakistan preliminary trial with ovatide in 1999 gave encouraging results in induced breeding of grass carp in Pakistan (Bhatti and Qureshi, 2000). Keeping in view the success of ovatide for spawning of grass carp, present study was planned to evaluate the efficacy of ovatide for spawning of other culturable carps.

MATERIALS AND METHODS

The experiments were conducted at Government Fish Seed Hatchery, Bahawalpur. The *Labeo rohita, Catla Catla, Cirrhinus mrigala, Ctenopharyngodon idella* and *Hypophthalmichthys molitrix* were used for the said experiment. The brood stock from the above said hatchery was utilized to administer various doses of ovaprim following standard method. Standard dose method recommended by Central Institute of Fisheries Education (CIFE), Mumbai, was adopted for ovatide. Both the hormones were injected intramuscularly in the caudal peduncle region above the lateral line with a 2.0 ml hypodermic syringe having 0.1 ml graduations and provided with a No. 22 needle.

Dose of ovatide used for female carps ranges from 0.20 to 0.45 ml/kg and for male is 0.1-0.2 ml/kg as reported by CIFE (1998). The dose of ovaprim for female ranges between 0.4 ml/kg to 0.6 ml/kg of fish body and for male is 0.2ml/kg as used by Nandeesha *et al*. (1991). Breeding trials were conducted in circular ponds. Most of the breeding trials were taken by the already available infrastructural facilities of the above said hatchery.

Water quality parameters, viz., temperature, dissolved oxygen, pH, CO_2 and were checked throughout the experiment to note the malfunction, if any. Weight of male, weight of male, fecundity, percentage of fertilization and hatching percentage were recorded for every pair to compare the efficacy and usefulness of both induced agents. The fertilization and hatching percentage were taken by using following formulae:

Percentage of fertilization =

$$\frac{\text{Average No. of fertilized eggs in sample}}{\text{Average total No. of eggs in a sample}} \times 100$$

Total No. of spawn = Average No. of spawn in a sample x total volume of spawn

Percentage of fertilization

$$= \frac{\text{total No. of spawn}}{\text{total No. of fertilized eggs}} \times 100$$

The results were analyzed statistically through ANOVA.

RESULTS

Keeping in view the success of ova tide for induced breeding of *Ctenopharyngodon idella* by Bhatti and Qureshi (2000), four major carps including *Labeo rohita, Labeo calbasu, Catla Catla* and *Cirrhinus mrigala* were used for the evaluation of the spawning response of ovatide. For the purpose of comparison and evaluation ovaprim was also used. All the four species *Labeo rohita, Labeo calbasu, Catla Catla* and *Cirrhinus mrigala* responded well to ovatide induction (Table I).

It is evident from the results that a dose of 0.4-0.45 ml/kg of ovatide is sufficient to achieve 100% complete spawning. Results for fecundity when analyzed through ANOVA, delineated that no significant difference was present in the efficacy of both ovatide and ovaprim.

The results of the experiments are summarized as fecundity, fertilization and hatching rates. During the experiment it was observed that fecundity rate (%) (Egg production) was better under the ovaprim treatment as compared to the ovatide. The ovaprim performed 1.34.-lac eggs/kg and ovatide as 1.37-lac egg/kg on average basis. Fecundity remained almost similar for ovaprim treated fish as compared to ovatide treated fish. The fertilization rate with ovaprim for *Labeo rohita* was calculated as 77% while for ovatide it remained as 80%. The ovatide performed better for fertilization of eggs. As the hatching percentage of eggs is concerned for the ovaprim, it remained as 58.66% and for ova tide as 64%, which revealed that ovatide gave 5.34% better performance towards hatching rate. No significant difference was observed between the number of eggs laid and the body weight of fish for both ovaprim and ovatide (F=1.51). The results for hormone used and the hatching percentage also remained non significant statistically (F=2.10). The latency period remained shorter in ovaprim treated fish as compared to ovatide treated ones. The difference in latency period may be due to the difference among potency, dose and viscosity of

the hormone. The results of the experiments do not indicate any superiority of the ovatide as compared to ovaprim. However ovatide is economically better when compared to ovaprim.

Table I: Comparison of spawning response of native major carps and Chinese carps to ovatide and ovaprim.

Fish Species	Temp. °C	No. of Female Fish	Total Wt. Kg. of Fish Kg.	Dose of Ovaprim ml/kg	Dose of Ovatide ml/kg	Spawning response time (hr)	No. Of Eggs (Lakh)	*Overall Fertilization (%)*	Overall Hatching (%)
Labeo rohita	29	3	8	0.3	----	8.30	2.0	55	30
		3	8	0.4	----	7.45	6.2	74	59
		4	10	0.5	----	7.30	7.0	86	75
		4	10	----	0.3	8.30	4.1	77	49
		4	11	----	0.4	8.0	7.1	79	61
		4	9	----	0.5	8.0	7.4	85	80
Labeo calbasu	28	3	4.50	0.3	---	8.0	1.5	58	43
		3	4.75	0.4	---	8.0	3.5	75	59
		3	5	0.5	---	7.0	3.9	90	79
		3	5	---	0.3	9.0	1.7	71	57
		3	5	---	0.4	8.30	2.3	79	65
		3	4.5	---	0.5	8.30	2.7	90	82
Cirrhinus mrigala	27	3	5.5	0.3		8.30	2.0	60	43
		3	9	0.4	---	7.30	3.9	75	65
		3	7.5	0.5	---	7.30	3.6	85	71
		3	6	---	0.3	9.0	1.6	54	41
		3	5	---	0.4	8.0	3.1	73	60
		3	5.5		0.5	8.0	3.9	89	77
Catla catla	28	3	11	0.3	---	8.0	3.5	54	47
		3	12	0.4	---	7.30	6.5	69	54
		3	10	0.5	---	7.30	6.0	84	79
		3	9	----	0.3	8.45	3.0	60	40
		3	10	----	0.4	8.30	7.0	85	72
		4	11	----	0.5	8.30	8.7	94	84

DISCUSSION

Ovatide a synthetic hormone has been tested successfully for native major carps including *Labeo rohita, Labeo calbasu, Cirrhinus mrigala* and *Catla catla* in a single dose. Spawning through a single dose of ovatide has been reported for *Ctenopharyngodon idella* by Bhatti and Qureshi (2000). The successful spawning through a single dose of ovaprim has also been reported in several carps (Das, 1994). The synthetic hormone ovatide launched by an Indian company (Hemmo pharma) has been successfully tested for ovulation of several fishes, including *Labeo rohita, Labeo calbasu, Cirrhinus mrigala, Catla catla* and *Clarias batrachus etc.* in India (Thakur and Reddy 1998). The ovatide is easy to store at room temperature, simple to use and less expensive as reported by CIFE (1997).

The results of the experiments when analyzed through ANOVA showed no significant differences in the number of eggs laid per kilogram of fish body weight and in hatching percentage of various fish species. The fecundity, fertilization and hatching also did not differ significantly under ovatide and ovaprim treatments. The results for ovatide treatment

were comparable to those described by Bhatti and Qureshi (2000) and Thakur and Reddy (1998). In the present study it was found that ovatide could induce 100% complete spawning in carps. The overall hatching % age varied from 59 to 68% in case of carps injected with ovatide. The low hatching % age may be due to the compromised conditioning factor of the brood fish because no hormone can induce spawning in fish unless the conditioning factor of brood fish is very good. Generally the results of the spawning experiments with ovatide were found to be satisfactory in terms of spawning success, fecundity, fertilization and hatching rates.

The significant difference between results of two hormones for latency period (spawning time) could not reveal any sound base. This may be due to the dose difference of two treatments. The dose of ovatide used was in the range of 0.3-ml/kg to 0.5ml/kg body weight of fish. The best dose of ovatide remained as 0.45 ml/kg of brood fish. Whereas the other hand dose of ovaprim was 0.5ml/kg of fish body weight as used by Khan *et al*. (1992). The results for ovatide were in line with the results reported by Central Institute of Fisheries Education (CIFE, 1997).

Latency period remained shorter in case of ovaprim (7.86 hours) and for ovatide latency period was 8.39 hours. This may be due to difference in handling of brood fish, water quality, temperature and other environmental conditions. As aforesaid the water quality parameters (alkalinity, DO, hardness), may also be different at two experimental sites of India and Pakistan. Spawning efficacy of two hormones remained statistically non-significant but economical efficacy is very much considerable. The ovatide is the low-priced hormone, and was calculated as 70% more economical as compared to ovaprim (Bhatti and Qureshi, 2000). This economically better position of ovatide suppresses the ovaprim giving an equal result of seed production at hatcheries where the hatchery managers are always to raise the economic position of the unit. If ovatide is made locally available at a competitive price, this hormone is likely to solve many constraints faced by the seed producers, as it is similar to that of ovaprim in action.

Long-term research and field trials are required to ascertain the efficacy of the synthetic hormones for breeding of the same brood stock. In addition trials are also required to assess the growth performance of spawns produced with these and new inducing agents. The trials conducted with ovatide under present study indicated no adverse effects on brood fish.

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