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Evaluation of a New Spawning Agent, *Ovopel* in Induced Breeding of Indian Carps

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Abstract

Fish breeding in India is no longer a complicated technique. Several hormones are being used for induced breeding of fish on commercial scale using Chinese type hatchery mostly at the private sector. A new spawning agent, Ovopel was evaluated for spawning of Indian carps in Assam, India. The paper highlights the results of successful breeding trials with ovopel. The study demonstrated the effectiveness of the new spawning agent, ovopel in inducing complete spawning in most of the species tested thus far.

A dose of 1 to 1.5 ovopel pellet•kg brood fish was found to be sufficient to achieve 100% complete spawning. Ovopel induced 100% complete spawning in majority of carp species tested under the study with a response time varying between 4 hrs 50 minutes to 9 hrs.

Introduction

Fish seed is the critical and basic input for successful fish culture operations and untill the 60's, a major part of the seed required for culture was being collected from the riverine sources in India. Spawning in the Indian major carps is precipitated by a consortium of environmental factors that prevail during the monsoon season (June-July as the peak period). However, in standing waters they develop the roe but do not spawn and this problem remains yet unsolved (Sinha et al. 1974). Under these conditions, the gonads undergo normal growth and development, but the final events of oocyte maturation and ovulation, and spermiation do not occur (Donaldson and Hunter 1983).

A breakthrough achieved during the 50's in induced breeding through hypophysation gave the thrust to mass production of quality spawn in a controlled environment thereby reducing the dependence on natural seed collection. In India the first successful induced breeding of carp was reported in 1957 (Choudhuri and Alikunhi 1957).

The technique of hypophysation practiced since 1957 has several inherent problems which have become constraints in its wider adoption for seed production; the major problems are: 1. varied potency of the pituitary glands as a result of which spawning success is uncertain, 2.difficulties involved in the collection and storage of glands, 3. non-availability of sufficient quantity of quality glands at the right time, 4.cumbersome process of extract preparation and 5. the necessity of administering the extract twice to female fish. Because of these constraints it is reported that only about 15% of the existing carp seed farms in the country employ hypophysation for carp breeding (Dehadrai 1984). Though pituitary still forms a major inducing agent for breeding various species of fish, research is continuing throughout the world to find effective substitutes for it. In this direction considerable progress has been achieved in the use of releasing hormones in combination with dopamine antagonists. A recent development in the technology of induced breeding is the stimulation of endogenous gonadotropin release from the pituitary of the treated fish by the use of synthetic analogue of gonadotropin releasing hormone, GnRH (Anon 1977). A number of studies conducted in breeding various species of cultured fish in China with LH-RH analogues led to the development of “Linpe method” (Peter et al. 1988) wherein the releasing hormone is combined with a dopamine antagonist for successful spawning. To facilitate the gonadotropin releasing activity of GnRH, it is combined with a dopamine receptor antagonist (Chang and Peter 1983). This method is gaining acceptance throughout the world (Horvath et al. 1986). The major breakthrough in fish breeding came in with the finding that dopamine acts as inhibitory factor for synthesis of gonadotropin (Peter et al.1986). This breakthrough led to the development of the spawning agent ovaprim. The first successful spawning through a single dose of Ovaprim has been reported in several species of fish in India (Nandeesha et al. 1990; Das et al. 1994). In addition to the age old hypophysation method (injection of pituitary extract), many seed producers today prefer Ovaprim for its ready to use formulation and reliability. Recently, another spawning agent Ovatide was launched by an Indian company (Hemmo pharma) which was successfully tested by CIFE (ICAR), Mumbai (Thakur and Reddy 1998) in several places. In recent years, the use of various synthetic formulations, including the ovaprim, has largely replaced the use of pituitary, making the induced breeding technology more farmer-friendly. Improvement in this regard include the administration of a single dose of ovaprim against double dose of pituitary extract reducing the stress on brood fish as well the costs. Khan et al. (1993), mentioned that ovaprim-c was superior compared to pituitary hormone in combination with human charionic gonadotrophin (HCG). In an earlier experiment, Dwivedi et al (1985), reported that human chorionic gonadotrophin with pituitary

hormone is more effective in breeding of silver carps *Hypophthalmichthys molitrix*.

In India, Manickam and Joy (1989) reported that simultaneous injections of pimozide (a dopamine antagonist) and the luteinizing hormone-releasing hormone analogue (LHRHa),des-Gly^{10}[D-Ala^{6}]–LHRH ethylamide, caused a high rate of ovulation(85.7%), while injections of either pimozide or LHRHa alone or the vehicle medium did not in the Asian catfish, *Clarias batrachus*. Kaul and Rishi (1986) reported successful spawning of *Cirrihinus mrigala* (Ham.) with LH-RH analogue [des-Gly^{10}-(D-Ala^{6})LH-RH ethylamide] at 10 micro gram•kg body weight. Although, their trials with pimozide at 10 mg•kg body weight resulted in 100% ovulation, spawning success was only 16.6%.

The new spawning agent tested in the present study is ovopel. The ovopel developed by the University of Godollo in Hungary, is a preparation containing mammalian GnRH analogue, D-Ala^{6},Pro^{9}NEt-mGnRH,and the water-soluble dopamine receptor antagonist, metoclopramide. The concentrations of D-Ala^{6}, Pro^{9} Net-mGnRH and metoclopramide are in the form of 18-20 micro gm•pellet and 8-10 mg•pellets respectively. The hormone is thus available in pellet form. Each pellet contains superactive gonadotropin releasing hypothalamic hormone analogue with an equal effect of which a 3 mg normal acetone-dried dehydrated carp hypophysis gland has. Induced propagation of fish had been shown to be more effective if the hormone was administered in two doses, prime dose and resolving dose, as reported by Szabo (1996). For cyprinids, successful results were reported when 2-2.5 pellets•kg were administered to female brood fish.

Horvath et al (1997), noted that induction of ovulation in fish by GnRH has been used for decades. GnRH analogue containing preparation (ovopel) was tested on four cyprinid species, the common, grass, silver and the tench and compared the effects of ovopel to those of pituitary treatment in relation to the ratio of ovulated to non-ovulated females. In case of common, grass and silver carp the resolving dose of ovopel was preceded by a priming dose of pituitary or ovopel. The tench received only one dose of ovopel or pituitary. In the common, silver and grass carp, the ovopel treatment resulted in high rate of responding females. Ovopel induced high rate of ovulation in the tench as well. A preliminary trial conducted in 1999 with ovopel gave encouraging results in inducing spawning of Indian carps in Assam, India (Das 2000a).

Materials and Methods

The required amount of ovopel was calculated on the basis of weight and condition of brood fish. The pellets were pulverized in a mortar and dissolved in distilled water to prepare a solution for injection. Since, intramuscular hormone administration to the carp brooder is a well-accepted method and is being practiced by most breeders in the country, the ovopel solution was injected using the same method. Single injection of ovopel was

given to both female and male brood at one time. In case of ovaprim, the same method was followed. However in case of pituitary, two injections were given to female brood in split doses. The trials were conducted in 1999 and 2000. For comparison, two other spawning agents viz. pituitary gland and ovaprim were also used following standard method as described by Choudhury and Alikunhi, (1957) and Nandeesha et al,(1990) respectively. The particulars of the breeders, hormone dose, egg production, fertilization and hatching rates were collected on the spot employing standard method. Experiments were conducted in a few Chinese carp hatcheries, randomly selected in the districts of Nagaon, Kamrup and Sonitpur of Assam. Breeding response was classified as complete, partial or no spawning based on the volume of residual eggs released on application of pressure on the abdomen. The fertilization rate was calculated by examining a minimum of three samples from each breeding experiment.

Results

Initially, a preliminary trial was conducted with the new hormone ovopel on a few carp species in 1999. Details are given in table 1. It was clear from the study that the new hormone, ovopel was effective at a dose of 1 to 2 pellets•kg body weight for Indian carps and catfish.

Encouraged by the initial trials, more trials with ovopel were conducted during the year 2000 to standardize the dose for this new hormone for all the cultivable carps in Assam. Table 2. gives the details of the experimental results. It was evident from the trial that a dose of 1 to 1.5 ovopel pellet•kg brood fish is sufficient to achieve 100% complete spawning.

Table 2 summarizes the results of induced breeding trials with the new spawning agent, ovopel. Through ANOVA no significant difference was recorded in the number of eggs laid•kg body weight (F=0.190) and in hatching percentage of various species (F=1.46).

Table 3 summarizes the result of spawning responses of carps to different hormones.

Table 1. Results of the preliminary trials with Ovopel conducted in July 1999

Species	Weight (Kg)	Dose of Ovopel (Pellet•kg)	Response
Labeo rohita	0.60	1.00	Ovulated
	0.30	2.00	Spawned
	0.30	0.50	No response
	0.30	0.50	No response
Cirrihinus mrigala	0.40	0.50	Ovulated
Labeo gonius	0.50	1.25	Spawned
	0.35	1.00	Spawned
	0.30	0.50	No response
Clarias batrachus	0.10	1.50	Ovulated

Note: Males were injected with 0.4 – 0.6 pellet•kg body weight based on the condition

Table 2. Induced breeding trials on various carps with new hormone, Ovopel

Fish species	Place	Month	Temp^{r} (°C)	No.of female brood fish	Total wt. of brood fish (kg)	Dose of Ovopel (Pellet•kg)	Type of response (%)			Response time (hr)	No. of eggs obtained (lakh)	Overall fertilization (%)	Overall hatching (%)	Hatchery unit
							CS	PS	NR					
Rohu and Mrigal*	Tuktuki	June	29-30	12	3.00	1.25	100	-	-	5.00	5.70	85.0	78	Chinese
Silver carp	(Nagaon)		31-32	3	1.35	1.50	100	-	-	6.00	1.62	90.0	82	Chinese
Grass carp			31-32	7	8.20	1.50	100	-	-	7.30	6.80	95.0	84	Chinese
Catla			31-32	1	2.30	1.30	100	-	-	4.50	2.14	87.0	80	Chinese
Silver carp			31-32	2	3.10	1.25	40	60	-	5.00	3.87	69.0	58	Chinese
Silver and Grass*			30-31	4	4.50	1.50	80	20	-	9.00	3.40	54.0	46	Chinese
Catla	Guwahati		31-32.5	1	2.10	1.25	100	-	-	8.30	2.90	88.0	72	Hapa
Silver carp	Tuktuki	July	29-30	2	4.50	1.25	100	-	-	8.00	2.03	90.0	76	Hapa
Catla	Tuktuki		28-29.5	1	3.00	1.40	100	-	-	9.0	5.10	90	82	Chinese
Rohu	(Kamrup)		31-31.5	1	1.50	1.50	100	-	-	8.0	2.60	90	85	Chinese
Rohu	Pabhoi		31-32.5	1	0.50	1.50	100	-	-	7.0	0.27	98	82	Chinese
Calbasu	Pabhoi		31-32.5	1	0.70	1.00	100	-	-	7.0	0.78	96	74	Chinese
Mrigal	Pabhoi (Sonitpur)		31-32.5	1	0.40	1.20	100	-	-	6.30	0.52	95	83	Chinese
Rohu	J.B.Garh (Nagaon)		32-33.5	2	1.70	1.00	100	-	-	8.30	2.64	90	81	Chinese

*Mixed spawning; CS: Complete spawning ; PS: Partial spawning ; NR: No response. F-test values : F=0.190 and F= 1.46

Table 3. A comparison on spawning responses of a few fast growing cultivable carps to Ovaprim, Pituitary and Ovopel hormones

Fish species	Place	Month	Temp[r] (°C)	No.of female brood fish	Total wt. of brood fish (kg)	Dose of Ovaprim (ml•kg)	Dose of Pituitary (mg•kg)	Dose of Ovopel (Pellet•kg)	Type of response (%)			Response time (hr)	No. of eggs obtained (lakh)	Overall fertilization (%)	Overall hatching (%)
									CS	PS	NR				
Catla	Nilbagan and Tuktuki (Nagaon)	July	28-29.5	4	7.20	0.30	-	-	100	-	-	8.30	12.12	75	60
				2	2.90	-	13	-	50	50	-	8.30	1.80	75	55
				1	3.00	-	-	1.5	100	-	-	8.00	2.60	90	85
Silver carp	-do-	July	27-29	3	4.80	0.50	-	-	66.67	33.33	-	10.30	5.25	85	75
				11	12.80	0.40	-	-	100	-	-	10.00	19.60	95	75
				4	6.30	0.30	17	-	25	75.00	-	10.30	1.68	85	75
				7	8.90	-	-	-	14.29	14.28	71.42	9.30	3.60	85	60
				2	4.50	-	-	1.4	100	-	-	9.00	5.10	90	82
Grass carp	-do-	July	28-30	6	6.90	0.40	-	-	50.00	33.33	16.67	7.0	4.80	95	78
				6	11.40	0.30	-	-	66.67	33.33	-	7.0	5.30	95	84
				9	9.50	-	14	-	22.22	44.00	33.33	9.0	6.60	92	82
				7	7.80	-	-	1.5	100	-	-	7.30	6.32	94	86

Note: CS: Complete spawning; PS: Partial spawning; NR: No response

Three species of carps were taken for the breeding experiment. All the three species viz. catla, silver carp and grass carp responded to ovopel induction. In the ovopel treated fish, 100% complete spawning was achieved. Whereas for the trials conducted with other two hormones viz. pituitary and ovaprim, the spawning responses varied widely. Through ANOVA (F-test) it was found that there was significant variation (F = 16.94**) between the spawning responses of various carps to different hormones. However, no significant variations (F = 1.71) were obtained between the number of eggs laid and body weight of the fish and between the hatching percentage and hormone used (F = 2.808). The results indicate the superiority of the new hormone, ovopel over pituitary and ovaprim.

Discussion

The hormone ovopel under the present study has been tested successfully with a single dose for all the Indian major carps and two Chinese carps. Successful spawning through a single dose of ovaprim has also been reported in several species of fish in India (Nandeesha et al 1990; Das et al 1994). The hormone, ovopel has been successfully tested for ovulation in several species of cyprinids, the Common carp, the Silver carp and the Tench in Europe (Horvath et al. 1997). Another study by Brzuska and Grzywaczewski (1999) suggest possibility of using this hormone again after a few days if the first stimulation has failed, while such a measure is not possible in the case of hypophysation (Szabo 1996). Ovulation was also reported in African cat fish (Brzuska 1998). In Assam the preliminary trials conducted on *Labeo rohita, Cirrihinus mrigala, Labeo gonius* and *Clarias batrachus* gave encouraging results as shown in table 1. This indicates the possibility of using this new hormone preparation for commercial production of fish seeds if made available locally to farmers at a competitive price. The new inducing agent ovopel is easy to store, simple to use and less expensive as reported by Szabo.(1996). The trials conducted in the year 2000, revealed that a dose of 1 to 1.5 ovopel pellet•kg brood fish is sufficient to achieve 100% complete spawning.

Through ANOVA, no significant difference was recorded in the number of eggs laid•kg body weight (F = 0.190) and in hatching percentage of various species (F = 1.46). Total eggs spawned and fertilization and hatching rates also did not differ significantly between two groups of ovulated bighead fish induced through LHRH-a + DOM and HCG + LHRH-a (Fermin 1991). Through the present study, it was found that ovopel induces 100% complete spawning in majority of carp species with a response time varying between 4 hrs 50 minutes to 9.0 hrs. Brzuska and Grzywaczewski (1999) reported higher percentage of spawning in common carp treated with ovopel. They also recorded an ovulation period of 7.5 to 10 hours in Common carp injected with ovopel. The overall hatching percentage varied from 65 to 80% in case of carps injected with ovopel (Fig. 1). The low hatching percentage may be due to several factors, such that the condition of brood fish selected for

breeding is very important as no hormone or hatchery can induce breed fish unless the condition of brood fish is good (Das 2000). However, overall hatching percentage of ovopel treated fish was uniformly good when compared with two other hormones (Fig. 2). Although, the analogues in combination with dopamine antagonists had proved to be useful, there were a number of problems in adopting this technique at farmers levels. The analogues administered in minute quantity required careful calculation in preparation, while the dopamine antagonists like pimozide or domperidone did not dissolve in water and hence the suspension had to be carefully injected to get the desired results. The introduction of ready to use ovaprim in solution form solved these problems. The percentage of spawning success, number of eggs obtained•kg, fertilization and hatching percentages remained consistently higher with ovopel and ovaprim treatment as compared to pituitary in the trials. One of the 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 two other hormones also appear to be responsible for comparatively better results with ovopel and ovaprim. The hormones ovopel and ovaprim are known to act at pituitary level leading to the secretion of endogenous gonadotropins, while in the case of hypophysation technique, exogenous gonadotropins are introduced into the

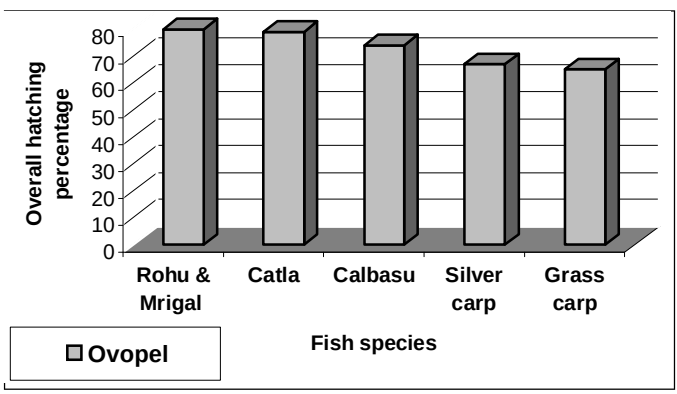

Fig. 1. Overall hatching percentage of different carps treated with Ovopel

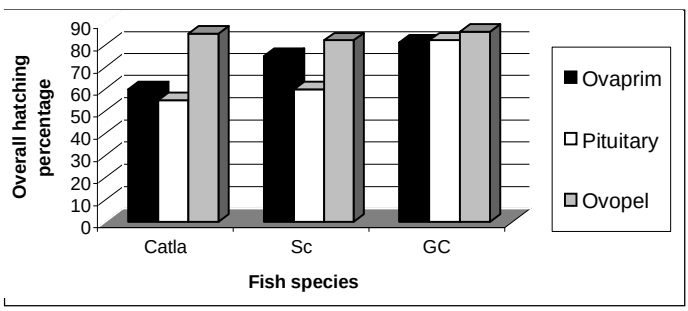

Fig. 2. Overall hatching percentage of three different carps treated with three different hormones.

body (Habibi et al. 1989). Endogenous gonadotropins appear to significantly enhance the secretion of the right type of steroids in abundant quantity enabling complete maturity of ova for spawning. Similarly, the new hormone under study, the ovopel is also expected to solve many of these problems. In general, the response of fish to ovopel was found to be good ,considering the percentage of spawning success, number of eggs released, percentages of fertilization and hatching.

Almost three decades of research efforts have led to the adoption of some promising alternate inducing agents for breeding carps. In recent years the hormone, ovaprim has solved many problems encountered in carp seed production. It's use not only saves considerable amount of time but helps in reducing post-spawning mortality to the minimum, since the brood fish is required to be handled only once (Nandeesha et al 1990). However, long-term research is required to ascertain the effect of synthetic hormones vis-à-vis breeding efficiency of the same brood fish. In addition, trials are required to assess the growth performance of spawn produced with these new inducing agents. The trials conducted so far with the ovopel under the present study indicated no adverse effect on brood fish or spawns. In a preliminary observation on the growth quality of carp spawn produced with ovaprim indicated no adverse effect (Nandeesha et al 1990). If Ovopel is made locally available at a competitive price, this new hormone is likely to solve many constraints faced by the seed producers as it is similar to that of ovaprim in action. Further studies are required to evaluate the growth performance of spawn produced with ovopel.

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