

**Journal of International  
Academic Research for Multidisciplinary**



**A Global Society for Multidisciplinary Research**

# Editorial Board

---

Dr. Kari Jabbour, Ph.D  
Curriculum Developer,  
American College of Technology,  
Missouri, USA.

Er.Chandramohan, M.S  
System Specialist - OGP  
ABB Australia Pvt. Ltd., Australia.

Dr. S.K. Singh  
Chief Scientist  
Advanced Materials Technology Department  
Institute of Minerals & Materials Technology  
Bhubaneswar, India

PROF.Dr. Sharath Babu,LLM Ph.D  
Dean. Faculty Of Law,  
Karnatak University Dharwad,  
Karnataka, India

Dr.SM Kadri, MBBS,MPH/ICHD,  
FFP Fellow, Public Health Foundation of India  
Epidemiologist Division of Epidemiology and Public Health,  
Kashmir, India

Dr.Bhumika Talwar, BDS  
Research Officer  
State Institute of Health & Family Welfare  
Jaipur, India

Dr. Tej Pratap Mall Ph.D  
Head, Postgraduate Department of Botany,  
Kisan P.G. College, Bahraich, India.

Dr. Arup Kanti Konar, Ph.D  
Associate Professor of Economics Achhruram,  
Memorial College,  
SKB University, Jhalda,Purulia,  
West Bengal. India

Dr. S.Raja Ph.D  
Research Associate,  
Madras Research Center of CMFR ,  
Indian Council of Agricultural Research,  
Chennai, India

Dr. Vijay Pithadia, Ph.D,  
Director - Sri Aurobindo Institute of Management  
Rajkot, India.

Er. R. Bhuvanewari Devi M.Tech, MCIHT  
Highway Engineer, Infrastructure,  
Ramboll, Abu Dhabi, UAE

Sanda Maican, Ph.D.  
Senior Researcher,  
Department of Ecology, Taxonomy and Nature Conservation  
Institute of Biology of the Romanian Academy,  
Bucharest, ROMANIA

Dr.Damarla Bala Venkata Ramana  
Senior Scientist  
Central Research Institute for Dryland Agriculture (CRIDA)  
Hyderabad, A.P, India

PROF.Dr.S.V.Kshirsagar,M.B.B.S, M.S  
Head - Department of Anatomy,  
Bidar Institute of Medical Sciences,  
Karnataka, India.

DR ASIFA NAZIR, M.B.B.S, MD  
Assistant Professor Dept of Microbiology  
Government Medical College, Srinagar, India.

Dr.AmitaPuri, Ph.D  
Officiating Principal  
Army Inst. Of Education  
New Delhi, India

Dr. Shobana Nelasco Ph.D  
Associate Professor,  
Fellow of Indian Council of Social Science  
Research (On Deputation),  
Department of Economics,  
Bharathidasan University, Trichirappalli. India

M. Suresh Kumar, PHD  
Assistant Manager,  
Godrej Security Solution,  
India.

Dr.T.Chandrasekarayya,Ph.D  
Assistant Professor,  
Dept Of Population Studies & Social Work,  
S.V.University, Tirupati, India.

## INDUCED BREEDING OF INDIAN MAJOR, MINOR AND CYPRINID CARPS IN INDIA: AN OVERVIEW

**DR. K. SHANKAR MURTHY \***  
**DR. M. VENKATESHWARLU \*\***  
**DR. B.R.KIRAN\*\*\***

\*Research & Teaching Assistant in Botany & Biotechnology, Directorate of Distance Education,  
Kuvempu University, Shankaraghatta, Karnataka, India

\*\* Director, Directorate of Distance Education & Professor, Dept. of Applied Zoology, Kuvempu University,  
Shankaraghatta, Karnataka, India

\*\*\* Research & Teaching Assistant in Environmental science, Directorate of Distance Education,  
Kuvempu University, Shankaraghatta, Karnataka, India

---

### ABSTRACT

Induced breeding of Indian major, minor carp and cyprinid fishes of India by various hormonal analogues is reviewed based on published literature. The comparative efficacy study of synthetic hormones viz., ovaprim, ovopel and ovatide used for the induced breeding of Indian minor, major carps and other cyprinid fishes revealed the effectiveness on breeding performance resulted in higher fecundity and fertilization rate in fresh water fishes. Ovaprim and ovatide are successfully being tested (in place of pituitary extract) for the induced breeding of fishes. Since, newly formulated inducing agents are also being tested for the induced breeding performance by various researchers in different parts of the country, under different climatic conditions, with varying degree of success. These synthetic hormones have following advantages over pituitary extract: ready to use in liquid form, consistent potency, stored at room temperature, stable with long shelf life and single dose requirement. Induced breeding is necessary to control timing and synchrony of egg production.

**KEYWORDS:** Major Carps, Minor Carps, Cyprinid Fishes, Induced Breeding, Synthetic Hormones.

### INTRODUCTION

Induced breeding is a technique whereby ripe fish breeders are stimulated by pituitary hormone or any other synthetic hormone introduction to breed in captive condition. The stimulation promotes timely release of sperms and eggs. The technique of induced breeding was first evolved in Argentina after producing pituitary extract by Houssay 1930 where viviparous fish was injected with the hormone to make premature birth. In the year 1934, Brazilians were succeeded in induced breeding by

pituitary extract. This technique was also followed in America (Merlin & Hubs) and in Russia (Gerebilisky). In India first attempt of induced breeding was made by Khan in 1937 on *Cirrhinus mrigala*. Later in 1955 Dr. Hiralal Choudhuri applied this technique in minor carps (*Esomus danricus*, *Pseudeotropius atherinoides*). Ramaswamy and Sunderaraj first induced to breed *Clarias batrachus* & *Heteropneustes fossilis*. The first successful induced breeding on major carps was done by Dr. Hiralal Choudhuri 1957 on *Cirrhinus mrigala*, *C. reba*, & *Labeo rohita*. Parameswaran & Alikuni successfully bred the exotic Chinese carps – *Hypophthalmichthys molitrix* & *Ctenopharyngodonidella* in 1963 (Monjit Paul, 2008).

Fish culture requires an adequate and reliable supply of fingerlings (fish seed). Collection of fingerlings from the wild, i.e. rivers, lakes, etc. is not reliable because the fingerlings may not be available when needed, or may not be of good quality. Fishes in particular, do not readily reproduce in fish ponds. Induced breeding therefore offers a reliable solution to the non availability of fingerlings ([http://mofa.gov.gh/site/?page\\_id=10279](http://mofa.gov.gh/site/?page_id=10279))

Many cultural farm fishes like Indian major carps do not breed in captivity. The reason may be environmental and consequently hormonal. Certain environmental parameters like photoperiods, rain, temperature, current of water influence the hormonal activity from pituitary and gonads. Disturbances arise in environment may cause the insufficient release of hormones in captive conditions and thus, the fish does not breed in captivity. Other factors like poor foods or insufficient natural foods, exposure to biocides and other pollutants badly affect the maturation of ovary. Commercially important freshwater fishes are commonly spawned with pituitary homogenate, human chorionic gonadotropin (HCG) and semi-purified fish gonadotropins. These preparations are often administered in two doses, a lower priming dose followed a few hours later by a higher resolving dose. Interval between the first and second injections may vary from 3 – 24 hours depending on the species. Variable doses are used even for the same species and may be due to variable potencies of the gonadotropin preparations. Synthetic analogues of luteinizing hormone-releasing hormone (LHRHa) are becoming widely used for inducing ovulation and spawning in a variety of teleosts. Although natural spawning is the preferred method for breeding cultivated fish, induced spawning may be necessary to control timing and synchrony of egg production for practical reasons (Marte, 1989).

Fish reproduction is a periodic phenomenon and is controlled by environmental (exogenous) as well as internal (endogenous) regulatory mechanism. The act of breeding occurs under optimal environmental conditions that are favorable to the survival of the young ones. Environmental stimuli are detected by sensory organs, relayed to brain-that triggers endogenous mechanism into action. Endogenous mechanism is mediated through cascade of various neurotransmitters and hormones secreted by tissues of brain-hypothalamus-pituitary-gonadal axis. The secretions of above axis are regulated through positive and negative feedback mechanisms involving specifically sensitive hormone receptors. The most important reproductive neuro-hormones are hypothalamic gonadotropin-releasing hormones (GnRH) and gonadotropin-release-inhibiting factors (hormones) (GRIF or GnRIH) that regulate secretions of pituitary gonadotropin hormones (GtH) which in turn, regulate the synthesis of gonadal steroids responsible for final maturation of gametes. An appropriate environmental stimulus may signal the arrival of optimal conditions for the fry, triggering spawning i.e. spermiation and ovulation. Fish in captivity may not always reproduce at the most favorable time. In this situation, hormones play a critical role in the reproductive processes. Hormone-induced spawning techniques influence this sequential mechanism at several steps, either by promoting or inhibiting the process. Induced reproduction in fish includes two main strategies. The first is the manipulation of culture environment to mimic important characteristics of natural spawning environment of that particular fish. The second strategy is the administration of one or more naturally occurring reproductive hormone or their synthetic analogs in brood fish through injection or dietary methods. Both these strategies are commonly used, sometimes in conjunction with one another. Numerous hormones have been used to induce reproduction. However, recent researches and commercial aquaculture practices suggest the emergence of two lines of hormone-induced spawning as the best for successful breeding at the least expense. These are i) injection or oral administration of GnRH analog (LHRH analog) with dopamine antagonist, and ii) injection of purified gonadotropin (e.g. human chorionic gonadotropin-HCG) either alone or mixed with common carp pituitary extract to improve its potency. In spite of these, application of steroids, pheromones and prostaglandins were also used, as a new emerging and less studied field and required more research before its commercial application to achieve captive spawning in majority of cultured fishes (Ram Singh and Akhilesh Kumar Gupta, 2011).

The technique of induced breeding gives very promising result in fishery point of view because

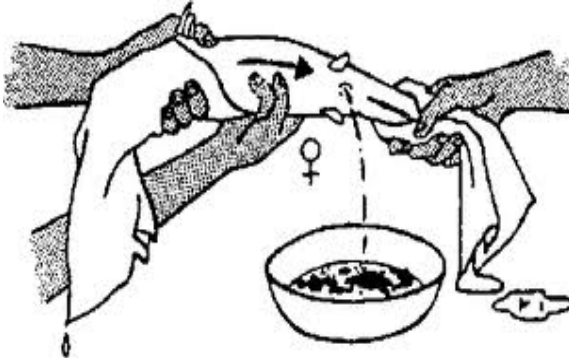
1. It gives pure spawn of certain species of fishes under cultivation. Spawn collected from natural water is not pure as because some undesirable wild species may come with them in culture pond. Sorting of pure seed is quite impossible in those stages. In later stages it is possible, but time consuming.
2. It assures timely available of pure seed, where as in nature the availability of seed is quite uncertain.
3. It can fulfil any quantity of demand in any time.
4. It also cuts short the holding potential spawners over long periods in uncertain hope of their breeding in time. Many carps take their full maturity in confined water but do not breed.
5. The technique is very simple and does not need too much technical assistance or knowledge. It can be easily learnt by a layman without much training.
6. The cost of expenditure is very low than the natural collections of spawns.

Based on the observations of Nandeesh et al, 1990 & 1993, the dosage of Ovaprim required for female brood fish of various species is as follows:

Catla catla	0.40 to 0.50 ml/kg
Labeo rohita	0.30 to 0.40 ml/kg
Cirrhinus mrigala	0.25 to 0.30 ml/kg
Hypophthalmichthys molitrix	0.50 to 0.70 ml/kg
Ctenopharyngodon idella	0.50 to 0.70 ml/kg
Big head carp	0.50 ml/kg
Labeo bata	0.50 ml/kg
Fringe-lipped carp	0.50 ml/kg

Although the dosage required for males of various species could not be standardized, it appears that males of most species will respond to 0.10 to 0.20 ml/kg. On several occasions of the present study, males could be induced with dosages of 0.10 to 0.15 ml/kg. At present, 100 ml of Ovaprim costs about 180 CAN dollars, while about 5 mg pituitary gland costs about Rs. 21, Considering these rates as base,

the economic analysis carried out has indicated that the use of Ovaprim more economical than pituitary. The post-spawning mortality of Ovaprim treated fish was negligible due to relatively less handling in comparison to pituitary treatment. Ovaprim does not require refrigerated storage and hence can be preserved at ambient temperature. Further trials are now in progress to confirm the results obtained during the last two years as well as to gather additional information on the eggs and hatchlings produced through Ovaprim treatment, such as their size, rate of growth, survival etc. Standardizations of dosages for other species is also being taken up. The results of these nation-wide experiments should go a long way in revolutionising the carp seed industry of India.



Hormone injection to Brooders and stripping of eggs

### Spawning

After injection to the brooders a set of brooders are released into breeding hapa. In hapa breeding the hapa is the fine netting, rectangular in shape and is held by four bamboo poles one at each corner. Closed meshed mosquito netting is preferred for that purpose, as its meshes will allow a good circulation of water and will also not let the laid eggs and milt escape through the meshes. The hapa measures the range of

3m × 1.5m × 1m for breeders weighing to 3 to 5kgs. The height of the hapa should remain about 20cm above to the level of water. The roof can be open or closed. The roof can be opened or closed.

The spawning takes place within 3-6 hrs following the second dose. It turns out the midnight if the second injection was given in the evening. Successful induced breeding results in the spawn of fertilised eggs. The fertilised eggs are transparent, pearl like where as unfertilised eggs are opaque or whitish.

#### **Factors influencing the breeding**

Light may help in early maturation and spawning of fish and turbidity of 100 ppm to 1000 ppm is optimum for breeding. Air temperature 24°C to 31°C with cloudy days and rainy periods. Light drizzling following heavy rains is ideal. In absence of rain artificial showers are used. Flowing water is preferred (Monjit Paul, 2008).

#### **HISTORY OF INDUCED BREEDING**

The present day concept of induced breeding of fish can be traced back from the work of Houssay (1930) of Argentina who attempted the application of pituitary hormones for spawning of fish. However, Brazil was the first country to develop the technique of hypophysation (Von Ihering, 1935). In India, Chaudhuri (1955) successfully induced spawning, for the first time, in an Indian major carp species using pituitary gland extract. He also bred *Pseudotropius atherinoides* by administering pituitary extract from *Cirrhinus reba*. Ramaswami and Sundararaj (1956, 1957) reported successful breeding of the catfishes, *Heteropneustes fossilis* and *Clarias batracus*, by hormone injection. In 1957, Chaudhuri and Alikunhi (1957), for the first time, succeeded in inducing breeding of IMCs, rohu and mrigal and minor carps by injecting them with carp pituitary extract. Since then, the application of this technique has spread widely and now, with modifications, forms a regular part of fish culture programmes all over the country. Induced breeding of Chinese carps was successful in 1962 by employing similar technique (Alikunhi, Sukumaran and Parameshwaran, 1963a, b). Chondar (1970, 1984) described in detail the induced breeding technique for the difficult-to-breed IMCs and Chinese carps. By judicious management of broodfish, he was able to make the specimens of several species of carps breed three times in the same season. Varghese et al. (1975) successfully bred carps with pituitary gland of marine catfishes, *Tachysurus thalassima* and *T. jolla* (Basavaraja, 2007).



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 study conducted by Das (2004) 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.

### **TECHNIQUES OF INDUCED BREEDING**

Carps breed in flowing waters like rivers. Naturally they never breed in confined waters. The seed collected from natural resources is generally a mixed stock with both desirable and undesirable varieties. Separation of desirable seed from mixed stock is a big problem. Due to the handling, the desirable varieties may die. If any predaceous fish seed is found, they injure desirable fish seed. Another big problem is never get required number in natural collection. Availability of pure seed is very difficult. To overcome all these problems induced breeding is several advantages with an excellent technique to get pure and required fish seed. With induced breeding pure seed of desirable species can be obtained. In induced breeding techniques, four main types of materials are used to give injections to fish - pituitary gland extractions, HCG, ovaprim and ovatide.

#### **Induced Breeding with Pituitary Extract & Human Chorionic Gonadotropin (H.C.G.)**

Fish breeding by pituitary gland extraction is an effective and dependable way of obtaining pure seed of cultivable fishes and is practiced today on a fairly extensive scale in India as well as many other countries in the world. It involves injecting mature female and male fishes with extracts of pituitary glands taken from other mature fish.

Today pituitary gland extraction is a well established technique for induced breeding all over the world. Its large scale use poses the following problems with regard to availability and quality of pituitary gland (P.G). Inadequate supply of P.G., high cost, variability in pituitary gonadotropin potency and cheating by unscrupulous P.G. suppliers.

To overcome these problems, Human Chorionic Gonadotropin (H.C.G) has been found as an alternative for pituitary gland. H.C.G. was discovered in beginning of 1927 by Aschheim and Zondek. They extracted good quality hormone with luteinising gonadotrophic activity from the urine of pregnant women. Russian workers first used chorionic gonadotropin in 1964 with a trade name as Choriogohin and got good results on Loach. A perusal of literature indicates that H.C.G. is effective either alone or in combination with P.G. extract in inducing various fishes all over the world.

Follicle stimulating hormone (FSH) and luteinizing hormone (LH) of the pituitary play an important role in the normal reproduction of fish i.e., in promoting the development of gonads, growth, and maturity and spawning. H.C.G is more or less similar in character and function to F.S.H and L.H. As pituitary gland is used for induced fish breeding, H.C.G can also be used for early ripening of gonads (Ravi Shankar Piska and Jithender Kumar Naik, 2007).

Superiority of H.C.G over P.G can be measured on the following grounds. Fish attains maturity faster with H.C.G ., the spawn of the breeding season can be increased with H.C.G ., H.C.G. ensures better survival of spawn, it reduces the time gap between preparatory and final doses, H.C.G is more economical and has a long shelf life, H.C.G is easily available from a standard source, hence is more reliable, periodical injections of H.C.G throughout the year ensure better health and increase in weight and gonadal development Potency of H.C.G is known (30 IU/ mg), available in neat packets of known weights, no preservation is involved, cannot be spurious, H.C.G treated fishes can be used more than once for induced breeding in the same season, mortality rate of hatchlings is negligible, consumption of the drug is less during induced breeding, H.C.G can be used as growth hormone and absorption of eggs at the end of the breeding season is comparatively less by the administration of H.C.G.

The crude H.C.G is in powder form and grayish white or light yellow in colour. It dissolves easily in water. The calculated quantity of crude H.C.G is taken into a tissue homogeniser and stirred for 5-10 minutes with measured distilled water. It is centrifuged for 3-5 minutes. The clear light yellowish supernatant liquid having the H.C.G hormones is taken and injected immediately. Any delay in use will result in the loss of the potency.

In case of silver carp, *Hypophthalmichthys molitrix* use of H.C.G is found to be quite successful. The dosage is 4-6 mg/kg. body weight of male, and 6-8 mg/kg

body weight of first dose and after about 6-7 hours, 10-12 mg/kg body weight of second dose for female which gave good results. Use of only H.C.G in the breeding of Indian major carps has not given successful results so far. A combination of 60-80% H.C.G and 40-20% P.G for Indian major carps and grasscarps (*Ctenopharyngodon idella*) is successful. Fishes which are induced to breed with H.C.G alone are mullets, *Cyprinus carpio*, *Lctalurus punctatus*, *Oreochromis nilotica*, *Aristichthys nobilis*, *Misgurnus fossilis*, *Esox lucius* and *Epinephelus tauvina*. Recent work shows that the combination of H.C.G and P.G. is more recommendable than H.C.G or P.G alone.

### **Induced Breeding with Ovaprim**

Due to the problem of varying potency of pituitaries, alternatives were tried. Attempts have been made in various countries to use the analogues of luteinizing hormones - releasing hormones (LH-RH) for induced breeding of fishes with varying degrees of success. However, the success achieved with LH-RH was not always consistent, apart from its higher dose requirement for induction of spawning.

Ovaprim is a ready to use product and the solution is stable at ambient temperature. It contains an analogue of 20 µg of Salmon gonadotropin releasing hormone (sGnPHa) and a dopamine antagonists, domperidone at 10 mg/ml. The dopamine antagonist, domperidone used in ovaprim is also reported to be better than another commonly used antagonist, pimozide. Ovaprim being a ready to use product and one which does not require refrigerated storage, appears to be the most convenient and effective ovulating agent (Ravi Shankar Piska and Jithender Kumar Naik, 2007).

This drug is administered to both female and male brood fish simultaneously in a single dose, unlike pituitary extract which is given in two split doses. This reduces not only the handling of brood fish but also helps in saving considerable amount of time and labour which will add on to the cost of seed production. The spawning response in treated species is found to be superior to the pituitary extract injected species (Ravi Shankar Piska and Jithender Kumar Naik, 2007).

The efficiency of ovaprim for induced breeding of carps have given highly encouraging results in catla, rohu, mrigal, silver carp, grass carp, big head, etc. The effective dose required for various species of carps is found to vary considerably. The common dose for all carps is 0.10-0.20 ml ovaprim/kg body weight of males and 0.25-0.80 ml ovaprim/kg body weight of females. Female catla is found to respond positively for a dose range of between 0.4-0.5 ml/kg, while rohu and mrigal respond

to lower doses of 0.35 ml/kg and 0.25 ml/kg respectively. Among exotic carps, silver carp and grass carp are bred at doses ranging between 0.40-0.60 ml/kg. Big head carp bred successfully at 0.50 ml/ kg. For males of Indian carps, 0.10-0.15 ml/kg and for exotic male carps 0.15-0.20 ml/kg of dosages are found to be optimum. The method of injection is the same as pituitary (Ravi Shankar Piska and Jithender Kumar Naik, 2007).

In many countries including our country, ovaprim is used on a large scale for induced breeding of all cultivable fishes successfully. In India, initial trials were conducted during 1988 in Karnataka, Andhra Pradesh and Tamil Nadu. Ovaprim has unique advantages over pituitary hormone - ready to use liquid form in 10 ml vial, consistent potency and reliable results, long shelf life, and can be stored at room temperature, formulated to prevent over dosing, male and female can be injected only once simultaneously, reduces handling and post breeding mortality, repeated spawning possible later in the season and high percentage of eggs, fertilization and hatching.

#### **Induced breeding with ovatide**

Ovatide is an indigenous, cost-effective and new hormonal formulation for induced breeding of fishes. The new formulation is having the base of a synthetic peptide which is structurally related to the naturally occurring hormone, gonadotropin releasing hormone (GnRH). GnRH is not a steroidal hormone and belongs to the class of organic substances called peptides. It is presented as a low viscosity injectable solution which is not only highly active but also cost-effective compared to other commercially available spawning agents. It is also effective in breeding major carps and catfishes. The doses for females are 0.20-0.40 ml/kg for rohu and mrigal, 0.40-0.50 ml/kg for catla, silver carp and grass carp and 0.20-0.30 ml/kg for calbasu. The dosages for males are 0.10-0.20 ml/kg for rohu, mrigal and calbasu, 0.20-0.30 ml/ kg for catla and 0.20-0.25 ml/kg for silver carp and grass carp (Ravi Shankar Piska and Jithender Kumar Naik, 2007).

The advantages of ovatide are: It is cost-effective hormonal preparation, it gives high fertilisation and hatching percentage (85-95%), it increases egg production through complete spawning, it produces healthy seed, it is easy to inject due to its low viscosity, it does not cause adverse effects on brood fish after injection, it can be administered in a single dose to brooders, it can be stored at room temperature, it is quite effective even under climatic adversities. It is cheaper than ovaprim. The selection of brooders and injecting methods are similar to pituitary extract.

### **Induced Breeding with Ovopel**

Ovopel, developed by the University of Godollo in Hungary, is a preparation containing mammalian GnRH and the water-soluble dopamine receptor antagonist, metoclopramide. The concentration of D-Ala<sup>6</sup>, Pro<sup>9</sup>NET-mGnRH and metoclopramide are in the form of 18-20 micro gm/pellets and 8-10mg/pellets respectively. The hormone is thus available in pellet form. Each pellet contains superactive gonadotropin releasing hypothalamic hormone analogue with an equal effect which a 3 mg normal acetone-dried dehydrated carp hypophysis gland has. For cyprinids successful results were reported when 2-2.5 pellets/kg were administered to female brood fish. However, preliminary trial with single injection of Ovopel gave encouraging result on a few species of Indian major carps and *Clarias batrachus* (Ravi Shankar Piska and Jithender Kumar Naik, 2007).

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. The trails were conducted in July-August of 1999. The new inducing agent. ovopel is easy to store, simple to use and less expensive, as reported by Szabo. T, 1996. However, in India, detailed studies to establish its efficacy and economic viability are required to be undertaken. In India, Ovopel was used with success in induced breeding of major carps in UP, Haryana and Punjab. In Assam the trials conducted recently on *Labeo rohita* (Rohu), *Cirrihinus mrigala* (Mrigal), *Labeo gonius* (Gonius) and *Clarias batrachus* (Magur) gave encouraging results. This indicates the possibility of using this new hormone preparation for commercial production of fish seeds if made available to farmers at a competitive price (Ravi Shankar Piska and Jithender Kumar Naik, 2007).

### **Other Substances used for Induced Breeding**

#### **LH-RH analogue**

Various analogues of Luteinizing hormone -releasing hormone (LH-RH) have been used for induced breeding of fishes. Investigations have revealed that the potential action of releasing hormone when dopamine antagonist is simultaneously used with the analogues is (10-100 µg/kg) used successfully in China. An analogue of teleost GNRH is found to be more potent than LH-RH. GNRH (Gonadotropin releasing hormone) stimulates GTH(Gonadotropin hormone) in teleosts (dosage 25-100 µg/kg) (Ravi Shankar Piska and Jithender Kumar Naik, 2007).

### Clomiphene

It is an analogue of the synthetic non-steroidal estrogen chlorotrianisene. It is known to have antiestrogenic effects in teleosts. It triggers the release of gonadotropins. The injections of clomiphene (10 µg/g) induced ovulation within 4 days in gold fish, whereas with same dosage, common carp spawned successfully after 40-64 hours (Ravi Shankar Piska and Jithender Kumar Naik, 2007).

Comparative account on induced breeding by ovatide in different fish species of India is showed in Table 1. Table 2 depicted induced breeding by synthetic hormones in various fish species in India by various authors.

**Table 1: Comparative account on induced breeding by ovatide in different fish species in India (Marimuthu et al., 2009)**

Species	Ovatide dose ml/ kg body weight	Latency period (hr)	Reference
Catla catla	0.2 - 0.5	7.4	Thakur & Reddy (1997)
Cirrhinus mrigala	0.2 – 0.4	9.32	Thakur & Reddy (1997)
Ctenopharyngodon idella	0.2 – 0.65	10.5	Thakur & Reddy (1997)
Labeo Calbasu	0.3 – 0.5	6.0	Thakur & Reddy (1997)
Labeo rohita	0.2 – 0.4	8.45	Thakur & Reddy (1997)
Heteropneustes fossils	0.4	10.0	Marimuthu etal. (2000)
Puntius javanicus	0.3 - 0.6	8.0	Thakur & Reddy (1997)

**Table 2: Comparative study on induced breeding by synthetic hormones in various fish species in India by various authors**

Fish species	Dosage of hormone (ml/kg)	Hormone	Total fertilized eggs %	Incubation period (Hrs)	Hatching % of fertilized eggs	Reference
Osteobrama belangeri	0.3-0.6	Ovatide	95.0	7.2 -8.15	88.8 ± 1.04	Devi et al. (2009)
Catla Catla	0.4-06	Ovaprim	94.20	10-12	92.05	More et.al. (2010)
Labeo rohita	0.4-0.6	Ovaprim	94.06	10-12	91.36	More et.al. (2010)
Cirrhinus mrigala	0.4-0.6	Ovaprim	92.89	10-12	88.34	More et.al. (2010)
Labeo rohita	0.2-0.5	Ovatide	75-85%	18-22	80-90	Saud et.al. (2013)
Labeo rohita	0.2-0.4	Ovaprim	71-78%	18-22	80-83	Saud et.al. (2013)

## INDUCED BREEDING OF INDIAN MAJOR CARPS

The technique of hypophysation of carps has been described by several workers. Induced breeding of IMCs, which normally spawn once a year either naturally or through hypophysation during monsoon, became successful within an interval of about two months (Bhowmick et al., 1977). Almost equal quantities of eggs were obtained in each of the two spawnings, thereby doubling the production of spawn. Nandeesh et al., (1990 b) reported positive response of mrigal to Ovaprim at a dose of 0.3 ml kg<sup>-1</sup> indicating a high potency of this drug in induced spawning.

Comparative efficacy of pituitary gland and Ovaprim as inducing agent was studied in *Clarias batrachus* by Basu et al.,(2000). They opined that Ovaprim yielded better result with higher percentage of fertilization and hatching. Induction with Ovaprim yielded 80% fertilization of eggs and 60% of their hatching, whereas induction with pituitary gland extract resulted in 45% fertilization and 25% hatching (Basu et al., 2000). However, percentage of fertilization achieved in the present experiment was 73.11% and percentage of hatching was 92.06% in breeding experiments with Ovaprim in induced breeding of *Anabas*. Induced breeding of Murrel, *Channa striatus* with various inducing agents was reported by Francis et al. (2000). They reported that among the different hormones used, Ovaprim showed better performance in terms of higher fertilization rate (93%) and lower latency period (21 hrs.). Latency period on Ovaprim induction in the present experiments was comparatively lower in *Anabas* (10-12 hrs). A similar spawning time (10-14 hrs) was reported by Nandeesh et al. (1990b) when *Labeo rohita* was induced bred with Ovaprim. Among the hormones studied by Nandeesh et al.(1993), the highest percentage of fertilization (93%) was observed in Ovaprim induced fish. The hormonal dose of Ovaprim recommended for carp is 0.3 ml to 0.4 ml kg<sup>-1</sup> (Nandeesh et al., 1993).

However for *Anabas* in the present experiment a much higher dose of 2ml kg<sup>-1</sup> body weight was required. Nandeesh et al. (1990b) also reported that *Labeo rohita* responded to higher dose of Ovaprim and presumed that this was because dopamine activity is higher in rohu. Peter et al. (1986) had reviewed dopamine activity in fish species and indicated that it may vary considerably between species. These supporting reports may suggest that higher dopamine activity of *Anabas* requires higher dose of Ovaprim. Unlike the carp pituitary stimulation, both males and females of *Anabas* were injected with Ovaprim only once in the present experiment. This is supported by

Nandeeshha et al. (1990b) who reported positive response of both male and female *Labeo rohita* to a single simultaneous injection of Ovaprim. In fact single simultaneous injection is very significant from the point of view of commercial fish seed production, as it saves a considerable amount of time and avoids excessive handling of brood fish. Single injection of another synthetic fish hormone, ovatide (GnRH $\alpha$  and a dopamine antagonist) resulted in successful induction of spawning in *Heteropneustes fossilis* (Marimuthu et al., 2000).

The applicability of Linpe method on induced breeding of *Anabas* has not been much exploited. Ghosh et al. (1999) reported successful maturation and ovulation of *Anabas testudineus* when injected by GnRH from *Channa punctatus*. The minimal effective standard dosage of 37.5 mg 100g<sup>-1</sup> body weight was administered to the female fish in two instalments. An additional dose of 25 mg 100g<sup>-1</sup> body weight of GnRH to the female fish was required for complete maturation and ovulation. The male fish on the other hand, received single injection of GnRH (25 mg 100g<sup>-1</sup> body weight) at the time of second injection to the female. According to several authors (Nandeeshha et al., 1993; Haniffa and Sridhar, 2002), ovaprim was the most potent in induced breeding of fish. However, in spite of good breeding results, the present experimental results indicated mortality of brooders from stress due to treatment of ovaprim containing Domperidone which may decrease haemoglobin in fish blood as in mammalian systems

Somashekarappa et al. (1990) succeeded in advancing maturity of catla by two months using a precooked diet formulated using black gram, horse gram, sunflower cake, rice bran, ground nut oil cake, broken rice and fish meal and monthly injection of the fish with HCG at 6 mg/kg body weight. Such fish could be bred once in April and again in July through hypophysation. Similarly, Gupta, Reddy and Pani (1990) successfully advanced maturity in IMCs and Chinese carps using a special feed and bred them during April-June.

Saud et al.,(2010) conducted breeding experiments on *Labeo rohita* using both the synthetic hormone analogues ovatide and ovaprim in Chinese circular Eco-hatchery system of agro-climatic condition of Assam. However use of ovatide at a dose of 0.5 — 0.4 ml/kg of body weight in female and 0.2-0.3 ml/kg of body weight in male found more successful than the ovaprim. Earlier, Pandey et al. (2002) also found a rate of 95-100% fertilization and 90-98% hatching success in *Labeo*



rohita using Ovatide at a water temperature ranging between 28°-31°C. Water temperature plays a crucial role in the breeding of carps.

Ovaprim-C was tested by Nandeesh et al. (1990) for its induced breeding efficacy in three species of Indian major carps, viz. catla (*Catla catla*), rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*). The drug, prepared based on the 'Linpe' method of breeding carps in China, contains an analogue of salmon gonadotropin releasing hormone and the dopamine antagonist, domperidone. Breeding response of the three carps was evaluated vis-a-vis that obtained in the controls, injected split doses of carp pituitary extract or a single dose of carp pituitary extract or a single dose of Ovaprim solvent. All the three species could be bred with a single intramuscular injection of Ovaprim at 0.5 ml/kg body weight. Mrigal responded positively to even lower dosages of 0.3 and 0.4 ml/kg, but 0.4 ml/kg was found to be the minimum dosage required for rohu. In all these trials, males were injected with carp pituitary extract at 3-4 mg/kg, 6 hr after injecting Ovaprim to females. In another trial, wherein female and male rohu were injected simultaneously with Ovaprim at 0.4 and 0.15 ml/kg, respectively, the spawning response was excellent. As against this, the control fish receiving pituitary extract in a single dose failed to respond. The percentage of fertilization in most cases ranged from 70 to 99%. The results of this investigation clearly indicate the suitability of Ovaprim-C for inducing breeding of the Indian major carps.

The comparisons of spawning success with different inducing agents in relation to different species revealed that the spawning success (fecundity and fertilization rate) of *C. catla* is more with ovaprim, whereas, the result were better with Ovatide in *L. rohita* and *C. mrigala*. Reddy and Mathur (2000) also reported higher success of ovatide in *L. rohita* and *C. mrigala* as compared to *C. catla*. Chauhan et al. (1999) reported the breeding success of *L. rohita* at par when induced to breed with ovaprim and ovatide

Bandyopadhyay (1999) made an attempt to induce spawning of the major carps (*Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*) enclosed in a battery of earthen pools consisting of two longitudinally built compartments. A single source of continuous water flow was maintained in the earthen pools till the starting of spawning. The experimental system was designed and operated in a fish farm at Komakantia village, Puri District, Orissa. The experiment was conducted twice during July in 1989 and 1990 with a continuous inflow of water to the pools till the starting

of spawning. The spawning was totally successful in the injected and non-injected females of *C. mrigala* while it was partial in the case of *C. catla* and *L. rohita*. The trials on induced breeding of Indian major carps (*Catla catla*, *Labeo rohita*, *Cirrhina mrigala*) were carried out by Asha Dhawan and Kamaldeep Kaur (2004) during 1999-2000 at the Fish Seed Farm of Ludhiana. Only one dose of either Ovaprim or Ovatide was injected (both @ 0.5 mg/kg female and 0.25 mg/kg male) intramuscularly to the male and female brooders in the evening. The fish was spawned and eggs were hatched in clothed hapas. The breeding performance revealed was estimated in relation to fecundity and fertilization rate

Experiments were conducted by Pramod Rokade et al.(2006) at hatchery complex of Aurangabad ( Maharashtra ) on males and females of major carp *Cirrhina mrigala* by injecting pituitary extract and ovaprim to observe the efficacy of ovaprim during induced breeding. The results were satisfactory and enhancing as ovaprim induced breeding in *C. mrigala* and the spawning took place within 9 hr with 91% overall fertilization. The present study suggests that ovaprim might be considered best substitute for pituitary extracts during induced breeding.

Rath et al. (2007) conducted experiments on Indian major carps, viz. *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* were induced bred in eco-carp hatcheries with 3 different GnRH based synthetic inducing agents, viz. ovaprim, ovatide, wova - FH (OOW) and carp pituitary extract (CPE). The breeding performance of the these agents are compared. Breeding response, mean spawning fecundity, mean spawn recovery rate of OOW ranged between 90 and 100%,  $1.30 \times 10^5$  and  $1.79 \times 10^5$ ,  $0.97 \times 10^5$  and  $1.34 \times 10^5$  respectively, Whereas the above parameters in the same sequence in CPE-administered fish were 77 and 84%,  $1.04 \times 10^5$  and  $1.30 \times 10^5$  and  $0.65 \times 10^5$  and  $0.90 \times 10^5$  respectively. The latency period (time between administration of inducing agent and initiation of spawning) of OOW and CPE-administered brood varied between 5-7 h and 10-13 h respectively. The effective spawning period for both the groups is worked out as 2-3 h and 5-6 h respectively. The GSI of the spent female on the day of spawning ranged between 2.18-2.58 in OOW administered individuals and 5.27-6.28 in CPE group. The histomorphology of spent ovaries retained more number of unspawned oocytes in the female brood of CPE- induced breeding than that of OOW. The pituitary cytophils of proximal pars distalis (PPD) was found to be hyperactive and degranulated in ovaprim, ovatide and wova-FH administered brood. Whereas CPE-administered pituitary was found at par with non-

induced individuals. More et al. (2010) during 2008-2009 observed the spawning response of ovaprim compared with pituitary extract in Indian major carps, at fish breeding center at Jaikwadi, Paithan Dist. Aurangabad (M.S) India. Total ten trial doses of ovaprim were used in induced breeding and ten trial doses of Carp Pituitary Extract (CPE) used for induced breeding in Indian major carps i.e Catla catla, Labeo rohita and Cirrhinus mrigala. The percentage of fertilization ranged (88.11 - 97.94%) was found with ovaprim treatment. and (53.19 - 85.48%) with pituitary extract treatment. The percentage hatchling ranged (74.70 - 95.92%) with ovaprim treatment and (60 -58.82%) with pituitary extract treatment.

### **INDUCED BREEDING OF INDIAN MINOR CARPS**

A trial on induced spawning and hatching of *Osteobrama belangeri* was conducted by Devi et al. (2009) using Ovotide an ovulating agent at Manipur state in Northeast India. Complete spawning was observed within 8 hours and larvae hatched in 26 hours at 28-29°C. Results showed  $95.0 \pm 1.05\%$  fertilization and  $88.8 \pm 1.04\%$  hatching rate. The successful induced breeding of *O. belangeri* with the help of Ovotide in traditional hapas indicates the possibility of adopting the technique by farmers with relative ease. The results of their breeding shows that commercial breeding programmes can be taken up for large scale commercial farming of this species as well as for stocking the natural waters as conservation strategy. Earlier breeding trials of this species were reported by Reddy (2000), Munilkumar and Nandeesh (2007) and Samar-jit and Basudha (2007). The state fisheries department has reported to have bred this fish with fish pituitary gland extracts.

*Cirrhinus reba* is a bottom feeding omnivore although the young feed voraciously on zooplankton and grow very quickly, even faster than the young of catla and mrigal. *C. reba* does not spawn in ponds even though they attain full maturity there. Currently, *C. reba* is considered as 'vulnerable in natural waters' due to a decline in its abundance, extent of occurrence, area of occupancy and habitat. Recently, *C. reba* has drawn attention as one of the potential new candidate species for aquaculture and captive breeding. *C. reba* is an annual breeder with a single spawning period restricted to south-west monsoons extending from May to July in Assam and Bangladesh, and June to August with a peak in June in West Bengal. The shallow waters affording the optimum range of temperature (approx. 28-30 C) may be a factor that induces the fish to spawn. The average fecundity was 420,000. The

average diameter of eggs was 2.24 mm and average weight was 0.0042 mg. *Labeo bata*, is a non-migratory fish and remains in one habitat throughout its life. The fish matures during monsoon season (April to July) in NE region and attains first maturity at 18.62 cm in total length. It breeds in floodplains during rainy season as Indian major carps. It spawns from May to July in at the range of water temperature 26.2-30.3°C (Datta, 2012).

*Puntius sarana* is a freshwater fish common in ponds and rivers, prefers shallow waters of floodplains for breeding, column-bottom dwelling nature and omnivorous in feeding habit, have good consumer demand as food fish for its high biological value. It is a prolific breeder and breeds during monsoon. In July and August it spawns in shallow water. However, poor survival of egg and larvae were noticed. Like many commercially important indigenous fish species *P. sarana* also needs urgent attention from conservation angles, as the population of this commercially important species is reported to be in drastic decline. Artificial propagation of seed of this commercially important fish species was done using Ovaprim @ 1 ml/kg weight of both male and female fish as an inducing agent with success. It was also found that a dose fish PG of 5 mg/kg to 6 mg/kg body weight of female was suitable for breeding (Datta, 2012).

*Puntius gonionotus* is an exotic species which breeds normally in streams and rivers like most tropical cyprinids. They can also breed in captivity like pond and tank water. Their breeding season starts from April and lasts till August. The natural source provides a negligible amount of fish seed because of higher mortality rate, mixture of cultivable and non cultivable species and shrinkage of spawning ground which impede the extension and expansion of fish culture. After the first attempt of induced spawning in early thirties this technique had been successfully tried in many countries of the world. The best ovulation, fertilization, hatching and survivability rates were achieved under higher dose of PG as used @2.00-5.00 mg/kg body weight (2 mg/kg weight of fish in first injection and 4 mg/kg in second injection) were found to be most effective for induced spawning of *P. gonionotus*. Slightly higher dose of hormone was required at the beginning and latter part of the spawning season and comparatively lower dose was required at the middle of the breeding season (Datta, 2012).

## INDUCED BREEDING OF CYPRINID AND OTHER CARPS

Bibha Chetia Borah et al. (1999) reported spawning performance of *Puntius sarana* (Ham.), a commercially important freshwater fish of India was studied by two parallel sets of induced breeding experiments viz., using ecohatchery and using nylon hapa fitted in cement cistern. In sharp contrast to its exotic counterpart *Puntius javanicus* (Sleeker) and major carps, *P. sarana* (Ham.) exhibited negative breeding response in ecohatchery. Inducing agent ovaprim at a dose of 0.3-0.5 ml/kg body weight of fish was found to be effective in bringing about ovulation in *P. sarana* in both the sets of experiments. However, spawning was successful in nylon hapa sets only, probably due to the presence of suitable substratum required for releasing the adherent eggs.

Successful breeding of *Labeo dyocheilus* (McClelland) by intramuscular administration of synthetic hormone, ovaprim with a latency period of 18 hr was reported by Sarkar et al. (2001). 90-95% fertilisation and high rate of hatching was observed.

Breeding of four major species of mahseer, *T. khudree*, *T. mussullah*, *T. tor* and *T. putitora*, by collecting the brooders from the breeding grounds and then stripping them is possible. In the effort to conserve mahseer resources artificial propagation of the fish by stripping the spawners is not always possible unless they are dependably obtainable from natural waters. To overcome this difficulty mahseer fingerlings of all the species can be raised to maturity in captivity (small ponds) by following improved aquacultural practices. Breeding of four major species of mahseer, with and without hypophysation, in brood fish ponds using manipulation of water flow, exercise and high protein palletized diet has also been successful. Stripping the ripe fish becomes necessary and for convenience and surety, two doses of pituitary extract or a single dose of ovaprim/ovatide is desirable. Mahaseer hatchery is simple but most successful and can be replicated in remote centers. Approximately 500 000 eggs are collected and fertilized every year by using different methods. Over 8.1 million fry/fingerlings have been produced in the last 30 years. Cross breeding of mahseer species and producing F1 and F2 generations was also successful. Mahseer breeding is no longer in its infancy but the commercial culture is. The breeding successes have raised new hopes for the prospects of mahseer fishery. However there exists the need to intensify these efforts by undertaking large-scale regular cage culture and a mahseer seed ranching programme. Fry and fingerlings of

major species are being distributed to many states of India and to angling associations in the country by the Tata Power Company as a measure of rehabilitation and conservation. Transport by air of eggs of mahseer in moist cotton has been successful. There is growing awareness about the need to conserve mahseer and there is ample scope for advancement in certain areas. The technique of cryopreservation of mahseer milt has been successfully developed and gene banking of endangered mahseer is technically feasible. Efforts on the induction of triploidy and gynogenesis in mahseer using heat shock treatment for manipulation of sex ratio are in progress (Ogale , 2002).

Five different carp species were tried for induced breeding trial viz. *Labeo rohita*, *Cirrhinus mrigala*, *Hypophthalmichthys molitrix*, *Puntius javanicus* and *Labeo calbasu* by Das (2003) during June-July 2000. It was observed that most carp fishes responded well at a dose ranging between 0.40 to 0.60 ml/ kg body weight. *Labeo calbasu* responded only partially at dose lower than 0.30ml/kg body weight. However, a dose of 0.3 ml / kg body weight of Ovaprim was sufficient for complete spawning in case of *Puntius javanicus*. Complete spawning of *Puntius javanicus* has previously been achieved with a single dose of 0.35 ml/kg ovaprim ( Das et al., 1994). Earlier Nandeesh et al (1990) recommended a dose range of 0.40 – 0.70ml/kg ovaprim for most carp species in India

Induced spawning of a threatened freshwater fish *Nandus nandus* (Hamilton-Buchanan) was conducted by Sarkar et al.(2009) using three commercially available synthetic GnRH preparations viz., wova- FH, ovaprim, and ovatide in different intensities. It was found that at different dosages (0.1 ml, 0.2, and 0.3 ml/kg of body weight) hormone wova-FH and ovaprim could induce the fishes to spawn. No spawning was observed by females treated with ovatide and in control set. The spawning time, fertilization rate, hatching rate, and survival rate were quantified in each set of experiment. The egg output/gm female was higher with the dosage of 0.3 ml in comparison to 0.1 ml/kg and 0.2 ml/kg of body weight of ovaprim and wova-FH. The statistical analysis showed significant effect ( $P < 0.05$ ) between hormonal doses with latency period, fertilization rate, incubation period, hatching percentage, and egg output. The present study suggests that wova –FH and ovaprim at 0.3 ml/kg body weight of fish are more effective in induced spawning of *N. nandus*.

Jhajhria, Anita ( 2011) were conducted twelve induced spawning exercises on *Cyprinus carpio*, *Labeo rohita* and *Cirrhinus mrigala* in the modified Hatchery unit in

Jodhpur using synthetic fish hormones ovaprim and ovatide. The eggs (1.00-3.45 lakhs ) were produced by varying the injection time from 7.15 to 4.30 pm. Inducement through ovatide yielded spawn production (1.9204-2.4394 Lakhs ) compared to ovaprim (0.4895-1.4509 Lakhs ). Studies indicated that ovatide is a more convenient less expensive , indigenous ovulating agent which required low dosage (0.3 ml/kg. brooder) compared to ovaprim(0.5 ml/kg brooder).

Ovaprim is highly effective in inducing ovulation of *L. dyocheilus*. The time taken for response in *L. dyocheilus* is lower (18 hours) compared to *Tor putitora* (24 hours) as reported by Pandey et al. (1998). The rate of fertilisation in the present experiment is higher (90-95%) compared to earlier reports in *T. putitora* (Pandey et al., 1998) using ovaprim. Sridhar et al. (1989) reported 75% fertilisation rate in endangered catfish *Ompok himaculatus* using ovaprim. The variation observed for incubation of fertilised eggs in different systems might be due to variations in temperature, dissolved oxygen, water flow, depth and other physico chemical factors.

In this study only a single dose of ovaprim and ovatide induced spawning within 9 hr while the carp pituitary extract were given two doses to female, still their spawning was delayed and the fertility by ovatide and carp pituitary extract was found less than those of the ovaprim injected. Peter (1986) had ascribed as self potentiating action of the releasing hormone to some drugs when given in two doses. In India, most of the breeders have been preferring ovaprim, as a survey showed that only 10 to 15 % of fish breeders use carp pituitary extract due to its complexity of technique (Dehadrai, 1986). Ovaprim is effective in induced spawning because it contains Salmon GnRH, native peptide found in most teleosts, also contains a dopamine inhibitor (brain neurotransmitter). Our results indicate that ovaprim might be considered best substitute over ovatide and carp pituitary extract during induced breeding.

Recently, Gurpreet Singh Tiwana and Sudhanshu Raman (2012) conducted induced breeding experiment on Indian major carp *Labeo rohita* at Amloh Distt. Fatehgarh Sahib (Punjab) India by using ovaprim, ovatide and Carp pituitary extract. According to their results the fertilization was found 61.30% with ovaprim, 58.50% with ovatide and 55.96% with carp pituitary extract treatment. The percentage hatchling was 72.20% with ovaprim, 66.37 % with ovatide and 59.25% with carp pituitary extract treatment. A comparison was carried out for fecundity, fertilization, and hatching rate during the induced spawning of *Labeo rohita* administered single

dose of ovaprim and ovatide while carp pituitary extract double dose to female brooder. Ovaprim performed much better than Ovatide and pituitary gland extract.

### **TECHNIQUE OF BREEDING MAJOR CARPS IN DRY BUNDH**

The mature carp brooders which are raised in perennial ponds elsewhere are introduced into the bundh at a ratio of 1:2 (Female:Male). The fish are left undisturbed for 2-3 days so that they get acclimatized to new environment. After this, 10-20 percent of the fish is given intramuscular injection of the pituitary extract or ready-to-use solution like ovaprim. Water current is created in the bundh by drawing water from a store tank. The following morning, the spent brooders are removed, eggs collected, water drained and the bundh dried for 2-3 days. The bundh is then utilized for the next breeding by releasing a fresh batch of breeders. Five to six spawning are generally conducted in each bundh during one season as opposed to only one spawning in a wet bundh. Silver carp and grass carp have been successfully bred in bundhs without stripping. Sinha et al.(1974) have reported natural spawning of both grass carp and silver carp in a dry bundh in Bankura District where they were able to spawn the two species without stripping. They consider dry bundhs to be one of the most reliable means of mass breeding of Chinese carps to meet the increasing demand of their seed ( Basavaraja,2007).

After spawning, eggs are collected from bundhs, after lowering the water level, by dragging a piece of mosquito net cloth (gamcha) and releasing the eggs hatching either in improvised pits or double-walled hatching hapas or cement hatcheries. Each pit may contain about 0.9-2.2 million eggs, of which 2.5-25 percent hatch successfully. A double-walled hapa, fixed in the bundh itself and consisting of an outer hapa (182 cm × 91 cm × 91 cm) and an inner hapa (152 cm × 76 cm × 46 cm), accounts for a spawn survival rate of 32 to 50 percent (Alikunhi et al., 1964). The provision of cement hatcheries (2.4 m x 1.2 m x 0.3 m) near the dry bundhs in Madhya Pradesh has aided in improving the survival of hatchlings to 97 %. A cement hatchery of Madhya Pradesh has three times more capacity than a double-walled hapa and is far more economical than the latter (Dubey, 1969; Basavaraja, 2007).

### **BREEDING OF POND-RAISED MAHSEER**

Efforts to propagate mahseer, especially Tor khudree, Tor tor and Tor mussullah at Lonavla lakes (Maharashtra) by Kulkarni (1971) and Tor putitora and Tor tor in



Kumaon lakes by Tripathi (1978) consisted of procuring the spawners and stripping them for artificial fertilisation. Although this is a sure method and has been successful at Lonavla fish farm producing half a million fertilised eggs annually for the past 30 years, it has its own drawbacks. The method of collecting spawners for stripping has limitations in the open hilly terrain or rivers. Since the conditions for the collection of spawners are unlikely to be encountered in many other places, the only reliable method to obtain fry and fingerlings is to grow mahseer juveniles in ponds and breed the resultant adults with the help of hormones. This step ensures proper growth of gonads in ponds. Following this, Kulkarni and Ogale (1986) demonstrated that pond-raised *Tor khudree* and *Tor tor* can be successfully bred through hypophysation after growing them for three years in ponds. Stripping was done after administering the second dose pituitary extract to the female, the male requiring only one dose. This success obviated the difficulty of obtaining spawners from widely spread spawning grounds (Ogale,2002).

Attempts to breed *Tor putitora* by hypophysation were first made by Sehgal and Kumar (1977) at Baintwali Mandi, Dehra Dun with little success. Pathani and Das (1978) also tried the induced breeding of *Tor putitora* without any success. Since the use of pond-reared brood stock of *Tor putitora* met with little success in induced spawning the induced breeding of natural stocks was resorted to. All efforts however remained unsatisfactory. Sehgal (1991) and Das (1992) reported that among the various species of mahseer, the golden mahseer was most affected and hence acquired the status of an endangered species. Kulkarni and Ogale introduced *Tor putitora* into the Lonavla lakes of the TPCL in January 1992 with the objective to breed the species both naturally and by hypophysation. Five hundred fingerlings were released and are thriving as evidenced by the catches of anglers in the lakes. However, no mature female was collected till 1997. As expected the golden mahseer adapted to the captive pond conditions and males started oozing in 1993 and were freely oozing by 1994. The females matured in 1995 (3 yrs) as could be seen from external features. The very first attempt to breed two pairs of golden mahseer at Lonavla with a single dose of Ovaprim was successful. Brooders were released in circular spawning tanks after injecting them with ovaprim. In both the experiments, stripping had to be done after 12 hours (Ogale, 1997). During the 1997 breeding season, *Tor khudree* and *Tor mussullah* were also bred with a single dose of ovaprim. Since then there has been a steady progress in the development of technique and the TPCL farm has produced

217 000 fry/fingerlings from 281 000 eggs of *Tor putitora* at Lonavla till May 2001. Hatching success has been over 90% (Ogale,2002).

### **Hybridization**

In addition to the propagation of *Tor khudree* and *Tor tor* at Lonavla, they were hybridized with each other by using males and females of either species. The characteristics in both cases were intermediate as regards color and body form. The rate of growth is similar or slightly better than the pure strains but the hybrids are more active and agile.

After the introduction of *Tor putitora* in 1992 into the Walwhan Lake and the proper identification of *Tor mussullah*, it was observed that any of the mahseer species could be hybridized. Even the F1 generation could be bred with hypophysation and sometimes even without it when provided with a proper protein diet, feed additives, exercise and running water. The eggs of the F1 generation could be fertilized successfully with the milt of any of the pure strains of mahseer to produce an F2 generation. Thus the Lonavla fish seed farm harbours most of the major species of mahseer and their hybrids and assumes the status of a National Center for Mahseer Studies (Ogale, 2002).

### **Conservation and protection of Brooders**

Action plan for conservation of fishes should include:

- Strict enforcement of fishing rules to prevent fishing with explosives, poisoning, etc.
- Prevention of killing of brood fish and juveniles.
- Replenishment of stock by artificial propagation.
- fish farm should be constructed in close proximity of every dam,
- a few tanks and ponds should be reserved in each farm.

### **CONCLUSION**

All the fresh water fish species are amenable to hypophysation, egg taking and artificial fertilization. At least one large size hatchery is required in each state. Efforts must be made to breed mahseer species on a large scale. Once the fish seed is available state governments of India and fish farmers can use fish fingerlings for river ranching, raceway ponds and running water culture. Hatchlings should be grown to the fingerling size and then released into reservoirs and downstream rivers. Thus if the suggested remedial measures are implemented in stages the mighty fresh water

fishes of India can be restored to its glory much to the delight of anglers and scientists in the country. Many species of fish will not readily reproduce under certain culture conditions. Others will, but not necessarily when the farmer desires. In these cases, induction of spawning can be of great value. Methods vary from species to species and situation to situation. However, at least two generalizations can be drawn. First, brooders are very vulnerable to rough handling. Care should always be used to avoid damaging these valuable animals. Second, a fish that does not have mature gametes will not produce viable eggs or sperm no matter how many times it is injected with hormones. Ripeness is the result of environmental factors working over a period of time, leading to maturation of the gonads and production of viable eggs. Many procedures have been developed for inducing fish to undergo the last steps of spawning. Farmers should thoroughly research the procedures that have been developed for their species of fish through experimentation, and select those that best suit the circumstances. In addition, once the fish have spawned, there are many techniques involved in incubating and caring for the eggs, and caring for the hatched fry. These too must be thoroughly researched (Minnesota Sea Grant, 2008).

The above findings of collected literature suggest the application of synthetic hormones for inducing ovulation and successful breeding and achieving quality egg and larval production for successful catfish cultivation in the country.

## REFERENCES

1. Alikunhi K H , Sukumaran KK and Parameshwaran S. (1963a). Induced spawning of the Chinese carps *Ctenopharyngodon idellus* (C and V) and *Hypophthalmichthys molitrix* (C and V) in ponds at Cuttack, India. *Current Science* 32: 103-26.
2. Alikunhi K H , Sukumaran KK and Parameshwaran S. (1963b). Induced spawning of the Chinese carps *Ctenopharyngodon idellus* (C and V) and the silver carp, *Hypophthalmichthys molitrix* (C and V) in ponds at Cuttack, India. In *Proceedings of the Indian Pacific Fisheries Council* 10(2): 181-204.
3. Alikunhi KH , Datta A , Singh VD and Dubey GP. (1964). Observation on the breeding of carps in bundhs, near Nowgong, Madhya Pradesh, during July-August 1964. *Bulletin of the Central Institute of Fisheries Education of Bombay* 1: 22.
4. Asha Dhawan and Kamaldeep Kaur. (2004). Comparative efficacy of ovaprim and ovatide in carp breeding. *Indian Journal of Fisheries* 51(2):227-228.
5. Basavaraja N. (2007). Freshwater fish seed resources in India, pp. 267–327. In: M.G.Bondad-Reantaso (ed.). *Assessment of freshwater fish seed resources for sustainable aquaculture*. FAO Fisheries Technical Paper. No. 501. Rome, FAO. 2007. 628p.
6. Bhowmick RM , Kowtal GV, Jana RK and Gupta SD. (1977). Experiments on second spawning of major Indian carps in the same season by hypophysation. *Aquaculture* 12(2): 149-56.

7. Basu D , Rana GC . Mondal BK, Sengupta KK and Dhar PK.(2000). Studies on the comparative efficacy of ovaprim, HCG and Piscine pituitary gland in Induced Breeding of *Clarias batrachus* (Lin). *Fishing Chimes*, 19 (10, 11):103-104.
8. Bandyopadhyay MK.(1999). Spawning of the Indian major carps in battery of earthen pools. *Indian J. Fish.*, 46(1) : 67-69.
9. Bibha Chetia Borah , Bhagowati AK and Biswas SP. (1999). Response of *Puntius sarana* (Ham.) to induce breeding by ovaprim administration. *Journal of Inland fisheries Society of India* .vol 31(1): 13-17.
10. Chauhans RS, Singh VK and Singh UP. (1999). Performance of Ovatide – A new spawning formulation in induced breeding of *Labeo rohita* in Tarai AgroClimatic region.National seminar on Sustainable quaculture,21-22, Jan,1999, Punjab Agricultural University,Ludhiana, p. 50.
11. Chaudhuri H. (1955). Successful spawning of the carp minnow, *Esomus danricus* by pituitary gland injection. University of Calcutta, Calcutta. (PhD thesis).
12. Chaudhuri H and Alikunhi KH. (1957). Observations on the spawning in Indian carps by hormone injection. *Current Science* 26(12): 381-382.
13. Chondar SL. (1970). Handbook of Breeding of Indian Major Carps by Pituitary Hormone Injections, Agra, India. Satish Book Enterprise. 100 pp.
14. Chondar SL.(1984). 'Bungla bundh' a simple unique device for mass breeding of Indian and Chinese carps. *Journal of Inland Fisheries Society of India* 16(1& 2): 37-41.
15. Das SK. (2004). Evaluation of a New Spawning Agent, Ovopel in Induced Breeding of Indian Carps. *Asian Fisheries Science* 17 : 313-322.
16. Das SK, Bhattacharjya BK and Sarma K.(1994). Induced spawning and hatching of *Tawes*, *Puntius javanicus* (Bleeker). *Asian Fisheries Science*.Philippines. Vol.7:191-194.
17. Das P. (1992). Ex-situ conservation of coldwater fish germplasm. National Workshop on R&D Needs in Cold Water Fisheries: pp. 30-31 and 35-43. Haldwani.
18. Das SK. (2003). Breeding of carps using a low-cost, smallscale hatchery in Assam, India – A farmer proven technology. *Aquaculture Asia*. Vol. 8( 1):8-10.
19. Datta MK. (2012). Diversification of Fish Species in Breeding in India. College of Fisheries, Central Agricultural University, Tripura, India. [Aquafind.com](http://Aquafind.com)
20. Devi GA, Devi GS , Singh OB , MunilKumar S and Reddy AK. (2009). Induced spawning and hatching of *Osteobrama belangeri* (Valenciennes) using Ovatide, an ovulating agent. *Asian Fisheries Science* 22(2009):1107-1115.
21. Dehadrai P V. (1986). *Carp seed production in India*, p. 33.
22. Dubey GP.(1969). Induced breeding of Indian carp dry bundhs. *FAO/UNDP Regional Seminar on Induced Breeding of Cultivated Fishes*, Calcutta. *FRIf.IBCF/21*: 10 p.
23. Francis T, Ramanathan N, Athilthan S and Cheryl HF. (2000). Induced breeding of murrel, *Channa striatus* using various inducing agents. *Fishing Chimes* 19 (10 &11): 191- 121.
24. Gurpreet Singh Tiwana and Sudhanshu Raman.(2012). An Economically Viable Approach for Induced Breeding of *Labeo Rohita* by Ovatide, Ovaprim And Carp Pituitary Extract. *IOSR Journal of Agriculture and Veterinary Science*. Volume 1, Issue 1 (Sep.-Oct. 2012) : 30-32.
25. Ghosh, C.; Ray, A. K.; Bhattacharya, S. and Bhattacharya S.1999. Effect of an organophosphate pesticide in the induced ovulation of maturing air breathing fish *Anabas testudineus* with hypothalamic gonadotropin releasing hormone from *Channa punctatus*. *Poll. Res.*, **18** (2):121-127.
26. Haniffa MAK and Sridhar S. (2002). Induced Spawning of spotted murrel (*Channa punctatus*) and catfish (*Heteropneustes fossilis*) using human chorionic gonadotropin and synthetic hormone (ovaprim). *Vet.Arshiv.*, **72** (1): 51-56.
27. Houssay BA. (1930). Accion sexual de la hipofisis en los perces y reptiles. *Rev. Soc.Agent. Biol.* 6: 686-688.

28. Jhahria Anita (2011). Application of induced breeding practices for conservation of fishes in the Thar desert. *Asian Journal of Environmental Science* 6(1): 42-45.
29. JeffMittelmarkand AnneKapusinski.(2008).[www.seagrant.umn.edu/aquaculture/induced\\_fish\\_reproduction](http://www.seagrant.umn.edu/aquaculture/induced_fish_reproduction). University of Minnesota.
30. Kulkarni CV and Ogale SN, (1986). Hypophysation (induced breeding) of Mahseer, Torkhudree (Sykes). - Pb. *Fish Bull.* 10(2): 23-26.
31. Munilkumar S and Nandeesh MC. (2007). Aquaculture practices in Northeast India: Current status and future directions. *Fish Physiology and Biochemistry* 33:399-412.
32. Marimuthu K , Haniffa MA and Aminur Rahman M. (2009). Spawning performance of native threatened spotted snakehead fish, *Channa punctatus* (Actinopterygii: Channidae: Perciformes), induced with Ovatide. *Acta Ichthyol. Piscat.* 39 (1): 1-5.
33. Marte CL. (1989). Hormone-induced spawning of cultured tropical finfishes. *Advances in Tropical Aquaculture*. Tahiti, Feb. 20 - March 4 . AQUACOP 1FREMERE Acres de Colloque 9 : 519 F39.
34. MonjitPaul.(2008).Aquaculture.[http://pics.livejournal.com/monjit\\_paul/pic/00004y8h/](http://pics.livejournal.com/monjit_paul/pic/00004y8h/).
35. More P R , Bhandare RY, Shinde SE, Pathan TS and Sonawane DL. (2010).Comparative Study of Synthetic Hormones Ovaprim and Carp Pituitary Extract Used in Induced Breeding of Indian Major Carps. *Libyan Agriculture Research Center Journal International* 1 (5): 288-295.
36. Marimuthu K, Muruganandam M, Jesu Arockia Raj A and Haniffa MA. (2000). Induced spawning of the Indian catfish *Heteropneustes fossilis* (Singhi) using a synthetic hormone,Ovatide. *Fishing Chimes*, 19 (10, 11): 105-106.
37. Nandeesh MC, Gopal Rao K, Jayanna RN, Parkar WC, Varghese TJ Keshavanath Pand Shetty HPC.(1990b). Induced spawning of Indian major carps through single application of ovarpim-C. In Hirano, R. and Hanyo, I. (eds) : Proc. second Asian Fisheries Forum. Asian Fisheries Soc. Manila, Philippines.
38. Nandeesh MC, Gopal Rao K, Jayanna RN, Parkar WC, Varghese TJ Keshavanath P and Shetty HPC. (1990). Induced spawning of Indian major carps through single application of Ovaprim-c. Hirano, R. and I. Hanyu, editors. 1990. The Second Asian Fisheries Forum. 991 p. Asian Fisheries Society, Manila, Philippines.
39. Nandeesh MC, Bhandraswami G, Patil JG, Varghese TJ, Kamal S and Keshavanath P. (1993). Preliminary results on induced spawning of pond varied mahseer, Tor Khudri J. *Aqua. Trop.*, 8: 55-60.
40. Nandeesh MC, Das SK, Nathaniel DE and Varghese TJ. (1990). Breeding of carps with ovaprim in India. Special Publ. No.4 (Asian Fisheries Society, Indian Branch, Mangalore), p 41.
41. Ogale SN.(2002). Mahseer breeding and conservation and possibilities of commercial culture. The Indian experience. In: Cold water fisheries in the trans-Himalayan countries. Fisheries and Aquaculture Department. FAO.
42. Ogale SN. (1997). Induced spawning and hatching of golden mahseer *Tor putitora* (Hamilton) at Lonavla, Pune District (Maharashtra) in Western Ghats. *Fishing Chimes*, June 1997: 27-29.
43. Ogale SN and Kulkarni CV.(1987). Breeding of pond-raised hybrids of Mahseer fish, Tor khudree (Sykes) and T. tor (Ham.). *J. Bombay nat. Hist. Soc.*, 84:332-335.
44. Pandey AK, Mahaptra CT, Kanungo G, Sarkar M, Sahoo GC and Singh BN.(2002). Ovatide induced spawning in the Indian major carp, *Labeo rohita* (Hamilton-Buchanan). *Aquacult.* 3: 1-4
45. Pandey AK, Patiyal RS , Upadhyay JC , Tyagi M and Mahanta PC. (1998). Induced spawning of the endangered golden mahseer. *Tor putitora*, with ovaprim at the state fish farm near Dehradun. *Indian J.Fish.*, 45(4): 457-459.
46. Pathani SS and Das SM. (1978). Induced breeding by hypophysation of Mahseer *Tor putitora* in Bhimtal. *Sci. & Cult.* 45: 209-211

47. Pramod Rokade, Ganeshwade RM and Somwane SR.(2006). A comparative account on the induced breeding of major carp *Cirrhina mrigala* by pituitary extract and ovaprim. *Journal of Environmental Biology* 27(2): 309-310.
48. Peter RE , Chang JP, Nahorniak CS , Omeljaniuk RJ , Sokolowska M, Shih SR and Billard R.(1986). Interaction of catecholamines and sGnRH in regulation of gonadotropin secretion in teleost fish. *Recent Prog. Horm. Res.* 42:513-548.
49. Ravi Shankar Piska and Jithender Kumar Naik S. (2007). Fresh water aquaculture Fisheries, II year Paper I ( Ed. Ravi Shankar Piska) Intermediate Vocational Course ,State Institute of Vocational Education and Board of Intermediate Education.
50. Reddy PVGK. (2000). Captive breeding of *Osteobrama belangiri* (Val.)- A threatened food species . In: *Fish Biodiversity of North East India.* (ed. A.G. Ponniah and U.K. Sarkar), pp 122-123. NATP Publ.2. National Bureau of Fish Genetics Re-sources, Lucknow, India.
51. Reddy AK and Mathur KB. (2000). Ovotide – A highly potent inducing agent for breeding of carps. First Indian Science Congress, 21-23, Sep. 2000, Panjab University, Chandigarh.
52. Ram Singh and Akhilesh Kumar Gupta. (2011). Control mechanism for induced spawning in fish reproduction. *J. Exp. Zool. India* Vol. 14, No. 1 : 1-13.
53. Ramaswamy LS and Sundararaj BI. (1956). Induced spawning in the Indian catfish *Science* 123(32):1080.
54. Ramaswamy LS and Sundararaj BI. (1957). Induced spawning in the cat-fish *Clarias*. *Naturwissenschaften* 44: 384.
55. Rath SC, Sarkar SK, Gupta SD and Mondal B. (2007). Gonadal development and induced spawning in spontaneously bred *Labeo rohita* (Ham). *Indian Journal of Fisheries* 54(2) :163-170.
56. Samarjit NS and Basudha C.(2007). Induced spawning of *Osteobrama belangeri* (Val.), a critically endangered fish in India using carp pituitary and Ovaprim. *Aquacult* (2):231-236.
57. Sarkar UK, Deepak PK , Negi RS and Lakra WS.(2009). Captive Breeding of a Gangetic Leaf-fish *Nandus nandus* (Hamilton-Buchanan) with Three Commercial GnRH Preparations, *Journal of Applied Aquaculture*, 21:4, 263-272.
58. Sridhar S , Vijay Kumar C and Haniffa MA. ( 1998). Induced spawning and establishment of a captive population for an endangered fish, *Ompok bimaculatus* in India. *Curr. Sci*, 7(10): 1066-1068.
59. Sehgal KL.(1991). Artificial propagation of Golden Mahseer *Tor putitora* (Ham.) in Himalayas. Special Publication. National Centre For Cold Water Fisheries, Haldwani.
60. Sarkar UK, Patiyal RS and Srivastava SM. (2001). Successful induced spawning and hatching of hill stream carp, *Labeo dyocheilus* (McClelland) in Kosi river. *Indian J. Fish.*, 48(4): 413-416.
61. Saud BJ , Hazarika R, Verma VK and Goswami MM. (2013). Induced Breeding Response of Two Synthetic Hormone Analogues In The Spawning of Indian Major Carp *Labeo rohita* (Hamilton-Buchanan) Under The Agro-Climatic Condition of Assam, India. [www.aquafind.com](http://www.aquafind.com).
62. Sehgal KL and Kumar K. (1977). Final project report on induced breeding and rearing of Mahseer *Tor putitora* (Ham.) seed in running water ponds. CIFRI.
63. Somashekarappa B, Chandrashekaraiyah HN and Nandeeshha MC. (1990). Advancement of maturity in an Indian major carp, *Catla catla* through improved diet and human chorionic gonadotropin administration, pp. 29-33. In P. Keshavanath & K.V.Radhakrishnan, eds. *Carp Seed Production Technology*. pp. 29-33. Mangalore, India, Asian Fisheries Society, Indian Branch. 102 pp.
64. Sinha VRP, Jhingran VG and Ganapathi SV.(1974). A review on spawning of the Indian major carps. *Arch. Hydrobiologia* 73(4): 518-536.

65. Teji KT and John Thomas K. (2006). Observations on the morphological abnormalities in induced bred larvae of some freshwater fishes. *Indian J. Fish.*, 53(3): 353-358.
66. Tripathi YR. (1978). Artificial breeding of *Tor putitora* (Ham.). *J. Inland Fish. Soc. India* 9: 161.
67. Thakur N.K., Reddy A.K. 1997. Repeat field trials with new hormonal preparation—Ovatide for fish breeding. Final Report, CIFE, Mumbai, India.
68. Varghese TJ, Satyanarayana Rao GP, Devaraj KV and Chandrashekhar B.(1975).Preliminary observation on the use of marine catfish pituitary glands for induced spawning of the Indian major carps. *Current Science* 44(3):75-77.
69. Von Ihering R. (1935). Die Wirkung von Hypophyseninjektion auf den Laichakt von Fischen. *Zool. Anz.* 11:273-279.
70. [www.fao.org](http://www.fao.org).
71. [www.aquafind.com](http://www.aquafind.com)
72. [http://mofa.gov.gh/site/?page\\_id=10279](http://mofa.gov.gh/site/?page_id=10279)