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Role of pituitary extracts and other inducing agents in induce breeding

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Abstract
Indian major carps comes under fishes which do not ordinarily spawn in confined water or stagnant water bodies such as ponds, lakes etc., but spawn usually in inundated terrains of rivers and streams during rainy season. Bulk of the Inland aquaculture production comes from farming of the three Indian Major Carps- Catla, Rohu and Mrigal also known as Gangetic Caps that inhabit the major river systems in the country. Induced breeding techniques have allowed farmers to profitably breed and culture carp fishes that do not naturally reproduce under captive conditions. Hormone administration is the most common method of induced breeding in fishes which the pituitary extract injected into the matured male and female fishes and the hormone induce them to spawn. Successful induced spawning depends upon the dosage of hormone. Injection, potency of the pituitary gland, gonodal maturity of the recipient fish and the prevailing environmental conditions. Such as temperature, water currents and rain. The important environmental factors responsible for induced breeding in Indian major carps are temperature, pH, dissolved oxygen and hardness. Proper care of brood stock is very important for assuring good production of eggs, hatchlings, fry and fingerlings in induced breeding of carp fishes. Several commercially available synthetic ovulating agents in a ready-made form which contained GnRHa and dopamine antagonist such as ovaprim, ovatide are becoming very popular nowadays and found to be efficient and successful spawning agents in different carp fishes.
The technique of spawning the fishes under controlled condition by administration of Pituitary hormone is known as Induced breeding. Induced breeding is also known as hypophysation.

Keywords: Pituitary extract, Induce breeding, Indian Major Carp, Ovaprim, Ovatide, HCG (Human Chronic Gonadotrophin)

Introduction
When the breeding is not allowed to occur naturally but it is induced artificially in fishes it is called induced breeding. Availability of required quantity of fish seed of desired species is one of the most important requisite for successful fish farming. The widely cultured Indian major carps in inland waters like Catla, Rohu, Mrigal and Chinese carps like silver carp, grass carp, mud carp, black carp etc. normally do not breed in confined waters. They do mature there, but only breed in the flooded shallow areas along the course of rivers during monsoon months which are their natural habitat. The Indian major carp do spawn in the specialized environments of bundhs, both wet and dry where lot of rain water is accumulated during monsoon period. Under such circumstances the fish culturists had to depend for fish seed collection from river systems and the collected fish seed consisted of not only of desired species, but also of uneconomic species including predators. The inability of Asiatic carps to breed in confined stagnant waters is owing to the lack of needed ecological stimuli to effect secretion of required quantity of gonadotropic hormones and so extraneous hormones such as pituitary extract or synthetic hormones are injected to brood fish to induce them to breed. The first success in induced breeding in fish in India was made by Hamid Khan in 1937 when khan tried to induce spawn C. Mrigal by the injection of mammalian pituitary gland. Later H.L Chaudhuri succeeded in induced spawning of small carp species *Esomus danricus* by administering the intra-peritoneal injection of Catla pituitary gland. The first success to inducing the Indian major carps was achieved in 1957 (Chaudhuri and Alikunhi, 1957) [13] and Silver and Grass carp introduced in India in 1959, in the year 1962 (Alikunhi *et al.*, 1963). Since then several experiments on induced breeding of fish have been carried out. The total fish production in Bihar is 8 lakh. 46 thousand metric tons. Bihar is the fourth inland fish producing States in India. Fish also provides proteins, fats, vitamins, essential amino acids and fatty acids. Above all, fish is rich in linolenic acid (omega-3) that helps in prevention of coronary heart diseases and other cardiovascular diseases.

For this reason, Greenland Eskimos and Japanese fisherman do not suffer from heart attacks as they consume fish daily (250g to 400g). The omega-3 fatty acid also prevent blood clotting and artherosclerosis. So cardiologists recommend that if fish is taken twice a week, it will prevent heart diseases. Now-a-days about 30-40% of the world population is suffering from protein deficiency. So, fish has special importance as a supplement to ill balanced cereal diets. Apart from improving the availability of fish food to the masses, a Growth can generate employment opportunities for the poor and Vulnerable sections of the society and will also check the flow of Money from Bihar to the state of Andhra Pradesh (production-34 lakh 50 thousand tons per annum) to buy fish. So Bihar is very weak in Compare to Andhra Pradesh. The first experiment of induced breeding of carp fishes was made by khan (1938) when he tried to induce spawn *Cirrhinus mrigala* by the injection of mammalian pituitary gland. The great success in induced breeding ofIndian Major carps (*Labeo rohita and Cirrhinus mrigala*) was in the year 1957 by chaudhuri and Alikunhi (1957) [13]. Alikuni successfully bred the exotic Chinese carps – *Hypophthalmichthys molitrix* & *Ctenopharyngodon Idella* in 1963. Dhawan and Kaur (2004) [18] found that Ovaprim was more effective than ovatide in breeding induction of *Catla Catla*, however, in *Labeo rohita* and *Cirrhinus mrigala*, ovatide resulted in high Fecundity and fertilization rate. Khan (2006) [33] started that ovatide was better than ovaprim in induced spawning of *Labeo rohita*. Naeem (2011) states that ovaprim is advantageous than ovatide in Inducing ovulation and fecundity. Spawn production perkg female body weight was found 0.88-1.0 lakh Rohu, 0.95 Lakh in catla and 1.1-1.3 lakh in bata as reported by Chakrabarti (2016) [8]. Mohapatra (2018) [42] conducted an experiment of Indian major carp seed Production through Induced breeding and reported fertilization rate was about 91.7-97.5% and spawn production was 1.02-1.18 lakh/kg body Weight. Of female in IMC. Ghosh (2019) [21] performed an experiment of induced breedingof C Catla.

Methodology adopted for induce breeding

Induced breeding technique

Removal of Gland

- Removal through foramen magnum – the foramen magnum was first exposed by removing vertebral parts adhering to skull. Fat is removed first by means of forceps and then cotton piece. A pair of forceps then inserted into foramen magnum dorsally to the brain and anterior part of the brain now detached and remaining is carefully lifted out through the foramen magnum. The gland is then located and removed.
- Removal of gland by dissecting head – This technique is not used commercially as because the heads are damaged by this process. The first method of removal is less time consuming and economical as the heads are used for human consumptions later. At first the head is dissected using sharp butcher's knife, a portion of scalp is chopped off in a clean cut with one stroke. Fat surrounding the brain is removed with the help of cotton. Olfactory and optic nerves are now severed, and then brain is lifted up and removed.

Preservation of Gland

- Glands can be preserved in 100% ethyl alcohol.
- Acetone can be used for preservation in temperate countries.
- Glycerin is also used as preservation media.

Preparation of Gland Extract

- Known amount of gland is taken by estimating the total quantity of fish to be breed.
- Gland is dried in air by using blotting paper.
- Gland is taken in tissue homogenizer with little amount of distilled water.
- The dilution rate is 0.2 ml/kg of body weight of the fish.
- The pituitary extract is then centrifuged and only the supernatant solution is used for injection.

Brooders Selection

- The brooders must be healthy enough and ripe.
- 2-4 years of age is generally selected.
- 1-5kg body weight is preferable.

Injection to the Brooders

- The pituitary extract is administered into the body of breeders by means of hypodermic syringe either intra muscular or intra peritoneal.
- Determination of correct dosage of pituitary extract to be given to the breeders is very important and depends upon the size and state of maturity of the recipient (breeders) as well as upon the state of maturity of the donor for the glands.
- Usually the female is given a preliminary dose of 2-3mg/kg of body wt. The preliminary dose is not given to the male. After an interval of time about 6hrs a second dose of 5-8mg are given perkg of body wt of female.

He techniques employed involved the use of the various synthetic agents for induced breeding exercises.

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.

Table 2 shows the results for the experiments done from year from July 2020 to August 2022. Overall 10 induced breeding exercises were done in rohu and mrigal in a hatchery of Bihar. WHICH involved ovaprim injection. The eggs produced varied from 1.00 Lacs to 3.20 Lacs and the fertilization rate was from 50 to 90%. The hatching rate was recorded above 79% in both the species. The spawn produced varied from 0.489 to 2,439 Lacs.

Table 1: Comparative study on induced breeding by synthetic hormones in various species in India by various authors

Fish species	Dosage of hormone	Hormone	Total fertilized eggs%	Incubation period (Hrs)	Hatching%	Reference
Catla catla	0.4-0.6	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	0.2-0.4	Ovaprim	71-78	18-22	80-83	Saud et.al (2013)

Table 2: The results in a hatchery showing of Ovaprim induced breeding exercises

Mrigal	(05 Exercises)	Rohu (05 Exercises)
Eggs production	1.92-3.20 Lacs	Eggs production 1.00-2.72
Fertilization%	70-90%	Fertilization% 50-85%
Hatching Time	79-88	Hatching Time 80-89
Hatching Rate%	79-88%	Hatching Rate% 80-89%
No. of Spawn Produced	1.224-2.439 Lacs	No. of Spawn Produced 0.489-1.942

Results and discussions

Brood fishes selection for induced breeding According to Hasan & Ahmed,6 three Indian major carps (rui, catla and mrigel) and three exotic carps (silver carp, grass carp and common carp) were the dominant fish species used as brood fish in most hatcheries of Bhuiyan *et al.*7 stated that healthy brood fish selection is important for induced spawning of *Labeo rohita*.

It was observed that fish hatcheries contribute to a remarkable part of inland fish production as well as to the aquaculture development of Bihar. This study also indicates the necessity of seed production by the induced breeding process or hypophysation. In the fisheries sector, induced breeding has opened the door of a new era throughout the world. For high quality and high quantity of fish production.

Other hormones used in induced breeding

Human chorionic gonadotropin (HCG) or the hypothalamic gonadotropin releasing hormones (LHRH, GnRH) and their synthetic analogues (LRH -A/LHRH-A). Synahorin (a mixture of mammalian hormones and mammalian pituitary extracts in combination with fish pituitary extracts (FPE) to give positive results. In Indian market HCG is sold under the name 'Sumaach'.

Luteinizing hormone – releasing hormones (LH-RH) and its analogues:-

- LH-RH is a synthetic decapeptide.
- Carps when injected with LHRH-A show greater success (78.5%) as against fish pituitary extracts (75%).

Progesterone

Derivatives of progesterone such as 17- Alpha- hydroxy-progesterone (17-alphaProg.) and 17-alpha-hydroxy-20-Beta-dehydroprogesterone (17-alpha-20-Beta-Prog.) are steroids that have been found in significant quantities in the blood plasma of maturing fishes. Injection with these steroids can bring about final maturation and ovulation in fishes.

Antiestrogens

These are synthetic, non-steroidal compounds (such as Clomiphene citrate and Tamoxifen) that are capable of competing with estrogen for binding sites on esttrogen receptors. They are used for inducing ovulation. Antiestrogens inhibit gonadotropin secration, when used in excessive dosage. It may work better in those fishes where vitel-logenesis is complete and blood estrogen are High.

Ovaprim (LINPE method)

The Chinese researchers have developed a technique, called the 'Linpe' method, where the female fish is induced to ovulate by injecting them with a combination of LHRH-Aand the drug domperidone. The drug inhibits the action of dopamine (a substance produced by the fish that inhibits ovulation). Therefore, stimulating the sex organs of the fish.

Conclusion

For induced breeding of the carp fish in captivity, both the pituitary and ovaprim hormone are used. It is found that the spawning response of the carp fish is poor when injected by the pituitary extract whereas the spawning results are satisfactory when the ovaprim hormone is used. Thus, ovaprim is preferred over pituitary for induced breeding of Indian Major Carps. Availability of adequate quantity of carp seeds of desired species at appropriate time is one of the prerequisites of successful induced breeding technique. The raising of seeds in the initial stages is associated with high rates of mortality due to several management problems. Thus, it is essential to follow standardized package of practices for higher growth and survival in intensive seed raising at higher stocking densities to avoid hypoxic conditions and competition for food and space.

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