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Hormone administration with induced spawning of Indian major carp

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Abstract
Induced Breeding (IB) is the most significant advancements in the field of aquaculture to induce reproduction in fish. It is a ttechnique to stimulate ripe fish breeders by pituitary hormone or any other synthetic hormone to breed in captive condition by promotion of timely release of sperms and eggs. Identification of donor fish can be done on the basis of Secondary Sexual characters which are prominent in the breeding season of fishes. The preparation of carp pituitary extract the glands are removed carefully from freshly killed the fish called donor fish. For best result the donor fish should be fully ripe and mature. Common carp is the best donor fish, because it breeds throughout the year and the individuals are available in all parts of the world.

Keywords: Induced breeding, Pituitary extract, Ova prim, Ovapel

1. Introduction
India is the third largest farmed fish producer ranking second globally. The current fish production in India has reached 8.3 million tonnes, which expected to reach 12.5 million tonnes by 2025. The first experiment in induced breeding of 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. Chaudhari succeeded in induced spawning on small carp species, *Esomus danricus* by administering the intra-peritoneal injection of catla pituitary gland. The first success in induced breeding of Indian Major Carps (*L. rohita* and *C. mrigala*) was in the year 1957 by H.L Chaudhari and K. H. Alikunhi [4] at Central Inland Fisheries Research Institute, substation, Cuttack (Orissa).
Induced Breeding (IB) is the most significant advancements in the field of aquaculture to induce reproduction in fish. It is a technique to stimulate ripe fish breeders by pituitary hormone or any other synthetic hormone to breed in captive condition by promotion of timely release of sperms and eggs. Farmed carp seed production has increased from 6,321 million fry in 1985-86 to over 45,000 million fry in recent year.
Indian major carp was induced bred in eco-carp hatchery with 4 different inducing agents, viz. carp pituitary extract, Ovaprim, Ovatide, Ovapel.

2. Why induced breeding is necessary
Spawn collected from natural water is not pure. The presence of some undesirable wild species and sorting of pure seed is quite impossible with availability of seed is quite uncertain but carps attain full maturity in confined water but do not breed. This method is easily learnt by layman without much training with cost of expenditure very low than the natural collections of spawns.

3. Technique of induced breeding
3.1 Reproductive hormones for induced breeding
1. Pituitary gland extractions
2. Ovaprim
3. Ovatide
4. Ovapel

4. Induced breeding with pituitary gland extraction
Very effective and dependable way to obtain pure seed of cultivable fishes practiced on extensive scale in India and other countries in the world. It involves injecting mature female

and male fishes with extracts of pituitary glands taken from other mature fishes.

4.1 Role of pituitary gland in induced breeding

Pituitary gland secretes the gonadotropins i.e., Follicle Stimulating Hormone (FSH), and Luteinising Hormone (LH) both hormones secreted throughout the year, but proportionally correlated with the cycle of gonadal maturity. FSH causes growth and maturation of ovarian follicles in females and spermatogenesis in the testes of males. LH cause Luteinisation in females and promote the production of testosterone in males. These hormones are not species specific, however, there is great variability in its effectiveness in different species.

4.2 Collection of pituitary gland

Proper selection of the donor fish is essential for success of IB and pituitary collected from fully ripe gravid fishes. Glands from immature or spent fishes do not give satisfactory results. The glands usually collected from freshly sacrificed fishes but ice-preserved specimens also used. However, May to July months, most suitable time in India for collection of pituitary glands of major carps.

4.2.1 Techniques for collection of pituitary glands

A. Open dorsal side of the skull
B. Open brain cavity through foramen magnum.

4.2.2 Pituitary gland preserved by two methods

i. Absolute alcohol preservation
ii. Acetone preservation

i. **Alcohol preservation**

After collection glands immediately put in absolute alcohol for defatting and dehydration After 24 hour's glands washed with absolute alcohol and kept again in fresh abs. alcohol Store in refrigerator up to 2-3 years or at room temperature up to 1 year.

ii. **Acetone preservation**

Glands kept in fresh acetone or in dry ice-chilled acetone inside a refrigerator at -20 ^{0}C for 36-48 hours 2-3 changes of acetone at about 8-12 hours intervals Glands are taken out of acetone, put on filter paper and dried at room temperature for one hour and largely practiced in USSR and USA.

4.3 Preparation of Pituitary Gland Extract

- Extract of the gland prepared just before injection
- Gland weighed and homogenized in distilled water or 0.3% saline
- Final volume should be 0.2ml/kg BW of the fish
- Centrifuged the suspension
- Supernatant used for injection

4.4 Preservation of Pituitary Gland Extract

Preserved extract in glycerin and kept in refrigerator for 24 hours and preserved in propylene glycol and kept in refrigerator for 30 days with Immersed in 1.5% TCA for 12 hours and kept in refrigerator for 10 days.

4.5 Technique of Breeding

- **Dosage of pituitary extract**
  - **Female given 2 doses**
    1. Initial dose: 2-3mg/kg body weight.
    2. Resolving dose / final dose: 6-8mg/ body weight.
  - Male given only 1 dose at the time of the 2nd dose given to female (2-3 mg/kg body weight).
  - For females of Indian major carps one initial and after 5-6 hours final dose given.

4.6 Method of Injection

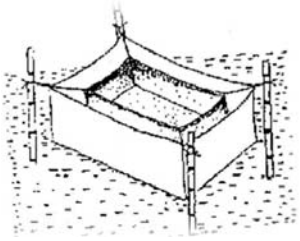
- Intra-cranial injections preferred in USSR and intra-peritoneal in USA and Japan.
- Intra-muscular injection is most common practice in India.
- Intra-muscular injection given at the caudal peduncle or shoulder regions near the base of the dorsal fin.
- Intra-peritoneal injections given at the base of the pelvic fin or pectoral fin.
- Injections given to the carps at an angle of 45^0.

4.7 Breeding hapa and spawning

After injection breeders released immediately inside breeding hapa. It is generally made of fine cloth closed in all the sides excepting a portion at the top and Size - 3.5 x 1.5 x 1.0 m for larger breeders and 2.5 x 1.2 x 1.0 m for breeders weighing less than 3 kg. One set of breeders released inside each breeding hapa. Spawning occurs within 3-6 hours after the second injection and Fertilized eggs of major carps appear like shining glass beads of crystal clear appearance and unfertilized eggs look opaque and whitish. The size of eggs from the same species of different breeders varies at least 4-5 hours after spawning to allow eggs to get properly water-hardened and stripping or artificial insemination also followed.

4.8 Technique of hatching the eggs

Eggs collected from breeding hapas transferred into the hatching hapas with consists of two separate smaller in size and fitted inside the outer hapa. The outer hapa made up of a thin cloth with standard size of 2 x 1 x 1 m and inner hapa made of round meshed mosquito net cloth in the dimension of 1.75 x 0.75 x 0.5 m. with 75,000 to 1, 00,000 eggs are uniformly spread inside each inner hapa and the eggs hatch out in 14-20 hours at a temperature range of 24-31 ^{0}C. After hatching, the hatchlings escape into the outer hapa through the meshes of the inner hapa and also inner hapa containing the egg shells and the dead eggs removed when the hatching complete.



4.9 Problems of hypophysation technique

- Farmer cannot measure the potency of the available gland
- Serious difficulties in large scale collection and storage of pituitary
- Large gap between the supply and demand of pituitary
- Basic equipment's like chemical balance, centrifuge and refrigerator normally not available in several farms
- Pituitary gland very costly in market.

5. Induced Breeding with Ovaprim

Dr. Lin of China and Dr. Peter of Canada, developed a reliable technology where in an analogue of LHRH combined with a dopamine antagonist. M/s Syndel Laboratories Limited, Canada manufactured a new drug called as ovaprim and in India marketed by Glaxo India Ltd., Bombay. Ovaprim consists of GnRH-a and dopamine receptor antagonist, domperidone.

Table 1: Recommended dose in fishes

Fishes	Male (ml/kg body wt.)	Female (ml/kg body wt.)
Catla	0.1 – 0.2	0.4 – 0.5
Rohu	0.1 – 0.2	0.3 – 0.4
Mrigal	0.1 – 0.2	0.25 – 0.3

5.1 Advantages of ovaprim treatment

Rates of fertilization and hatching higher and size of eggs after water hardening always considerably bigger in Ovaprim treated fish with hatchlings obtained healthier. It's more economical than pituitary however post-spawning mortality of fish negligible little or no effects on reproductive cycles and not require refrigerated storage and preserved at ambient temperature.

6. Induced Breeding with Ovatide

- Synthetic compound launched by Hermmopharma, Bombay
- Combined of GnRH analogue with dopamine antagonist pimozide

Table 2: Recommended dose in fishes

Fishes	Male (ml/kg body wt.)	Female (ml/kg body wt.)
Catla	0.2-0.3	0.4-0.5
Rohu	0.1-0.2	0.2-0.4
Mrigal	0.1-0.2	0.2-0.4

6.1 Specific Advantages of Using Ovatide

Use of Ovatide represents the most modern and advanced technology for spawning of fish at considerably low cost. Fishes injected with Ovatide produce increased number of eggs through complete spawning with high fertilization and hatching percentage with low viscosity of Ovatide makes it easily injectable. Administrated in a signal dose without causing any adverse effect on brood fish after injection male and female brood fish are injected simultaneously with effective even under adverse climatic condition. Ovatide produce healthy fish seed of good growth rate.

7. Induced breeding with Ovopel

Ovapel developed by university of Godollo in Hungary, Combined of mammalian GnRH analogue and dopamine receptor antagonist, Lactosum, Carriers and recommended dose 1-2 pellet/kg of fish in catla and Rohu.

7.1 Use

- Weight of brood stock should be measured before propagation.
- The required amount of ovopel is 1 pellet for 1 kg of brood stock + 0, 1 pellet for losses during powdering and dissolving.
- When, fish are ready for spawning, pellet should be powdered and dissolved in salt solution (0.65% Nacl) 12 hour befor planned ovulation then injected in to the abdomen fish.

7.2 Other Substances used for Induced Breeding

Other substances like LH-RH analogues, steroids, HCG and clomiphene also used for IB

Environmental factors like temperature, water condition, light, meteorological conditions, etc. are important factors controlling the reproduction of fish.

8. Conclusion

- Fish hatchery operators should be trained on better brood fish management, hatchery management and nursery management to produce quality fish seed.
- Government or financial institutions should sponsor setting up of field laboratories for assessing and monitoring fish seed quality.
- More emphasis should be laid on multiple spawning of carps so as to ensure the availability of seed over a longer duration in a year
- Greater support (technical as well as financial) from government agencies needed for sustainable fish seed production
- Production of seed of valuable species like catfish and murrels, which command a good price in several parts of the country
- The Government of India should explore the possibility of having a uniform fish seed grading system and pricing for the entire country.

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