INDUCED BREEDING OF FISH:

- Houssay (1930) of Argentina was the first to attempt induced breeding of fish by using pituitary extract on a viviparous fish.
- He was successful in obtaining premature birth of young fish.
- Subsequently, based on the lines of Houssay, Von Ihering and his team of Brazil, in 1934, successfully induced bred a catfish with pituitary hormones and hence credit for the present day concept of induced breeding of fish goes to Brazilians.
- In India, Chaudhuri and Alikunhi (1957) successfully induced major carps to spawn through hypophysation technique.
- Since then, the technique has been standardized and refined for the large-scale production of fish seed.
- The Indian Major Carp, which normally spawn once a year either naturally or through hypophysation during monsoon, were successfully induced bred twice within an internal of about two months.
- Chondar (1984;1990) described a method for the mass scale breeding of IMC(Indian Major Carp) and silver carp in `Bangla bundh' through Human

Chorionic Gonadotropin (HCG) and its combination with pituitary extract.

Environmental factors concerned with breeding of fishes:

Environmental factors concerned with fish breeding are

1. Light
2. Temperature
3. Ecological factors
4. Meteorological conditions

These factors are known to play important roles in stimulating the release of pituitary gonadotropins, thereby controlling reproduction in fish.

1.Light

- It is an important factor that controls reproduction in fish.
- Early maturation and spawning of fish as a result of enhanced photoperiodic regimes.
- In India, Cirrhinus reba was found to attain early maturity when subjected to artificial day lengths longer than natural day even at a low temperature of the winter months, viz. 19-20oC.

- The resorption of gonads in C. reba was delayed and spawning conditions could be maintained up to November.

2.Temperature:

- The role of environmental temperature on sexual maturation and spawning of fish in India has been studied.
- All observations show that there are optimum temperature ranges for induced breeding of cultivable fishes and critical temperature limits, above and below which fish will not reproduce.
- The Indian Major Carps are found to breed within a range of 24-31oC. Beyond this range fish do not spawn.
- The Chinese silver and grass carp have been successfully induced bred at temperatures 28.2oC to 34oC.
- It was observed natural spawning of pituitary injected grass carp at a water temperature varying between 28.9 and 31.1oC, the optimum being 27oC, as in the case of Indian Major Carps.

3. Ecological factor:

- It was opined that fresh rainwater and flooded condition in a tank are the primary factors in triggering the spawning of carps.
- The presence of repressive factors may be responsible for inhibiting spawning of carps in confined waters, but when this repressive factor is sufficiently diluted by the onrush of floods in bundhs or ponds, spawning occurs.

4. Meteorological condition:

- Some workers suggested that it is the sudden drop in the electrolytes level in the environment caused by heavy monsoon rain or water current which induces gonadal hydration, resulting in natural spawning of carps.
- Rain water and weather condition are important factors for induced breeding of fish.
- Successful spawning in the majority of fishes has been induced on cloudy and rainy days, especially after heavy showers.
- The carps are known to breed at a fairly wide range of pH and dissolved oxygen content.

Sympathetic breeding:

- Sympathetic breeding refers to the breeding of uninjected fish at the sympathy of injected fish.
- This is common in bundh breeding, wherein, only 10-20 brooders are injected with either pituitary extract or synthetic spawning agent and the rest are not injected.
- After an interval of about 8-10 hours, the injected brooders first start spawning and subsequently the uninjected brooders are also stimulated to spawn, thereby leading to the complete spawning of all the brooders.
- Sympathetic spawning leads to lesser use of hormone and reduced handling of brooders.
- By this method, natural spawning of both grass carp and silver carp is possible in a dry bundh of Bankura District where they spawned naturally, without stripping.
- Some consider sympathetic breeding as one of the reliable means of mass breeding of Chinese carps to meet the increasing demand of their seed.

Fish Pituitary gland:

- Pituitary gland is an endocrine (ductless) gland situated on the ventral side of the brain.
- It is a small, soft, whitish body whose size and shape vary with species.
- It is more or less round in carps; oval in catla and rohu and pear-shaped in mrigal.
- The pituitary is located in a concave cavity known as Sella turcica and enclosed by a thin membrane known as duramater.
- It may be attached to the brain by a short stalk called the Infundibular stalk.

Types of pituitary glands:

Based on the presence or absence of the stalk, the pituitary is classified into :

i.Leptobasic pituitary (with stalk)– eg. Carps and catfishes.

ii.Platybasic pituitary (without stalk)– eg. Murrels and glassfish (Ambasis species).

The teleost pituitary comprises of two parts-

a.The glandular part (the adenohypophysis).

b.The nervous part (the neurohypophysis).

Collection of pituitary gland:

Fish pituitary gland can be collected by dissecting and removing a portion of the scalp or through the Foramen magnum.

(1) Dissecting and removing a portion of the scalp:

- In this method, the brain case (cranium) is obliquely cut using a butcher's knife/hand saw/bone cutter and the scalp removed.
- The brain is then exposed by removing grey matter and fatty substance with forceps and cotton.
- The anterior end (optic and olfactory nerves) of the brain is cut and the entire brain is lifted up and laid back, thus exposing the pituitary under a membrane.
- After removing the membrane and the fluid, the pituitary is lifted up by inserting the blunt end of the forceps and carefully transferred to a vial containing a preservative.

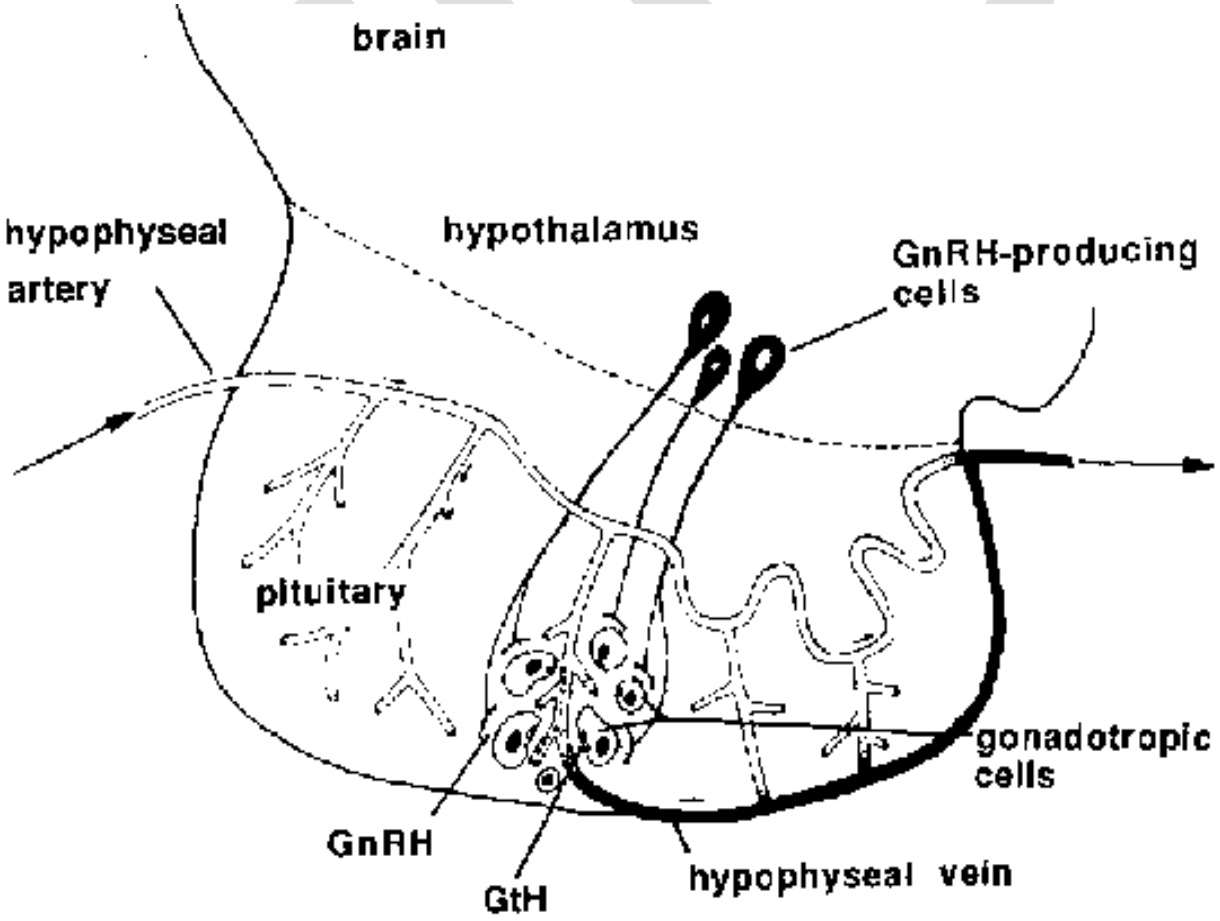
brain
hypophyseal artery
hypothalamus
GnRH-producing cells
pituitary
gonadotropic cells
GnRH
GtH
hypophyseal vein

Figure: Making an oblique cut in the cranium (left) and fatty tissues and grey matter being exposed. (right).

Figure: Making an oblique cut in the cranium Fatty tissue and grey matter exposed.

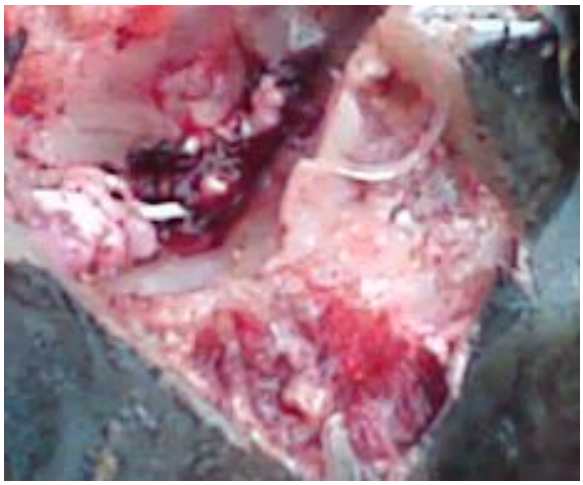

Figure: The brain being exposed the pituitary seen as a small whitish body.

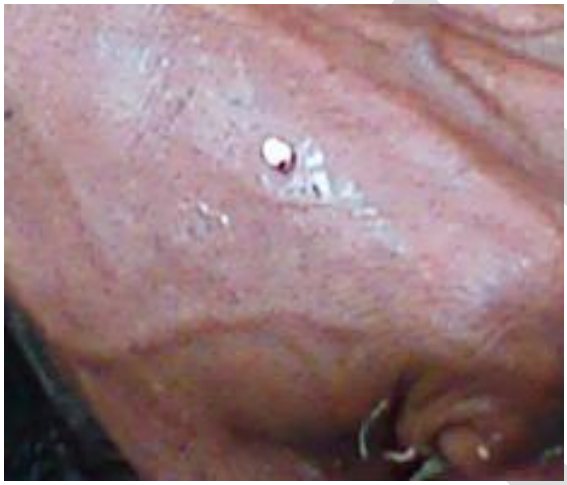

Figure:The pituitary mounted on to a wrist.

(2) Through the Foramen magnum:

- Foramen magnum is a large posterior aperture of the skull through which the spinal cord passes.

- The grey matter and fatty substance are first removed with the help of forceps and cotton (they are pulled out posteriorly).
- The brain is then exposed.
- After this, the anterior end (optic and olfactory nerves) of the brain is cut and the entire brain is lifted up and laid back, thus exposing the pituitary.
- After removing the fluid the membrane, the pituitary is lifted up by inserting the blunt end of the forceps and carefully transferred to a vial containing a preservative.

Remark:

The first method is commonly practiced even though the second method is less time consuming and a large number of glands can be collected within a short time, with a good resale value of the fish.

Preparation of fish pituitary extract for injection:

1. The extract preparation should be carried out just before injection.
2. The required quantity of glands is taken out of vial and they are dried on a filter paper by allowing the alcohol to evaporate.

3. The glands are then homogenized with distilled water or saline in a tissue homogenizer.
4. If acetone-dried glands are used, they can directly be taken for maceration.
5. One-third of the media is used for homogenization, while the remaining two-third is used for rinsing the homogenizer and the glass rod.
6. Recommended dilution rate is 20-30 mg in 1 ml of the media.
7. The extract is centrifuged at 5,000 rpm for 5 minutes.
8. The clear supernatant solution containing gonadotropins is taken in syringe for injection.

Types of injection:

1. Homoplastic injection: Injecting pituitary from one fish to another fish closely related to the donor fish. E.g. carp pituitary gland extract to carps.

2. Heteroplastic injection: Injecting pituitary from one fish to another fish distantly related to the donor fish. E.g. carp pituitary gland extract to catfish and vice versa.

Methods of injecting fish brooders:

There are three methods of injecting brooders. They are :

1. Intra-muscular injection:

- It is administered into the muscle on the caudal peduncle or behind the dorsal fin, but above the lateral line.
- It is most effective, convenient, simple and less risky.
- It is widely practiced.

2. Intra-peritoneal injection:

- It is give through the soft regions of the body, generally at the base of the pelvic fin or the pectoral fin.
- It is risky as it may damage the gonads or liver.

3. Intra-cranial injection:

- In this method, the injection is given through the cranium and is also risky as it may damage the brain.
- The pituitary extract is administered through a glass or disposable syringe, 2.0 ml capacity, having 0.1 ml graduation.
- The size of the needle depends upon the weight of the brooder to be injected.
- Needle number 22 is used for fish weighing 1-3 kg, No. 19 for larger fish and No. 24 for smaller fish.

- When two injections are given, one is given on the side that did not receive the first injection.

Preservation of pituitary gland:

1. Preservation in absolute alcohol:

- In this method, the gland, after collection, is immediately transferred to a vial/phial containing fresh absolute alcohol (ethanol).
- After 24 hours, the alcohol is removed and fresh alcohol is added and stored at room temperature or in a refrigerator.

2. Preservation in acetone:

- Immediately after collection, the pituitary gland is kept in ice-chilled acetone and stored in a refrigerator for 2-3 days.
- After this period, the acetone is changed and the gland stored in a refrigerator.
- Both absolute alcohol and acetone have de-fattening and dehydrating effect.

3. Immediate freezing:

- In this method, the collected glands are frozen immediately and stored in a freezer.

Dosage of pituitary extract:

- Assessment of proper dosage is most important for successful spawning. In practice, the female receives two injections, while the male receives only one injection, i.e. at the time of second injection to the female.
- I Dose or Provocative or preliminary dosage and II Dose or effective or resolving dosage.
- The interval between the two doses is 6 hours.

Carp glands to major carps:

	Female	Male
I Dose	2-3 mg/kg b.w.	nil
II Dose	5-8 mg/kg b.w.	2-3 mg/kg b.w.

Carp glands to exotic carps:

	Female	Male
I Dose	4-6 mg/kg	nil

	b.w.	
II Dose	10-16 mg/kg b.w.	4-6 mg/kg b.w.

Catfish glands to major carps:

	Female	Male
I Dose	10 mg/kg b.w.	nil
II Dose	20 mg/kg b.w.	10 mg/kg b.w.

Catfish glands to exotic carps:

	Female	Male
I Dose	20 mg/kg b.w.	nil
II Dose	40 mg/kg b.w.	20 mg/kg b.w.

Synthetic hormones for induced breeding of fishes:

- Studies conducted by numerous investigators on induced breeding of fishes have indicated the superiority of several ovulating agents over fish pituitary extract.
- Although fish pituitary extract was initially used extensively for fish breeding all over the world, synthetic spawning hormones are now being increasingly used due to their efficacy and convenience.
- Banerjee et al. (1989) succeeded in the purification of pituitary gonadotropic hormone from Channa punctatus and Catla catla.
- Mammalian pituitary hormones in combination with fish pituitary gland extract precipitated spawning in fish.
- Of all the mammalian hormones tested on fish, chorionic gonadotropin (CG) has given successful result in inducing fish to breed, probably because CG behaves primarily as a luteinising hormones (LH).
- Synahorin (a mixture of CG and mammalian pituitary extract) in combination with pituitary gave positive results when injected to rohu.

- Sinha (1969) reported the fractionisation of pituitary extract from carps and tilapia. He obtained success in spawning of carps.
- Bhowmick et al. (1979) found mammalian hormones antuitrin-s, leutocyclin and RH-LH ineffective when injected singly or in combination with carp pituitary extract.
- The CIFRI, Barrackpore undertook detailed studies on the use of LH-RH alone or in combination with progesterone and obtained breeding success which ranged between 25-49% in carps and 100% in catfish.

Synthetic spawning agents:

- The stimulation of pituitary gonadotropin secretion by synthetic LH-RH has been demonstrated in a number of teleosts.
- Since LH-RH (natural or synthetic) alone is not very effective in inducing spawning in fish, a combination of LH-RH-a (GnRH-a) and a dopamine antagonist for induced ovulation and spawning in cultured fish is a highly effective procedure called the Linpe method.
- Some workers reported successful spawning of catla, rohu and mrigal with LH-RH analogue at 10-20

mg/kg b.w. and also obtained 100% ovulation with pimozide at 10mg/kg b.w.

- Parameswaran et al. (1988) achieved successful spawning in mrigal with LH-RH-a, buserelin acetate in combination with progesterone.
- Investigations of Jose et al. (1989) with LH-RH-a indicated successful breeding of mrigal and Labeo fimbriatus.
- The Linpe method and ovaprim.
- Both of these rapidly gained acceptance in fish farms in China and India and has now been commercialized by Syndel Laboratories, Inc., Vancouver, British Columbia, Canada, under the tradename ovaprim.
- The ovaprim spawning kit is especially formulated for use with salmonids, cyprinids and other freshwater cultured fish.
- It has been used successfully in a number of species in several countries and is gaining wide acceptance as the preferred method for induced ovulation and spawning of cultured freshwater fish.
- For example, in India, based on field trials (during 1988-90) with ovaprim for induced spawning of Indian major carps, fringe lipped carp, silver carp, bighead carp and grass carp in various fish farms

located in different agro-climatic regions, Nandeesha et al. (1990, 1991) concluded that in economic terms, the use of ovaprim is advantageous.

- The spawning success, quantity of eggs obtained, the fertilization rate and hatching percentage remained consistently higher with ovaprim as compared to carp pituitary extract (CPE) or human chorionic gonadotropin (HCG) in almost all instances.
- The results also indicate that nearly 40% more fry can be obtained by using ovaprim in place of commercial CPE.
- Most of the carps tested generally spawned within 10-14 hours after injection. Ovulation and spawning has been successfully induced in India by the Linpe method in the Asian catfish, Clarias batrachus (Manickam and Joy, !989) and Indian catfish, Heteropneustes fossilis (Manickam, 1992).
- Similarly, indigenous preparations, viz. Ovatide (M/s. Hemmopharma Ltd., Mumbai) and WOVA-FH (M/s. WOCKHARDT Ltd., Mumbai) are also being used commonly for the commercial spawning of carps and other fishes in India.

- A combination of busereline (LHRH-a) and domperidone has been successfully used for the spawning of IMC (Basavaraja et al., 2007).

Induced breeding of Indian major carps:

Breeding of fish with pituitary gland (hypophysis) extract is termed as Hypophysation.

The credit for developing the technique of hypophysation in the world goes to the Brazilians, while the pioneers of hypophysation of Indian major carps are H.L.Chaudhary and K.H.Alikunhi.

Induced breeding refers to inducing fish to release gametes through the application of pituitary extract or hormones or chemicals.

Identification of sex of brooders:

- Identification of sex is a prerequisite to induced spawning of the fish.
- Fish is sexually dimorphic and sexual dimorphism is exhibited primarily by gonads and their ducts and this involves killing of fish.
- Alternatively, the sex is identified based on certain morphological/external characteristics which include size, length, weight, colouration, fin characteristics,

modification in the head in the form of nuptial dress, genital opening, width of mouth, etc.

- Carps are sexually dimorphic i.e. mature male and female are morphologically different.
- Some of the external morphological characters which are developed during breeding season could be used to identify sex in major carps which mature during their 2nd or 3rd year.

Characteristics	Male	Female
1. Scale, Operculum and pectoral fins	Rough to touch, particularly the dorsal surface of pectoral	Pectoral smooth to slippery
2. Abdomen	Round and firm	Swollen and soft
3. Genital opening swollen	Elongated slit, white in colour, not swollen	Round and pink
4. When pressure applied on abdomen opening	milky white fluid oozes through genital opening	a few ova may ooze through genital

5. Shape of body and size	Body linear, swollen	stouter, slightly larger

Breeding technique:

- Induced breeding of carps starts with the onset of south-west monsoon, June.
- The male and female brooders are conditioned for a few hours prior to injection.
- Sets of brooders are formed, each consisting of 1 : 2 (female : male) ratio.
- The injected brooders are released in the breeding hapa.

Breeding hapa:

- A Breeding hapa is a box-shaped cloth enclosure made of long cloth, generally of size 2 x 1 x 1 m with provision to close its top after releasing brooders.
- The upper flap is attached to one side and the other sides are either tied or buttoned.
- The hapa is fixed in a canal or pond or cement cistern.

- The four bottom and four top corners are tied to four poles such that the bottom of the hapa should not touch the ground and one-third of the hapa remain above the water level.

Injection of brooders:

1. Intra-muscular injection:

- It is administered into the muscle on the caudal peduncle or behind the dorsal fin, but above the lateral line.
- It is most effective, convenient, simple and less risky.
- It is widely practised.

Figure : A major carp being injected with a spawning agent for induced breeding.

2. Intra-peritoneal injection:

- It is give through the soft regions of the body, generally at the base of the pelvic fin or the pectoral fin.
- It is risky as it may damage the goads or liver.

3. Intra-cranial injection:

- In this method, the injection is given through the cranium and is also risky as it may damage the brain.

Syringe & Needle:

- The pituitary extract is administered through a glass or disposable syringe, 2.0 ml capacity, having 0.1 ml graduation.
- The size of the needle depends upon the weight of the brooder to be injected.
- Needle number 22 is used for fish weighing 1-3 kg, No. 19 for larger fish and No. 24 for smaller fish.

Remark:

Intra-muscular injection is commonly practiced.

Site of Injection:

The hormone injection (pituitary/ovaprim/ovatide) is given at the caudal peduncle region in between posterior

end of dorsal fin and base of caudal fin, above the lateral line, avoiding the lateral line.

Spawning:

- After releasing the brooders in the hapa, they should not be disturbed.
- After about 6 hours, splashing will commence for breeding and be involved in courtship which will continue for one hour.
- At the climax of the courtship, both the partners will be seen in an embrace with their bodies twisted around each other. This exerts pressure on the abdomen, resulting the extrusion of gametes.
- The following morning, the spent brooders are removed and then the eggs are collected and transferred for hatching in a suitable hatching device.

Examination of eggs:

- After the eggs are water-hardened, a sample of eggs is taken in a beaker for assessing quality and quantity.
- The fertilized (good) eggs are transparent with a clearly visible nucleus at the centre and look-like pearls.

- The unfertilized (bad) eggs are opaque white and the nucleus disintegrate within one hour.