

## Control of Fish Reproduction in Captivity

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**Abstract**— Globally, fish production in the wild is decreasing and different aquaculture systems are presently used for the development of broodstock in captivity. Broodstock raised in captivity undergoes severe reproductive dysfunction at the level of the brain-pituitary-gonad (BPG) axis. To stimulate growth and maturation of gametes, different techniques like hypophysation and LIPNE based methods targeting BPG axis are used. The paper highlights the problems of reproductive dysfunction in captivity and methods used in induced breeding.

**Keywords:** Reproductive Dysfunction, Hypophysation, LINPE, GnRH

### I. INTRODUCTION

Aquacultured fish fail to complete the reproductive cycle in captivity and exhibit different forms of reproductive dysfunction (Lam, 1982). In females, the two major problem encountered are failure to complete the vitellogenesis and the other is failure to undergo final oocyte maturation and ovulation, after completion of vitellogenesis. In few species, captive male fails to undergo spermiation. In addition, few species of captive females and males take longer duration to undergo gonadal growth and maturation. To overcome these problems, several hormonal preparations have been used and found to show prominent results (Zohar and Mylonas, 2001).

Gonadotropin-releasing hormone (GnRH) producing neurons are localized in specific regions of the brain, particularly preoptic-hypothalamic region innervating anterior pituitary regions, where pituitary gonadotropins (GtHs), follicle-stimulating hormone (FSH) and luteinizing hormone (LH) synthesizing cells are localized. These FSH and LH are released into the blood streams, which act on the gonadal somatic cells to produce sex steroids. These sex steroids act on gonadal somatic cells to regulate germ cell proliferation, growth and maturation. Most of the commercial hormone preparations such as human chorionic gonadotropin (hCG), GnRH analogue (GnRH<sub>a</sub>), Ovaprim, and Ovatide target the above hormonal pathways to affect gonadal growth and maturation. In recent years, kisspeptins have been shown to act as novel upstream regulator of GnRH and few in-vivo studies indicated their possible application for aquaculture (Munoz-Cueto et al., 2020). This paper presents the major application of methods for manipulation of gonadal growth and maturation in captive conditions.

### II. HYPOPHYSATION

Breeding of fish using pituitary gland extract (PE) is termed as hypophysation. It was Houssay, B.A. of Argentina in 1930 who injected the pituitary gland extract prepared from migratory characin (*Prochilodus platensis*) into a viviparous catfish *Cresterodon decemmaculatus* that resulted in premature birth of young. Following this, Von Ihering and his team in 1934 succeeded in inducing fish to spawn using hypophysation. Later, Russian scientists in 1937 succeeded in inducing surgeons (*Acipenser stellatus*) to spawn. In India,

Khan, H in 1937 was the first to induce Mrigal, *Cirrhinus mangala* to spawn using mammalian pituitary extract. This was followed by Chaudhuri, H in 1955 to succeed in inducing Indian flying barb, *Esomus fabrics* to spawn using pituitary gland of Catla. Chaudhuri and Alikunhi in 1957 successfully induced Indian major carps to spawn using carp pituitary (Aktar and Islam, 2015).

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. Depending on the size, body shape and fish species, commercially available needles and syringes, sold in the pharmacy shops can be utilized for administration of inducing agents. The optimum dosage of the pituitary extract depends mainly on the degree of gonadal ripeness in the recipients and the prevailing environmental and climatic conditions during the breeding period. Doses are given as fresh or dry weights of pituitary gland per unit body weight of the broodfish or in dose units, defined as the ratio of the body weight of the donor and the body weight of the recipient (Lam, 1982).

The common carp (*Common carpio*) is a good donor and glands from this species can be collected throughout most of the year for induced breeding of carps. Generally, pituitary glands are collected from maturing and mature fish for preparation of pituitary extract. Fishes which have been induced to spawn through administration of pituitary extract are also used as pituitary donors within 6-8 h of their spawning. Fully ripe males and females are selected for hypophysation. The female is given a priming dose of 2-3 mg/kg body weight and, after an interval of 4-6 h, a final dose of 5-8 mg/kg body weight. It is at the time of the second injection of the female that the male is also injected at 2-3 mg/kg. The knockout doses are usually 10-14 mg/kg body weight for females IMCs and 15-20 mg/kg body weight for the ripe females of silver and grass carps (Aktar and Islam, 2015).

The supply of pituitary glands is a problem and although crude or partially purified pituitary extracts with assayed gonadotropin potency are commercially available. The problems of standardization and cost of hormone preparations are partly solved with the use of mammalian gonadotropin preparations. Two are available in purified form, human chorionic gonadotropin (HCG) and pregnant male serum (PMS). When given alone, these are ineffective in Indian major carps. Human chorionic gonadotropin (HCG) and Synahorin (a mixture of HCG and mammalian anterior pituitary extract) have been found to be successful in the induced spawning of rohu at a rate of 25 rabbit units/kg after priming with 2-4 mg/kg of carp pituitary extract and they reduce the total carp pituitary requirement by about 50-60%. A single injection of 250 IU/kg HCG together with 6 mg/kg CPE is also effective for spawning Indian major carps (Lam, 1982; Aktar and Islam, 2015).

### III. LINPE TECHNIQUE

Injecting a gonadotropin-releasing hormone analogue (GnRH<sub>a</sub>) followed by (or in combination with) a dopamine antagonist has been called the Linpe method, after Lin and Peter the researchers who started it (Lin and Peter, 1986). Most of the research work resulting in the Linpe method was done on cyprinids, and there is strong evidence for these fish that the method is effective when GnRH analogue is administered along with dopamine antagonist.

The LinPe method involves administering a single injection of luteinizing hormone releasing hormone analogue, (LHRH<sub>a</sub>, [D-Ala<sup>6</sup>-Pro<sup>9</sup>NH<sub>2</sub>-LHRH]) or salmon gonadotropin releasing hormone analogue, (sGnRH<sub>a</sub>, [D-Arg<sup>6</sup>-Pro<sup>9</sup>NH<sub>2</sub>-LHRH]) together with one of the dopamine antagonists. LHRH or GnRH, a hypothalamic decapeptide and its synthetic analogues have been shown to stimulate gonadotropin secretion in teleosts. Gonadotropin release inhibiting factor (GRIF's) inhibitory effect on gonadotropin (GtH) release is blocked by administration of dopamine receptor antagonists such as domperidone, pimozide, metoclopramide, or reserpine (Gorban and Sower, 2003).

The required doses of LHRH<sub>a</sub> and dopamine antagonist differ for the different species and range between 10-100 µg/kg for LHRH<sub>a</sub>, 1-15 mg/kg for domperidone and 1-10 mg/kg for pimozide. The combination of domperidone and sGnRH<sub>a</sub> is more potent than pimozide+LHRH<sub>a</sub> for spawning the same species of carp. Ovulation in the three species occurs from 8-12 hours after the injection (Aktar and Islam, 2015).

#### A. Gonadotropin-Releasing Hormone (GnRH)

Teleost fish of the order Perciformes were the first group of vertebrates in which three GnRH forms were found: salmon GnRH (sGnRH; GnRH III), chicken GnRH-II (cGnRH-II; GnRH II), and seabream GnRH (sbGnRH; GnRH I) (Amano et al., 1991, 1998, 2002; Fernald and White, 1999). Multiple GnRH forms existing in the brain of vertebrates are known as GnRH I, GnRH II, and GnRH III on the basis of molecular characterization (Munoz-Cueto et al., 2020). In recent years, three GnRH forms have been confirmed in the elasmobranchs including sharks, skates and rays (Gillard et al., 2018).

GnRH I is the hypophysiotropic form, distributed in the neuronal population of the preoptic area (POA) and the hypothalamus (HYP). These neurons are shown to be involved in the stimulation of pituitary gonadotrophic hormones (GtH), follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Teleosts share the mammalian GnRH (mGnRH) form in the GnRH I group with other vertebrates. The other GnRH forms found only in the teleosts include seabream GnRH (sbGnRH), medaka or pejerrey GnRH (mdGnRH or pjGnRH), whitefish GnRH (wfGnRH), catfish GnRH (cfGnRH), and herring GnRH (hrGnRH). GnRH2 is the midbrain tegmentum (MT) form, with a neuronal population in the MT region, mainly in nuclei of the medial longitudinal fasciculus (nMLF). GnRH2 is represented in all vertebrates examined to date by chicken GnRH-II (cGnRH-II) form. GnRH3 is a teleost-specific form, expressed in neuronal populations mainly in the olfactory bulb (OB), terminal nerve ganglion (TNG) region, and POA (Gothilf et al., 1996; Gorban and Sower, 2003).

### IV. DOPAMINE

GnRH is considered as the major hypothalamic factor controlling pituitary gonadotrophins; however, its stimulatory action opposed by the potent inhibitory actions of dopamine in teleosts. This dual neuroendocrine control of reproduction by GnRH and dopamine has been demonstrated in several teleosts, where dopamine plays an inhibitory role in the neuroendocrine regulation of the last steps of gametogenesis.

Both *in vivo* and *in vitro* experiments, as validated by molecular and biochemical studies indicate that the dopamine D<sub>2</sub>-like, but not D<sub>1</sub>-like, receptors inhibit gonadotropin secretion directly in the pituitary. This led to the development of methods to induce maturation and spawning in aquaculture, using a combined treatment with a GnRH agonist and a dopamine-D<sub>2</sub> receptor antagonist such as domperidone. This method called LinPe is widely used in aquaculture.

#### A. GnRH based analogues

- 1) Ovaprim: Ovaprim, the product developed based on LINPE method and an analogue of salmon GnRH<sub>a</sub> combined with a dopamine receptor antagonist, domperidone. Ovaprim manufactured by M/s Syndel Laboratories Limited and in India, marketed by Glaxo India Ltd., Mumbai. The recommended dose varies between species. Recommended single dose of ovaprim is 0.5 ml/kg of fish. In Indian major carps, male brood fish administered with 0.1-0.2 ml/kg body weight and in females, vary between 0.25-0.5 ml/kg body weight.
- 2) Ovatide: Ovatide, launched by Hermmopharma, Mumbai consist of GnRH analogue with dopamine antagonist, pimozide. The recommended dose varies between species. In Indian major carps, male brood fish administered with 0.1-0.3 ml/kg body weight and in females, vary between 0.2-0.5 ml/kg body weight.
- 3) Ovopel: Ovopel, developed by University of Godollo, Hungary, the product based on mammalian GnRH analogue and dopamine receptor antagonist metoclopramide. The recommended dose in catla and rohu is 1-2 pellet/kg of fish in rohu and mrigal.
- 4) WOVA-FH: WOVA-FH, the product developed and marketed by Wockhardt (Biostat Agrisciences, Wockhardt Life Sciences Ltd., Mumbai, India). The product based on synthetic GnRH analogue and recommended dose is 0.5 ml per kg body weight of fish.

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