



Rapid review on the use of new age induced breeding agent 'LHRHa' in Indian finfish seed production sector

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Abstract

A focused review on the use of LHRHa in induced breeding of fishes in India was made. Use of LHRHa is mainly restricted to high value brackish water and marine fish species whose market value generally exceeds INR 300-400/kg (USD 4.5-6/kg). Published information on use of LHRHa in induced breeding of fishes in India could document only twelve species out of which nine were brackishwater or marine fish. Besides length and weight, the egg diameter of female fishes (>0.45–0.7 mm) is an important criterion for treating the fishes with LHRHa. LHRHa is either used alone or in combination with HCG (most popular), PGE, 17 α -MT and Pimozide. Dosage of 20-70 μ g/kg body weight for females and 10-40 μ g/kg body weight for males with a latency period of 24-36 hours is most common in India. Experiments with LHRHa are being carried out mainly by ICAR-CIBA, Chennai (dominant) and ICAR-CMFRI, Kochi. Standardization and optimization of LHRHa dosage has been attained in Asian Seabass (*Lates calcarifer*), Cobia (*Rachycentron canadum*), Grey mullet (*Mugil cephalus*) and Spotted scat (*Scatophagus argus*) while in Grouper (*Epinephelus tauvina*), Moonfish (*Monodactylus argenteus*), Milk fish (*Chanos chanos*), Crescent Bass (*Terapon jarbua*) and Silver Pompano (*Trachinotus blochii*), it is under progress.

Keywords: LHRHa; induced breeding; finfish; hormone dose; broodstock eligibility; latency period; India

INTRODUCTION

India has moved from the collection of natural (wild) fish seeds from rivers to the practice of induced breeding in hatcheries in order to ascertain the growth of its aquaculture sector. For most of the cultured species, wild fish seed from natural sources is often insufficient to supply the requirements for culture. The seeds obtained from wild are often impure, contaminated with pollutants and pathogens or contain mix of other unwanted fishes and aquatic organisms. Moreover, the natural fish seed supply is dependent on the season, and fluctuates with environmental and climatic conditions. This signifies the importance of induced breeding operations in any aquaculture industry which is capable of ensuring

adequate and timely supply of pure fish seed. The practice of induced breeding too experienced pronounced transitions *i.e.* from the use of old school pituitary gland homogenates (pituitary gland extract) to the use of new age induced breeding agents like Ovaprim, Ovotide, WOVA-FH, Ovopel and the recent improved synthetic potentiated analogues of LHRH that follow the LINPE method of reproductive physiology manipulation in fishes (ICAR 2011; Thomas *et al.* 2003).

SUMMARY OF REPRODUCTIVE PHYSIOLOGY IN FISHES

Ovulation and spawning in teleosts as in other vertebrates are controlled by several interacting factors. The environmental and pheromone stimuli are received

by the specific receptors of Central Nervous System (CNS) through many of the sensory organs. These stimuli are translated by the brain into neural signals which result in the release of gonadotropin releasing hormone (GnRH) and/or inhibition of the release of gonadotropin release inhibiting factor (GnRIF) causing the pituitary to secrete gonadotropins (GtH) (Peter 1982, 1983a; Peter *et al.* 1986; Lin and Peter 1986). Actually, GnRH stimulates the gonadotrophs of pituitary to produce the gonadotropins (GtH). Fishes have two types of gonadotropins namely GtH I and GtH II. GtH-I is secreted during vitellogenic phase of oocyte maturation in females. GtH-II is secreted during post vitellogenic phase of oocyte maturation in females. When a certain GtH level is reached, vitellogenic oocytes undergo the process of final oocyte maturation: the germinal vesicle migrates to the periphery; theca and granulosa cells of the follicle are stimulated to secrete a maturation-inducing steroid (MIS); and the MIS induces germinal vesicle breakdown (GVBD) (Nagahama 1983; Fostier and Jalabert 1983; Goetz 1983). The Gonadotrophic inhibiting hormone/factor (GiH) which is essentially Dopamine is responsible for suppressing gonadotropin secretion from pituitary gland and is the primary physiological obstacle for attainment of sexual maturity or spawning. To bypass this obstacle, the modern strategy in induced breeding involves the use of GnRH-a/LHRH-a along with a dopamine antagonist like Domperidone, Pimozide or Reserpine. This strategy is known as LINPE's method of induced breeding (Marte 1989; Thomas *et al.* 2003).

ABOUT LUTEINIZING HORMONE RELEASING HORMONE ANALOGS (LHRHa)

LHRH, a hypothalamic decapeptide and its synthetic analogues have been shown to stimulate gonadotropin secretion in teleosts (Crim *et al.* 1987; Peter 1983a, 1983b; Lin and Peter 1986). LHRHa injected together with pimozide or other dopamine antagonists is highly effective in inducing ovulation in these species. The use of LHRHa alone or together with dopamine antagonists in spawning various species of cultured fish was earlier reviewed by Crim *et al.* (1987). Synthetic analogues of luteinizing hormone-releasing hormone (LHRHa) are becoming widely used for inducing ovulation and spawning in a variety of teleosts. For marine species such as milkfish, mullet, sea bass, and rabbitfish, a single LHRHa injection or pellet implant appears to be effective. In a number of freshwater fishes such as the cyprinids, LHRHa alone however has limited efficacy. Standardized methods using LHRHa together with the dopamine antagonists pimozide, domperidone and reserpine have been developed for various species of carps (Marte 1989). LHRHa Acts on the pituitary gland to stimulate the release of gonadotrophins (GtH). LHRHa are the preferred

hormones for spawning induction in fish and are available commercially and available in purified form. According to Lucas and Southgate (2012), LHRHa is more effective than HCG (Human Chorionic Gonadotropin) and PGE (Pituitary Gland Extract) in bringing about oocyte maturation. It is usually used at a dosage of 10–50 µg/ kg fish. Initially, a mid-range dose is used, and the optimum dose is determined by trial and error method. If the dose is too low, it will fail to induce a spawning. Too high a dose will cause final oocyte maturation to occur too rapidly and will result in poor egg quality or egg plugging. A very low dose will be effective if the oocytes are very mature (determined by cannulation). A schematic diagram explaining the mode of action of LHRHa in fish reproduction is given in Figure 1 (adopted from Lucas and Southgate, 2012). The LHRHa treatment involves less handling stress of the fish (Bruzuska 1999).

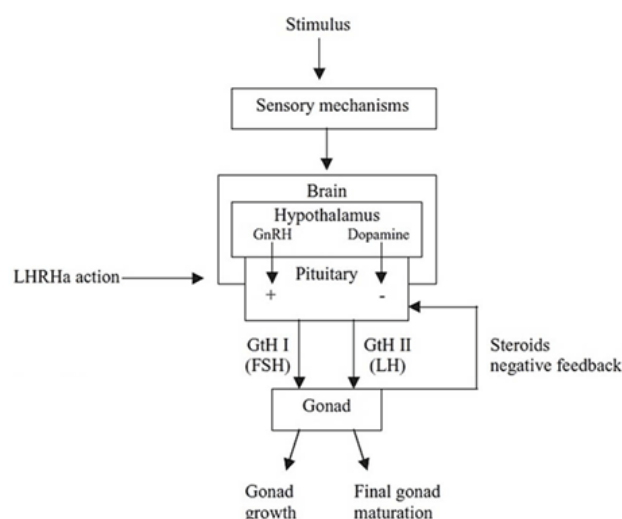


Figure 1: Mode of action of LHRH-a in fish reproduction (adopted from Lucas and Southgate, 2012)

Analogues of LHRH (LHRHa) are synthesized by substituting amino acids at position 6, 7 or 10 of the naturally existing LHRH decapeptide molecule (template). The high potency of LHRHa that are modified at 6th, 7th and 10th positions have been attributed to higher receptor affinity and relative resistance of peptide to degradation (Thomas *et al.* 2003). For induced breeding of fin fishes, [des-Gly¹⁰, D-Ala⁶]-LH-RH ethylamide acetate salt hydrate is the most widely used synthetic analog of LHRH (Lucas and Southgate 2012). LHRHa is commercially available with Sigma-Aldrich chemicals, USA; ARGENT chemicals, USA; Syndel laboratories, USA; Western Chemical Inc., USA; etc (Lee *et al.* 1986; Thirunavukkarasu *et al.* 2009). It occurs in an anhydrous powder form and its price ranges between INR 2186-6985 per 1 mg pack, as on January 2016.

METHODOLOGY OF LHRHa APPLICATION

The hormone in the vial (normally 1 mg) is dissolved in distilled water of known volume (5 ml). Care should be taken so that hormone is thoroughly dissolved. If the 1 mg vial is dissolved in 5 ml distilled water, each ml will have hormone concentration of 200 µg. Say, a brood fish weighs 4 kg and the standard dosage level is @60 µg/kg body weight, then the hormone requirement will be: 60 x

4 = 240 µg for that fish. So from the vial containing dissolved hormone @200 µg/ml, (1/200) x 240 = 1.2 ml has to be drawn through a hypodermic syringe for the purpose of injection. A needle no. 19 or 20 is recommended for injecting large brood fish (>2 kg) and needle no. 21 or 22 is recommended for injecting smaller broods (<1 kg). Intramuscular injection is generally preferred.

Table 1: Application of LHRHa in induced breeding of fishes: Indian scenario

Fish Species	Hormone	Brood stock eligibility	LHRHa Dosage (µg/kg body weight)	Latency period (hours)	Fertilization & Hatching (%)	Reference
Mrigal (<i>Cirrhinus mrigala</i>)	LHRHa (In-Ala ⁶ , Pro ⁹ NHET)	N/A	♀: 10 ♂: N/A	N/A	F: 80 H: 70	Kaul and Rishi (1986)
	LHRHa Buserelin acetate	♀ and ♂: Age 2+ years	♀: 100-200 ♂: N/A	10-16	F: 5-87 H: NA	Parameswaran et al. (1988)
Freshwater shark (<i>Wallago attu</i>)	LHRHa Buserelin acetate	♀ and ♂: Age 2+ years	♀: 150 ♂: N/A	11-15	F: 35-80 H: NA	Parameswaran et al. (1988)
Stripped murrel (<i>Channa striatus</i>)	LHRHa + Pimozide	♀: >600 g ♂: >520 g	♀ & ♂: 40 to 60 + Pimozide @5 mg/kg	18-20	F: 75.3-80 H: N/A	Haniffa (2000)
Asian Seabass (<i>Lates calcarifer</i>)	LHRHa	♀: 4-7 kg ♂: 2-3 kg	♀: 60-70 ♂: 30-35	30-36	F: 70-90 H: N/A	Thirunavukkarasu et al. (2009)
	LHRHa	N/A	N/A	N/A	F: 20-90 H: 35-85	CIBA (2014)
	LHRHa	N/A	♀: 60-70 ♂: 30-35	N/A	F: 38-86 H: 60-92	CIBA (2010)
Cobia (<i>Rachycentron canadum</i>)	LHRHa	♀: 10-15 kg. >3 years age. Eggs >0.7 mm diameter. ♂: at least 10 kg. >1.5 years age.	♀: 20 ♂: 10	12-24	N/A	Gopakumar (2009)
	LHRHa + HCG	♀: >12 kg with ova diameter 0.7 mm. ♂: >12 kg.	♀: 10 + 300 IU/kg HCG ♂: 5	20	F: 30 H: N/A	Arasu (2014)
	LHRHa	♀: 0.7 mm ova diameter. ♂: N/A	♀ and ♂: around 20	N/A	Comparable with natural spawning	Tamilmani (2014)
	LHRHa + HCG	♀ and ♂: 13-16 kg. Ova diameter 0.49-0.85 mm during Apr to Nov	♀ and ♂: 20 +100-300 IU/kg	N/A	N/A	CIBA (2012)
	LHRHa + HCG	♀: 15-18 kg. Ova diameter >0.7 mm during Oct-Dec ♂: 8-15 kg	♀: 20 ♂: 10	20	F: 30 H: N/A	CIBA (2011)
Grouper (<i>Epinephelus tauvina</i>)	LHRHa + HCG	♀ & ♂: 2-3 kg or more. >2 years age. Females with ova diameter >0.45 mm.	♀ & ♂: 40 + HCG @750-1000 IU/kg	72-144	N/A	Arasu (2014)

Table 1: Continued

<i>Fish Species</i>	<i>Hormone</i>	<i>Brood stock eligibility</i>	<i>LHRHa Dosage (µg/kg body weight)</i>	<i>Latency period (hours)</i>	<i>Fertilization & Hatching (%)</i>	<i>Reference</i>
Grey mullet (<i>Mugil cephalus</i>)	LHRHa + PE + HCG	♀: Ova diameter around 0.57 mm during Sep to Nov. ♂: N/A	♀: 50 + PE @2mg/kg + HCG @1000 IU/kg ♂: None	24	F: 1% H: N/A	CIBA (2011)
	LHRHa + HCG	♀: 0.3-1.5 kg. Ova diameter 0.58-0.6 mm attained during Oct - Jan. ♂: 0.3-1.5 kg.	♀ & ♂: 40-50 +HCG @1000 IU/kg	N/A	N/A	Arasu (2014)
	LHRHa + HCG / 17α-MT	♀ and ♂: 0.95-2.9 kg. Ova diameter 0.48-0.6 mm during Nov-Dec.	NA	48	F: N/A H: Failed	CIBA (2014)
Spotted scat (<i>Scatophagus argus</i>)	LHRHa + HCG	♀ & ♂: 72 to 320 g.	N/A	N/A	N/A	CIBA (2013)
	LHRHa + HCG	♀: 150-350 g with ova diameter between 0.42-0.52 mm ♂: N/A	♀ & ♂: 100 + HCG @5000-6000 IU/kg	32-38	F: 15-40 H: 10-45	CIBA (2012)
	LHRHa + HCG	♀ and ♂: 48-254 g. Ova diameter >0.42 mm during Feb-Mar	♀: 100 + HCG @600 IU/kg ♂: 50 + HCG @600 IU/kg	48	F: 20 H: 40	CIBA (2011)
	LHRHa + HCG	♀: 426 g. Ova diameter >0.51 mm. ♂: 47-310 g	♀ and ♂: 35 + 500 IU/kg	24	N/A	CIBA (2010)
Moonfish (<i>Monodactylus argenteus</i>)	LHRHa	♀: >75 g. Ova diameter above 0.4 mm. ♂: 45-75 g.	♀ and ♂: 20	16-20	F: 50-77 H: 60-63	CIBA (2013)
	LHRHa + HCG	♀ and ♂: 8-12 g. Ova diameter 0.38-0.52 mm attained during Jan-Feb	♀ & ♂: 50 + HCG @100-300 IU/kg	18-19	F: 1 H: N/A	CIBA (2012)
Milk fish (<i>Chanos chanos</i>)	LHRHa	♀ and ♂: 2.5-7 kg.	♀ and ♂: 50	N/A	N/A	CIBA (2015)
Crescent bass (<i>Terapon jarbua</i>)	LHRHa	♀: Ova diameter >0.46 mm. ♂: N/A	♀: 75 ♂: 37.5	36	N/A	CIBA (2015)
Silver pompano (<i>Trachinotus blochii</i>)	LHRHa	♀ and ♂: 750 gm – 1.5 kg. Ova diameter >0.5 mm	♀ and ♂: around 20	36-48	Comparable with natural spawning	Nazar (2014)

Abbreviations and Symbols - ♀: Female; ♂: Male; F: Fertilization rate; H: Hatching rate; N/A: Data Not Available; 17 α-MT: 17-alpha-methyltestosterone; HCG: Human Chorionic Gonadotropin; IU: International Units; LHRHa: Luteinizing Hormone Releasing Hormone Analog; PE: Pituitary Gland Extract; g: gram; kg: kilogram; mm: millimeter; µg: microgram

APPLICATION OF LHRHa IN INDUCED BREEDING OF FISHES: INDIAN SCENARIO

In India, use of LHRHa for induced breeding of fishes does not count under a popular option although it is gaining momentum in recent years. In this context, Tamilmani (2014) claimed that natural induced breeding agents like purified GnRH, heteroplastic Pituitary Gland Extracts (PGE), Human Chorionic Gonadotropin (HCG) and other naturally derived hormones are generally large molecules which may provoke immunization reaction in the recipient fishes, and as a result, fish treated with such agents may not respond when treated repeatedly with the same hormone. However, this issue can be successfully avoided by using analogs of LHRH as they are small molecules with just 10 peptides. Other synthetic induced breeding agents like Ovaprim, Ovotide, Wova-FH containing Gonadotropin Hormone Releasing Hormone (GnRH) and dopamine antagonists dominate the Indian fin fish seed production sector. These are cheap and more widely known. A focused review on the use of LHRHa in induced breeding of fishes in India over the last three decades (1985-2015) revealed the following points.

(1) Use of LHRHa is mainly restricted to high value brackish water and marine fish species whose market value generally exceeds INR 300-400/kg. Application of the same in freshwater fishes is nearly absent as the freshwater fishes are comparatively cheaper and easy to breed as cited in Marte (1989). Induced breeding in freshwater fishes is accomplished through more popular agents like Ovaprim, Ovotide, Wova-FH and CPE. Moreover, hassles involved with procurement of LHRHa through external agencies and its high pricing is hindering its dissemination or widespread use among the less liberally spending fisher folk of India. Presently published information on use of LHRHa in induced breeding of fishes in India could document only twelve species out of which nine were brackishwater or marine fish.

(2) Besides age and weight of brood fishes which are generally considered during induced breeding, the egg diameter of female fishes during their spawning season is an important deciding factor or criterion for treating the fishes with LHRHa. Gopakumar (2009) pointed out that the timing of LHRHa injection must coincide with the optimal egg diameter of females in order to obtain high egg yield, fertilization and hatching rate comparable to that of natural spawning in a species. Accordingly, it may be seen from Table 1 that the dosage and brood stock eligibility of many fishes especially in cobia (*Rachycentron canadum*), grey mullet (*Mugil cephalus*), grouper (*Epinephelus tauvina*), spotted scat (*Scatophagus argus*), moon fish (*Monodactylus argenteus*) and crescent bass (*Terapon jarbua*) has been adjusted over the years

revolving around an optimal range of egg diameter for each species *i.e.* cobia (>0.7 mm), grey mullet (>0.47 mm – 0.58 mm), grouper (>0.45 mm), spotted scat (>0.42 mm – 0.51 mm), moon fish (>0.38 mm – 0.40 mm), crescent bass (>0.46 mm), pompano (>0.5 mm).

(3) LHRHa is either used alone or in combination with a wide array of other induced breeding agents like HCG, PGE, 17 α -MT and Pimozide. When used alone LHRHa has proved its efficacy beyond any reasonable doubt. But taking the cost factor into consideration and in order to economize the breeding, LHRHa is used as a resolving dose followed by a priming dose with any other induced breeding agent as given above (Arasu 2014; Tamilmani 2014). Among all the combinations experimented alongside LHRHa, HCG seems to be the most feasible option.

(4) Lots of efforts have been made to establish minimum effective dose of LHRHa either alone or in combination with other agents in each species over the past years. The range of dosage varies quite widely from 10 to 100 μ g/kg body weight of the brood fish. It is clear that inter-specific/inter-generic variation in requirement of LHRHa exists. However, dosage of LHRHa in a range of 20-70 μ g/kg body weight for females and 10-40 μ g/kg body weight for males is most common in India. This range of effective LHRHa dosage is also applicable in a number of other fishes reported from other parts of the world as given in an earlier published review on application of LHRHa in aquaculture (Marte 1989). Experiments on standardization of LHRHa dosage in induced breeding of various fishes are mostly being carried out in only two premier fisheries research institutes of India *i.e.* ICAR-Central Institute Brackishwater Aquaculture, Chennai (dominant) and ICAR-Central Marine Fisheries Research Institute, Kochi. As evident from Table 1, standardization and optimization of LHRHa dosage has been attained in Asian seabass (*Lates calcarifer*), cobia (*R. canadum*), grey mullet (*M. cephalus*) and spotted scat (*S. argus*) while in grouper (*E. tauvina*), moon fish (*M. argenteus*), milk fish (*C. chanos*), crescent bass (*T. jarbua*) and silver pompano (*Trachinotus blochii*), it is under progress.

(5) Although variations in latency period, *i.e.* spawning time after LHRHa injection, exists among different fishes. Under Indian conditions the most common latency period appears to be 24-36 hours.

(6) Data deficiency on various performance aspects of LHRHa in terms of fish breeding *i.e.* latency period, fertilization rate and hatching rate was encountered. Comprehensive information does not exist and it often appears some specific information relevant to LHRHa performance in fin fish breeding is being withheld either intentionally or unintentionally. Shadowy or grey areas exist for some parameters especially fertilization and

hatching rate without which the efficiency of an LHRHa dependent induced breeding experiment with a specific brood stock cannot be ascertained or recommended. Therefore it is imperative to include the information on these aspects especially in institutional annual reports.

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