Indian Journal of Science and Technology, Vol 8(17), DOI: 10.17485/ijst/2015/v8i17/65166, August 2015
ISSN (Print) : 0974-6846
ISSN (Online) : 0974-5645

Role of GnRH, HCG and Kisspeptin on Reproduction of Fishes

P. Elakkanai, T. Francis*, B. Ahilan, P. Jawahar, P. Padmavathy, N. Jayakumar and A. Subburaj

Fisheries College and Research Institute, Tamil Nadu Fisheries University, Thoothukudi - 628 008, Tamil Nadu, India;
t_franciz2000@yahoo.com, elakkanai@gmail.com, kamahilan@yahoo.com, jawaharphd@gmail.com, padmarengan1968@yahoo.co.in, jknap@rediffmail.com, arunmari.raj3@gmail.com

Abstract

The technique of breeding fish by other than its natural course is known as induced breeding. Induced breeding techniques have been developed for production of quality fish seed of cultivable varieties. These techniques have allowed farmers to profitably breed and raise species that do not naturally reproduce in captivity, and to manipulate the timing of reproduction to suit production cycles. Induced breeding is necessary to control timing and synchrony of egg production. Various synthetic hormones like ovatide, ovaprim, wova-FH, ovopel, HCG and LHRH are used in induced breeding of fishes. Newly formulated inducing agents are also being tested for the induced breeding performance by various researchers in different parts of the country, under different climatic conditions, with varying degree of success. These synthetic hormones have several advantages which include ready to use in liquid form, consistent potency and stable with long shelf life. The present review focuses on the effect of GnRH, HCG and Kisspeptin hormone on induced breeding of fishes.

Keywords: Fish breeding, GnRH, HCG, Inducing Agents, Kisspeptin

1. Introduction

Fishes form an important element in the economy of many nations as they have long been a stable item in the diet of many people. The breeding period of fishes in India is variable. Environmental factors play an important role in regulating reproduction in fishes. Fish reproduction is a periodic phenomenon and is controlled by environmental (exogenous) as well as internal (endogenous) regulatory mechanism. The act of breeding occurs under optimal environmental conditions that are favorable to the survival of the young ones. Environmental and hormonal manipulation of ovulation in the fish have become of practical importance in the fish farming industry for two main reasons; to solve the problem of spawning asynchrony which necessitates frequent broodstock handling[1] and for accelerating or delaying gametogenesis in captive broodstock, spawning may be scheduled to yield fry whenever needed[2]. Use of exogenous hormones is an effective way to induced reproductive maturation and produce fertilized eggs[3]. Numerous factors can affect the quality of semen and seminal plasma composition. This factor includes season, temperature, nutrition, stress, hormonal stimulation, milt contamination and short-term storage[4].

Choosing a successful synthetic hormone involves the selection of effective hormone formulations, proper duration of hormonal treatment and timing of the hormone administration. In fish, as with all higher animals, hormones play a critical role in the reproductive process.

*Author for correspondence

Hormones are chemical messengers released into the blood by specific tissues, such as the pituitary gland. The hormones travel through the bloodstream to other tissues, which respond in a variety of ways. One response is to release another hormone, which elicits a response in yet another tissue. The primary tissues involved in this hormonal cascade are the hypothalamus, pituitary gland, and gonads[5].

Fish propagation is increasingly dependent on artificial reproduction. New hormonal preparations permit improving techniques for the controlled reproduction of species that have been propagated successfully for many years. It is also possible to apply these hormonal preparations in the reproduction of species that, until recently, were not frequently studied, for example rheophilic cyprinid fishes[6,7] or species that are under protection[8,9] .

2. The Role of GnRH in Fish Reproduction

The commercially available synthetic inducing hormones in readymade form containing GnRH and dopamine blocker receptor (Ovaprim, Ovopel, Dagin and Aquaspawn) are becoming very popular and found to be efficient in successful spawning of fishes[10-13]. GnRH plays a central role in the coordination of the reproductive endocrine axis in all vertebrates examined to date[14]. Released from the hypothalamus in the brain GnRH targets the pituitary gland (also in the brain) in all vertebrates to stimulate the production and release of follicle stimulating hormone and leutinising hormone in mammals or the equivalent of these peptide hormones in fishes. The release of GnRH from the hypothalamus targets the pituitary to stimulate the release of sex hormones that ultimately regulate gamete activity and the production of sex steroids (androgens and estrogens) by the developing gametes[15].

Administration of GnRH analogues has successfully been used in a number of sturgeon species, including the white sturgeon, *Acipenser transmontanous*[16], the Siberian sturgeon, *Acipenser baerii*[17] and the stellate sturgeon, *Acipenser stellatus*[18]. Three freshwater fishes, namely *Heteropneustes fossilis* (stinging catfish), *Anabas testudineus* (climbing perch), *Mystus vitattus* (striped dwarf catfish) were induced bred and morphological studies of the larvae were carried out[19].

The application and usefulness of Ovopel in controlled fish reproduction has been confirmed in numerous scientific studies[20,21]. Ovaprim is a new preparation in Polish aquaculture and reports on its effectiveness in fish reproduction are still few[22,23]. This preparation is, however, applied successfully in Asian countries[24] and in the USA[25].

An experiment was conducted on *Clarias gariepinus* using carp pituitary extract or ovopel[26]. The carp pituitary at the dose of 4 mg/kg body weight or ovopel a preparation that contains a mammalian GnRH analogue D-Ala^6, Pro^9 NEt-mGnRH (1 pellet/kg body weight) and dopamine receptor antagonist metoclopramide-10 mg/kg being used as ovulation stimulators. The application of ovopel showed significant ($P \leq 0.01$) higher weight of eggs per kg female body weight and the significant ($P \leq 0.05$) higher quality of eggs after 24 hr incubation in comparison with the effects of hypophysation. Statistically significant ($P \leq 0.05$) correlation was found between the percentage of egg fertilization and that of living embryos, the determined correlation coefficient being higher after the application of ovopel than after the carp pituitary homogenate.

Gonadotropin Releasing Hormone (GnRHa) is one of the most commonly used hormones which stimulates the pituitary and releases GTH, thus subsequently milt production. Also GnRHa have been used to induce spermiation in European cat fish *Silurus glanis*[27], Atlantic halibut *Hippoglossus hippoglossus*[28], Green back flounder *Rhombosolea tapirina*[29] and Deccan mahseer *Tor khudree*[30]. Breeding with hormones is a common practice among edible fishes. Considering the demand and need to produce these fishes at specified period and place, induced breeding merits due research. Ovaprim[31-33] though claimed to yield good performance in breeding.

The spawning response of ovaprim compared with pituitary extract in Indian Major Carps[34], at fish breeding center Jaikwadi, Paithan Dist. Aurangabad (M.S) India. Total ten trial doses of ovaprim were used in induced breeding and ten trial doses of Carp Pituitary Extract (CPE) used for induced breeding Indian major carps i.e *Catla catla, Labeo rohita* and *Cirrhinus mrigala*. The percentage hatchling ranged between 74.70 - 95.92 % with ovaprim treatment and 60 - 58.82 % with pituitary extract treatment.

A study was conducted to provide detailed information about the embryonic and larval development of *Ompok pabo*[35] during December 2007 to November, 2010. Artificial breeding of *Ompok pabo* (Hamilton-Buchanan) were carried out at Goalpara College, Assam, India. They examined fertilized eggs till the end of larval developmental period to each and every stage of embryonic and larval development.

Out-of-season spawning of threatened weatherfish, *Misgurnus fossilis* was studied using commercial preparations containing GnRH analogues[36]. The eggs from 83% of females and the sperm from all the males were obtained 12 hours after Ovopel injection. The relative weight of the eggs ranged from 3.2 to 7.8 % of the female body weight and was significantly higher than eggs provided in the course of Ovaprim stimulation ($p<0.05$). The time of latency strongly varied in the case of Ovaprim stimulated fish. After Ovaprim treatment ovulation was induced in all the females, however the ovulation time ranged from 36 to 42 hours. The sperm was obtained from 80% of males 24 to 36 hours after Ovaprim injection.

Neurosteroids play a vital role in governing the physiology of reproduction next to neuropeptides and neurotransmitters. Gonadal development influences the steroid synthesis in Central Nervous System (CNS) and the CNS regulates the gonadal steroid production. It is well known that the receptors of estrogen modulate the production of GnRH, and serotonin, dopamine and GABAergic neurons modulate the steroidogenic enzyme. A detailed study was conducted to identify the various steroids present in the total and regions of Tilapia brain which resulted in the quantitative difference in E2, T, 11-Ketotestosterone (11-KT), Androstenedione (A), DHEA, and 21-Hydroxyprogesterone (21-P). The reproductively active female fish brain showed the high quantity of testosterone when compared with the male brain. The steroidal production in the incubated regions of Tilapia brain highlighted the augmented presence of 5α-or 3α-reductase evidence the elimination pathway[37]. The quantitative expression of mRNA analysis of 3α-HSD, 3β-HSD, Cyp17, Cyp19 and Cyp21 substantiated the variation in sex and maturation of gonadal stages. Aromatase indicated the shift in the sex dependent pathway. The sulphated steroids of pregnenalone and DHEA indicated the presence of Hydroxysteroid Sulfotransferase (HST) for purging action. The study suggested that the sexual modulation can be done at CNS through manipulating the steroidal receptors more particularly at thalamus region of brain.

3. The Role of HCG in Fish Reproduction

The discovery of HCG is traditionally ascribed to Aschheim and Zondex[38] who in 1927 demonstrated in the blood and urine of pregnant woman, a substance which induces ovarian hypennia, corpus luteumfomlation and vaginal estrous in immature female mice. For human chorionic gonadotropin, doses vary from 45 IU/kg to 12500 IU/kg again depending on the species[39,40]. Human Chorionic Gonadotropin (HCG) was also used in experiments on fish reproduction. It was shown that this hormone stimulated Germinal Vesicle Breakdown (GVBD) in oocytes of several fish species[41-43] and steroid production in vitellogenic and full-grown ovarian follicles[44].

Application of HCG for stimulation showed good results in reproduction of *Cyprinus carpio* L.,[45] and Silver carp[46]. Human chorionic gonadotropin is the most common purified gonadotropic hormone used for induced spawning. HCG and pituitary gland extract was injected to the silver carp and the bighead carp during the breeding season[46-48]. It was found that a mixture of HCG and pituitary gland found better than either HCG or pituitary gland separately in induce spawning of females.

Human chorionic gonadotropin is purified gonadotropin hormone used for induced spawning[49]. Over recent years, HCG has been increasingly employed in spawning induction trials of many fish species. PG and HCG has been used to induce spermiation in Japanese eel *Anguilla japonica*[50], mullet *Mugil cephalus*[51], Bream *Abramis brama*[52], Pangasiid cat fish[53] and European eel *Anguilla Anguilla*[54]. Weekly injections of HCG induced spermiation in farmed male European eels. The milt volume increased from the 5th to 12th weeks[54]. Sea bass *Dicentrarchus labrax* was examined for the potential of HCG to increase spermiation in precocious males and examined the potential of HCG to induce spermatogenesis and spermiation in non-precocious 1-year-old males[55].

Gonadotropins[56-60] such as luteinizing hormone and human chorionic gonadotropin have also been used with varying success in sex inversion and maturation of various

fishes. Many hormonal treatments such as carp pituitary homogenate, human chorionic gonadotropin and different luteinizing Kabarelin have been used for stimulation of gamete maturation in commercial cyprinid culture[61-64].

The use of HCG alone, shown to be successful in inducing ovulation/spawning in captive milkfish *Chanos chanos* in Taiwan[65]. Degani and Boker[66] examined *in vitro* effect of 17α-hydroxyprogesterone (17-P), 17α, 20β-dihydroxy-4-pregnen-3-one (17, 20-P), and Human Chorionic Gonadotropin (HCG) on vitellogenesis and the induction of Germinal Vesicle BreakDown (GVBD) in oocytes of the female *Trichogaster trichopterus*. An experiment conducted using the Human Chorionic Gonadotropin for increasing spawning rates in Nile Tilapia[67]. The dosage of 500-3500 IU of HCG/kg of fish given through intramuscularly into the sexually mature females and 2000-3500 IU of HCG/kg fish showed better effectiveness.

The injection of different inducing agent in fish breeding is adopted for successful ovulation and collection of eggs in different cultivable fish species. Human chorionic gonadotropin is one among them and is reported successful in catfish[68, 69] during induced ovulation. Human chorionic gonadotropin was also used to induce ovulation and may be combined with carp pituitary. Synthetic leutinizing hormone-releasing analogue (LHRH-a) was found to be effective in bighead carp[70]. A series of experiments were conducted to test synthetic ovulation stimulators on carp[71].

Investigation conducted to study the gonadal development, Gonado Somatic Index (GSI), oocyte diameter, fecundity, histology and level of serum steroid hormones (testosterone and estradiol-17β) in captive striped murrel (*Channa striatus* Bloch) implanted with Human Chorionic Gonadotropin (HCG) capsules and cholesterol capsules as control for a period of five months after implantation[72]. They observed that, HCG implants induced a significant increase in the GSI of male and female fish. They revealed variation in ova diameter of control and HCG implanted fishes and presence of large size ova in HCG implanted fishes throughout the study period. Similarly, a significant increase in the fecundity of HCG implanted fishes was also observed. The fecundity was highest (15415 eggs) in HCG implanted fish, whereas only 2245 eggs were observed from control fish. In control fish, only perinucleolar and primary oocytes were observed throughout the study period.

4. The Role of Kisspeptin in Reproduction

Kisspeptins are a group of peptides that stimulate GnRH release and are required for puberty and maintenance of normal reproductive function. Studies in teleosts have revealed the presence of multiple kisspeptin forms (Kiss1, Kiss2) in the brain. Neurons expressing Kisspeptin are direct targets of the steroids feedback action, both positive and negative, which differently regulate the mRNA expression in several brain areas and have again a relevant role in the establishment of puberty. It has been suggested that there is a double site of Kisspeptin action in the brain, either in the hypothalamic-hypophyseal region or in the median eminence, an area located outside the blood brain barrier.

The KISS1 gene was first discovered in the context of cancer, specifically melanoma, where it was demonstrated to be a suppressor of metastasis[73,74]. More recently, the KISS1 gene and a paralog termed KISS2 have been identified in teleost fishes[75-84]. The products of the KISS1 gene are termed kisspeptins, which bind to KISS1R[85-87]. The full-length KISS1 protein is 54 amino acids and is termed KP54 (alternatively named metastin) which can be proteolytically cleaved into shorter fragments (e.g., KP10, KP13, and KP14) representing the C-terminus of KP54, and which signal through KISS1R with presumably equal activity[85]. Exogenous kisspeptins have been administered to numerous vertebrate animals including humans and rodents, and have been shown to stimulate gonadotropin release including Luteinizing Hormone (LH), Follicle-Stimulating Hormone (FSH), and testosterone[88-91].

The major peptide product of the *KiSS-1* gene appears to be a 54-amino-acid peptide, largely secreted by the placenta and termed metastin or kisspeptin 54. The important role of Kiss 1r was also shown in teleost fish[92,93] kiss1 mRNA is expressed in hypothalamic regions that regulate gonadotropin secretion, including the anteroventral periventricular nucleus, the periventricular nucleus and the arcuate nucleus[94].

KISS1 and KISS2 gene sequences are dissimilar; however, they have some sequence similarity at the amino acid level (60–80 %) of the smallest known kisspeptin, the decapeptide[95] KP-10. This disparity in amino acid sequences could result in different efficacies on the KISS receptor(s)[96].

In goldfish *Carassius auratus*, intraperitoneal administration of KISS1 resulted in an increase in serum LH, while KISS2 treatment showed little effect[80]. Alternatively, in European sea bass, *Dicentrarchus labrax*, intramuscular injections of KISS2 exerted superior effects in terms of LH secretion over KISS1[78]. In orange-spotted grouper, *Epinephelus coioides*, intraperitoneal injection of KISS2 decapeptide significantly increased gnrh1 transcript levels in the hypothalamus and follicle-stimulating hormone beta (fsh b) transcript levels in the pituitary at 6 and 12 h post-injection[82].

Kiss1 mRNA levels in the brain gradually increased in female zebra fish (*Danio rerio*)[97], during the first 2-8 weeks of life to peak in fish with large mature vitellogenic follicles at 12 weeks. Both kiss1 and kiss2 mRNA levels in the brain peaked 30 days after fertilization and remained high during puberty and adulthood in grass pufferfish (*Takifugu niphobles*), expressing only kiss2, mRNA levels peaked in the brain and pituitary of adult mature and spawning females[79]. The highest kiss2 mRNA expression in the forebrain and midbrain either before or during the spawning season was noticed in Atlantic cod (*Gadus morhua*)[98]. However, in kiss2 mRNA expression in the brain was elevated in the vitellogenic females. Based on these results, it has been concluded that increased kiss mRNA levels in the brain are likely involved in the regulation of Final Ovarian Maturation (FOM) and ovulation in chub mackerel. The brain of goldfish (*Carassius auratus*), only kiss2 neurons in the preoptic area were shown to be up-regulated by ovarian estrogen. Interestingly, in the prepubertal zebrafish, estradiol treatment was shown to enhance expression of both kiss1 and kiss2. Studies on the gonadal maturity of *Channa striatus* by the administration of kisspeptin-10 (mammalian kisspeptin-10) indicated that kisspeptin-10 (natural) played major role in gonadal maturity of *Channa striatus*[99].

Daily spawning activity in chub mackerel females after one week of GnRH agonist administration suggested that the endogenous hormonal pathway of Brain-Pituitary-Gonad (BPG) axis is involved in the regulation of multiple spawning, without the influence of externally administered GnRH agonist[100]. Interestingly, an increase in *kiss1*, *kiss2*, and *gnrh1* levels was observed during the Post-Ovulation (POV) stage. Histologically, females sampled at POV period (06.00 h), showed the presence of 6- to 8-h old post-ovulatory follicles in their ovarian tissue, which indicated that these fish had undergone spawning on the previous night. Moreover, during the POV period, it is most likely that the standing stock of late vitellogenic oocytes in the ovary is ready to undergo Germinal-Vesicle Migration (GVM) for the next spawning. This study proposed that an increased level of *kiss1*, *kiss2*, and GnRH1 in the brain regulates spawning cycle in female chub mackerel.

5. Conclusion

Reproduction in fishes is regulated by both internal mechanisms within the fish and external environmental factors. The environmental factors trigger the internal mechanisms into action. The internal mechanism that controls the process of reproduction in fish is the brain-hypothalamus-pituitary gonad chain. Hormone-induced spawning techniques influence this sequential mechanism at several levels, by either promoting or inhibiting the process. Many procedures have been developed for inducing fish to undergo the last steps of spawning. The successful induced breeding of fish may reduce the uncertainty and unavailability of fish seed/fry, may increase the large scale production for export purpose and may be a potential sector for meeting the national demand and help to increase the foreign exchange earnings.

6. Acknowledgements

The authors wish to thank the authorities of Tamil Nadu Fisheries University, Nagapatinam and The Dean, Fisheries College and Research Institute, Thoothukudi for their constant encouragement and support.

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