A mini-review on kisspeptin hormone as an inducing agent in fish breeding

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Abstract
Kisspeptin system is involved in the control of reproduction in vertebrates, including teleost fish. Neuroanatomical distribution of kisspeptin neurons has confirmed their distribution in the preoptic area and hypothalamus of several teleost fish. In few species including chub mackerel, kisspeptin system has been shown to stimulate GnRH neurons in regulating the reproductive processes. Expression changes of kiss and gnrh mRNAs in teleost fish have demonstrated increased expression during the reproductive cycle. Interestingly, like mammalian species kisspeptin mediates the positive feedback effect of sex steroids in sexually mature fish including medaka and goldfish. In several teleosts, pharmacological administration of synthetic kisspeptin peptides affects gene expression of gnrh1, fshβ and lhfβ, and also induces gonadal development in immature fish, suggesting their possible application in captive reproduction. This review highlights the importance of selection of suitable mature peptides of fish kisspeptins for induced maturation in captivity.

Keywords: kisspeptins, Kiss1, Kiss2, reproduction, teleosts

1. Introduction
Kisspeptin system are considered as gatekeeper of reproductive function, including sexual differentiation, puberty onset, seasonal gonadal development, maturation and spawning in various vertebrate species (Gottsch et al., 2004; Castellano et al., 2009; Pasquier et al., 2014; Cao et al., 2019; Feng et al., 2019) [16, 6, 54, 5, 13]. In placental mammals, the kisspeptin is encoded by a single Kiss1 gene; however, in teleosts encoded by two kiss genes, kiss1 and kiss2 with the exception of puffer fish. Senegalese sole, three spined stickleback (Akazome et al., 2010; Mechaly et al., 2009, 2011; Nagler et al., 2011; Somoza et al., 2020) [1, 34, 35, 40, 72]. Also, in red seabream, a premature stop codon was present upstream of kisspeptin-10 region (Shimizu et al., 2012) [49]. In contrast to the situation in mammals where the anatomy and physiology of kisspeptin systems are well understood, studies in non-mammalian vertebrates particularly teleosts, where species differences have been observed, making the kisspeptin system more complex in fish reproductive physiology (Alvarado et al., 2016; Somoza et al., 2020) [2, 72].

Before kisspeptins demonstration on significant involvement in the reproductive function, gonadotropin-releasing hormone (GnRH) was thought to be the upstream modulator of the brain-pituitary-gonad (BPG) axis (Kah et al., 2007; Kauffman et al., 2007) [17, 23]. Kisspeptins act through kisspeptin receptor, primarily expressed by preoptic and hypothalamic GnRH neurons (Lee et al., 2009; Um et al., 2010; Akazome et al., 2010; Matsuyama et al., 2013; Zhao et al., 2014) [28, 76, 1, 32, 82]. These GnRH neurons regulate the synthesis and secretion of pituitary gonadotropins (GtHs), follicle stimulating hormone (FSH) and luteinizing hormone (LH). Pituitary GtHs, in turn stimulate the production of sex steroids, responsible for the progression of gonadal growth and maturation (Yaron et al., 2003) [80]. In mammals, KISS1 precursor protein is shown to be proteotically cleaved into several mature peptides, including KISS-54, -14, -13 and -10 (Kotani et al., 2001; Gottsch et al., 2004) [27, 46]. All these peptides possess a distinct structural Arg-Phe-amide motif in their C-termius and are shown to activate kisspeptin receptor with equal biopotency. In teleosts, multiple mature peptides have been reported in recent years (Ohga et al., 2020) [46].
2. Neuroanatomical Distribution of Kisspeptin Neurons

Using in-situ hybridization, several studies have demonstrated the kiss1 and kiss2 expressing cells in the brain of teleost fish (Ogawa et al., 2013) [41]. In the adult zebrafish brain, kiss1 cells are exclusively localized in the ventromedial habenula and the periventricular hypothalamic nucleus. The kiss2 mRNA was observed in the preoptic area (POA), mediobasal hypothalamus, posterior tuberal nucleus and the periventricular hypothalamic nucleus (Kitahashi et al., 2009; Servilli et al., 2011) [26, 61]. In the mature medaka, distribution of kiss1 expressing cells was restricted to habenula and hypothalamic regions, nucleus ventral tuberis (NVT) and nucleus posterioris periventricularis (NPPv). NVT kiss1 neurons in medaka exhibit sexual dimorphism, with male neurons being more than female ones (Kanda et al., 2008) [18].

In the medaka, kiss2 expressing cells are localized in the similar regions as that of zebrafish (Mitani et al., 2010) [38]. Like zebrafish, kiss1 expression was recorded in the habenula and hypothalamic regions, and kiss2 expression in the POA, nucleus lateralis tuberis (NLT) and nucleus recessus lateralis (NRL) of goldfish (Kanda et al., 2012) [20, 21]. In the striped bass, neurons expressing kiss1 are found to be dorsal (NRLd) and ventral (NRLv) subdivisions of the lateral nucleus of the recess, and the posterior tuberal nucleus (NPT) of the hypothalamus. The kiss2 expression was found in the similar regions of kiss1 expression, NRLd and NRLv (Zmora et al., 2012) [83]. In the European seabass, kiss1 expressing cells are found in the habenula and rostral mediobasal hypothalamus, with kiss2 distribution in the preoptic area and dorsal hypothalamic hypothalamus, above and under the lateral recess (Escobar et al., 2013) [9]. The kiss1- and kiss2-expressing neurons were mainly localized in the NRL and the nucleus of the posterior recess (NRP) in the hypothalamus of club mackerel (Ohga et al., 2017) [84]. In the red seabream expressing only kiss2, neurons that express kiss2 are distributed in the dorsal (NRLd) and ventral (NRLv) parts of nucleus recessi lateralis in the hypothalamus (Shimizu et al., 2012) [49]. In this study, the authors found that number of kiss2 expressing neurons in the NRLd was larger during the spawning season in both males and females, compared to post-spawning fish. Similarly, the number of kiss2 neurons in NRLd of maturing male was higher than post-spawning male. The kiss2 mRNA expressing cells are localized in the nucleus of lateral recess in the hypothalamus of Nile tilapia (Ogawa et al., 2013) [43].

Using immunocytochemistry, it was confirmed that kiss1 producing neurons are only localized in the habenular nucleus and project to the interpeduncular and raphe nuclei. In contrast, kiss2 producing neurons are mostly present in the dorsal and ventral hypothalamus and project widely into the subpallium, preoptic area, thalamus, ventral and caudal hypothalamus and the mesencephalon (Servilli et al., 2011) [65]. Similarly, in the European seabass, using specific antibodies raised against preprokiss2, it was found that kiss2 neurons are mainly located in the hypothalamus and project widely to the subpallium and pallium, preoptic area, thalamus, pretectal area, optic tectum, mediobasal medial and caudal hypothalamus and the neurohypophysis (Escobar et al., 2013) [9]. In the striped bass, kiss1- and kiss2-immunoreactive neurons were localized in the NLT and innervated the neurohypophysis, suggesting direct regulation of GnRH in this species (Zmora et al., 2013) [83].

3. GnRH Neurons Express Kisspeptin Receptors

Parhar et al. (2004) first demonstrated the co-localization of kisspeptin receptor mRNA with the three grnhr mRNAs in the Nile tilapia brain. Later, it was confirmed that expression of kissr mRNA peaks during puberty in cobia and grey mullet (Mohamed et al., 2007; Nocilllado et al., 2007) [41]. Furthermore, Khan et al. (2008) confirmed that kissr protein colocalized with multiple GnRh forms in Atlantic croaker. In the African cichlid fish, kissIr was localized in the GnRH1 and GnRH3 neurons (Grone et al., 2010) [44]. Using antibodies raised against the C-terminus of zebrafish preproKiss1 and preproKiss2, only Kiss2 fibers profusely innervated the ventral forebrain and notably made close apposition with GnRH neurons, suggesting direction regulation of kisspeptin on GnRH in zebrafish (Servilli et al., 2011) [60]. In contrast, in the medaka kisspeptin receptors did not co-localized in GnRH neurons, suggesting indirect regulation in this species. In this species, kisspeptin receptors are expressed in POA surrounding the GnRh neurons, and the study found that isotocin and vasotocin neurons in POA express kisspeptin receptors (Kanda et al., 2013) [19]. Similarly, in the European seabass and Nile tilapia, GnRh1 neurons did not appear to express kisspeptin receptors (Escobar et al., 2013; Ogawa et al., 2013) [8, 9]. The kiss2 expressing neurons are tyrosine hydroxylase, neuropeptide Y and neuronal nitric oxide producing neurons, suggesting indirect regulation of kisspeptin on GnRh (Escobar et al., 2013) [8, 9]. In the striped bass, kissr2 was colocalized in POA GnRh1 neurons (Zmora et al., 2012) [83]. Using immunocytochemistry in the striped bass, it was confirmed that kiss1-immunoreactive neurons directly innervate into pituitary regions where luteinizing hormone producing cells are localized. Further, kiss2 innervations were prominent in the NLT region and the neurohypophysis, forming large axonal bundles and intermingling with GnRh1 axons, suggesting direction regulation of kisspeptin to GnRh in this species. Espigares et al. (2015) indicated that the forebrain-midbrain acts as functional endocrine signaling pathway of kiss2/GnRh1 system controlling the gonadotroph activity in the European seabass and kiss2 is a potent regulator of pituitary Fsh and Lh secretion via paracrine/autocrine signaling. Similarly, in the club mackerel POA, GnRh neuron coexpresses kiss1r, suggesting kisspeptin direct regulation on GnRh in this scromboid fish (Ohga et al., 2017) [81]. These studies in different species clearly indicate that kisspeptin may regulate directly or indirectly GnRH neurons to modulate reproductive events.

4. Kisspeptin Neurons Express Estrogen Receptors

Gonadal sex steroids have been identified as important regulators of preoptic and hypothalamic kisspeptin systems. Double label in situ hybridization found that medaka NVT kiss1 neurons coexpress estrogen-receptor-α (ERα), whereas NRL kiss2 neurons do not. The study found that kiss1 neurons in the NVT decreased after ovarioectomy, and recovered after estrogen treatment (Kanda et al., 2008) [18]. The authors concluded that NVT kiss1 neurons are positively regulated by ovarian estrogen via their coexpressed ERα and are directly involved in the central regulation of reproduction in medaka (Kanda et al., 2008; Mitani et al., 2010) [18, 38]. Estrogen treatment of juvenile zebrafish with estradiol caused an increase in kiss1 and kiss2 expression, particularly at the periphery of the anterior tuberal nucleus and in the caudal hypothalamus (Servilli et al., 2011) [63]. In the goldfish, up-regulation of gene expression by ovarian steroids was observed only in the kiss2 neurons of the POA, and this
observation coincided with the expression of estrogen receptor in these kiss2 neurons (Kanda et al., 2012; Kanda and Oka, 2012) [20-21]. The authors found that in breeding females, kiss2 expression was significantly higher in the POA and NLT, whereas there was no significant difference between these conditions in NRL. Wang et al. (2013) demonstrated that the goldfish kisspeptin neurons co-express the estrogen receptors, with eral and erbl in the habenula kiss1 neurons and eral, era2, and erbl in the preoptic and hypothalamic kiss2 neurons. Interestingly, the study confirmed that estrogen (17β-estradiol, E2) treatment enhances the promoter activities of two kiss genes in the presence of ERα, suggesting that E2 is capable of exerting positive feedback regulation on the expression of kiss1 and kiss2 in the goldfish. In the European seabass, most kiss1 expressing cells of the mediobasal hypothalamus strongly express ERα (Escobar et al., 2013) [8-9]. The study did find any coexpression of kiss2 and ERα or ERβ1. In the European seabass and striped bass, mediobasal hypothalamus acts as a major site for sex steroid actions on kisspeptins in this species (Zmora et al., 2013; Alvarado et al., 2016) [84-82]. These studies clearly indicate that though species specific differences exist, the kisspeptin system is involved in mediating sex steroid regulation in teleosts.

5. Expression Changes of Kisspeptin mRNAs During the Reproductive cycle

In the chub mackerel, expression changes of kisspeptin and gnrh mRNAs were analyzed during different stages of reproductive periods: early development, sexual differentiation, puberty, seasonal reproductive and spawning cycles (Selvaraj et al., 2010; 2015; Ohga et al., 2013, 2015) [55, 59, 45, 47]. Expression changes of kiss mRNAs during early development (0-30 dpfs) and gonadal sex differentiation periods (37-60 dpfs) indicated that kiss, kissr and gnrh mRNA levels were higher between 0 and 15 dpfs, in comparison to other early developmental periods. During sexual differentiation periods, kiss2, kissr1, and kissr2 mRNA levels were higher at 37 dpf, in comparison to 45 and 60 dpfs. These expression profiles indicated that kisspeptin systems are involved in the early larval development and sexual differentiation of the brain of chub mackerel (Selvaraj et al., 2015) [59]. During pubertal onset in male fish, kiss2, kissr1, kissr2 levels increased significantly at 14 weeks post-hatch (wph), synchronously with an increase in type A spermatogonial populations in the testis; kiss2 and gnrh1 levels significantly increased at 22 wph, just before the onset of meiosis in the testis. In female fish, kiss1 and kiss2 levels increased significantly with an increase in kissr1, kissr2 and gnrh1 levels at 24 wph, just before the appearance of vitellogenic oocytes in the ovary (Ohga et al., 2015) [45]. These result clearly indicated positive involvement of kisspeptin-GnRH system in the pubertal onset in the chub mackerel. During seasonal reproductive cycle, kiss1 levels in the males were higher during immature stage when the testis is mainly occupied with spermatagonia, and lower during post-spawning stage, when the testis was contained of residual spermatoozoida. In contrast, kiss1 levels in the females did not show any significant fluctuations; however, the mRNA levels were 2-fold higher than males at different stages analyzed (Selvaraj et al., 2010) [58]. The kiss2 expression profiles were similar in males and females with levels higher during early gametogenic periods compared to later stages. Like kiss1, kiss2 levels in the females were 1.5-fold higher than males, suggesting their involvement in the seasonal reproductive cycle of chub mackerel. Seasonal expression changes of kisspeptin receptors showed higher expression levels of both kissr1 and kissr2 in the brain of the females during early vitellogenic period; however, no significant differences were found in the brain of males. Increased expression of kiss2 coincided with kissr1 and kissr2 in female brain, suggesting their dominant involvement in the female reproductive cycle (Ohga et al, 2013) [47]. During the spawning season, both kiss1 and kiss2 levels were higher compared to seasonal reproductive cycle stages. Particularly, kiss1 and kiss2 levels were higher during the final oocyte maturation (FOM) stages, germinal vesicle migration and hydration stages compared to the late vitellogenesis in females. These peaks coincided with circulating estradiol levels in the blood plasma (Matsuyama et al., 2005; Selvaraj et al., 2012) [33, 57]. Surprisingly, expression of both kisspeptin receptor genes significantly decreased during FOM, suggesting rhythmic expression of kiss/kissr during the spawning season are involved in the regulation of LH surge (Ohga et al., 2017) [44]. These studies in chub mackerel have clearly demonstrated the involvement of kisspeptin-GnRH system in the regulation of reproductive cycle (Ohga et al., 2018) [50].

Studies in other fish species expressing two kiss genes have also indicated increased expression of elements of BPG axis including kisspeptins. Kitahashi et al. (2009) found a significant increase in zebrafish kiss1, kiss2, gnrh2, gnrh3 mRNA levels at the start of the pubertal phase and remained high in adulthood, suggesting involvement of kisspeptin-GnRH systems in the regulation of pituitary gonadotropins. In this species, temperature differentially regulates the two kisspeptin systems in the brain, with kiss1/kissr1 system sensitive to low temperature and kiss2/kissr2 system sensitive to high and low extremes of temperature (Shahjahan et al., 2013) [60]. In the brain of mature female striped bass, both kiss1 and kiss2 mRNAs, including levels of their receptors kissr1 and kissr2, were found to be significantly increased in comparison to juvenile and prepubertal fish (Zmora et al., 2012) [60]. Both kiss1 and kiss2 mRNAs were detectable at 1 day post fertilization (dpf) and then increased during the first week of life (Zhao et al., 2014) [83]. In Indian major carp, rohu, kiss1 and kiss2 expression was elevated during prespawning and spawning periods (Saha et al., 2016) [56]. In pejerrey, all members of the kisspeptin system are expressed during early period, and the increase of kiss2 transcripts at week 4 suggested as their involvement in the differentiation of the brain-pituitary axis in mature development (Bohórquez et al., 2017) [51]. In the golden mahseer, expression of kiss1 and kiss2 mRNAs was comparatively higher during the initial stages of gonadal development, than that of spermatiation or ovulation stage (Shahi et al., 2017) [64]. In the spotted snakehead, expression profile of kiss1, kissr1 and kissr2 revealed sexual dimorphism depending on tissues, and insignificant correlation was observed by the authors between the expression of kiss1 and its receptors in the brain (Bakshi and Umesh, 2019) [5].

Expression profile has been demonstrated in a number of fish expressing only kiss2. In the grass pufferfish, kiss2 and kissr1 mRNAs were significantly elevated during the spawning period in the brain and pituitary of both sexes, indicating a strong positive correlation between the amounts of kiss2/kissr1 and gnrh1 mRNAs in the brain over the spawning season (Shahjahan et al., 2010) [66]. In the orange spotted
grouper exhibiting protogynous hermaphroditism, kiss2 expression was higher in females compared to males, and in the first week after methyl testosterone implantation, transcript levels of kiss2 and kiss1 in the hypothalamus reduced significantly. Interestingly, kiss2 expression increased on the fourth week, in accordance with the expression pattern of gnrh1 mRNA in the hypothalamus suggesting involvement of kisspeptin system in the sex reversal in orange spotted grouper (Shi et al., 2010) [68]. In female Senegalese sole (Solea senegalensis), Mechaly et al. (2012) found highest kiss2 mRNA expression in the forebrain and midbrain either before or during the spawning season. In the Atlantic cod, elevation in kiss2 in vitellogenic females and spermatiating males and spikes in kiss4 during early vitellogenesis in females and spermatogenesis in males was observed (Cowan et al., 2012) [71]. Administration of thyroid hormone significantly increases kiss2 and gnrh1 mRNAs in the sexually mature males of Nile tilapia (Ogawa et al., 2013) [42]. The kiss2 mRNA in male seahorse brain increased significantly at the early pubertal stage, and decreased significantly during pregnancy (Zhang et al., 2018) [81]. Both kiss2 and kissr are highly expressed in the brain regions of sexually mature black porgy (Ma et al., 2019) [31]. At the onset of sexual maturation in the threespine stickleback, kiss2 and kissr mRNA levels were higher, suggesting their possible involvement in pubertal onset (Shao et al., 2019) [60]. Likewise, changes in expression patterns of kiss2 mRNA during different developmental stages indicated its potential role in embryonic development of singhi, a freshwater catfish of India (Kumari et al., 2020) [29].

6. Functional Kisspeptin Peptides in Teleost Fish

Based on the position of dibasic amino acid residues, upstream to kisspeptin-10 regions, Kiss1-10 and Kiss2-10 of teleosts are suggested to produce Kiss1 pentadecapeptides (Kiss1-15) and Kiss2 dodecapeptides (Kiss2-12), respectively (Table 1 and Table 2). However, differences have been noted in the position of dibasic amino acid residues, upstream to kisspeptin-10 regions in few species. Dibasic amino acid residues (KR) have been found five position upstream of Kiss1-10 in medaka (Kitahashi et al., 2009) [26], zebrafish (Kitahashi et al., 2009) [26], European seabass (Felip et al., 2009) [32], chub mackerel (Selvaraj et al., 2010) [30], goldfish (Li et al., 2009) [30], striped bass (Zmora et al., 2012) [83], black rockfish (Song et al., 2015) [73], catla (Rather et al., 2016) [50], rohu (Saha et al., 2016) [66], pejerrey (Bohórquez et al., 2017) [5] and Atlantic bluefin tuna (Ohga et al., 2020) [48], suggesting pyroglutamated Kiss1 pentadecapeptide as mature form in these fish. In chub mackerel and grouper species, it is likely that Kiss1 hexadecapeptides are mature forms, based on the position of dibasic amino acid residues (Kang et al., 2012; Ohga et al., 2013) [22, 47]. Interestingly, in the chub mackerel reporter gene assays have indicated higher potency for receptor activation of Kiss1-16 peptides compared to Kiss1-15 and pyroglutamated Kiss1-15 peptides (Ohga et al., 2017) [44]. Also, Ohga et al. (2020) performed alanine scanning of Kiss1-15 peptides of chub mackerel, and highlighted the importance of specific residues in receptor binding. Surprisingly, the study found that primary structure of the functional peptide might be species-specific even within species of the family Scombridae, and demonstrated five types of putative mature Kiss1 peptides from sixteen scombridae species (Table 1).

In contrast to Kiss1, dibasic residues (RR) have been found two positions upstream to Kiss2-10 region in zebrafish (Kitahashi et al., 2009) [26], medaka (Kitahashi et al., 2009) [26], European seabass (Felip et al., 2009) [32], orange spotted grouper (Shi et al., 2010) [68], chub mackerel (Selvaraj et al., 2010) [30], red seabream (Shimizu et al., 2012) [69], Atlantic cod (Cowan et al., 2012) [71], striped bass (Zmora et al., 2012) [83], Nile tilapia (Ogawa et al., 2013) [43], grass pufferfish (Shahjahan et al., 2010) [66], catla (Rather et al., 2016) [55], rohu (Saha et al., 2016) [66], pejerrey (Bohórquez et al., 2017) [5], golden mahseer (Shahi et al., 2017) [64], seahorse (Zhang et al., 2018) [81] and black porgy (Ma et al., 2019) [31], suggesting Kiss2 dodecapeptide as mature form in these species. However, in the salmon immunoaffinity purification and mass spectrometric analysis have indicated mature peptide of kiss1 gene as tridecapeptide (Osugi et al., 2013) [80] (Table 2). Recently, putative mature form of Kiss2 was confirmed to be Kiss2 dodecapeptide in sixteen species of family scombridae (Ohga et al., 2020) [48].

Surprisingly, dibasic amino acid residues are located five and six positions upstream to Kiss2-10 in few species (Table 3). In zebrafish, goldfish, catla, rohu, golden mahseer, singhi and Chinese rare minnow, dibasic residues are located five position upstream to Kiss2-10 region, suggesting possibility of Kiss2 pentadecapeptide (Kiss2-15) as mature form in these species (Kitahashi et al., 2009; Li et al., 2009; Rather et al., 2016; Saha et al., 2016; Shahi et al., 2017; Kumari et al., 2020) [26, 30, 55, 56, 64, 29]. In the rainbow trout, dibasic amino acid residues are found six position upstream to Kiss2-10 region, indicating possibility of Kiss2 hexadecapeptide as mature form (Genbank Accession No. JX122506).

| 1  2  3  4  5  6  7  8  9  10  11  12  13  14  15  16 |
|----------------------------------------------------------|
| Zebrafish, Goldfish, Catla, Rohu, Golden mahseer       |
| – Gln Asn Val Ala Tyr Tyr Asn Leu Asn Ser Phe Gly Leu Arg Tyr |
| Goldfish                                                |
| – Gln Lys Val Ala Tyr Tyr Asn Leu Leu Ser Phe Gly Leu Arg Tyr |
| Medaka                                                  |
| – Gln Asp Leu Ser Ser Tyr Asn Leu Leu Ser Phe Gly Leu Arg Tyr |
| European seabass, Striped bass, Black rockfish, Pejerrey |
| – Gln Asp Val Ser Ser Tyr Asn Leu Asn Ser Phe Gly Leu Arg Tyr |
| Atlantic Bluefin tuna                                   |
| – Gln Asp Met Ser Ser Tyr Asn Phe Asn Ser Phe Gly Leu Arg Tyr |
| Chub Mackerel                                           |
| His Gln Asn Met Ser Ser Tyr Asn Leu Asn Ser Phe Gly Leu Arg Tyr |
| Longtooth grouper                                      |
| His Gln Asn Val Ser Ser Tyr Asn Leu Asn Ser Phe Gly Leu Arg Tyr |

Table 1: Kiss1 pentadecapeptides/hexadecapeptides in teleost fish
Table 2: Kiss 2 dodecapeptides/tridecapeptides in teleost fish

|   | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|---|---|---|----|----|----|----|
| Zebrafish, Medaka, Catla, Rohu, Golden mahseer, Pejerrey, Singhi | Ser | Lys | Phe | Asn | Tyr | Asn | Pro | Phe | Gly | Leu | Arg | Phe |
| Chub Mackerel | Ser | Asn | Phe | Asn | Phe | Asn | Pro | Phe | Gly | Leu | Arg | Phe |
| Orange spotted grouper, European seabass, Striped bass, Grass Puffer, Senegalese sole, Red seabream, Black porey, Atlantic bluefin tuna | Ser | Lys | Phe | Asn | Tyr | Asn | Pro | Phe | Gly | Leu | Arg | Phe |
| Goldfish | Gly | Lys | Phe | Asn | Tyr | Asn | Pro | Phe | Gly | Leu | Arg | Phe |
| Nile tilapia | Ser | Asn | Phe | Asn | Tyr | Asn | Pro | Leu | Ser | Leu | Arg | Phe |
| Atlantic bluefin tuna | Ser | Lys | Phe | Asn | Tyr | Asn | Pro | Phe | Gly | Leu | Arg | Phe |
| Grass pufferfish | Ser | Lys | Phe | Asn | Val | Asn | Pro | Phe | Gly | Leu | Arg | Phe |
| Atlantic cod | Ser | Pro | Phe | Asn | Tyr | Asn | Pro | Phe | Gly | Leu | Arg | Phe |
| Masu salmon, Kokanee salmon | Thr | Ser | Lys | Phe | Asn | Ser | Pro | Phe | Gly | Leu | Arg | Phe |

Table 3: Possibility of Kiss2 pentadecapeptides/hexadecapeptides in teleost fish

|   | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|---|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|
| Zebrafish | Leu | Ala | Arg | Ser | Lys | Phe | Asn | Tyr | Asn | Pro | Phe | Gly | Leu | Arg | Phe |
| Goldfish | Leu | Pro | Arg | Gly | Lys | Phe | Asn | Tyr | Asn | Pro | Phe | Gly | Leu | Arg | Phe |
| Catla, Rohu, Golden mahseer, Singhi, Chinese rare minnow | Leu | Thr | Arg | Ser | Lys | Phe | Asn | Tyr | Asn | Pro | Phe | Gly | Leu | Arg | Phe |
| Rainbow trout | Leu | Thr | Arg | Thr | Ser | Lys | Phe | Asn | Val | Asn | Pro | Phe | Gly | Leu | Arg | Phe |

6.1. Short-term effects of kisspeptin peptides on gene expression

Several studies have demonstrated that administration of kisspeptin peptide through different routes induces changes in the elements of reproductive axis. Filby et al. (2008) found that intraperitoneal administration of mammalian kisspeptin-10 at a dose of 2 nmol/g body weight in early-mid pubertal fish induces grn1h and kissl1r expression in the brain after 10 hours post-injection. Intramuscular administration of seabass Kiss1-10 and Kiss2-10 at a dose of 250 ng/g body weight in prepubertal seabass evoked significant elevations in circulating LH levels at 120 minutes after injection, with Kiss1-10 and Kiss2-10 eliciting 2 fold increase and 4 fold increase, respectively. Similarly, intramuscular administration of seabass Kiss1-10 and Kiss2-10 at a dose of 250 ng/g body weight revealed that only Kiss2-10 induces an increase of circulating LH levels at 120 minutes after injection (Felip et al., 2009) [12]. In zebrafish, intraperitoneal administration of Kiss1-10 and Kiss2-10 at a dose of 2 nmol/g body weight to sexually mature females showed that Kiss2-10 induces significant increase in pituitary fshβ (2.7 fold) and lhfβ (8 fold) (Kitahashi et al., 2009) [28]. Incubation of primary cultures of goldfish pituitary cells with goldfish Kiss1-10 (100 nM) induced an increase in luteinizing hormone (LH), growth hormone (GH) and prolactin (PRL) in 30 min. duration; short-term incubation with Kisspeptin-10 did not alter LH, GH and PRL mRNAs expression but elevation in mRNA level for the hormones were observed by prolonging the kisspeptin-10 treatment to 24 hours (Yang et al., 2009) [79]. Li et al. (2009) found that in-vitro action of goldfish Kiss1-10 and Kiss2-10 in primarily culture of pituitary cells did not stimulated LH release; however, intraperitoneal administration of Kiss1-10 significantly increased serum LH levels in a dose-dependent manner (0.01-1 μg/g). In orange spotted grouper, intraperitoneal administration of Kiss2-10 at a dose of 2 nmol/kg body weight significantly increases gnr1h mRNA levels in the hypothalamus, and fshβ mRNA levels in the pituitary at 6 and 12 h post-injection (Shi et al., 2010) [60]. Intramuscular administration of Kiss1-15 and Kiss2-12 were potent in inducing the pituitary LH release in striped bass; however, the responses showed dose-dependent and reproductive stage differences. In prepubertal fish, only Kiss2-12 increased LH levels by 4.5-7 fold at doses of 5 and 25 nmol/kg body weight, while 100 nmol/kg body weight induced LH blood levels by only 2.5 and 3.5 fold at 4 and 24 h post-injection, respectively. In adult fish of midgonadal development phase, the response was less prominent compared to prepubertal fish. Kiss1-15 at doses of 50 and 100 nmol/kg body weight induced LH plasma levels by 1.7 and 2.5 fold at 24 h post-injection; Kiss2-12 at doses of 5 and 25 nmol/kg body weight increased LH levels significantly at 24 h post-injection (Zmora et al., 2012) [83]. In chub mackerel, intracerebroventricular administration of synthetic chub mackerel Kiss1-15 and Kiss1-12 peptides showed that in female fish, gnr1h levels decreased in the presence of both kisspeptin peptides at 12 h postinjection; only Kiss2-12 significantly increased fshβ and lhfβ mRNAs at 12 h post-injection (Ohga et al., 2014) [49]. In vitro studies using brain slices of striped bass demonstrated that only Kiss2 can upregulate the expression of hypophysiotropic gnr1h, which was subsequently diminished by kisspeptin antagonists, pep 234 and pep 359 (Zmora et al., 2015) [84]. In the cinnamon
clownfish, treatment with 0.1 and 0.5 µg/g body mass of mammalian kisspeptin-10 significantly increased the mRNA levels of growth hormone (GH) in the pituitary. Similarly, under in vitro condition, treatment with Kiss significantly increased GH mRNA level, especially at 48 h after treatment (Kim et al., 2014, 2015) [24-25]. This studies suggested that kisspeptin plays a role in modulating growth and artificially induced rapid growth in cinnamon clownfish. Park et al. (2016) found that intraperitoneal administration of tilapia Kiss2-10 into immature male and female Nile tilapia at a dose of 200 pmol/g body weight increases the expression of gnrhl, fshβ, and lhβ mRNAs in the brain and increased estradiol-17β and 11-ketotestosterone levels in the blood plasma. In fish injected with Kiss2-10 twice weekly for a total of 8 times in Nile tilapia; fish at late vitellogenesis accounts for 30%, while fish at pre-vitellogenesis only for 20%, in the control group, fish at late vitellogenesis accounts for 16.6% and at pre-vitellogenesis 25%. Intrapерitoneal injection of porgy Kiss2-10 stimulated gene expression of kissr, gnrhl, gnrh3, fshβ, lhβ, p450c17, star, and ar, and the serum testosterone level in male black porgy (Ma et al., 2019) [31]. Nile tilapia pituitaries cultured with high concentration of Kiss2-10 more than 0.1 µM for 3 hours exhibited a significant increase of fshβ mRNA expression, but not lhβ mRNA; expression of both fshβ and lhβ mRNAs increased after 6 hours in 0.1 µM of Kiss2-10 medium (Park et al., 2020) [31]. In goldfish, kisspeptins found to be a more suitable inducing hormone than GnRH based analogue, Ovaprim for accelerating and synchronizing oocyte maturation (Valipour et al., 2020) [73]. These studies clearly indicate that kisspeptin peptides can stimulate reproductive axis.

6.2. Long-term effects of kisspeptin peptides on growth and maturation of gonads

Repeated bi-weekly injections (over 7 weeks) of European sea bass Kiss1-10 and Kiss2-10 peptides (250 ng/g body weight) accelerated pubertal onset in basses of the genus Morone species; in sexually mature basses, an increase in gonadosomatic index value, and advancement in gonadal development was observed (Beck et al., 2012) [46]. Nocillado et al. (2013) showed that administration of yellowtail kingfish Kiss 1-10 and Kiss2-10 peptides in prepubertal fish during the breeding (50 µg/kg) and non-breeding season (100 µg/kg) showed that pituitary expression of fshβ and lhβ was upregulated only with Kiss1-10 treatment regardless of the season; gonadal development was stimulated in male fish with either Kiss1–10 or Kiss2–10, with Kiss2–10 being more effective during the non-breeding period. In chub mackerel, continuous administration of synthetic chub mackerel Kiss1-15 using mini-osmotic pumps stimulated an increase in GSI values of adult male fish on day 45 post-injection; spermatogenic male fish showed significantly higher levels of pituitary fshβ and lhβ mRNAs and circulating 11-ketotestosterone; yolk vesicles were observed in the oocytes of Kiss1-15 treated fish with higher levels of pituitary fshβ and circulating estradiol-17β (Selvaraj et al., 2013a) [60]. Similarly, subcutaneous administration of synthetic chub mackerel Kiss1-15 using injection for three times (biweekly) over 6 weeks, stimulated an increase in GSI values of prepubertal male fish on day 45 post-injection; testicular histology revealed higher percentage of advanced stages of germ cells in comparison to other treatments; levels of circulating sex steroids, 11-ketotestosterone and estradiol-17β were higher in Kiss1-15 treated fish (Selvaraj et al., 2013b) [62]. Also, subcutaneous administration of synthetic chub mackerel Kiss1-15 using injection for three times (biweekly) over 6 weeks, stimulated an increase in the oocyte diameter of previtellogenic oocytes; testosterone and estradiol-17β levels were significantly higher in Kiss1-15 injected fish (Selvaraj et al., 2015) [61]. These studies have demonstrated the potential of using synthetic kisspeptin peptides for inducing gonadal development at different reproductive stages.

7. Conclusion

Recent isolation of cDNAs encoding kisspeptins in different teleost fish has confirmed the presence of different mature peptides as previously demonstrated for mammalian kisspeptins. Reporter gene assays have demonstrated that shorter form of kisspeptin (Kiss-10) exhibit lower activity in activating kisspeptin receptors compared to larger forms (Kiss1-15, Kiss1-16, Kiss2-12), at least in marine scombrids. Selection of suitable mature peptide would be important in inducing growth and maturation of fish gonads as shorter peptides would undergo faster clearance in peripheral circulation. In cyprinids like goldfish and Prussian carp, synthetic kisspeptin peptides have been shown to be superior than existing GnRH analogues, when administered along with dopamine antagonists. Recently, in marine scambrid fish, functional mature peptide of Kiss1 and Kiss2 has been demonstrated highlighting the importance of selection of suitable mature peptides in commercial aquaculture.

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