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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 *lhβ*, 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 kisseptins 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) [69]. 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) [29, 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, 16]. All these peptides possess a distinct structural Arg-Phe-amide motif in their C-terminus 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) [43]. 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, 63]. In the mature medaka, distribution of *kiss1* expressing cells was restricted to habenula and hypothamic 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 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 chub mackerel (Ohga *et al*., 2017) [51]. 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) [69]. 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) [42].

Using immunocytochemisty, 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 (Servili *et al*., 2011) [63]. 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 Kissspeptin Receptors

Parhar *et al*. (2004) first demonstrated the co-localization of kisspeptin receptor mRNA with the three *gnrh* 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; Nocillado *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, *kiss1r* was localized in the GnRH1 and GnRH3 neurons (Grone *et al*., 2010) [14]. 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 (Servili *et al*., 2011) [63]. 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) [9, 42]. The *kissr2* 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 chub mackerel POA, GnRh neuron coexpresses *kissr1*, suggesting kisspeptins direct regulation on GnRh in this scombrid fish (Ohga *et al*., 2017) [51]. 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 insitu 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 ovariectomy, 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 (Servili *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 *era1* and *erb1* in the habenula kiss1 neurons and *era1*, *era2*, and *erb1* in the preoptic and hypothalamic kiss2 neurons. Interestingly, the study confirmed that estrogen (17β-estradiol, E_2) treatment enhances the promoter activities of two *kiss* genes in the presence of ERα, suggesting that E_2 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, 2]. 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) [58, 59, 45, 47]. Expression changes of *kiss* mRNAs during early development (0-30 dphs) and gonadal sex differentiation periods (37-60 dphs) indicated that *kiss*, *kissr* and *gnrh* mRNA levels were higher between 0 and 15 dphs, in comparison to other early developmental periods. During sexual differentiation periods, *kiss2*, *kissr1*, and *kissr2* mRNA levels were higher at 37 dph, in comparison to 45 and 60 dphs. 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 spermatogonia, and lower during post-spawning stage, when the testis was contained of residual spermatozoa. 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) [65]. 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) [83]. 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) [82]. 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 male development (Bohórquez *et al*., 2017) [2]. In the golden mahseer, expression of *kiss1* and *kissr* mRNAs was comparatively higher during the initial stages of gonadal development, than that of spermiation 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) [3].

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 *kissr1* 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 spermiating males and spikes in *kissr4* during early vitellogenesis in females and spermatogenesis in males was observed (Cowan *et al*., 2012) [7]. 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) [67]. 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) [28].

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 to Kiss1-10 in medaka (Kitahashi *et al*., 2009) [26], zebrafish (Kitahashi *et al*., 2009) [26], European seabass (Felip *et al*., 2009) [12], chub mackerel (Selvaraj *et al*., 2010) [58], 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) [55], rohu (Saha *et al*., 2016) [56], golden mahseer (Shahi *et al*., 2017) [64], 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) [12], orange spotted grouper (Shi *et al*., 2010) [68], chub mackerel (Selvaraj *et al*., 2010) [58], red seabream (Shimizu *et al*., 2012) [69], Atlantic cod (Cowan *et al*., 2012) [7], striped bass (Zmora *et al*., 2012)[83], Nile tilapia (Ogawa *et al*., 2013) [42], grass pufferfish (Shahjahan *et al*., 2010) [66], catla (Rather *et al*., 2016) [55], rohu (Saha *et al*., 2016) [56], 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 *kiss2* gene as tridecapeptide (Osugi *et al*., 2013) [50] (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, 28]. 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).

Table 1: Kiss1 pentadecapeptides/hexadecapeptides in teleost fish

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	Asn	Ser	Phe	Gly	Leu	Arg	Tyr
Medaka															
–	Gln	Asp	Leu	Ser	Ser	Tyr	Asn	Leu	Asn	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	Asp	Met	Ser	Ser	Tyr	Asn	Phe	Asn	Ser	Phe	Gly	Leu	Arg	Tyr
Longtooth grouper															
His	Gln	Asp	Val	Ser	Ser	Tyr	Asn	Leu	Asn	Ser	Phe	Gly	Leu	Arg	Tyr

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 porgy, Atlantic bluefin tuna												
–	Ser	Lys	Phe	Asn	Phe	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	Phe	Asn	Pro	Phe	Gly	Leu	Arg	Phe
Grass pufferfish												
–	Ser	Lys	Phe	Asn	Leu	Asn	Pro	Phe	Gly	Leu	Arg	Phe
Seahorse												
–	Asn	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	Phe	Asn	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 induce 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 *grnh3* and *kiss1r* expression in the brain after 10 hour 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 min. 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 minute 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 *fsh*β (2.7 fold) and *lh*β (8 fold) (Kitahashi *et al*., 2009) [26]. 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 h (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/g body weight significantly increases *gnrh1* mRNA levels in the hypothalamus, and *fsh*β mRNA levels in the pituitary at 6 and 12 h post-injection (Shi *et al*., 2010) [68]. 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 h 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, intracebroventricular administration of synthetic chub mackerel Kiss1-15 and Kiss1-12 peptides showed that in female fish, *gnrh1* levels decreased in the presence of both kisspeptin peptides at 12 h postinjection; only Kiss2-12 significantly increased *fsh*β and *lh*β 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 *gnrh1*, 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 *gnrh1*, *fsh*β, and *lh*β 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%. Intraperitoneal injection of porgy Kiss2-10 stimulated gene expression of *kissr, gnrh1, 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 *fsh*β mRNA expression, but not *lh*β mRNA; expression of both *fsh*β and *lh*β mRNAs increased after 6 hours in 0.1 µM of Kiss2-10 medium (Park *et al*., 2020) [53]. 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) [77]. 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) [4]. 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 *fsh*β and *lh*β 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; spermiating male fish showed significantly higher levels of pituitary *fsh*β and *lh*β mRNAs and circulating 11-ketotestosterone; yolk vesicles were observed in the oocytes of Kiss1-15 treated fish with higher levels of pituitary *fsh*β 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), atleast 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 scombrid fish, functional mature peptide of Kiss1 and Kiss2 has been demonstrated highlighting the importance of selection of suitable mature peptides in commercial aquaculture.

8. References

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