Endocrine Regulation of Fish Reproduction

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Introduction – Overview of the Brain–Hypophyseal–Gonadal Axis

Environmental signals, in conjunction with social cues, are conveyed by sensory signals to various brain centers culminating in the hypothalamus. In the absence of hypophyseal portal system, hypothalamic nerve fibers branch throughout the adenohypophysis to regulate the synthesis and release of the gonadotropic hormones, follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Dopamine secreted by the hypothalamus may inhibit LH release. FSH and LH, in turn, bind to their respective receptors in the gonads (FSH-R and LH-R, respectively).

The gonads respond by secretion of sex steroid hormones; estradiol 17β (E2) in females that promotes oogonial proliferation and vitellogenesis, and progestogens such as 17α,20β dihydroxy-4-pregnene-3-one (DHP) that promotes initiation of germ cell meiosis and follicular maturation and ovulation (Figure 1(a)). In males, these are androgens, mainly 11 ketotestosterone (11-KT), that regulate spermatogenesis and spermiogenesis, as well as DHP that initiates the meiotic division of spermatogonia and controls the spermatooza maturation and spermiation. DHP in its free or conjugated forms serve as pheromones as well. Generally, fertilization occurs in the water, either in specialized nests guarded by one or both parents or in the open water. Several teleost fish such as the guppy (Poecilia reticulata) are viviparous.

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Glossary

Follicle-stimulating hormone (FSH) A glycoprotein hormone secreted by the pituitary; stimulates early phases of gametogenesis: spermatogenesis in males and early development of oocytes in females.

Gonadotropin-releasing hormone Neuropeptide hormone produced in the hypothalamus, released at the anterior pituitary gland to regulate gonadotropin secretion and is a key regulator of reproduction.

Luteinizing hormone (LH) A dimeric glycoprotein hormone released from the anterior pituitary gland that acts through gonadal membrane receptors to stimulate steroidogenesis and gametogenesis. LH is a gonadotropin.

Oogonia Female germ cells at the beginning of their development toward becoming an oocyte and egg prior to meiotic division.

Spermatogonia Male germ cells at the beginning of their development toward becoming spermatooza and prior to meiotic division.

Introduction

Oogenesis

Oogonial Proliferation and Meiosis

Oogonia proliferate mitotically under the stimulation of E2 (Figure 2). Meiotic division commences in a subpopulation of the oogonia that can be visualized by the presence of synaptonemal complexes (a hallmark feature of homologous chromosomal synapses). Another marker of meiosis is Spo11, a protein involved in the creation of double-stranded breaks in the DNA at early stages of meiotic recombination. Addition of DHP to the medium of cultured ovarian fragments results in increased abundance of synaptonemal complexes and increased expression of Spo11, indicating that DHP acts to induce meiosis in the oogonia of teleost ovary (Figure 2).

Oocyte Growth

As the oocyte starts growing during fish puberty, it is still arrested at the prophase of the first meiotic division...
(Figure 2). At this phase, it sequesters yolk precursors (vitellogenins) and chorionic proteins (choriogenins) mainly produced by the liver under estrogenic stimulation. The yolk accumulates in the oocyte which increases in diameter. FSH regulates both the secretion of estradiol and the incorporation of vitellogenins into the oocytes (Figure 1(a)).

**Mutual Regulation of the Follicle Cells and the Oocyte**

The granulosa cells surrounding the oocyte and the oocyte itself maintain a mutual communication system by which molecules of the epithelial growth factor...
(EGF) family secreted by the oocyte affect the adjacent granulosa cells. Activin, a major mediator of fish pituitary gonadotropins, is released by the somatic cells of the follicle and affects the oocyte through activin receptors present in this germ cell.

**Oocyte Maturation**

The post-vitellogenic oocyte may remain quiescent for several months but following environmental, social, or pheromonal cues, it begins the process of final maturation, that is, the resumption of meiosis (Figure 2). This will commence with a surge in gonadotropin-releasing hormone (GnRH), with or without a decrease in dopaminergic inhibition, followed by a rise in circulating LH. Upon binding of LH to its receptors on the granulosa cells, the ovarian follicle starts the process of maturation, beginning with the production of the maturation-inducing steroid (maturation-inducing steroid (MIS) such as DHP or 17α-20β,21 Trihydroxy-4-pregnen-3-one (20/3-S)). Binding of the MIS to its receptors on the oocyte plasma membrane is followed by activation of the maturation-promoting factor (MPF), a complex consisting of existing cdc2-kinase and newly synthesized cyclin B. (Figure 1(b)). The process of oocyte maturation is reflected morphologically by the migration of the germinal vesicle (GV) toward the animal pole (GV migration) and the disintegration of its membrane, a stage known as GV breakdown (GVBD) (Figures 1(b) and 2). The chromosomes then condense, a spindle is formed, and the first polar body is extruded which marks the end of the first meiotic division (Figure 2). At this stage the oocytes absorb water and inflate; this is especially pronounced in marine fish with pelagic eggs, the pressure within the follicle increases, the follicular wall is ruptured, and the oocyte is released (ovulated) into the ovarian lumen or to the coelomic cavity. The meiosis is arrested again at metaphase II. Completion of the second meiotic division and extrusion of the second polar body are further delayed and will proceed only if the egg is fertilized (Figure 2).

**Spermatogenesis**

**Endocrine Regulation of Spermatogenesis**

Spermatogenesis consists of several successive processes, each regulated by a distinct set of hormones. Mitotic divisions of spermatogonia that lead to germ cell renewal are regulated by E2 secreted by the interstitial Leydig cells under gonadotrophic stimulation, mainly FSH. Upon binding of E2 to their receptors in the Sertoli cells, the latter secrete the spermatogenic stem-cell renewal factor (possibly gonadal soma-derived growth factor (GSDF)) that stimulates spermatogonial mitotic proliferation (Figure 3).

Experimental increase or decrease in the FSH and FSH-Rs expression in the testis of African catfish (Clarias gariepinus) resulted in respective changes in Sertoli cell proliferation and testicular growth, suggesting that in fish, as in mammals, FSH in cooperation with androgens is probably the regulator of Sertoli cell number.

Gonadotropic stimulation in the Japanese eel (Anguilla japonica) shifts a subpopulation of spermatogonia germ cells from a renewal line to a proliferation line ensuing by meiosis. Gonadotropins (mainly FSH) elicit a surge in the secretion of 11-KT from Leydig cells to stimulate in Sertoli cells the production of several mediators such as activin B, insulin-like growth factor I (IGF-I), and anti-Müllerian hormone (AMH). The action of 11-KT on this spermatogonial line is positively mediated by activin B and IGF-I, and negatively by AMH (Figure 3). However, the initiation of meiotic division leading to the formation of spermatids is induced by DHP, which is produced by the germ cells themselves, exerting their effect on the germ cells in a paracrine or autocrine manner (Figure 3). The need for DHP to initiate meiosis was demonstrated by 11-KT stimulating DNA replication and meiosis in organ culture of eel testes and anti-DHP serum blocking this effect. Moreover, addition of DHP resulted in the expression of two meiosis-specific markers DMC1 and Spo11, and the appearance of synaptonemal complexes in testicular sections.

Immature spermatozoa undergo sperm maturation, which is also regulated by DHP acting directly on spermatozoa to activate carbonic anhydrase. This enzymatic activation is followed by an increase in the seminal plasma pH, which augments intrasperm cAMP levels to enable their motility (Figure 3).

**Gonadal Steroids**

**Formation of Gonadal Steroids in Fish**

Fish gonadal steroids are mainly synthesized through pathways similar to those in other vertebrates with a few exceptions (Figure 4). Testosterone in fish testis is hydroxylated at carbon 11 by P450-11β and, after oxidation by 11β-hydroxysteroid dehydrogenase (11β-HSD), is converted into 11-KT that is the potent androgen of teleost fish. In certain fish, testosterone hydroxylation occurs in an extra-testicular tissue, the liver. The conversion of 17α-hydroxyprogesterone by 20α-HSD produces DHP that acts as the MIS in many fishes. However, in a number of marine perciform fish, the MIS is 17α,20β,21-trihydroxy-4-pregnen-3-one (21β–S). This steroid too derives from
17-hydroxyprogesterone that is hydroxylated at carbon 21 by 21 hydroxylase (P450c21) to form 11-deoxycortisol. The latter is oxidized by 20/β-HSD to form 20/β-S (Figure 4).

The Shift from Estradiol to Progestogens

At the onset of the periovulatory phase, the LH surge is followed by a dramatic shift in the steroidogenic pattern. There is an increase in the formation of DHP concurrently with a decline in E2 level. The shift in the steroidogenic pattern from the formation of C18 and C19 steroids (estrogens and androgens, respectively) toward the formation of C21 steroids (progestogens or corticosteroids) involves a decrease in 17–20 lyase activity and a rise in that of 20/β-HSD.

Two genes encoding 17-hydroxylase (P450c17) occur in fish. One is P450c17-I which is similar to that in tetrapod gonads and displays lyase activity resulting in C19 steroids that serve as precursors for androgens and estrogens. P450c17-I is expressed in the ovarian granulosa cells during the vitellogenic phase. The other gene, P450c17-II, encodes a 17-hydroxylase that is devoid of lyase activity and is fully expressed in the oocytes of the Nile tilapia (Oreochromis niloticus) only during final oocyte maturation, increasing the production of progestogens instead of androgens and estrogens (Figure 4).

Irrespective of the mechanism leading to the steroidogenic shift, estradiol and its seven-transmembrane receptor (GPR30) in the oocyte maintain meiotic arrest. Therefore, the decrease in estradiol is essential for the resumption of meiosis during oocyte maturation (Figures 1 and 2).

In addition to the aforementioned free steroid compounds, teleost gonads produce a number of reduced and conjugated steroids, especially in the periovulatory phase. The glucuronidated testosterone and sulfated or glucuronidated progestogens, when released into the water, may act as sex pheromones.

The formation of sex steroids in the vitellogenic ovary of the salmon and probably in other fish as well is a two-step process in which the steroidogenic conversions from cholesterol to testosterone are carried out in the theca cells. The androgen diffuses into the surrounding and is taken up by the granulosa cells possessing P450arom that aromatizes testosterone to form E2 (Figure 4).

In a parallel manner, in the ovary approaching final oocyte maturation, all the steroid conversions from cholesterol to 17-hydroxyprogesterone is carried out in the theca of the post-vitellogenic ovary while the conversion
to DHP is performed by the granulosa cells possessing 20β-HSD (Figure 4).

Gonadotropins and Their Regulation

The fish pituitary produces and secretes two gonadotropins: FSH and LH. Both are heterodimeric glycoproteins formed by a shared α-subunit (GPa) noncovalently linked to a specific β-subunit. Each subunit, α, FSHβ, and LHβ, is encoded by a distinct gene.

Molecular cloning techniques allowed so far the isolation of the genes encoding the gonadotropin subunits for 56 fish species belonging to 14 teleost orders. The phylogenetic relationships of fish gonadotropins, together with their structural and biological characteristics, provided the evidence for their classification as the piscine counterparts of the mammalian FSH and LH.

The α-subunit is the most conserved among fish species and contains two potential sites for N-glycosylation and 10 conserved cysteines. LHβ and FSHβ subunits each contain 12 conserved cysteines linked by six disulfide bridges. This structure is conserved in fish LHβ subunits, but not in FSHβ, which is the least conserved gene.

All LHβ gene promoters isolated from fish contain putative response elements for Sf-1 and Pitx1, but unlike their mammalian counterparts, the teleost proximal promoters do not contain an early growth response factor 1 (Egr-1). The latter appears to have been replaced by the estrogen receptor (ER). Comparison of FSHβ5′ flanking region in the fish studies so far revealed that they all share several putative response elements, such as GSE, CRE, half sites of ERE, and activating protein 1 response element (AP1), the recognition site for the Fos and Jun transcription factors.

Recombinant Gonadotropins

Fish gonadotropin research has traditionally relied on the laborious isolation and incomplete purification of the
native hormones extracted from thousands of fish pituitary glands. The isolation and cloning of cDNAs encoding piscine gonadotropin subunits enabled the production of recombinant hormones (rFSH and rLH) through their expression in heterologous systems. However, to endow the products with the correct biological activity, they have to be glycosylated, folded, and assembled as a heterodimers. All recombinant LH and/or FSH produced so far in teleosts can bind and activate the respective receptors, and stimulate sex-steroid output from isolated gonadal tissue. The lack of cross-contamination of these recombinant hormones enables the study of LH and FSH differential functions. In addition, it is anticipated that recombinant gonadotropins will find use in spawning induction in aquaculture.

**Regulation of Gonadotropin Synthesis and Secretion**

The hypothalamus regulates synthesis and release of gonadotropins through multiple neurohormones. The major ones are GnRH in its various forms, kisspeptins and dopamine, and potentially other hormones such as γ-aminobutyric acid (GABA), pituitary adenylate cyclase-activating peptide (PACAP), norepinephrine, neuropeptide Y (NPY), serotonin, secretoneurin, ghrelin, leptin, and glutamate (Figure 5). Of special interest are hormones associated with growth and metabolism (IGF-1, glutamate, leptin, and ghrelin), which signifies a relationship between reproduction, energy reserves, and body mass of the fish (Figure 5).

**Gonadotropin-releasing hormone**

GnRH stimulates the production and release of gonadotropins from the pituitary of teleosts as in other vertebrates. It increases the amounts of mRNA encoding gonadotropin subunits in the pituitary. The response of Gα and LHβ mRNAs to GnRH implantation in maturing females or males is higher than that of FSHβ, indicating differences in the hypothalamic regulation of the two gonadotropins.

GnRH effects vary with a fish’s reproductive stage. In maturing salmonids sGnRH elevates pituitary mRNAs encoding for Gα and FSHβ, but not that of LHβ. The response of the gonadotropin subunit mRNAs to GnRH in common carp (*Cyprinus carpio*) too is differential and depends on the gender and reproductive stage. Thus, information regarding the reproductive stage is potentially conveyed via the steroid hormone profile that can modulate the response and make it specific to each phase (Figures 1 and 5). The three forms of GnRH present in the brain of gilthead seabream (*Sparus aurata*) and Nile tilapia (*sbgGnRH = GnRH1; cGnRHI = GnRH2; sGnRH = GnRH3*) all have LH-stimulating activity in mature females. However, the form most abundant in the pituitary of mature fish, GnRH1, is the least potent in inducing LH release.

**Dopaminergic inhibition of gonadotropins**

Dopamine inhibits both basal and GnRH-stimulated LH secretion (Figure 5). In vitro experiments, as validated by molecular studies, show that dopamine D2-like, but not D1-like, receptors inhibit gonadotropin secretion directly in the pituitary. In fact, cyprindin spawning induction in aquaculture uses dopamine D2 antagonists, such as domperidone, pimozide, or metoclopramide, to facilitate the stimulation by GnRH of LH release and ovulation. Nevertheless, the dopaminergic inhibition does not operate in all fish and is lacking altogether in
Atlantic croaker (*Micropogonias undulatus*) and gilthead sea bream.

**Kisspeptin regulation of gonadotropins**

Kisspeptin, a member of the RF amide peptide family, has important roles in mammals in timing of puberty, maintaining gonadal functions, photoperiod control of seasonal breeding, metabolic gating of fertility, and insulin secretion. Kisspeptin has been proposed to be a novel gatekeeper for the gonadotropic axis of fish mainly due to its potent stimulation of gonadotropin secretion (Figure 5).

Kiss1 was cloned in several fish species and its involvement in gonadotropin release and signaling of sexual maturation/puberty has been confirmed so far in medaka (*Oryzias latipes*), zebrafish (*Danio rerio*) and the European sea bass (*Dicentrarchus labrax*). In addition, a second Kiss gene, namely Kiss2, exists in teleost fish. Apparently, the two Kiss genes arose by gene duplication early in vertebrate evolution, and Kiss2 gene might have been lost in the mammalian lineage (Figure 6). Kiss1 and Kiss2 in zebrafish are regionally expressed within the hypothalamus, and may exert differential or distinct effects on LH and FSH expression and secretion.

Fish possess two Kiss receptors: kiss1ra and kiss1rb. Sequence identity and genome synteny analyses indicate that zebrafish kiss1ra is a human KISS1R ortholog, whereas kiss1rb is a specific fish form. Both kisspeptins and their receptors are abundantly expressed in the brain, notably in the hypothalamus, suggesting that these ligand–receptor pairs have neuroendocrine and neuro-modulatory roles. Fish kisspeptins differ in their signal transduction pathways, tissue distribution, and their gene expression pattern toward puberty.

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**Figure 6**  Phylogenetic analysis of kisspeptins. Unrooted phylogenetic trees of kisspeptin sequences generated with a MEGA version 4. Numbers at nodes indicate the bootstrap values as percentages obtained from 1000 replicates. Scale bar indicates the substitution rate per amino acid. Hamster – Siberian hamster (*Phodopus sungorus*); mouse – House mouse (*Mus musculus*); cow (*Bos taurus*); sheep (*Ovis aries*); human (*Homo sapiens*); xenopus (*Xenopus tropicalis*); opossum – gray short-tail opossum (*Monodelphis domestica*); platypus (*Ornithorhynchus anatinus*); goldfish (*Carassius auratus*); zebrafish (*Danio rerio*); medaka (*Oryzias latipes*); European sea bass (*Dicentrarchus labrax*); grouper – orange-spotted grouper (*Epinephelus coioides*); grass lizard – Japanese grass lizard (*Takydromus tachydromoides*); sockeye salmon – sockeye salmon (*Oncorhynchus nerka*).
Regulation by gonadal steroids
Steroids released from gonads can feed back and regulate the production and release of FSH and LH. This type of feedback effect (positive or negative) varies with the gonadal phase of reproductive development. Positive or negative feedback mechanisms operate either indirectly through certain hypothalamic nuclei, or directly on the pituitary cells. Negative feedback of gonadal steroids on gonadotropin secretion was demonstrated using gonadectomy and steroid-replacement protocols in certain teleost species. In rainbow trout (Oncorhynchus mykiss), the negative feedback of estrogens on the pituitary is direct or indirect through GnRH inhibition, and potentially by E2 upregulation of dopamine receptor mRNA. Nevertheless, a positive feedback of gonadal steroid also operates in fish, but only early in gonadal development. Estrogens and aromatizable androgens can stimulate LHβ-subunit gene expression and increase pituitary LH protein levels in juveniles of several fish species.

Regulation by gonadal peptides
Activins are peptides composed of two β-subunits that form either hetero- or homodimers: activin A (βα + βα), activin BA (βα + ββ), and activin B (ββ + ββ). Activins A and B increase FSHβ and reduce LHβ mRNA levels in cultured pituitary cells. Furthermore, follistatin, an activin-binding protein, can reverse the effects of exogenous activin on FSHβ and LHβ expression, and also stimulate basal expression of FSHβ gene. In goldfish (Carassius auratus), Smad3 is likely the principal signal transducing molecule involved in activin stimulation of FSHβ expression. While activin was first identified as an FSH stimulator and its effect on FSH biosynthesis has been well studied in a variety of fish, the details of its action mechanism are poorly understood.

Gonadotropin Receptors
In line with the gonadotropic duality, two distinct receptors occur in fish gonads: the FSH-R and the LH-R. Both show different expression profiles during follicular development. Expression of FSH-R is associated predominantly with vitellogenesis, while the LH-R is prevalent during oocyte maturation and ovulation.

Studies on gonadotropin receptors selectivity in representatives of three teleost orders (salmoniformes, siluriformes, and cypriniformes) indicated that FSH-Rs not only show a preference for FSH but also respond to LH, whereas LH-Rs specifically respond to LH only. Amago salmon (Oncorhynchus rhodurus) is the only known exception to this scheme.

The FSH-Rs and LH-Rs are G-protein-coupled receptors (GPCRs) whose large extracellular domain contributes to the recognition and specific binding of the hormone. The transmembrane domain is the most conserved part of these receptors, both in structure and amino acid sequence. It has seven α-helices composed of 22–28 hydrophobic amino acids, with each crossing the lipid bilayer and being connected by three intracellular and three extracellular loops. The transmembrane domain is responsible for receptor activation and signal transduction of the hormone.

Generally, the cellular localization of the gonadotropin receptors agrees with their steroidogenic biopotencies in mature fish. In situ hybridization studies in African catfish revealed FSH-R expression in Sertoli cells, but in contrast to other vertebrates, FSH-R is expressed in the interstitial Leydig cells too, together with LH-R. In European sea bass, FSH-R transcripts are detected in the follicular cells surrounding the pre- and early vitellogenic oocytes but not on fully grown oocytes, indicating the involvement of FSH-R in the recruitment of new oocyte generation and initiation of vitellogenesis.

FSH-R was localized in the theca layer and intensely on granulosa cells of vitellogenic ovaries of coho salmon (Oncorhynchus kisutch), whereas in the preovulatory follicle, it occurred in the theca and interstitial connective tissue, but not in the granulosa. At all stages of oogenesis, only granulosa cells of the preovulatory follicle exhibited LH-R, which is in line with LH function in oocyte maturation.

The expression of LH-R in males is consistently correlated with spermatiation and spawning in all fish species studied so far.

Unorthodox Sites for Kiss, GnRH, and Gonadotropins
Recently, evidence is accumulating that the gonads produce and release hormones, and contain receptors that originally were thought to be restricted to the hypothalamus and pituitary. Oocytes of the gilthead sea bream produce and release GtHs, processes that can be enhanced by GnRH and reduced by an GnRH antagonist. In the African catfish, GnRH-1 and GnRH-2 mRNAs are expressed in the testis, and GnRH-2 mRNA in the ovary. In addition, kiss1 and kiss1r occur in the ovary and testes of various fish. These local regulatory axes could finely tune the activity of hypothalamic–pituitary–gonadal axis. Nevertheless, further research is still required before an updated comprehensive model for this endocrine regulation of fish reproduction can be formulated.

See also: Hormones in Communication: Hormonal Pheromones. The Pituitary: Pituitary Gland or Hypophysis. The Reproductive Organs and Processes: Anatomy and Histology of Fish Testis; Regulation of Spermatogenesis; Vitellogenesis in Fishes.
Further Reading


