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Steroidogenesis and its regulation in teleost-a review

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Abstract Steroid hormones modulate several important biological processes like metabolism, stress response, and reproduction. Steroidogenesis drives reproductive function wherein development and differentiation of undifferentiated gonads into testis or ovary, and their growth and maturation, are regulated. Steroidogenesis occurs in gonadal and non-gonadal tissues like head kidney, liver, intestine, and adipose tissue in teleosts. This process is regulated differently through multi-level modulation of promoter motif transcription factor regulation of steroidogenic enzyme genes to ultimately control enzyme activity and turnover. In view of this, understanding teleostean steroidogenesis provides major inputs for technological innovation of pisciculture. Unlike higher vertebrates, steroidal intermediates and shift in steroidogenesis is critical for gamete maturation in teleosts, more essentially oogenesis. Considering these characteristics, this review highlights the promoter regulation of steroidogenic enzyme genes by several transcription factors that are involved in teleostean steroidogenesis. It also addresses different methodologies involved in promoter regulation studies together with glucocorticoids and androgen relationship with reference to teleosts.

Keywords Gonadal steroids · Testosterone · Estradiol · Testis · Ovary · Promoter

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Introduction

In recent times, more emphasis was given on gene expression studies including promoter level regulation. Several key pathways and mechanisms have regulatory checkpoints (e.g., cell cycle check points) wherein the gene expression is stimulated or suppressed at specific times or tissues. Steroids control important processes like metabolism, inflammation, immune functions, gonadal development, and maturation, and hence, regulation of steroidogenesis is extremely critical. Though few reports showed the promoter level regulation of steroidogenic enzyme genes, comprehensive analysis of all the enzyme genes in teleosts were not available, and hence, the present review attempted to highlight the transcriptional regulation of steroidogenesis in teleosts by emphasizing on the promoter level regulation. Owing to the wide variation in regulation of teleosts, more general and comprehensive view is given to provide a clear perspective.

Steroid hormones regulate embryonic development, gonadal differentiation, neuroprotection, stress response, and gametogenesis in teleosts as in other

vertebrates (Mayer et al. 1990; Borg 1994; Baroiller et al. 1999; Devlin and Nagahama 2002; Handa et al. 2008; Tokarz et al. 2015). The major sites of steroidogenesis include gonadal and non-gonadal tissues like head kidney, liver, intestine, and adipose tissue (Nagahama 1994; Jiang et al. 1998; Rasheeda et al. 2010a, b; Swart et al. 2013). In teleosts, steroidogenesis occurs in specific cell types of gonads, wherein cholesterol is processed into pregnenolone and subsequently into different steroids such as estrogens, androgens, and progestins, while conversion to corticosteroids and mineralocorticoids occur at peripheral tissues (Borg 1994; Mommsen et al. 1999; Senthilkumaran et al. 2004; Blazquez and Somoza 2010; Scott et al. 2010). Owing to its diverse role in several biological processes, tissue specific expression, and their intrinsic regulation, it is essential to study the regulation of steroidogenic enzyme genes at promoter motif level. In addition, steroidogenesis is regulated by multiple means like substrate limitation, co-factor preference, allosteric mechanism, and expression of steroidogenic enzymes genes (Stocco 2000; Zhou et al. 2005; Guiguen et al. 2010; Rasheeda et al. 2010a; Raghuveer et al. 2011; Rajakumar and Senthilkumaran 2014a, b, 2015, 2016). This regulation is critical with respect to stage or reproductive cycle, tissue, season, and sex-specific biosynthesis of steroids.

Gonadal steroidogenesis is primarily regulated by hypothalamo-hypophysial axis, while autocrine and/or paracrine regulation and steroidogenic cell niche (microenvironment) are also critical for stage and sex-specific steroidogenesis (Peter et al. 1991; Nagahama 1994; Senthilkumaran and Joy 1996; Goos et al. 1999; Ge 2005; Scott et al. 2010). Another important aspect is their involvement in gonadal differentiation, development, and growth (Devlin and Nagahama 2002; Vizziano et al. 2007; Guiguen et al. 2010; Raghuveer et al. 2011; Sudhakumari and Senthilkumaran 2013). In the case of mammals, sex determination and gonadal development are strictly genetic (Wilhelm et al. 2007) and shows limited or no gonadal plasticity (Tanaka and Nishinakamura 2014). Conversely, teleost gonads show plasticity, often retaining the ability to change gonadal sex at different stages of development or even in adulthood in various species, which can be directed to undergo complete reversal from testis to ovary and vice-versa through hormonal treatments irrespective of genetic/chromosomal sex or through environmental cues (Francis 1992; Baroiller et al. 1999; Kobayashi et al. 2003; Sudhakumari and Senthilkumaran 2013; Kobayashi et al. 2013). Further, the levels of hormones during critical period of gonadal differentiation determine the development of undifferentiated gonad into either testis or ovary (Devlin and Nagahama 2002; Baroiller et al. 2009; Guiguen et al. 2010). Consequently, two hypotheses had been proposed, the first one considers the differential expression of aromatase (cytochrome P450, family 19, subfamily A, polypeptide 1a [*Cyp19a1a*/ ovarian aromatase] gene) for ovarian development and elevated temperature and/or cytochrome P450 family 11 subfamily B member 1 (*cyp11b1/11β-hydroxylase*)/ *11β-hydroxysteroid dehydrogenase* (*hsd11b*) gene expression for testicular development (Baroiller et al. 2009; Blasco et al. 2010; Fernandino et al. 2012, 2013; Guiguen et al. 2010; Nakamura et al. 2010). Recent study using gene editing methodologies (TALEN and CRISPR) showed that the knockout of *Cyp19a1a* results in all-male offspring in zebrafish (Lau et al. 2016). While the second hypothesis perceives androgens do not participate in early testicular differentiation, active expression of *cyp19a1a* induces ovarian differentiation and its inhibition alone is sufficient for testicular differentiation (Fernandino et al. 2012, 2013; Guiguen et al. 2010; Hattori et al. 2009; Sudhakumari and Senthilkumaran 2013; Yamaguchi et al. 2010). In gonochoristic fishes like pejerrey or the Japanese flounder, elevated temperature and other environmental stressors result in increased cortisol levels and decreased aromatase, which leads to activation of androgen pathways, increased *hsd11b* expression, and gonadal masculinization (Fernandino et al. 2012, 2013; Hattori et al. 2009; Yamaguchi et al. 2010). Regulation of steroidogenic enzyme genes seems to play central role in gonadal differentiation. Hence, the role of sex steroids and regulation of steroidogenic enzyme genes by certain transcription factors during the critical stages of gonadal differentiation have a direct impact on gonadal fate and sex of the organism as well as the reproductive cycle.

In addition to sex steroids, glucocorticoids play an important role in gonadal differentiation in few teleosts like pejerrey and the Japanese flounder by downregulating cyp19a1a, thereby inducing testicular differentiation (Hattori et al. 2009; Yamaguchi et al. 2010). Differential expression of specific steroidogenic enzyme genes during gonadal differentiation/development, maturation, and seasonal cycle results in the production of pertinent sex steroids. However, very little is known about their promoter regulation and transcription factor

interaction in teleosts. Any detailed analysis on these aspects will unravel their role in androgen and estrogen production *vis-à-vis* gonadal differentiation. On these perspectives, the current review focused on the transcriptional regulation of steroidogenesis in teleosts, based on scientific literature available till date.

Steroidogenic enzyme gene expression and their regulation

Steroidogenesis starts with the rate-limiting transport of cholesterol into the mitochondria mediated by steroidogenic acute regulatory protein (StAR), where it is converted into pregnenolone, a first precursor in the steroidogenic cascade and it is the rate-limiting step in steroidogenesis (Stocco 2000). However, existence of additional rate-limiting steps in steroidogenesis is not clear. Cyp11a1 is the only enzyme involved in the conversion of cholesterol to pregnenolone, which thereby initiates the whole process of steroidogenesis (Stocco 2000; Rajakumar and Senthilkumaran 2014a; Tokarz et al. 2015) after the initial stint from StAR. Sequential action of several steroidogenic enzymes results in the conversion of pregnenolone into active steroids like 17α,20β-dihydroxy-4-pregnen-3-one (17α,20β-DP), testosterone (T), 11-Ketotestosterone (11-KT), and estradiol-17β (E_2) as well as corticosteroids (Senthilkumaran 2011; Rajakumar and Senthilkumaran 2014a, b; Tokarz et al. 2015). Maturation-inducing steroids (MISs), 17α,20β-DP and 17α,20β,21-trihydroxy-4-pregnen-3-one, have been implicated in the final oocyte maturation and to some extent in sperm maturation of teleosts (Senthilkumaran et al. 2004; Sreenivasulu et al. 2012a; Trant and Thomas 1989). Interestingly, 11-hydroxytestosterone (11-OHT) and 11-KT (a potent androgen in fishes) are present in mammals, except that it was detected when induced with human chorionic gonadotropin (hCG; Yazawa et al. 2008; Senthilkumaran et al. 2009; Scott et al. 2010; Sreenivasulu et al. 2012a; Rajakumar and Senthilkumaran 2013; Rege et al. 2019). However, 5α-dihydrotestosterone (DHT), a potent androgen of mammals, was detected in plasma of fathead minnow and found to have androgenic potency (Margiotta-Casaluci and Sumpter 2011; Margiotta-Casaluci et al. 2013). Further studies are needed to understand the implications of 11-oxygenated androgens in mammals and DHT in fishes during testicular development. Studies using specific chemical blockers or targeted gene silencing provide reason for varied pattern of androgen metabolism to act more potentially than the natural potent androgen, T in reference to evolution. In the case of Japanese eel, immature testes have the ability to produce 11-KT, in the presence of 11-OHT, and it was proposed that synthesis of 11-KT is arrested in immature testis earlier in the steroidogenic pathway other than the step from 11-OHT to 11-KT (Ozaki et al. 2006). Hence, cohesive study of all other steroidogenic enzyme genes and their stringent control might provide novel leads in unraveling the critical steps of testicular steroidogenesis. The steroidogenic enzyme genes, cyp11a1, cyp17, and cyp19a1a, are best characterized in the pathway, because they constitute three important regulatory inputs in the steroidogenesis. Steroidogenesis is strictly regulated by gonadotropins (GTHs)/hCG/cAMP, wherein acute and chronic stimulation of Mouse Leydig cells (with 8-bromo cAMP in culture containing synthetic serum-free medium containing 0.1% BSA and insulin (500 μg/ml) for up to 15 days) is required for the steady-state expression of steroidogenic enzyme genes which in turn stimulate the production of sex steroids (Payne and Youngblood 1995). A recent study using FreeStyle 293-F cell lines producing recombinant Japanese eel Follicle-stimulating hormone (reFsh) and Luteinizing hormone (reLh) and gonadotropin receptors-expressing COS-7 cells indicated reFsh stimulated its cognate receptor; meanwhile, reLh activated both receptors. Both reFsh and reLh induced testicular 11-KT production in a dose- and time-dependent manner by upregulating expression of steroidogenic enzyme genes (Suzuki et al. 2019). Owing to the receptor sharing of luteinizing hormone (LH) and hCG (Bogerd et al. 2001; Choi and Smitz 2014; Vischer et al. 2003), and promoter regulation, several researchers used hCG for functional studies on hormonal profiles and on the expression of steroidogenic enzyme genes (Choi and Smitz 2014; Rajakumar and Senthilkumaran 2015). Vischer et al. (2003) used human follicle stimulating hormone, hCG, and human LH, in 25 μl of Hepes-modified Dulbecco's modified Eagle's medium containing 0.1% bovine serum albumin and 0.1 mM 3-isobutyl-1-methylxanthine in 96-well plate for ligand stimulation and receptor binding assays. Production of E_2, T, and 11-KT was stimulated by hCG by binding to luteinizing hormone receptor (LHR) and both directly and indirectly through an increase in the expression of steroidogenic enzyme genes like *cyp11a1* (Rajakumar and Senthilkumaran 2014a), *17β-*

hydroxysteroid dehydrogenase 1 (*hsd17b1*) and *hsd17b12* (Rajakumar and Senthilkumaran 2014b), *cyp19a1a* and *cyp19a1b* (brain aromatase; Rasheeda et al. 2010b), *cyp11b1* (Jiang et al. 1996; Rajakumar and Senthilkumaran 2015), and *hsd11b* (Jiang et al. 2003; Ozaki et al. 2006; Rasheeda et al. 2010a; Rajakumar and Senthilkumaran 2016). These reports provided detailed information on the regulation of all steroidogenic enzyme genes comprehensively with changes in the gene expression during gonadal differentiation, development, and gametogenesis in teleosts. In fact, it has been well established that hCG binds to LHR and complements LH action in gonads at different time points of active reproduction in teleosts including advancing the gonadal maturation (Kagawa et al. 2009; Murugananthkumar et al. 2017). Nevertheless, it will be ideal to check the effect of teleostean LH independently as hCG shows differential response in teleosts.

Cyp11a1

Cholesterol is converted by cholesterol side-chain cleavage enzyme, P450scc or cyp11a1, into pregnenolone, which is the slowest step, and thus, controls the rate of synthesis of steroid hormones. Hence, the regulation of *cyp11a1* together with cholesterol mobilization is very important in controlling overall steroidogenesis (Rajakumar and Senthilkumaran 2014a). Though the expression changes during gonadal development, maturation, and gametogenesis were studied in teleosts (Hsu et al. 2002; Hu et al. 2004; Kazeto et al. 2006; Rajakumar and Senthilkumaran 2014a), promoter motif regulation seems to be less understood except for zebrafish. In zebrafish, Ff1b (homolog of steroidogenic factor 1 [Ad4BP/Sf-1 referred as SF-1]) binds to two conserved FF1 response elements (FRE) on the putative promoter of *cyp11a1* and activates transcription (Quek and Chan 2009). Deletion and mutagenesis studies revealed that only the proximal FRE was essential for transcriptional activation, which critically regulates *cyp11a1* expression (Quek and Chan 2009). Chromatin immunoprecipitation (ChIP) and electrophoretic mobility shift assays (EMSAs) further confirmed the importance of Ff1b in the transcriptional activation of *cyp11a1* (Table 1; Fig. 1). Further, *cyp11a1* promoter drives the EGFP expression specifically to the internal gland and genital ridge when transiently expressed in microinjected zebrafish embryos (Quek and Chan 2009).

Cyp17

The conventional type of cyp17 (cyp17a1) and a novel type of cyp17 (cyp17a2) were identified in tilapia (Zhou et al. 2007a), medaka (Zhou et al. 2007b), and Japanese eel (Kazeto et al. 2000; Su et al. 2015) and these two types of Cyp17 are encoded by two different genes (Zhou et al. 2007a). The cyp17a1 showed both 17α-hydroxylase and 17,20-lyase activities (Fig. 1) while cyp17a2 possesses unique 17α-hydroxylase activity, without any 17,20-lyase activity which involve in 17α,20β-DP production (Fig. 1). *Cyp17a2* was expressed not only in the gonads, but also in the head kidney, while *cyp17a1* was exclusively expressed in the gonads (Zhou et al. 2007a, b). It is well known that T and E_2 are involved in gametogenesis and decreased during gamete maturation during which progestin levels might get elevated (Scott et al. 2010; Sreenivasulu et al. 2012a; Rajakumar and Senthilkumaran 2013). The specific molecular mechanisms are not clearly understood. One possibility is the differential expression of *cyp17a2* in few teleosts, yet specific promoter regulation of *cyp17* gene is not studied in any teleosts to validate the contention. In other teleosts, cyp17a1 through its 17α-hydroxylase and 17, 20 lyase activities synthesizes androgen from progestins (Scott et al. 2010; Sreenivasulu and Senthilkumaran 2009). Comprehensive studies on gene expression and enzyme activity analysis in catfish warrant the cyp17 is indeed involved in androgen production, and the presence of second isoform has been ruled out (Sreenivasulu and Senthilkumaran 2009). The expression of *cyp17a2* was significantly decreased in the testis of the GTH receptor knockout zebrafish (Chu et al. 2015). Incidentally, in the commercially important Japanese eel, *Anguilla japonica*, the MIS, 17α,20β-DP is generated from its precursor by cyp17 which has both 17α-hydroxylase and 17, 20 lyase activities. In order to elucidate the regulatory mechanism underlying the steroidogenic shift from E_2 to 17α,20β-DP, and the mechanistic basis for the failure of this shift in natural versus artificially induced eel will be rewarding. Taken together, specific activity of cyp17a1 and cyp17a2 as well as targeted gene silencing might provide relevant information to establish the specific role of these enzymes in MIS and androgen production as well as shift in steroidogenesis.

Table 1 Summary of regulation of steroidogenic enzyme genes in teleosts

Sl. no	Name of the gene	Transcription factor/orphan nuclear receptor involved	Methods used	Species studied	Reference
1.	Cyp11a1	Ad4BP/Sf-1 (Ff1b)	Luciferase assay, SDM, EMSA and ChIP	*Danio rerio*	Quek and Chan (2009)
2.	Cyp17	–	–	–	Not studied
3.	Hsd3b	–	–	–	Not studied
4.	Hsd17b	–	–	–	Not studied
5.	Cyp11b	Ad4BP/Sf-1	Luciferase assay	*Clarias batrachus*	Unpublished data
6.	Hsd11b	Sox3 Wt-1	Luciferase assay, SDM, EMSA and ChIP Luciferase assay (weak activity)	*Clarias batrachus*	Rajakumar and Senthilkumaran (2016)
7.	Cyp19a1a	Foxl2 Ad4BP/Sf-1/FTZ-F1	Luciferase assay, SDM and EMSA	*Oreochromis niloticus*	Watanabe et al. (1999); Yoshiura et al. (2003); Govoroun et al. (2004); Wang et al. (2007)
8.	Hsd20b	CREB	Luciferase assay, SDM and EMSA	*Clarias gariepinus* and *Oncorhynchus mykiss*	Sreenivasulu et al. (2012b), Senthilkumaran et al. (2015)

SDM site directed mutagenesis, *EMSA* electrophoretic mobility shift assay, *ChIP* chromatin immunoprecipitation

3β-hydroxysteroid dehydrogenase (Hsd3b)

Regulation of *hsd3b* (Δ5-Δ4 isomerase) gene at the promoter level is not studied in any teleost. In mouse Leydig cell culture, cAMP induction of gonadotropin regulation was observed in *Hsd3b* (Payne and Youngblood 1995). In the case of Nile tilapia, two forms of hsd3b were reported to be similar to human (Senthilkumaran et al. 2009). GTHs regulate hsd3b expression in Protogynous Orange-Spotted Grouper, *Epinephelus coioides* (Huang et al. 2019) and several other fish species (Levavi-Sivan et al. 2009). The expression of *hsd3b* was significantly decreased in the testis of the GTH receptor knockout zebrafish (Chu et al. 2015).

In the case of human, gene regulation of *hsd3b* is complex and involves several different factors including a number of endocrine and paracrine regulatory mechanisms. The enzyme, hsd3b, is involved in the synthesis of several natural steroid hormones like progesterone and T and the hepatic degradation of the pheromone androstenone (Rasmussen et al. 2013). Transcriptional activity of *hsd3b* was influenced by several signaling and regulatory pathways like JAK-STAT, LH/hCG, estrogen receptor alpha, androgen receptor, Ad4BP/Sf-1, and peroxisome proliferator-activated receptor alpha in mammals (Rasmussen et al. 2013). Whether similar phenomenon exists in the case of teleosts remains to be elucidated.

Hsd17b

The enzyme hsd17b isoforms are involved in the interconversion between 17β-hydroxy- (active) and 17-keto- (inactive) steroids, which thereby regulate the level of specific substrates required for sex steroid biosynthesis (Adamski and Jakob 2001; Moeller and Adamski 2009; Rajakumar and Senthilkumaran 2014b). Various forms of hsd17bs were reported in teleosts (14 forms were reported in mammals), which are involved in several processes leading to the production of sex steroids. The enzyme hsd17b is required for the production of essential sex steroids like T and E_2, and thus, is indirectly involved in the process of sex differentiation and gametogenesis in fish (Guiguen 2000; Mindnich et al. 2004; Zhou et al. 2005).

Incidentally, hsd17b1 is a key enzyme involved in E_2 synthesis together with cyp19a1a. It is predominantly involved in interconversion of estrone (E1) to E_2 in teleosts (Zhou et al. 2005). The enzyme hsd17b2 is involved in the inactivation of estrogens and androgens while hsd17b3 is androgenic which is essential for testicular production of T (Adamski and Jakob 2001; Moeller and Adamski 2009). Hsd17b12 is able to reduce E1 to E_2 (Mindnich et al. 2004). The enzyme is the most recent addition to this family which shares close relationship to hsd17b3 and was shown to be an ancestor of hsd17b3 by phylogenetic analysis (Mindnich et al. 2004). In zebrafish, hsd17b12-like was able to convert cortisone to 20β-hydroxycortisone, and hence, it was named as hsd20b2 (Tokarz et al. 2012). Transcriptional regulation of *hsd17b* was not studied for any of the isoforms identified in teleosts. Owing to the presence of different isoforms and substrate specificity and its role in T production, it is relevant to study the gene regulation of all the hsd17b subtypes, which might provide interesting data on promoter level control of tissue and stage-specific expression in teleosts.

Cyp19a1a

The enzyme cyp19a1a is responsible for the formation of C18 steroids and is thus the most important enzyme with reference to hormonal control of sexual development in teleosts (Rashid et al. 2007; Rasheeda et al. 2010b; Mills et al. 2014). Though the importance of *cyp19a1a* in female sexual differentiation is known, regulation of expression of *cyp19a1a* within the developing gonads remains to be elucidated (Ijiri et al. 2008). In fish, as well as in other vertebrates, T acts as essential substrate for cyp19a1a to produce E_2 in granulosa cells (Tanaka et al. 1992; Nagahama et al. 1995; Senthilkumaran et al. 2004). Steroidogenic shift that occurs at the completion of vitellogenesis in female involves loss of stimulatory effects of FSH and Igfs on *cyp19a1a* expression and inhibition of *cyp19a1a* transcription by LH (Nakamura et al. 2016).

Promoter characteristics of *cyp19a1a* were described in few species (Kazeto et al. 2001; Tchoudakova et al. 2001; Valle et al. 2002). In the Nile tilapia and the Japanese medaka, an orphan nuclear receptor protein, fushi tarazu-factor I (FTZ-F1) named as Ad4BP/Sf-1, plays an important role in the transcriptional activation of *cyp19a1a* expression (Table 1; Fig. 1) and enzyme activity (Watanabe et al. 1999; Yoshiura et al. 2003). Forkhead family of transcription factor, forkhead box l2 (Foxl2), is shown to be involved in the transcriptional regulation of *cyp19a1a* expression as a co-regulator (Govoroun et al. 2004; Wang et al. 2007). Though the expression pattern and levels of their transcripts vary between the transcription factors, foxl2 and nuclear receptor subfamily 5 group A member 1 (nr5a1) are both thought to be involved in the regulation of steroidogenesis. These factors, FTZ-F1 and foxl2, are also involved in the transcriptional activation of *cyp19a1b* expression which were authenticated using ChIP and EMSA methodologies (Sridevi et al. 2012). Transcription of cyp19a1b is positively regulated by E_2 in zebrafish (Cheshenko et al. 2007; Diotel et al. 2010).

Cyp11b

Cyp11b is involved in the biosynthesis of 11-hydroxyandrostenedione (11-OHA) and 11-OHT, precursors for 11-KT production (Borg 1994; Lokman et al. 2002; Rajakumar and Senthilkumaran 2015). It is also involved in the production of corticosterone and cortisol in interrenal cells of kidney of several teleosts (Jiang et al. 1998). Various splice variants of *cyp11b* were reported in catfish showing its pivotal role in androgenesis (Rajakumar and Senthilkumaran 2015). Transcriptional regulation of *cyp11b* has not been studied in any teleost till date except for a preliminary study in catfish, *Clarias batrachus* (unpublished data), which revealed the regulation of *cyp11b1* expression by Ad4BP/Sf-1 (Table 1; Fig. 1).

Hsd11b

The enzyme hsd11b2 plays a key role in the synthesis of 11-oxygenated androgens and glucocorticoids (Borg 1994; Lokman et al. 2002; Jiang et al. 2003; Rasheeda et al. 2010a; Rajakumar and Senthilkumaran 2016). The other form, hsd11b1 (reductive), was not well characterized in teleosts.

Hsd11b2 is involved in the conversion of cortisol to cortisone as well as 11-oxygenated androgen synthesis pathway (Borg 1994; Lokman et al. 2002). Hence, it was suggested to protect the testicular tissue from circulating cortisol in addition to their role in 11-KT production (Kusakabe et al. 2003). A strong correlation exists between androgen and glucocorticoid pathways (Fernandino et al. 2012); however, the specific mechanism and regulation during different circumstances are

unclear. In the next section, the relationship of glucocorticoid with androgen metabolism was described in detail based on the available information in teleosts. In spawning fishes, 11-KT is produced and released in higher levels, which shows that testicular expression of the enzyme is responsible for the conversion of 11-OHT to 11-KT; hsd11b2 would increase specifically during the spawning season. Similarly, in catfish, higher levels of *hsd11b2* expression were evident during spawning followed by pre-spawning phase (Rajakumar and Senthilkumaran 2016). There is a correlation between *hsd11b2* and sox3 in catfish (Rajakumar and Senthilkumaran 2016). Detailed analyses using site-directed mutagenesis (SDM), luciferase assay, EMSA, and ChIP revealed that sox3 binds to *hsd11b2* gene promoter (Fig. 1; Table 1) and transactivates its transcription by binding to its specific promoter motifs in catfish (Rajakumar and Senthilkumaran 2016). Partly, *hsd11b2* is also moderately regulated by Wt-1 (Rajakumar and Senthilkumaran 2016; Murugananthkumar and Senthilkumaran 2016). Further, in a previous work, cortisol was shown to induce *hsd11b2* expression (Fernandino et al. 2012).

Hsd20b

Hsd20b is involved in the production of MIH, i.e., 17α,20β-DP, which has been explicitly shown in few teleosts using recombinant protein in combination with radiometric assays using ovarian tissues, implicating their pivotal role in final oocyte maturation (Senthilkumaran et al. 2002, 2004; Sreenivasulu et al. 2012a). Incidentally, the work has also been extended with gene promoter motif analysis in teleosts (Sreenivasulu et al. 2012a). On the other hand, based on the work on masu salmon and medaka, the production of MIH is different in relation to steroidogenic enzymes in other teleosts (Zhou et al. 2007a, b). Recent detailed study in masu salmon showed that hsd 17b12-like (not hsd20b) is responsible for MIH synthesis by granulosa cells during final oocyte maturation (Ijiri et al. 2017). The enzyme hsd20b2 (hsd17b12-like) was able to convert cortisone to 20β-hydroxycortisone in zebrafish (Tokarz et al. 2012). In view of these, the production of MIH seems to operate differently in teleosts with differential gene regulation mechanism. 17α,20β-DP is essential for the initiation of meiosis in the spermatocyte, milt production, and sperm mobility and acts as a pheromone in cyprinids (Miura et al. 1992; see review by Scott et al. 2010; Schulz et al. 2010; Sreenivasulu et al. 2012a; Rajakumar and Senthilkumaran 2013). Promoter motif regulation of *hsd20b* was explored in detail in both catfish and rainbow trout (Sreenivasulu et al. 2012b) wherein the importance of cAMP-responsive element binding protein (CREB) and Ad4BP/Sf-1 was shown explicitly (Table 1; Fig. 1). Incidentally, differential expression of CREBs was shown in tilapia and catfish gonads wherein their pivotal role in gonadal growth and maturation was demonstrated with special emphasis on final oocyte or meiotic maturation (Senthilkumaran et al. 2015). Taken together, hsd20b and CREB seem to be essential for MIH production in few teleosts.

Important aspects of steroidogenic enzymes are the existence of isoforms, and it is essential to probe this phenomenon to delineate specific function. The next section of the review will highlight these with reference to functional perspectives and genome duplication.

Isoforms and variants role in regulation of steroidogenesis

Teleost-specific genome duplication has occurred around 350 million years ago, which provides several different evolutionary processes and adaptive mechanisms in teleosts (Meyer and Schartl 1999; Meyer and Van De Peer 2005). After genome duplication, the duplicated gene copies had been attributed to different or modified functions in different species or no function at all (Meyer and Schartl 1999). Several isoforms or variants of steroidogenic enzyme genes are detected in several teleosts: *cyp11a1* (Parajes et al. 2013), *cyp17* (Zhou et al. 2007a, b), *hsd3b* (Senthilkumaran et al. 2009), and *cyp11b1* (Zhang et al. 2010; Rajakumar and Senthilkumaran 2015). Specific role for each isoform has been shown in some teleosts, yet the role of different variant forms was least understood for example *hsd3b* variants in tilapia (Senthilkumaran et al. 2009). The gene duplication in teleost fishes is different from the human and hence, several duplicated genes were reported in teleosts. This opens the possibility for diverse regulatory processes in teleosts like tissue-specific and/or developmental stage-specific effects.

Germ and Sertoli cell steroidogenesis Leydig cells of testes and thecal and granulosa cells of ovaries

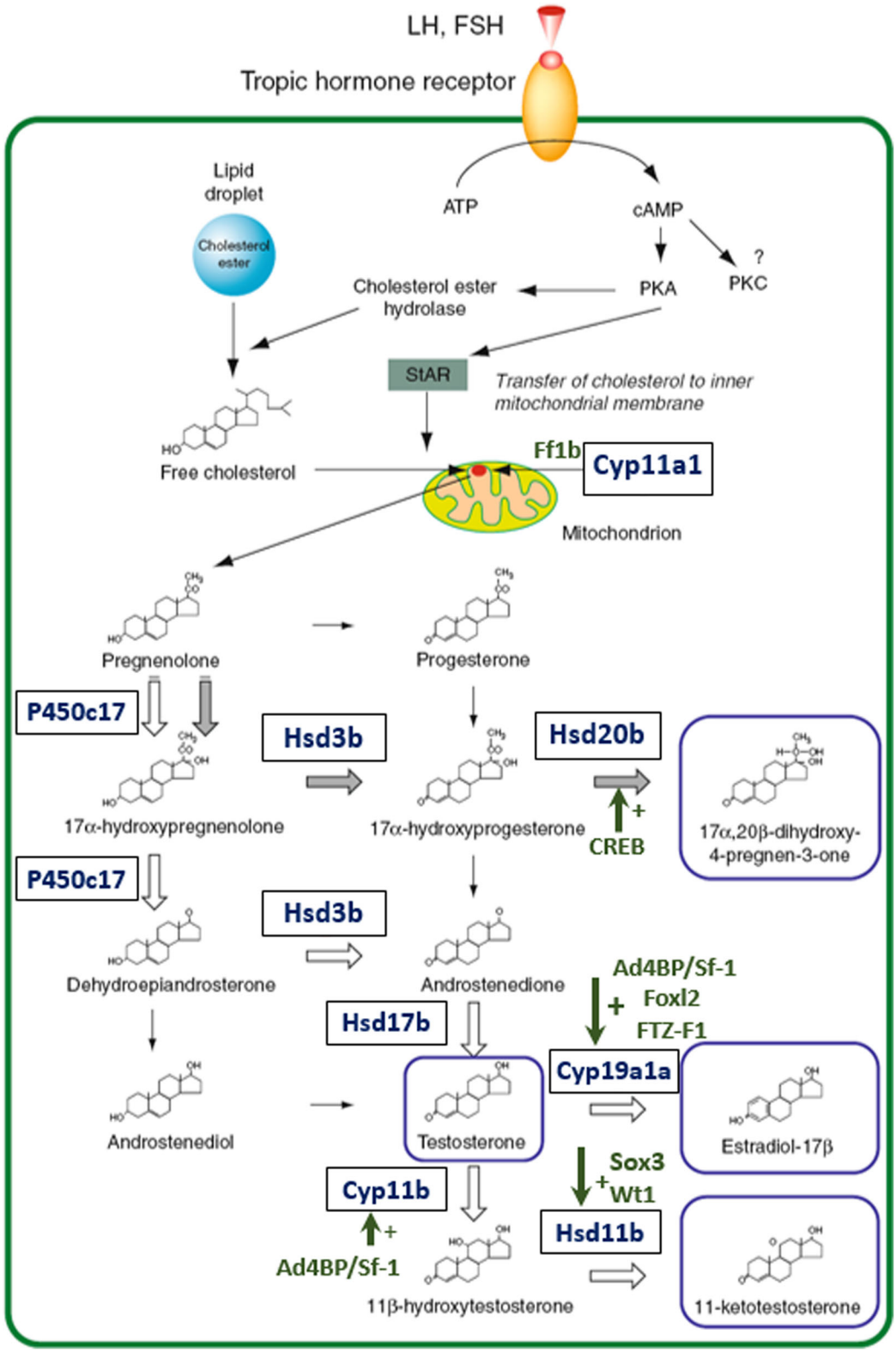

Fig. 1 A schematic pathway of steroidogenesis and regulation of steroidogenic enzyme genes with its transcription factors in the gonads of teleosts. + indicates stimulation; − indicates suppression. White arrows indicate the proposed androgen synthesis pathway. Gray arrows indicate progestogen synthesis pathway (Adapted from Young et al. 2004)

contribute largely for steroidogenesis. Androstenedione (A) and T are converted into E_1 and E_2, respectively, by the cyp19a1a localized in the granulosa cells. However, recent studies in several fish species have revealed the presence of different steroidogenic enzymes in the germ (Spermatogonia/Spermatocyte) and Sertoli cells of testis (Hinfray et al. 2013; Rajakumar and Senthilkumaran 2015). In chondrichthyes, Sertoli cells producing steroids were reported (Sourdaine and Garnier 1993; Prisco et al. 2008). In the spotted ray testis, hsd3b and hsd17b were localized in Sertoli and Leydig cells and these cells were indirectly involved in the hormonal control of spermatogenesis (Prisco et al. 2008). Likewise, expression of steroidogenic enzyme genes is well characterized in zebrafish and *cyp19a1a* mRNA was detected in presumptive granulosa cells surrounding oocytes (Wang and Orban 2007).

Glucocorticoids and androgens relationship Stress hormones can interfere with the reproductive signaling by accelerating, delaying, and/or inhibiting reproduction (Nematollahi et al. 2009; Schreck et al. 2001; Schreck 2010). Inversely, sex steroids can influence the stress response (Fuzzen et al. 2011) thereby maintaining reproductive cycle. Incidentally, the role of androgen and cortisol crosstalk involved in the male pathway is not clearly understood. In the case of pejerrey and the Japanese flounder, cortisol acts directly by downregulating *cyp19a1a* expression (Hattori et al. 2009; Yamaguchi et al. 2010). Further, in the Japanese flounder cortisol binds to glucocorticoid receptor, which acts as a transcription factor by binding to glucocorticoid responsive element in the upstream of *cyp19a1a* promoter and thereby suppresses its transcription (Yamaguchi et al. 2010). In the European sea bass, hyper-methylation of *cyp19a1a* promoter was reported, which leads to the downregulation of *cyp19a1a* gene expression (Navarro-Martin et al. 2011). Androgens are involved in testicular differentiation, which was shown by the treatment of androgen or androgenic analogues (Devlin and Nagahama 2002; Raghuveer and Senthilkumaran 2009). Involvement of 11-oxygenated androgens and the expression of steroidogenic enzyme genes were reported during the critical period of sex determination/differentiation in several teleosts (Liu et al. 2000; Blazquez et al. 2001; Hattori et al. 2009; Fernandino et al. 2012; Rajakumar and Senthilkumaran 2014a, 2015, 2016). The main enzymes involved in the pathways are cyp11b1 and hsd11b2. The enzyme cyp11b2 converts A and T to 11-OHA and 11-OHT, respectively (Borg 1994; Lokman et al. 2002). Interestingly, in pejerrey, it was reported that *hsd11b2* is regulated by cortisol thereby promoting the synthesis of 11-KT in temperature-dependent sex determination (Fernandino et al. 2012).

In fish, 11-OHA is considered as the principal steroid produced by the gonads whereas 11-KT is the predominant androgen (Borg 1994; Cavaco et al. 1997). Nonetheless, the expression of gene that codes for cyp11b showed an increase at later stages of morphological gonadal differentiation in males only (Socorro et al. 2007; Blasco et al. 2010; Raghuveer et al. 2011).

As already stated, hsd11b plays a key role in the synthesis of 11-oxygenated androgens and also known to be involved in the metabolism of glucocorticoids (Oppermann et al. 1997). Recently, it was proposed that hsd11b is the key enzyme involved in the warm temperature-induced masculinization in pejerrey and was also suggested that the enzymatic machinery necessary for the local production of 11-oxygenated steroids was already active in the undifferentiated gonads during this critical period (Fernandino et al. 2012). Enzymes hsd11b2 and hsd20b2 were suggested to consecutively metabolize cortisol to 20β-hydroxycortisone, which subsequently might be glucuronidated or sulfated and excreted from fish (Tokarz et al. 2012). Environmental stress-induced testicular differentiation was reviewed in depth with other intrinsic factors by Fernandino et al. (2013).

An elaborate view on steroidogenesis (steroidogenic enzyme genes expression and enzymatic activity) and the steroid hormone receptors, impact of duplicated genome on these processes, and influence of anthropogenic endocrine-disrupting compounds on steroid hormone were described in detail by Tokarz et al. (2015). An overview on the impact of pesticides on the reproduction, endocrine signaling and the resulting adverse physiological effects on teleost fishes was illustrated by Senthilkumaran (2015).

Approaches for studying regulation of gene expression at the promoter level

Function of every genome involves regulatory sequences, such as enhancers, co-regulators, and promoters. Instructions for when, where, and to what level each gene should

be expressed in an organism are encoded by these correlates. Transcription factors binding to its target promoter/enhancers is a tightly controlled process, which governs the connectivity of gene networks which coordinately regulates complex spatiotemporal and gender-specific gene expression with modulating effects from co-regulators (Geertz and Maerkl 2010; Rajakumar and Senthilkumaran 2016). In principle, experimental methods can be divided into either in vitro or in vivo approaches. In vitro methods like EMSA and in vivo methods like ChIP provide evidences for DNA-protein interaction. However, each method has their own advantages and limitations in studying different aspects of transcription factor binding to their target motifs which can be an enhancer or regulator or promoter. Combining selective methods for understanding research problem can provide robust results. ChIP being an in vivo technique provides more reliable data in native condition on DNA-protein interaction than EMSA (Yan et al. 2004; Gade and Kalvakolanu 2012), but specific bp region cannot be ascertained using basic ChIP. EMSA is a rapid and sensitive method which provides evidence for specific DNA binding site for transcription factors on the designated oligos to ascertain functional promoter motifs (Hellman and Fried 2007). The main drawback of EMSA is that it is an in vitro technique, wherein the samples are not in chemical equilibrium during EMSA gel run and also many complexes are significantly more stable in the gel than at free solution (Gade and Kalvakolanu 2012). Single bp resolution can be achieved using Chip-Seq and other related methodologies, which cannot be done in general using basic ChIP methodology. ChIP is a versatile technique wherein analysis of proteins interacting within a native chromatin environment can be ascertained, and it also provides unbiased observations into the chromatin changes occurring in response to extracellular signals and/or hormone stimuli (Yan et al. 2004; Gade and Kalvakolanu 2012) and hence it is a better method for hormone interaction studies. Moreover, ChIP has been used to determine the allele-specific transcription factor-binding patterns (Heckman and Boxer 2002). Therefore, it is very essential to use all the available methods to obtain more credible data on DNA-protein interaction with respect to the analysis on steroidogenic enzyme gene regulation.

Future prospects

There is still limited knowledge on the neuroendocrine mechanisms, dietary, and environmental conditions that control the regulation of steroidogenesis to modulate gonadal development and maturation in teleosts. Identification of new or novel factors in relation to gametogeneis as well as steroidogenesis broadens the cross talk and interaction. Hence, understanding these aspects through molecular level studies integrated with systemic approach might delineate the importance of their cohesive regulation on teleost reproduction.

Though steroidogenesis is relatively well understood in teleosts, species-specific variation together with functions of all the reported isoforms/variants of genes is poorly deciphered. Further studies are needed to understand the role of miRNA interaction and epigenetic regulation of essential genes related to steroidogenesis. In addition, identification of new genes from evolutionarily/mechanistically diverse teleost species might provide novel leads on their functional relevance and overall survival of the organism. These understandings together with information on regulation of steroidogenic enzyme genes are crucial to unravel the evolutionary significance of several reproductive modes such as gonochorism, protandry, protogyny, true hermaphroditism, gynogenesis, androgenesis, and basic biology of this group of organisms. Current and the future research should address the lacunae in the information on altered endocrine conditions either naturally through genetic means or through anthropogenic sources as in the case of endocrine disruption for the sustainable environment leading to better aquaculture practice and seed production.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

References

Adamski J, Jakob FJ (2001) A guide to 17β-hydroxysteroid dehydrogenases. Mol Cell Endocrinol 171:1–4. https://doi.org/10.1016/S0303-7207(00)00383-X

Baroiller JF, Guiguen Y, Fostier A (1999) Endocrine and environmental aspects of sex differentiation in fish. Cell Mol Life Sci 55:910–931. https://doi.org/10.1007/s000180050344

Baroiller J, D'Cotta H, Bezault E, Wessels S, Hoerstgen-Schwark G (2009) Tilapia sex determination: where temperature and genetics meet. Comp Biochem Physiol A 153:30–38. https://doi.org/10.1016/j.cbpa.2008.11.018

Blasco M, Fernandino JI, Guilgur LG, Strussmann CA, Somoza GM, Vizziano-Cantonnet D (2010) Molecular characterization of cyp11a1 and cyp11b1 and their gene expression profile in pejerrey (*Odontesthes bonariensis*) during early gonadal development. Comp Biochem Physiol A 156:110–118. https://doi.org/10.1016/j.cbpa.2010.01.006

Blazquez M, Somoza GM (2010) Fish with thermolabile sex determination (TSD) as models to study brain sex differentiation. Gen Comp Endocrinol 166:470–477. https://doi.org/10.1016/j.ygcen.2009.10.004

Blazquez M, Felip A, Zanuy S, Carrillo M, Piferrer F (2001) Critical period of androgen-inducible sex differentiation in a teleost fish, the European sea bass. J Fish Biol 58:342–358. https://doi.org/10.1111/j.1095-8649.2001.tb02257.x

Bogerd J, Blomenrohr M, Andersson E, van der Putten HH, Tensen CP, Vischer HF, Granneman JC, Janssen-Dommerholt C, Goos HJ, Schulz RW (2001) Discrepancy between molecular structure and ligand selectivity of a testicular follicle-stimulating hormone receptor of the African catfish (*Clarias gariepinus*). Biol Reprod 64:1633–1643. https://doi.org/10.1095/biolreprod64.6.1633

Borg B (1994) Androgens in teleost fishes. Comp Biochem Physiol C 109:219–245. https://doi.org/10.1016/0742-8413(94)00063-G

Cavaco JEB, Vischer HF, Lambert JGD, Goos HJT, Schulz RW (1997) Mismatch between patterns of circulating and testicular androgens in African catfish, *Clarias gariepinus*. Fish Physiol Biochem 17:155–162. https://doi.org/10.1023/A:1007716514816

Cheshenko K, Brion F, Le Page Y, Hinfray N, Pakdel F, Kah O, Segner H, Eggen R (2007) Expression of zebra fish aromatase cyp19a and cyp19b genes in response to the ligands of estrogen receptor and aryl hydrocarbon receptor. Toxicol Sci 96:255–267. https://doi.org/10.1093/toxsci/kfm003

Choi J, Smitz J (2014) Luteinizing hormone and human chorionic gonadotropin: origins of difference. Mol Cell Endocrinol 383:203–213. https://doi.org/10.1016/j.mce.2013.12.009

Chu L, Li J, Liu Y, Cheng CH (2015) Gonadotropin signaling in zebrafish ovary and testis development: insights from gene knockout study. Mol Endocrinol 29:1743–1758. https://doi.org/10.1210/me.2015-1126

Devlin RH, Nagahama Y (2002) Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. Aquaculture 208:191–364. https://doi.org/10.1016/S0044-8486(02)00057-1

Diotel N, Page YL, Mouriec K, Tong S-K, Pellegrini E, Vaillant C, Anglade I, Brion F, Pakdel F, Chung BC et al (2010) Aromatase in the brain of teleost fish: expression, regulation and putative functions. F Neuroendocrinol 31:172–192. https://doi.org/10.1016/j.yfrne.2010.01.003

Fernandino JI, Hattori RS, Kishii A, Strussmann CA, Somoza GM (2012) The cortisol and androgen pathways cross talk in high temperature-induced masculinization: the 11β-hydroxysteroid dehydrogenase as a key enzyme. Endocrinology 153:6003–6011. https://doi.org/10.1210/en.2012-1517

Fernandino JI, Hattori RS, Moreno Acosta OD, Struessmann CA, Somoza GM (2013) Environmental stress-induced testis differentiation: androgen as a by-product of cortisol inactivation. Gen Comp Endocrinol 192:36–44. https://doi.org/10.1016/j.ygcen.2013.05.024

Francis RC (1992) Sexual lability in teleosts: developmental factors. Q Rev Biol 67:1–18. https://doi.org/10.1086/417445

Fuzzen MLM, Bernier NJ, Van Der Kraak G (2011) Differential effects of 17β-estradiol and 11-ketotestosterone on the endocrine stress response in zebrafish (*Danio rerio*). Gen Comp Endocrinol 170:365–373. https://doi.org/10.1016/j.ygcen.2010.10.014

Gade P, Kalvakolanu DV (2012) Chromatin immunoprecipitation assay as a tool for analyzing transcription factor activity. Methods Mol Biol 809:85–104. https://doi.org/10.1007/978-1-61779-376-9_6

Ge W (2005) Intrafollicular paracrine communication in the zebrafish ovary: the state of the art of an emerging model for the study of vertebrate folliculogenesis. Mol Cell Endocrinol 237:1–10. https://doi.org/10.1016/j.mce.2005.03.012

Geertz M, Maerkl SJ (2010) Experimental strategies for studying transcription factor-DNA binding specificities. Brief Funct Genomics 9:362–373. https://doi.org/10.1093/bfgp/elq023

Goos HJT, Senthilkumaran B, Joy KP (1999) Neuroendocrine integrative mechanisms in the control of gonadotropin secretion in teleosts. In: Joy KP, Krishna A, Haldar C (eds) Comparative endocrinology and reproduction. Narousa Publishing House, New Delhi, pp 113–136

Govoroun MS, Pannetier M, Pailhoux E, Cocquet J, Brillard JP, Couty I, Batellier F, Cotinot C (2004) Isolation of chicken homolog of the FOXL2 gene and comparison of its expression patterns with those of aromatase during ovarian development. Dev Dyn 231:859–870. https://doi.org/10.1002/dvdy.20189

Guiguen Y (2000) Implication of steroids in fish gonadal sex differentiation and sex inversion. Curr Top Steroid Res 3:127–143

Guiguen Y, Fostier A, Piferrer F, Chang CF (2010) Ovarian aromatase and estrogens: a pivotal role for gonadal sex differentiation and sex change in fish. Gen Comp Endocrinol 165:352–366. https://doi.org/10.1016/j.ygcen.2009.03.002

Handa RJ, Pak TR, Kudwa AE, Lund TD, Hinds L (2008) An alternate pathway for androgen regulation of brain function: activation of estrogen receptor β by the metabolite of dihydrotestosterone, 5α-androstane-3β,17β-diol. Horm Behav 53:741–752. https://doi.org/10.1016/j.yhbeh.2007.09.012

Hattori RS, Fernandino JI, Kishii A, Kimura H, Kinno T, Oura M, Somoza GM, Yokota M, Strussmann CA, Watanabe S (2009) Cortisol induced masculinization: does thermal stress affect gonadal fate in pejerrey, a teleost fish with temperature-dependent sex determination? PLoS One 4:e6548. https://doi.org/10.1371/journal.pone.0006548

Heckman CA, Boxer LM (2002) Allele-specific analysis of transcription factors binding to promoter regions. Methods 26: 19–26. https://doi.org/10.1016/S1046-2023(02)00004-X

Hellman LM, Fried MG (2007) Electrophoretic mobility shift assay (EMSA) for detecting protein-nucleic acid interactions. Nat Protoc 2:1849–1861. https://doi.org/10.1038/nprot.2007.249

Hinfray N, Nobrega RH, Caulier M, Baudiffier D, Maillot-Marechal E, Chadili E, Palluel O, Porcher JM, Schulz R, Brion F (2013) Cyp17a1 and Cyp19a1 in the zebrafish testis are differentially affected by oestradiol. J Endocrinol 216: 375–388. https://doi.org/10.1530/JOE-12-0509

Hsu HJ, Hsiao P, Kuo MW, Chung BC (2002) Expression of zebrafish cyp11a1 as a maternal transcript and in yolk syncytial layer. Gene Expr Patterns 2:219–222. https://doi.org/10.1016/S1567-133X(02)00059-5

Hu MC, Hsu HJ, Guo IC, Chung BC (2004) Function of Cyp11a1 in animal models. Mol Cell Endocrinol 215:95–100. https://doi.org/10.1016/j.mce.2003.11.024

Huang M, Chen J, Liu Y, Chen H, Yu Z, Ye Z, Peng C, Xiao L, Zhao M, Li S, Lin H, Zhang Y (2019) New insights into the role of follicle-stimulating hormone in sex differentiation of the protogynous orange-spotted grouper, *Epinephelus coioides*. Front Endocrinol (Lausanne) 10:304. https://doi.org/10.3389/fendo.2019.00304

Ijiri S, Kaneko H, Kobayashi T, Wang DS, Sakai F, Paul-Prasanth B, Nakamura M, Nagahama Y (2008) Sexual dimorphic expression of genes in gonads during early differentiation of a teleost fish, the Nile tilapia *Oreochromis niloticus*. Biol Reprod 78:333–341. https://doi.org/10.1095/biolreprod.107.064246

Ijiri S, Shibata Y, Takezawa N, Kazeto Y, Takatsuka N, Kato E, Hagihara S, Ozaki Y, Adachi S, Yamauchi K, Nagahama Y (2017) 17β-HSD type 12-like is responsible for maturation-inducing hormone synthesis during oocyte maturation in masu salmon. Endocrinology 158:627–639. https://doi.org/10.1210/en.2016-1349

Jiang JQ, Kobayashi T, Ge W, Kobayashi H, Tanaka M, Okamoto M, Nonaka Y, Nagahama Y (1996) Fish testicular 11β-hydroxylase: cDNA cloning and mRNA expression during spermatogenesis. FEBS Lett 397:250–252. https://doi.org/10.1016/S0014-5793(96)01187-8

Jiang JQ, Young G, Kobayashi T, Nagahama Y (1998) Eel (*Anguilla japonica*) testis 11β-hydroxylase gene is expressed in interrenal tissue and its product lacks aldosterone synthesizing activity. Mol Cell Endocrinol 146:207–211. https://doi.org/10.1016/S0303-7207(98)00147-6

Jiang JQ, Wang DS, Senthilkumaran B, Kobayashi T, Kobayashi HK, Yamaguchi A, Ge W, Young G, Nagahama Y (2003) Isolation, characterization and expression of 11β-hydroxsteroid dehydrogenase type 2 cDNAs from the testes of Japanese eel (*Anguilla japonica*) and Nile tilapia (*Oreochromis niloticus*). J Mol Endocrinol 31:305–315. https://doi.org/10.1677/jme.0.0310305

Kagawa H, Kasuga Y, Adachi J, Nishi A, Hashimoto H, Imaizumi H, Kaji S (2009) Effects of continuous administration of human chorionic gonadotropin, salmon pituitary extract, and gonadotropin-releasing hormone using osmotic pumps on induction of sexual maturation in male Japanese eel, *Anguilla japonica*. Aquaculture 296:117–122. https://doi.org/10.1016/j.aquaculture.2009.07.023

Kazeto Y, Ijiri S, Todo T, Adachi S, Yamauchi K (2000) Molecular cloning and characterization of Japanese eel ovarian P450c17 (CYP17) cDNA. Gen Comp Endocrinol 118:123–133. https://doi.org/10.1006/gcen.1999.7449

Kazeto Y, Ijiri S, Place AR, Zohar Y, Trant JM (2001) The 5′-flanking regions of CYP19A1 and CYP19A2 in zebrafish. Biochem Biophys Res Commun 288:503–508. https://doi.org/10.1006/bbrc.2001.5796

Kazeto Y, Ijiri S, Adachi S, Yamauchi K (2006) Cloning and characterization of a cDNA encoding cholesterol side-chain cleavage cytochrome P450 (CYP11A1): tissue distribution and changes in the transcript abundance in ovarian tissue of Japanese eel, *Anguilla japonica*, during artificially induced sexual development. J Steroid Biochem Mol Biol 99:121–128. https://doi.org/10.1016/j.jsbmb.2005.12.004

Kobayashi T, Kajiura-Kobayashi H, Nagahama Y (2003) Induction of XY sex reversal by estrogen involves altered gene expression in a teleost, tilapia. Cytogenet Genome Res 101:289–294. https://doi.org/10.1159/000074351

Kobayashi Y, Murata R, Nakamura M (2013) Physiological and endocrinological mechanisms of sex change in the grouper. In: Senthilkumaran B (ed) Sexual plasticity and gametogenesis in fishes. Nova Biomedical, Waltham, pp 221–234

Kusakabe M, Nakamura I, Young G (2003) 11β-hydroxysteroid dehydrogenase complementary deoxyribonucleic acid in rainbow trout: cloning, sites of expression, and seasonal changes in gonads. Endocrinology 144:2534–2545. https://doi.org/10.1210/en.2002-220446

Lau ES, Zhang Z, Qin M, Ge W (2016) Knockout of zebrafish ovarian aromatase gene (*cyp19a1a*) by TALEN and CRISPR/Cas9 leads to all-male offspring due to failed ovarian differentiation. Sci Rep 6:37357. https://doi.org/10.1038/srep37357

Levavi-Sivan B, Bogerd J, Mananos EL, Gomez A, Lareyre JJ (2009) Perspectives on fish gonadotropins and their receptors. Gen Comp Endocrinol 165:412–437. https://doi.org/10.1016/j.ygcen.2009.07.019

Liu S, Govoroun M, D'Cotta H, Ricordel MJ, Lareyre JJ, McMeel OM, Smith T, Nagahama Y, Guiguen Y (2000) Expression of cytochrome P450 (11β) (11β-hydroxylase) gene during

gonadal sex differentiation and spermatogenesis in rainbow trout, *Oncorhynchus mykiss*. J Steroid Biochem Mol Biol 75: 291–298. https://doi.org/10.1016/S0960-0760(00)00186-2

Lokman PM, Harris B, Kusakabe M, Kime DE, Schulz RW, Adachi S, Young G (2002) 11-oxygenated androgens in female teleosts: prevalence, abundance, and life history implications. Gen Comp Endocrinol 129:1–12. https://doi.org/10.1016/S0016-6480(02)00562-2

Margiotta-Casaluci L, Sumpter JP (2011) 5α-Dihydrotestosterone is a potent androgen in the fathead minnow (*Pimephales promelas*). Gen Comp Endocrinol 171:309–318. https://doi.org/10.1016/j.ygcen.2011.02.012

Margiotta-Casaluci L, Courant F, Antignac JP, Le Bizec B, Sumpter JP (2013) Identification and quantification of 5α-dihydrotestosterone in the teleost fathead minnow (*Pimephales promelas*) by gas chromatography-tandem mass spectrometry. Gen Comp Endocrinol 191:202–209. https://doi.org/10.1016/j.ygcen.2013.06.017

Mayer I, Borg B, Schulz R (1990) Seasonal changes in and effect of castration/androgen replacement on the plasma levels of five androgens in the male three spined stickleback, *Gasterosteus aculeatus* L. Gen Comp Endocrinol 79:23–30. https://doi.org/10.1016/0016-6480(90)90084-Y

Meyer A, Schartl M (1999) Gene and genome duplications in vertebrates: the one-to-four (-to eight in fish) rule and the evolution of novel gene functions. Curr Opin Cell Biol 11: 699–704. https://doi.org/10.1016/S0955-0674(99)00039-3

Meyer A, Van De Peer Y (2005) From 2R to 3R: evidence for a fish-specific genome duplication (FSGD). BioEssays 27: 937–945. https://doi.org/10.1002/bies.20293

Mills LJ, Gutjahr-Gobell RE, Zaroogian GE, Horowitz DB, Laws SC (2014) Modulation of aromatase activity as a mode of action for endocrine disrupting chemicals in a marine fish. Aquat Toxicol 147:140–150. https://doi.org/10.1016/j.aquatox.2013.12.023

Mindnich R, Moller G, Adamski J (2004) The role of 17β-hydroxysteroid dehydrogenase. Mol Cell Endocrinol 218: 7–20. https://doi.org/10.1016/j.mce.2003.12.006

Miura T, Yamauchi K, Takahashi H, Nagahama Y (1992) The role of hormones in the acquisition of sperm motility in salmonid fish. J Exp Zool 261:359–363. https://doi.org/10.1002/jez.1402610316

Moeller G, Adamski J (2009) Integrated view on 17β-hydroxysteroid dehydrogenases. Mol Cell Endocrinol 301: 7–19. https://doi.org/10.1016/j.mce.2008.10.040

Mommsen TP, Vijayan MM, Moon TW (1999) Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Rev Fish Biol Fish 9:211–268. https://doi.org/10.1023/A:1008924418720

Murugananthkumar R, Senthilkumaran B (2016) Expression analysis and localization of wt1, ad4bp/sf-1 and gata4 in the testis of catfish, *Clarias batrachus*: impact of wt1-esiRNA silencing. Mol Cell Endocrinol 431:164–176. https://doi.org/10.1016/j.mce.2016.05.006

Murugananthkumar R, Prathibha Y, Senthilkumaran B, Rajakumar A, Kagawa H (2017) *In vivo* induction of human chorionic gonadotropin by osmotic pump advances sexual maturation during pre-spawning phase in adult catfish. Gen Comp Endocrinol 251:74–84. https://doi.org/10.1016/j.ygcen.2016.09.015

Nagahama Y (1994) Endocrine regulation of gametogenesis in fish. Int J Dev Biol 38:217–229

Nagahama Y, Yoshikuni M, Yamashita M, Tukumono T, Katsu Y (1995) Regulation of oocyte growth and maturation in fish. Curr Top Dev Biol 30:103–145. https://doi.org/10.1016/S0070-2153(08)60565-7

Nakamura S, Kobayashi K, Nishimura T, Higashijima S, Tanaka M (2010) Identification of germline stem cells in the ovary of the teleost medaka. Science 328:1561–1563. https://doi.org/10.1126/science.1185473

Nakamura I, Kusakabe M, Swanson P, Young G (2016) Regulation of sex steroid production and mRNAs encoding gonadotropin receptors and steroidogenic proteins by gonadotropins, cyclic AMP and insulin-like growth factor-I in ovarian follicles of rainbow trout (*Oncorhynchus mykiss*) at two stages of vitellogenesis. Comp Biochem Physiol A Mol Integr Physiol 201:132–140. https://doi.org/10.1016/j.cbpa.2016.06.035

Navarro-Martin L, Vinas J, Ribas L, Diaz N, Gutierrez A, Di Croce L, Piferrer F (2011) DNA methylation of the gonadal aromatase (cyp19a) promoter is involved in temperature-dependent sex ratio shifts in the European sea bass. PLoS Genet 7:1–15. https://doi.org/10.1371/journal.pgen.1002447

Nematollahi MA, van Pelt-Heerschap H, Komen J (2009) Transcript levels of five enzymes involved in cortisol synthesis and regulation during the stress response in common carp: relationship with cortisol. Gen Comp Endocrinol 164:85–90. https://doi.org/10.1016/j.ygcen.2009.05.006

Oppermann UC, Persson B, Filling C, Jornvall H (1997) Structure/function relationships of SDR hydroxysteroid dehydrogenases. Adv Exp Med Biol 414:403–415. https://doi.org/10.1007/978-1-4615-4735-8_48

Ozaki Y, Higuchi M, Miura C, Yamaguchi S, Tozawa Y, Miura T (2006) Roles of 11β-hydroxysteroid dehydrogenase in fish spermatogenesis. Endocrinology 147:5139–5146. https://doi.org/10.1210/en.2006-0391

Parajes S, Griffin A, Taylor AE, Rose IT, Miguel-Escalada I, Hadzhiev Y, Arlt W, Shackleton C, Müller F, Krone N (2013) Redefining the initiation and maintenance of zebrafish interrenal steroidogenesis by characterizing the key enzyme Cyp11a2. Endocrinology 154:2702–2711. https://doi.org/10.1210/en.2013-1145

Payne AH, Youngblood GL (1995) Regulation of expression of steroidogenic enzymes in Leydig cells. Biol Reprod 52:217–225. https://doi.org/10.1095/biolreprod52.2.217

Peter RE, Trudeau VL, Sloley BD (1991) Brain regulation of reproduction in teleosts. Bull Inst Zool Acad Sin Monogr 16:89–118

Prisco M, Liguoro A, Ricchiari L, Del Giudice G, Angelini F, Andreuccetti P (2008) Immunolocalization of 3β-hsd and 17β-hsd in the testis of the spotted ray *Torpedo marmorata*.

Gen Comp Endocrinol 155:157–163. https://doi.org/10.1016/j.ygcen.2007.04.016

Quek SI, Chan WK (2009) Transcriptional activation of zebrafish cyp11a1 promoter is dependent on the nuclear receptor Ff1b. J Mol Endocrinol 43:121–130. https://doi.org/10.1677/JME-09-0029

Raghuveer K, Senthilkumaran B (2009) Identification of multiple dmrt1s in catfish: localization, dimorphic expression pattern, changes during testicular cycle and after methyltestosterone treatment. J Mol Endocrinol 42:437–448. https://doi.org/10.1677/JME-09-0011

Raghuveer K, Senthilkumaran B, Sudhakumari CC, Sridevi P, Rajakumar A, Singh R, Muruganantkumar R, Majumdar KC (2011) Dimorphic expression of various transcription factor and steroidogenic enzyme genes during gonadal ontogeny in the air-breathing catfish, *Clarias gariepinus*. Sex Dev 5:213–223. https://doi.org/10.1159/000328823

Rajakumar A, Senthilkumaran B (2013) Sperm maturation in teleosts: role of androgens and progestins in our present understanding to emerging new concepts. In: Senthilkumaran B (ed) Sexual plasticity and gametogenesis in fishes. Nova Biomedical, Waltham, pp 377–400

Rajakumar A, Senthilkumaran B (2014a) Expression analysis of cyp11a1 during gonadal development, recrudescence and after hCG induction and sex steroid analog treatment in the catfish, *Clarias batrachus*. Comp Biochem Physiol B 176:42–47. https://doi.org/10.1016/j.cbpb.2014.07.007

Rajakumar A, Senthilkumaran B (2014b) Molecular cloning and expression analysis of 17β-hydroxysteroid dehydrogenase 1 and 12 during gonadal development, recrudescence and after *in vivo* hCG induction in catfish, *Clarias batrachus*. Steroids 92:81–89. https://doi.org/10.1016/j.steroids.2014.09.009

Rajakumar A, Senthilkumaran B (2015) Dynamic expression of 11β-hydroxylase during testicular development, recrudescence and after *in vivo* and *in vitro* hCG induction in the catfish, *Clarias batrachus*. Gen Comp Endocrinol 211:69–80. https://doi.org/10.1016/j.ygcen.2014.11.010

Rajakumar A, Senthilkumaran B (2016) Sox3 binds to 11β-hydroxysteroid dehydrogenase gene promoter suggesting transcriptional interaction in catfish. J Steroid Biochem Mol Biol 158:90–103. https://doi.org/10.1016/j.jsbmb.2016.01.003

Rasheeda MK, Kagawa H, Kirubagaran R, Dutta-Gupta A, Senthilkumaran B (2010a) Cloning, expression and enzyme activity analysis of testicular 11β-hydroxysteroid dehydrogenase during seasonal cycle and after hCG induction in air-breathing catfish *Clarias gariepinus*. J Steroid Biochem Mol Biol 120:1–10. https://doi.org/10.1016/j.jsbmb.2010.02.014

Rasheeda MK, Sridevi P, Senthilkumaran B (2010b) Cytochrome P450 aromatases: impact on gonadal development, recrudescence and effect of hCG in the catfish, *Clarias gariepinus*. Gen Comp Endocrinol 167:234–245. https://doi.org/10.1016/j.ygcen.2010.03.009

Rashid H, Kitano H, Lee KH, Nii S, Shigematsu T, Kadomura K, Yamaguchi A, Matsuyama M (2007) Fugu (*Takifugu rubripes*) sexual differentiation: CYP19 regulation and aromatase inhibitor induced testicular development. Sex Dev 1:311–322. https://doi.org/10.1159/000108935

Rasmussen MK, Ekstrand B, Zamaratskaia G (2013) Regulation of 3β-hydroxysteroid dehydrogenase δ5-δ4 isomerase: a review. Int J Mol Sci 14:17926–17942. https://doi.org/10.3390/ijms140917926

Rege J, Garber S, Conley AJ, Elsey RM, Turcu AF, Auchus RJ, Rainey WE (2019) Circulating 11-oxygenated androgens across species. J Steroid Biochem Mol Biol 190:242–249. https://doi.org/10.1016/j.jsbmb.2019.04.005

Schreck CB (2010) Stress and fish reproduction: the roles of allostasis and hormesis. Gen Comp Endocrinol 165:549–556. https://doi.org/10.1016/j.ygcen.2009.07.004

Schreck CB, Contreras-Sanchez W, Fitzpatrick MS (2001) Effects of stress on fish reproduction, gamete quality, and progeny. Aquaculture 197:3–24. https://doi.org/10.1016/S0044-8486(01)00580-4

Schulz RW, França LR, Lareyre JJ, LeGac F, Chiarini-Garcia H, Nobrega RH, Miura T (2010) Spermatogenesis in fish. Gen Comp Endocrinol 165:390–411. https://doi.org/10.1016/j.ygcen.2009.02.013

Scott AP, Sumpter JP, Stacey N (2010) The role of the maturation-inducing steroid, 17,20β-dihydroxypregn-4-en-3- one, in male fishes: a review. J Fish Biol 76:183–224. https://doi.org/10.1111/j.1095-8649.2009.02483.x

Senthilkumaran B (2011) Recent advances in meiotic maturation and ovulation: comparing mammals and pisces. Front Biosci 16:1898–1914. https://doi.org/10.2741/3829

Senthilkumaran B (2015) Pesticide- and sex steroid analogue-induced endocrine disruption differentially targets hypothalamo–hypophyseal–gonadal system during gametogenesis in teleosts – A review. General and Comparative Endocrinology 219:136–142. https://doi.org/10.1016/j.ygcen.2015.01.010

Senthilkumaran B, Joy KP (1996) Effects of administration of some monoamine synthesis blockers and precursors on ovariectomy-induced rise in plasma gonadotropin II in the catfish *Heteropneustes fossilis*. Gen Comp Endocrinol 101:220–226. https://doi.org/10.1006/gcen.1996.0024

Senthilkumaran B, Sudhakumari CC, Chang XT, Kobayashi T, Oba Y, Guan G, Yoshiura Y, Yoshikuni M, Nagahama Y (2002) Ovarian carbonyl reductase-like 20β-hydroxysteroid dehydrogenase shows distinct surge in messenger RNA expression during natural and gonadotropin-induced meiotic maturation in Nile tilapia. Biol Reprod 67:1080–1086. https://doi.org/10.1095/biolreprod67.4.1080

Senthilkumaran B, Yoshikuni M, Nagahama Y (2004) A shift in steroidogenesis occurring in ovarian follicles prior to oocyte maturation. Mol Cell Endocrinol 215:11–18. https://doi.org/10.1016/j.mce.2003.11.012

Senthilkumaran B, Sudhakumari CC, Wang DS, Sreenivasulu G, Kobayashi T, Kobayashi HK, Yoshikuni M, Nagahama Y (2009) Novel 3β-hydroxysteroid dehydrogenases from gonads of the Nile tilapia: phylogenetic significance and expression during reproductive cycle. Mol Cell Endocrinol 299:146–152. https://doi.org/10.1016/j.mce.2008.11.008

Senthilkumaran B, Sreenivasulu G, Wang DS, Sudhakumari CC, Kobayashi T, Nagahama Y (2015) Expression patterns of CREBs in oocyte growth and maturation of fish. PLoS One 10:e0145182. https://doi.org/10.1371/journal.pone.0145182

Socorro S, Martins RS, Deloffre L, Mylonas CC, Canario AV (2007) A cDNA for European sea bass (*Dicentrachus labrax*) 11β-hydroxylase: gene expression during the thermosensitive period and gonadogenesis. Gen Comp Endocrinol 150:164–173. https://doi.org/10.1016/j.ygcen.2006.07.018

Sourdaine P, Garnier DH (1993) Stage-dependent modulation of Sertoli cell steroid production in dogfish (*Scyliorhinus canicula*). J Reprod Fertil 97:133–142. https://doi.org/10.1530/jrf.0.0970133

Sreenivasulu G, Senthilkumaran B (2009) A role for cytochrome P450 17alpha-hydroxylase and c17-20lyase during shift in steroidogenesis occurring in ovarian follicles prior to oocyte maturation. J Steroid Biochem Mol Biol 115:77–85. https://doi.org/10.1016/j.jsbmb.2009.03.004

Sreenivasulu G, Senthilkumaran B, Sridevi P, Rajakumar A, Rasheeda MK (2012a) Expression and immunolocalization of 20β-hydroxysteroid dehydrogenase during testicular cycle and after hCG induction, *in vivo* in the catfish, *Clarias gariepinus*. Gen Comp Endocrinol 175:48–54. https://doi.org/10.1016/j.ygcen.2011.09.002

Sreenivasulu G, Senthilkumaran B, Sudhakumari CC, Guan G, Oba Y, Kagawa H, Nagahama Y (2012b) 20β-Hydroxysteroid dehydrogenase gene promoter: potential role for cyclic AMP and xenobiotic responsive elements. Gene 509:68–76. https://doi.org/10.1016/j.gene.2012.07.017

Sridevi P, Chaitanya RK, Dutta-Gupta A, Senthilkumaran B (2012) FTZ-F1 and FOXL2 up-regulate catfish brain aromatase gene by specific binding to promoter motifs. Biochim Biophys Acta 1819:57–66. https://doi.org/10.1016/j.bbagrm.2011.10.003

Stocco DM (2000) The role of the StAR protein in steroidogenesis: challenges for the future. J Endocrinol 164:247–253. https://doi.org/10.1677/joe.0.1640247

Su T, Ijiri S, Kanbara H, Hagihara S, Wang DS, Adachi S (2015) Characterization and expression of cDNAs encoding P450c17-II (cyp17a2) in Japanese eel during induced ovarian development. Gen Comp Endocrinol 221:134–143. https://doi.org/10.1016/j.cbpa.2016.03.012

Sudhakumari CC, Senthilkumaran B (2013) Expression profiling of various marker genes involved in gonadal differentiation of teleosts: molecular understanding of sexual plasticity. In: Senthilkumaran B (ed) Sexual plasticity and gametogenesis in fishes. Nova Biomedical, Waltham, pp 401–421

Suzuki H, Kazeto Y, Gen K, Ozaki Y (2019) Functional analysis of recombinant single-chain Japanese eel Fsh and Lh produced in FreeStyle 293-F cell lines: binding specificities to their receptors and differential efficacy on testicular steroidogenesis. Gen Comp Endocrinol 285:113241. https://doi.org/10.1016/j.ygcen.2019.113241

Swart AC, Schloms L, Storbeck KH, Bloem LM, Toit TD, Quanson JL, Rainey WE, Swart P (2013) 11β-Hydroxyandrostenedione, the product of androstenedione metabolism in the adrenal, is metabolized in LNCaP cells by 5α-reductase yielding 11β-hydroxy-5α-androstanedione. J Steroid Biochem Mol Biol 138:132–142. https://doi.org/10.1016/j.jsbmb.2013.04.010

Tanaka SS, Nishinakamura R (2014) Regulation of male sex determination: genital ridge formation and Sry activation in mice. Cell Mol Life Sci 71:4781–4802. https://doi.org/10.1007/s00018-014-1703-3

Tanaka M, Telecky TM, Fukada S, Adachi S, Chen S, Nagahama Y (1992) Cloning and sequence analysis of the cDNA encoding P-450 aromatase (P450arom) from a rainbow trout (*Oncorhynchus mykiss*) ovary: relationship between the amount of P450arom mRNA and the production of oestradiol-17β in the ovary. J Mol Endocrinol 8:53–61. https://doi.org/10.1677/jme.0.0080053

Tchoudakova A, Kishida M, Wood E, Callard GV (2001) Promoter characteristics of two cyp19 genes differentially expressed in the brain and ovary of teleost fish. J Steroid Biochem Mol Biol 78:427–439. https://doi.org/10.1016/S0960-0760(01)00120-0

Tokarz J, Mindnich R, Norton W, Möller G, Hrabé de Angelis M, Adamski J (2012) Discovery of a novel enzyme mediating glucocorticoid catabolismin fish: 20β-hydroxysteroid dehydrogenase type 2. Mol Cell Endocrinol 349:202–213. https://doi.org/10.1016/j.mce.2011.10.022

Tokarz J, Möller G, Hrabě de Angelis M, Adamski J (2015) Steroids in teleost fishes: a functional point of view. Steroids 103:123–144. https://doi.org/10.1016/j.steroids.2015.06.011

Trant JM, Thomas P (1989) Isolation of a novel maturation-inducing steroid produced *in vitro* by ovaries of Atlantic croaker. Gen Comp Endocrinol 75:397–404. https://doi.org/10.1016/0016-6480(89)90174-3

Valle LD, Lunardi L, Belvedere CP (2002) European sea bass (*Dicentratus labrax* L) cytochrome P450arom: cDNA cloning, expression and genomic organization. J Steroid Biochem Mol Biol 80:25–34. https://doi.org/10.1016/S0960-0760(01)00170-4

Vischer HF, Granneman JC, Noordam MJ, Mosselman S, Bogerd J (2003) Ligand selectivity of gonadotropin receptors role of the beta-strands of extracellular leucine-rich repeats 3 and 6 of the human luteinizing hormone receptor. J Biol Chem 278:15505–15513. https://doi.org/10.1074/jbc.M300634200

Vizziano D, Randuineau G, Baron D, Cauty C, Guiguen Y (2007) Characterization of early molecular sex differentiation in rainbow trout, *Oncorhynchus mykiss*. Dev Dyn 236:2198–2206. https://doi.org/10.1002/dvdy.21212

Wang XG, Orban L (2007) Anti-mullerian hormone and 11β-hydroxylase show reciprocal expression to that of aromatase in the transforming gonad of zebrafish males. Dev Dyn 236:1329–1338. https://doi.org/10.1002/dvdy.21129

Wang DS, Kobayashi T, Zhou LY, Paul-Prasanth B, Ijiri S, Sakai F, Okubo K, Morohashi K, Nagahama Y (2007) Foxl2 up-regulates aromatase gene transcription in a female-specific manner by binding to the promoter as well as interacting with

ad4 binding protein/steroidogenic factor 1. Mol Endocrinol 21:712–725. https://doi.org/10.1210/me.2006-0248

Watanabe M, Tanaka M, Kobayashi D, Yoshiura Y, Oba Y, Nagahama Y (1999) Medaka (*Oryzias latipes*) FTZ-F1 potentially regulates the transcription of P450 aromatase in ovarian follicles: cDNA cloning and functional characterization. Mol Cell Endocrinol 149:221–228. https://doi.org/10.1016/S0303-7207(99)00006-4

Wilhelm D, Palmer S, Koopman P (2007) Sex determination and gonadal development in mammals. Physiol Rev 87:1–28. https://doi.org/10.1152/physrev.00009.2006

Yamaguchi T, Yoshinaga N, Yazawa T, Gen K, Kitano T (2010) Cortisol is involved in temperature-dependent sex determination in the Japanese flounder. Endocrinology 151:3900–3908. https://doi.org/10.1210/en.2010-0228

Yan Y, Chen H, Costa M (2004) Chromatin immunoprecipitation assays. Methods Mol Biol 287:9–19. https://doi.org/10.1385/1-59259-828-5:009

Yazawa T, Uesaka M, Inaoka Y, Mizutani T, Sekiguchi T, Kajitani T, Kitano T, Umezawa A, Miyamoto K (2008) Cyp11b1 is induced in the murine gonad by luteinizing hormone/human chorionic gonadotropin and involved in the production of 11-ketotestosterone, a major fish androgen: conservation and evolution of the androgen metabolic pathway. Endocrinology 149:1786–1792. https://doi.org/10.1210/en.2007-1015

Yoshiura Y, Senthilkumaran B, Watanabe M, Oba Y, Kobayashi T, Nagahama Y (2003) Synergistic expression of Ad4BP/SF-1 and cytochrome P- 450 aromatase (ovarian type) in the ovary of Nile tilapia, *Oreochromis niloticus*, during vitellogenesis suggests transcriptional interaction. Biol Reprod 68:1545–1553. https://doi.org/10.1095/biolreprod.102.010843

Young G, Kusakabe M, Nakamura I, Lokman PM, Goetz FW (2004) Gonadal steroidogenesis in teleost fish. In: Melamed P, Sherwood N (eds) Hormones and their receptors in fish reproduction. World Scientific, New Jersey, pp 155–223

Zhang WL, Zhou LY, Senthilkumaran B, Huang BF, Sudhakumari CC, Kobayashi T, Nagahama Y, Wang DS (2010) Molecular cloning of two isoforms of 11β-hydroxylase and their expressions in the Nile tilapia, *Oreochromis niloticus*. Gen Comp Endocrinol 165:34–41. https://doi.org/10.1016/j.ygcen.2009.05.018

Zhou LY, Wang DS, Senthilkumaran B, Yoshikuni M, Shibata Y, Kobayashi T, Sudhakumari CC, Nagahama Y (2005) Cloning, expression and characterization of three types of 17β-hydroxysteroid dehydrogenases from the Nile tilapia, *Oreochromis niloticus*. J Mol Endocrinol 35:103–116. https://doi.org/10.1677/jme.1.01801

Zhou LY, Wang DS, Kobayashi T, Yano A, Paul-Prasanth B, Suzuki A, Sakai F, Nagahama Y (2007a) A novel type of P450c17 lacking the lyase activity is responsible for C21-steroid biosynthesis in the fish ovary and head kidney. Endocrinology 148:4282–4291. https://doi.org/10.1210/en.2007-0487

Zhou LY, Wang DS, Shibata Y, Paul-Prasanth B, Suzuki A, Nagahama Y (2007b) Characterization, expression and transcriptional regulation of P450c17-I and -II in the medaka, *Oryzias latipes*. Biochem Biophys Res Commun 362:619–625. https://doi.org/10.1016/j.bbrc.2007.08.044