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Cloning and expression of *follistatin* gene in half-smooth tongue sole *Cynoglossus semilaevis* during the reproduction cycle*

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Abstract Follistatin (FST) is a monomeric glycoprotein highly enriched in cysteines and belongs to TGF-β superfamily. FST can suppress the secretion of follicle-stimulating hormone and plays a vital role in the reproduction of vertebrates. We used rapid amplification of cDNA ends technology to clone the *FST* gene of half-smooth tongue sole, *Cynoglossus semilaevis*. We characterized its phylogenetic context and expression patterns to elucidate its function in the breeding season. The full-length sequence of *FST* is 1 455 bp and encodes a protein of 321 amino acids. We investigated the expression pattern of *FST* in *C. semilaevis* at different stages of reproduction using reverse transcription-polymerase chain reaction (RT-PCR). *FST* mRNA was expressed in all 13 tissues analyzed, and was expressed at high levels in gonad and at slightly lower levels in gill and brain. During the reproductive cycle of *C. semilaevis*, the transcript level of *FST* was the highest in the perinucleolus stage, decreased in the primary yolk stage, slightly increased in the tertiary yolk stage, and then reduced to a minimal level in the atretic follicles stage of the ovary. We concluded that FST suppressed follicle-stimulating hormone, which stimulated oocyte development. However, no significant variation was observed across all stages of testis development, although the expression level in the spermatogenesis stage was relatively low, which may result from the regulation of FST by aromatase.

Keyword: *Cynoglossus semilaevis*; follistatin (FST); cloning; gene expression

1 INTRODUCTION

Follistatin (FST), also called follicle-stimulating hormone (FSH) suppressing protein (FSP), is a monomeric glycoprotein highly enriched in cysteines that belongs to TGF-β family. FST was first isolated from bovine and porcine follicular fluid and is expressed in a wide variety of tissues (Robertsona et al., 1987; Ueno et al., 1987). Initially, FST was found to be associated with reproductive functions in vertebrate animals (Patel, 1998). However, subsequent studies showed that it is also important in embryogenesis during all stages of post-natal life (Patel, 1998). The FST gene encodes a TGF-β superfamily binding protein. Two isoforms, FST-315 and FST-288 result from alternative mRNA splicing (Sidis et al., 2006).

Many studies have demonstrated that FST functions through a family of proteins, the inhibins. FST and inhibins have the same inhibitory activity on FSH release; however, they have no sequence homology. There are also factors, the activins, that stimulate FSH secretion in ovarian follicular fluids. FST is a binding protein of activin, and FST is involved in diverse range of regulatory actions of activin and can inhibit the release of pituitary follicle-stimulating hormone (FSH) indirectly (Nakamura et al., 1990). Interactions between FST, inhibin, and activin regulate a variety of reproductive processes within the body. FST has

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emerging roles as a potent tissue regulator in gonad, pituitary gland, pregnancy membranes, vasculature and liver (Phillips and de Kretser, 1998). In addition to activin, FST also binds other members of the TGF-β superfamily, including myostatin (MSTN), and some bone morphogenetic proteins (BMPs) and regulates their physiological and homeostatic activities (Sidis et al., 2006).

Many studies have described the structure and function of the *FST* gene in mammals, such as human and pig, but few sudies in fish have been published. The *FST* cDNA sequence has been cloned in some teleosts, including common goldfish (*Carassius auratus*), grass carp (*Ctenopharyngodon idella*), globefish (*Takifugu rubripes*), zebrafish (*Danio rerio*) (Bauer et al., 1998), channel catfish (*Ictalurus punctatus*), blue catfish (*I. furcatus*), white catfish (*Ameiurus catus*) (Gregory et al., 2004), largemouth bass (*Micropterus salmoides*) (Li et al., 2007) and large yellow croaker (*Larimichthys crocea*) (Liu et al., 2014). In the juvenile stage of zebrafish and large yellow croaker, *FST* is weakly detected in the gastrula stage and is expressed in later stages of development only, showing that *FST* is not involved in early physiological activities (Bauer et al., 1998; Liu et al., 2014). Nicol et al. (2013) described the expression of *FST* during ovarian differentiation, indicating its important function in folliculogenesis and oocyte maturation. *FST* was expressed in most tissues, such as gonad, brain, spleen and kidney, indicating that it is a multifunctional protein. Numerous studies have shown that increased *FST* expression levels can promote the muscle growth of aquaculture animals (de Santis et al., 2012). Liu et al. (2014) performed a fasting response experiment to show that *FST* expression varied greatly in most tissues, and also was influenced by age and gender. But the function and expression patterns of *FST* in the reproductive cycle have not been systematically reported. FST suppresses the release of FSH, so it is meaningful to study the characterization of FST in these species to further understand its physiological function.

Half-smooth tongue sole, *Cynoglossus semilaevis*, is an important commercial seawater flatfish, which belongs to the batch-spawning fish (Chen et al., 2010b). The females of the species grow one to two times faster than males (Chen et al., 2007). A large number of *C. semilaevis* genes have been cloned and studied, including sex-related genes, *AMH*, *SOX9* and *FOXL2* (Dong et al., 2011; Liu et al., 2013) and growth-related genes, *GHRH*, *PACAP*, *GH* and *GHR* (Ji et al., 2011; Ma et al., 2012a, b). Song et al. (2012) constructed a high-density genetic linkage map and many sex-linked and growth-related simple sequence repeat markers were identified. The identification of genes and molecular markers associated with economically important traits will aid marker-assisted selection. The whole genome sequence of *C. semilaevis* has recently been published by Chen et al. (2014), providing a foundation for the study of *C. semilaevis* genetic improvement and breeding. Compared with other bony fishes, less is known about FST biology in *C. semilaevis*. Therefore, we cloned and characterized the full-length cDNA of *FST* from the *C. semilaevis*. It is important to clarify the expression pattern of *FST* during testis and ovary development in order to evaluate the relationship of FST with other relevant hormones, such as FSH and LH.

2 MATERIAL AND METHOD

2.1 Experimental fish and tissue sampling

About 70 samples, including phenotypic male and female *C. semilaevis* at first sexual maturity, were obtained from an aquatic farm in Yantai, Shandong, in different months. Female samples were taken in April (perinucleolus stage, PN), July (primary yolk stage, PY), October (tertiary yolk stage, TY), and the following January (atretic follicles stage, AF) (Chen et al., 2010a). Male samples were obtained in March (immature), June (late spermatogenesis), September (late spermatogenesis), and December (spermiation) (Zhang, 2009). Twelve females (41.9–54.9 cm, 528.1–1 194.6 g) and twelve males (23.7–37.1 cm, 89.2–352.8 g) at the age of two or three years were used in this experiment. After fostering fish for 2–3 days, gonad, liver, brain, pituitary, intestine, heart, kidney, spleen, stomach, head kidney, gill and muscle were excised and snap-frozen in liquid nitrogen, then stored at -80°C for RNA extraction. A sexually mature female *C. semilaevis* was bought at Qingdao Nanshan Market and used for cloning the full-length FST cDNA.

2.2 Total RNA isolation and reverse transcription

Total cellular RNA was isolated using RNAiso Plus Extraction Kit according to the manufacturer's protocol (TaKaRa, Japan). The concentration of extracted total RNA was spectrophotometrically measured. Reverse transcription was carried out using a PrimeScript™RT reagent Kit with gDNA Eraser

Table 1 Primers used for *C. semilaevis FST* cloning and mRNA expression analysis

Primer	Sequence (5'→3')	Amplification target
FP1	ACRTGYGAYAAYGTGGACTGYGG	For partial sequence of *FST*
FP2	TABGTGGYGTTRTCGCTGGC	
F5-1	CCGTAGGTGACCCCGTCGTTGCCAC	For 5' RACE
F5-2	GGCACGTTTTCTTGCACTTTCCCTG	
F3-1	CGCTGGGAAGAAGTGTTTGTGGGATGCT	For 3' RACE
F3-2	CGAAGTGTTTGTGGGATGCTCGCAT	
FF	CGGGAAGACCTACAAGGACGAAT	Expression of *FST*
FR	TGCGAGCATCCCACAAACAC	
18SF	CCTGAGAAACGGCTACCACATC	Expression of *18S*
18SR	CCAATTACAGGGCCTCGAAAG	

(Perfect Real Time) (TaKaRa, Japan) following the manufacturer's instructions. The synthesized cDNA was stored at -20°C for later use.

2.3 Molecular cloning and sequencing of *FST* cDNA

A pair of degenerate primers (FP1 and FP2) were designed to amplify a *FST* fragment according to highly conserved *FST* cDNA sequences from *Danio rerio* (NM001039631), *Salmo salar* (DQ186633), *Takifugu rubripes* (NM001037858), *Ictalurus punctatus* (AY534327) and *Ctenopharyngodon idella* (DQ340765). PCR reactions consisted of cDNA 1 μL, 10×buffer 2.5 μL, dNTP 2 μL, FP1 0.5 μL, FP2 0.5 μL, Taq DNA Polymerase 0.2 μL. PCR cycling conditions: 94°C 5 min, followed by 38 cycles of 94°C 30 s, 61°C 35 s, 72°C 40 s, ending with 5 min at 72°C for extension. According to the FST fragment sequence, PCR was conducted with 5' and 3' RACE primers and nested primers (Table 1) that were designed by Primmer 5.0. The 5' and 3' RACE reactions used the SMART™ RACE cDNA amplification kit (Clontech, USA). PCR was carried out in a final volume of 50 μL containing 2 μL of cDNA and followed the manufacturer's instructions (TaKaRa). PCR cycling conditions were 5 min at 94°C, 36 cycles of 30 s at 94°C, 30 s at 60°C for F3-1 and universal primer, or 30 s at 62°C for F3-2 and universal primer, or 30 s at 67°C for F5-1 and universal primer, or 30 s at 60°C for F5-2 and universal primer, 1 min at 72°C and a final extension at 72°C for 10 min. Products of PCR and RACE reactions were separated by agarose gel electrophoresis and bands of the expected size were purified with a TIAN Gel Extraction Kit (TIANGEN, China). The purified fragments were then cloned into pGM-T vector (TIANGEN), propagated in *Escherichia coli* DH5 and then sequenced by BGI (China).

2.4 Phylogenetic analysis and sequence analysis

Homology searches of *C. semilaevis FST* cDNA were performed with the basic local alignment search tool (http://www.ncbi.nlm.nih.gov/BLAST). The signal peptide region and potential N-glycosylation sites were predicted using SignalP 3.0 Server (http://www.cbs.dtu.dk/services/SignalP/). Multiple protein sequence alignments of *FST* were conducted with DNAMAN software. Phylogenetic analyses were conducted using the Neighbour-Joining algorithm (Saitou and Nei, 1987) (1 000 runs) combined with Clustal X, MEGA4.0 based on FST amino acid resources from GenBank (Altschul et al., 1990).

2.5 Tissue distribution and expression patterns of *C. semilaevis FST* during the reproductive cycle of *C. semilaevis*

The level of *FST* expression was evaluated using semi-quantitative RT-PCR assays. Total RNA was extracted from testis, liver, brain, pituitary, intestine, heart, kidney, spleen, stomach, head kidney, gill and muscle of a male fish and ovary of a female fish to explore the spatial expression of *FST*. Different stage samples of ovaries and testes were chosen to investigate the temporal expression during the reproductive cycle. To avoid genomic contamination, extracted RNA was treated with DNase-I before reverse transcription. A pair of specific primers FF and FR (Table 1) were designed according to the *FST* cDNA; the length of the expected product was 379 bp. RT-PCR was carried out using Takara Taq™ (TaKaRa) according to the manufacturer's guidelines. The PCR cycling program was, 94°C for 5 min, 34 cycles of 94°C for 30 s, 61°C for 30 s, 72°C for 30 s, and a final extension at 72°C for 5 min. Tissue expression was normalized using 18S rRNA as an internal control (primers are listed in Table 1). The PCR program was 94°C for 5 min, 23 cycles of 94°C for 30 s, 62°C for 30 s, 72°C for 30 s, and a final extension at 72°C for 5 min. Five μL product of each reaction was examined by agarose gel electrophoresis and visualized on a Gel system. The relative expression levels were determined by comparing the brightness of bands using ImageJ software (Ghaia et al., 2010).

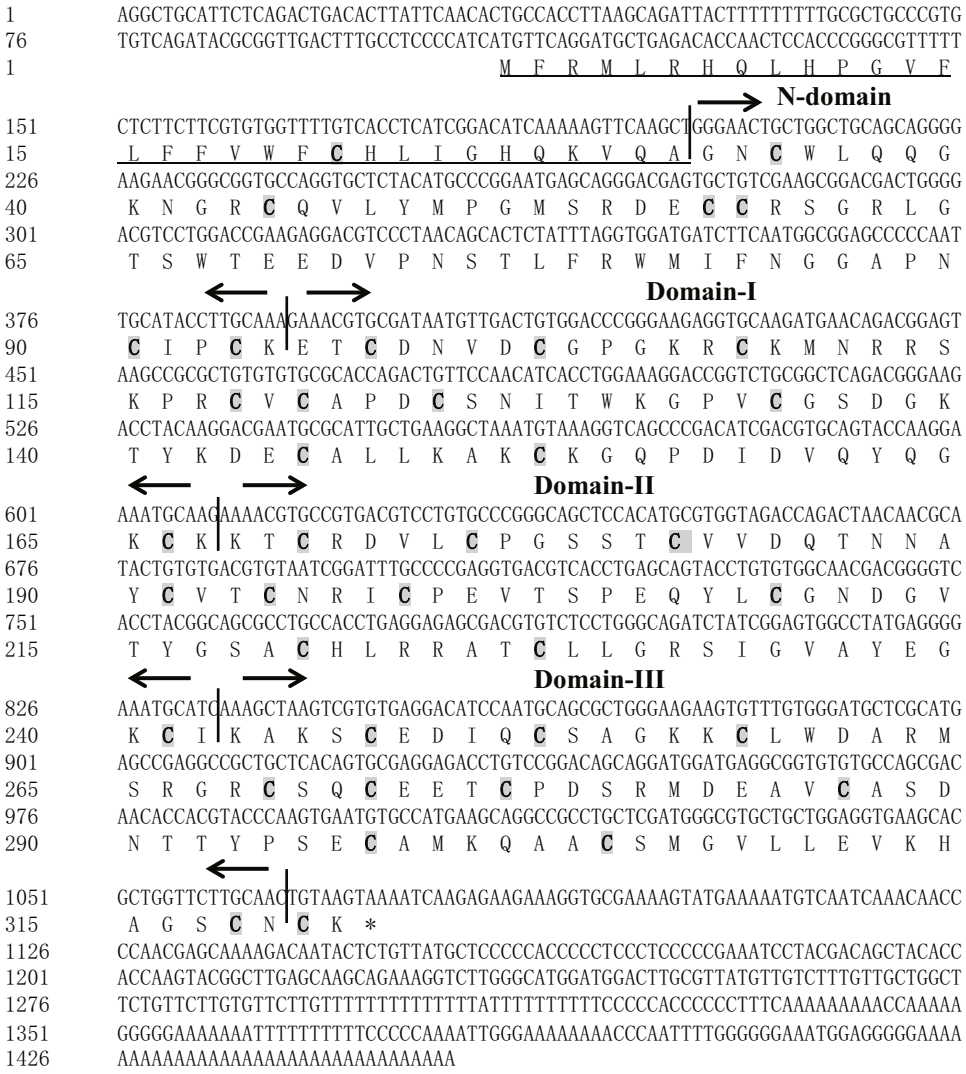

Fig.1 Complete sequence of *C. semilaevis FST* and analysis of deduced amino acid sequence

The nucleotide sequence is depicted in the upper row and the amino acid sequence in the lower row. The signal peptide sequence is underlined. The four domains are marked by arrows. The cysteine residues are shown in bold with a gray background. The termination codon (TAA) is indicated by an asterisk.

2.6 Statistical analysis

All data are represented as the mean ± the standard error (SE). Gene expression levels of *FST* in gonads during different gonadal stages were analyzed by one-way ANOVA followed by Duncan's multiple comparison range test in SPSS 13.0. Histograms were generated with Excel. Statistical significance was considered as $P<0.05$, and different letters in figures represent significant differences between different gonadal stages.

3 RESULT

3.1 Isolation and characterization of *C. semilaevis FST* cDNA

The full-length cloned *C. semilaevis FST'* cDNA was 1 455 bp, which contained a 108 bp 5'-untranslated terminal region (UTR), a 380 bp 3'-UTR and a 963 bp open reading frame (ORF) encoding a predicted 321 amino acid protein (GenBank accession No. JF423304). N-domain, Domain I, Domain II, and Domain III were identified in the protein sequence (Fig.1). The N-domain had six cysteine residues, Domain I, Domain II, and Domain III all had 10 cysteine residues. By comparing the FST N-domain of *C. semilaevis* with that of Japanese flounder, cows and other species, we found that the N-domain was highly conserved in these species. Alignment of the N-domain of *C. semilaevis FST'* to that of *Danio rerio*, *Ctenopharyngodon idella*, *Sparus aurata*, *Paralichthys olivaceus*, *Solea senegalensis*, *Mus musculus* and *Bos taurus* is shown in Fig.2. *C. semilaevis FST'* had the

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Paralichthys olivaceus      MFRMLK-HHLHPGIFLFFIWLCHLMEHQKVQAGNCWLQQGKNGRCQVLYMPGMSREECCRSGRLGTSWTEEDVPNSTLFRWMIFNGGAPNCIPCK
Micropterus salmoides       MFRMLK-HHLHPGIFLFFIWLCHLMEHQKVQAGNCWLQQGKNGRCQVLYMPGMSREECCRSGRLGTSWTEEDVPNSTLFRWMIFNGGAPNCIPCK
Solea senegalensis          -FGKLK-HQLHPGIFLFFIWLCHLMEHQKVQAGNCWLQQGKNGRCQVLYMPGMSRDECCRSGRLGTSWTEEDVPNSTLFRWMIFNGGAPNCIPCK
Cynoglossus semilaevis      MFRMLR-HQLHPGVFLFFVWFCHLIGHQKVQAGNCWLQQGKNGRCQVLYMPGMSRDECCRSGRLGTSWTEEDVPNSTLFRWMIFNGGAPNCIPCK
Danio rerio                 MLRMLKRQQLHPGMILLLFWLCYLIEDQKVQAGNCWLQQGKNGRCQVLYMPGMSREECCRSGRLGTSWTEEDVPNSTLFRWMIFNGGAPNCIPCK
Oncorhynchus mykiss         ----------------------MEDQKVQAGNCWLQQGKNGRCQVLYVPGMSREECCRSGRLGTSWTEEDVPNSTLFRWMIFNGGAPNCIPCK
Mus musculus                -----CARHQPGGLCLLLLLLCQFMEDRSAQAGNCWLRQAKNGRCQVLYKTELSKEECCSTGRLSTSWTEEDVNDNTLFKWMIFNGGAPNCIPCK
Homo sapiens                ---MVRARHQPGGLCLLLLLLCQFMEDRSAQAGNCWLRQAKNGRCQVLYKTELSKEECCSTGRLSTSWTEEDVNDNTLFKWMIFNGGAPNCIPCK
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Fig.2 Alignment of the N-domain of FST from several species

The N-domain is underlined. Asterisks and dots indicate identical and similar amino acids, respectively.

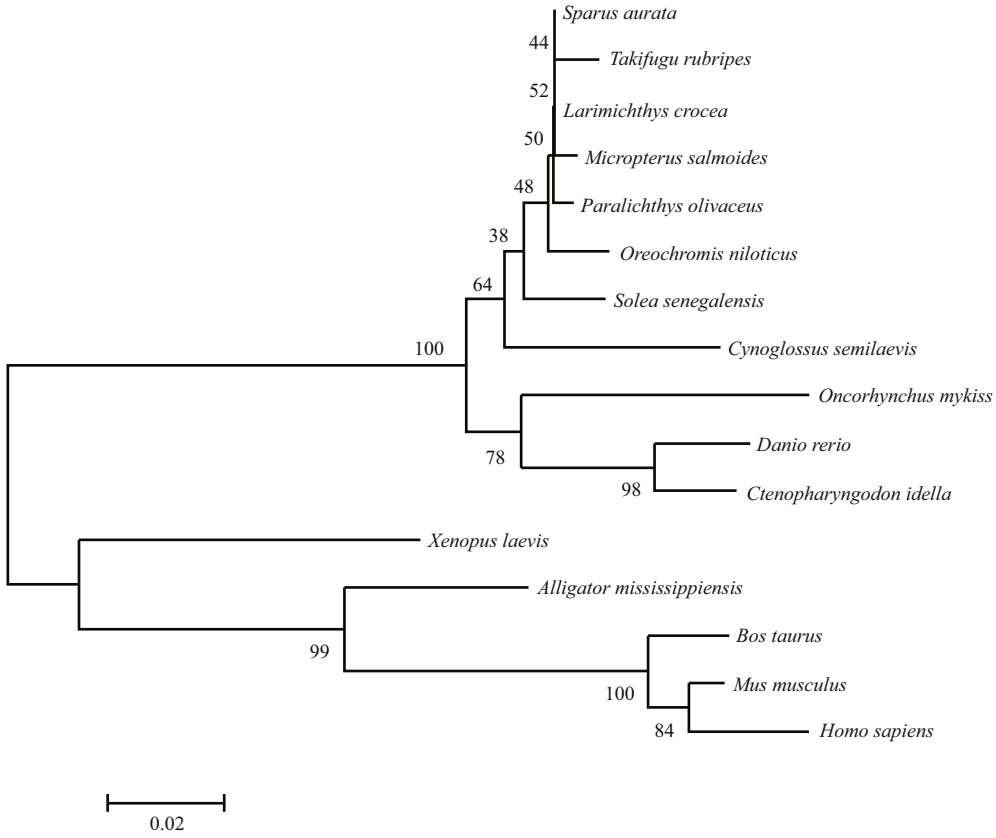

Fig.3 Phylogenetic tree constructed for FST amino acid sequences

The phylogenetic tree is inferred from the neighbor-joining method. Bootstrap values are indicated (1 000 replicates).

highest homology (94%) with *Paralichthys olivaceus* (GenBank accession No. ABP04247), *Sparus aurata* (GenBank accession No. AAT09419) and *Micropterus salmoides* (GenBank accession No. ABL95955).

3.2 Alignment and phylogenetic tree analysis

A phylogenetic tree was constructed using the deduced amino acids to identify the evolutionary position of *C. semilaevis FST'*. GenBank accession numbers of FST amino acid sequences used in this study are shown in Table 2. The topology (Fig.3) shows that the FSTs are divided into two main groups. Bony fish clustered as a branch, amphibians, reptiles and mammals formed the second main branch. FST of *C. semilaevis* is most similar to that of Senegal sole and had high similarity with Japanese flounder and Nile tilapia, showing common evolutionary status features. FST is highly conservative in evolution.

3.3 Tissue distribution

FST expression patterns in different tissues of sexually mature *C. semilaevis* were studied by RT-PCR. *FST* was widely expressed in every tissue of *C. semilaevis*. *FST* mRNA was highly expressed in testis and ovary with levels slightly less in gill and brain. High expression levels were also found in liver, pituitary, intestine, heart, kidney, spleen, stomach, head kidney, and the lowest expression level was detected in muscle (Fig.4).

3.4 *FST* expression in gonads during the reproductive cycle of *C. semilaevis*

The temporal transcription levels of *C. semilaevis FST* mRNA during the reproductive cycle of gonads in males and females are shown in Fig.5. *C. semilaevis FST'* was expressed throughout the reproductive cycle in both males and females. The *FST* expression level was significantly decreased during the reproductive cycle of females (Fig.5a). The expression level was highest in the perinucleolus stage (PN), and declined in the primary yolk (PY) and tertiary yolk (TY) stages, then expression significantly and consistently decreased in the atretic follicles (AF) stage (P<0.05).

For males, *FST* mRNA levels did not change during the testis reproductive cycle (Fig.5b). The expression levels in March (immature) and in June (late spermatogenesis) were similar; the average values of relative expression were 0.616 and 0.592, respectively, and then dropped to 0.474 in September (late spermatogenesis). Expression then clearly increased from September to December (spermiation).

Table 2 GenBank accession numbers of FST amino acid sequences

Species	GenBank accession number
Sparus aurata	AAT09419
Larimichthys crocea	AEC13716
Takifugu rubripes	NP_001032947
Micropterus salmoides	ABL95955
Paralichthys olivaceus	ABP04247
Solea senegalensis	ACL12509
Oreochromis niloticus	XP_003451067
Cynoglossus semilaevis	AEF32782
Oncorhynchus mykiss	ACZ54280
Danio rerio	AAD09175
Ctenopharyngodon idella	ABC72407
Xenopus laevis	AAB30638
Alligator mississippiensis	AAZ31476
Mus musculus	CAA58291
Bos Taurus	AAA30522
Homo sapiens	CAG46612

4 DISCUSSION

We have isolated and sequenced the *FST* gene from *C. semilaevis* and analyzed its expression among different tissues and in different gonadal development stages of healthy males and females. The amino acid sequence of *C. semilaevis* FST shared high sequence identity with that of *Sparus aurata*, *Paralichthys olivaceus* and *Micropterus salmoides* (all 94%). This indicated that the nucleotide sequence we cloned was *FST*, and that it has been highly conserved during

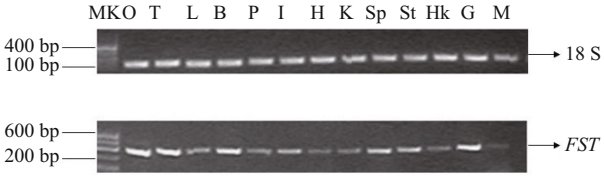

Fig.4 Tissue distribution of *FST* expression in adult *C. semilaevis*, assayed by RT-PCR

T: testis; O: ovary; L: liver; B: brain; P: pituitary; I: intestine; K: kidney; H: heart; Sp: spleen; St: stomach; Hk: head kidney; G: gill; M: muscle. The integrity of the RNA from each tissue was ensured by uniform amplification of *18S* transcripts (lower panel).

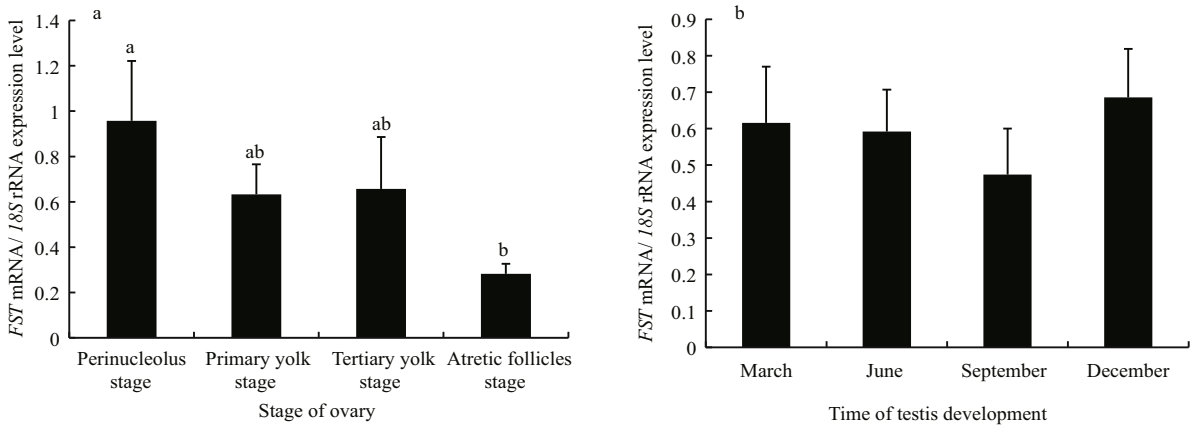

Fig.5 *FST* mRNA levels in ovary (a) and testis (b) during the *C. semilaevis* reproductive cycle

Different letters indicate significant difference (P<0.05, one-way ANOVA, followed by Duncan's test).

evolution. In some species, *FST* produces two isoforms from alternatively spliced mRNAs, for example in zebrafish, grass carp (Zhong et al., 2013), *Xenopus* (Bauer et al., 1998), rat (Nakamura et al., 1990), cow (Sugino et al., 1993), and human. These two isoforms, FST-288 and FST-315, have different affinity to activin, but there is no evidence for alternative splicing of *FST* in catfish (Gregory et al., 2004) or Japanese flounder (Liu et al., 2007). In this study, we found only one *FST* cDNA. It has been proposed that FST has evolved to produce two kinds of *FST* mRNA in animals, with FST function divided between the two different isoforms in higher animals (Liu et al., 2007). Comparative protein analysis showed that *C. semilaevis* FST had high homology with other species. The N-domain had six cysteine residues and Domain I, Domain II, and Domain III all had 10 cysteine residues. The cysteine residue content of FST was highly conserved among species. This shows that these cysteine residues play an important role in the function of FST, such as maintaining stable protein structure (Liu et al., 2007). The N-terminal domain of human FST combines with MSTN specifically to inhibit its physiological functions and to gain muscle (Kocams et al., 2004; Tsuchita, 2005). The N-terminal domain is located in the most important active region of human FST. Cash et al. (2012) revealed the structure of the N-terminal domain of FST-like 3 interacting with MSTN in human. There are three key amino acids Trp, Trp, and Phe located at positions 4, 36, and 52, respectively, and substitution or loss of any one of them affects the biological activity of FST (Sidis et al., 2001). The positions of these three amino acids in *C. semilaevis* were consistent with those in human, and this indicated that FST can inhibit MSTN in other species. This speculation was confirmed in gilthead bream and rat. In addition, the N-terminal domain of FST can specifically combine with BMP and affect its function (Sidis et al., 2001).

FST is involved in the reproductive cycle, embryo development, muscle growth and development (Patel, 1998) and a primary physiological function of FST is the binding and neutralization of activin. *FST* was widely expressed in *C. semilaevis*, in every tissue examined, indicating that FST has extensive physiological functions. *FST* expression levels in the gonads were significantly higher than in other tissues and similar results were found in catfish (Gregory et al., 2004) and rainbow trout (Nicol et al., 2013). The high level of *FST* expression in gonads demonstrated that one of the important functions of FST is regulating gonad physiology. *FST* expression was not detected in liver or muscle in adult catfish (Gregory et al., 2004) or Japanese flounder, but *FST expression* could be detected in these tissues in large yellow croaker and *C. semilaevis*. It has been speculated that the tissue-specific expression of *FST* is different in various fishes (Liu et al., 2014).

It is well known that FST regulates the release of FSH in the pituitary and other tissues and organs of the adult body related to reproductive functions. In the reproductive cycle of the female *C. semilaevis*, *FST* expression was the highest in the preparation period of the ovary, and then significantly decreased with the ovarian development. In April, oocytes were at the perinucleolus stage. FST antagonizes the effects of activin and inhibits the secretion of FSH indirectly and may regulate oocyte maturation. As an FSH suppressing protein (FSP), FST was expressed at a high level in the perinucleolus period, which would suppress FSH activity and inhabit oocyte development indirectly. Subsequently, oocytes were in the primary yolk stage in July, and the expression level of *FST* decreased significantly during yolk accumulation in oocytes ($P<0.05$), and the inhibitory effect of FST on FSH was weakened. At the tertiary yolk maturation stage in October, the expression of *FST* had decreased further and was accompanied by oocyte maturation as the inhibitory effect on FSH was further weakened. After ovulation in January the following year, the ovary entered the atretic follicles stage of yolk absorption, and *FST* mRNA levels were at their lowest, suggesting that the *C. semilaevis* ovary secreted FST, which is consistent with the synthesis of FST in human ovarian granular cells (Sadatsuki et al., 1993). Chen et al. (2010c) studied the changing trends of *FSHR* and *LHR* in the same samples from female *C. semilaevis*, and showed that the highest expression of *FSHR* was consistent with the lowest expression of *FST* in October. *FSHR* had the lowest expression level in January, indicating that *FSH* was expressed at a lower level, while *FST* expression was also at a low level, which resulted from follicle atresia at the end of the breeding season. FST inhibited *FSHβ* but enhanced *LHβ* expression in the goldfish and eel pituitary (Yuen and Ge, 2004; Aroua et al., 2012), while there was no obvious correlation in the change of *FST* and *LHR* expression in our study according to the data of Chen et al. (2010c).

In this study, no significant variation in *FST*

mRNA levels was observed during the breeding cycle of male *C. semilaevis*. *FST* was lowly expressed in late spermatogenesis in June and September, while serum testosterone (T) and estrogen (E2) levels in this stage were far higher than those in immature (March) and spermiation (December) stages. This may involve high-level of aromatase activity. Aromatase P450c19 is a key enzyme and a rate-limiting enzyme in E2 synthesis from arachidonic acid, while FST can inhibit the aromatase activity in a dose-dependent manner (Simpson et al., 2002). Moreover, P450c17, possessing 17α-hydroxylase and 17, 20-lyase activities, plays an important role in testosterone production (Chen et al., 2009). It is well known that testosterone is the precursor of E2 synthesis, and a high level P450c17 catalyzes the conversion of testosterone to E2; therefore, high levels of testosterone lead to high levels of E2. However, the expression level of *Crassostrea angulata FST* has a similar trend to the level of E2 during the reproductive cycle of the oyster (Ni et al., 2012). The distinction between mammals, fish and oyster in the regulatory mechanism of ovarian *FST* expression is still unknown, and further studies in different animal models will provide convincing evidence about the evolution and species diversity of the regulation. Bony fish have two types of P450c17. In our previous study of the same batch of male *C. semilaevis* (Chen et al., 2010b, 2012), we found the expression level of *P450c17-I* had no relationship with that of *FST*, while *P450c17-II* had the same change trend with that of *FST* in the reproductive cycle. In large yellow croaker, the expression of *FST* has the opposite trend at different development stages and in different genders, which indicates that *FST* transcripts were regulated by gender at different ages (Liu et al., 2013). A similar result is reported in our study, which implies that the correlation between *FST* transcription and gender needs to be further investigated.

5 CONCLUSION

The full-length cDNA coding for *FST* was cloned from the half-smooth tongue sole (*Cynoglossus semilaevis*) ovary. *FST* is expressed widely in at least 13 tissues and organs, indicating the extensive physiological function of FST. The *FST* expression pattern was studied during the breeding cycle of *C. semilaevis* for the first time and results show that *FST* may play an important role in ovary development and that in male *C. semilaevis* FST may be associated with P450c17-II in the breeding cycle. These findings can be used to further understand the function of FST during the reproductive cycle in teleosts.

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