ISSN: 2347-5129
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2017; 5(1): 23-28

www.fisheriesjournal.com
Received: 06-11-2016
Accepted: 07-12-2016

Avinash Pradhan
ICAR-Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar, Odisha, India

Suresh Chandra Rath
ICAR-Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar, Odisha, India

Ashis Saha
ICAR-Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar, Odisha, India

Correspondence
Ashis Saha
ICAR-Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar, Odisha, India

Molecular characterization and expression analysis of gonadotrophic α-subunit hormone in rohu (*Labeo rohita*)

Avinash Pradhan, Suresh Chandra Rath and Ashis Saha

Abstract
Reproduction in fish is controlled by two pituitary gonadotropin hormones, follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Rohu (*Labeo rohita*) is a commercially important seasonal breeder freshwater fish species, but the information regarding molecular regulation of reproduction in this important carp species is very scanty. In the present study we identified, cloned and characterized gonadotropin α-subunit (GTHα) full-length cDNA by RACE (rapid amplification of cDNA ends), and analyzed their basal expressions in different tissues by quantitative real time PCR (qRT-PCR) assay. Full-length rohu GTHα cDNA encodes 118 amino acids and in the adult fish, they were widely expressed in pituitary, brain, gonads, muscle, liver, intestine, gill, kidney, eye and heart.

Keywords: GTH, rohu, gonad, pituitary

1. Introduction
Two gonadotropins (GTHs), follicle-stimulating hormone (FSH) and luteinizing hormone (LH) have been demonstrated as the key hormones of the brain–pituitary–gonadal axis (BPG) that regulate gametogenesis and gonad steroidogenesis in fish [1, 2]. For vertebrates, these hormones are non-covalently bound heterodimeric glycoproteins composed of a common α-subunit, essential for signal transduction, protein folding and heterodimer stabilization, and a hormone specific β-subunit that is involved in determining the metabolic clearance rate being responsible for eliciting a particular biological response [3-5]. The α-subunit amino acid sequence is highly conserved, with fishes of the same order showing 90–100% identity among them and 55–70% identity when compared to other teleosts [6-9]. The cDNA cloning and sequencing of these subunits, including GTHα, FSHβ and LHβ have been reported in a number of teleost species, such as common carp *Cyprinus carpio* [10], European eel *Anguilla anguilla* [11, 12], killifish *Fundulus heteroclitus* [13], skipjack tuna *Katsuwonus pelamis* [14], striped sea-bass *Morone saxatilis* [15], channel catfish *Ictalurus punctatus* [16, 17], coho salmon *Oncorhynchus kisutch* [18, 19] European sea-bass *Dicentrarchus labrax* [20], red sea bream *Pagrus major* [21], and Atlantic halibut *Hippoglossus hippoglossus* [22]. The subunits are long peptide chains (around 100 amino acids) highly complex in structure, which are determined by the presence of different O- and N-linked oligosaccharide chains and the formation of cross-linked disulfide bonds between cysteine residues [23]. The non-covalent association of two subunits (α and β) determines the formation and release of the bioactive dimeric hormone [24, 25]. Like in mammals, the fish GTH α-subunit contain two potential sites for N-glycosylation and 10 conserved cysteines located in identical sequence positions that form five intra-molecular disulfide bridges [26]. Generally, FSH is involved in the control of puberty and gametogenesis, whereas LH mainly regulates final gonadal maturation and spawning [1, 2].
Rohu (*Labeo rohita)* belongs to cyprinidae family, and is a seasonal spawner in the rivers of subtropical and tropical region of Indian sub-continent and breeds during monsoon season. Though breeding and seed production technique has been standardized in this important fresh water carp species but the information regarding molecular regulation of reproduction in this important carp species is very scanty. As a first step to ascertain the involvement of gonadotropin in the regulation of reproduction in rohu, we cloned and characterized GTH α-subunit and investigated its basal expression in different tissues.

2. Materials and Methods

2.1 Fish and tissue sampling

Rohu broods were maintained in the brood-rearing pond of the Fish Nutrition and Physiology Division at the ICAR-Central Institute of Freshwater Aquaculture (ICAR-CIFA), Bhubaneswar, India. For the present study, different tissues, namely brain, pituitary, ovary, testis, muscle, liver, intestine, gill, kidney, eye and heart from adult rohu were quickly dissected and snap-frozen in liquid nitrogen and kept at deep freezer (-80 ºC) for further use for RNA isolation.

2.2 RNA extraction and cDNA preparation

Total cellular RNA from the collected tissues was extracted with Trizol (Invitrogen, USA) following the manufacturer's protocol and was treated with RNase free DNase I (Fermentas) to remove contaminating genomic DNA. The integrity of total RNA was checked by observing the band intensity of 28S and 18S ribosomal RNA on 2.0% agarose gel. The purity and concentration of total RNA was measured with a Nanodrop spectrophotometer (Thermo Scientific). First-strand cDNAs were synthesized from 1 µg of total RNA using RevertAid^{TM} First Strand cDNA synthesis kit (Fermentas) according to the manufacturer's protocol.

2.3 Molecular cloning of cDNA for GTHα

The cDNA of rohu pituitary were PCR amplified with different sets of primers (Table 1) designed from the GTHα gene sequence of available fish species in the GenBank database. PCR was carried with 1 µl of cDNA in a 25 µl of reaction volume under the conditions of initial denaturation at 95 ºC for 2 min, followed by 35 cycles of 95 ºC for 30s, 54 ºC for 30s, 72 ºC for 45s and a final extension at 72 ºC for 5 min. PCR products were separated on 1.2% agarose gel and desired bands were purified using gel extraction kit (Qiagen). The purified fragments were T-A cloned into pGEM^{T} easy vector (Promega, Madision, USA) and then transformed into chemically competent DH5α cells. The positive inserts were sequenced at both strands with T7 and SP6 primer using an automated ABI 310 genetic analyzer (Perkin-Elmer Applied Bio system). Obtained sequences were confirmed by BLAST. Rapid amplification of cDNA ends (RACE) were performed to obtain 5′ and 3′ end of the GTHα transcript using Smarter RACE cDNA amplification Kit (Clontech, USA) as per manufacturer's protocol. Gene specific primers (Table 1) were designed from the partial DNA sequence of rohu GTHα obtained previously. Touch down PCR was carried out for 3′ and 5′ RACE using different primers sets (Table 1) under the conditions of 1 cycle of initial denaturation at 94 ºC/2 min; followed by 5 cycles of 94 ºC/30 s then 72 ºC/3min; 5 cycles of 94 ºC/30s, 70 ºC/30s, 72 ºC/3min; next 27 cycles of 94 ºC/30s, 68 ºC/30s, 72 ºC/3min. The amplified DNA fragments were cloned and sequenced as described above. The sequence was submitted to the GenBank database.

Table 1: Primers used for cDNA cloning and quantitative real time PCR analysis of GTHα-subunit in rohu (*Labeo rohita*).

Primers	Nucleotide sequence (5'-3')	Purpose
GTHαF	GCTTATTCGTCTTGGACAACTG	Partial Cloning
GTHαR	AGTAGCAGGTGCTGCAGTGG	Partial Cloning
GTHα RACE 5'	CTTGGACCTCAGTGGCGTGGGGTAAGC	5' Race primer
GTHα RACE 3'	CTTACCCCACGCCACTGAGGTCCAAGA	3' Race primer
QELF1F	GAACTGTACCTGTGGGTCGTG	qPCR primer
QELF1R	CAACGTTGTCACCAGGAGTG	qPCR primer
QGTHαF1	TGGATGTGAGGAGTGCAAAC	qPCR primer
QGTHαR1	GCACCCGCTTAACTTCTTTG	qPCR primer

2.4 Sequence analysis

The cDNA sequences of rohu GTHα was verified by BLAST program at NCBI (http://www.ncbi.nlm.nih.gov/blast). The amino acid sequence was deduced by ExPASy translate tool (http://expasy.org/tools/dna.html) and verified using BLASTP program of NCBI. The Open Reading Frame (ORF) sequences of rohu GTHα were predicted by ORF Finder (http://www.ncbi.nlm.nih.gov/gorf.html). The amino acid alignments of the sequences with other known GTHα sequences were performed using Clustal Omega program (www.ebi.ac.uk/Tools/msa/clustalo). The signal peptide was predicted at the server SMART (http://smart.embl-heidelberg.de/smart). The potential N-glycosylation sites were predicted at NetNGlyc1.0 server (http://www.cbs.dtu.dk/services/NetNGlyc/). Phylogenetic analysis of full-length amino acid sequences of GTHα with other amino acid sequences was performed using MEGA 4 program [27]. The phylogenetic tree was constructed using Neighbor Joining consensus statistical method with 1000 replicates for bootstrap analysis.

2.5 Quantitative Real Time PCR (qPCR)

To determine GTHα mRNA expression levels in different tissues of rohu, quantitative real-time PCR (qRT-PCR) analysis was performed in a Light Cycler 480 II real-time PCR detection system (Roche, Germany). The qRT-PCR was performed in 10µl reaction volume containing 5 µl of 2x Light Cycler 480 SYBR Green I master mix (Roche, Germany),1 µl of cDNA, 0.25 µl FW and RV primer (0.2 mM each) (Table 1) and 3.5 µl of PCR grade water. The thermo cycling conditions were as follows: initial denaturation at 95 ºC for 5 min, followed by 40 cycles of 94 ºC for 10s, 55 ºC for 10s, and 72 ºC for 20s. The reaction carried out without cDNA was used as negative control. The PCR efficiency were determined by analysis of serial dilution of cDNA and efficiencies were closed to 100% allowing the use of $2^{-\Delta\Delta C_T}$ method [28] for calculation of the relative gene expression of the target gene with that of reference gene elongation factor-1 alpha (elfα).

2.6 Data representation and statistical analysis

All data are shown as mean ±SEM (standard errors of the mean) and analyzed by one way ANOVA followed by Duncan multiple-range test using SPSS16.0 software.

3. Results

3.1 Cloning and sequence analysis of rohu GTHα-subunit

We cloned full length cDNA of GTHα-subunit from rohu pituitary using RACE technique. Rohu GTHα cDNA (GenBank KU743471) was 857 bp, with a 5′ untranslated

region (UTR) of 10 bp, 3′ UTR of 490 bp and an open reading frame (ORF) of 357 bp encoding a precursor protein of 118 amino acids (aa) with a predicted signal peptide of 23 aa (Fig. 1). A putative polyadenylation signal (AATAAA) was noted 179bp upstream of the poly-A tail (Fig. 1). The rohu GTHα-subunit consisted of two N-linked glycosylation sites (^{79}NITS, ^{104}NHTD) as predicted by NetNGlyc 1.0 server and contained 10 conserved cysteine residues respectively (^{34}C, ^{37}C, ^{55}C, ^{58}C, ^{59}C, ^{86}C, ^{87}C, ^{108}C, ^{110}C, ^{113}C). The sequence alignment at the protein level revealed that rohu GTHα-subunit is highly homologous to the reported cyprinid fish species, especially *Carassius auratus* (96%), *Procypris rabaudi* (95%) and *Gobiocypris rarus* (93%) (Fig. 2)

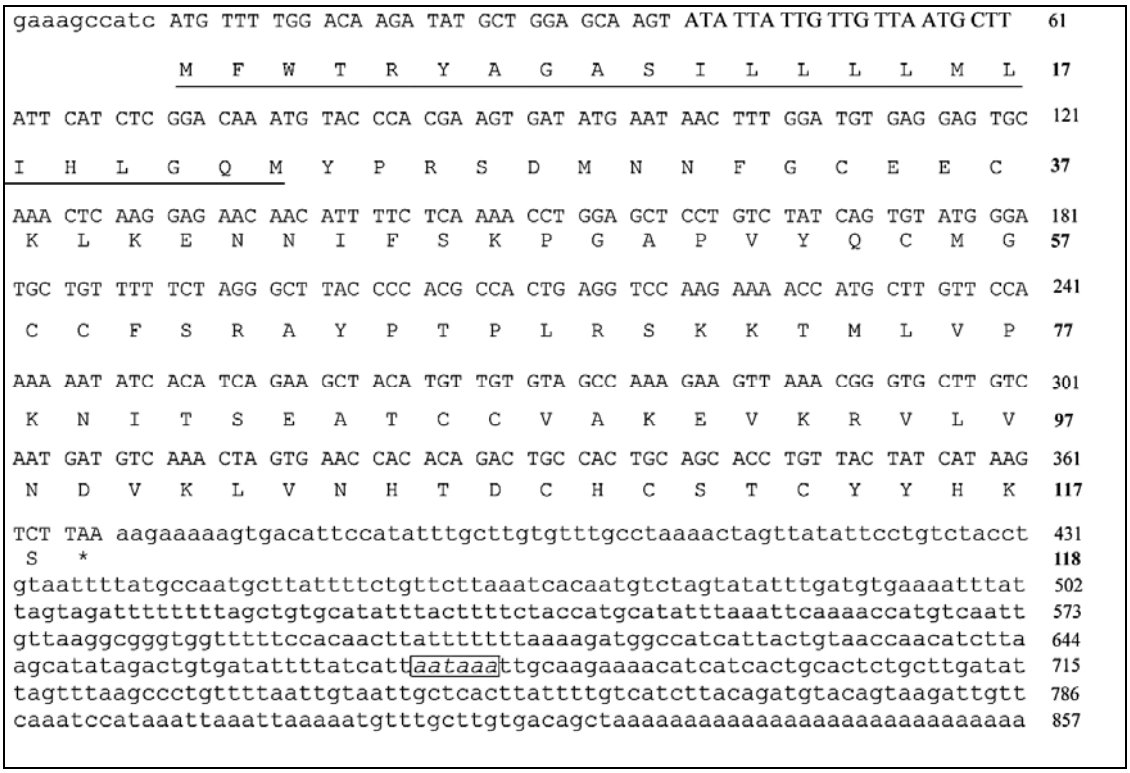

Fig 1: Nucleotide and deduced amino acid sequence of the cDNA encoding the GTHα-subunit of *Labeo rohita.* The putative signal peptide is underlined. The stop codon (TAA) is indicated by asterisks (*). The polyadenylation signal (AATAAA) is boxed.

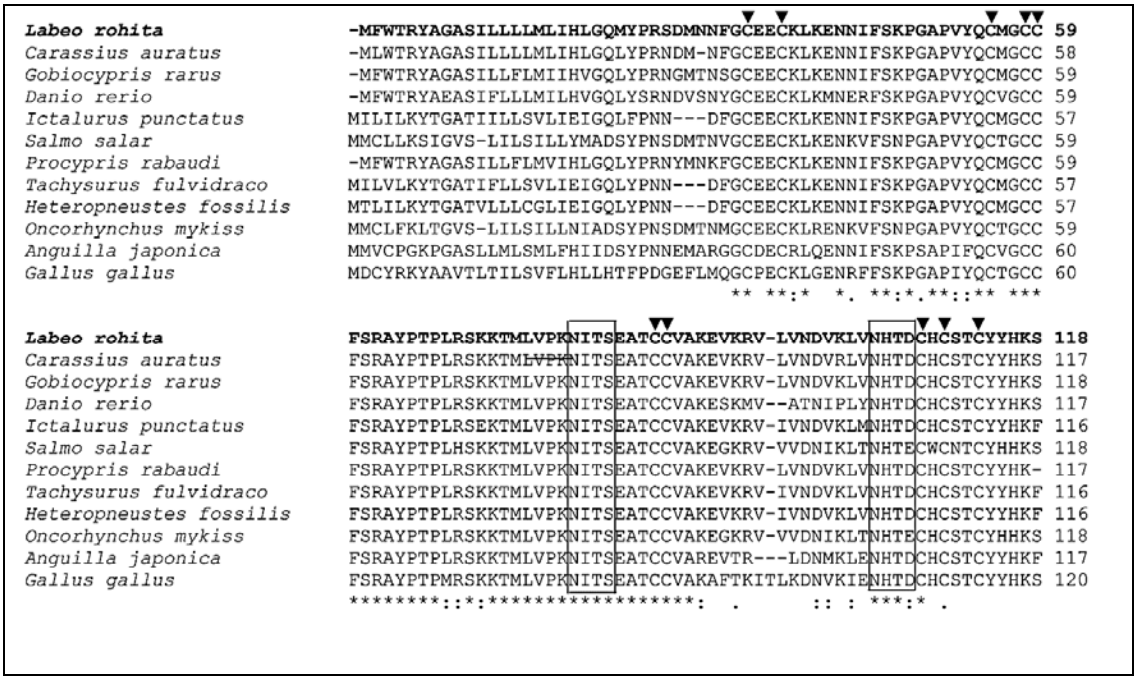

Fig 2: Clustal W alignment analysis of the amino acid sequences of *Labeo rohita* GTHα-subunit with other vertebrates. Identical sequences are indicated by asterisks (*). The putative N-linked glycosylation sites are boxed. The conserved cysteines residues are shown by black inverted triangles.

3.2 Phylogenetic analysis

The neighbor-joining phylogenetic tree was constructed based on the amino acid sequences of fish GTHα-subunit. Bootstrap analysis and consensus trees obtained from Neighbor-joining analysis showed that rohu GTHα-subunit exhibits the highest homology with *Carassius auratus*, *Procypris rabaudi* and *Gobiocypris rarus* which cluster in the same branch with a high bootstrap value (Fig. 3).

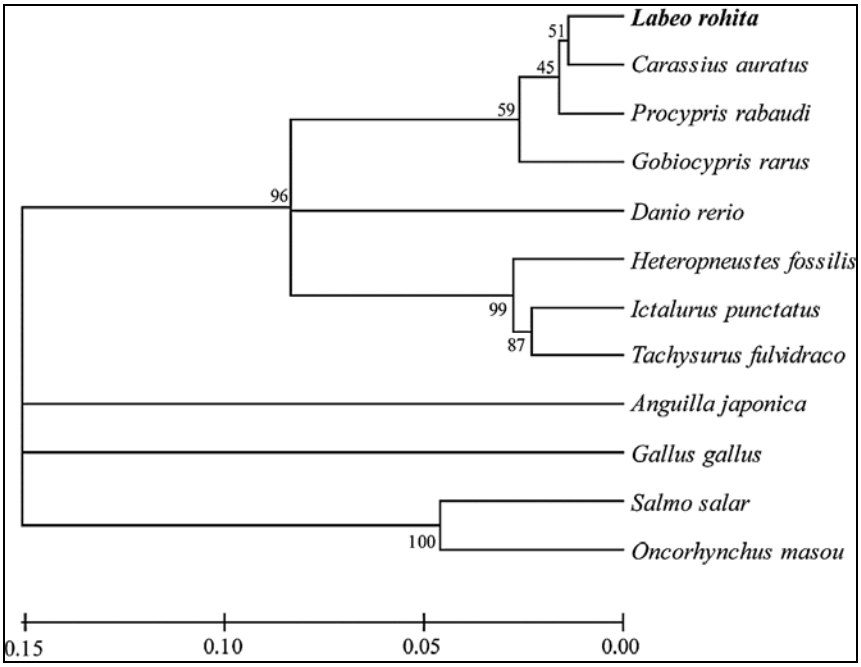

Fig 3: Phylogenetic relationship of deduced amino acid sequences of rohu GTHα-subunit with vertebrate species. The consensus tree was inferred using the Neighbor-joining algorithm and the branch points were validated by 1000 bootstrap replications. The position of rohu GTHα – subunit was written in bold letters. The GenBank accession numbers of sequences for analysis are as follows: *Labeo rohita* (KU743471); *Carassius auratus* (AAX18859), *Gobiocypris rarus* (AFA42949) *Tachysurus fulvidraco* (ACX37700), *Heteropneustes fossilis* (HB20168), *Ictalurus punctatus* (NP_001187007), *Danio rerio* (NP_991250), *Salmo salar* (NP_001139928), *Anguilla japonica* (BAD14301), *Procypris rabaudi* (AGC00409), *Oncorhynchus masou* (AAB30421), *Gallus gallus* (NP_001264950)

3.3 Expression analysis of GTHα in different Tissues

The result of qRT-PCR analysis showing GTHα mRNA expression in different tissues, namely brain, pituitary, testis, ovary, muscle, liver, intestine, kidney, fin, eye and heart of adult spermiating male and late vitellogenic female are presented in Fig. 4. It was observed that GTHα mRNA transcript was constitutively expressed in all the tissues examined. The highest level of expression was noticed in pituitary followed by brain and ovary and testis (Fig. 4). However, among non-reproductive tissue higher level of expression was recorded in muscle and liver (Fig. 4). In other tissues, low levels of expression were observed (Fig. 4).

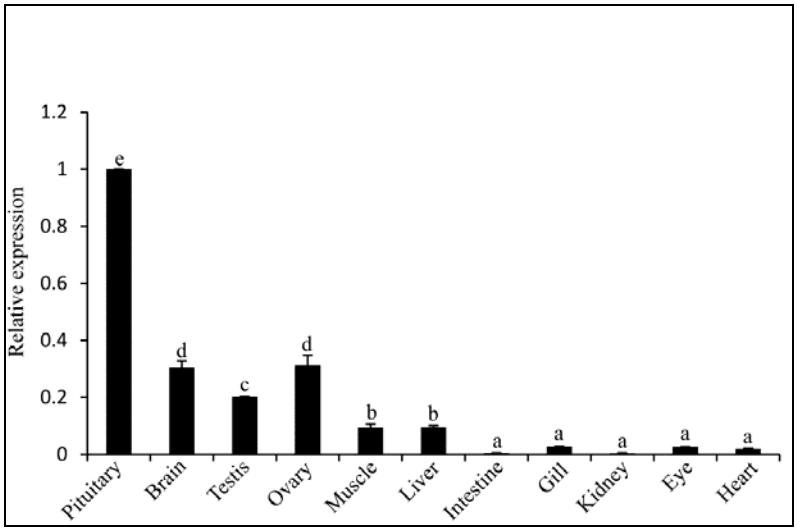

Fig 4: Expression analysis of GTHα gene transcripts in different tissues of adult rohu. Expressions of GTHα mRNA transcripts were represented as a ratio relative to elfα (internal control) levels in the same sample. Pituitary was chosen as the calibrator and the expression of GTHα in other tissues was represented as fold changes from the calibrator. Values are mean ± SEM (n=3 for each sample). Columns sharing different letters show significant differences.

4. Discussion

In the present study, we cloned and characterized full length cDNA of GTHα-subunit in rohu, and examined their basal expression in different tissues of adult fishes. Alignment of the deduced amino acids of GTHα revealed a high homology in fish, including 10 cysteine residues and the two putative N-linked glycosylation sites. These conserved structures are suggested to be involved in the processes of subunit assembly and receptor binding in vertebrates including fish [21, 23, 29, 30]. The fish GTHα is more conserved than FSHβ and LHβ which is likely due to the fact that GTHα-subunit is shared by all pituitary glycoprotein hormones, including FSH, LH and TSH, and hence it is probably under higher selective pressure during vertebrate evolution [31]. N-Glycosylation site is important to the sugar chains for the bioactivity of the GTHs, specifically on stimulation of the post-receptor binding functions upstream from G-protein activation and second messenger stimulation [32]. Like that of other fish species, rohu GTHα also contain 10 cysteine residues which may be responsible for forming 5 disulfide linkages. The region from 58 to 87 residues, consisted of two paired adjacent cysteines and the first putative N-linked glycosylation site, was suggested to be involved in the processes of subunit assembly and/or receptor binding in fish [21, 33] and in higher vertebrates [29].

Although pituitary is considered as the main tissue producing and releasing the gonadotropin conventionally, many studies have demonstrated that GTHα also exist in other tissues. Like rohu, the expression of gonadotropins besides pituitary had been reported in brain in Nile tilapia [34] and zebrafish [31], in ovary in Southern catfish [35], in kidney and liver in zebrafish [31]. Based on these data, it was believed that the extra pituitary ectopic site expression of gonadotropin may be a common phenomenon which is further supported by our results. However, the physiological significance of the extra pituitary gonadotropins expression remains unknown.

5. Conclusion

In conclusion, rohu GTHα cDNA was identified and sequenced in this study and its putative mature peptide sequence compared to those of other teleosts. The characterization of rohu GTHα is the first step necessary for the biotechnological production of rohu-FSH and rohu-LH, extremely important hormones for gonadal development and spawning induction for this very important carp species.

6. Acknowledgement

This is the part of the PhD research work of first author. The authors would like to thank the Director, ICAR-CIFA for providing necessary facility to carry out this research. This research was financially supported by Science and Engineering Research Board, Department of Science and Technology, Govt. of India (Project NO.SR/FT/LS-19/2011).

7. References

1. Schulz RW, Bogerd J, Bosma PT, Peute J, Rebers FEM, Zandbergen MA *et al*. Physiological, morphological, and molecular aspects of gonadotropins in fish with special reference to the African catfish, *Clarias gariepinus*. In: Goetz, F.W., Thomas, P. (Eds), Reproductive Physiology of Fish. Fish Symp. Austin. 2001a, 2-6.
2. Schulz RW, Vischer HF, Cavaco JE, Santos EM, Tyler CR, Goos HJ *et al.* Gonadotropins, their receptors, and the regulation of testicular functions in fish. Comparative Biochemistry Physiology B. 2001b; 129:407-417.
3. Pierce JG, Parsons TF. Glycoprotein hormones: structure and function. Annual Review of Biochemistry. 2001a; 50:465-495.
4. Bousfield GR, Butnev VY, Walton WJ, Nguyen VT, Huneidi J, Singh V *et al*. All-or- none N-glycosylation in primate follicle-stimulating hormone β subunits. Molecular and Cellular Endocrinology. 2007; 260:40-48.
5. Carvalho CM, Oliveira JE, Almeida BE, Ueda EKM, Torjesen PA, Bartolini P *et al.* Efficient isolation of the subunits of recombinant and pituitary glycoprotein hormones. Journal of Chromatography A. 2009; 1216:1431-1438.
6. Gen K, Maruyama O, Kato T, Tomizawa K, Wakabayashi K, Kato Y. Molecular cloning of cDNAs encoding two types of gonadotrophin alpha subunit from the masu salmon, Oncorhynchus masou: construction of specific oligonucleotides for the alpha 1 and alpha 2 subunits. Journal of Molecular Endocrinology. 1993; 11:265-273.
7. Kobayashi M, Kato Y, Yoshiura Y, Aida K. Molecular cloning of cDNA encoding two types of pituitary gonadotropin alpha subunit from the goldfish, *Carassius auratus*. General and Comparative Endocrinology. 1997; 105:372-378.
8. Han YS, Yu JYL. Molecular cloning and sequence analysis of the cDNAs for pituitary glycoprotein hormone alpha subunits from two species of Synbranchiformes, *Monopterus albus* and *Ophisternon bengalense*. Fish Physiology and Biochemistry. 2002; 26:111-120.
9. Cerda J, Chauvigne F, Agulleiro MJ, Marin E, Halm S, Martinez- Rodriguez G *et al.* Molecular cloning of Senegalese sole (*Solea senegalensis*) follicle-stimulating hormone and luteinizing hormone subunits and expression pattern during spermatogenesis. General and Comparative Endocrinology. 2008; 156:470-481.
10. Chang YS, Huang CJ, Huang FL, Lo TB. Primary structures of carp gonadotropin subunits deduced from cDNA nucleotide sequences. International journal of peptide and protein research. 1988; 32:556-564.
11. Querat B, Jutisz M, Fontaine YA, Counis R. Cloning and sequence analysis of the cDNA for the pituitary glycoprotein hormone alpha-subunit of the European eel. Molecular and Cellular Endocrinology. 1990; 71:253-259.
12. Degani G, Goldberg D, Tzchori I, Hurvitz A, Din SY, Jackson K. Cloning of European eel (*Anguilla anguilla*) FSH-beta subunit, and expression of FSH-beta and LH-beta in males and females after sex determination. Comparative Biochemistry Physiology B. 2003; 136:283-293.
13. Lin YW, Rupnow BA, Price DA, Greenberg RM, Wallace RA. *Fundulus heteroclitus* gonadotropins 3. Cloning and sequencing of gonadotropic hormone (GTH) I and II β subunits using the polymerase chain reaction. Molecular and Cellular Endocrinology. 1992; 85:127-139.
14. Koide Y, Itoh H, Kawauchi H. Isolation and characterization of two distinct gonadotropins, GTH I and GTH II, from bonito (*Katsuwonus pelamis*) pituitary glands. International journal of peptide and protein research. 1993; 41:52-65.
15. Hassin S, Elizur A, Zohar Y. Molecular cloning and sequence analysis of striped bass (*Morone saxatilis*) gonadotropin-I and -II subunits. Journal of Molecular Endocrinology. 1995; 15:23-35.
16. Liu Z, Li P, Argue BJ, Dunham RA. Gonadotropin alpha subunit glycoprotein from channel catfish (*Ictalurus*

punctatus) and its expression during hormone-induced ovulation. Molecular marine biology and biotechnology. 1997; 6(3):217-227.
17. Liu Z, Kim S, Karsi A. Channel catfish follicle-stimulating hormone and luteinizing hormone: complementary DNA cloning and expression during ovulation. Marine Biotechnology. 2001; 3(6):590-599.
18. Dickey JT, Swanson P. Effects of sex steroids on gonadotropin (FSH and LH) regulation in coho salmon (*Oncorhynchus kisutch*). Journal of Molecular Endocrinology. 1998; 21:291-306.
19. Dickey JT, Swanson P. Effects of salmon gonadotropin-releasing hormone on follicle-stimulating hormone secretion and subunit gene expression in coho salmon (*Oncorhynchus kisutch*). General and Comparative Endocrinology. 2000; 118:436-449.
20. Mateos J, Mananos E, Martinez-Rodriguez G, Carrillo M, Querat B, Zanuy S. Molecular characterization of sea bass gonadotropin subunits (alpha, FSH beta, and LH beta) and their expression during the reproductive cycle. General and Comparative Endocrinology. 2003; 133(2):216-232.
21. Gen K, Okuzawa K, Senthilkumaran B, Tanaka H, Moriyama S, Kagawa H. Unique expression of gonadotropin-I and -II subunit genes in male and female red seabream (*Pagrus major*) during sexual maturation. Biology of Reproduction. 2000; 63(1):308-319.
22. Weltzien FA, Norberg B, Helvik JV, Andersen O, Swanson P, Andersson E. Identification and localization of eight distinct hormone-producing cell types in the pituitary of male Atlantic halibut (*Hippoglossus hippoglossus* L.). Comparative Biochemistry Physiology A. 2003; 134:315-327.
23. Hearn M, Gomme P. Molecular architecture and bio-recognition processes of the cystine knot protein superfamily: Part I. The glycoprotein hormones. Journal of Molecular Recognition. 2000; 13:223-278.
24. Combarnous Y. Molecular basis of the specificity of binding of glycoprotein hormones to their receptors. Endocrine Reviews.1992; 13:670-691.
25. Lapthorn AJ, Harris DC, Littlejohn A, Lustbader JW, Canfield RE, Machin KJ *et al*. Crystal structure of human chorionic gonadotropin. Nature. 1994; 369: 455-461.
26. Querat B, Tonnerre-Doncarli C, Genies F, Salmon C. Duality of gonadotropins in gnathostomes. General and Comparative Endocrinology. 2001; 124:308-314.
27. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Molecular Biology and Evolution. 2007; 24:1596-1599.
28. Livak KJ, Thomas SD. Analysis of relative gene expression data using real time quantitative pcr and the of $2^{-\Delta\Delta C}{}_T$ method. METHODS. 2001; 25:402-408.
29. Xia H, Chen F, Puett D. A region in the human glycoprotein hormone alpha subunit important in holo-protein formation and receptor binding. Endocrinology. 1994; 134:1768-1770.
30. Zenkevics H, Vose V, Vosekalne I, Bucena A. Partial deglycosylation of alpha subunit modifies sturgeon gonadotropin function. Acta Biochimica Polonica. 2000; 47:815-819.
31. So WK, Kwok HF, Ge W. Zebrafish gonadotropins and their receptors. II. Cloning and characterization of zebrafish follicle stimulating hormone and luteinizing hormone subunits—their spatial-temporal expression patterns and receptor specificity. Biology of Reproduction. 2005; 72:1382-1396.
32. Beitins IZ, Padmanabhan V. Bioactivity of gonadotropins. Endocrinology and Metabolism Clinics of North America. 1991; 20:85-120.
33. Hurvitza A, Degani G, Goldberg D, Yom Dina S, Jackson K, Levavi-Sivana B. Cloning of FSHβ, LHβ, and glycoprotein α subunits from the Russian Sturgeon (*Acipenser gueldenstaedtii*), β-subunit mRNA expression, gonad development, and steroid levels in immature fish. General and Comparative Endocrinology. 2005; 140:61-73.
34. Parhar IS, Soga T, Ogawa S, Sakuma Y. FSH and LH-β subunits in the preoptic nucleus: Ontogenic expression in teleost. General and Comparative Endocrinology. 2003; 132:369-378.
35. Wu F, Zhang X, Zhang W, Huang B, Liu Z, Hu C *et al*. Expression of three gonadotropin subunits in Southern catfish gonad and their possible roles during early gonadal development. Comparative Biochemistry and Physiology A. 2009; 153:44-48.