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EXPRESSION PROFILING OF FSH RECEPTOR (FSH-R) AND DOPAMINE RECEPTOR (D-R) GENES OF INDIAN CATFISH, *CLARIUS MAGUR* (HAMILTON, 1822)

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Key words: *C. magur, Brain, Gonad, FSH-R, Dopamine-R, Expression, RT-PCR.*

Abstract–The Gene expression analysis provides a better understanding of the biological processes like Maturation; Reproduction in which the genes involved in complex regulatory networks of the physiological processes. Quantitative real-time polymerase chain reaction (qRT-PCR) is one of the most specific, precise and also easy to operate technique to examine the target genes' expression. Expression profiles of magur FSH receptor (FSH-R) and dopamine receptor (D-R) genes were examined in the brain and gonads of pre-mature and mature, 6 and 16 h post-injection with Ovatide TM of both sexes. Magur D-R registered highest level of expression in the brain at 6h post-Ovatide injection followed by matured brain and brain at 16h post Ovatide in male fish. *C. magur* FSH-R registered highest expression levels in the male brain at 6h post-Ovatide injection followed by the female brain at 16h post Ovatide. The study showed that D-R is mainly expressed in the mature male brain and FSH-R is also expressed in mature male brain. Ovatide TM injection for induced spawning resulted in significant increase in D-R and FSH-R gene expression in the male brain at 6 hpi.

INTRODUCTION

Aquaculture in India has an industry since the late eighties, with several entrepreneurs taking up aquaculture with carps, catfish, and prawn, etc. as their main product. Government of India has reviewed the research and development in freshwater aquaculture and suggested that emerging areas like catfish culture be given the emphasis for diversification of cultural practices and also identified catfish farming as a National priority in Indian aquaculture.

Catfishes are commercially important for fisheries and aquaculture industry due to several attributes such as good taste, nutritional, and medicinal value. Amongst the catfishes, *Clarias magur* (commonly called magur), an obligatory air-breathing catfish is the most preferred indigenous catfish in India. Catfishes, being air-breathing in nature, can survive in water conditions that are unsuitable for carps. *Clarias magur*, an air-breathing indigenous catfish, commands a good market value (Sinha *et al*., 2014).

C. magur attains sexual maturity after one year weighing 150g (Talwar and Jhingran, 1991). Generally, a high density of 50,000-70,000 fingerlings/ha is recommended for the culture of magur, but a further reduction of stocking density can be adopted for higher growth of fish. It breeds only once in a year, i.e., from June to August, peak period being July. The fecundity of this fish is very low as it ranges from 15000 to 20000. Eggs are dark brown and are demersal and adhesive. *C. magur* is widely distributed in India, Ceylon, Bangladesh, Pakistan, Burma, Malaya, Thailand, Indochina, Philippines, Hongkong, and South China (Thomas, 2003). Natural sources of *C. magur* have been sharply declining due to ecological imbalances in their native breeding grounds (Sinha *et al*., 2014). These imbalances are caused by the increasing use of pesticides in the paddy fields, which are the main breeding grounds for this fish. Therefore, IUCN (2010) has declared this fish species as endangered.

Synthetic hormones are the key to induced breeding, and they are widely used for seed production of carp. However, *C. magur* male does

not release milt even when the hormone is administered and has to be sacrificed for milt collection. Also, this catfish is known to exhibit a peculiar mating behavior. In the wild, male fish show spotting in the dorsal fin but sex identification can be done by examining the genital papillae of the fish. The genital papilla is elongated and pointed in the male magur and shorter and blunted in the female. Female fish have much more rounded belly than males. Before spawning body contact between the pair increases, and they can be observed swimming side-by-side (Talwar and Jhingran, 1991). Therefore, it is necessary to know the molecular mechanisms underlying maturation and spawning in this commercially and ecologically famous catfish. Expression analysis of reproduction-related genes will help in understanding the molecular mechanisms underlying maturation and spawning. The expression profiles of these crucial genes will enable us to determine their possible role in maturation and spawning.

MATERIALS AND METHODS

Site of Experiment

The research work was carried out in the Molecular Biology laboratory of Fish Genetics and Biotechnology Division, ICAR-Central Institute of Fisheries Education (Mumbai) during 2017-19.

Sample Collection

C. magur specimens of ~130g to ~150g were collected from College of Fisheries, Raha (Assam), Powerkheda CIFE Centre, and CIFE Balabhadrapuram station of Kakinada Centre. Few samples of the fish were brought in live condition. The fish were anesthetized using clove oil (50µL/ L water) and aseptically dissected for the collection of brain, pituitary, testis, and ovary tissues. The tissues were stored in RNAlater™ solution (Qiagen, Netherlands) and transported to Fish Genetics and Biotechnology lab, CIFE, Mumbai. The samples were stored at -80°C until RNA isolation was done.

Analysis of Gene Expression

Expression of reproduction-related genes (FSH-R and DP-R) were analyzed in *C. magur* at four different maturity stages namely preparatory, mature, 6 & 16-hour post-OvatideTM injection (a commercial formulation of sGnRH analog along with dopamine antagonist) in brain, testis, and ovary. For this, the individuals were sampled for each tissue at each maturation stage. Total RNA was isolated from all the sampled tissues using Trizol™ reagent (Invitrogen, USA) according to the manufacturer's guidelines. The total RNA isolated from all the samples is normalized and equal quantity (2.5 µg) was used to synthesize first strand cDNA (Thermo Scientific, USA) for relative quantification by real-time PCR and cDNA samples were kept at -20 °C.

Real-time PCR

Real-time PCR amplifications were carried out in a LightCycler® Real-Time PCR detection system (Roche, Switzerland). Expression analysis of selected reproduction-related genes was carried out in the brain, testis, and ovary of *C. magur*. Mature male and females of 1+ year old were injected with Ovatide™ @ 1.0 ml/Kg body weight and kept in tanks. After 6 and 16 hours, brain, testis and ovary were dissected out and stored in RNA later™ solution at -20 °C until RNA isolation. Total RNA was extracted from different tissues using Trizol™ reagent (Invitrogen, USA). Extracted RNA was subjected to DNase I treatment (Thermo Scientific, USA) according to the manufacturer's protocol. First strand cDNAs were synthesized from DNase I treated RNA (5 µg) using RevertAid reverse transcriptase (Thermo Scientific, USA) as per the manufacturer's instructions. Specific primers for real-time PCR analysis were designed for all the genes. The volume of the reaction was kept 10 µL containing 5µl of 2× Maxima™ SYBR Green qPCR Master Mix (Thermo Scientific, USA), 1 µl of (10 pmol) each gene-specific primer and 1 µL of cDNA. For each sample, cDNA from three different individuals was pooled, and three well replicates were used. No template controls were included to detect cross-contamination if any. The default thermal profile was used for PCR amplification, and it consisted of initial denaturation at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 10s, annealing at 65 °C for 10s and extension at 72 °C for the 20s. β-actin was used as reference control as it showed stable expression across all the experimental samples. Melting curve analysis of amplification products was performed at the end of each PCR reaction to confirm that only one PCR product was amplified and detected. Comparative C_T method was used to estimate the relative expression of mRNA. Briefly, ÄCt was calculated by subtracting Ct value of internal control from the target gene (ΔCt = target gene Ct–β-actin Ct) and

then mean ΔCt was calculated from this normalized ΔCt value. Relative expression was then calculated according to the equation $2\text{-}\Delta^{Ct}$.

Analysis of data

Data were expressed as the arithmetic mean ± standard error. The significance of differences was determined using one-way analysis of variance (ANOVA) using SPSS version 16. P value of less than 0.05 were accepted as significant.

RESULTS

Purity and Yield of Total RNA

The total RNA isolated from brain, testis, and ovary tissues of *Clarias magur* at various stages of sexual maturation using the Trizol method. The total RNA concentration of each sample was quantified by Nanodrop spectrophotometer and found to be in the range of 500 to 1000 ng/µl, and the 260/280 ratio ranged from 1.9-2.0 for different samples indicating that the isolated RNA is of good quality. The quality of isolated RNA was also checked on 2% agarose gel, which showed the characteristic 28S rRNA, 18S rRNA, and 5.8S rRNA bands (Fig. 1). The total RNA was subjected to DNase I treatment before first strand cDNA synthesis. In an earlier study, three reference genes (Beta-actin, GAPDH, and EF1a) were tested for stability in selected *C. magur* tissues and maturity stages where beta-actin was found to be more stable (Varghese *et al.*, 2018) Hence, the quality of the cDNA was checked by PCR amplification of the β-actin gene (Fig. 2).

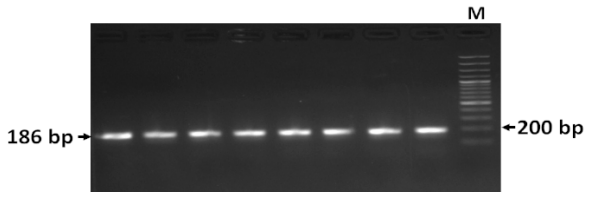


Fig. 1. Total RNA isolated from various tissues of *Clarias magur.*
Lanes 1: Testis; L 2: Ovary; L 3-4: Brain

Expression studies on reproduction-related genes

Expression profiling of reproduction-related genes, namely FSH-R and D-R were performed by real-time PCR. The cDNA sequences of *C. magur* housekeeping genes, namely β-actin, EF1α, and GAPDH, were also taken from NCBI GenBank (EU527190, AB916539, and KC414932, respectively).

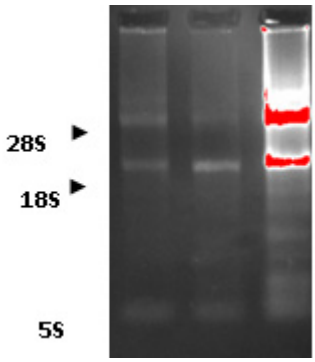


Fig. 2. The first strand cDNA quality checked by PCR amplification of β-actin gene. M: 100 bp+ ladder

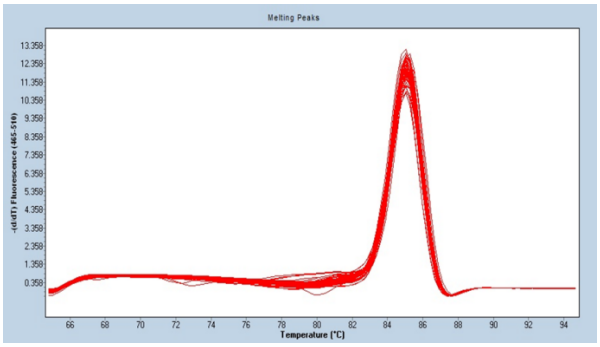
In an earlier study, 3 reference genes (Beta-actin, GAPDH, and EF1a) were tested for stability in selected magur tissues and maturity stages where beta-actin was found to be more stable (Varghese *et. al.*, 2018). Hence, beta-actin was used as a reference gene in the present study. Based on this sequence, oligonucleotide primers for the qPCR analyses were designed to generate amplicons of ~186 bp size. Melting curve analysis was performed to test the specificity of the amplification for all the genes, and it was found that only specific sequence has been amplified in real-time PCR (Fig.3). Expression analysis was carried out in the brain and gonads at different maturity stages, namely premature, mature, 6 & 16 h post-Ovatide™ injection. Relative quantification of gene expression was performed using â-actin as a reference gene. Firstly, the relative expression of the selected 3 genes were determined in the male brain, female brain, ovary, and testis at 4 different maturation stages. The fold changes in expression of these genes were calculated by using values in premature animals as a control to estimate changes in mature fish, 6 h and 16 h post-Ovatide™ treatment. One way ANOVA was performed using the software SPSS, the mean deviation was plotted against different tissues studied.

Validation of reference genes

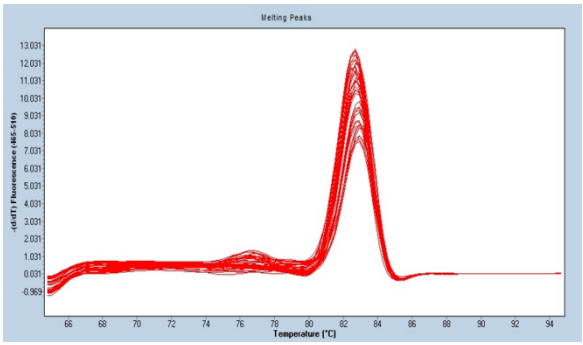
Selection of a suitable reference gene whose expression is stable in all the experimental samples is a pre-requisite for any relative quantification of gene expression. In this study, β-actin was used as internal control.

FSH-R

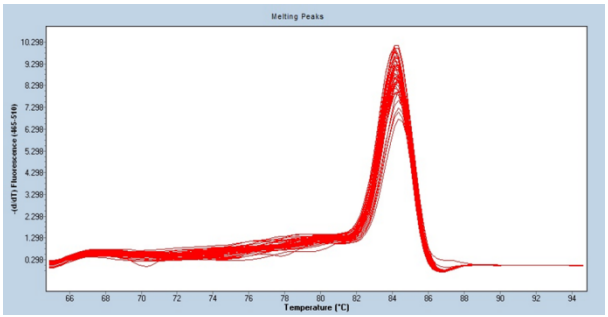
The preparatory stage was taken as a control to calculate fold change in expression. 11-fold upregulation was observed in the male brain at 6h



(A)



(B)



(C)

Fig. 3. Melting peak analysis

A) β-actin; B) FSH-R; and C) D-R amplicons to verify the specificity ofamplification.

post Ovatide injection. In case of the female brain, 6-fold upregulation was seen at 16h post Ovatide injection. No significant change in expression could be seen in gonads (Fig. 4).

D-R

The preparatory stage was taken as a control to calculate fold change in expression. 4.5-fold upregulation was observed in the male brain at 6h post Ovatide injection. No significant change in expression could be seen in the female brain and gonads (Fig. 5).

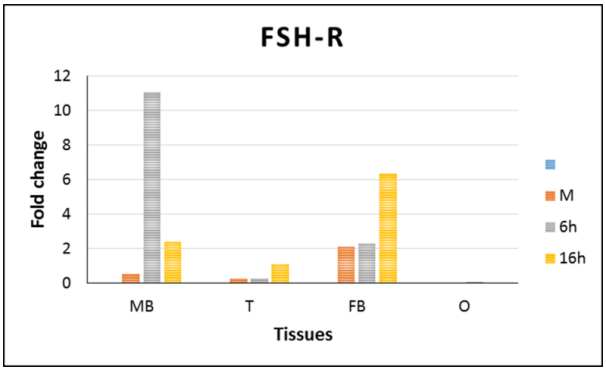


Fig. 4. Real-time PCR analysis of FSH-R gene

FB: Female brain; MB: Male brain; O: Ovary; T: Testis; PM: Premature; M: Mature, 6h: after 6 hours ovatide injection, 16h: after 16 hours ovatide injection.

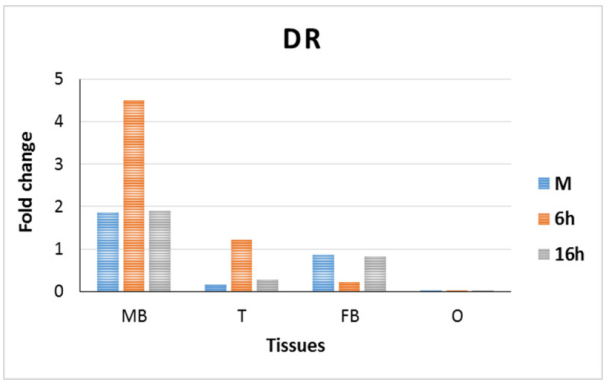


Fig. 5. Real-time PCR analysis of D-R gene

FB: Female brain; MB: Male brain; O: Ovary; T: Testis; PM: Premature; M: Mature, 6h: after 6 hours ovatide injection, 16h: after 16 hours ovatide injection

RESULTS AND DISCUSSION

In this work, the expression profile of *C. magur* dopamine receptor (D-R) and FSH receptor (FSH-R) genes were studied in brain and gonads of premature and mature fishes of both sexes. Ovatide™ is a commercial formulation containing sGnRH analog and dopamine antagonist that is commonly used for induced breeding in carps and catfish. However, it is reported that these synthetic hormone formulations are not as effective in catfish as carps (Binte *et al*., 1988; Ngamvongchon *et al*., 1988). Therefore, an attempt has also been made to see the effect of Ovatide™ injection on the expression levels of these 2 genes at 6 and 16h post-injection.

Magur D-R registered highest expression levels in the brain at 6h post-Ovatide injection followed by matured brain and brain at 16h post Ovatide in male fish. 4.5-fold upregulation was observed in the male brain at 6h post Ovatide injection, and no significant

change was seen in expression in the female brain and gonads. According to Mrzljak *et al*. 1996, among the D2-like family of DRs, the D4 DR (D4R) has been of specific interest because it is densely expressed in the frontal cortex, a brain region where dopamine signaling may modulate working memory and the establishment of memory fields. RT-PCR analysis showed that the expression of the DA2 receptor gene was distributed in various tissues, and high expression levels were observed in the cranial ganglia and the thoracic ganglia (Yang *et al.*, 2018). This gene or its transcript has not been sequenced from African catfish that is closely related to *C. magur*.

C. magur FSH-R registered highest expression levels in the male brain at 6h post-Ovatide injection followed by the female brain at 16h post Ovatide. 11-fold upregulation was observed in the male brain at 6h post Ovatide injection. In the case of the female brain, 6-fold upregulation was seen at 16h post-Ovatide injection, and no significant change in expression could be seen in gonads. In coho salmon, FSH receptor was identified by autoradiography on the surface of the supporting cells in the gonads. Oba *et al*. (1999) reported that the FSHR is expressed on the Sertoli cells. The FSH and LH receptors belong to the rhodopsin receptor subfamily of G protein-coupled receptors. They are characterized by the presence of a particularly large extracellular N-terminal domain (ECD), primarily responsible for hormone recognition and high-affinity hormone binding, joined to a transmembrane domain (TMD) and an intracellular C-terminal domain coupled to a G protein (Vassart *et al*., 2004).

Yaron and Levavi-Sivan, 2011 have informed that if Sertoli cells numbers are too low, germ-cell apoptosis will be increased and sperm production will be reduced. They have observed the elevated FSH blood levels during the start of seasonal testis growth, that is, when the numbers of spermatogonia and their accompanying Sertoli cells start expanding. In mammals, FSH is the most important stimulator of Sertoli-cell proliferation. Sertoli cells provide physical, nutritional, and regulatory (via growth factors) support for developing germ cells. In the spermatogenic tubules, Sertoli cells are the only phagocytotically active cell type, and they remove apoptotic germ cells and cellular remnants discarded by developing germ cells. Eventually, Sertoli cells express receptors for sex steroid hormones as well as FSH receptors.

CONCLUSION

In this work, the expression profile of magur FSH receptor (FSH-R) and dopamine receptor (D-R) genes were studied in brain and gonads of pre-mature and mature of both sexes. An attempt has been also made to see the effect of Ovatide™ injection on the expression levels of these 2 genes at 6 and 16 h post-injection. Magur D-R registered highest expression levels in the brain at 6h post-Ovatide injection followed by matured brain and brain at 16h post Ovatide in male fish. 4.5-fold upregulation was observed in the male brain at 6h post Ovatide injection, and no significant change was seen in expression in the female brain and gonads. Among the D2-like family of DRs, the D4 DR (D4R) has been of specific interest because it is densely expressed in the frontal cortex, a brain region where dopamine signaling may modulate working memory and the establishment of memory fields (Mrzljak *et al.*, 1996). *C. magur* FSH-R registered highest expression levels in the male brain at 6h post-Ovatide injection followed by the female brain at 16h post Ovatide. 11-fold upregulation was observed in the male brain at 6h post Ovatide injection. In the case of the female brain, 6-fold upregulation was seen at 16h post-Ovatide injection, and no significant change in expression could be seen in gonads. In coho salmon, FSH receptor was identified by autoradiography on the surface of the supporting cells in the gonads. Oba *et al.* (1999) reported that the FSHR is expressed on the Sertoli cells and the FSH and LH receptors belong to the rhodopsin receptor subfamily of G protein-coupled receptors.

In conclusion, the present study of Expression profiling showed that D-R is mainly expressed in the mature male brain and FSH-R is also expressed in mature male brain. Ovatide™ injection for induced spawning resulted in significant increase in D-R and FSH-R gene expression in the male brain at 6 hpi. This has implications in the better understanding of the maturation and reproduction processes in catfish and *C. magur*, in particular cases.

REFERENCES

Binte, S.T., Ahmad, A.B.O. and Sulaiman, M.Z.B. 1988. Induced spawning techniques practised at Batu Berendam, Melaka, Malaysia. *Aquaculture.* 74(1-2): 23-33.

Mrzljak, L., Bergson, C., Pappy, M., Huff, R., Levenson, R. and Goldman-Rakic, P.S. 1996. Localization of

dopamine D4 receptors in GABAergic neurons of the primate brain. *Nature.* 381(6579): 245-248.

Ngamvongchon, S., Pawaputanon, O., Leelapatra, W. and Johnson, W.E. 1988. Effectiveness of an LHRH analogue for the induced spawning of carp and catfish in Northeast Thailand. *Aquaculture.* 74(1-2): 35-40.

Oba, Y., Hirai, T., Yoshiura, Y., Yoshikuni, M., Kawauchi, H. and Nagahama, Y. 1999. The duality of fish gonadotropin receptors: cloning and functional characterization of a second gonadotropin receptor cDNA expressed in the ovary and testis of amago salmon (*Oncorhynchus rhodurus*). *Biochemical and Biophysical Research Communications*. 265(2): 366-371.

Sinha, M., Mahapatra, B.K., Saha, D. and Maitra, N.J. 2014. Mass scale seed production of Magur: *Clarias batrachus* at farm level through improvised modifications. *International Journal of Fisheries and Aquatic Studies.* 2(2): 210-214.

Talwar, P.K. and Jhingran, A.G. 1991. *Inland Fishes Of India and Adjacent Countries.* Volume 2. A.A. Balkema, Rotterdam.

Thomas, P.C. 2003. *Breeding and Seed Production of Fin Fish And Shell Fish.* Daya Books.

Vassart, G., Pardo, L. and Costagliola, S. 2004. A molecular dissection of the glycoprotein hormone receptors. *Trends in Biochemical Sciences.* 29(3): 119-126.

Varghese, T., Chanu, T.I. and Jain, K. 2018. Reference gene selection for quantitative real-time RT-PCR normalization in *Clarias magur* at different larval developmental stages. *Indian Journal of Animal Sciences.* 88(3) : 380-382.

Yang, X., Huang, G., Xu, M.J., Zhang, C., Cheng, Y. and Yang, Z. 2018. Cloning and functional characterization of the DA2 receptor gene in Chinese mitten crab (*Eriocheir sinensis*). *PloS One*. 13(3): e0193999.

Yaron, Z. and Levani-Sivan, B. 2011. Endocrine regulation of fish reproduction. *Encyclopedia of Fish Physiology: From Genome to Environment.* 2: 1500-1508.