EXPRESSION OF MIS RECEPTORS IN THE OOCYTE OF INDIAN MAJOR CARP, *CIRRHINUS MRIGALA*

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Introduction:

Many actions of steroids are too rapid to be readily explained be the classical genomic mechanism of steroid action mediated by activation of nuclear steroid receptors. The receptors mediating these rapid steroid actions have been studied extensively in many laboratories over the past 30 years. The binding moieties with the characteristics of progestin membrane receptors have been demonstrated in fish and amphibian oocytes and some other vertebrate tissues. Maturation-inducing steroid (MIS) receptors are potential intermediaries in meiotic maturation of oocytes. 17α,20β-dihydroxy-4-pregnen-3-one (17α,20β-DHP) has been identified as the MIS in many teleosts, and induces oocytes to enter into final meiotic maturation leading to ovulation. The MIS receptors are membrane progestin receptors (mPR) and are responsible to mediate rapid non-genomic progestin action. The mPRs are mainly consisting of three forms such as mPRα, mPRβ and mPRγ. An attempt has been made to identify the receptors encoding gene in an Indian major carp, *Cirrhinus mrigala*.

Methods:

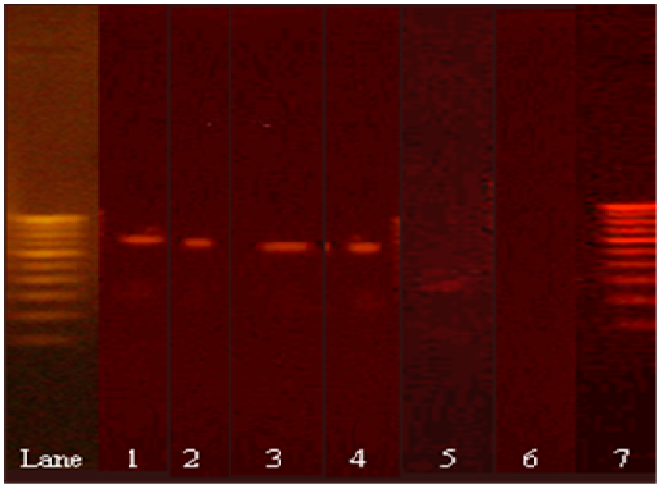
The immature and mature oocytes of *C. mrigala* were collected during the month of May (vitellogenic stage) and August (gravid stage) in RNA later, and total RNA was extracted using guanidine thiocyanate method. The extracted RNA was reverse transcribed to cDNA by M-MuLV, RT-PCR kit (Medox). Specific primers (Sigma, USA) were constructed for mPRα, mPRβ and mPRγ and applied to catch the specific gene. The primers are mPRα - sense 'CTGTCCTGTACGGGCTG', and antisense 'CTCCTGCTTGTCTTCTAGATACGC', mPRβ - sense 'ACTGGTTTCCCCGTCTACCT', and antisense 'GTACAGGACAGCCAGGCCAGGA', mPRγ sense 'AACTCCTCGGATCCCAAAC', and antisense 'TGTGATAGCACAGCCGAGAC'. The PCR products were visualized by gel electrophoresis using ethidium bromide. The PCR amplified product was quantified and sequenced (Genei, India).

Results and Discussion:

The mPRα does not show any difference between the vitellogenic and gravid stage of oocytes (lane 1 and 2) and the mPRβ has the band intensity difference between the two stages (lane 3 and 4) whereas mPRγ could not be identified in the gravid stage (lane 6) in reference with the 100bp DNA marker (lane 7). The results confirm the possible expression of membrane progestin receptors mPRα and mPRβ in the matured oocytes and mPRγ gene expression in the mid vitellogenic stage of *C. mrigala*. This is the first demonstration of MIS receptor gene expression in the oocytes of *C. mrigala.*

In previous studies Yukinori et al. [1] reported that in channel catfish mPRα transcripts gradually increased during oocyte growth, mPRβ varied slightly throughout the reproductive cycle whereas in zebrafish mPRβ level increased during the follicular development stage. In sea trout, Zhu et al. [2] reported that mPRα was expressed in the plasma membrane, mPRβ brain and oocyte, and mPRγ was expressed in the oocyte as well as kidney. The present result also suggests that the mPRγ is not playing any role in the final maturation, however mPRα and mPRβ transcription is seasonally varied with maturity of oocytes. The partially sequenced genes (mPRα-979bp, mPRβ-981bp and mPRγ-521bp) were used to construct phylograms which indicate that *C. mrigala* is closely related to *Carassius auratus* and *Danio rerio* in comparison with other teleosts.

Fig. 1. The transcripts of mPRα, β and γ of vitellogenic (lane 1, 3 and 5) and gravid (lane 2, 4 and 6) oocytes of *C. mrigala*.



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References:

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