Consolidated summary
Present study concentrated on studying the expression pattern of three important terminal enzymes (cytochrome P450 ovarian aromatase-
cyp19a1, cytochrome P450 11β-hydroxylase-11β-H and 11β-hydroxysteroid dehydrogenase-11β-HSD2) during gonad differentiation, by real-time PCR which might be controlling the estrogen and androgen ratio during gonad formation and thereby assisting in initiating and establishing the differentiation of the bipotent gonads to develop either as male or female. Further present study also aimed to investigate the role of these steroidogenic enzymes during puberty and reproductive cycle in catfish by analyzing their expression and activity at various stages of gamete development and maturation. Additionally attempts were made to understand the interplay of steroidogenic enzyme genes and their modulators such as gonadotropins and thyroxine during various stages of gametogenesis. These aspects have been studied as four major chapters and the result and important findings are summarized below.

Chapter 1: Molecular cloning, expression and enzyme activity of ovarian aromatase (cyp19a1) during ovarian development and oogenesis in air-breathing catfish Clarias gariepinus and in vivo hCG-modulation of cyp19a1 during female reproductive cycle

To investigate the specific role of cytochrome P450 ovarian aromatase (cyp19a1) during ovarian development and annual reproduction in air-breathing catfish C. gariepinus we initially cloned full length cDNA of cyp19a1 from the catfish ovarian tissue containing 1551 bp of open reading frame (ORF), which displayed 79% homology with channel catfish cyp19a1. Characterization of the encoded protein in non–steroidogenic COS-7 cells illustrated that cyp19a1 ORF could efficiently catalyze the aromatization reaction by producing estradiol-17β (E₂) from testosterone. Tissue distribution pattern revealed the
predominance of ovarian form in the ovary with trace amount being detected in other tissues including brain. The seasonal expression profile of *cyp19a1* measured using relative real-time PCR in connection with the different ovarian follicular stages revealed, high expression in the prespawning phase when compared to spawning, preparatory and regressed phases which corroborated with the measured serum E$_2$ levels. The enzymatic activity assessed by means of a sensitive radiometric assay in ovarian tissues collected during different phases recorded results consistent with the expression levels. Ontogeny results displayed sexual dimorphism, with relatively early expression of ovarian form in ovary than the testis clearly indicating its role in female sex differentiation. Further to understand the role of gonadotropins in modulating the *cyp19a1* transcripts and enzyme activity, we performed an *in vivo* study by injecting human chorionic gonadotropin (hCG) in adult catfish at three phases and observed phase-dependent stimulatory effect of hCG in the preparatory and prespawning phases. However, in spawning phase induction of transcripts was not sustained suggesting that the stage of oocyte during ovarian cycle was crucial and gonadotropins could not override the induction of *cyp19a1* transcripts once the follicles have undergone or undergoing meiotic maturation. These results demonstrates specific role of *cyp19a1* during ovarian differentiation by displaying dimorphic expression during the period of gonadal differentiation in catfish, and a distinct expression pattern and activity during ovarian cycle, indicating its involvement in maintaining the periodicity of the ovarian cycle by modulating the levels of E$_2$.

**Chapter 2: Cloning, expression and enzyme activity analysis of testicular 11β-hydroxysteroid dehydrogenase during seasonal cycle and after hCG induction in air-breathing catfish *Clarias gariepinus***
A full length cDNA encoding 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) was cloned from testis of air-breathing catfish, *C. gariepinus* which showed high sequence homology to zebrafish and eel. 11β-HSD2 ORF was then transfected to COS-7 cells. The transfected cells converted 11β-hydroxytestosterone (11-OHT) to 11-ketotestosterone (11-KT) at a considerable rate than mock transfected cells. Tissue distribution analysis by RT-PCR revealed prominent expression in testis, anterior kidney and liver. Expression of 11β-HSD2 in the testes was assayed by real-time PCR during four testicular phases (preparatory, prespawning, spawning and resting phase) and was found to peak during the prespawning phase and gradually decline during the spawning and resting phases. With NAD⁺, testicular microsomes oxidized 11-OHT with apparent $K_m$ of 56 ± 4 nM and $V_{max}$ of 55 ± 6 pmol/h/mg-protein, respectively. Seasonal 11β-HSD2 dehydrogenase activity in testicular tissues revealed highest production of 11-KT during the prespawning phase. Serum 11-KT levels corroborated well with the levels of transcripts and activity of 11β-HSD2. *In vivo* hCG administration enhanced 11β-HSD2 expression in the testis, especially during the prespawning phase, at 4, 8, 12 and 24h after induction. It also augmented 11-KT production by testicular microsomes at 8 and 24h. Ontogeny study indicated that this enzyme is expressed after the fate of gonad is determined. However, levels of 11β-HSD2 transcripts were significant during testicular differentiation. Thus the spatiotemporal expression results supported with dehydrogenase activity and circulating 11-KT. Based on these observations, present study clearly indicated a major role for 11β-HSD2 during testicular differentiation and seasonal testicular cycle in catfish.

**Chapter 3: Cloning and expression analysis of testicular 11β-hydroxylase during seasonal cycle and after hCG induction in air-breathing catfish Clarias gariepinus**
Cytochrome P450 11β-hydroxylase (11β-H) gene is involved in production of 11β-OHT, the precursor for the synthesis of 11-KT, a potent androgen for several male fishes. In the present study, a partial 11β-H cDNA sequence (768bp) was cloned from air-breathing catfish *C. gariepinus* which shared high homology with zebrafish (76%) and rainbow trout (72%). Tissue distribution analysis revealed expression of 11β-H in most of the tissues with predominance in testis, anterior kidney, liver and gills. Ontogeny study by real-time PCR from 45 days post hatch (dph) till 260 dph in male and female catfish larvae, detected the expression of 11β-H from 55 dph suggesting that 11-KT might not be required during testis formation but subsequent increase in transcript levels clearly indicated a crucial role of 11β-H in advancing testicular growth and development. Bleak expression was detected in female catfish larvae from 150 dph. Expression of 11β-H correlated well with testicular recrudescence, displaying maximum expression in the prespawning phase. Administration of hCG elevated 11β-H mRNA levels from 4h in the prespawning testis while priming was ineffective in the resting phase. These results tend to suggest that onset of spermatogenesis during testicular recrudescence is marked by corresponding increase in the mRNA levels of steroidogenic enzymes 11β-H and 11β-HSD2 (Chapter 2).

**Chapter 4: Thiourea-induced alterations in the expression of some steroidogenic enzymes in air-breathing catfish *Clarias gariepinus***

Previous study from our laboratory on thiourea-induced thyroid hormone depletion in mature male demonstrated that thyroid hormones play a significant role in testicular function of catfish. In the present study, we aimed to analyze the changes in the expression pattern of several steroidogenic enzyme genes using semi quantitative RT-PCR after
thyroid hormone depletion by thiourea in adult male and female catfish. There was a marked decrease in the $11\beta H$ expression in the testis while no changes were observed in kidney. A marked decrease in $11\beta$-HSD2 transcript level in testis, liver and kidney were observed in the thiourea-treated males. The observed results corroborate our earlier findings on testicular regression after thyroid hormone depletion. In females, expression of cyp19a1 increased in the experimental group when compared to control. No significant changes were observed in the transcript levels of 3$\beta$-hydroxysteroid dehydrogenase, cytochrome p450c17$\alpha$ enzyme, and 20$\beta$-hydroxysteroid dehydrogenase in both males and females. Thus, thyroid hormones might regulate expression of terminal steroidogenic enzyme genes and thereby reproduction in catfish.

In conclusion, present study demonstrated crucial role of cyp19a1 in catfish ovarian development and oogenesis, with early expression during ovarian differentiation and marked correlation in the seasonal expression, enzyme activity and plasma estradiol-17$\beta$ levels during different follicular stages. On the other hand, $11\beta$-H and $11\beta$-HSD2 genes were not detected at the time of testis formation but their levels increased with the onset of spermatogenesis. Further seasonal variations in $11\beta$-H and $11\beta$-HSD2 transcripts vis-à-vis levels of 11-KT during various phases of reproductive cycle attribute specific role for these enzymes in the entrainment of testicular cycle. Thyroid hormone depletion using thiourea and gonadotropin (hCG) exerted phase-dependent effects during gonadal recrudescence by modulating the expression of these steroidogenic enzyme genes.