12. Histoarchitectural modulation encountered in the gonads of Cadmium (metalloestrogen) exposed *Oreochromis mossambicus*

12.1 Introduction:

An organism express its routine natural as well as unusual events through many responses such as morphological (internal & external), physiological, biochemical (including enzymes and hormones) and histological means. The symptomatic changes be the ideal diagnostic tool to understand the impairments in the body. It would help to recover the individuals to its originality or suggest for managerial purposes.

The phenomenon of endocrine disruption leads widespread concern because of its potential to cause deleterious physiological effects in animals. A variety of chemicals found as contaminants in freshwater and marine environments have greater implications, as potential endocrine disruptors. About 80,000 chemicals have been introduced into the environment within the last 50 years (Curtis and Skaar, 2002). There are mounting evidences that some of these chemicals may pose extensive, even global, threats to inhabiting animals, wildlife and humans (Curtis and Skaar, 2002, Fox, 2001 and Vos et al., 2000). One of the most complex and heterogeneous classes of these compounds are those that might act upon the endocrine system, thereby adversely affecting reproduction and development (Leino et al, 2004). Examination of gonadal anatomy and histology could be much beneficial in understanding and assessing the effects of potential endocrine disrupting chemicals in organisms including fish. Therefore, the present study describes the normal gonadal histology of tilapia (*Oreochromis mossambicus*), the test organism, with respect of endocrine disrupting chemicals with its different modes/mechanisms of action on the histological structure of the ovaries and testes in the sub adult /early maturing fishes due to the induction of exposed cadmium (metalloestrogen). Endocrine disrupting chemicals (EDCs) even in small quantity may yield a greater impact, in subtle ways they affect behavior
and secondary sexual characters as well as the gonads themselves. It has been suggested that a variety of biomarkers and bioassays in the laboratory and in semi-field or field studies can be used to determine the consequences of potential EDCs (Ankley et al., 2001; Gray et al., 2002; Parrott and Wood, 2002; Van der Oost et al., 2003; Vos et al., 2000). Fishes are the ideal and potential target to evaluate the environmental endocrine disruption ultimately useful data have been obtained from biomarkers assays as was suggested (Van der Oost et al., 2003). Effects of different EDCs in relation to gonadal histopathology have not been as thoroughly characterized as of some other diagnostic end points (Leino et al, 2005).

12.2 Materials and methods
The cadmium treated experimental fishes gonadal tissue ovary and testis were dissected out and anatomically analysed (see chapter.10) then they were processed for cytomorphological observation under the microscope. The processing techniques were given elsewhere in chapter 7.

12.3 Result
12.3.1 Histological stages of *O.mossambicus* ovarian development
1. Primary growth Oogonia and primary oocytes
   - Oocytes in nests; small cytoplasmic volume
   - Oocytes larger, out of nests, surrounded by follicle cells; many pleiomorphic nucleoli bordering the nuclear envelope (Photo.8)
2. Cortical alveolus Appearance of cortical alveoli and scattered small lipid droplets
3. Early vitellogenic Appearance of yolk bodies: initially few and small; ultimately many and variably-sized; centrally located germinal vesicle is round to oval with several peripheral nucleoli.(100 ppt Juvenile)
4. Late vitellogenic Germinal vesicle loses nucleoli, moves towards the periphery and breaks down; yolk bodies frequently fill the entire center of the oocyte and a germinal vesicle may not be evident.

5. Mature/spawning oocyte Germinal vesicle breakdown complete; yolk bodies fuse and may become larger than cortical alveoli. (150 ppt juvenile)

12.3.2 Histological stages of *O. mossambicus* testicular development. (Photo.9)

1. Spermatogonia
   - Primary spermatogonia: large cells near edges of tubule; have a lightly staining nucleus with a prominent nucleolus
   - Secondary spermatogonia: clusters of medium-sized cells with a round, lightly basophilic nucleus; cluster or cyst is the result of several mitotic divisions of a primary spermatocyte

2. Spermatocytes
   - Primary spermatocytes: smaller cells with smaller, more basophilic nuclei than spermatogonia: will undergo meiosis I to produce secondary spermatocytes.
   - Secondary spermatocytes: small cells with smaller, more basophilic nuclei than primary spermatocytes: will undergo meiosis II to produce spermatids

3. Spermatids and some spermatozoa in lumen of seminiferous tubule; small tubule lumen Spermatids have a small, intensely basophilic nucleus; they mature into spermatozoa (100 & 150 ppt juvenile)

4. Abundant sperm in an expanded lumen (Adult, control)

In the case of 100ppt and have Early vitellogenic and late vitellogenic germinal vesicle loses nucleoli, moves towards the periphery and breaks down; yolk bodies frequently fill the entire center of the oocyte are seen. 150ppt treated groups ovary have different stages of ovarian follicles were found oocyte Germinal vesicle breakdown complete; yolk bodies fuse and may become larger than cortical alveolar stages were found (Fig.8).The abundance of primary and secondary spermatocytes and also early spermatids are seen in the case 100 ppt cadmium
Photo-8 Cytomorphology of cadmium exposed ovary in *O.mossambicus*

100 ppt Juvenile

Final oocyte maturation

150 ppt juveniles

Post-ovulatory Stage
Photo-9 Cytomorphology of Cadmium exposed Testes O.mossambicus

- 100 ppt juvenile testis
- Spermatids
- Spermatic maturation phase
- Control male (adult)
- Convoluted seminiferous
- Cavity of tunica vaginalis
- 150 ppt adult testis
- 100 ppt male
- 150 ppm
- 150 juvenile testes
- Spermatogonia
exposures whereas the 150 ppt treated have spermatids and some spermatozoa in lumen of seminiferous tubule and small tubule lumen Spermatids (Photo.9).

12.4 Discussion

The anatomical and cytomorphological analysis of the ovaries and testis revealed the effect of EDC that profoundly influenced the testicular maturation. Percentage of testicular stages present such as primary and secondary spermatogonia and spermatocytes, can provide information as to whether any of these stages has a typical distribution. Unlike ovaries, the relatively small and more numerous testicular germ cells are difficult to count properly without an ocular grid or similar device. Smith (1978; Leino et al, 2005)

Spermatogonia are located in small peripheral cysts in the tubule; these cysts enlarge and extend toward the tubule lumen as spermatogenesis proceeds. Five stages of germ cell development are readily identified in the fathead minnow: (1) primary spermatogonia, (2) secondary spermatogonia, (3) primary spermatocytes, (4) secondary spermatocytes, and (5) spermatids and spermatozoa (Grizzle, 1979, Jensen et al., 2001 and Smith, 1978). The presence or absence of these stages in a histological section, then, can be used to judge the state of testicular maturity. However, a better idea of how many sperm are being produced may be obtained by considering the relative size and sperm content of the seminiferous tubules (Gimeno et al., 1998, Leino et al., 1990 and Smith, 1978). Seminiferous tubules with different types of germ cell cysts (from spermatogonia to spermatids) and apoptotic spermatids are depicted in the 50 days old treated juveniles (Photo.9). They were observed predominantly close to the tunica albuginea. Primary or mature (differentiated) spermatogonia. were also found more often close to the tunica, but showed a less restricted distribution pattern than immature spermatogonia. Compared to the cells in mammals, these cell types could be considered, respectively, as mature (differentiated) and immature (undifferentiated) type A spermatogonia (Schulz et al, 2005) In nile tilapia spermatogenic cysts form when Sertoli cells enclose a single primary
spermatogonium (Pudney, 1993). The germ cells derived from a single primary spermatogonium then divide synchronously to constitute an isogenic germ cell clone that is bordered by the cytoplasmic extensions of a single layer of Sertoli cells. Hence, in cystic spermatogenesis, a Sertoli cell is usually in contact with only a single germ cell clone that is accompanied through the different stages of spermatogenesis by its associated group of Sertoli cells. Thus, life-long exposures to very low and environmentally relevant concentrations of environmental estrogen have severe and deleterious effects on reproductive success for breeding populations of *O. mossambicus* and there is evidence that these strong effects will occur in other species at similar concentrations (Balch et al. 2004; Lange et al. 2001). Furthermore, these effects occur at concentrations that are at least an order of magnitude lower than for short-term exposures of mature fish proximate to spawning time; (Seki et al. 2002, Van den Belt et al. 2002, and Zillioux et al. 2001). Nash et al. (2004); provide other examples of lower sensitivity to adult-only exposure. Early primary oocytes have a round or oval nucleus with a few variably sized nucleoli. The germinal epithelium of *O. mossambicus* has an apparently random distribution of spermatogonia along the entire length of the tubule, the so-called “unrestricted” type of testis as described (Grier, 1981 and Jensen et al., 2001). In the case of control no such evidence were seen because the gonad is not developed. Therefore, this evidences are clearly show the early stages exposure of EDCs leads to very drastic affect in the reproductive system of the organism. The different reproductive components have differential sensitivities to endocrine disruption and that these sensitivities are dependent on the length of exposure and timing of exposure related to development and maturity(Thorpe et al, 2003; Nash et al, 2004). Full life-long exposures had a strong impact on reproductive success at a concentration that was at least one order of magnitude less than when fish were given short-term exposures proximate to spawning (Andersen et al, 2003; Balch et al, 2004; Thorpe et al, 2003).