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IV DISCUSSION AND CONCLUSION

4.0 Introduction:

The present investigation is undertaken to study the histology and histochemistry of gonads of some Larvivorous fishes in Yerala and Krishna river in Sangli district.

The result observed are derived by employing well accepted methods and techniques in histology and histochemistry.

In the present chapter, these results and observation are compared to those of available literature to derive a define conclusion.

4.1 Guppy (*Poecilia reticulata* Peters):

Now a days guppy fishes and mosquito fishes (*Gambusia affinis*) are gaining much importance in public heath and hygene. The guppy fishes play an important role in a control of insect vectors of wide range diseases such as elephantiasis, malaria, dengue fever and chikun guniya (Chatterji, 1985), however, uptil now, the histology and histochemistry of gonads in guppy fishes was least studied (Leatherland and Pandey, 1969; Pandey, 1969a, Rosen and Gordon 1963; stanely et.al 1965) hence for the present investigation, the histogoly & histochemistry of gonads in *P. reticulata* is studied.

4.1.1 Testis and gonopodium :

In the guppy, *Poecilia reticulata,* an ovoviviparous cyprinodont, gonadal sex differentiation occurs at some period during the prenatal life

active engagement in the steroid biosynthesis and the spermatogenesis. The strong alcinophillic staining in the interstitial cells or leydig cells also suggestive of the role of mucins in the testicular differentiation. The positivity of sperm heads with alcian blue pH 2.5 indicate the presence of enzyme Hyaluronidaze (vibhute, 1982).

The problem of nourishment of sperms inspite of any accessory male reproductive gland is being solved by testicular sperm ducts. The primary intercalated sperm ducts among the testicular follicles played important role in the nourishment and maturation of spermatozoa.

This structure of sperm duct is comparable with epidydamis of mammals (Mote, 1982). The sertoli cells since to be separated from testicular folliculi and aggregated into secretory epithelium of the sperm ducts. A very intense basophilic staining was obtained in the cells of sertoli. Few of the sertoli cells may have detached from the secretory epithelium and added to spermatic Milieu. The secretory material may be the albumen like proteins and other mucins of acidic nature, as it revealed by strong AB pH 2.5 reaction in the sertoli cells and mucous secreting cells,.

The sertoli cells of sperm ducts intensly reacted with PAS, not only suggestive of supportive function of sertoli cells but the nutritive role also. The PAS positive granular staining in sertoli cells clearly indicate the presence of glycogen in the cells, however the second epithelial cell

type is a steeriociliated type which might involved in the sweeping down of the sperms through the sperm duct.

The strong acidophillic staining in the basement membrane of testicular follicle indicative of presence of myoepithelium for the rhythmetic contraction and the expulsion of the sperms.

Gonopodium:

The evolution of sex organs and copulatory organs is the subject of much discussion. The structure of claspers in the Elasmobranch fishes and the modification of pelvic fins have been will illustrated in many textbooks of vertebrate zoology (Kotpal, 1984, Dhami 1983, Ovr, 1976, Gupta and Gupta 2006, Khanna 1985, Lagler 1962, Rosen and Gordon 1963, Hopper 1965, Turner 1941) The gonopodium is formed from the modified anal fin, however the rectangular guard plates of injecting canal might had evolved from the modified cycloid scales.

The working mechanism of gonopodium in poeciliids has well illustrated by Rosa-Molinar et al 1998; Rosen and Gorden, 1953. Recently it has observed in our laboratory that the radially arranged bundles of smooth muscles play an important role in steering mechanism of gonopodium with the help of apical hook, the gonopodium of is injected suddenly in the female genitalia. Thus it works like a hypodermic syringe.

4.1.2 Ovary of Guppy (*P. reticulata*):

In the viviparous teleosts where the ovary produces eggs, stroes sperm, serves as a site for fertilization and provides nourishment for the development of embryo along with yolk sac up to advanced stage hence, the histological structure of ovary is complicated, it serves site for fertilization, vitellogenesis, and the development of follicular placenta. The ovarian structure and the presence of corpus atreticum or corpus luteum had been reported in the guppy ovary. (Lambert, 1970). The similar presence of corpus luteum has also reported in the gold fish. (Khoo, 1975).

Even the site of hormone production in ovary of guppy had earlier reported (Lambert, 1966). The ovarian structure reported by Jaski, 1939. Histoendocrinology of gestation in the guppy and the role of corpus luteum in steroid biosynthesis had reported by stolk (1950).

The histology, histochemistry and the physiological relations of ovary with developing embryo in viviparous teleost is rather like that of one act play drama. The germinal epithelium is proliferated after the surge of pituitary gonadotropins (Leatherland and Pandey, 1969).

The two ovarian horns work alternatively for the division of physiologival labour. The functional horn is characterized by the presence of primary oocytes, maturing oocytes, and fertilized zygotes since, the fertilization in guppy is in situ or internal. The positive staining of Alcian

Blue in the functional ovary has revealed in the follicular cells and perinuclear cytoplasmic granules, rather these are the stages of vitellogenesis. The early young oogonia devoid of any cytoplamic inclusions but it was noticed after vitellogenesis that the yolk deposited in the ovarian cytoplasm. The follicular cells of oocytes stain intensely with HE and Alcian Blue pH 2.5 and hence. It is predicted that or belived that follicular cells involved in the process of viteliogenesis and the biosynthesis of ovarian steroid (Guraya, 1976; Pawar, 1978). The perinuclear cytoplasmic secretion may aid in the formation of proteneous yolk bodies and the cortical granules. (Mote, 1988). The similar theory of cellular secretion (GIRL) reported in the typical mammalian ovum and even in non-mammalian vertebrates for the mucins (Nagbhushanam et al.1975).

The presence of corpus luteum and its involvement in steroid biosynthesis and pregnancy is well illustrated in guppy and other higher mammanls. (Lambert, 1970; Mote 1986, Bjersing, 1969; Mirecka 1973) The strong PAS positive staining reactivity in corpus luteum has been reported in the microchiropteran bats (Patil, 1982), the lysosomal enzyme activity in the corpus luteum of mircochiropteran bat (Kanase, 1978; Dhange 1982) the corpus luteum and alternate functioning of the uterine horns in the ovaries of Indian fruit bats is well illustrated by Gopalkrishna (1987).

In guppy gestation period extends for a month, a follicular placenta is found in live bearing guppy (Hoar 1969). The cite of embryonic implantation developed into follicular placenta, a strong reaction with Alcian Blue for ovarian connective tissue, blood vessels and the placental site indicate the presence of sulphated mycopolysuccharides and the chondratin sulphate (Nalwade 1975, Spicer et al 1967). The site of follicular implantation is characterized by the presence of the mucins, lipids and the glycogen granules. The same site had reported by Sastry, 2004 and Srivastava, 1999.

The process of parturation is dynamically controlled by the sudden surge of pituitary gonadotropins in the blood. It is also partly might be controlled by the ovarian estrogen.

After delivery the ovarian follicle is converted into the corpus luteum. The corpus luteum of previous generation prevent the fertilization of ovum in the same horn (Gopalkrishna, 1986). The freshly hatched youngone may be predated upon by the female, so the female is quickly separated from the yougone or the vice versa. (Scrimshaw, 1944) when embryo practically completes the development, the yolk sac gradually reduced (Agarwal, 1985; Balensky, 1970 Lagler et al 1977) and absorbed into gut prior to hatching, however the process of complete development and the complicated control of ovarian follicular gestation yet to be

illucided & needs further fine electron microscopic research work on ovary and endocrinological aspects.

The newly hatched fry is well provided with the hundreds of star shaped melanophores in interorbital region, might have role in the absorption of solar radiations and heat production for the development of embryo. (Chen and Chavin, 1986; Chavin 1956; Fox, 1957; crozier, 1974, Cott, 1940).

4.2 *Chanda ranga* (Ambassis):

The glass fishes (Ambassis) *Chanda ranga* are very important for the biological control of guinea worms, rotifers and mosquito larvae, (Job; 1941) hence this fish type is selected for this research work.

At the beginning the fish was studied for its habitat and wide range of distribution but later on the sexual dimorphism and for more details the gonadal histomorphology and histochemistry.

4.2.1 Sexual dimorphism and Exoskeleton:

This glass fish has wide range of habitat, it is not only found in water stagnancies of many rivers but it was observed for its ample population in the estuarine water also (personal observation at Bhagwati port-Ratnagiri) .

The sexual diamorphism is not distinct however by close examination of the fishes, it is found that the female is two fold larger

than the male. However, clear cut sexual dichromatism is found to be absent.

Exoskeleton:

There are very thin closely applied obliquely and linearly arranged cycloid scales in the integument present. The interscallular connective tissue is rainforced by the presence of cementing substance, chondratine, since the interstitial space was strongly positive with AB pH 1.

Only single type of chromotophores are found, their shades are vary with the colour of the natural fresh water hence important for comouflage. The chromatophores are specifically arranged at the interscallular spaces. Such a type of exoskeleton quite adaptable with the pond water and prevent the attack of microorganisms. (Khanna, 1985; Gupta & Gupta, 2006; Fox; 1957; Crozier, 1974)

4.2.2 Testis :

In teleost, the structural anatomy of testis is quite different in different fishes. In most of the carp fishes the testis become ribbon like or filamentous (Khanna, 1985, Lagler et al, 1977; Kotpal, 1992) but in few catfishes the testis become filamentous, differentiating into the secretory part and the generative part.

In the *Chanda ranga* the testis become pyriform slightly pointed at anterior side but all the while rounded testis of both the sides are connected with intertesticular septa and the common sperm duct in

between two. The sexual breeding period is seasonal, the sexual quicent period falls during December, to February. The prebreeding during the March-April, active breeding period (May to September) and post breeding during the October-November. The gonadal size regresses during the winter months of season.

Each testis is elliptical in size covered with thick connective tissue capsule called tunica albuginea, the testis is internally divided with radially arranged septa. Thus the testis is divided into many seminiferous lobules.

A pair of bilobed, rounded testis are found in the visceral mass near pectoral fin might for the balancing during the swimming. Both the testes are connected with muscular bridge. Two sperm ducts arised from each testis are combined together to form a common sperm duct. As usual HE staining pattern was obtained in the T.S. of testis, the staining was found in hypertrophied germinal epithelium, septa and dividing spermatogonia in the seminiferous lobules (Khanna, 1985, Hoar et al 1983, Billard, 1970, Munro, 1990, Smith, 1978, Nagbhushanam et al 1993, Upadhyay and Guraya 1973).

An intense staining with AB pH 1 in the connective tissue frame work of the testis, and in tunica albuginea was more intense indicate the presence of chondratin sulphate (Nalwade, 1975, Spicer, 1967). A strong Alcian Blue pH 2.5 staining was revealed in the dividing spermatogonia

and the interstitial cells of testicular septa, similar staining has reported in interstitial cells in testis of tilapia(Fartade 1982).

These interstitial cells are equivalent to leydig cells and can elaborate the rising titer of androgen. (Khanna 1985, Marshall, 1960), PAS positive staining in the cells of sertoli indicates its nutritive and supportive role in the maturation of sperms. The cells might also helps in the nourishment of spermatids and the sperms. Since both the elements are in structural in vicinity of sertoli cells (Mote, 1981).

The regressed testis in post breeding reduced in size, the few degenerative figures or sperm debris are found, such a type of sperm debris has reported in the testis of *Tilapia mosambica* (Faratade, 1982, Dhange, 1982, Kanase 1978).

The presence of light cells and dark cells in the spermatic ducts revealed for its PAS positive staining might have role in the nourishment of the cells while light cells, with steriocillia can swift down the sperms down to the exterior. (Mote & Nalwade, 1982).

4.2.3 Ovary :

The staining pattern in the ovary varies according to their breeding period. During early ovarian preparatory period (June- July) the dividing oogonia are formed in the ovigirous lamellae. Only eosinophillic staining in the cytoplasm of oocytes and their mitotic figures suggestive of the proliferative stage of the ovary (Khanna, 1985).

The follicular cells or nurse cells in the developing follicles are important for vitellogenesis. (Srivastava, 1999). The ultra structure of nurse cells had also reported for the ovarian steriod production (Guaya 1973, Faratade, 1982). The hormone released from follicular cells might be involved in the process of ovulation in the teleost (Gupta & Gupta, 2006).

The post ovulatory corpus atreticum or corpus luteum like structure, (Battle, 1940, Sastry, 2004, Khan, 1938) also played important role in yolk absorption (Rai, 1966, Livni N. (1971) .

The ovarian interstitial cells or stromal cells might be playing important role in steroid biosynthesis and differentiation of the follicles (Saidapur 1978; Guraya 1973, Lang 1 1881a, Lang 1 1881 b, Yamazaki and Donaldson, 1968b).

The yolk vesicles surrounded with granules of glycogen are found in the maturing follicles, the process of vitellogenesis is correlated with secretion of perinuclear proteins and the semi-lunar staining pattern of nucleus with the Alcian Blue pH 2.5 and periodic schiff reagent related with synthesis of nuclear histoproteins.

The typical staining pattern of nucleus, presently can not be interpritated, however such a staining can be well correlated with vitellogenesis in the follicles. The staining might be due to

heterochromatin or the nuclear histoproteins as the proteins stained with PAS also and Alcian Blue pH 2.5.

The two types of follicular atresia involved in the teleost ovary (Khanna, 1985).The various type of follicular atresia reported in *Wallago attu* (Dixit, 1965), *M. Seenghala Clarias vatrachus* (Lehri, 1967), *Glyptothorax Pectinopterus* (Pant, 1969).

In the ovary of *Chanda ranga* non-hypertrophic atresia was found, suggestive of breakdown of viteline membrane, the membrane become velvet, disappearance of the nucleus and the coagulation and the vacuolization of yolk. It might be reabsorptive follicular atresia of the ovarian economic importance (Rai, 1966; Hoar, 1957) Beach, 1959; Chieffi (1961, 62, 67)

4.3 *Puntius sophore* (Ham.):

4.3.1 Testes :

The structure of the testis undergoes seasonal variations and hence. The interstitial cells or lobule boundry cells in *puntius* undergoes hypertrophy during the preparatory period and the active breeding period. Such a types of leydig cells have been reported in testis of several teleost as *Gasterosteus, Tilapia, Lebistes, Cymatogaster,* as well as in cyclostomes, elasmobranches. Dipnoi and *Latimeria* (Marshall 1960).

During the hypertrophy of germinal elements or seminiferous lobules showed gradually increased staining with haematoxylene. The

staining intensity has dependant on the chromatin compactness of spermatogonia, spermatiocytes and the spermatids (Khanna, 1985).

During the spermatogenesis the histological and histochemical staining in various elements of seminiferous lobule had reported in several teleosts (Billard, 1970, Van Deurs, 1975). The presence of non-granular PAS staining in tunica albugenea, interstitial connective tissue and sertoli cells indicate the presence of neutral mucins, of testicular infrastructure are supportive. The granular staining with PAS in germinal epithelium and sertoli cell indicates the presence of glycogen (Bratanov et al 1971).

The staining with AB pH 2.5 in lobule boundry cells or interstitial cells and sertoli cells indicate the presence of sialomucins. Actually, perhaps the site of the steroidogenic biosynthesis. (Nagbhushanam et al 1993; Nalwade, 1975; Spicer 1967; Goyal and Sastry, 2004).

The testis is degenerated during the post breeding period by detaching sertoli cells and clarifying the luminal sperm debris, the mechanism so called steatogenesis, might be involved (Mote, 1981; Dhange, 1982).

4.3.2 Ovary:

The *Puntius sophore* produced enomorous eggs during their active breeding period cycle (June- September). During early breeding season gonads springs into activity due to the prolonged photoperiod and

stimulation of pituitory. The HE staining was revealed in perinuclear secretary granules of ovarian follicles (De. Robertis, 1980) .

The staining also revealed in follicular hypertrophied follicular cells well suggestive of process of vitellogenesis (Pawar, 1978) and steroid biosynthesis (Guraya, 1973). The basophilic staining of haematoxylene was revealed in dozen of the nucleoli. These nucleoli along with m-RNA migrate through nuclear pore and may play a role in the process of vitellogenesis in a formation protein yolk nuclei. The two types of yolk granules revealed in the mature oocytes as in ovaries of other teleost viz. Chromophobic oil droplets and mucoid yolk granules stained with PAS and Alcian Blue pH 2.5 technique.

The non-hypertrophied follicular cells were recorded in ovary of *Puntius sophore* in which ovarian connective tissue stroma was hypertrophied. The nucleus disappears, nuclear membrane also distorted, atrophied follicular cells broken, zona radiat became wavy. The structure if at all retain in the ovary it will become converted into basophillic corpus luteum or corpus atreticum involved yolk reabsorption rather than steroid biosynthesis.