CHAPTER - I

INTRODUCTION

Presence of fatty substances were firstly reported in the testicular cells of some animals by Leydig (1850). Today these cells are known as interstitial cells or Leydig cells. The first sequential analysis of lipids in the spermatozoa was carried out by Miescher (1878), who proved that the lipids are concentrated in the sperm tails and concluded that sperm tail in their composition resemble the grey matter of the nervous system. Von Ebner (1888), firstly studied the phenomenon of a flow of lipid globules directed first from the basal to the apical parts of the Sertoli cells.

Testis :

The research work on testicular lipids gradually accelerated since the beginning of the present century. Loisel (1903) demonstrated testicular lipids in the intertubular areas in cat, dog, bat, rat. Hanes and Rosenbloom (1911), reported that the lipids in swine testis accumulated close to the membranapropria in cryptorchidism. Sertoli (1871) described elongated cells attached to the basement membrane of the seminiferous tubules in mouse testies. Similar cells have been reported in other vertebrates such as fish, amphibians, reptiles etc. and now known as Sertoli cells. During the early days these cells were considered as nutritive cell or nurishing cells. Champy (1908) believed that Leydig cells provide nutrients to the developing sperms. Recently it is known that the Sertoli and Leydig cells act as endocrine cells and pituitary gonadotropins controls their functioning.

During the past, attention was focussed mainly on the reproductive phenomena of the mammals. From the last three or four decades however more attention is being paid to the reproduction in submammalian vertebrates also. The overall data indicate that the testicular pattern in the annual cycles in the whole vertebrate series practically follows an identical pattern. The female reproductive organs are mainly concerned with reduction, in the number of eggs and in mammals as well as in other viviparous vertebrates to provide protection and nutrient supply to the developing embryo. On the other side, male reproductive organs are mainly concerned with the production of large number of spermatozoa which is essential for the perfection of the internal fertilization. The vertebrate testes are the organs which produce in addition to the sperms, internal secretions which play a role in appearance and maintanance of secondary sexual characters.

Eventhough the testes - a male reproductive organ have been studied from various points of view such as histology, cyclical histological changes, process of spermatogenesis, testicular blood supply, presence of various chemical elements, viz. proteins, enzymes, carbohydrates, nucleic acid etc. Up-to-date much is to be known and the work is in progress. The present dissertation correlates with the work on lipids in the male and female reproductive organs of two different species of fishes during the annual breeding/spawning cycle. Kulkarni and Sathyanesan (1985), studied the cyclical changes in the lipids content

of Leydig cells and lobule boundary cells of teleostei fish, and concluded that the lipids content of Leydig cells correlates with the gonadal cycle. Ezenwa (1985), studied the seasonal changes in the gonads of cat fish, and he found that during reproductive cycle male and female gonads show clear different stages. It is clear that in the initial period especially before 1940, the attention was mainly paid on the mammalian sex organs probably because of the richness of the accessory glands and duct system in comparison with the lower vertebrates. But after 1940, intensive researches were started on sub-mammalian, vertebrates in the shake of cellular localization of lipids, quantitative estimations, purification and identification of lipids in these organs by fractionation, thin layer chromatography, gas-chromatographic separation and their metabolism, localization of enzymes involved in lipid metabolism. The brief summary of fish testicular lipids is as follows.

Bioassay studies on testicular lipids :

After a critical review of the existing literature on the biochemical studies on testicular lipids in the fishes, indicates that there exists very scanty literature on the fishes as compared to the other sub-mammalian vertebrates and mammals.

Lizenka et al. (1973), described the contents of the total lipids and their fractional composition determined by thin layer chromatography (TLC) of male and female gonads of the fish, coregonusalbula. They further reported that the

quantity of fat was lower in the males than the females and the gonads of larger males contained more total lipids than those of the younger ones. Takada et al. (1979), observed the high concentration of lipids (TG) in ovary of some fishes, the same occurs in less quantity in the testis. Singh and Mondal (1982), studied the detection and localization of phosphates and bound lipids in the testis of teleost fish, Anabas testudineus, and found that, the five morphologically and cytologically different male germ line cells (spermatogonium, spermatocytes, secondary spermatocyte, spermatid and spermatozoon) from the testis and four different periods of the testicular activities of the year (growth phase, maturation phase, discharge phase and resting phase) were correlated with the localization and distribution of phosphatases and bound lipids.

Seasonal changes in testicular lipids :

The literature based on the alterations in testicular lipids during the seasonal breeding of sub-vertebrates is reviewed critically by Pawar (1978), it can be seen that very scanty literature exists on seasonally breeding lower vertebrates. Moreover, it is evident that the studies have been carried out employing mainly histochemical techniques and there is a little work employing biochemical techniques.

Marshall and Lofts (1956), studied the cyclic variations in the lobule boundary cells in Esox lucius and Salvelinus willughbii and showed that they become seasonally modified into glandular cells. These cells around spent lobules develop and diposite

the cholesterol positive lipids in their cytoplasm. Lateron Lofts and Marshall (1957), confirmed the previous investigation in a teleost fish, E.lucius, in which cyclic variations were also observed. All these events include the post-nuptial appearance of cholesterol positive lipid material, which accumulates in the seminiferous tubules and it's gradual disappearance at the time of the annual pre-breeding assembly. Similar observations were made by Chan and Phillips (1967), in gonads of teleost fish. Monopterusalbus after the reversal of sex from male to female. Ahsan (1969) also reported that the boundary cells from the seminiferous tubules of fish exhibit increase in lipids (cholesterol) quantity before discharge of seminal content. Potter and Hoar (1954) concluded that the depletion of cholesterol positive material prior to discharge of seminal content indicated that this material might be used as precursor of androgen synthesis in fishes. The similar observations were made by Kulkarni and Sathyanesan (1985) in the testis of the Puntius-sophore. This observation indicate changes in the lipid content of the Leydig cells and the lobule boundary cells could be correlated with its seasonal breeding cycle.

Hormonal effects on testicular lipids :

Larsen (1965) showed that hypophysectomy of a fish river lamprey, Lampetra-fluviatilis, creates an abnormal diposition of lipids into the interstitial cells and in the cells of lobule wall. There is no any feasible change in the cholesterol of the

lobule of the operated animals. Saksena and Devendranath (1980), reported that there is a relation between pituitary gland and reproductive cycle of fresh water fish goby, Glossogobius-guiris, they further explained that the proximal pars-distalis secretes various hormones which brought about the changes within testicular lipids.

Lipid metabolism in testis :

Appearance and maintainance of male sex accessory reproductive organs and the male secondary sexual characteristics are carried out by the hormones androgens. Leyding cells from the testis secretes androgens. Christensen and Mason (1965) become successful in separating Leyding cells from seminiferous tubules in rat testis and also able to prove that Leydig cells convert the progesterone to androgens in vitro more efficiently than the tubules. Beside much information is available on biosynthesis and metabolism of androgens in mammals. Some data on sub-mammalian vertebrates gives an indication that the pathways of metabolism are generally similar.

Lipids in Spermatozoa :

Occurance of lipids into the spermatozoa is firstly analysed by Miescher (1878). He found that ether extractable material from salmon spermatozoa composed of 50% lecithin, 14% cholesterol, and 35% fat. Investigation of Mathews (1897) also indicates the presence of lecithin. Sano (1922), carried a research work on sperms of herrings, salmon, pargy and codfish,

also indicated the occurance of sphingomyelin, cephalin, lecithin and small quantities of other lipids.

Ovary :

After a critical analysis of the available literature on the biochemistry of the ovarian lipids of fish, it is found that the ovary of higher vertebrates is studied extensively, especially mammals. But, very scanty information is available on regarding the ovary of lower vertebrates especially fish. The following is the brief summary of the existing literature on the ovarian lipids of fish.

Lipid biochemistry of the ovary :

Presence of fatty oil in the eggs of Salmon, Oneorhynchus-keta was firstly reported by Koichi et al. (1958-59, a). Later on Koichi et al. (1958-59,b) compaired the conjugated the conjugated lipids in unfertilized and fertilized eggs of the above species of fish. They estimated 38.5 and 25.3 gm lecithin, 0.65 and 0.55 gm cephalin and 0.93 and 0.76 gm sphingolipids and small amount of acetylated lipids in crude lecithin fraction. The lecithin consisted the phosphatidyl-serine, phosphatidyl-ethanolamine and small amount of inositol while the spingolipids consisted of mainly sphingomyelin and cerebrosides. Zunivici (1969) estimated 17.55 to 36% of the total lipids in the mature ovaries of Chipeonella-cultriventries. Lizenko et al. (1972) found that the phospholipids, were much less affected than triacylglycerides by seasonal changes during the ovagenesis in a

teleost fish, C.albula. Lizenko et al. (1973) critically analysed the content of total lipids by thin layer chromatography (TLC) in male and female gonads of C.albula, and he observed that the eggs contained the more glycerides and cholesterol esters. The ratio of cholesterol ester to cholesterol ranged 0.3 for males, whereas it is 3.6 for females. Similarly phospholipids were found to be higher in the eggs. Gastaud (1975) analysed and compared the values of total lipids in younger and older Oocytes and concluded that the concentration of total lipids is higher in older Oocytes than in the younger ones, while they contained low level of cholesterol and triacylglycerides. Singh and Singh (1980) concluded that lipids levels in the ovary of fresh water teleost fish, Heteropneustes fossilis, showed increase during the pre-spawning phase while decreases at post-spawning phase.

A) Reasons that led to undertake the present investigation :

A critical evaluation of the existing literature on the lipids from testes and ovaries with special reference to fishes, reveals that there are several avenues open for young scientists working in the field of lipid-biochemistry in different organs (testis, ovary, liver, brain) of fishes. The literature on gonadal lipids significantly shows that -

i) The testes and ovaries of higher vertebrates, especially mammals have been studied more details with respect to mucin, enzymes, proteins and lipids. On the other hand such studies were comparatively less reported in lower vertebrates with special reference to fishes. Of course

some reports on testicular and ovarian lipids of some selected fishes are available but compared to higher vertebrate, it is very scanty.

ii) Lofts and his co-workers studied the testis of few fishes, their main work was at histochemical level, others studied the ovarian lipids either histochemically or biochemically. Biochemically, they studied one of the component of lipids such as TG or CHO.

iii) Research work on fish gonadal lipids indicated that the gonads either testes or ovaries studied at particular stages and not through out annual breeding cycle.

iv) Some workers reported either testicular or ovarian lipids but not in both testicular and ovarian lipids, hence clear picture of gonadal lipids is yet to be clear.

v) The available literature indicates that the workers either reported some neutral lipid components such as TG or CHO. But the complete picture of neutral lipids composition is not clear.

vi) In some species of fishes few components of testicular or ovarian phospholipids were reported such as PC and PE. But overall picture of gonadal phospholipid is poorly understood.

vii) Seasonal alterations in the gonadal lipids of fishes were poorly reported, hence detail study in this respect is essential.

Keeping the above facts in view, it was decided to investigate lipids in testes and ovaries in two selected fishes. The care was taken to choose the fishes for present investigation were true seasonal breeders. Such a selection of two seasonal breeders was hope to afford an opportunity to discuss the lipid contents, composition, quantitative and seasonal alterations in them in annual breeding at a comparative level.

Ablation techniques were purposely avoided to get an insight into the probable hormonal control over the lipids in the gonads, and instead naturally occuring hormonal variations were made use of for discussing this problem. To attain a technical and methodological perfection it was decided to employ biochemical techniques. Each organ (testis and ovary) of the selected fishes was studied biochemically through out the course of their annual breeding/spawning cycles, monthly observations being recorded regularly. All the lipids and their various constituents were investigated.

B) Choice of the seasonally breeding fishes :

While selecting the seasonally breeding fishes, it was decided to select two fishes. While making a choice of these seasonal breeders the following points were kept in mind :-

i) They show clear season dependant reproductive cycles involving a pre-breeding/pre-spawning period of gametogenesis/Ogenesis, a breeding period/spawning period, a post-breeding

period/post-spawning period, involving gonadal recession and last a period of sexual quiescence.

ii) Their testes and ovaries undergo clear histological changes.

iii) Their availability in required number through out the course of their annual cycle.

Keeping in view these requirements the following fishes seasonal breeders were selected.

(a) Rasbora daniconius (Ham.)

(b) Cirrhina fulungee (Sykes.)

Of the above two vertebrates absolutely no information exists on the lipids in gonads of R.daniconius and C.fulungee. Even the breeding cycle of both species are not known. So the first positive point of the present investigation is to report the breeding cycles of the above two species of fishes.

C) Choice of the techniques :

To attain a technical and methodological perfection biochemical techniques were selected for the study of lipids. In the biochemical techniques total lipids were determined gravimetrically, whereas for neutral lipid and phospholipid components were separated by using TLC employing silica gel-G and H, respectively. The various neutral lipid and phospholipid components isolated by TLC were assayed by employing well known and recent assay techniques. For studying the histological changes the usual eosin haematoxylene technique was employed.

D) Critical evaluation of the observations :

It was decided to critically analyse the observations made on the lipid content, composition and seasonal variations in the testes and ovaries, in relation to :-

i) Lipid content and composition of the testes and ovaries as observed in the present investigation and to compare these with the available information in each of the organs (testes and ovaries) in other class with a view to find similarities and dissimilarities, if any, and comment upon them from point of views of class characteristics, if any, in lipids of the gonads and species diversity, if any.

ii) To study the lipid alterations in the gonads, during seasonal breeding cycle of the vertebrates and to relate them to the reproductive events occuring in their organs during such cycles.

iii) To compare the observations made on the seasonal alteration in the lipids in the respective with the available information on similar aspect in other vertebrates of a particular class and find out similarities and dissimilarities, if any, in the lipid alterations in the annual reproductive cycle of various species within a class and to comment upon them from the class characteristics and species diversities, if any.

iv) To project some ideas about the functional significance of such lipid alterations on the physiology of reproduction of the vertebrates under investigation.

v) To project ideas on the hormonal influence over lipid alterations in the gonads in light of comparison of the observed lipid alterations and the known hormonal variations in the breeding cycle of this vertebrates, taking into consideration the available information on this aspect.

vi) Finally to compare the lipid content, composition and alterations in various lipid components in two species, with a view to get an insight into the comparative lipid metabolism in testes and ovaries.

E) Presentation of the dissertation :

It was selected to divide the present dissertation into four chapters, the first chapter being on introduction giving a review of the literature on the lipids in gonads of the vertebrates, reasons that stimulated the present investigation and plan of proposed work. The second chapter is devoted to a detailed description of the materials and methodoloty as well as techniques employed in the present investigation. The third chapter dealing with detailed report of biochemical observations on the gonadal lipids (both testes and ovaries) of R.daniconius and C.fulungee. In this chapter the text of the observations is well supported by tabular data, graphical data and TLC separation plates. The fourth and last chapter deals with general discussion in which the lipids in the gonads of the two species have been considered from the point of view of comparative lipids in the gonads of their content,

composition and seasonal alterations, as well as their probable functional significance and hormonal control. At the end of this chapter some concluding remarks containing some ideas for future work on lipids in reproductive organs are listed in brief. The dissertation ends with a bibliography of literature cited in the investigation.