Introduction
1.1 Review of Literature

Among mammalian male accessory sex organs, the epididymis plays a vital role in maintaining the fertility, as it is involved in sperm maturational events (see Bedford, 1963a,b). The epididymis is a highly convoluted ductal system located on the dorsolateral aspects of the testis, extending from the cranial to the caudal pole of the testis (Hoskins et al., 1978). The length of epididymal duct varies in different species; it ranges from 2 metres in rat to 40 metres in the bovine; in man, the length of the tubules may be 3-4 metres (Turner and Howards, 1978). The epididymal duct is folded, tightly packed, surrounded by a loose fibrocytic adventitia, and enclosed by a firm connective tissue sheath known as tunica albuginea (Baumgarten et al., 1971; see Hafez, 1977).

The epididymis is generally divided into caput (head), corpus (body) and cauda (tail) epididymes (see Hamilton, 1975). However, it may be subdivided into proximal and distal caput, central corpus and proximal and distal cauda with specific epithelial cell types (Hoskins et al., 1978).

The caput epididymidis is lined by high columnar epithelium with prominent cilia and minimal intertubular stromal tissue in rat, rabbit, sheep and rhesus monkeys. The
cauda epididymidis has the characteristic feature of low columnar-cuboidal epithelium with short cilia and abundant intertubular stromal tissue. The columnar epithelium with minimal intertubular stromal tissue is also seen in corpus epididymidis (Riar et al., 1973).

Two types of cells, principal and basal cells are present in the epididymis (Allen and Slater, 1957; Reid and Cleland, 1957). In addition, clear, halo and apical cells are also seen in rat and mouse epididymis (Reid and Cleland, 1957).

Apart from its role on sperm maturation, epididymis is also involved in sperm transport, storage and concentrating mechanisms (Turner, 1979). Sperm transport through the epididymis takes 8.1 days in rats, 10.5 days in rhesus monkeys and 12 days in man (Amann et al., 1976). The efferential ducts from the rete testis facilitate sperm transport by ciliary movement and spontaneous contraction (Mac Millian et al., 1960).

Hydrostatic pressure inside the epididymal tubule plays a vital role in sperm transport (Johnson and Howards, 1975). There is a gradual increase in hydrostatic pressure from caput towards cauda epididymidis due to increase in thickness of the tube wall (Johnson and Howards, 1976). As
the distal cauda epididymidal sperm are expelled into the vas deferens during ejaculation, sperm from the corpus and proximal cauda epididymides move down the tubule (Amann and Almquist, 1962; Turner and Howards, 1977).

In rats, intratubular sperm concentration increases from caput to cauda epididymidis as the luminal fluid gets absorbed along the entire length of the duct (Crabo, 1965; Levine and Marsh, 1971; Wong and Yeung., 1977; Hinton and Setchell, 1980). Removal of water is considered as a consequence of active transport of sodium chloride out of the epididymal luminal fluid (Salisbury and Cragle, 1956; Turner and Howards 1977; Wong and Yeung, 1978). Sodium ions are replaced in part by secretion of potassium ions into the epididymal luminal fluid (Turner, 1984).

Sperm maturation takes place mainly within the caput and corpus epididymides while, the cauda epididymidis serves as a storage area (Young, 1929; see Bedford, 1975; Turner and Howards, 1978). The attainment of motility precedes fertilizing capacity of sperm. Initially, sperm display only vibratory movements, followed by movements in tight circles and finally forward progression (Gaddum, 1968). The initial vibratory movement is associated with increased intracellular concentration of cyclic adenosine monophosphate (c-AMP) in sperm and there is enhancement of forward motility protein in
sperm as they develop forward progression (Acott and Hoskins, 1978; Brandt et al., 1978).

The sperm undergo a number of structural, biochemical and physiological changes during epididymal transit. These include modification of sperm dimensions (Bedford, 1963a), migration and loss of the cytoplasmic droplet (Redenz, 1924), increased susceptibility to cold shock (Quinn and White, 1967), increased surface negative charge (Bedford, 1963b), reduction in whole cell isoelectric point (Hammerstedt et al., 1979), increased disulfide cross linking (Bedford, et al., 1973), changes in the composition of lipids (Scott et al., 1967), proteins (Voglmayr et al., 1980) and antigens (Killian and Amann, 1973), modified enzymatic activity (Purvis et al., 1982) and altered binding of lectins to the cell surface (Nicolson et al., 1977).

Sperm surface antigens associated with species specific ovum - sperm recognition process get modified during epididymal transit of sperm. Such modifications are due to addition, substraction or changes in the chemical composition of surface antigens and facilitate sperm specific receptor binding to ovum (Lea et al., 1978; Garberi et al., 1979). Epididymal specific glycoproteins EP1 - EP6 bind to sperm and increase their fertilizing ability (Gonzalez Echeverria et al., 1982; Thomas et al., 1984).
Secretion is another important function of the epididymis. The principal cells are associated with synthesis, secretion and absorption of proteins and glycoproteins (Kopency and Pech, 1977; Flickinger, 1979). Basal and apical cells contribute little or nothing to the overall synthetic activity (see Hamilton, 1972). Clear cells have been implicated in the synthesis and secretion of glycerophosphoryl choline (Martan, 1969).

Mammalian epididymis also contains enzymes involved in glycoprotein synthesis and metabolism. Glycosyl, galactosyl and mannosyl transferases which are involved in the transfer of sugar moieties to the nacent polypeptide chain have been identified in the epididymis (Hamilton, 1980; Isuem et al., 1984). Epididymis is also a rich source of glycosidases (Conchie et al., 1959) which catalyze the hydrolysis of glycosyl bonds of sperm surface glycoproteins (Bamberg et al., 1975; Hamilton, 1980; Verheijen et al., 1982). Epididymal principal cells are the major source of glycosidases (Allison and Hartee, 1970; Shur and Hall, 1982), like β-galactosidase, β N-acetyl glucosaminidase and β-N-acetyl galactosaminidase (Chapman and Killian, 1984).

High activity of glucosamine-6-phosphate synthase associated with sialic acid synthesis was also reported to be present in rat epididymal tissue (Rukmini and Reddy, 1980).
Epididymal sialic acid is involved in sperm transport (Riar et al., 1973), maintenance of osmotic balance in the cauda region and in the stabilization of acrosomal membrane (Rajalakshmi et al., 1976). The sialic acid content of rat epididymal tissue increases during maturity (Rajalakshmi and Prasad, 1969).

The epididymal luminal fluid also influences sperm maturation. The epididymal fluid contains significant amount of proteins, free and bound sialic acid and lipids (mostly phospholipids). The concentration of protein, phospholipids and free sialic acid are maximum in the caput epididymal fluid, whereas bound sialic acid and non polar lipids are maximum in the cauda epididymal fluid of rats (Arora et al., 1975; Jones, 1978).

Marquis and Firtz (1965) demonstrated high level of carnitine in the epididymis of rat. The intracellular concentration of carnitine in the spermatozoa increases during epididymal transit (Casillas, 1973). The activity of carnitine acetyl transferase, the enzyme involved in the conversion of carnitine to acetyl carnitine, is more in the spermatozoa than in epididymal tissue of rats (see Brooks, 1980).

One of the main features of sperm maturation is a decrease in the permeability of plasma membrane to carnitine.
(Casillas, 1972; Hutson et al., 1977). Epididymal carnitine is principally located in the luminal fluid and in the cauda epididymal tissue (Brooks, 1980). The epididymis cannot synthetise carnitine from trimethyl aminobutyrate and hence is taken up from blood and concentrated by the epididymis (Casillas and Erickson, 1975).

Epididymis is capable of synthetising glycerol phosphoryl choline (GPC) which may have a role in maintaining the osmotic pressure (Wales et al., 1966). The concentration of GPC in the mammalian epididymal tissue is much higher than testis (Dawson and Rowland, 1958; Hinton and Setchell, 1980). In rat, the caput and corpus epididymides contain more amount of GPC than the cauda epididymidis (Dawson and Rowland, 1958; Scott et al., 1963). Epididymal phosphatidyl choline may be a major source of fatty acids and GPC synthesis (Brooks et al., 1974; Brooks, 1978). Sperm cannot metabolize GPC (Wales et al., 1966).

The spermatozoa acquires fertilizing ability during its sojourn through epididymis, partly by modified lipid composition of its plasma membrane (Nicolson et al., 1977). The cholesterol : phospholipid ratio increases as the spermatozoan matures and this might result in decreased fluidity of sperm membrane (Parks and Hammerstedt, 1985). Lavon et al. (1970) showed diminished total lipid level in
bovine sperm during epididymal maturation and attributed it to dehydration and a decrease in dry matter content of sperm due to the utilization of phospholipids as a source of energy. Accumulation of lipids may occur when a particular portion of the epididymis becomes devoid of spermatozoa and testicular fluid due to non utilization of the same by sperm (Turner and Johnson, 1971).

Almost all phospholipid classes except, choline, plasmalogen were found to be decreased during maturation of ram spermatozoa, suggesting the utilization of fatty acid side chain of phospholipids as energy source (Scott et al., 1967; Poulos et al., 1975). While the caput epididymidis provide 75% of lipids, the remaining is supplied by cauda epididymidis (Brooks, 1980).

Testicular androgens are the major regulators of epididymal structure and function (see Orgebin-Crist et al., 1975; Brooks, 1981; Amann, 1987; Robaire and Hermo, 1988). Testicular androgens reach the epididymis through retetesticular fluid (see Mooradian et al., 1987).

Androgen binding protein (ABP) secreted by Sertoli cells serves as the carrier protein for the transport of testosterone from the testis to the epididymis. The rat ABP is a 8500d acidic glycoprotein with a high binding affinity
for testosterone and dihydrotestosterone (DHT), the major androgen acting on the epididymis (Musto et al., 1980; Feldman et al., 1981). Turner et al. (1989) showed that intraluminal androgen - binding protein is an important factor in transepithelial androgen movement.

Testosterone moves into the epididymal lumen against a concentration gradient (Turner, 1988). Androgen concentrations in epididymal tissue are higher than serum level (Aafjes and Vreeburg, 1970; Vreeburg, 1975; Pujol et al., 1976; Boujard et al., 1982). The luminal fluid of caput epididymis contain very high concentrations of androgens (Turner et al., 1984, 1985).

There is evidence for the de novo synthesis of androgens by the epididymis. The de novo synthesis of cholesterol and testosterone from acetate has been demonstrated in all regions of the epididymis of rat, ram and rabbit in vitro (Hamilton et al., 1969; Hamilton and Fawcett, 1970; see Hamilton, 1972). On the contrary, Frankel and Eik-Nes (1970) were unable to demonstrate conversion of acetate or cholesterol into pregnenolone, testosterone or any of the intervening metabolites in rabbit epididymis. Nevertheless, conversion of pregnenolone, 17α-hydroxy progesterone and dehydroepiandrosterone into subsequent products in the steroid metabolic pathway was readily demonstrated (Inano et al., 1969).
Epididymis can convert testosterone into its active metabolite, DHT by the action of the enzyme 5α-reductase (Klinefelter and Amann, 1980). Testosterone and DHT can act on the epididymis through their specific cytosolic and nuclear receptors which have been clearly demonstrated in rat and rabbit (Ritzen et al., 1971; Hansson et al., 1973; Danzo et al., 1973; Danzo and Eiler, 1975). The ram epididymal androgen receptor is highly specific for DHT (Tekepetey and Amann, 1988). Schleicher et al., (1984) reported DHT receptors in the principal cells of rat epididymis.

Androgens induce gene transcriptions associated with the synthesis of specific epididymal proteins and glycoproteins (see Main-Waring, 1977; Darnell, 1982). Androgen dependent mRNAs are present in rat epididymal tissue (Brooks et al., 1986; Brooks, 1987). The principal cell protein involved in sperm maturation has been shown to be androgen dependent (Amann, 1987, 1988; Robaire and Hermo, 1988). Androgens are also involved in the regulation of protein degradation (Brooks and Higgins, 1980; Higgins et al., 1981).

Testicular androgens appear to play a significant role in the regulation of epididymal lipids (Umapathy et al., 1980). However, this report also reveals that the effects of
testicular androgen on epididymal lipids is specific one and not a general one.

Movement of Na\(^+\)-K\(^+\) ions across the epididymal epithelium is androgen-dependent (Wong and Yeung, 1978). The activity of carnitine transport pump was increased by androgen (Yeung et al., 1980).

Estrogens were also found to have influence on epididymis. Estrogen receptors have been identified in the epididymis and was found to be more in the cauda than in the caput epididymidis (Tekpetey and Amann, 1988).

Corticosterone was also considered essential for the maintenance of Na\(^+\) and water reabsorption in the caudal epididymis of rat (Au et al., 1978). Hypercorticosteronism was found to be associated with accumulation of lipids in the epididymis of rats (Balasubramanian, 1984). Corticosterone was shown to have an inhibitory effect on epididymal enzymes of the pentose phosphate pathway, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (Balasubramanian et al., 1982, 1983).

Prolactin also influences the epididymal structure and function. In rat, prolactin was found to increase the nuclear uptake of testosterone by the caput epididymidis (see Baker et al., 1977). A recent study from this laboratory
showed that the conversion of testosterone into DHT by the enzyme 5 α-reductase is inhibited in the epididymis of hyperprolactinemic mature bonnet monkeys. Hyperprolactinemia may also modify lipid utilization as it diminished carnitine level through the entire length of epididymis of these monkeys (Jayakumar, 1989).

Apart from steroids and prolactin, thyroid hormones are also known to have appreciable influence on mammalian male reproductive system. The influence of thyroid on reproductive system is known for a long time. (see Myant, 1964; see Ingbar, 1985). Nevertheless, the specific effect of thyroid hormones on the mammalian male reproductive physiology is reckoned with only recently. Despite improvement in male infertility with thyroid hormone therapy it has been believed that thyroid hormones cannot directly negotiate with the functions of testis and accessory sex organs in mammals. (see Myant, 1964). However, studies conducted during the last two decades have proved that thyroid hormones have specific influence on the growth, structure, biochemistry and function of testis, epididymis and other accessory sex organs (Aruldhas, 1981; Aruldhas et al., 1982a,b; 1983, 1984, 1986a,b; Pereira et al., 1983a,b; 1984a,b; Chandrasekar et al., 1985a,b; Chowdury and Arora, 1984; Geraldine et al., 1988; Priyadarsini, 1989; Rose, 1989; Senthilkumaran et al., 1990).
Primary hypothyroidism is associated with hypogonadism in males and females (see Longcope, 1986a; Buchanan et al., 1988; Pringle et al., 1988). Talbert (1962) reported that testicular maturation in rats may be accelerated by thyroxine treatment. Similarly T₄ was found to accelerate gonadal development in congenitally hypothyroid mice as evident from increase in seminiferous cell number and sperm count (Bocabella, 1963; Matsushima et al., 1986).

Arrest of spermatogenesis was reported in perpubertal, hypothyroidism (De la Blaze et al., 1962). Thyroidectomy of immature and mature rats were shown to be associated with disruption of spermatogenesis (Aruldhas, 1981; Chowdhury and Arora, 1984). Thyroid replacement to thyroidectomised immature and mature rats were found to maintain normal spermatogenesis, suggesting reversible adverse effect of hypothyroidism on spermatogenesis (Aruldhas, 1981).

Amin and El-sheikh (1977) reported abnormal changes in spermatogenic and interstitial cells under chronic hypothyroidism. Hypothyroidism leads to decreased weight of testis, seminal vesicles, ventral prostate and epididymis (Karkun and Mukherjee, 1965, 1967) in mice.

Thyrotoxicosis also has been reported to be associated with impairment of spermatogenesis, low sperm
count, impotence and loss of libido (Clyde et al., 1976; Monson et al., 1988). Hyperthyroidism has been shown to be associated with oligospermia and infertility, as well as less volume and density of semen and modified sperm morphology and forward motility in men (Buitrago and Diez, 1987).

Elevation of follicle stimulating hormone (FSH) and prolactin are frequently associated with primary hypothyroidism in girls and boys (Pringle et al., 1988). Primary hypothyroidism alters the pulsatility of gonadotrophins (Buchanan et al., 1988; Pringle et al., 1988). Bioactivity of luteinizing hormone (LH) and the level of serum testosterone are decreased in hypothyroid rats (Bruni et al., 1975) and rams (Chandrasekhar et al., 1985a). Unaltered serum LH also has been reported in hypothyroid rats (Baksi, 1973; Kalland et al., 1978; Aruldhas et al., 1982a, 1986a).

Hyperprolactinemia is associated with primary hypothyroidism (Buchanan et al., 1988). However, thyroidectomy induced hypothyroidism is associated with a decreased concentration of serum prolactin in male rats (Tang et al., 1986).

Hyperthyroidism lead to increased serum FSH, LH and testosterone in men (Chopra and Tulchinsky, 1974; Clyde et
Monson et al. (1988) showed higher rate of LH secretion in Graves' disease. Hyperthyroid men were found to have basal LH, FSH, PRL, testosterone and estradiol but with increased LH and FSH response to GnRH (Rojdmark et al., 1988). However, hyperthyroidism has no effect on gonadotrophin responsiveness to exogenous GnRH in rams (Chandrasekhar et al., 1985a). However, hyperthyroidism in ram lambs was associated with reduced LH pulse frequency, and basal LH (Chandrasekhar et al., 1985b).

Thyroxine-induced hyperthyroidism for 30 days decreased the serum levels of FSH, LH and testosterone in prepubertal, pubertal and adult rats (Aruldhas et al., 1982b). Howland and Ibrahim (1973) reported no adverse effect of T3 induced hyperthyroid on gonadotrophin secretion in rats.

In the presence of ovary, T4 has stimulatory effect on LH synthesis and release (Erfurth and Hedner, 1987). T4 counteracts the effects of estradiol in the regulation of LH secretion (Wang, 1988). Hudler et al. (1980) reported that estrogen increases extra thyroidal conversion of T4 into T3, since T3 is more potent than T4.

Thyroid hormones are essential for the expression of the androgen dependent submandibular gland activity in adult mice (Minetti et al., 1987). T3 increases the peripheral
aromatization of testosterone (Southern et al., 1974). Cold induced atrophy of male accessory sex organs was correlated to increased release of thyroid hormones and testosterone was shown to prevent the atrophy (Das and Peerault, 1971; 1974).

Thyrotoxicosis was found to be associated with increased sex hormone binding globulin in man (Yosha et al., 1984a, 1984b; Monson et al., 1988). Administration of T₄ to hypothyroid subjects was also found to enhance serum sex hormone binding globulin (Gow et al., 1987).

Testicular enzymes involved in the Embden-Meyerhof and hexose monophosphate pathways of glycolysis and pyruvate malate cycle were shown to be specifically influenced by thyroid hormones in immature and mature rats (Aruldhas et al., 1982 a,b; 1983, 1984). These authors also showed that thyroid hormones have a direct effect on testicular NADPH-generating system. Accumulation of testicular lipids were reported in rats subjected to short term hypothyroidism (Aruldhas et al., 1986a) and an opposite effect was seen in hyperthyroid rats (Aruldhas et al., 1986b).

Morphological difference has been observed in epididymal caput and corpus cells of hypothyroid rats (Del Rio and Quiros, 1983). Insufficient vascularization was one of the features observed in the epididymis of hypothyroid
rats (Delrio and Quiros, 1979). Recently thyroidectomy was shown to decrease the weight of epididymis in immature rats (Valle et al., 1985).

Pereira et al., (1982) from this laboratory reported that thyroid hormones have a stimulatory influence on sialic acid content in the epididymis of male albino rats. Accumulation of epididymal lipids was observed in rats subjected to hypothyroidism for 20 days (Pereira et al., 1983a). Whereas hyperthyroidism for the same duration did not alter the major lipid classes (Pereira et al., 1984). These authors also proved that the specific stimulatory effect of thyroid hormones on some epididymal glycolytic enzymes (Pereira et al., 1983b,c). These reports suggested diminished activities of epididymal enzymes of the HMP shunt pathway, which were resistant to hypothyroidism (Pereira et al., 1983 b,c).

1.2 Scope of the present study

Thyroid disorder is one of the major endocrine diseases, next only to diabetes. Male infertility accompanying hypo- and hyperthyroidism is known for a long time. Nevertheless, the mechanism responsible for male infertility associated with thyroid diseases remain obscure.
Despite the pivotal role played by the epididymis in sperm maturation and fertility, not much attention has been paid to the status of this male accessory sex organs in relation to thyroid status. Recent studies from this laboratory (Pereira et al., 1981, 1983a,b,1984) and others (Del Rio and Quiros, 1979, 1983) have crystal clearly shown that thyroid hormones can modify epididymal structure and biochemistry. In the present study, an attempt is made to understand the modification in various classes of epididymal lipids under chronic hypo- and hyperthyroid conditions, as information on these lines are lacking.

The results of the present study are expected to endow us with deep knowledge of modified epididymal biochemistry due to thyroid diseases. This may help to have an erudition of the mechanism by which thyroid disorders alter male fertility as epididymal lipids have an essential role in sperm maturational processes.