CHAPTER - 1

INTRODUCTION
1. Introduction

Male fertility is a complex physiological event, which depends upon a synergy of physiological, genetic, behavioral, and environmental factors. Each part of male reproductive system has its own functions and importance. The development, differentiation and functions of male reproductive organs are chiefly regulated by signaling systems, the neuroendocrine system. The male reproductive system (Fig. 1.1) consists of a pair of testis that produces sperms (or spermatozoa), ducts that transport the sperm to the penis and glands that add secretions to the sperms to make semen. Historical interest and research in male fertility have been motivated by several goals, including diagnosis and treatment of infertility, improvements in contraceptive technology and, more recently, concerns of environmental and occupational exposure.

1.1. Male reproductive system

1.1.1. Testis

The testes are a pair of oval-shaped glands that are suspended in the scrotal sacs and considered very important for the normal functioning of the male reproductive system. The testes serve the dual function of producing sperm and hormones. The testis is principally made up of two types of cells, the Sertoli cells and the Leydig cells which are involved in spermatogenesis (production of sperm) and steroidogenesis (biosynthesis of testosterone), respectively.

1.1.1a Overview of spermatogenesis

Spermatogenesis is a process facilitating transmission of the genetic patrimony and, thus, the perpetuation of the species. Mammalian spermatogenesis is classically divided into three phases. The first phase is known as proliferative or mitotic phase: the primitive germ cells or spermatogonia undergo a series of mitotic divisions. In the second phase, the spermatocytes undergo meiotic division to produce the haploid spermatids. Finally, in the third phase spermiogenesis occurs where spermatids differentiate into spermatozoa. The entire process is regulated by paracrine, autocrine and endocrine pathways (Cheng, 2008). The Sertoli cell transfers essential molecules to the germ cells from the interstitial fluid, synthesizes essential substrates for germ cell metabolism. Spermiation (release of the sperm) is an active process that requires the separation of the specialized junctions between Sertoli cell
and spermatid. The newly formed sperm are released into the seminiferous tubule fluid, which itself is a product of the Sertoli cell, is transported to the epididymis to attain maturation. Thus Sertoli cells play pivotal roles to nourish the developing sperm cells, destroy defective sperm cells, and secrete fluid that helps in the transport of sperm into the epididymis and to release the hormone inhibin that helps in regulation of sperm production.

Spermatogenesis relies on the coordinated support and interactions of the germ cells, Sertoli cells, Leydig cells, peritubular cells, interstitial macrophages, and the blood vasculature. Apart from the cells and blood, overall regulation of the process is mediated through the hypothalamic-pituitary-Leydig cell endocrine axis, but equally important is the local regulation of cellular function through paracrine factors, secreted by one cell affecting its neighbors, or through autocrine factors produced by a cell to regulate its own function (Hess and de Franca, 2008).

1.1.1b Overview of steroidogenesis

Testicular steroidogenesis is one of the evolutionary conserved processes which is mainly involved in the biosynthesis of testosterone. Leydig cells play an important role in the production of testosterone, male androgen. It consists of a series of steroid precursors of testosterone and the respective enzymes necessary to synthesize each precursor from the previous one. Androgens are synthesized from cholesterol through the actions of the steroidogenic enzymes like cytochrome P450 side chain cleavage (P450scc), 3β-hydroxy steroid dehydrogenase, cytochrome P450 17α-hydroxylase and 17β-hydroxysteroid dehydrogenase/17-κetosteroid reductase. In the cascade of steroidogenesis, 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase (17β-HSD) are the two important testicular enzymes which mediate sequential steps involved in the conversion of cholesterol to testosterone. 3β-HSD belongs to the family of oxidoreductases, specifically acting on the CH-OH group of donor with NAD⁺ or NADP⁺ as acceptor. This enzyme participates in C21-steroid hormone metabolism and androgen and estrogen metabolism. The reaction catalysed by 3β-HSD is as follows:

\[
3\beta\text{-hydroxy-\(\Delta^5\)}\text{-steroid} + \text{NAD}^+ \rightleftharpoons 3\text{-oxo-\(\Delta^5\)}\text{-steroid} + \text{NADH} + \text{H}^+
\]

The 17β-hydroxysteroid dehydrogenases are a group of alcohol oxidoreductases which catalyse the dehydrogenation of 17-hydroxysteroids in steroidogenesis.
There are two distinct populations of Leydig cells: fetal Leydig cells, which originate and function in the fetus but largely regress soon after birth, and adult Leydig cells. Fetal Leydig cells produce high levels of testosterone required for the stimulation of male sexual differentiation and testis descent. Adult Leydig cells are formed from precursor cells during postnatal life. The adult cells produce high levels of testosterone required for maintaining spermatogenesis and male secondary sexual characteristics in adult life. A number of environmental toxicants have been shown to disrupt fetal Leydig cells function, adult Leydig cells development, and/or adult Leydig cells function. Exposures to toxicants have been shown to have a variety of pathological consequences, including hypospadia and cryptorchidism, resulting from exposures of fetal Leydig cells; delayed puberty, resulting from Leydig cell precursor cell postnatal exposures; or hypogonadism and infertility, resulting from adult Leydig cell exposures (Chen et al., 1994). So, it seems apparent that Sertoli cells and Leydig cells of testis are the important endowed apparatus of male reproductive machinery from prenatal life to adult stage of male.

1.1.2 Accessory sex organs and their functions

1.1.2a Epididymis

The epididymis is one of the important accessory sex organs. On arrival of the mature sperm from the testis to the epididymis, the sperm undergoes further maturation processes which are under the control of hormonal-dependent secretory, resorptive, and storage functions. Epididymis is a highly coiled duct that connects the seminiferous tubules in the testis and the vas deferens. The epididymis consists of three parts: the head (cap-out), the middle (corpus) and the tail (cauda) part. The spermatozoa are stored in the tail of epididymis where they remain viable for a month and they become motile and acquire the capacity to fertilize. Most of this maturation process involves reorganization of the molecular architecture of the sperm plasma membrane. These modifications take place as the sperm progress from caput to caudal region. The secretory and reabsorptive function of the epididymal epithelium provides an appropriate microenvironment for proper maturation of sperm (Kirchhoff, 1998). Thus, the resorption of fluid through the efferent ductules, as well as maturation of sperm during their passage through the epididymis, is fundamental for adequate sperm content of the ejaculate and for fertilizing capabilities.
1.1.2b Vas deferens

The main function of the vas deferens is transport and ejaculation of mature sperms. The vas deferens is also connected to nutrient providing centers for the mature sperms, seminal vesicles, and the prostate glands.

1.1.2c Seminal vesicles

Seminal vesicles are paired, bag shaped glands and the internal surface consists of intricate system of folds to form irregular diverticula. The seminal vesicles secrete a viscous fluid, which is expelled along with the sperms. The fluid is rich in nutrients that may be important as an energy source for sperm.

1.1.2d Ventral prostate

The ventral prostate is a bi-lobed gland wherein its secretions protect the seminal fluid and feeds sperm. It has numerous small ducts through which the secretions are discharged directly to the urethra. The secretions contain several nutrients and also serve as a lubricant for the semen.

1.1.2e Cowper’s gland

Bolbo urethral (Cowper’s) glands are of pea shaped located below the prostrate gland. Its secretions serve as a lubricant and its alkalinity helps to protect the sperm from the acid present in the male urethra and female vagina and there by increases sperm motility.

The formation of the copulatory plug, which retains the sperm in the female vagina, requires the additional secretions of the coagulating gland. Ejaculation and emission of semen into the female genital tract requires effective mounting, penile erection, and intromission. These functions are dependent on a mixture of hemodynamic and androgenic factors as well as central and autonomic nervous input. Interference with any of these processes has the potential to reduce fertility of the male, but in such cases histopathology is unlikely to show any adverse effects. This emphasizes the importance of using multiple endpoints to monitor the diverse functions of the different parts of the reproductive tract.

1.2. Hormonal regulation of male reproduction

Hormones control and coordinate complex physiological processes in animal systems including reproduction. In mammals, male fertility is mainly regulated by hormones of hypothalamo-pituitary-testicular (HPT) axis.
1.2a Hypothalamus

Hypothalamus is the master controlling gland of HPT axis. It synthesizes gonadotropin releasing hormone (GnRH), a decapeptide in a pulsatile manner, which is mainly responsible for the production and release of gonadotropins from anterior pituitary gland.

1.2b Pituitary gland

It is well acknowledged that pituitary gland plays an important role at least in part in all biological processes in the body. Whereas, in particular, the pulsatile GnRH production signals the gonadotroph cells in the anterior pituitary to produce two classical gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH). These two hormones (LH and FSH) are then released into the body's general circulation and act primarily at the level of the gonads (testis). LH and FSH through signal transduction pathways affect the Leydig cells and the Sertoli cells, respectively. LH binds to receptors on the surface of Leydig cells in the testis and stimulates the production of testosterone, a steroid hormone that diffuses into the seminiferous tubules. Within the seminiferous tubules only Sertoli cells possess receptors for testosterone and FSH and thus these cells are the major targets of the ultimate hormonal signals that regulate spermatogenesis (Walker and Cheng, 2005).

1.2c Testis

It is well established that testicle performs two important functions a) production of sperms and b) biosynthesis of testosterone. Leydig cells produce the bulk of testicular testosterone and that the testosterone biosynthetic enzymes are localized in Leydig cell mitochondria and smooth endoplasmic reticulum. In the adult, testosterone regulates sexual behavior, accessory sex organ function, epididymal sperm maturation, and spermatogenesis (Ewing et al., 1977). LH plays an important role in the regulation of biosynthesis of testosterone in the Leydig cells. LH binds with its LH-receptor on Leydig cells in a receptor mediated fashion. This binding allows activation of second messenger (cAMP) production. It is also believed that trophic hormones play an important role in the cholesterol transport into the mitochondria of Leydig cells. The delivery of cholesterol molecules into the inner mitochondrial membrane is generally accepted to be the rate-determining step in steroidogenesis (Stocco and Clark, 1996). The cholesterol transport is critically
mediated by two proteins viz., steroidogenic acute regulatory proteins and peripheral-type benzodiazepine receptor. The former plays a key role as initiator of cholesterol transport and the latter act as a gate for cholesterol entry into mitochondria (Stocco, 2000). Once cholesterol reaches the inner mitochondrial membrane, it is immediately converted into pregnenolone. This reaction is one of the rate-limiting in steroidogenesis and catalysed by the cytochrome P450 enzyme CYP11A1 and specific electron transferring proteins, localized on the inner mitochondrial membrane. Pregnenolone then leaves the mitochondria to the smooth endoplasmic reticulum, where it is converted by 3β-HSD to progesterone. This steroid is then metabolized by cytochrome P450c17 to androstenedione, which is converted to testosterone by 17β-HSD (Svecchnikov et al., 2009). Testosterone is considered crucial for spermatogenesis, and also to sustain structural and functional integrity of male reproductive organs.

Hence, proper functioning of the mammalian testis is dependent upon an array of hormonal messengers acting through endocrine, paracrine, and autocrine pathways. Within the testis, the primary messengers are the gonadotrophins, follicle stimulating hormone and luteinizing hormone, and the androgens. Abundant evidence indicates that the role of the gonadotrophins is to maintain proper functioning of testicular somatic cells. It is the androgens, primarily testosterone, which act through the somatic cells to regulate germ cell differentiation. Therefore, toxic agents which inhibit hormones of HPT axis may have a profound effect on myriad processes required for the timely deposition of viable spermatozoa into the female reproductive tract.

1.3. Environmental contaminants and male reproduction

Industrialized society is currently facing serious problems regarding the exposure of its population to environmental pollutants. In the field of reproductive environmental health there remain many unanswered questions regarding the impact of the environment on male reproductive health. The evidence of the past few decades has shown disturbing trend in male reproductive health hazards due to indiscriminate use of these chemicals which caused detrimental effects on different organs. Therefore, broad-spectrum irreversible toxic actions at cellular and molecular level were observed mainly on reproductive system of human and experimental animals (Chowdhury, 2009). It has been demonstrated that, environmental
contaminants at least in part affects male reproductive health by disrupting the endocrine system and/or altering the pro-and antioxidant status of animal systems (Sharpe, 2001; Aitken and Roman, 2007).

1.3.1. Endocrine disrupting activities of environmental pollutants

Most of the chemicals introduced into the environment are hormonally active chemicals that have potentially hazardous effects on male reproductive axis resulting in subfertility and on other hormonal dependent reproductive functions causing erectile dysfunction. Such chemicals are called as "endocrine disruptors". The effects of endocrine disrupting chemicals are mainly due to (i) mimicking natural hormones, (ii) inhibiting the action of hormones, and/or (iii) altering the normal regulatory function of the endocrine circuits. Besides reduced fertility and erectile dysfunction, other consequences viz., testicular and prostate cancers, abnormal sexual development, alteration in pituitary and thyroid gland functions, immune suppression, and neurobehavioral effects are also possible due to such endocrine disruption in the male.

Epidemiological research has indicated a causal connection between human exposure to contaminants and endocrine disrupting effects such as poor sperm quality (Swan et al., 2003) and increased incidence of cryptorchidism (Weidner et al., 1998). Studies conducted on workers exposed to pesticides occupationally have shown that exposure to various pesticides increased incidences like abortion, stillbirth, male infertility, neonatal deaths, congenital defects, testicular dysfunction and other reproductive abnormalities (Kumar et al., 2000). Toxicants can attack the male reproductive system at one of several sites or at multiple sites. These sites, and assays associated with their respective functions include the testes, accessory sex organs and the HPT axis.

1.3.1a Target sites for endocrine disruptors

Reproductive toxicants can be categorized into heavy metals, agricultural chemicals, and industrial chemicals.

(i) Testis

Male fertility largely depends on normal spermatogenesis. Within the testes are Sertoli cells, or nurse cells, that form a continuous and complete lining within the tubular walls which envelope the developing sperm during spermatogenesis.
Production of sperms is a complex process by which immature germ cells undergo a cascade of steps including division; differentiation and meiosis to give rise to haploid elongated spermatids. Several environmental contaminants are known to interfere at various stages of germ cell development and thereby causing reduced sperm count (Sikka and Wang, 2008). Thus, many irregularities of spermatogenesis due to interference by endocrine disruptors may reflect changes in the function of the Sertoli cell population and not necessarily by pathology in the germ cells themselves.

Leydig cells are also major target site for many endocrine disruptors. Steroidogenesis is a classical pathway which occurs in the Leydig cells that produce testosterone from cholesterol via a series of enzymatic pathways and steroidal intermediates under the control of luteinizing hormone (LH) from the pituitary. Recent reports demonstrate that reductions in testosterone due to exposure to environmental toxicants have the potential to adversely affect normal sexual development in humans (Sikka and Wang, 2008). Thus, it is clear that both Sertoli cells and the Leydig cells are the major targets of a wide array of toxicants including heavy metals.

(ii) Accessory sex organs

Spermatozoa after completing the testicular phase of maturation along with its surrounding fluid enter the epididymis. The basic step in the sperm concentration is absorbance of 90% of testicular fluid within the efferent ductules before entering the epididymis, thereby ensuring that a large number of spermatozoa are being released upon ejaculation (Clulow et al., 1998). Epididymis is one of the androgen dependent organs (Mann, 1974). It is plausible that any toxic insult to Leydig cell will cause androgen deprivation in blood and testicular fluid, which will subsequently hamper the epididymal sperm maturation and fertility. The effects of various environmental toxicants on the efferent ducts and epididymis, with primary focus on compounds that targets the efferent ducts and seminiferous epithelium has been reviewed (Hess, 1998). It has been speculated that environmental contaminants mentioned above result in some form of androgen deprivation, leading to decrease in epididymal sperm transit time. Toxicant-induced accelerated sperm transition through the epididymis affects not only the number of sperm available for ejaculation, but also the quality by compromising the process of epididymal sperm maturation, and in turn, the fertility of sperm.
The role of seminal vesicles in male fertility is well known (Gonzales, 2001). Seminal vesicles secrete proteins that enhance sperm motility, increase stability of sperm chromatin, and suppress the immune activity in the female reproductive tract. There are only a few reports on the effects of toxicants on accessory sex glands in mammals. However, studies in rats have shown suppressive effect of environmental contaminants on the functional activity of accessory sex glands as revealed by decrease in the seminal vesicle and prostatic weight, changes in acid phosphatase activity, fructose content and decrease in the testicular testosterone production following inhibition of pituitary gonadotropins release (Sujatha et al., 2001; Joshi et al., 2003).

1.3.1b Effect of endocrine disruptors on wildlife and humans

In wildlife and humans, there is concern that male reproductive health has progressively declined in the past 50 years (Carlsen et al., 1992; Olsen et al., 1995; Toppari et al., 1996), since many pollutants primarily target the endocrine centers of brain and testis (Sikka and Wang, 2008).

(i) Male reproductive effects in Wildlife

In wildlife, in addition to decreasing sperm counts, increased incidence of testicular cancer and cryptorchidism were reported in the recent past (Giwereman et al., 1993). Evidence also indicated that deleterious effects on males were found with low sperm counts and cryptorchidism (Face mire et al., 1995). Male alligators in lake Apopka in Florida demonstrated reproductive abnormalities including reduced penis size, abnormal testis morphology and decreased testicular steroidogenesis (Guillette et al., 1994). In seagulls, altered sex ratio and feminization of sexual behavior in males were observed (Fry and Toone, 1981). Inter sex and testis abnormalities have been reported in a high proportion of male fish sampled in rivers, estuaries and coastal waters in the United Kingdom (Lye et al., 1997). These alarming reports led to the hypothesis that decline in sperm counts and related disorders of the male reproductive system in wild life could have arisen because of exposure to xenoestrogens during prenatal or neonatal life (Sharpe and Skakkebaek, 1993; Colborn et al., 1993).
(ii) Male reproductive effects in Humans

The major male reproductive disorder, testicular dysgenesis syndrome (TDS) is observed in human beings in recent years. TDS is a common name given to male reproductive abnormalities like fallen sperm counts (Carlsen et al., 1992), testicular cancer (Bergstrom et al., 1996; Toppari et al., 1996) and congenital disorders like cryptorchidism (failure of testicular descent into the scrotum) and hypospadias (Urethral opening is not at the tip of the penis) (Paulozi et al., 1997). TDS is associated with abnormal function of gonocytes (fetal germ cells), Sertoli cells and Leydig cells (Sharpe, 2000).

1.3.1c Mechanism of action of endocrine disruptors

Complex interactions are involved in normal gonadal function and hormonal communication. There are multiple loci that could be involved mechanistically in a toxicant’s endocrine-related effects. Impairment of such hormonal control could occur as a consequence of altered hormone biosynthesis, storage/release and transport/clearance, receptor recognition/binding, and/or post receptor responses. The possible mechanism of action of endocrine disruptors is as follows:

(i) Altered hormone biosynthesis: A number of agents possess the ability to inhibit the biosynthesis of various hormones or specific enzymatic steps in the biosynthetic pathway of steroidogenesis. Environmental estrogens and antiandrogens further alter pregnane biosynthesis induced by gonadal steroids through a series of signals at transcriptional and translational levels (Manavathi and Kumar, 2006). Both estrogen and testosterone have been shown to affect pituitary hormone synthesis directly or through changes in the glycosylation of LH and FSH (Wilson et al., 1990). A decrease in glycosylation of these glycoproteins reduces the biological activity of the hormones. Any environmental compound that mimics or antagonizes the action of these steroid hormones could presumably alter glycosylation.

(ii) Altered hormone storage and/or release: Steroid hormones do not appear to be stored intracellularly within membranous secretory granules. For example, testosterone is synthesized by the Leydig cells of the testis and released on activation of the LH receptor. Thus, compounds that block the LH receptor or the activation of the 3',5'-cyclic AMP (cAMP) dependent cascade involved in testosterone biosynthesis can rapidly alter the secretion of this hormone (Sikka and Wang, 2008).
(iii) Altered hormone transport and clearance: Steroid hormones are transported in the blood by specialized transport (carrier) proteins known as steroid hormone-binding globulin or testosterone-estrogen-binding globulin. Thus any toxic insult if interferes with binding of steroid hormones with their respective proteins could affect bioavailability of steroid hormones.

(iv) Altered hormone receptor recognition/binding: Hormones elicit responses from their respective target tissues through direct interactions with either intracellular receptors or membrane-bound receptors. A number of environmental agents may alter this process by mimicking the natural ligand and acting as an agonist or by inhibiting binding and acting as an antagonist (White et al., 1994; Kelce et al., 1995).

(v) Induction of oxidative stress

Apart from endocrine disruption, another mechanism that has emerged in the last two decades is the discovery of reactive oxygen species (ROS) and the role of associated oxidative stress in the etiology of defective sperm function and male infertility (Bustos-Obregón and Hartley, 2008). Oxidative stress (OS) is theoretically the result of an improper balance between ROS generation and intrinsic scavenging activities. Adequate levels of superoxide dismutase, catalase, and probably glutathione peroxidase and reductase normally maintain the free radical scavenging potential in the testes. This balance can be referred to as oxidative stress status and its imbalance may play a critical role in inducing testicular toxicity and infertility (Sikka, 1996). Many contaminants have been reported to disturb the pro-oxidant/antioxidant balance leading to excessive generation of ROS (Sikka and Wang, 2008). Sperms are the major targets of OS; this is because the PUFAs that are present in the sperm plasma membrane are very susceptible to ROS attack, resulting in decrease in sperm motility and viability leading to infertility. These free radicals are produced as a result of electron leakage due to interaction of steroid products or other pseudosubstrate with the enzymes. The inability of the pseudosubstrate to be oxygenated promotes the release of ROS (Peltola et al., 1996). Some environmental contaminants are reported to produce ROS through the above-mentioned mechanism and thereby produce oxidative changes in testis (Sujatha et al., 2001). Decreased activity of steroidogenic enzymes and SOD, which has been shown to act as an alternate regulatory switch in testicular steroidogenesis have been linked to the possible role of ROS in reduced steroidogenesis.
1.4. Metals and male reproductive health

Many heavy metals are classical testicular toxicants, though the mechanism of their action may differ. Metals are chemical elements with specific gravity that is at least five times the specific gravity of water (Passow et al., 1961; Hawkes, 1997). Examples of heavy metals commonly found in the environment include lead, cadmium, mercury, zinc, arsenic, bismuth etc. These metals are particularly dangerous because they tend to bio-accumulate in the body tissues and organs (Babalola et al., 2005). Reproductive hazards from metal exposure in males are one of the fastest growing areas of concern in toxicology today. Exposure to different heavy metals causes irreversible toxic insult to male reproductive system. Metals produce cellular impairments at structural and functional level in male reproductive system. The effect of heavy metals, such as lead, mercury, cadmium, chromium and arsenic on male reproduction has been studied in detail in various experimental species. Data on effect of heavy metals on humans are steadily building up. Metals could interfere with the gametogenic cells or Leydig cell or spermatozoa directly in semen. These effects may result in reduced fertility or associated with pregnancy wastage, congenital malformation associated with genetic diseases.

1.4.1 Lead

Among a gamut of metals, lead is a ubiquitous and versatile metal which has been used by mankind for many years. It ranks as one of the most serious environmental poisons amongst the toxic heavy metals all over the world. Since antiquity, mankind started using lead in wide variety of applications.

(i) Sources

Most of lead contamination came from human activities like mining, manufacturing, and the burning of fossil fuels. Lead is used as a construction material for equipment used in sulfuric acid manufacture, petrol refining, halogenation, sulfonation, extraction and condensation. It is used in storage batteries, alloys, solder, ceramics and plastics. It is also used in the manufacture of pigments, tetraethyl lead and other lead compounds, in ammunition, and for atomic radiation and X-ray protection. Lead is used in aircraft manufacture, building construction materials (alloyed with copper, zinc, magnesium, manganese and silicon), insulated cables and wiring, household utensils, laboratory equipment, packaging materials, reflectors,
paper industry, printing inks, glass industry, water purification and water-proofing in the textile industry.

(ii) Human exposure

Human exposure to lead is from numerous sources and a myriad of pathways including air, food, dust, soil and water (Gillillan, 1965; Herman et al., 2007). The important sources of lead exposure include gasoline additives, food can solder, lead-based paints, ceramic glazes, drinking water system, cosmetics and plastic recycling industries. Research has also shown that lead is present in tobacco, cigarettes contain 2.4 μg of lead and 5% of this occurs in ash and side stream smoke (Mussalo-Rahumaa et al., 1986). In the recent past, lead toxicity has emerged as an important global problem with public health consequences, particularly in children, due to its serious impact on brain functions. A higher incidence of acute intoxication among children than adults has been reported and children are exposed to higher levels of lead than of adults because of behavioral patterns (for example, characteristic mouthing of objects). Also, exposures to lead from sources such as air, food and water are higher as per kilogram of body weight basis for children than for adults (Kamala and Kumar, 1998; Gidlow, 2004; Sikka and Wang, 2008; Meyer et al., 2008).

1.4.2 Metabolism

Lead is absorbed by ingestion and inhalation. Absorption varies from individuals to individuals and depends on the chemical form of lead and type of exposure. For example, about 99% of the amount of lead taken into the body of an adult will leave in the waste within a couple of weeks, but only about 32% of the lead taken into the body of a child will leave in the waste (Barry, 1975). Once lead enters the body, it travels through the blood to soft tissues such as the liver, kidneys, lungs, brain, spleen, muscles, and heart. The alimentary and respiratory tracts are the main portals of entry for lead into the body. The half-life of lead varies from about a month in blood, 1-1.5 months in soft tissue, and about 25-30 years in bone. Lead passes through the placenta easily and fetal blood has almost the same lead concentration as maternal blood (Laureys et al., 1978; Carpenter, 1974; Ong and Lee, 1980). 90% of the ingested lead is excreted in the stool and urine whereas the inhaled lead is excreted through the renal pathway. Lead is also eliminated through sweat and mother’s milk (Jensen, 2006).
1.4.3 Lead poisoning

(i) Acute poisoning

In acute poisoning, typical neurological signs are pain, muscle weakness, paraesthesia, and rarely, symptoms associated with encephalitis (Pearce, 2007). Abdominal pain, nausea, vomiting, diarrhea, and constipation are other acute symptoms. Gastrointestinal problems, such as constipation, diarrhea, poor appetite, or weight loss, hemolysis (the rupture of red blood cells), anemia and decreased urination due to damage to kidneys are also symptoms of acute poisoning.

(ii) Chronic poisoning

Chronic exposure to lead affects multiple target systems like gastrointestinal, neuromuscular, and neurological (Pearce, 2007; Kosnett, 2007). Central nervous system and neuromuscular symptoms usually result from intense exposure, while gastrointestinal symptoms usually result from exposure over longer periods. The symptoms of chronic exposure include loss of short-term memory or concentration, depression, nausea, abdominal pain, loss of coordination, and numbness and tingling in the extremities, problems with sleep, headaches, stupor, slurred speech, and anemia are also found in chronic lead poisoning (Pearce, 2007; Patrik, 2006). Another important feature of chronic exposure to lead is appearance of “lead hue” on the skin (Needleman, 2004) and a blue line along the gum, with bluish black edging to the teeth (Rambousek, 2008). It has also been reported that chronic exposure to lead in children may have hyperkinetic or aggressive behavior disorders (Pearce, 2007).

1.4.4 Target sites of lead

Lead targets all most all parts of the body and exerts its toxicity. Since, lead interferes with a variety of body processes and is toxic to the body systems including the cardiovascular, reproductive, haematopoietic, gastrointestinal, renal, hepatic and nervous systems (Meyer et al., 2008; Nolan and Shaikh, 1992; Gajawat et al., 2006). Lead mainly affects heme-synthesis in both adults and children. It has been reported that increased lead levels elevate serum erythrocyte protoporphyrin and also urinary excretion of coproporphyrin and δ-aminolaevulinic acid. Whereas at lower levels, lead inhibits the enzymes δ-aminolaevulinic acid dehydratase and dihydrobiopterin reductase (Arai and Yamamura, 1990).
Lead impairs learning, memory and audio-visual functions in children (Cohn et al., 1993). Lead has been shown to be associated with impaired neurobehavioral functioning in children. (Ernhart, 1980). It has been shown that blood lead levels as low as 10μg/dl have been associated with developmental delays, deficits in intellectual performance and neurobehavioral functioning (Davis and Svendsgaard, 1987; Mushak et al., 1989), decreased stature (Schwartz et al., 1986, Schwartz and Otto, 1987) and diminished hearing acuity (Schwartz and Otto, 1991). Nephrotoxic effects include proximal renal tubular damage, characterized by generalized aminoaciduria, hypophosphataemia with relative hyperphosphaturia and glycosuria accompanied by nuclear inclusion bodies, mitochondrial changes and cytomegaly of the proximal tubular epithelial cells. Tubular effects are noted after relatively short-term exposures and are generally reversible, whereas sclerotic changes and interstitial fibrosis, resulting in decreased kidney function and possible renal failure, require chronic exposure to high lead levels (Lin et al., 2003).

Lead-induced cardiotoxic effects seem to be indirect and occur via the autonomic nervous system; it has no direct effect on the myocardium (Chen et al., 2005). Evidence suggests that lead exposure is associated with high blood pressure, and studies have also found connections between lead exposure and coronary heart disease, heart rate variability, and death from stroke (Ana Navas-Acien et al., 2007). The carcinogenic effect of lead has been receiving increasing attention (Silbergeld et al., 2000). Research has shown that lead causes oxidative stress in the body by inducing the generation of free radicals (Gurer and Ercal, 2000). Many studies have also demonstrated that lead is also implicated in immunological problems. It apparently impairs antibody production and decreases immunoglobulin plaque-forming cells. An increased percentage and increased absolute count of B lymphocytes were seen in workers with blood lead levels of >50 μg/100 ml (Gidlow, 2004).

Effect of lead on reproductive systems is also well documented. Both human (Winder, 1989) and animal (Foster et al., 1993) studies suggest that lead affects both steroidogenesis and spermatogenesis and thereby decreases the testosterone production and in females, menstrual irregularities, preterm deliveries and still births.
1.4.5. Lead and male reproductive health in animal system

Several toxicological studies have addressed the possible relationship between reproductive toxicity and exposure to lead but the results are controversial and the effect of lead on male reproduction and male-mediated developmental toxicity is still unclear. Lead targets all most all compartments of reproductive system (Retto de Queiroz and Waissmann, 2006). It is intriguing that lead exposure causes age specific reproductive effects at all developmental stages viz., adult stage (Naha and Chowdary, 2005), pubertal and prepubertal stage (Sokol and Ucmal, 1991), neonatal stage (Klien et al., 1981), and prenatal stage (Buchet et al., 1977).

Reproductive dysfunction has been described in men exposed to lead at the workplace, including oligozoospermia and dose-dependent astenozoospermia (Sikka and Wang, 2008). A study in which men were exposed to lead in the workplace showed that increased blood lead levels were associated with decreased libido and an increase in semen abnormalities (Retto de Queiroz and Waissmann, 2006). Histological observation of testicular sections of lead treated mice reveals germ cell disorganization, epithelial vacuolization and cell loss (Batra et al., 1998). According to Adhikari et al. (2001), high doses of lead elicit apoptosis of germ cells which is a common mechanism of action of many toxic chemicals, including lead. Although testicular biopsies reveal peritubular fibrosis, vacuolation, and oligospermia, suggesting that lead is a direct testicular toxicant (Braunstein et al., 1978). Lead exposure is also known to disrupt the hormonal feed-back mechanism at the hypothalamic-pituitary level (Sokol, 1987).

Earlier studies have demonstrated that lead can pass through the blood-testis barrier, accumulate in the testis and/or epididymus and affect the germinal cells at different levels of differentiation (spermatogonia, primary spermatocytes, spermatids or spermatozoa) (Apostoli et al., 1999). Acute and chronic lead poisoning is associated with severe damage in various organs, particularly the testes, in both humans and animals (Buerger et al., 1986; Fair and Ricklefs, 2002; Snoeijis et al., 2004). Gennart et al. (1992) reported that long-term exposure to lead causes asthenospermia and oligospermia. Lead-exposed battery factory workers showed a decrease in sperm count, density, motility and semen volume (Bonde et al., 2002). In contrast, Xu et al. (2006) observed no correlation between quality of sperm and seminal plasma lead concentration. Lead can alter prostate secretory function.
concentration of zinc, acid phosphatase, and citric acid in the seminal fluid). Donovan et al. (1980) showed that in animals lead, like other divalent cations, can inhibit the binding of dihydrotestosterone to specific receptors in the prostate and seminal vesicle. It is apparent that lead-induced reproductive-toxicity affects all most all compartments of reproductive organs.

1.4.6. Mechanism of action of lead-induced toxicity

1.4.6a Effect of lead on HPT axis

Lead exposure can disrupt the hormonal feed-back mechanism at the hypothalamic-pituitary level (Sokol, 1987). A significant decrease in testicular weights with reduced androgen secretion as a result of exposure to lead was observed indicating that testicular endocrine function had been compromised (Xu et al., 2006). Since spermatogenesis and fertility are critically dependent upon the maintenance of adequate levels of testosterone, the ability of lead to reduce serum testosterone levels might contribute to the reduction in spermatogenesis and fertility after exposure to lead. Lead effects on HPT axis are confounding. In some studies, LH and FSH levels were not significantly affected after lead treatment despite the increase of LH level observed in all lead-treated groups; this suggests that there was no obvious alteration of hypothalamic-pituitary function. No changes in serum LH and FSH levels were observed in rats, mice or monkeys (Sokol, 1989, 1990; Foster et al., 1993; Pinon-Lataillade et al., 1995; Wadi and Ahmad, 1999), while some studies demonstrated a decrease in serum FSH and LH concentrations (Batra et al., 2004; Biswas and Ghosh, 2004). Concerning testosterone levels, some experimental groups suggested that serum testosterone level was decreased after lead exposure (Sokol, 1989; Thoreux-Manlay et al., 1995; Biswas and Ghosh, 2004; Rubio et al., 2006), but in contrast others demonstrated that the serum testosterone level was unaffected (Johansson and Wide, 1986; Foster et al., 1993; Pinon-Lataillade et al., 1995; Foster et al., 1988).

1.4.6b Lead induced oxidative stress

Generation of highly reactive oxygen species in the aftermath of lead exposure may result in systematic mobilization and depletion of the cell’s intrinsic antioxidant defenses. When formation of reactive oxygen intermediates outstrips the scavenging capacity of antioxidant defense mechanisms, harmful free radicals accumulate and increase the likelihood of oxidative damage to critical biomolecules, such as
enzymes, proteins, DNA, and membrane lipids. Several mechanisms have been proposed to mediate the oxidative stress caused by lead, mostly associated with disrupted prooxidant/antioxidant balance (Hsu and Guo, 2002). Biochemically, the generation of ROS such as superoxide radicals, hydrogen peroxide and hydroxyl radical is known to result in cellular oxidative damage: lipid peroxidation leads to loss of membrane integrity causing increased cell permeability to electrolytes (Halliwell and Gutteridge, 1999). This free radical mediated process may also cause enzyme inactivation, structural damage to DNA and cell death. If hydroxyl radicals are generated close to DNA, they can attack the purine and pyrimidine bases and cause DNA base changes. Mammalian spermatozoa are known to be highly sensitive to injuries caused by high oxygen concentration (Cummings and Laskey, 1993). The production of abnormal levels of ROS is now believed to be involved in many aspects of human male infertility where spermatozoa are rendered dysfunctional by lipid peroxidation, altered membrane function and impaired metabolism. Recent studies suggest that lead poisoning disrupts the prooxidant /antioxidant balance and could contribute at least partially to this element toxicity by affecting membranes, DNA and antioxidant defense systems of cells. Indeed, an increase of ROS production was observed after lead exposure in sperm or in the reproductive organs in rats (Hsu et al., 1997; Marchlewicz et al., 2007) and in human (Kasperczyk et al., 2008). Thus it is clear that though lead alters endocrine mediated functions and also pro-and anti-oxidant status, the results are conflicting. There are, however, many questions to be elucidated clarify with regard to the effect of metal on the male reproductive system. Therefore, more experimental data is necessary to confirm the exact mechanism of action of lead on male reproductive health.

Although a large number of studies have been reported on detrimental and deleterious effects of lead on reproduction (Semczuk and Semczuk- Sikora 2001), the damaging effects of lead on developmental toxicity has not attracted the attention of researchers. Thus, critical information is needed to determine the minimum effective exposure level of lead causing the developmental restraints on puberty-related hormones and the onset of puberty, as well as the developmental stage(s) of exposure when the detrimental effects of lead occur. A few toxicological studies have addressed the possible relationship between reproductive toxicity and exposure to lead but the results are controversial and the effect of lead on male-mediated
developmental toxicity is still unclear. There have been no systemic reports dealing particularly with lead-induced male reproductive toxicity in adults and in F1 generation adults exposed to lead during perinatal period. These two time-points are primarily selected because they give immense scope to study the toxicity of lead on reproductive system in rats at different time points, a firmly shaped male reproductive system (adult rats) and developing/shaping male reproductive system (perinatal period).

Another concern is towards the restoration of lead-induced deteriorated reproductive health. Experiments are designed to investigate whether supplementation of testosterone reduces the risk of male reproduction in rats exposed to lead during pubertal period and/or perinatal period. It is well established that testosterone, a potent hormone plays a crucial role in the development and differentiation of the male reproductive organs. Since, hormones play a crucial role in male reproduction; hormone replacement therapy is a commonly used approach to treat male reproductive disorders. Hypothalamus-pituitary-testicular axis is a vulnerable target to a range of pollutants including lead (Sant Ana et al., 2001; Biswas and Ghosh, 2004) and thus, exogenous supplementation of testosterone is generally used to support male fertility. It has long been known that testosterone replacement therapy restores partially or completely many of the adverse pathophysiological events which occur in androgen deficiency, including a regain of libido and erectile function.

In view of this, the present study is aimed at evaluating the 1) effect of exposure to lead acetate on male reproductive health in pubertal rats and 2) effect of exposure of male to lead acetate during gestation and lactation on reproductive performance in rats at their adulthood. The studies are also extended to know the effect of testosterone supplementation on lead-induced suppressed male reproduction in rats exposed during pubertal and/or perinatal period.
Figure 1.1: Male reproductive system of rat

LT: Left testis   RT: Right testis  E: Epididymis
CP: Cap out     CD: Cauda part    VD: Vas deferens
SV: Seminal vesicles  PG: Prostate gland  P: Penis