REVIEW
OF
LITERATURE
Reproduction is a key biologic event for all the living beings. Declining fertility is of serious concern since defects passed on to the progeny are detrimental to the very existence of human species. Further, it is important to realize the fact that reproductive processes entail more than just proliferation of the organism. It involves fusion of gametes and mixing of genetic material from two different individuals. Therefore, hereditary material should be passed unaffected to the progeny. Any threat to reproductive health evokes significant response not only from the scientific community but also from the public media.

Lack of awareness and indiscriminate use of chemicals in all spheres of life has increased the burden of toxic chemicals in the environment. Of the thousands of new chemicals introduced each year, only few are tested for reproductive toxicity. Persistence in the environment and body burden raises the very important question regarding their effects on future generations. In this frenzied pace of development, we are risking the safety of the environment and indirectly the existence of life.

The principal components of the male reproductive system are testis, accessory sex glands such as seminal vesicles, prostate gland, and bulbourethral or Cowper's gland and the duct system which includes the efferent ducts, epididymis, ductus deferens and ejaculatory duct. Reproduction is a complex process that consists of a series of sequential steps right from sperm production to capacitation and acrosome reaction to sperm oocyte fusion.
The mammalian testis has evolved to perform dual functions of being both exocrine and endocrine. Its primary function is to produce sperms, and other is to secrete male sex hormones, the androgens, which regulate spermatogenesis, development and differentiation of accessory reproductive organs and synchronize their functional physiology. The seminiferous tubule contains two types of cells. An interdependent mass of the proliferating cells, the germ cells produce sperm. The second type of cells are the non-proliferating group of irregularly shaped sessile Sertoli cells. They provide architectural support to the tubules and also a microenvironment for the germ cells undergoing the process of sperm formation. The vascularized interstitial tissue contains the Leydig cells, which produce androgens. Spermatogonia are dormant until after birth and up to the time of puberty when they begin to increase in number through mitosis and produce primary spermatocytes, which further divide meiotically to form two haploid secondary spermatocytes, that further divide to form four spermatids. The sperms then begin their journey through the rete testis and efferent ducts into the epididymis. The sperm undergo maturation in the epididymis where they acquire motility and are stored there until ejaculation. The accessory sex glands contribute most of the volume to the semen, and their secretions may be responsible for effective transport, survival, and function of the sperm through the female reproductive tract. The duct system and the accessory glands are androgen-dependent, and can be affected by agents that alter androgen levels.

Mammalian spermatozoa are small, motile and consist of two principal parts, head and the tail. The tail consists of four components such as the neck, mid-piece, principal piece and end piece (Phillips, 1975). The main part of the head is occupied by the nucleus, which largely consists of closely packed chromatin material. The narrow region, which connects the sperm head with the middle piece, is known as the neck. The middle piece contains the mitochondria in the form of a sheath around the mid piece. These organelles are arranged in a lightly coiled spiral, surrounding the contractile fibrils, which provide locomotion and originate in the neck of the spermatozoa and pass through the tail. The acrosome is a membrane bound
cap like structure covering the anterior portion of the sperm nucleus. It is formed by the golgi apparatus during spermatogenesis. Acrosomal reaction is the release of materials capable of lysing the zona pellucida surrounding the ovum.

Successful regulation and function of the male reproductive system depends upon a balanced interplay among the hypothalamus, anterior pituitary, and testis. Production of gonadotrophin releasing hormone (GnRH) by the hypothalamus stimulates the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary. This in turn permits release of testosterone from the Leydig cells, which is negatively regulated by increased levels of testosterone. Environmental agents might disrupt the reproductive process directly/indirectly by affecting either the hypothalamic-pituitary axis, or pituitary-testicular axis, testis or accessory sex glands. Some toxicants might be functioning as hormonal agonist or antagonist. Toxic agents can alter endocrine function by affecting any part of the CNS-pituitary-gonadal axis. If a compound affects the CNS or pituitary then serum LH and FSH may be altered leading to decreased testosterone concentrations. On the other hand, severe interference with Sertoli cell function or spermatogenesis would be expected to elevate serum FSH levels due to diminished secretion of inhibin.

Changes in the levels of reproductive hormones may be helpful in identifying sites or mechanisms of toxic action. Thus a proper regulation between spermatogenesis, hormone production and release as well as biochemical secretions from the accessory sex glands is necessary for the successful maturation of sperm and sperm oocyte fusion. If toxicants affect any of these events/processes, results will reflect in the deterioration of semen quality, reproductive function and outcome. Exogenous chemicals might influence the organs and processes that are involved in male reproductive sexual maturation function directly or indirectly. Reproductive toxicants might act either directly by altering the functioning of the target organ, by affecting a critical cell or cellular component, and/or indirectly by producing alterations in physiologic control mechanisms, e.g. enzyme
induction or inhibition or hormonal production and regulation. One of the probable mechanisms of toxic action of environmental chemicals in male reproductive function is shown in Fig-A. It is rational that when a chemical/toxicant enters the body, it is absorbed, distributed, biotransformed and excreted either a parental compound or its metabolite in urine or other biological fluid including semen. Alternatively compounds can also be adsorbed to sperm and be introduced directly into the egg at fertilization (Chapin et al. 2004). These contaminants would then be transferred to the female via the sperm or by the seminal fluid and may produce adverse reproductive outcome.

Reduced male fertility can also be the result of congenital and acquired urogenital abnormalities, infection of the genital tract, increased scrotal temperature, endocrine disturbances, genetic abnormalities and immunological factors (WHO, 2000). No causal factor is found in 60-75% of cases of idiopathic male infertility. These men present with no previous history associated with fertility problems and have normal findings on physical examination and endocrine laboratory testing. Semen analysis reveals a decreased number of spermatozoa (oligozoospermia), decreased motility (asthenozoospermia) and many morphological abnormal forms (teratozoospermia). These unexplained forms of male infertility may be caused by several factors such as chronic stress, endocrine disruption due to environmental pollution, reactive oxygen species and genetic abnormalities (Dohle et al. 2004). Heavy metals induce modifications of neurotransmitters in the CNS and impair the pulsatile hypothalamic release of GnRH. This will lead to alteration in the reproductive hormone regulation. Dancher et al. (1990) also reported that a number of harmful substances such as mercury are stored in the pituitary gland and affect the production of gonadotropins, which may affect reproductive function.

In recent years a debate has been raised over a new form of hazard known as "endocrine disruption" in the form of altered sex differentiation, immune function and malformation in reproductive functions that might be associated with exposure to certain persistent chemicals. These chemicals...
Fig A: Schematic diagram of possible role of environmental toxicants in male reproduction.
are called endocrine disruptors. These endocrine disruptive chemicals can interfere with the body’s own hormones production and release and jeopardize health including reproduction. A large number of environmental chemicals have the potential of causing adverse effects and/or alter the functions of endocrine system, which might cause adverse health effects to an organism or its progeny. These synthetic chemicals and their metabolites or byproducts have entered into the environment due to industrial revolution. In 1991, a hypothesis was formulated suggesting that numerous xenobiotic chemicals used in everyday commerce or natural chemicals released into the environment by human activity had the potential to disrupt the endocrine system of wildlife and humans at ecologically relevant concentrations; this hypothesis has become known as the endocrine disrupting contaminants (EDCs) hypothesis (Guillette, 2005). An early documented case concerning the effect of endocrine disrupting chemicals on reproduction was the report of Fox (1992). He reported that newly hatched gull chicks collected from lake Ontario, USA, a region highly contaminated with DDT had altered reproductive system. Further reduction in penis size and serum testosterone levels in juvenile population of alligators of lake Apopka, Florida, USA have also been attributed the contamination of the lake by DDT metabolite p,p-DDE (Guillette et al. 1996). Studies that clearly indicate exposure and effects measures especially in human are scanty on environmental endocrine disruptors. Scientists believe that hormone-disrupting chemicals have contributed to various health and reproductive problems in men and women, including a rising incidence of breast, prostate and testicular cancers, decline in sperm count, an increasing incidence of endometriosis (a disease of women where uterine endometrial tissue grows outside the uterus), and increasing genital defects. Major debates, in the scientific and public arena, continue to arise around the issues of EDC-induced human health effects, especially breast cancer, human semen quality and birth defects of the genitalia and reproductive system (McLachlan, 2001). Although experimental animal studies demonstrate that high-level in-utero exposure to certain environmental persistent chemicals can impair fertility and increase the rate of spontaneous abortion, human studies that clearly indicate exposure level and effects measures are scanty on environmental
endocrine disruptors. Thus, the relationship between observed reproductive effects and endocrine disruption remains speculative. Wada (2001) reviewed the toxicity studies on the major endocrine disruptors and reported that only dioxins, which are endocrine in broad sense, cause toxicity in humans, while toxicity data for the other agents have only been obtained in animal experiments. Metals such as lead, cadmium and mercury are also included in the list of endocrine disruptors.

A number of reports have appeared in literature regarding the occupational exposure and reproductive dysfunction among male. However, in occupational situation, workers are exposed to raw materials, byproducts as well as finished products during manufacture of various chemicals and other products and also in industrial process. General population is also exposed to several thousands of exogenous chemicals daily due to modern industrial process and other developmental activities. However, our forefathers or people of earlier century might not have faced such type of polluted environment during their life span. A large number of mutagenic or potentially toxic chemicals are currently in use in various industries or produced for various other use or domestic purposes and workers or users may be exposed to these chemicals in various ways. Each year industrial facilities release hundreds of millions of pounds of chemicals into the environment, which are linked to cancer, developmental and reproductive problems and neurological and respiratory disorders. In USA, more than 50 million pounds of chemicals related to reproductive disorders were released into air and water in 2000 alone (Dutzik et al. 2003). Some of the chemicals even travel hundreds or thousands of miles away from the source of exposure. For example, dioxin from the incinerator smoke can travel through air or on dust or water particles and eventually fall on the ground, sometimes as far as hundreds or thousands of miles away from the incinerator that released it (IATP, 2001). Thus environmental pollution is not a regional problem but a global problem and has a role in the well being of all the living organisms.
There are several recent reports of declining human sperm counts over the last fifty to sixty years. A systematic meta-analysis of 61 studies was undertaken by Carlsen et al. (1992) It showed a significant decrease in sperm concentration and semen volume over the period 1938-1990. However, other investigators reported no such changes in semen quality (Fisch et al. 1996, Paulsen et al. 1996). Even some of the investigators criticized the pro-decrease paper on statistical point of view (Olsen, 1995). Swan et al. (1998) mentioned that there was no evidence of a decline in semen quality in non-western countries while they observed a significant decline in Europe and United States. Heinze (1998) reported that recent studies from Europe and the United States indicated large interregional differences in sperm density. He noted that interregional differences in the United States (New York city vs Los Angeles, CA) were as large as the reported differences in mean sperm density in 1938 versus 1990. A couple of studies from the Indian subcontinent also reported a decline in semen quality (Mehta and Anand Kumar, 1997, Gopalkrishnan, 1997). Orejuela et al. (1998) emphasized that generalization of a world wide declining trend of semen quality is still risky and highlighted the need for innovative new prospective studies with good quality data to address this important issue related to human reproduction. It has been documented recently that a global trend for declining semen quality is not supported by the current data (IPCS, 2002). Some studies show declines in certain regions or cities, whereas others have not found a decline, suggesting there may be regional trends but not a global trend. Very recently, Kumar (2004) b) suggested that looking at the increasing evidence of declining trend in semen quality, growing rates of testicular and other disorders of male reproductive organs, one can hypothesize that hazardous substances in the environment adversely affect the male reproductive system. It has been postulated by Sharpe and Skakkebaek (1983) that the apparent drop in sperm count may be due to the developmental exposure to estrogenic xenobiotics. Later Skakkebaek et al. (2001) summarized existing evidence advocating a new concept that poor semen quality, testicular cancer, undescended testis and hypospadias are symptoms of one underlying entity, the testicular dysgenesis syndrome (TDS) which may be increasing due to adverse
environmental influence. Still an intense debate is going on among the scientific community about the real picture regarding the decline of semen quality. However, positive findings encourage scientists to take up further studies on this important issue of human reproduction.

A number of studies are available which indicate that some chemicals with diverse structures as well as physical agents such as radiation and temperature affect the male reproductive system of various animal species and some of them have a potential threat to human male reproductive system. The origin of male reproductive toxicity can be traced back as early as 1775 when English physician Percival Pott reported a high incidence of scrotal cancer in chimney workers (Schrader, 1998). However, this area of human toxicology was firmly established after Whorton (1977) studied the adverse effects of the pesticide di-bromo chloropropane (DBCP) on male workers. Since then, a number of reports have appeared on increasing prevalence of various abnormalities of the reproductive system of occupationally exposed workers to chemical and physical agents generated by industrial and agricultural activities (Bonde, 1996; Multigner and Spira, 1997). In the last decade, various reviews have been published that reported higher incidences of reproductive abnormalities due to environmental chemicals in wildlife and humans (Toppari, 1996; Kumar, 2004a). However, in the present scenario of alarming elevation in the environmental pollution, concern is growing among the scientific community whether the deterioration in male reproductive capacity is happening among the general population as observed among workers as a result of occupational exposure to chemicals.

Pesticides represent a major portion of persistent organic pollutants. It is rational to believe that pesticides, which are toxic to pests, might produce some adverse health effect including reproductive toxicity on living beings. A large number of workers are engaged in agricultural sector all over the world including India. These workers are being exposed to various pesticides through different route while handling or spraying. A sizeable number of family members even pregnant women and children are exposed to these chemicals during spraying, mixing or accidentally through ingestion. The
general population is also exposed to these pesticides or their metabolites to some extent through the food chain, as some of the pesticides are persistent in the environment and bio-accumulative.

A classical example of reproductive toxicant is 1,2-dibromo-3-chloropropane (DBCP), which was in use since the mid-1950 as nematocide. Its spermatotoxic effects in rats were discovered in the earlier 60's, but its deleterious effects on human spermatogenesis were discovered only in 1977. It was noted that there was paucity of children among the workers of a DBCP plant in California, after they had started to work in DBCP production (Whorton et al. 1977). They further reported that occupational exposure to DBCP caused reduction in sperm concentration in ejaculates among the exposed workers (Whorton, 1979). Wyrobek et al. (1981), reported harmful effects of carbamate pesticide (carbaryl) on sperm morphology. Significantly higher level of asthenozoospermia and teratozoospermia were found in 2,4-D (2,4-dichloro phenoxy acetic acid) exposed workers engaged in spraying as compared to unexposed control subjects (Lerda and Rizzi, 1991). A few studies are also available on effects of multiple pesticides exposure on the reproductive system of male workers, which might affect the reproductive outcome. In a study carried out in India, male agriculture workers exposed to various pesticides such as DDT, BHC, endosulfan; organophosphorus pesticides i.e. malathion, methyl-parathion, dimethote, monocrotophos, phosphamidon and quinalphos; synthetic pyrethroid like fenvelrate and cypermethrin during mixing and spraying, displayed adverse reproductive performances due to chromosomonal aberrations in germ cells (Rupa et al. 1991). However, Larsen et al. (1998) observed no overall effect of pesticides on male fecundability in their retrospective study among Danish farmers. Later, Thonneau et al. (1999) also reported no relation between fertility (time to pregnancy) and male exposure to pesticides.

Organic solvents represent another major source of chemical exposure in working population. Exposure to solvents can occur in manufacturing process, dry-cleaning, degreasing, painting and paint removal and during printing etc. They are volatile and lipophilic in nature and exposure may
occur via inhalation or dermal route. In humans, occupational exposure to some organic solvents has been related to various disorders of reproductive health. Potential reproductive effects from occupational exposure to ethylene glycol ether are of major concern since these organic solvents have been used widely in industry. 2-methoxyethanol (2-ME) and 2-ethoxyethanol (2-EE) have been shown to affect male fertility (Hardin, 1983). Workers exposed to another solvent ethylene dibromide (EDB) were found to have more sperm with tapered head and fewer sperm per ejaculate than did control (Ratcliffe et al. 1987). Later, Schrader et al. (1988) also reported that long-term EDB exposure resulted in decline of sperm motility and viability, suggesting that the short-term exposure may slow sperm velocity, but longer exposures may cause immotility and cell death. Lancranjan (1972) observed a significant higher frequency of asthenospermia, hypospermia and teratospermia in CS₂ poisoned workers as compared to control. Oliva et al. (2001) reported that environmental factors, particularly exposure to pesticides and solvents, might contribute to the severity of sperm parameters.

The rapid technological advancement is also diminishing manual work at a high pace and an ever-increasing proportion of the population spends more time in a sitting position in transportation and office like surroundings. It is known that moderate increase in scrotal temperature associated with the sitting position impairs testicular function and infertility (Bonde, 1999). Temperature plays an important role in the spermatogenesis of human beings. Therefore, nature has also kept the scrotum outside the body cavity so that the temperature of the testes may be lower than that of the body temperature. Occupational exposure to high temperatures adversely affects testicular function causing partial or complete spermatogenic arrest. This leads to oligoasthenoteratozoospermia (OAT) and azoospermia (Dada et al. 2001). It has been reported that active sperm production is dependent on an environment that is 4°C lower than the normal body temperature (Lahdetie, 1995). Figa-Talmanca et al. (1992) studied the effects on sperm production of chronic occupational exposure to high temperature in ceramic industry. They indicated a higher prevalence of pathologic sperm profile among the
exposed subjects compared to control subjects. Exposure to high temperature results in adverse effect on sperm morphology (Wang et al. 1997) Thus, it is hypothesized that exposure to high temperature probably elevates intratesticular temperature, which might disrupt the normal process leading to the induction of morphologically abnormal sperms with impaired motility However, the extent of damage depends on the severity of the temperature as well as duration of exposure to heat

The advent of industrialization has also resulted in a simultaneous increase in air pollution. The rising demand for energy, food, housing, transport, industries has led to extensive fossil fuel burning and therefore releasing toxic pollutants such as SO₂, NOₓ, particulates, hydrocarbons etc into the air. It has been demonstrated that continuous exposure to traffic pollutants impairs sperm quality in young/middle-aged men (Rosa et al. 2003) Metabolites of PAHs (polycyclic aromatic hydrocarbons) associated with industrial air pollution have been reported to alter male reproductive function in test species (Ford and Huggins, 1963, MacKenzie and Angevine, 1981) Further, metals such as lead and cadmium that are present in the particulate fraction of air pollution have been associated with decrements in human semen quality.

Workers are exposed to various toxic metals and their oxides in a number of occupations. The effect of different heavy metals such as mercury, lead, cadmium, chromium etc. on male and female reproduction has been studied in detail in experimental system However, detailed studies on human reproduction with reference to heavy metals are scanty, but lead is an exception. All the living beings including human are exposed to lead to some extent due to contaminated air, water and food. The general population may also be exposed to lead through leaded pipes that supply drinking water, lead based paints, lead glazed ceramics, plants grown in lead contaminated soil, ayurvedic medicines, toys, pencils, etc. Further, industrial workers are exposed to high doses of lead in various occupations such as lead based paints, construction, battery manufacturing or recycling, automobile repair, electronics, printing, welding and soldering, jewellery
making and repair, etc. Earlier, vehicular exhaust used to be a major source of lead exposure for the general population, but nowadays due to the use of unleaded gasoline, the problem has subsided to some extent. However, leaded petrol is still in use in many of the underdeveloped areas of the world.

A number of experimental studies are available on the effects of lead on various animal species. Studies carried out in mice indicated that high exposure to lead caused testicular atrophy and impairment of spermatogenesis (Eyden et al. 1978). Recently, Gautam et al. (2001) studied the toxic effects of lead acetate on testicular tissues and sperm morphology of Swiss mice after three different doses of lead acetate. They reported conspicuous degenerative changes in testicular tissues and elevation in sperm head shape abnormalities. Several investigators have reported that oral administration of lead to animals even at doses in the \( \mu g /\text{kg} \) range can cause changes in spermatogenesis (Egorova et al. 1966; Golubovic et al. 1968). It has been reported very recently that an administered dose of 0.1 ppm via drinking water ingestion by neonatal male mice sufficient to produce PbB of 20-60 \( \mu g/dL \) compromised reproductive function in these mice as adults (Pace et al. 2005).

Epidemiological and case studies are available which indicated that occupational exposures to lead have adverse effects on human reproduction. A study on workers of a newspaper printing press carried out by Roychowdhury et al. (1986) indicated that the average sperm counts were significantly lower and lesser proportion of them where found to be motile in the exposed subjects as compared to control. These changes were associated with the level of lead in blood. In a review on male reproductive toxicity of lead in animals and humans, the authors reported that human studies focused mainly on semen quality, endocrine function, and birth rates in occupationally exposed subjects. They mentioned that exposure to inorganic lead greater than 40 \( \mu g/dL \) in blood impaired male reproductive function by reducing sperm count, volume, and density or changing sperm motility and morphology. However, no relevant effects were detected on
endocrine profile (Apostoll et al. 1998). Further, there is also a report, which indicates that moderate exposure to Pb (Blood Pb < 40 µg/dl) and Cd (Blood Cd < 10 µg/L) could significantly reduce human semen quality without conclusive evidence of impairment of male reproductive endocrine function (Telisman et al. 2000). Later, Bonde et al. (2002) reported the adverse effects of lead on sperm concentration and susceptibility to acid induced denaturation of sperm chromatin are unlikely at blood lead concentrations below 45 µg/dL. Very recently, mean lead levels as high as 48.5 µg/dL have been reported among traffic police officers in Peru where leaded gasoline was used. Authors found that sperm motility and viability differed significantly between the < 40 µg/dL and ≥ 40 µg/dL categories and decrease in sperm motility and viability with increasing Pb in simple linear regression (Eibensteiner et al. 2005). In addition, on the basis of a cross sectional study among men employed at a lead smelter unit, Alexander et al. (1998) concluded that blood lead concentrations below the currently accepted workers protection criteria seem to adversely affect spermatogenesis. Benoff et al. (2003) suggested that increased lead levels may contribute to the production of unexplained male infertility.

Lead has been reported to cause oxidative cellular damage in reproductive tissues of adult male rats, which may be closely associated with the reactive oxygen species (ROS) production (Marchlewicz et al. 2004). Earlier, Agarwal and Said (2003) emphasized that ROS plays an essential role in the pathogenesis of many reproductive processes. In male factor infertility oxidative stress attacks the fluidity of the sperm plasma membrane and the integrity of DNA in the sperm nucleus. Successful binding of the sperm on the egg induces an event called mannose-induced acrosome reaction, which depends on the mannose receptors located on the head of the human sperm. Benoff et al. (2000) found that higher lead levels in the seminal plasma correlated with low expression of mannose receptors and with inability of sperm to undergo mannose-induced acrosome reaction. Conversely, higher lead levels were associated with premature (or spontaneous) acrosome reaction that occurs before sperm-egg contact, also
blocking fertilization. Available studies suggest that blood lead levels above 40 μg/dL might have adverse effects on male reproductive function. However, the lower level of Pb at which toxicity is occurring is still under debate among the scientific community and needs further investigation.

Cadmium (Cd) is an ubiquitous element and exposure can occur through contaminated air, water and food. Occupational exposure to cadmium occurs in industries such as metal plating, semiconductor manufacture, wire, plastic, or battery manufacture, welding, soldering, ceramics etc. Food, and cigarette smoke are the biggest sources of cadmium exposure for the general population (ATSDR, 1998) Cadmium can reach in to the body by ingestion with food, such as fish and rice, from areas with contaminated groundwater. Cadmium has been been found to bioaccumulate in reproductive organs of fish and disrupt important endocrine processes, especially those involved in synthesis, release and metabolism of hormones (Tilton et al. 2003) Effects of cadmium on the testis have been extensively reviewed by Gunn and Gould (1970) Laskey et al. (1984) documented decreased testicular weights and decline in testosterone levels in rats at very low doses after oral administration. Semen quality and testicular characteristics were measured in male rabbits exposed to cadmium chloride at 12 or 27 weeks of age The results indicated that Cd with all the three modes of exposure (s.c., orally and i.v.), resulted in depressing sperm output of these males when adults Histopathological examination in the cadmium-treated animals revealed the presence of lesions in the Sertoli cells, the seminiferous tubules, primary and the secondary spermatocytes and spermatids, whereas no significant lesions in the Leydig cells were observed (Lymberopoulos et al 2000).

The experimental studies available points that cadmium causes testicular necrosis in several animal species, although there is scanty data on its possible effects in humans. Semen cadmium levels have been correlated with sperm motility ($r = 0.53$, $p < 0.02$) and curvilinear velocity ($r = 0.64$, $p < 0.002$) (Noack-Fuller et al 1993) Significant correlations have
been reported between blood cadmium levels and volume of semen, midpiece defects, and immature forms of spermatozoa (Chia et al. 1992). Later, Xu et al. (1993) reported a significant inverse correlation between blood cadmium level and sperm density among oligospermic men and between seminal plasma cadmium and semen volume among men without known occupational exposure to cadmium. A significant inverse correlation was found between Cd and sperm density, sperm number per ejaculum in a study carried out in China among non-smokers, which indicated that Cd in seminal plasma could affect semen quality and oxidative DNA damage in human spermatozoa (Xu et al. 2003). Saaranen (1987) found higher levels of cadmium among infertile men compared to fertile men. However, in a study carried out among men with proven fertility (group I) and normozoospermic patients (group II) as well as unselected patients of an infertility clinic (group III) and industrial workers with occupational exposure to cadmium where no significant correlation between seminal cadmium concentrations and conventional semen parameters or between cadmium concentration and the fertility status of the patients was observed (Keck et al., 1995).

Cadmium is also associated with deleterious effects on the gonadal function and with changes in the secretory pattern of hormones like prolactin, adreno cortico trophic hormone (ACTH), growth hormone (GH) or thyroid stimulating hormone (TSH). The available data indicates the existence of a disruption in the regulatory mechanisms of the hypothalamic-pituitary axis by cadmium (Lafuente, 1999). Mason (1990) found no difference between cadmium-exposed men in a manufacturing plant and unexposed controls in serum testosterone, FSH and LH levels. Based on the experimental data available coupled with positive findings in human on cadmium and reproductive impairment, it can be inferred that Cd might have adverse effect on reproduction. The dose and duration of exposure is a matter of discussion for such effects. Further, a recent report has highlighted the potential of Cd to mimic the effects of estrogen in various tissues (Henson and Chedrese, 2004). Hence, it can be speculated that cadmium
has endocrine disruptive effects and might affect reproduction through these mechanisms also.

Trace elements are required in small concentration as essential components of biological enzyme systems or of structural portions of biologically active constituents. These trace elements include iron, iodine, fluorine, copper, zinc, chromium, cobalt, molybdenum, selenium, tin, vanadium, nickel and silicon. Copper is an essential trace element found in its elemental form and also as a component of many minerals. Copper is essential for various physiological functions and it is also required by some cellular enzymes and other biomacro molecular components for their normal function. A large number of enzymes are copper containing proteins e.g. Cytochrome oxidase, erythrocyte superoxide dismutase, lysyl oxidase, skin tyrosine oxidase and diamine oxidase. The levels of copper in serum vary because of interaction with other metal such as zinc, molybdenum, cadmium, iron and calcium. Several elements such as zinc and cadmium reduce copper absorption thereby reducing the serum/plasma copper levels. Exposure to copper has been shown to be linked to a decreased sperm count and to be a cause of terato-and asthenozoospermia (Lahdetie, 1995). Significant correlations between copper concentrations in semen and sperm concentration \( r = 0.32, p < 0.001 \), percentage progressive motility \( r = 0.23, p < 0.005 \) and normal morphology \( r = 0.22, p < 0.005 \) have been reported (Jockenhovel et al. 1990). Similarly, blood plasma copper levels were also significantly correlated with sperm motility (Wong et al., 2001). Both Zn and Cu are linked with the structure/activity center of SOD, which is closely related to sperm motility. Decreases in the Zn and Cu levels will naturally lead to the lowering of the superoxide dismutase (SOD) activity as reported by Zhang et al. (2000). In vitro studies have also reported the toxic effect of copper on sperm motility. Incubation with the metal caused a fall on the percentage of motile sperm, which was directly related to the surface area of copper employed and to the copper content of whole semen. These changes were accompanied by a decrease in semen zinc levels and an uptake of copper by individual sperm cells (Battersby et al. 1982). The significance of copper in seminal plasma therefore becomes a matter of interest in order to
understand the role of copper in human reproduction. Though the beneficial role of copper in reproduction is accepted, it is also not clear as to what level of copper is essential for reproduction and what dose produces toxicity.

Zinc is necessary for growth, sexual maturation and reproduction. A number of animal studies are available on various zinc compounds. Low (12 mg/kg b.wt) or medium (120 mg/kg b.wt) dose of zinc appeared to enhance reproductive function whereas high dose (240 mg/kg b.wt) of zinc appeared to lower reproductive function (Wei et al. 2003). On administration of Zn and Cr together to mice, Afonne et al. (2002) found that greater solubility and higher concentration of zinc forced its absorption rather than that of chromium. The protective role of zinc against lead and cadmium has recently been documented in rats by Batra et al (2004). Based on animal studies it is rational to believe that zinc is having a beneficial role in reproductive function. However, very high levels of zinc might have a detrimental effect on reproduction.

Zinc is secreted by the prostate in small vesicles called protasomes and seems to play an important role in the physiology of spermatozoa. Further, it is also reported to play a significant role in the oxidant defence system (Bray and Bettger, 1990). There are conflicting reports regarding the levels of zinc in seminal plasma and different semen quality parameters. It has been reported that seminal zinc level has a significant positive correlation with sperm count and sperm motility (Madding et al. 1986, Chia et al. 2000). However, Carreras and Mendoza (1999) reported a negative correlation of zinc with sperm motility. These studies indicate that zinc has an important role in maintaining the normal male reproductive function and at certain higher level may have adverse effects on male reproduction.

There are reports which indicate that some of the toxic metals like lead and cadmium, etc have adverse effects on zinc metabolism and availability. Very recent study suggests that Pb may affect sperm chromatin by altering zinc availability (Hernandez-Ochoa et al. 2004). Further, it has...
been reported earlier that co-exposure to Pb and Cd through polluted environment may be more injurious to the functioning of male gonads, however, the effect can be counteracted by simultaneous administration of zinc (Saxena et al. 1989) Human exposures to Pb and Cd are often accompanied by considerable exposure to zinc (and vice versa), which may act as antagonist and thus mask Pb and Cd-related effects, both Pb and Cd can adversely affect zinc metabolism, and possibly Cu metabolism and Cu and Zn can antagonistically influence each others absorption and metabolism (Telisman, 1995).

In addition to environmental exposure to toxic metals, pesticides, etc., certain lifestyle factors also contribute human exposure to toxicants. Tobacco smoke is the best-studied environmental exposure (Benoff et al. 2000) There is a lot of concern about the potential adverse reproductive effect of tobacco consumption, which includes both smoking as well as chewing tobacco A number of studies have shown that smoking detrimentally affects sperm concentration, motility, morphology and damages DNA (Kunzle et al. 2003, Saleh et al. 2002, Stillman et al. 1986, Vine et al. 1996) Cigarette smoking impairs fertility through the putative effect of cadmium in causing testicular endothelial injury and production of antisperm antibodies (Omua et al. 1998) Though there is plenty of data on the effect of smoking on semen quality, there is scanty data on the effect of tobacco chewing and its relation to male reproductive function. Recently, Said et al. (2005) reported a significant decrease in semen quality associated with chewing tobacco However, earlier Dikshit et al. (1987) had reported no significant difference in semen parameters between tobacco consumers and non-users. Alcohol consumption leads to deficits in attention-allocation, prospective cognition, autobiographical memory and disinhibition as well as emotional mechanisms such as depression and aggression (O'Connell and Lawlor, 2005). Impotence, gynecomastia and loss of sexual interest are often associated with alcoholism in men (Boyden, 1983). There are several reports on the effect of alcohol in human semen quality (Kuchena, 1985;
Goverde et al. 1995). Earlier, Brzek et al. (1987) also reported that alcohol consumption decreases semen volume density and motility. Recently Muthusami and Chinnaswamy (2005) reported that alcohol consumption has a detrimental effect on male reproductive hormones and on semen quality.

The weight of evidence from clinical studies suggests that while assessing factors associated with semen quality, in addition to consideration of occupational and environmental exposure and personal habits, the role of age should not be ruled out. Age is associated with a decline in semen parameters reported by various workers (Schwartz et al. 1983; Kidd et al. 2001). Recently, Chen et al. (2003) also observed that semen volume, sperm concentration, total sperm count, motility, total motile sperm, and morphology significantly decreased as age increased. In addition, as age increased, the percentage of sperm with tail defects increased.

It is a known fact that the process of spermatogenesis is highly complex and the mechanisms underlying reproductive toxic alteration are still being worked on. The impact of toxic exposure on the male reproductive system is most often studied with the help of semen quality which is based on an evaluation of several parameters, including semen volume, pH, sperm concentration, sperm motility, and sperm morphology. Bonde et al. (2002) reported that the probability of conception increased with increasing sperm concentration up to 40 million/ml, but any higher sperm density was not associated with additional likelihood of pregnancy. Sperm motility has been shown to be a good predictor of human male fertility in vivo and in vitro (Auger et al. 1994). Bonde et al. (1999) also observed that proportion of sperm with normal morphology was strongly related to likelihood of pregnancy, independently of sperm concentration.

Assessment of sperm vitality is one of the basic elements of semen analysis, and is especially important in samples where many sperm are immotile, to distinguish between immotile dead sperm and immotile live sperm (BjoErndahl et al. 2003). The functional and structural integrity of the sperm membrane are crucial for the viability of the spermatozoa (Lechniak et
In addition, the most important mechanisms of fertilization such as capacitation, acrosome reaction and binding of the spermatozoa to the egg surface are believed to depend on the functional integrity of the sperm membrane (Tartagni et al. 2002). The hypoosmotic swelling test (HOS) is supposed to be a useful assay in assessing the integrity of sperm membrane. This test is based on the semi permeability of the intact sperm cell membrane. HOS scores have been reported to be significantly lower (p < 0.001) among oligoasthenozoospermics compared to group having normal motility (Carpino et al. 1998). However, there have been mixed reports on the value of the HOS test in predicting outcome following in vitro fertilization (Check, 1995). Some researchers have even disputed the fact that HOS scores can be used to predict fertilization rate (Milingos et al. 1996).

The interest to assess the chromatin quality of human sperm has immense significance since DNA damage in sperm from infertile men has been associated with infertility in numerous studies. It is the sperm head that carries the nuclear material, and any damage or alteration in the DNA might cause defect in the spermatozoa, which might lead to subfertility or infertility. Agarwal and Said (2003) mentioned the importance of the assessment of sperm chromatin/DNA, which is considered an independent measure of sperm quality that may yield better diagnostic and prognostic approaches than standard sperm parameters (concentration, motility and morphology). The acrosomal region of the spermatozoa is very important as it contains a number of enzymes, which play a decisive role in the penetration of the spermatozoa through the oocyte.

Biochemical analysis of the seminal plasma provides insights into the function of the accessory sex glands (Schrader, 1998). Chemicals that are secreted primarily by each of the glands of this system are typically selected to serve as a marker for each respective gland. For example, the epididymis is represented by alpha glucosidase, the seminal vesicles by fructose, and the prostate gland by zinc. Seminal plasma, in addition to spermatozoa, consists of secretions from various glands—the seminal vesicles, prostrate, and the epididymis. Seminal plasma contributes importantly to the normal
coitus-fertilization scenario. It serves as a vehicle for sperm transport, a buffer from the hostile acidic vaginal environment and an initial energy source for the sperm. Alpha-glucosidase activity is reported to be a good indicator of epididymal function (WHO, 1999). Further, sperm maturation also takes place in the epididymis. Thus studying the relationship of alpha-glucosidase activity with sperm count, motility will be constructive to assess epididymal function. Mahmoud et al. (2002) reported that alpha glucosidase measurement in seminal plasma might be helpful for the differential diagnosis of certain cases with azoospermia. Further, Zopfgen et al. (2000) studied biochemical markers in infertile men and found a close statistical relationship with a correlation coefficient in case of neutral alpha-glucosidase to assess epididymal function as compared to other markers such as free carnitine and total carnitine. Fructose is secreted by the seminal vesicles and is the major carbohydrate source in seminal plasma and essential for normal sperm motility (Buckett and Lewsi-Jones, 2002). Level of fructose in seminal plasma indicates the functional status of the seminal vesicles.

The overall functioning of the reproductive system is controlled by the nervous system and the hormones produced by the endocrine glands. These hormones act upon multiple interdependent target cells, directing the development of gametes as well as their transport, release, fertilization, implantation and gestation. The reproductive neuroendocrine axis of males involves principally the CNS, the anterior pituitary gland and the testis. Gonadotropins (FSH and LH) and testosterone are the prime regulators of germ cell development. Abnormal spermatogenesis is often associated with altered serum gonadotropins and testosterone. GnRH secreted from the hypothalamus stimulates the pituitary gland to secrete the gonadotropins-follicle stimulating hormone (FSH) and luteinizing hormone (LH). One of the roles of follicle stimulating hormone (FSH) is to stimulate Sertoli cells to produce androgen-binding protein (ABP) to transport androgens to differentiating germ cells. The role of luteinizing hormone (LH) is to stimulate and maintain the continual synthesis of androgens in interstitial cells (Hayes, 1984). The testis has a dual role involving both hormonal and spermatogenic functions. Testosterone, the major androgen in men, is necessary for fetal
male sexual differentiation, pubertal development, and the maintenance of adult secondary sex characteristics and spermatogenesis. Disorders of any endocrine systems, involves both over and under active hormone secretion, the effects of which may extend to many different organs and functions. Toxicants that damage the Leydig cells can lead to reduce secretion of testosterone, which in turn affect the Sertoli cell function and spermatogenesis. Most of reproductive toxicants are thought to act directly on the testis. There are some indications that substances interacting with the pituitary secretion of gonadotropin (FSH, LH) and hypothalamic neuroendocrine releasing factors may also play an important role in deterioration of semen quality. Though the role of FSH, LH and testosterone has been well documented, there are few reports on the levels of these hormones in seminal plasma, which can be used as markers of reproductive dysfunction due to toxicants. The relationship of hormone levels in seminal plasma with semen indices with respect to toxicants remains largely unexplored.

Evidence is accumulating on the elevated levels of PRL, hyperprolactinemia (hyperPRL), can interfere with testicular function, libido, potency, and fertility (Bartke, 2004). There are reports associating higher serum prolactin levels with low sperm counts in men (Gonzales et al., 1992). Very recently Arowojolu et al. (2004) also reported that serum prolactin levels were significantly correlated to sperm motility. Earlier, Zepp et al. (1973) showed that prolactin increased the uptake of testosterone by the anterior and dorsal robes of the rat prostate. Stimulatory effects of prolactin on seminal vesicles have also been reported (Antliff et al., 1960; Bartke, 1974). However, there is scanty literature on prolactin levels in seminal plasma and semen parameters, and till date there has been no conclusive finding. Anbarg and Sukcharoen (1996) found significant differences in blood and semen lead levels, LH levels and prolactin levels in workmen who were exposed to lead.

Keeping in view of deleterious reproductive hazards associated with certain occupations in which exposure is much higher than chronic environmental exposure, the same effects might also be possible with
environmental exposure to such chemicals. Action is needed to protect people from hazards and to prevent disorders of reproduction. However, occupational/environmental reproductive disorders can be successfully controlled only through aggressive prevention programs and may even be controlled eventually. Prevention of occupational disorders of reproduction, is much more problematic due to gaps in the knowledge as well as other non-occupational factors, which cannot be separated from occupational factors. Because of scanty data and gaps in the existing knowledge, the prevention strategy should focus on research to find out reproductive toxicants by conducting well-planned animal studies. Prevention of most disorders of reproduction in humans, however, will require successful research programs to improve our understanding of reproductive and developmental biology and to identify etiologic agents and populations at risk. The risk assessment to human is absolutely necessary for such chemicals that already proved toxic to reproductive system in animal studies.

Occupations, which are hazardous to reproduction, are generally assessed on the basis of year of services, possible exposure agent based on the chemical used in the industry as well as industrial environmental monitoring and adverse reproductive outcome such as abortion, impaired semen quality, congenital malformations, etc. However, the biological exposure monitoring data along with effect parameters are scanty which provide exact compound/chemical/factor responsible for the reproductive impairment. Various confounding factors such as malnutrition, infections, smoking, indoor pollution, etc. are also involved in reproductive dysfunction. Thus in the present study both the approaches have been considered and information on exposure to various toxicants, life style factors, work and home environment of the subjects were collected on predesigned proforma through questionnaire and Pb, Cd and Cu were measured in blood and seminal plasma. Feldman et al. (1989) and Kelly et al. (1990) reported that the ability of a person to recall specific exposure was good to excellent for some environmental exposure. However, some problems exist such as possible interviewer bias and selective memory of respondents.
In the present study the relationship of Pb, Cd, Zn, Cu with parameters of semen quality and reproductive endocrine function in men was assessed. The possible influences of age, smoking habits, chewing tobacco and alcohol consumption on reproductive parameters were evaluated bearing in mind that they are common confounding factors associated with reproductive function.