DISCUSSION
Reproduction is one of the essential functions of plants, animals, and even single cell organisms, as necessary for the preservation of the species as eating is for the preservation of the individual. Most multicellular animals and plants undergo a complex form of sexual reproduction in which especially differentiated male and female reproductive cells (gametes) unite to form a single cell, known as a zygote, which later undergoes successive divisions to form a new organism. In this form of sexual reproduction, half the genes, the carriers of inheritable characteristics, in the zygote come from one parent and half from the other parent. The father, like the mother, can transmit genetic defects to his offspring that are detrimental for normal development and healthy life. Sperm DNA is known to contribute one half of the genomic material to the offspring. Thus, normal sperm genetic material is required for successful fertilization, normal embryonic and fetal development and postnatal child well being. Normal functioning of the male and female reproductive systems depends on complex hormonal communication signals between the endocrine system and the sexual organs (ovaries in women and the testes in men).

The etiologies of male reproductive dysfunction are complex and involve interplay of hundreds of cellular and subcellular processes. The process of spermatogenesis involves a series of complex steps (stem cell replication, meiosis, and spermiogenesis) over approximately 74 days in humans (Clermont, 1963; Heller and Clermont, 1964). Therefore, it becomes difficult to point to a particular cellular or subcellular process affected by the environmental toxicant. Although it might seem that spermatozoa have to fulfill a single role namely to achieve syngamy, this can only happen if
Multiple cell functions operate synchronously and flawlessly during events proceeding, before and after fertilization (Varghese et al. 2005).

Male reproductive system is vulnerable to the effects of chemical and physical factors. This might be because sensitive events take place during spermatogenesis and persistent environmental chemical or physical factors may affect these events to some extent (Kumar, 2004). It is not possible to pinpoint which sex is more sensitive to environmental persistent chemicals. But more reproductive toxicity data on male with environmental chemicals are available than female. This may be due to the fact that male reproductive toxicity end points can be studied more easily as compared to female. Still we do not know why occupational exposure to suspected reproductive toxicants appears to have a greater effect on male fecundity than do female occupational exposures (Spinelli et al. 1997) or why men are more sensitive than women to environmental exposure to metals (Staessen et al. 1991; Dickman et al. 1998) or organic toxicants (Welch et al. 1988; Schrader, 1997).

A number of studies have documented variations in human spermatozoa and their risk factors. Physiological stress, genetic, environmental, lifestyle and psychosocial factors appear to affect sperm production in human (Sharpe and Franks, 2002). These factors can act by themselves, but they can also interact in a complex manner. Hence, understanding the effect of these factors and their potential interactions may provide new information concerning the physiology of sperm production and variables that may affect this process. There are many reasons for the lack of information on the influence of occupational exposure to chemical and physical materials on physiology of sperm production and associated with sperm abnormalities. In occupational and environmental exposures, people are exposed to a number of pollutants rather than to a single agent. Animal studies generally test the toxic effects of a single agent/factor. This standard approach makes it difficult to come to a conclusion about the interactions of different factors (Sheiner et al. 2003). When available, human data rare often inconsistent and, except in a few instances, refer to broad categories or to
groups of agents (Tas, 1996). Therefore, well-planned animal studies are necessary in order to predict the reproductive toxicity of the chemical. The infertile cases due to DBCP exposure is one such example, where data on reproductive toxicity in animals was available two decades before the human reports of infertility were detected. However, the risk assessment to human is absolutely necessary for a chemical that proved to be toxic to the reproductive system in animal studies. Besides, variations in the environment and multifactorial control mechanisms and long latent periods, it is often difficult to predict whether reproductive toxicity will occur.

Rising rate of reproductive health problems is one of the major concerns today. More and more reports in the last decade focused on the increasing rate of infertility, hypospadias, cryptorchidism, testicular cancers, etc (Toppari, 1996; Tas, 1996; Kumar, 2004). An alarming report on declining semen quality by Carlsen et al. (1992) suddenly alerted the scientific community or even public attention towards this phenomenon. It has been reported very recently that as many as 15% of all couples have difficulty in conceiving a child (Agarwal and Prabhakaran, 2005). More than 30% of the cause lies with the male. Further, infertility is not just a medical problem; it can have serious psychological and social implications for the concerned persons.

Various occupational (physical and chemical) agents have been shown to affect male reproductive function in animal studies, but large differences in reproductive function and/or xenobiotic pharmacokinetics or metabolism between various species limit extrapolation to humans. However, animal studies provide useful information about the toxic potential of reproductive toxicants which are in use normally. Synthetic chemicals are an essential part of modern life. The expanding use of these chemicals in agriculture, industry, medicine and in day to day life has had a major role in enhancing the quality of life as well as life expectancy. The shift in the global pattern of rapid industrialization is reflected in the changing burden of reproductive abnormalities and related functional defects. Thus there is an urgent need to address this important issue of human reproduction by
conducting research and also imparting education. Because of the long time lapse between the exposure and onset of inevitable wave of effects that follow, the full effect of today's globalization and indiscriminate use of chemicals will be felt in future decades to come.

Lifestyle factors play an important role in the etiology of various diseases and have also been implicated to cause reproductive impairment. Data on personal habits revealed a higher number of chewers of tobacco and arecanut in comparison to smokers and alcoholics. The chewing tobacco and arecanut is mainly confined to South East Asian countries, especially the Indian subcontinent. Therefore, less attention has been paid on the role of chewing quid (Areca nut and tobacco) on reproduction by western countries. The higher number of chewers in the present study with respect to smokers might be due to the fact that smoking is banned in public places and more and more smokers have switched over to tobacco chewing. As a large number of men smoke worldwide and also the fact that cigarette smoke contains known mutagens and carcinogens, there has been much concern that smoking may have unfavorable effects on male reproduction (Zhang, 2000). However, in regard to the clinical studies on the relationship between smoking and male reproduction, the literature results have been non-conclusive. Some investigators have reported that smoking lowers sperm motility and normal morphology and has adverse effects on male fertility (Sofikitis et al. 1995). However, some authors reported no such finding (Aziz et al. 2004). In the present study, no significant effect was noted between smokers and non-smokers with respect to sperm count, progressive motility and sperm morphology. However, on comparison of sperm count, motility and morphology among the oligozoospermic subjects, it was found that there was a non-significant lowering of sperm count and rapid progressive motility between smokers and non-smokers. This shows that these men may be more susceptible to the effect of tobacco smoke. In addition, the number of oligozoospermics in the study group was only 18.3%, which explains the non significant results obtained in this study. Zenses et al. (1995) also reported that subfertile or infertile men might be more sensitive to the deleterious effects of cigarette smoke than fertile men.
Similar findings were also reported by other workers who found that total sperm count and motility were lower in oligozoospermic men who smoked than oligozoospermic non-smokers (Rantala and Koskimies, 1987; Vine et al. 1996). This suggests that infertility patients may be predisposed to and may respond more strongly to environmental exposure than the population in general. In addition, Oldereid et al. (1994) reported that smoking lowers the zinc content in the seminal plasma which in turn might diminish the Zn content of chromatin, and thereby the affecting the stability of the sperm chromatin; this may in turn contribute to reproductive failure or have consequences in fetal development. In addition, smoking increases the production of free radicals that will impair the synthesis and/or augment the consumption of SOD (Oldereid et al. 1994).

The relationship between tobacco chewing and male infertility remains unclear and data specifically addressing this issue is rare. A recent report documented a decrease in sperm quality among tobacco chewers (Said et al. 2004). However, an earlier report by Dikshit et al. (1987) found no such effect of tobacco chewing on male reproductive function. In the present study, we found no significant change in sperm count, motility or morphology between chewers and non-chewers. Earlier, a study carried out by Banerjee et al. (1993) reported adverse effect of tobacco chewing on motility and total sperm count of tobacco chewers as compared to control subjects. Experimental studies conducted by Kumar et al. (2003) reported that the long-term chronic exposure to panmasala (mixture of tobacco, arecanut, lime and other ingredients) might be responsible for the elevation in sperm head shape abnormality with respect to control. They further suggested that the higher number of sperm head shape abnormalities may be due to the adverse effect of various ingredients of pan masala especially arecanut alkaloids and tobacco specific N-nitrosamine (TNSA) on the reproductive system. One of the reasons for the non-significant result obtained in this study may be because the mean duration of chewing tobacco among the subjects was only about six years. We speculate that adverse effects of chewing quid on human semen parameters may be observable only if the duration of chewing is longer.
At present the effects of alcohol consumption on male reproductive function remain under discussion (Martini et al. 2004). Some workers have reported a reduction in sperm concentrations and percentage of normal morphology among chronic alcohol consumers (Kucheria et al. 1985; Goverde et al. 1995). In the present study, we found deterioration in all the three semen parameters – count, motility and morphology among alcohol consumers who had oligozoospermia. However, these results did not reach statistical significance. One of the reasons for the non significant results might be the small number of alcoholics in the study population. Earlier reports have suggested that moderate drinking does not affect the male gametes quality (Curtis et al. 1997; Chia et al. 2000). Martini et al. (2004) reported that alcohol or cigarette consumption alone did not alter the seminal parameters. However, when they compared the patients with these two habits to those without these habits, a significant increase of non-motile gametes was detected. In the present study, when the risk of subfertility was assessed, the subjects that indulged in all the three habits were found to have a higher risk of subfertility. This indicates that indulgence in lifestyle factors such as smoking, chewing tobacco and alcohol consumption should be included as chemical exposures as they aggravate the toxicity among the already susceptible subgroups.

Vegetarians and non-vegetarians were compared on the basis of seminal characteristics as it has been reported that persistent environmental chemicals have the ability to bio accumulate in the food chain and can affect reproduction. Recently, higher xenoestrogen concentrations were found among fish eaters (Rozati et al. 2002). In the present study, we compared the semen parameters between vegetarians and non-vegetarians on the basis of sperm count, rapid progressive motility and morphology. No significant difference was found between the two groups regarding the above parameters.

Body mass index has been reported to influence fertility. Although few studies are available, data suggests that subjects with higher Body Mass Index values are at risk for having poorer semen quality (Jensen et al. 2004).
However, no appreciable change with respect to the seminal parameters was observed either in the high or low BMI group as compared to subjects having BMI within the reference range. In the present study, majority of the subjects (69%) had BMI values within the recommended range of 18.5 to 24.9 (NIN, 1991) while about 13.6 and 17% of the subjects had BMI in the lower and higher range respectively. Mean BMI was 21.3 in the study group. This value is nearly the same as that reported by an earlier study where the authors found mean BMI of 21.8 ± 3.8 kg/m² among urban men in Mumbai, India (Shukla et al. 2002).

Most of the research on the effects of chemicals on biologic systems is conducted on one chemical at a time. However, in the real scenario people are exposed to chemical mixtures, not single chemicals. Although various substances may have totally independent actions, in many cases two substances may act at the same site in ways that can be either additive or nonadditive (Carpenter et al. 2002). One of the major places where exposure to toxicants occurs is at the workplace. This important fact cannot be ignored since any normal person spends about 8-14 hours at the workplace. During this period he may be exposed to a number of chemical or physical agents, which might cause reproductive and developmental disturbances, neurological and immunological effects, cancer and other health effects. At the National Institute for Occupational Safety and Health Registry, approximately 104,000 chemical and physical agents existing in workplaces are listed. The toxicity of most of these materials is not yet known or has only been partially studied (Gold et al. 1994). There is a significant public health concern about the potential effects of occupational exposure to toxic substances on reproductive outcomes. Many toxicants with reported reproductive and developmental effects in animal studies are still in regular commercial or therapeutic use and thus pose potential threat to ecosystem and humans. Examples of these include heavy metals (lead, cadmium, mercury, etc.), organic solvents (glycol ethers, toluene, vinyl chloride, etc.), pesticides (DDT, endosulfan, etc.) and herbicides (ethylene dibromide, etc.), and sterilizing agents, anesthetic gases, and anticancer
drugs. In the present study, occupational exposure to toxic agents was assessed on the basis of self-reported exposure. Earlier Tielemans et al. (1999a) also reported that questionnaires have provided good estimates of exposure and their association with reproductive toxicity. In the present study, exposure to toxicants was found to have a significant effect on sperm count. This decline in sperm count may be due to the adverse effects of toxicants on spermatogenesis and hormonal regulation. A reproductive toxicant might exert its effect at testicular level, or at hormonal level affecting the balance of reproductive hormones or spermatogenesis. Toxic damage directly to the testicular tissues can result in various effects, namely, reduced sperm production, the production of defective spermatozoa, and impaired androgen production (Oliva et al. 2001). This can lend support to the fact that FSH levels were significantly elevated among exposed persons in comparison to unexposed subjects in the present study. Since FSH is a marker of Sertoli cell function, it may be assumed that these toxicants might affect the secretion of inhibin, which in turn affects the feedback mechanism resulting in significant elevation in circulating FSH levels. When individual toxic agents were analyzed for their effects on fertility, it was found that subjects exposed to pesticides had an increased risk of developing subfertility. This indicates the potential role of pesticide exposure in altering semen quality. Kumar et al. (2000) detected significant amounts of hexachlorocyclohexane (HCH) and its isomers, dichlorodiphenyl trichloroethane (DDT) and its metabolite 1,1,1-trichloro-2,2-bis (P chlorophenyl ethane (pp'-DDE) and low values of 1,1,2-dichloro-2,2-bis (P chlorophenyl ethane) (pp'-DDD), aldrin and endosulphan in human seminal plasma. Earlier Rupa et al. (1993) reported that male workers who were exposed to various pesticides during mixing and spraying showed male mediated adverse reproductive outcomes such as abortion, still births, neonatal deaths, and congenital defects as well as reduced fertility as compared to controls. Very recently, Jager De et al. (2006) reported that non-occupational exposure to the pesticide DDT, was associated with poorer semen parameters in men indicating adverse effects on testicular function and/or reproductive hormone regulation. Thus exposure to pesticides may play a role in deterioration of semen quality and male reproductive function.
Exposure to pesticides might also have a potential role in reproductive dysfunction among general population as people are unknowingly exposed to these toxic chemicals not only at their workplace but also through other sources such as contaminated food and water.

Industrialization has led to a simultaneous increase in air pollution, primarily due to burning of fossil fuels. Various chemicals present in polluted air such as PAH’s, SOx, NOx, CO, etc cause adverse health effects. Reproductive health studies were prompted by reports that rates of conception and incidence of congenital anomalies were affected by seasonal increases in air pollution (Lewtas, 2000). Selevan et al. (2000) studied alterations in sperm quality in young men exposed to air pollution and they found significant associations between elevated pollutant levels and sperm motility, morphology, abnormal head shape as well as abnormal chromatin. A progressive decline in sperm production in Greece was also reported during a time period when air pollution increased (Adampoulos et al. 1996). In the present study, a non-significant effect of exposure to air pollution on semen quality was observed. Further, when levels of metals in blood were compared between exposed and non-exposed subjects, it was found that persons exposed to air pollution had significantly higher levels of toxic metals — lead, cadmium and copper than non-exposed individuals. These metals have been reported to affect semen parameters (Benoff et al. 1997; Alexander et al. 1998; Huang et al. 2000). Thus chronic exposure to air pollutants might have effect on sperm quality. The results of this study show a clear exposure effect relationship between toxic environmental chemicals and parameters of reproductive function. Earlier, Mehta and Anandkumar (1997) reported a decline in sperm count in a study of population from Bangalore, India and they correlated this decline with the changes in various pollution indices such as suspended particulate matter, sulphur dioxide and lead. Telisman et al. (2000) also reported that metals such as lead and cadmium that are present in the particulate fraction of air pollution have been associated with decrements in semen quality.
It is known that active production of sperm requires a temperature about 3-4 degrees lower than normal body temperature. The effect of chronic occupational exposures to heat has been observed in various occupations such as welding and ceramic industry. Earlier reports have shown heat exposure as an independent risk factor for male infertility (OR 4.5, p<0.05) using multivariate analysis (Velez de la Calle et al. 2001). In the present study, however, we did not observe statistically significant effect of heat on semen quality, although sperm count was marginally lower among heat-exposed workers compared to non-exposed individuals. This shows that exposure to heat has adverse effect on sperm production.

It is clear from the above results that persons exposed to toxicants such as metals, pesticides, air pollutants, etc. suffered from adverse effects on fertility. Among individual toxicants, apart from metals, pesticides were found to pose higher risk for subfertility. Male reproductive function was affected at hormonal level since higher FSH levels were found among exposed subjects in respect to non-exposed individuals. Elevated FSH levels possibly signify an effect on Sertoli cell function. We speculate that these toxic substances might target their toxicity at Sertoli cells along with other cellular and subcellular targets, which would lead to an observed effect on impaired semen quality.

Exposure to heavy metals during critical period of development and in adulthood might pose a significant risk to human reproduction. There are few studies that demonstrate the capacity of semen and sperm to accumulate these metals (Bench et al. 1999). Toxic effects of heavy metals, especially lead have been documented earlier. Other metals implicated in reproductive toxicity include cadmium, mercury, copper, etc. However, dose at which toxicity occurs is still debated among scientific community and also the exposure circumstances including synergistic interaction of various pollutants, which might be associated with reproductive dysfunction.

Some 45000 measurements in European industrial settings on lead workers spanning smelters, battery manufacturers and foundries, have been
carried out by Bonde and Apostoli (2005). Bonde (1999) reported that the average concentration of lead in blood steadily declined from 68 \( \mu \text{g/dL} \) in 1970 to 35 \( \mu \text{g/dL} \) in 1995. Most of the studies available deal with occupational exposure and reproductive impairment, very few studies have reported exposure of the general population to lead. Further, studies in rats and other rodents indicate that blood lead concentrations above 30-40 \( \mu \text{g/dL} \) are associated with impairment of spermatogenesis and reduced concentrations of androgens- although some rat species and strains are quite resistant (Apostoli et al. 1998). WHO has set a limit of 40 \( \mu \text{g/dL} \) of blood lead levels, however, awareness and technology have reduced lead exposures at work and in the environment. Further, only a handful of studies have been carried out in the Indian subcontinent that specifically deal with human exposure to lead and effects on male reproductive function. Moreover, a very recent report stated that there is definitely a need to keep open this line of research (Bonde and Apostoli, 2005). Various investigators have determined the levels of heavy metals in various biological fluids such as blood, spermatozoa and seminal plasma. Earlier exposure to lead was mainly due to its presence in automobile exhaust. Nowadays, most of the developed and developing countries have switched over to unleaded petrol so the problem has subsided to some extent. Higher levels of lead present in blood and seminal plasma of the subjects in this study are a result of other sources of lead in the environment. Mean seminal plasma lead levels in the present study are higher than those reported by other researchers (Noack Fuller et al. 1993, Jockenhovel et al. 1990, Pant et al. 2003). This might be due to the fact that Ahmedabad is mainly an industrial city. There is predominance of small scale units engaged in manufacturing of textile processing chemicals, foundries, dyes and dye intermediates, engineering, steel rolling mills, etc. However, the present results on lead level are in accordance with earlier findings (El-Zohairy et al. 1996; Umeyama et al. 1996). Benoff et al. (2003) reported mean lead levels of 35.9 \( \mu \text{g/dL} \) among patients undergoing IVF and reported that increased lead levels may contribute to the production of unexplained male infertility. In the present study, mean seminal plasma lead level was 18 ± 1.3 \( \mu \text{g/dL} \) and mean blood
lead level was $24.8 \pm 2.1 \, \mu g/dL$. Considering that these men were from general population and were not specifically subjected to exposure to lead in their occupations, lead levels seem to be on the higher side. Benoff et al. (1998b) also observed that greater than 40% of their subjects who were not exposed to Pb2+ in their workplace and who did not smoke cigarettes exhibited blood and seminal plasma lead concentrations that were above the permissible limit in men non-occupationally exposed to Pb2+.

A number of epidemiological studies indicated that occupational exposures to lead have adverse effects on human sperm. High lead exposure (blood lead levels exceeding 70 $\mu g/dL$; 0.34 $\mu$ mole/dL) still exists among battery assemblers and lead smelters and relatively low exposures (40-50 $\mu g/dL$; 0.19-0.24 $\mu$M/dL) are found for large groups in the metal manufacturing and consuming industries. In a cross-sectional survey of semen carried out among 503 men in the United Kingdom, Italy and Belgium, the authors reported that median sperm concentration was reduced by 49% in men with blood lead concentration above 50 $\mu g/dL$ (Bonde et al. 2002). A study on workers of a newspaper printing press in Ahmedabad, India also indicated that the average sperm counts were significantly lowered and lesser proportion of them were found to be motile in the exposed subjects as compared to controls. These changes were associated with dose dependent blood lead levels (Roy Chowdhury et al. 1986). Telisman et al. (2000) reported Pb-related decrease in sperm density, in counts of total motile and viable sperm in the percentage and count of progressively motile sperm, in parameters of prostrate secretory function and an increase in abnormal sperm head morphology, serum testosterone and estradiol. Mean seminal plasma lead levels were lower in the group having normal count and motility as compared to the oligoasthenospermic group. Saaranen et al. (1987) also reported that the seminal fluid lead concentration was significantly ($p$ less than 0.001) higher in infertile than in fertile men.

In the present study, a negative effect of lead on sperm count and sperm morphology was observed. This is in accordance with other reports
among workers occupationally exposed to lead who were found to exhibit decreased sperm density and a high rate of teratozoospermia (Lancranjan et al. 1975; Lerda, 1992; Xuezhi et al. 1992; Robins et al. 1997). It is clear from the present study coupled with earlier reports that an association exists between higher lead levels and deterioration in semen quality. Earlier, Xu et al. (1993) found no correlation between seminal plasma lead and semen quality parameters in subjects with low lead exposure (7.7 ± 3.3 μg/dL). However, Bonde et al. (2002) found significant effect of lead among industrial workers (PbB 12 to 66 μg/dL). According to CDC, a PbB concentration ≥ 25 μg/dL in an adult is considered an elevated exposure. The mean blood lead level obtained in the present study is almost 25 μg/dL, which explains some of the adverse effects of lead exposure on semen quality obtained in the present study. On analysis of data (based on lead levels < 30 μg/dL and ≥ 30 μg/dL, results showed lower values of seminal parameters among the subjects with higher lead levels. Lead levels above 30 μg/dL were also found to be associated with lower HOS test score, which reflects impairment in sperm membrane integrity. Recently, Kumar et al. (2003) also reported lower HOS scores among welders exposed to toxic metals along with other toxicants during welding operation. This suggests that lead level above ≥ 30 μg/dL might also have adverse effect not only on sperm count but also sperm membrane integrity and sperm motility. Earlier blood lead level of 40 μg/dL was recommended safe among exposed subjects. However, from the present results, it can be postulated that even lead levels ≥ 30 μg/dL might lead to deterioration in semen parameters.

In addition to sperm count, motility and morphology, there are reports of associations between lead and certain accessory sex gland markers. In a study carried out in Lucknow, India, the authors found associations between lead and fructose, acid phosphatase and gamma-glutamyl transpeptidase. They concluded that lead might be one of the pollutants indirectly affecting semen quality by altering the functions of accessory sex glands (Pant et al. 2003). However, we did not find any statistically significant relationship between lead levels in seminal plasma and accessory gland markers.
Association of exposure to lead with endocrine dysfunction has been studied earlier. However, the results are inconclusive. It is not clear whether lead acts directly on the testicular tissues or testicular production of androgens or indirectly through its effects on hypothalamo-pituitary axis production and secretion of gonadotrophins (Ng et al. 1991). It has been observed in animals that lead appears to disrupt the hypothalamic-pituitary-testicular axis (Sokol et al. 1985; 1994; Sokol, 1987; Kempinas et al. 1994). Lancranjan et al. (1975) found no effect of lead exposure on endocrine status of exposed workers. But, Gennart et al. (1992) found a positive correlation between serum follicle stimulating hormones and blood lead. However, they could not detect any effect at levels below 47 μg/dL. In the present study, we observed no effect of lead on FSH and LH, however negative effect of lead was observed on circulating testosterone levels after adjusting for confounding factors. This indicates that lead might affect testicular function by affecting testosterone secretion. Braunstein et al. (1987) also reported similar finding in 10 lead intoxicated men who complained of impotence, where plasma testosterone concentrations were reduced, gonadotrophin and prolactin concentrations were normal. Reduction in testosterone levels indicates damage to testicular function to some extent. Further, Ng et al. (1991) found that in men exposed to lead for 10 or more years had normal FSH and LH and low testosterone. They concluded that lead had a direct toxic action on the testis thus leading to reduced production of testosterone. Based on the findings of the present study, and earlier reports, we hypothesize that reproductive function is affected by the direct toxic effect of lead on the gonads and indirectly through hypothalamopituitary system.

Men with normal spermograms may still be infertile; the cause could be related to abnormal sperm DNA (Alvarez, 2003). The interest to assess the chromatin quality of human sperm has increased since DNA damage in sperm from infertile men has been associated with infertility. Its importance has become more obvious in the context of increasing use of assisted
reproductive techniques (ART’s) in infertility treatment. The chromatin contained in the nuclei is extremely stable and compact structure. This tight packaging minimizes the volume of the sperm nuclei for transport through the female system and for protection of genetic material from any kind of damage. DNA integrity in the sperm is essential for the accurate and successful transmission of genetic information and maintenance of good health in future generation. Detection of sperm nuclear DNA integrity is thus, a potential tool for evaluation of semen samples prior to their use in ART. This test detects the ability of sperm nuclear chromatin to resist denaturation, which has been associated with male fertility potential. In the present study, we found significant negative correlation between seminal plasma lead levels and percentage of sperms with normal DNA. The mean percentage of sperms with normal DNA was significantly higher among subjects with seminal plasma lead level below 30 µg/dL.

The negative correlation between lead levels and sperm DNA test indicates that lead may have some effect on the chromatin structure. However, Bonde et al. (2002) reported that 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, Hernandez-Ochoa et al. (2004) reported that 48% of urban men in Mexico showed higher values of nuclear chromatin condensation associated with Pb in semen. It is reported that sperm with abnormalities of DNA and/or chromatin are able to fertilize and transmit abnormal DNA to the conceptus (Chapin et al. 2004). It is also evident from this study that exposure to lead is capable of causing damage to DNA which may affect pregnancy outcome.

One remarkable finding of the present study is that all the observed effects on semen quality found to correlate well with higher seminal plasma lead level instead of blood lead levels. Most of the epidemiological studies have focused on relationships between blood lead levels and semen parameters. It is our hypothesis that although blood lead levels indicate recent exposure to lead, considering the results of this study, seminal
plasma lead level might reflect lead in the reproductive system and might be a better indicator of lead induced reproductive toxicity than blood lead level. This is in agreement with the theory of Alexander et al. (1998) who suggested that distribution of lead in the male reproductive tract is not reflected by PbB levels; and that Pb in semen or semen compartments may better assess the amount of Pb at the site of its effects in the male reproductive tract. In the present study also, appreciable levels of lead were detected in seminal plasma of subjects from the general population. It can be inferred from the present study that environmental lead exposure may cause deterioration in semen quality.

Cadmium is a toxic heavy metal that occurs widely in nature. It is non-biodegradable, has a biological half life of 200 days in rats (Webb, 1975) and is a cumulative toxicant (Li and Heindel, 1998). In the present study mean cadmium levels in blood and seminal plasma were found to be 14.6 (0.2 - 71.6) and 8.8 μg/L (0.02 - 40.6). These levels were higher than those reported earlier by Chia et al. (1992) and Pleban and Mei (1983). However, data of the present study can be correlated with the study of Umeyama et al. (1986) who reported mean cadmium levels 13 ± 12 μg/L among infertile men and also with Telisman et al. (2001), who found blood cadmium levels in the range of 0.2 to 11.3 μg/L among men without occupational exposure to cadmium. However, in a recent Indian study, authors reported very high cadmium levels (Mean 5.0 ± 3.6 μg/dL) among men in the general population (Pant et al. 2003). Favino et al. (1968) found no significant decrease in fertility or urinary androgen excretion in cadmium exposed workers in comparison to unexposed subjects. Keck et al. (1995) also found no significant difference in the seminal plasma cadmium concentrations of fertile and infertile men. However, higher cadmium concentrations have been reported in infertile men compared to fertile men by other investigators (Saaranen et al. 1987a; Umeyama et al. 1986). Chia et al. (1992) also reported significant correlations between blood cadmium levels and volume of semen, midpiece defects, and immature forms of spermatozoa. Noack Fuller et al. (1993) also found significant correlation between semen
cadmium levels and sperm motility. Telisman et al. (2000) indicated that blood cadmium less than 10 μg/L can also increase abnormal sperm morphology but not seminal plasma cadmium. In the present study, when we divided subjects based on cadmium levels above and below 10 μg/L, no significant differences were observed among the semen parameters between the higher and lower Cd group. This finding is in accordance with most experimental studies, which failed to find any significant effect on the male reproductive organs in animals chronically exposed to cadmium at low doses (Einder, 1986). Cadmium is also associated with deleterious effects on the gonadal function and with changes in the secretory pattern of other pituitary hormones like prolactin, ACTH, GH or TSH (Lafuente, 1999). The accumulative data indicates the existence of a disruption in the regulatory mechanisms of the hypothalamic-pituitary axis by cadmium. However, in the present study, we found no effect of cadmium on hormone levels in serum or seminal plasma. Toxicity of cadmium is dependent on the duration of exposure and dose. In the present study, very few subjects had blood cadmium levels above 10 μg/L and these subjects might not have been exposed long enough to observe detectable toxicity. However the observed sperm count, fast progressive motility and normal morphology were marginally lower when cadmium levels were above 10μg/L.

The genetic damage to sperm cells was also evident in the form of higher percentage of sperms with abnormal DNA with rising lead levels. The same was noted for cadmium also even though the trend was statistically non-significant. This study indicates that toxic chemical exposure either through occupation or through the environment have deleterious effects on male reproductive system. In the present study, considerable levels of lead and cadmium were detected in blood and seminal plasma. This might be because of various industrial activities releasing these metals in the air. This can also be explained by our results which show that lead and cadmium levels in blood were significantly higher among subjects having exposure to air pollution. Further, duration of exposure to toxicants might also have a role in reproductive dysfunction as these chemicals might accumulate in the
body. In the present study also, a significant positive correlation was obtained between blood lead and duration of exposure and blood cadmium level and duration of exposure. Similar findings were also reported by Robins et al. (1997), where they found higher blood lead levels correlated with the exposure duration. Another source of lead and cadmium in the environment is cigarette smoke. It has been reported that a single cigarette contains 0.6-2.0 μg Pb2+ (Chiba and Masironi, 1991) and 1-4.5 μg Cd2+ (Chia et al. 1994). Although Cd levels in blood and seminal plasma did not correlate significantly with any of the semen parameters studied. However, reduced sperm counts observed among smokers in asthenozoospermic group might be a result of cadmium exposure through smoking along with other factors. Earlier, Chia et al. (1994) also indicated that cadmium in cigarettes could be a possible causative agent for the low sperm density among smokers. Irrespective of the source, it becomes increasingly clear from the data available that xenobiotics find their way from the environment into the human body including blood and semen and might affect spermatogenesis/oogenesis and subsequently any event at the time of conception or the development stage of the embryo.

Certain heavy metals occur in the body in trace amounts and are essential for development, growth and health. Many of these elements produce toxic effects following excessive exposure, while characteristic pathologies can result in deficiencies. One such example is that of copper. Copper is an essential metal that plays an important functional role as cofactor for several enzymes. However, the reproductive toxicity of copper has not been studied in detail. Various workers have determined levels of copper in seminal plasma. Exposure to copper has been linked to a decreased sperm count and a cause of terato and ashtenozoospermia (Lahdetie, 1995). In the present study, a significant deterioration in sperm morphology and motility with increasing serum copper levels was observed. However, the relationship of seminal plasma copper levels with motility and morphology was statistically non-significant. Stanwell Smith et al. (1998) also
found higher concentration of blood plasma copper among infertile men than those of proven fertility, but no relation with seminal plasma copper level.

Skandhan (1992) reviewed literature on copper toxicity and reported that the level of copper in seminal plasma appears to fall in cases of azoospermia and increase in oligo and asthenozoospermia. This statement is completely in agreement with the results of the present study. However, mean serum copper level was higher among oligozoospermics and asthenozoospermics compared to normal subjects. Huang et al. (2000) also found higher copper levels in asthenospermia. They reasoned that these changes may be related to semen quality and that lipid peroxidation may be involved in the loss of sperm motility. In an in vitro study, the authors suggested that higher copper level caused a fall on the percentage of motile sperm (Battersby et al. 1982). This deterioration in sperm motility might be due to uptake of metallic copper by spermatozoa. One of the hypotheses is that copper reduces the oxidative process and glucose consumption, which reduces or abolishes sperm motility (Skandhan, 1992). The results suggest that copper has a toxic effect on sperm morphology and motility and it might be exerting its effect by disturbing the antioxidant defence system. Also, the present findings reveal that serum copper is a better marker for assessing copper toxicity than seminal plasma copper.

Studies have shown a possible role of zinc in sperm production and/or viability, in the prevention of spermatozoa degradation. A recent pilot study examined the relationship between concentration of zinc in seminal plasma and semen quality and the authors reported that mean zinc levels in seminal plasma were lower among azoospermics as compared to oligozoospermic and normospermic groups. A recent study carried out at our laboratory indicated a significant correlation was observed between zinc levels and sperm count ($r=0.22$, $p<0.05$). A significant relationship between zinc level in seminal plasma and alpha-glucosidase, which is an epididymal marker, was also observed (Mankad et al. 2006). In the present study, however, no statistically significant relationship between zinc and sperm motility was found. The mean zinc level was 88.9 mg/L in subjects with normal motility,
whereas it was slightly higher (101.7 mg/L) in asthenospermic subjects. The correlation between zinc levels and fast progressive motility was also not statistically significant. Earlier, Lewis-Jones et al. (1997) also reported no significant relationship between the motile sperm concentration and zinc in seminal plasma. However, it has been reported that extremely high levels of zinc might inhibit sperm motility and the function of the mannose receptor on the sperm head (Lin et al. 2000). It has also been reported that high seminal Zn concentrations even have a suppressing effect on progressive motility of spermatozoa (Sorenson, 1999). Fuse et al. (1999) found a positive correlation of Zn with sperm motility ($r = 0.22$). They concluded from their study that although adequate amount of zinc is essential for normal sperm motility, an excessively high zinc concentration is apparently related to defective motility in asthenozoospermic patients. It can be concluded from the present results coupled with available studies that zinc plays an important role in spermatogenesis, although high zinc levels may cause inhibitory effect on sperm motility. One of the reasons could be that zinc is closely related to superoxide dismutase (SOD) and higher zinc levels might affect SOD activity, causing oxidative damage, which manifested in the form of poor motility.

In the present study, zinc levels were lower in samples with hyperviscosity compared to semen samples having normal viscosity. However, the changes were not statistically significant. Recently, Elzanaty et al. (2002) also reported that lower level of zinc was found in hyperviscous samples. They concluded that zinc might be one of the factors contributing to the liquefaction process of semen, assisting in the breakdown of proteins associated with coagulation. Hyperviscous semen has been found to be more frequent among infertile men compared to fertile men (Mankad et al. 2006). Higher viscosity might to be associated with lower motility. The present study also indicated that there was a positive correlation between the activity of the α-glucosidase level and sperm count; the lowest level was found in the azoospermic group compared to oligozoospermic and normozoospermic groups. The level of α-glucosidase in seminal plasma reflects the functional state of the epididymis. Its low activity has been
reported in cases of epididymal obstruction (Krause and Bohring, 1999). Mahmoud et al. (1998) reported that α-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 the case of neutral α-glucosidase to assess epididymal function compared to other markers such as free carnitine and total carnitine. Based on the relationship between alpha glucosidase and sperm count obtained in the present study as well as other data available, it can be inferred that α-glucosidase plays an important role in fertility and its levels are positively correlated with sperm count. Earlier, Elzanaty et al. (2002) reported a significant positive correlation between seminal levels of neutral α-glucosidase and percentage of motile sperm. In the present study, the levels of α-glucosidase and fast progressive sperm motility showed a linear relationship, but the difference was not statistically significant.

The present data along with earlier studies suggest that both zinc and α-glucosidase have a role in sperm production; however, their effects on sperm motility need further research. In the present study, we found a significant correlation between zinc and neutral α-glucosidase ($r = 0.46$, $p<0.05$). Earlier, it has been suggested that the levels of biochemical parameters in seminal fluid were closely related to each other (Elzanaty et al. 2002). Available studies clearly suggest that semen quality is reflected not merely by the number of sperms or sperm motility; it is a complex mixture containing secretions from various glands in the correct proportions that predicts overall fertility. The present data coupled with earlier studies suggests that alpha glucosidase can act as a useful biomarker of epididymal function in male reproductive impairment. However, no relationship was noted between the levels of toxic metals and biochemical secretions in the present study. However, Pant et al. (2003) concluded that lead and cadmium exert their toxicity by affecting biochemical secretions (alpha glucosidase, fructose and zinc) from accessory sex glands. Thus more data are needed on this aspect to reach any firm conclusion.
FSH, LH and testosterone are prime regulators of germ cell development. The quantitative production of spermatozoa generally requires the presence of these hormones. FSH acts directly on the seminiferous tubules whereas LH stimulates spermatogenesis indirectly via testosterone. In the present study mean serum FSH levels were higher among azoospermics as compared to oligospermics and normal. Similar trend was observed in LH values in serum samples. This indicates alteration to the hypothalamic pituitary axis affecting spermatogenesis. A significant correlation was observed between mean serum FSH and serum LH levels. It has been reported that higher concentration of FSH is considered to be a reliable indicator of germinal epithelial damage and has been shown to be associated with azoospermia and severe oligozoospermia (Bergman et al. 1994). Earlier, de Kretser et al. (1979) reported elevated levels of serum FSH with increasing severity of seminiferous epithelium destruction. Further, Babu et al. (2000) mentioned that in infertile males with abnormal histopathology (Seroli cell only syndrome, hypospermatogenesis and spermatid arrest) the mean FSH levels were significantly elevated compared to the control group. Our results are in accordance with previous reports of Zabul et al. (1994) and Weinbauer and Nieschlag (1995) who also showed elevated levels of both FSH and LH in infertile men. Mean testosterone concentrations in serum were lower among azoospermics as compared to subjects with normal count. Present study also indicated the effect of metals like lead on circulating testosterone levels. FSH levels were also significantly higher among exposed subjects compared to non exposed individuals. In addition, prolactin levels were also altered among subjects exposed to toxicants. Prolactin has earlier been reported to correlate with sperm concentration (Aiman et al. 1988) and sperm motility (Gonzales et al. 1989). Therefore, the change in prolactin levels in seminal plasma following exposure to toxic substances also cannot be ignored. Lower levels of testosterone coupled with elevated FSH levels in samples with abnormal semen quality confirm the fact that androgen balance is important for normal spermatogenesis.
The data from the study indicated adverse effects of lead exposure on the male reproductive function in the form of lower sperm count and lesser percentage of morphologically abnormal forms among subjects having higher seminal plasma lead levels. In addition, lower testosterone concentrations among subjects with higher seminal plasma lead indicate direct toxicity on the testis at cellular level. Oxidative stress (OS) has elicited enormous interest in researchers in recent period. Reactive oxygen species are continuously produced by various metabolic and physiologic processes. When the balance between ROS production and antioxidant capacity of the organism is distorted, oxidative stress occurs. Oxidative stress affects male fertility in many ways. Because the reactions occur quickly and are a part of complex chain reactions, we usually can detect only their “footprints.” One of the major attributes and probably causes of defective sperm function is oxidative stress created by excessive ROS generation by the spermatozoa and/or the disruption of antioxidant defense systems in the male reproductive tract. Excessive free radical generation frequently involves an error in spermiogenesis resulting in the release of spermatozoa from the germinal epithelium exhibiting high levels of cytoplasmic retention (Aitken and Sawyer, 2002). They further suggested that excess cytoplasm contains the enzymes that fuel the generation of ROS by the spermatozoa’s plasma membrane redox systems. The consequences of such oxidative stress include a loss of sperm motility, and fertilizing potential and induction of DNA damage in the sperm nucleus. In the present study, higher DNA damage was noted among the subjects with higher seminal plasma lead levels. Lead and cadmium have been implicated in catalysis of ROS formation (Agarwal and Prabhakaran, 2005). An important source of ROS is immature spermatozoa. Earlier study has suggested that the defect in spermiogenesis that results in retention of cytoplasmic droplet is a major source of ROS (Gomez et al. 1996). In the present study, mean number of spermatozoa with cytoplasmic droplet was higher among subjects having higher seminal plasma lead levels. We hypothesize that lead along with other toxicants might be responsible for oxidative stress to the spermatozoa which indirectly causes sperm dysfunction. It has also been reported earlier that as the concentration of immature spermatozoa in the human ejaculate increases,
the concentration of mature spermatozoa with damaged DNA rises (Gil-Guzman et al. 2001). In the present study, we also observed a higher percentage of immature spermatozoa subjects with higher lead levels as well as significant correlation between DNA damaged sperm and higher seminal plasma lead levels. Regarding the relationship between cadmium and its semen quality, although non-significant results were obtained in the present study, all semen parameters were found to be lower among the group with higher cadmium levels indicating role of cadmium in causation of reproductive dysfunction. We suspect that deterioration in semen quality might be due to the environmental toxicants and these toxicants exert their effect by causing oxidative damage, which affects the process of spermatogenesis. The final outcome would thus show in terms of a defect in sperm number, morphological structure, membrane damage or DNA integrity, any of which would affect the normal course of events in the reproductive cycle.

It is evident that exposure to metals play an important role in the etiopathogenesis of various sperm abnormalities such as defective sperm count, morphology, etc. Although changes in sperm count, motility or morphology are not ultimate decisive factors for infertility, but probably the result of a number of factors which might influence subsequent events in reproductive function such as acrosome reaction, capacitation and sperm oocyte fusion. The higher levels of metals observed among exposed persons indicate that more stringent measures need be adopted at workplace. The general population too was found to have higher metal levels in blood and seminal plasma indicating that these metals persist in the environment. Deteriorating semen quality is not an effect that should be ignored any longer since it is the ultimate vehicle that travels to the egg and determines the type of offspring. Moreover, disturbances in hormone levels observed among exposed individuals warrants the need for future studies as well as remedial measures by policy makers and government agencies that should aim to reduce these exposures.
In conclusion, the study clearly indicates that exposure to toxic substances leads to disturbance in normal hormonal balance which in turn might affect the subsequent chain of events involved in normal reproduction. Lead levels above 30 μg/dL affect sperm count, normal sperm morphology. It also causes an increase in the number of immature spermatozoa with a concomitant decrease in the percentage of sperm with normal DNA. Cadmium levels are also elevated following exposure to air pollution and a significant correlation was observed between cadmium levels in blood and duration of exposure. Studies on essential metals revealed a positive role of zinc in spermatogenesis. Higher copper levels in serum in cases of asthenozoospermia and teratozoospermia indicated copper toxicity on sperm motility and morphology. Overall summation reveals that lead, cadmium and copper are toxic to the human sperm and might exert their effect either by affecting the hormonal balance or through indirect effect on the oxidative defence mechanism.

Inadequate knowledge about the etiology of male factor infertility renders any effort to treat infertile men futile. The trauma and burden of an already distressed couple coping with infertility are often heightened by an unexplained diagnosis that eludes treatment. It is imperative that toxic factors in the environment that may result in this condition be identified and eliminated (Rozati et al. 2002). Intervention studies will also be useful to understand the causative factors associated with reproductive impairment. Earlier, Vine et al. (1996) observed an improvement in semen quality when subfertile men quit smoking. Viskum et al. (1999) reported similar observations among lead exposed workers when workers were removed from lead exposure. As more information becomes available, so our concepts of how spermatozoa control their intricate functions will be refined and a consensus emerge. Most importantly, these fundamental advances in our understanding of sperm biochemistry will create a platform from which we can launch new initiatives in contraception, toxicology and reproductive medicine (Aitken, 1997).
In the absence of a mechanism for global ban or phase outs, reproductive toxicants such as metals (lead, mercury, etc.), DDT, use of leaded gasoline, etc. that are banned in some industrialized countries are still being manufactured for various purposes and export. Due to migration of these toxins via air, a global mechanism offers the only means for protecting the health of ecosystem and humans including reproductive health. Further, Information Education and Communication (IEC) will help in reducing exposure level at workplace leading to minimum exposure level, which in turn would help in reducing toxicant exposure at workplace or in the environment and also might lead to a reduced risk to the ecosystem and human health.