Discussion
Evolution is the genetic turnover of the individuals in every population from generation to generation (Mayr, 2001). In natural populations, individuals vary in their ability to survive and reproduce, and thus differ in how many copies of their genes they pass on to the next generation. Selection acts on differences in lifetime reproductive success between individuals, which are the result of a complex array of phenotypic traits, which in turn result from an interaction between genetic and environmental factors. Throughout evolution, heritable traits, which enhance individual reproductive success, will be selected over many generations and will spread (Gomendio et al., 2007). It is a fact that both animals and humans produce healthy sperm and ova for continuing their species, which needs formation and maturation of spermatozoa, ova, fertilization, development and production of offsprings. A number of exogenous and endogenous favorable conditions are necessary for the continuation of the species. However, a number of exogenous, both lifestyle and environmental factors, responsible for declining fertility and increasing reproductive disorders have also been reported. Any risk to the reproductive health results in the significant response not only from the scientific community but also from the public media.

Male reproductive function in the general population has attracted increasing attention in recent years due to growing reports suggesting that the occurrence of several biological problems affecting the male genital tract have increased during the last 50 years (Toppari et al., 1996). These include an increased incidence of testicular cancer and some congenital anomalies, such as cryptorchidism, and an apparent decline of sperm production in the overall male population. Male reproductive system is vulnerable to the effects of chemical and physical factors that might be because of sensitive events taking place during spermatogenesis and persistent environmental chemicals or physical factors may affect these events to some extent (Kumar, 2004). There is an immense research going on in the field of male infertility as in many cases, the underlying causes are still unknown. Various
studies have explained the genetic causes of male infertility using mouse models (O'Bryan and de Krester, 2006) as well as through candidate gene sequencing using genomic DNA from infertile men (Miyamoto et al., 2003). A number of studies have documented various risk factors with respect to male reproductive health. Physiological stress, genetic, environmental, lifestyle and psychosocial factors appear to affect sperm production in human (Sharpe and Franks, 2002) These factors can act independently and can also interact in a complex manner. Various lifestyle and environmental factors may impair male fertility by interfering with spermatogenesis, spermiogenesis, motility, sperm DNA and chromatin integrity, hormonal regulation and/or by reducing the fertilizing capacity of the spermatozoa. Therefore, the effect of these factors as well as their potential interactions may provide new information concerning the risk assessment to male reproductive health and to understand the variables that may affect this process. The expanding use of synthetic chemicals in agriculture, industry, medicine and in day to day life as well as the changing lifestyle pattern has played a major role in enhancing the quality of life but in the same time might have also been resulted in the increased reproductive abnormalities and associated functional defects as well as other health impairments.

The results on the subject characteristics revealed that most of the subjects were from the urban area. This could be explained based on the enrollment of subjects as the study was conducted at the hospital situated in the urban area. The subjects indulged in the habit of tobacco smoking/chewing and/or alcohol was considered as lifestyle exposed whereas the subjects exposed to toxic harmful substances such as metals, pesticides, solvents etc. based on history were considered as toxicant exposed. About 31.7% of the subjects were non-exposed to any lifestyle and/or toxicant factors whereas 68.3% of the subjects were having the exposure of lifestyle and/or toxicant factors. Among the subjects with both lifestyle and/or toxicant exposure, about 15.9% subjects were only toxicant exposed while 46.3% subjects were exposed to only lifestyle factors while the rest 37.8% subjects were exposed to both lifestyle as well as toxicant factors.

The data were further categorized with respect to their individual or combined lifestyle habits like tobacco smoking/chewing and/or alcohol. About 42.5% of the subjects were having no such lifestyle habit whereas the rest 57.5% of the subjects were undertaking atleast one lifestyle habit. Among these 2.9%, 6.5% and 49.3%
were only alcoholics, only smokers and only chewers respectively whereas 32.6% were having the combination of two such habits and 8.7% of the subjects were indulged in all the three habits.

Subjects were classified on the basis of seminal parameters i.e., sperm count, motility and morphology. Further, for the analysis purpose, the subjects were grouped into fertile and subfertile. Fertile group included subjects having normal sperm count, motility and morphology within the recommended values whereas subfertile included azoospermics, oligozoospermics, asthenozoospermics, teratozoospermics as well as the combination of these conditions. On comparing the semen parameters among fertile and subfertile subjects, it was observed that sperm count, total progressive motility, normal sperm morphology and viability were significantly higher among the fertile group as compared to subfertile subjects.

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. In the present study, the semen parameters, when compared between vegetarians and non-vegetarians showed no significant difference with respect to total progressive motility and normal sperm morphology. However, it was reported that frequent intake of lipophilic foods like meat products may negatively affect semen quality in humans, whereas some fruits or vegetables may maintain or improve semen quality (Mendiola et al., 2009).

Humans are exposed to many environmental agents that may be hazardous to their reproductive capacity. Male reproductive function is known to be highly sensitive to many chemical and physical agents generated by industrial or agricultural activities (Bonde, 1996; Spira and Multigner, 1998). Such agents are commonly present in some occupational activities and in the general environment. Many toxicants with reported reproductive and developmental effects in animal studies are still in regular commercial or therapeutic use that poses potential threat to ecosystem and humans. Examples of these include heavy metals (lead, cadmium, etc.), organic solvents (glycol ethers, toluene, vinyl chloride, etc.), pesticides (DDT, endosulfan, etc.), herbicides (ethylene dibromide, etc.), and sterilizing agents, anesthetic gases and anticancer drugs. The public health concern about the potential adverse effects of toxic substances on reproductive health outcomes has been
increased significantly. The involvement of environmental factors in male infertility and the suspected increased incidences of male-related infertility induced by such chemicals are of great concern. Toxic chemicals damage the testes resulting in the reduced sperm quality, the production of abnormal spermatozoa and hormonal imbalance.

In the present study, exposure to toxic agents was assessed on the basis of self-reported history of exposure among the subjects. Earlier, Tielemans et al., (1999) had also reported that questionnaires provide good estimates of exposure as well as their association with reproductive toxicity. In the present study, it was found that the subjects ever exposed to toxic chemicals have increased risk of subfertility (OR: 1.14, 95% CI: 0.62 to 1.79). This may be due to the direct or indirect adverse effect of toxicants on spermatogenesis and hormonal regulation. Toxicants damages directly the testicular tissues, which can result in various adverse effects, namely reduced sperm count, the production of defective spermatozoa, and impaired androgen production (Oliva et al., 2001). It is also 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 also, exposure to heat was found to increase the risk of subfertility (OR: 1.90; 95% CI: 0.45-3.86).

The prevalence of azoospermia and oligozoospermia in the metropolitan cities of Mumbai, Bangalore and Jalandhar (Mehta et al., 2006) were similar to those reported in most other parts of the world (Thonneau et al., 1991; Mazzilli et al., 2000). The present study demonstrated that the prevalence of azoospermia and asthenozoospermia was significantly higher among the exposed subjects as compared to non-exposed subjects. Mehta et al., (2006) had also suggested the higher prevalence of azoospermia in Jodhpur and Kurnool, hypothesized to be caused due to fluoride levels in drinking water and pesticide exposure respectively.

The subjects were categorized into three different groups on the basis of age i.e. 20-29, 30-39 and ≥ 40 years. About 50% of the subjects were in the age group of
20-30 years In the present study, age showed significant negative correlation with sperm count and viability. However, the recent findings by Winkle et al., (2009) suggested that neither the routinely assessed semen parameters nor the amount of spermatozoa with fragmented DNA were affected by male age. However, earlier Plastira et al., (2007) showed that the increased age in infertile patients to be associated with an increase in sperm DNA fragmentation and poor chromatin packaging as well as with a decline in semen volume, sperm morphology and motility. In the present study, DNA damage increased whereas seminal antioxidant activity decreased with increasing age, though the results were statistically non-significant. Singh et al., (2003) found by means of comet assay that male age may predispose for DNA double strand breaks. In contrast Schmid et al., (2007), using the same method, reported that male age only influences single stranded breaks, which do not have any influence on fertilization, because single stranded breaks can be repaired by the oocytes. Luetjens et al., (2002) reported that men of advanced age still wanting to become fathers do not have significantly higher risk of producing offsprings with chromosomal abnormalities compared with younger men.

The data on the relationship between male body weight index and the reproductive potential of humans are scanty. In the present study, majority of the subjects (61.7%) had BMI values within the recommended range of 18.5 to 24.9 kg/m² while about 16.6% and 22.7% of the subjects had BMI in the lower and higher range respectively. Mean BMI was 22.21 ± 0.26 kg/m² in the study group, which was 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). Body mass index has been reported to influence fertility Some authors have reported the adverse effect of elevated BMI on sperm quantity and quality (Kort et al., 2006; Hammoud et al., 2008), while other did not observe any relationship with BMI (Pauli et al., 2008). It was evident from the results obtained in the present study that sperm count, % normal morphology and viability were significantly lower among the subjects of higher BMI as compared to normal BMI subjects. Further, the incidence of oligoasthenoteratozoospermia was significantly higher among the subjects with higher BMI This corroborates with the earlier findings of Hofny et al., (2010). They showed that the mean BMI was inversely correlated with sperm concentration, motility as well as normal morphology and further suggested that serum leptin mediates a link between obesity and male infertility. Earlier, Jensen et al., (2004)
reported higher prevalence of oligozoospermia in overweight and obese men than in normal weight men with a significant association between sperm count and BMI. In the present study, sperm chromatin/DNA damage was found to be non-significantly higher among the overweight subjects. However, Rybar et al., (2011) had concluded that the impact of elevated BMI on the parameters investigated for assessing chromatin integrity and condensation could not be established. The results of the present study also demonstrated significant negative correlation between BMI and serum testosterone levels. Several studies have publicized reductions in testosterone level with respect to obesity (Roudebush et al., 2005; Fejes et al., 2006). It has been reported that reduction in total testosterone production in obese men can result in decreased intra-testicular testosterone levels, thereby affecting the function of the seminiferous epithelium and the synchrony of spermatogenesis (Varghese et al., 2008). The possible reasons for variation in spermatogenesis or decreasing semen quality among the subjects with higher BMI could be due to the deposition of fat in supra-pubic area and thighs which alters the hormone profiles particularly reproductive sex hormones which can impair the expression of genes involved in spermatogenesis pathways leading to impaired semen parameters (Ashok and Sigman, 2007; Hofstra et al., 2008) and also increase in scrotal temperature that can lead to erectile dysfunction (Andersen et al., 2008; Yassin et al., 2008). Numerous authors have noted that obesity and several of its causative agents, namely, insulin resistance and dyslipidemia, are associated with increased oxidative stress (Dandona et al., 2005, Davi and Falco, 2005), which is most likely to be the result of elevated metabolic rates that are required to maintain normal biological processes and an increased level of stress in the local testicular environment, both of which naturally produce ROS. The same has been revealed from the present study in which higher BMI showed significant association with decreasing activity of SOD and thiols, resulting into an increased oxidative stress by depleting the antioxidant level. It can be hypothesized from the present study coupled with available data that excess weight might be related directly or indirectly to several biological changes that could increase the risk of male infertility.

Lifestyle factors play an important role in the etiology of various diseases and have also been implicated to cause reproductive impairment. Approximately, one-third of the adult population in the world uses tobacco in some form and half of them die prematurely. According to an estimate by the WHO, 4.9 million people worldwide
died in 2000 as a result of their addiction to nicotine (WHO, 2002). Moreover, WHO predicted that tobacco deaths in India might exceed 1.5 million annually by 2020 (Murray and Lopez, 1996). In the present study, on the basis of exposure to lifestyle and/or toxicant factors, subjects were categorized into three categories i.e. only lifestyle exposed, only toxicant exposed and the subjects exposed to both factors. It was found that the total progressive motility and normal sperm morphology were significantly lowered among the only lifestyle exposed subjects. The relationship between tobacco chewing and male infertility remains unclear and data specifically addressing this issue is rare even though tobacco smoking might have adverse effects on semen quality. Data on personal habits in the present study, revealed a higher number of chewers of tobacco and areca nut in comparison to smokers and alcoholics. The chewing tobacco and areca nut is mainly confined to south-east Asian countries, especially in 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. The condition of oligoasthenoteratozoospermia was found to be significantly higher among the chewers as compared to subjects having no lifestyle habit. In a study conducted by Said et al., (2005), the incidence of increasing trend of oligoasthenoteratozoospermia has been reported from mild to moderate to severe with respect to addiction of tobacco chewing. They further observed that the incidence of oligoasthenoteratozoospermia was highly significant in the severely addicted group as compared to mild and moderate tobacco chewers. In the present study, the chewing of mixture containing areca nut and tobacco as one of the main components was found to be associated with increased risk of subfertility (OR: 1.26; 95% CI: 0.66 to 1.84). However, an earlier report by Dikshit et al., (1987) found no such effect of tobacco chewing on male reproductive function. In the present study, significant decrease was observed in fast progressive motility and % normal morphology among chewers as compared to non-chewers. Further, the fast progressive motility decreased significantly with increase in chewing frequency (quids/day) and duration in years. On comparison of the semen parameters among oligozoospermic subjects, it was found that total progressive motility (p<0.05) and normal sperm morphology (p<0.001) decreased significantly among chewers as compared to 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) also reported that the long-term chronic exposure to panmasala (mixture of tobacco, areca nut, 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 areca nut alkaloids and tobacco specific N-nitrosamine (TSNAs) on the reproductive system. Further, one of the important finding of the present study is the significant effect of chewing areca nut and tobacco on the sperm DNA damage as assessed by comet assay. Sperm DNA damage was found to be significantly higher among the subjects with the habit of chewing areca nut and tobacco. Further, a significant dose-duration dependent increase in the sperm DNA damage was observed with respect to chewing habit.

As a large number of men smoke worldwide and also the fact that cigarette smoke contains various known mutagens and carcinogens, there has been much concern that smoking may have unfavorable effects on male reproduction (Zhang et al., 2000) Studies indicated that cigarette smoking is associated with modest reduction in semen quality including sperm concentration, motility and morphology (Pasqualotto et al., 2006), semen volume and acidity (Zhang et al., 2000) and could cause specific lesions in the development of spermatozoon, and it might be directly or indirectly toxic to spermatogenesis in rat (Rajpurkar et al., 2002) However, in regard to the clinical studies on the relationship between smoking and male reproduction, the literature results have been inconclusive. Some investigators have reported that smoking lowers sperm motility and normal morphology and has adverse effects on male fertility (Sofikitis et al., 1995), whereas some author reported no such finding (Aziz et al., 2004). In the present study, no statistically significant effect was noted among smokers with respect to total progressive motility, viability and normal sperm morphology as compared to subjects having no lifestyle habit. However, on comparison of these semen parameters among the oligozoospermic subjects, it was found that there was a non-significant lowering of sperm count and total progressive motility between smokers and non-smokers. This shows that these men might be susceptible to the effect of tobacco smoke and supports the earlier findings. Zenses (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 infertile patients may be predisposed to and may be more susceptible to lifestyle or environmental exposure than the general population. 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 affecting the stability of the sperm chromatin; this may in turn contribute to reproductive failure or have consequences in fetal development. This was observed in the present study also in which the level of both serum and seminal plasma zinc were lowest among the subjects having both lifestyle and toxicant exposure and thereby the sperm chromatin integrity was also found to be diminished among the subjects having both lifestyle and toxicant exposure as compared to the non-exposed subjects. It has also been reported that smoking increases the production of free radicals that will impair the synthesis and/or augment the consumption of SOD (Oldereid et al., 1994). In the present study, the subjects having the habit of smoking alone showed the lowest activity of seminal SOD and CAT as compared to the subjects having no lifestyle habit, resulting in an increased oxidative stress.

The effects of alcohol consumption on male reproductive function have remained under discussion (Martini et al., 2004). Some workers have reported a reduction in sperm concentration 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 sperm count, total progressive motility and normal sperm morphology among alcohol consumers who had oligozoospermia. However, these alterations did not reach statistical significance. One of the reasons 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). The present study further indicated that the subjects having the habit of alcohol consumption were found to have the higher risk of subfertility as compared to non-alcoholics (OR: 1.11; 95% CI: 0.53 to 2.04). It can be inferred that chronic alcohol consumption might have detrimental effect on male reproductive system and thereby affecting reproductive organs directly or indirectly via hormonal production and regulation.
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 (OR: 1.70). This indicates that indulgence in lifestyle factors such as tobacco smoking/chewing and alcohol consumption plays an important role in the deterioration of semen quality.

The use of cell phones has increased many folds worldwide and data are limited on reproductive system of users of cell phone, which emits the electromagnetic radiation. The evaluation of the effect of cell phone on the reproductive health of human is a difficult issue, as it is hard to distinguish the effect caused by various lifestyle and environmental factors from the particular one that may be due to electromagnetic waves emitted by cell phones. It has been further shown that people who talk on the phone, to a greater degree may be exposed to stress, which by affecting the level of cortisol, prolactin and testosterone may contribute to the decrease in the sperm count (Sheiner et al., 2003). Recently, Kesari et al., (2011) investigated the effect of mobile phone on fertility pattern in male Wistar rats and concluded that radiofrequency wave from commercially available cell phones might affect the fertilizing potential of rat spermatozoa. The result in the present study suggests that semen quality was lowered among the cell phone users, both in terms of frequency of daily use as well as duration of possession, as compared to non-users but the alterations were non-significant. Further, no alterations were observed in the other semen or hormonal parameters with respect to the cell phone use. Earlier, Agarwal et al., (2009) based on an in vitro study, concluded that radio-frequency electromagnetic waves emitted from cell phone may lead to oxidative stress in human semen. Various contradictory studies on the effect of cell phone use with respect to male reproductive health have been reported. Dasdag et al., (2003) reported no effects of cell phone use on the testis of rats, whereas Davoudi et al., (2002) observed declining levels of rapid progressive spermatozoa among a small study group of cell phone users. The use of cell phones has become widespread and hence more prospective as well as controlled studies in the population with longer duration of use needs to be done to affirm the effect of cell phone use on deteriorating semen quality.
The pathophysiology of male infertility could be explained by a cascade of molecular and biochemical events which is mostly represented by abnormal semen parameters. Growing evidence indicates that imbalance between peroxidative and antioxidative substances in semen leads to metabolic and functional disorders of male germ cells and may be a primary cause of some types of infertility (Fraczek and Kurpisz, 2007). Aerobic metabolism of human sperm produces different potentially harmful reactive oxygen species (ROS) against the sperm plasma membrane with its high content of polyunsaturated fatty acids (Jones et al., 1979; Storey, 1997). An increase in the seminal ROS level has been reported in 40% of the infertile men. Although antioxidant defense system is active in the semen, its activity is limited as the amount of cytoplasm of the sperm cell is low (Lewis et al., 1997). Spermatozoa are highly sensitive to injuries caused by high generations of ROS concentration such as superoxide anion, hydrogen peroxide, and the hydroxyl radical which can result in the damage to cell membrane (Aitken and Clarkson, 1987).

It is known that declining semen quality affects fertility as the adequate level of sperm number, motility, and morphology are necessary for the reproductive success. The imbalance between ROS production and ROS degradation has been hypothesized as a cause of oxidative stress in semen with peroxidative injury to the sperm membrane and a consequent impairment of the related functional properties, such as sperm motility (Sharma and Agarwal, 1996). Excessive generation of ROS in semen may be associated with reduced sperm fertilizing potentials, impaired metabolism, morphology, and motility (Cummins et al., 1994). Recently, it has been suggested that insufficient antioxidants and increased oxidative stress may attribute to the risk of declining semen quality (Murarka et al., 2011).

Seminal plasma malondialdehyde (MDA) assay, which is the stable lipid peroxidation product, is a simple method to evaluate the effect of lipid peroxidation on sperm (Geva et al., 1998). Lipid peroxidation triggers the loss of membrane integrity, causing increased cell permeability, enzyme inactivation, structural damage to DNA, and cell death (Halliwell, 1994). Studies on the peroxidation of phospholipids in mammalian sperm had demonstrated that peroxidation reaction causes membrane damage, which leads to loss of motility and membrane integrity (Engel et al., 1999). In the present study, LPO was found to have significantly negative correlation with sperm count, motility and morphology. Oligozoospermic and azoospermic subjects
showed significantly higher MDA content as compared to normozoospermics. Similarly, a significant increase in the MDA levels was observed in asthenozoospermics and teratozoospermics as compared to progressively motile and morphologically normal groups, respectively. However, Suleiman et al., (1996) showed no significant negative correlation between seminal plasma level of MDA and sperm count as well as motility but they observed that spermatozoal MDA concentration was higher with decreased sperm motility. The results of the present study are in agreement with the earlier findings (Kobayashi et al., 1991, Fraczek et al., 2001; Hsieh et al., 2006) Our results further demonstrate that lipid peroxide level were higher in the seminal plasma of subjects who were exposed to lifestyle and/or environmental factors, which may be due to increased oxidative stress, exerted by these factors.

It is known that, in addition to lipids, free radicals can also react with proteins. Protein carbonyl (PC) formation is known to be an early marker for protein oxidation (Reznick and Packer, 1994). Sperm normal morphology was observed to have significant negative correlation with protein carbonyls in the present study. An inverse relationship of PC level in seminal plasma was found with sperm concentration and motility, though the results were statistically non-significant. The source of cytotoxic oxygen radicals is frequently intracellular, as in the case of oligozoospermic males whose spermatozoa generate particularly high levels of ROS (Gomez et al., 1996). Oxidative stress frequently represented as an MDA value and protein oxidation formation has been recognized in accordance with male fertility (Aitken et al., 1989; Agarwal et al., 2003; Aydemir et al., 2007).

Seminal plasma is well endowed with an array of antioxidant defense mechanism to protect spermatozoa against oxidants that compensates for the deficiency in cytoplasmic enzymes in the spermatozoa (Agarwal et al., 2003). SOD scavenges both intracellular and extracellular superoxide radical and prevents the lipid peroxidation of plasma membrane. It should be conjugated with catalase or glutathione peroxidase in order to prevent the action of hydrogen peroxide (Agarwal and Prabhakaran, 2005). Catalase detoxifies both intracellular and extracellular H$_2$O$_2$ to water and oxygen (Baker et al., 1996). Sperm count, motility and morphology showed significant positive relationship with the seminal SOD levels and it was found to be higher with the elevation in sperm count, which indicated that decline in SOD
might be involved in the abnormal deterioration of semen quality. Alvarez et al., (1987) showed that the SOD activity of a semen sample appears to be a good predictor of the lifetime (up to the complete loss of motility) of that particular sample. Some infertility cases reveal intensive aberrations in chromosomes 13, 20, and 21, which contain sequences coding for superoxide dismutase (Brown, 1995). It has also been reported that SOD activity survey in seminal plasma could be a useful tool for determining sperm fertilization potential and could improve the diagnosis of male infertility (Murawski et al., 2007). However, Zini et al., (1993) and Hsieh et al., (2002) observed no significant difference in the SOD activity measured in seminal plasma and semen quality. Khosrowbeygi et al., (2004) observed that both catalase activity and total antioxidant capacity (TAC) were significantly correlated with sperm motility and morphology. In the present study also, significant positive correlation of CAT was observed with sperm count, motility and morphology. Further CAT activity was found to be significantly lowered among the subjects exposed to lifestyle and/or environmental factors indicating the role of these factors in deterioration of semen quality.

The free radical scavenging system, SOD and catalase are present in all oxygen metabolizing cells and their function is to provide a defense against the potentially damaging activities of superoxide and H$_2$O$_2$ (Gupta and Ray, 2004). Depletion in the enzymatic activity observed in the present study may be due to decreased synthesis of enzyme or oxidative inactivation of enzyme protein. Decrease in the SOD activity suggests that it may not be able to completely remove or cope up with the free superoxide radical generated by various lifestyle and/or toxicant exposure. Further, reduction in catalase activity reflected inability to eliminate H$_2$O$_2$ produced by lifestyle and/or toxicant exposure. Decreased status of these antioxidant enzymes could contribute to the imbalance of ROS.

Another mode of protection from ROS is glutathione antioxidant system that plays a fundamental role in the cellular defence against the reactive free radicals and the other oxidant species. It consists of GSH and an array of functionally related enzymes of which GRD is responsible for the regeneration and the synthesis of GSH whereas GST work together with GSH in the decomposition of H$_2$O$_2$ and other hydroperoxides (Mak et al., 1996). GPx and GST activity were observed to have significant positive correlation with sperm count, which might be a compensatory
reaction against the lifestyle and/or toxicant exposure induced oxidative damage. Further GST activity increased significantly with sperm count, motility and morphology, which could possibly reduce oxidative stress as the enzyme is involved in the detoxification mechanism.

Glutathione is the major intracellular antioxidant involved in the maintenance of the thiol moieties of proteins and reduced forms of many other molecules (Meister, 1984). In the present study, GSH and thiol concentrations were more or less similar in all the groups based on sperm count, motility and morphology. When present in the extracellular space, reduced glutathione (GSH) is able to react directly with cytotoxic aldehydes produced during lipid peroxidation, and thus protect the free sulphydryl groups on the sperm plasma membrane. Moreover, GSH acts to preserve SH groups of protein in the reduced state by means of disulfide interchange (Eskiocak et al., 2005). Despite its role as an antioxidant, GSH also serve as a source of amino acid for spermatogenesis. Supplementation of GSH from Sertoli cells is required for spermatogenic cells as GSH content of spermatozoa is very low (Darmani and Al-Hiyasat, 2005). The altered semen quality by lifestyle and/or toxicant exposures suggest that GSH is not responsible for the changes as no considerable alteration was noticed in GSH level with respect to semen quality. Earlier, Garrido et al., (2004) also did not observed any difference in total ejaculate GSH between fertile and subfertile men.

Protein oxidation can lead to loss of critical thiol groups (Pready et al., 1998). Thiols and ROS are implicated in human reproduction. Thiols are scavenging ROS and are therefore suggested to be important in sperm function and fertilization as well (Rousseaux and Rousseaux, 1995). The maintenance of free protein sulphydryl groups is important in the proper folding and activity of protein. The DNA in the spermatozoa head is intensely compacted as a result of disulphide bridges between oxidized Cys residues in protamine molecules which are important during the maturation of spermatozoa in the epididymis. The oxidation of thiols is also important for the stabilization of the tail structure, sperm motility, and the protection of sperm DNA against physical or chemical damage (Rousseaux and Rousseaux, 1995). According to Ebisch et al., (2006), ROS produced by spermatozoa leads to upregulation of thiol synthesis in order to protect sperm from oxidative damage. In the present study, total thiols in the seminal plasma showed no significant difference
between different groups with respect to sperm count, motility and morphology that is in agreement with Lewis et al., (1997) However, it has been reported earlier that seminal plasma free sulphydryl in infertile patients were significantly lower than those in the control groups (Alkan et al., 1997). Furthermore, in the stress condition, seminal plasma free sulphydryl levels were shown to be significantly lowered than those found in the non-stress condition (Eskiocak et al., 2005).

Ascorbic acid, a major water-soluble antioxidant, acts as a major scavenger for a wide range of ROS. It is present at approximately 10-fold higher concentrations in seminal plasma compared with blood plasma, suggesting its physiological role in seminal plasma (Lewis et al., 1997). In the present study, no relationship was observed between ascorbic acid and seminal parameters. However, earlier, it was reported that a defect of ascorbic acid level is a possible mechanism of sperm DNA damage in infertile men, leading to ROS overproduction and increased consumption of ascorbic acid in seminal plasma (Song et al., 2006).

In addition, sperm concentration was positively correlated with progressive motility, percent of normal sperm morphology, SOD, CAT, glutathione levels and thiols, while negatively correlated with LPO and PC. The increasing ranges of sperm count showed negative correlation with MDA and PC, whereas positive correlation with SOD, CAT, and thiol levels. All these results suggests that the semen samples consisting of better sperm count, motility, and normal morphology were observed to be associated with improved antioxidant activity, which might reveal enhanced scavenging activity against the generation of free radicals.

One half of the genomic material to the offspring is known to be contributed by sperm DNA Thus, normal sperm genetic material is required for successful fertilization, normal embryonic and fetal development as well as postnatal child well being. The interest to assess the chromatin quality of human sperm has increased since DNA damage in sperm has been associated with infertility. The chromatin content in the nuclei is extremely stable and is compact in structure. This tight packaging minimizes the volume of the sperm nuclei for transport through the female genital 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 of future generation. Detection of sperm nuclear DNA integrity is thus, a potential tool for evaluation of semen
samples prior to their use in assisted reproductive techniques (Sharma et al., 2004). In the present study, DNA/chromatin damage in sperm was observed by comet assay as well as AB, TB, AO and CA3 staining test.

Although many methodologies are available to assess DNA fragmentation in spermatozoa, the neutral comet assay has been technically simple and cost-efficient method to measure the double-stranded (ds) DNA breaks in sperm cells (Singh et al., 2003). The neutral comet assay seems to be more sensitive and reliable for the detection of ds DNA breaks than the alkaline comet assay because the alkaline conditions inherently induce the formation of DNA damage, a phenomenon that is not observed with the neutral comet assay (Chi et al., 2008). In the present study, the sperm DNA damage was significantly higher in the subfertile semen samples compared to normal semen samples. Shamsi et al., (2009) reported a similar statistically significant increase in DNA damage among oligozoospermic (20%), asthenozoospermic (24%), teratozoospermic (28%) and oligoasthenoteratozoospermic (OAT) semen samples (43%) when compared to control samples (8%). In addition, other authors also reported that oligozoospermic (Tomsu et al., 2002), teratozoospermic (Kalthur et al., 2008), leukocytospermic (Fariello et al., 2009), and OAT semen samples (Nili et al., 2009) showed a significant increase in the amount of DNA damaged sperm. Therefore, sperm DNA integrity might serve as a good biomarker to evaluate semen quality and to diagnose male infertility in conjunction with semen analysis. Moreover, in the present study, DNA fragmentation and poor chromatin packaging appeared to be inversely correlated with conventional semen parameters, a fact highlighted in several studies (Shen and Ong, 2000, Irvine et al., 2000, Moskovtsev et al., 2005). Damaged DNA may not prevent fertilization from occurring but may lead to fetal abnormalities, which will only be apparent later. Infertility may be linked to DNA damage, as the sperm DNA of infertile patients has been shown to be more susceptible to damage in vitro than DNA from fertile men (Hughes et al., 1996).

The integrity of sperm chromatin structure in turn is influenced by certain endogenous and exogenous factors, such as peroxidative damage of DNA, which may arise from infectious or toxic agents (Irvine et al., 2000). As sperm head consists mainly of nucleus, damage induced by exogenous factors may be present in DNA and/or chromatin packaging. In the present study, the sperm heads with abnormal
versus normal chromatin structure were specified as orange-red versus green by the AO method, blue versus unstained by the AB method, purple-violet versus light stained by the TB method and bright green versus dull green by the CA3 method. A good correlation for the proportion of sperm heads with abnormal chromatin structure was found among all the methods used, which exemplified all four techniques as sensitive enough to estimate sperm DNA/chromatin integrity. It can be further concluded that the AB and TB methods are inexpensive as well as simple and in addition have the advantage of providing permanent preparations, which can be observed under light microscope. In contrast, AO and CA3 are comparatively expensive techniques that uses fluorescent microscope. Moreover, results on deterioration in sperm chromatin integrity suggested that lifestyle and/or toxicant exposure could lead to improper packaging of sperm DNA as revealed by AB, TB and CA3 test, though the results were statistically non-significant. Further, Acridine orange staining test and comet assay results respectively suggested that lifestyle and/or toxicant exposure could cause single as well double stranded DNA break(s) in human sperm. Zini et al., (2009) also suggested that sperm head abnormalities might, in part, be due to incomplete sperm chromatin condensation. Oxidative stress has been associated to DNA-protein cross-links (Nackerdien et al., 1991), DNA strand breaks (Hughes et al., 1996), DNA base damage (Dizdaroglu et al., 1991) in the presence of iron and copper ions, and chromosomal aberrations (Emerit, 1994). Oxidative damage to DNA might be responsible for the increased occurrence of genetic abnormalities as well as other diseases in the offsprings. The present results further insinuate that ROS is the most possible source of lifestyle and/or toxicant induced alterations which affect sperm count, motility, morphology and DNA/chromatin damage. Further, oxidative stress marker i.e. lipid peroxidation showed significant positive correlation with olive tail moment that suggests the possible role of oxidative stress in the induction of sperm DNA damage. It can also be hypothesized that excessive generation of ROS can lead to impairment in the cellular macromolecules such as DNA, protein and membrane lipids, leading to probable damage in the functioning of the sperm cells that plays a crucial role in fertilization. There is also strong clinical evidence that sperm DNA damage in association with increased abnormalities in conventional semen parameters have a pronounced negative effect on reproductive outcome (Li et al., 2006; Zini and Libman, 2006). Furthermore, for a threshold value of DNA fragmentation above 10%, a significant negative correlation to the fertilization rate has been reported by
Benchaib et al., (2003). Moreover, in the present study, the sperm aneuploidy assessed by Fluorescent in situ hybridization was carried out among the representative number of subjects. The results indicated that sperm aneuploidy was increased in the subjects with oligozoospermia as well as asthenozoospermia as compared to normozoospermics and progressive motile subjects respectively. Further, the habit of chewing areca nut and tobacco also showed to increase sperm aneuploidy. But the results pertaining to sperm aneuploidy were statistically non-significant.

Spermatozoa have two defense mechanisms against oxidative attack of their DNA; first is the packaging arrangement of the DNA that reduces exposure to free radical attack and the second one is the antioxidant capacity of its seminal plasma (Sidney and Grimes, 1986; Lewis et al., 1997). During spermiogenesis the nucleus elongates and the chromatn becomes highly condensed, with somatic type histones being replaced by basic proteins which are in turn replaced by protamines enriched in arginine (Sidney and Grimes, 1986). The DNA is organized in loops which are also stabilized by disulphide bonds (Ward, 1993, Barone et al., 1994), causing the condensed chromatin to be inert and resistant to the standard lysis agents. The present study in which thiols negatively correlated with chromatin damage markers i.e., AB, TB and CA3 positive sperms, lend supports the above view. Further, a significant negative correlation was observed between thiol levels and TB positive sperms indicating the role of thiols in preventing chromatin damage.

Endogenous mediators, such as ROS, can decide the mode of cell death via apoptosis. The presence of immature sperms may contribute to increased levels of apoptotic markers in ejaculated sperm (Cayil et al., 2004). Apoptosis is a mechanism regulating spermatogenesis in humans, and there are clear differences in molecular markers of apoptosis between males with normal sperm parameters and those with abnormal sperm parameters (Sakkas et al., 1999). This is consistent with the results from the present study that percent apoptosis was lower in men with normal count compared to oligozoospermic subjects. The DNA diffusion assay is a highly sensitive method for apoptosis assessment. Singh (2000) observed the sensitivity of the assay to be greater than 98%. In the present study, the percent apoptosis in ejaculated sperm ranged from 0.2 to 17.0%. The results are similar to those of Singh et al., (2003), who showed 0.3% to 23% apoptosis of human sperm cells and Chen et
al., (2006) showing 1 to 20% of apoptotic cells, using the DNA diffusion assay. In the present study, the percent of apoptosis did not showed any significant correlation with sperm count, motility or morphology. The literature to date has inconsistent results on the relationship between sperm apoptosis and measures of sperm quantity (Gandini et al., 2000; Oosterhuis et al., 2000, Shen et al., 2002). Apoptotic bodies declined with age in the study, which might be due to the fact that the damaged sperm are preferentially eliminated. The present study coupled with the data available indicated that the use of the DNA diffusion assay to measure apoptosis in human ejaculated sperm is a simple technique and can be useful in the assessment of male reproductive potential.

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 (Anderson et al., 1997). In the present study mean serum FSH and LH levels were significantly higher among azoospermics and oligospermics as compared to normozoospermics. A significant correlation was observed between mean serum FSH and LH levels. This indicates alteration to the hypothalamic pituitary axis affecting spermatogenesis. Elevated FSH levels possibly signify an effect on Sertoli cell function. Further, it can be hypothesized that toxic substances might target sertoli cells along with other cellular and subcellular targets, which could impair semen quality. It has also 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 (1979) reported elevated levels of serum FSH with increasing severity of seminiferous epithelium destruction. Further, Babu et al., (2004) mentioned that in infertile males with abnormal histopathology (sertoli cell only syndrome, hypospermatogenesis and spermatid arrest) the mean FSH levels were significantly elevated compared to the control group. The results of the present study corroborates 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 oligozoospermic and normozoospermics. Further, present study also indicated the significant effect of metals like lead on circulating FSH levels. 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 thyroid hormones thyroxine (T4) and triiodothyronine (T3) are iodinated amino acid derivatives formed by oxidative coupling of two iodinated tyrosine residues in the thyroid protein thyroglobulin. These hormones play a role in the stimulation of metabolic rate and regulation of growth, development, and differentiation (Nunez, 1999). Thyroid hormones appear to regulate the duration of Sertoli cell proliferation, affecting adult Sertoli cell number and hence the capacity of the testis to produce sperm (Buzzard et al., 2000). T3 and T4 influence the duration of proliferation of Sertoli cells (Francavilla et al., 1991; Van Haaster et al., 1992; 1993). Thyroid hormones as well as steroid hormones interact with a superfamily of cytoplasmic "zinc finger" protein receptors (Rush et al., 1999). Neurotransmitters enable transmission from one neuron to the next across synaptic gaps (Rush et al., 1999). In the present study, no significant alterations were observed in the levels of thyroid hormones with respect to sperm count. Thyroid hormones increase metabolic activity and oxygen consumption causing oxidative stress in exposed cells (Wilson et al., 1989; Oppenheimer et al., 1996). Also, during the normal catabolic pathway of oxidation of noradrenaline, ROS and hydrogen peroxide are produced (Graham, 1984). The low levels of thyroid hormones and noradrenaline normally present in the human body might not produce any genetic damage. However, unusual concentrations of thyroid hormones in the human body may be caused by the administration of some drugs. Dobrzynska et al. (2004) suggested that thyroid hormones triiodothyronine (T3) and L-thyroxine sodium salt (T4) induce oxidative stress and subsequent DNA damage due to reactive oxygen species and possibly due to reactive hormone derivatives created during their redox cycling.

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 measurements in European industrial settings on lead workers spanning smelters, battery manufacturers and foundries, have been carried out by Bonde and Apostoli (2005). They reported that the average concentration of lead in blood steadily declined from 68 μg/dL in 1970 to 35 μg/dL in 1995 (Bonde, 1999). 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, awareness and technology has reduced lead exposures at work and in the environment. 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. In the present study population, the mean blood lead levels were found to be 5.05 ± 0.35 μg/dL. The low levels of lead found in this study might be due to the above reasons.

A number of epidemiological studies indicated that occupational exposures to lead have adverse effects on human sperm. 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, percentage of total motile and viable sperm, progressively motile sperm, in parameters of prostate secretory function and an increase in abnormal sperm head morphology, serum testosterone and estradiol. In the present study, the Pb levels were significantly higher among azoospermics as compared to the normozoospermic subjects. Further Pb levels were also significantly higher among the subjects having only toxicant exposure as compared to non-exposed population.

In the present study, a negative effect of lead on sperm count was observed while cytoplasmic droplets showed significant positive correlation with Pb levels. This is in accordance with earlier 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 higher lead levels are associated with deteriorating semen quality. In addition, there are reports showing the associations between lead and certain accessory sex gland markers. In a study carried out in Lucknow, India, the authors found lead to be negatively associated with fructose, acid phosphatase and gamma-glutamyl transpeptidase. They further 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). In the present study, blood Pb level was found to affect the levels of alpha glucosidase and fructose activity in seminal plasma.

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 hypothalamic-pituitary axis production and secretion of gonadotrophins (Ng et al., 1991). In the present study, lead was found to have significant positive correlation with FSH levels and non-significant positive correlation with LH levels, however negative association of lead was observed on circulating testosterone levels. This indicates that lead might affect testicular function. 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, it can be hypothesized that reproductive function is affected by the toxic effects of lead on the gonads and indirectly through hypothalamic-pituitary system.

Men with normal spermiograms may still be infertile; the cause could be related to abnormal sperm DNA (Alvarez, 2003). In the present study, we found significant positive correlation between blood lead levels and percentage of sperms with abnormal DNA. The lead levels were found to have significant positive
correlation with comet length and abnormal sperm DNA assessed by acridine orange test, which indicates that lead, may have negative effect on chromatin structure. Hernandez-Ochoa et al., (2005) 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 the present study that exposure to lead is capable of causing damage to DNA/chromatin structure which may affect pregnancy outcome. It can be inferred from the present study that lead exposure may cause deterioratin in semen quality.

Cadmium is a toxic heavy metal that occurs widely in nature and also produced during tobacco smoking. 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., 1987; 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. Telisman et al., (2000) indicated that blood cadmium less than 10 μg/L could also increase abnormal sperm morphology. In the present study, no statistically significant effect of cadmium was observed with respect to sperm count, motility as well as normal morphology. However, the sperm count and motility showed decreasing trend whereas the abnormal sperm morphology showed increasing trend with respect to higher cadmium levels. 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 et al., 1999). The accumulative data indicates the existence of a disruption in the regulatory mechanisms of the hypothalamic-pituitary axis by cadmium. In the present study, blood cadmium was found to have significant negative effect on testosterone levels whereas positive effect on T₄ levels. The genetic damage to sperm cells was also evident in the form of higher percentage of sperm DNA damage with rising lead and cadmium levels. The present study also indicates that toxic chemical exposure either through occupation or through the environment have deleterious effects on male reproductive system.
Oxidative stress (OS) has elicited enormous interest among researchers in recent period. Reactive oxygen species are continuously produced by various metabolic and physiological processes. 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 which results in retention of cytoplasmic droplet is a major source of ROS (Gomez et al., 1996). In the present study, mean percentage of spermatozoa with cytoplasmic droplet was significantly higher among subjects having higher blood lead levels. Hence it can be hypothesized that lead along with other toxicants might be responsible for oxidative stress to 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 among lead exposed subjects as well as significant correlation between DNA damaged sperm with higher lead levels. Cadmium and its relationship with semen quality, although showed non-significant results, however all semen parameters were found to be deteriorated among the group with higher cadmium levels indicating role of cadmium in causation of reproductive dysfunction. It can be suspected 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.

Certain heavy metals occur as trace elements in the body but are essential for the development, growth and health. Many of these elements produce toxic
effects following excessive exposure, and 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. Exposure to copper has been linked to a decreased sperm count and a cause of teratozoospermia and asthenozoospermia (Lahdetie, 1995). In the present study, serum copper levels showed negative relationship with increasing sperm count, motility and morphology. However, the relationship of seminal plasma copper levels with motility and morphology showed only minor variation. Stanwell Smith et al., (1983) also found higher concentration of blood plasma copper among infertile men than those of proven fertility, but no relation with seminal plasma copper level. Huang et al., (2000) found higher copper levels in asthenozoospermia subjects. 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 in the percentage of motile sperm (Battersby et al., 1982), which is also evident in the present study. This deterioration in sperm motility might be due to uptake of metallic copper by spermatozoa. One of the hypotheses also suggests that copper reduces the oxidative process and glucose consumption, which reduces or abolishes sperm motility (Skandhan, 1992). Serum copper level was also associated with significant decline in the levels of SOD and ascorbic acid. The results propose that copper has a toxic effect on sperm count, motility and morphology and it might be exerting its effect by disturbing the antioxidant defense system. Also, the present findings reveal that serum copper is a better marker for assessing copper toxicity than seminal plasma.

Studies have shown a possible role of zinc in sperm production and/or viability, in the prevention of spermatozoa degradation. The present study reported that the mean zinc levels in serum were lower among azoospermics as compared to oligozoospermic and normozoospermic groups. However, no statistically significant relationship between zinc and sperm motility was found. Earlier, Lewis-Jones et al., (1996) also reported no significant relationship between the motile sperm concentration and zinc in seminal plasma. Further, it has been reported that extremely high levels of zinc might inhibit sperm motility and 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 et al., 1999). Fuse et al., (1999) found a positive correlation
of Zn with sperm motility and concluded 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, serum zinc correlated positively with SOD as well as CAT activity.

The level of α-glucosidase in seminal plasma reflects the functional status of the epididymis. Its low activity has been reported in cases of epididymal obstruction (Krause and Bohring, 1999). The present study also indicated a positive correlation between the activity of α-glucosidase and sperm count; low level of α-glucosidase was found in the azoospermic and oligozoospermic groups as compared to normozoospermic groups. Thus, it can be interpreted from the results obtained so far that the α-glucosidase activity in seminal plasma might prove to be useful for the differential diagnosis of oligozoospermia as well as 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. Earlier, Elazanaty 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 total progressive sperm motility showed a significant linear relationship. Based on the relationship between alpha glucosidase and sperm count as well as available data indicates that α-glucosidase plays an important role in identifying the epididymis functioning of an individual as well as its role in male reproductive impairment.

In the present study, a significant correlation between seminal plasma zinc and neutral α-glucosidase has been observed, which is in accordance with earlier report of Mankad et al., (2007). 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 not merely the number of sperms or sperm motility reflects semen quality; it is a composite mixture of secretions from various
glands in the correct proportions that predict overall fertility. The present data along with earlier studies suggest that both zinc and α-glucosidase have a positive role in sperm production. Pant et al., (2003) concluded that lead and cadmium exert their toxicity by affecting biochemical secretions (α-glucosidase, fructose and zinc) from accessory sex glands. In the present study also, significant negative correlation was observed between blood lead levels and fructose levels.

The last decade has seen an exceptional growth in the field of male infertility mainly due to the increased understanding of reactive oxygen species and oxidative stress which has lead to the development of various enzymatic and non-enzymatic antioxidants. The treatment of male infertility using various antioxidants might prove to be an important aspect in the field of reproductive medicine. In conclusion, the study clearly indicates that exposure to lifestyle and/or toxic substances leads to disturbance in semen quality as well as normal hormonal balance, which in turn might affect the subsequent chain of events involved in normal reproduction. The chewing of areca nut and tobacco might be associated with increased sperm DNA damage. Studies on essential metals revealed a positive role of zinc in spermatogenesis. Overall results revealed that lead, cadmium and copper might be toxic to the human sperm and might exert their effect either by affecting the hormonal balance or through indirect effect on the oxidative defense mechanism.