Review Of Literature
Infertility is a disorder that results in much trauma, emotional instability and psychological stress, which in turn has an adverse consequences on the physiology and psychology of the individual, particularly in an Indian social set-up, with a strong emphasis on child-bearing. Infertility affects approximately 15% of all couples trying to conceive. Male infertility is the sole or contributing factor in roughly half of these cases and no identifiable cause can be found in over 25% of infertile males (Sharlip et al., 2002).

SEMEN QUALITY

Demographers have been studying fertility in populations for many years, but it was not until the last 3-4 decades of the 20th century that epidemiologists and toxicologists took interest on this aspect (Bonde, 1999). There are several recent reports of declining human sperm counts over the last fifty to sixty years as well as an apparent drop in semen quality from different parts of the world particularly from western countries. A systematic meta-analysis of 61 studies, including 14,947 normal men was undertaken by Carlsen et al., (1992) which showed a significant decrease in sperm concentration from 113 to 66 millions/ml and decline in semen volume from 3.40 to 2.75 ml, over the period 1938-1990. Following years, many research papers had supported the theory (Bromwich et al., 1994; Suominen and Vierula, 1993) whereas some investigators disagreed with the Carlsen's theory (Fisch et al., 1996; Paulsen et al., 1996) Olsen et al., (1995) have reviewed the declining trend in sperm counts by substantiating the findings with reliable statistical models. During the past 20 years, Auger et al., (1995) showed a decline in the concentration and motility of sperm and in the percentage of morphologically normal spermatozoa in fertile men that were independent of the age of the men. Swan et al., (1998) mentioned that there was no evidence of a decline in semen quality in non-western countries while they observed a significant decline in Europe and United States Heinze (1998) reported that studies from Europe and the United States indicated large interregional differences in sperm density. He noted that interregional
differences in the United States (New York city vs Los Angeles, CA) were as large as the reported differences in mean sperm density in 1938 versus 1990. Bonde et al., (1998) demonstrated an association between year of birth and falling sperm counts in a meta-analysis of 10 Danish occupational studies. Earlier, Aitken (1997) also suggested that the widely publicized decline in human sperm counts reported in several European countries is happening at a too fast rate to be genetic and probably involves environmental factors. Andolz et al., (1999) found a statistically significant decline in semen volume whereas a non-significant increase was observed in sperm concentration among 20,411 infertile men Orejuela et al., (1998) emphasized that generalization of a worldwide declining trend of semen quality is still risky and highlighted the need for innovative new prospective studies with good quality data to address this important issue related to human reproduction. It has also been documented that a global trend for declining semen quality is not supported by the current data (IPCS, 2002). Later, Lackner et al., (2005) reported the declining trend in sperm concentration from $27 \times 10^6$/ml to $10 \times 10^6$/ml between 1986 and 2003. Sripada et al., (2007) also found a decrease in sperm concentration of men in infertile relationship from $75 \times 10^6$/ml in 1994 to $55 \times 10^6$/ml in 2005. Later, Feki et al., (2009) found a decline in the sperm count and morphology over a 12-year period among men in infertile relationships from the south of Tunisia, suggesting that the reported worldwide decline in semen quality is also real in certain areas of the African continent.

A couple of studies from the Indian subcontinent also reported a decline in semen quality (Mehta and Anand Kumar, 1997, Gopalkrishnan, 1997). Adiga et al., (2008) provided the first evidence for the deterioration in human semen quality in the southern part of India over the years, probably due to environmental, nutritional, lifestyle or socioeconomic causes. The large-scale study consisting of 3,729 men by Mukhopadhyay et al., (2010) confirmed a significant decline in the sperm motility parameters and seminal volume between the two distinct decades, that is, between 1981-85 and 2000-06 with no change in overall sperm concentration. However, Pal et al., (2006) did not support the contention of a decrease in the semen quality in men from northern India. Also, the study on the trend in semen parameters of males from Ahmedabad city (Gujarat) by Highland et al., (2010) showed no significant alteration in sperm count, motility, viability and morphology over the period of 20
years. Some studies showed decline in certain regions or cities, whereas others have not found a decline, suggesting there may be regional trends in the country.

**OXIDATIVE STRESS (OS)**

By definition, a free radical is of any chemical compound with one or more unpaired electrons responsible for inducing oxidative stress. The free radicals that have been associated with infertility are oxygen and oxygen-derived oxidants, which include single oxygen, hydrogen peroxide, superoxide anion radicals, peroxyl radicals and hydroxyl radicals. Excessive production of free radicals or reactive oxygen species (ROS) can damage sperm and ROS have been extensively studied as one of the mechanisms of infertility. Superoxide anion, hydroxyl radical and hydrogen peroxide are some of the major ROS present in seminal plasma. Cells living under aerobic conditions constantly face the oxygen (O\(_2\)) paradox – O\(_2\) is required to support life, but its metabolites such as ROS can modify cell functions, endanger cell survival, or both (Agarwal and Saleh, 2002); hence, any excess ROS must be continuously inactivated in order to maintain normal cell function. This function is taken up by the antioxidants present in the seminal plasma. When there is an excessive production of ROS or impaired antioxidant defense mechanisms, oxidative stress occurs, which is harmful to spermatozoa. OS is one of the major causes of male infertility; it damages spermatogenic cells, the spermatogenic process and sperm function. Recent advances in redox biology have revealed the signaling role of ROS that are generated by cells. While highly reactive oxidants, such as the hydroxyl radical, exert largely deleterious effects, hydrogen peroxide can feasibly serve as a signal mediator because it is moderately reactive and membrane permeable and because it can oxidize only limited number of functional groups of biological molecules (Fujii and Tsunoda, 2011).

Poor sperm quality is linked to increased ROS generation as a consequence of the presence of excess residual cytoplasm. Spermatozoa undergo a remarkable transformation during the final stage of sperm differentiation and lose their cytoplasm to become mature spermatids. Following spermiation, any residual cytoplasm associated with spermatozoa is retained in the mid-piece region as an irregular cytoplasmic mass (Aziz et al., 2004). If this residual cytoplasm occupies more than one-third of the sperm head, it is termed a cytoplasmic droplet. Under these circumstances, the spermatozoa that are released after spermiation are thought to be
immature and functionally defective. They are capable of producing increased amounts of ROS (Sikka, 2001)

**Role of Reactive Oxygen Species (ROS)**

ROS are produced in the body by many pathways and other physiological processes in which they act as vital signalling molecules. They are products of natural oxygen metabolism and represent approximately 1 to 2% of metabolized oxygen (Fulbert and Cals, 1992). Studies have indicated that male germ cells at various stages of differentiation have the potential to generate ROS. ROS can have beneficial or detrimental effects on sperm functions depending on the nature and the concentration of the ROS as well as the location and length of exposure to ROS (Agarwal and Saleh, 2002). During epididymal transit, sperm acquire the ability to move progressively; however, they acquire the ability to fertilize in the female tract through a series of physiological changes called 'capacitation' (Visconti and Kopf, 1998). Under physiological conditions, spermatozoa produce small amounts of ROS, which are needed for capacitation and acrosomal reaction. Superoxide anion appears to play a role in this process (Agarwal et al., 2003).

**Sources of ROS**

The sources of free radicals in the living body are respiratory chain, phagocytes, arachidonic acid metabolism, cytophosphokinase, non-enzymatic reaction of oxygen and ionizing radiations. These compounds sometimes working together with some common metals like copper, iron and cobalt attack an important group of tissue constituents, notably lipids—creating a number of deleterious effects. Stress, anesthesia, anti-cancer drugs, painkillers and even normal energy metabolism will also release free radicals in varying amounts (Ogbuewu et al., 2010). ROS are also undoubtedly produced by spermatozoa (Aitken et al., 1992; Hendin et al., 1999), mainly through their mitochondrial system (Plante et al., 1994), as well as round cells during the spermatogenic process and epithelial cells. The production of ROS in ejaculated spermatozoa is initiated in immature germ cells (Gil-Guzman et al., 2001) and continues in the epididymis when the surface of the spermatozoa is remodelled. When the mitochondrial capsule is assembled, chromatin undergoes condensation and motility is acquired for the capacitation of spermatozoa (Fisher and Aitken, 1997, Agnihotri et al., 1999). However, O₂⁻ production by spermatozoa has been questioned (Richer and Ford, 2001) on the basis that no free radical signal can
be detected by electron paramagnetic resonance (EPR) spectroscopy. Electron leakage from complexes I and II of the mitochondrial transport chain has been proposed as a source of superoxide in male gametes (Vernet et al., 2001).

Numerous studies have shown that human sperm exhibit the capacity to generate ROS such as superoxide anion, hydrogen peroxide and hydroxyl radicals (Aitken et al., 1989; 1994). The production of ROS by human sperm is due to a membrane-bound nicotinamide adenine dinucleotide (NADH) oxidase system (Aitken et al., 1989). Excessive generation of ROS in semen by leukocytes as well as by abnormal spermatozoa could be a cause of infertility (Sharma and Agarwal, 1996). Virtually every human ejaculate is contaminated with potential sources of ROS such as leukocytes and abnormal spermatozoa. It follows that some spermatozoa will incur oxidative damage and a concomitant loss of function. The impact of ROS on male fertility is, therefore, a question of degree rather than the presence or absence of the pathology (Agarwal et al., 2003). Gomez et al., (1998) demonstrated that levels of ROS produced by spermatozoa were negatively correlated with the quality of sperm in the original semen. However, pathological levels of ROS detected in the semen of infertile men are more likely to be a result of increased ROS production rather than reduced antioxidant capacity of the seminal plasma (Zini et al., 1993).

**Damage to Lipids and Proteins**

A free radical prefers to steal electrons from the lipid membrane of a cell, initiating a free radical attack on the cell known as lipid peroxidation. Polyunsaturated fatty acids (PUFAs) are abundant in cellular membranes and in Low-Density Lipoproteins (LDL) as reported by Dekkers et al., (1996). Spermatozoa are vulnerable to ROS because their plasma membrane and cytoplasm contain large amounts of polyunsaturated fatty acids (Alvarez and Storey, 1995). The PUFAs allow for fluidity of cellular membranes. ROS targets the carbon-carbon double bond of PUFA. Peroxidation of PUFAs in sperm cell membranes is an autocatalytic, self-propagating reaction, which can give rise to cell dysfunction associated with loss of membrane function and integrity. Hydrogen peroxide is the major ROS producer in human spermatozoa. Moderately-elevated concentrations of hydrogen peroxide do not affect sperm viability but cause sperm immobilization, mostly via depletion of intracellular adenosine-triphosphate (ATP) and the subsequent decrease in the phosphorylation of axonemal proteins (Kemal Duru et al., 2000; Misro et al., 2004).
High concentrations of hydrogen peroxide induce lipid peroxidation and result in cell death.

**DNA damage and Apoptosis**

Oxidative damage can cause base degradation, DNA fragmentation and cross-linking of proteins (Sharma et al., 2004). Spermatozoa with damaged DNA lose their ability to fertilize the oocyte. A study by Sun et al., (1997) found a negative correlation between the percentage of spermatozoa with damaged DNA and the fertilization rate. Apoptosis, or programmed cell death, is a physiological phenomenon characterized by cellular morphological and biochemical alterations that cause a cell to die. Apoptosis appears to be strictly regulated by extrinsic and intrinsic factors and can be triggered by a wide variety of stimuli. Examples of extrinsic stimuli that are potentially important in testicular cell apoptosis are irradiation, chemotherapy and toxin exposure.

Mitochondria play a key role in the mechanism of apoptosis. The integrity of mitochondria is established by the presence of cytochrome C in the inner membrane space. High levels of ROS disrupt the inner and outer mitochondrial membranes. This results in the release of cytochrome C protein from the mitochondria, which activates the caspases and induces apoptosis (Wang et al., 2003). Studies in infertile men showed that high levels of cytochrome C in seminal plasma indicate significant mitochondrial damage by ROS. Considerable evidence suggests that disruption of mitochondrial functions (e.g., loss of transmembrane potential, permeability transition and release of cytochrome C leading to impaired electron transport) are important events in many apoptotic cell deaths (Yang et al., 1997). Apoptosis in sperm may also be initiated by ROS independent pathways involving the cell surface receptor called Fas or CD 95. Fas is a type I membrane protein that mediates apoptosis. When Fas ligand or anti-Fas antibody bind to Fas, apoptosis occurs (Lee et al., 1999). Moustafa et al., (2004) determined that infertile patients had high ROS levels in their seminal plasma and a higher percentage of apoptosis than normal healthy donors. A positive relationship between increased sperm damage by ROS and higher levels of the pro-apoptotic molecules such as cytochrome C, caspase 9 and caspase 3 was also reported in patients compared with healthy donors, indicating mitochondrial injury, increased apoptosis and ultimately, DNA damage (Wang et al., 2003).
Antioxidants

Since ROS has both physiological and pathological roles, an array of antioxidants maintains a steady state of ROS in the seminal plasma. Antioxidants act as free radical scavengers to protect spermatozoa against ROS. These antioxidants are superoxide dismutase (SOD), catalase and glutathione peroxidase (GPX). In addition, semen contains a variety of non-enzymatic antioxidant molecules such as vitamin C, vitamin E, pyruvate, glutathione and carnitine (Agarwal et al., 2004). These antioxidants compensate for the loss of sperm cytoplasmic enzymes as the cytoplasm is extruded during spermiogenesis, which, in turn, diminishes endogenous repair mechanisms and enzymatic defenses (Aitken et al., 1989). Recently, Gharagozloo and Aitken (2011) has made an extensive review of the results presented in published clinical trials conducted to evaluate the overall impact of oral antioxidants on measures of sperm oxidative stress and DNA damage. They further suggested the requirement of adequately powered, placebo-controlled comprehensive clinical trials to establish a clear role for antioxidants in the prevention of OS in the male germ line, such that the clinical utility of the oral antioxidant therapy becomes established once and for all.

Enzymatic and Non-enzymatic Antioxidants

Superoxide dismutase (SOD) scavenges both extracellular and intracellular superoxide anion and prevents lipid peroxidation of the plasma membrane. In order to act against H$_2$O$_2$, it must be conjugated with catalase or glutathione peroxidase (Jeulin et al., 1989). SOD also prevents premature hyperactivation and capacitation induced by superoxide radicals before ejaculation (Lamirande and Gagnon, 1995). This system forms an excellent protection against lipid peroxidation of plasma membrane of spermatozoa. It scavenges lipid peroxides thereby arresting the progressive chain reaction of lipid peroxidation. It also scavenges H$_2$O$_2$, which is responsible for the initiation of lipid peroxidation. Glutathione reductase (GRD) stimulates the reduction of glutathione disulfide (GSSG) to reduced glutathione (GSH). This ensures a steady supply of the reductive substrate NADPH to GPX. Glucose-6-phosphate dehydrogenase (G6PD) is required for the conversion of NADP$^+$ to its reduced form, NADPH. Catalase detoxifies both intracellular and extracellular H$_2$O$_2$ to water and oxygen (Baker et al., 1996). In addition, catalase activates nitrous oxide (NO$^-$) induced sperm capacitation, which is a complex mechanism involving H$_2$O$_2$ (Lamirande et al., 1997).
In vitro studies show that vitamin E is a major chain breaking antioxidant in the sperm membrane and it appears to have a dose-dependent protective effect (Hull et al., 2000). Cryopreservation and thawing procedures are associated with significant reduction in sperm motility induced by OS and these effects can be avoided by adding vitamin E to cryoprotectants. In a randomized, double-blind controlled trial, asthenozoospermic patients received oral vitamin E which significantly decreased the MDA (marker for lipid peroxidation) concentration in spermatozoa and improved sperm motility (Suleiman et al., 1996). Vitamin E and selenium supplementation lead to a significant decrease in MDA concentrations and improved sperm motility (Keskes-Ammar et al., 2003). Disparate results in sperm quality and quantity were observed in those reports using doses of vitamin E below 400 mg (Geva et al., 1996). In contrast, patients taking vitamin B showed no change in sperm motility, but a small decrease in the MDA concentration was observed. Selenium could potentially protect against oxidative DNA damage in human sperms, but the experience with this trace element is scarce (Lerda, 1992).

Vitamin C (ascorbate) is another important chain breaking antioxidant contributing up to 65% of the antioxidant capacity of the seminal plasma. Vitamin C concentration in seminal plasma exceeds 10 times more than that in blood plasma (364 compared with 40 μM/L) as reported by Lewis et al., (1997). A dose-dependent effect of vitamin C on sperm motility has been demonstrated. At a dose of 1,000 μg/L, vitamin C positively influenced the motility of the spermatozoa, however, above that level, vitamin C reduced the motility. On the other hand, doses under 200 mg of vitamin C did not provide any benefit (Abel et al., 1982). Vitamin C has been shown to improve sperm quality in smokers. Dawson et al., (1992) recruited 75 men (20 - 35 years) who were randomly divided into one of three supplementation groups – placebo, 200 mg and 1,000 mg vitamin C. They observed no improvement in sperm quality in the placebo group, while the groups receiving vitamin C showed improvement in sperm quality, with the highest improvement in the 1,000 mg group. Fraga et al., (1991) studied in two groups, the oxidative damage to DNA in relation to the seminal fluid vitamin C levels in the semen samples provided by healthy donors. In a group of 24 individuals, high levels of 8-OHdG were correlated with low seminal vitamin C. The second group of individuals was maintained on a controlled diet that varied only in vitamin C content. When dietary vitamin C was decreased, vitamin C in seminal fluid decreased by half and the level of 8-hydroxydeoxyguanosine (8-OhdG)
in sperm DNA increased by 91%. Repletion of dietary vitamin C for 28 days caused a doubling in seminal vitamin C and reduced 8-OHdG by 36%. This indicates that dietary supplementation protects human sperm from endogenous oxidative DNA damage, thereby decreasing the risk of genetic defects, particularly in populations with low vitamin C levels, such as smokers (Fraga et al., 1991).

Glutathione is the most abundant non-thiol protein in mammalian cells (Irvine, 1996). Its deficiency can lead to instability of the mid-piece resulting in defective motility (Hansen and Deguchi, 1996; Ursini et al., 1999). It protects plasma membrane from lipid peroxidation, scavenges superoxide and prevents O$_2^-$ formation. In a study of infertile men with unilateral varicocele or genital tract inflammation, glutathione led to significant improvement in sperm quality (Lenzi et al., 1994; 2004). Molecules such as N-acetyl L-cysteine, carotenoids, coenzyme Q10 and carnitines provide excellent antioxidant support. N-acetyl L-cysteine is a precursor of glutathione that improves sperm motility and reduces ROS-induced DNA damage (Oeda et al., 1997; Lopes et al., 1998). Carotenoids play an important role in protecting the cells and organisms by scavenging the superoxide radicals (Sies and Stahl, 1995). Co-enzyme Q10 protects lipids against peroxidative damage (Frei et al., 1990). It scavenges superoxide anion as well as peroxides. Carnitine promotes membrane stability and plays an important role in sperm maturation and development (Agarwal and Said, 2004).

**LIFESTYLE FACTORS**

**Tobacco Smoking/Chewing**

Tobacco is one of the most addictive substances. Tobacco smoke contains approximately 4,000 compounds such as alkaloids, nitrosamines and inorganic molecules and many of these substances are reactive oxygen or nitrogen species (Carrasquedo and Fraga, 1996). Early initiation of tobacco use by adolescents is a major public health concern. Globally, nearly 5 million people die every year from tobacco-related illnesses, with disproportionately higher mortality occurring in developing countries (Peto and Lopez, 2001). By 2030, it is estimated that 10 million people per year will die from tobacco use, with 70% of these will occur in developing countries (Stewart and Kleihues, 2003). Tobacco chewing is more common in south east Asian region while smoking is more prevalent in western countries.
Several studies suggested role of tobacco smoking in deterioration of seminal quality. Reina et al., (2007) demonstrated alterations in sperm concentration and morphology with an elevation of immature forms, in men with idiopathic infertility having habit of tobacco smoking. Smoking more than 20 cigarettes a day or longer than 10 years were shown to have deleterious effects on the semen volume, sperm motility and morphology as well as to decrease sperm DNA integrity and nuclear maturation (Niu et al., 2010). Lower sperm penetration assay scores and greater number of leukocytes in the seminal fluid were also noticed among smokers (Close et al., 1990, Saleh et al., 2002a). Smokers had decreased results of hypo osmotic swelling test and increased concentration of leukocytes than non-smokers among infertile subjects whereas no differences were found in sperm concentration, percentage normal forms, different sperm defects, induced acrosomal reaction and chromatin damage by aniline blue staining test. Thus, cigarette smoking deteriorates semen quality, which could worsen fertilizing capability in infertile men (Taszarek et al., 2005). It has also demonstrated that smokers can suffer from some degree of impotence or reduction in their sexual frequency (Zavos and Zavos, 1999). Poor semen quality was also observed in men with a prenatal exposure to tobacco smoke (Ramlau-Hansen et al., 2007a). Further, tobacco smoking significantly lowered zinc levels, necessary for the stability of the sperm chromatin, in the ejaculates of smokers than in non-smokers (Oldereid et al., 1994).

Shen et al., (1997) suggested that cigarette smoking enhances the extent of DNA damage in sperm. The extent of oxidative damage among smokers was associated with decrease in the antioxidant defenses in the sperm of infertile males (Kiziler et al., 2007; Pasqualotto et al., 2008). Chohan and Badawy (2010) showed that the rate of sperm respiration to be significantly lower in smokers and further suggested that the negative impact of cigarette smoking on sperm aerobic metabolism might explain the lower rate of fertility in smokers. A study by Saleh et al., (2002b) demonstrated that smoking increases ROS levels and decreases seminal antioxidants. Cigarette smoking may also increase the risk of aneuploidy for certain chromosomes and those men may have different susceptibilities to aneuploidy in germ cells (Shi et al., 2001). A significant positive association has been found between active smoking and sperm DNA fragmentation (Sun et al., 1997) as well as axonemal damage (Zavos et al., 1998). Smoking is also associated with a decreased sperm count (Vine et al., 1996). Fraga et al., (1996) found the level of 8-
OHdG – a marker of DNA fragmentation to be 50% higher in smokers compared with non-smokers

Tobacco smoking is reported to alter various sex hormones. Serum estradiol (E2) and prolactin were increased in smokers as compared to non-smokers (Attia et al., 1989). It is believed that smoking affects spermatogenesis by increasing the production of norepinephrine, which increases the conversion of testosterone to estrogen causing decreased testosterone levels (Pasqualotto et al., 2004). In a recent study, serum follicle stimulating hormone (FSH) was higher among non-smokers as compared to smokers whereas no significant differences were found for inhibin B, testosterone, sex hormone-binding globulin (SHBG), luteinizing hormone (LH) and estradiol (Richthoff et al., 2008). However, earlier a positive dose–response relationship between smoking and testosterone, LH and the LH/free testosterone ratio was observed (Ramlau-Hansen et al., 2007b). Various studies reported incompatible findings on the effects of smoking on serum hormones. There may be some effect of tobacco smoking on testosterone level as Kapoor and Jones (2005) hypothesized that the effects of smoking on testosterone levels are due to changes in plasma-binding capacity rather than a direct effect of nicotine on androgens. Recently it has been noticed that smoking in patients undergoing IVF (in vitro fertilization) and GIFT (gamete intra-fallopian transfer) can have negative impact on treatment outcome (Klonoff-Cohen et al., 2001). The data on tobacco chewing and semen quality are scanty. A recent report indicated that the percentage of men with azoospermia and oligoasthenoteratozoospermia rose with the level of addiction to chewing tobacco (Said et al., 2005). The data suggest the role of tobacco smoking and chewing in deteriorating semen quality or even might have effect in IVF outcome.

Alcohol

Humans have been consuming alcoholic beverages since pre-historic times, for a variety of reasons. Alcohol is the second most addictive substance after nicotine. Alcohol is reported as direct testicular and Leydig cell toxin (Van Thiel et al., 1975; Lipsett, 1980). It has been observed that chronic alcohol use was common among infertile men (Tsujimura et al., 2004). Excessive alcohol consumption has the potential to decrease an already low percentage of sperm with normal morphology (Goverde et al., 1995; Guo et al., 2006). Martini et al., (2004) also found significant reduction in seminal volume, sperm concentration, percentage of motile spermatozoa
and a significant increase of the non-motile viable gametes among men with habits of alcohol and smoking (Vine et al., 1997). In alcohol users, higher numbers of leukocytes were also found in the seminal fluid with respect to nonusers (Close et al., 1990). Progressive deterioration in semen quality has shown to be related to increasing quantity of alcohol intake and cigarettes smoked. Further heavy alcoholics and smokers showed asthenozoospermia, teratozoospermia as well as oligozoospermia (Gaur et al., 2010). Alcohol use affects the hypothalamic-pituitary-gonadal (HPG) axis, a system of endocrine glands and hormones. Alcohol use is associated with low testosterone and altered levels of FSH and LH and hence can interfere with hormone production (Emanuele and Emanuele, 1998). In alcoholics, FSH, LH and estradiol (E2) levels significantly increased, whereas testosterone, semen volume, sperm count, motility and number of morphologically normal sperm significantly decreased (Muthusami and Chinnaswamy, 2005). But studies among healthy male volunteers with habituation of alcohol showed no significant effect on sperm nuclear size, shape, or chromatin texture and sperm concentration, motility, viability and normal morphology (Vine et al., 1997; Stutz et al., 2004). However, majority of studies reported adverse effects of alcohol use on semen quality.

**Age**

The societal trend to have children at mature ages raises public health concern about age-associated risks of abnormal pregnancies and outcomes. It is well known that female fecundity declines precipitously by the fourth decade of life due to oocyte loss and that older mothers have increased risks of miscarriage, trisomies and chromosomal defective offspring (Lansac, 1995). Male fecundity also seems to decline with age, although spermatogenesis continues well into male senescence and some men of advancing age can also father children (Kidd et al., 2001; Sloter et al., 2004). However, the risks of abnormal pregnancies and heritable effects associated with advancing paternal age are poorly understood. There is suggestive epidemiological evidence that the incidence of abnormal reproductive outcomes and heritable defects increases with paternal age (Tarn et al., 1998; de la Rochebrochard and Thonneau, 2002), including pregnancy loss (Risch et al., 1987; de la Rochebrochard and Thonneau, 2002), developmental and morphological birth defects (Lian et al., 1986), gene mutations (Crow, 2000; Tiemann-Boege et al., 2002), various aneuploidy and chromosomal syndromes (Sloter et al., 2004) and diseases of complex aetiology such as prostate cancer (Zhang et al., 1999).
However, the epidemiological studies of abnormal reproductive outcomes require large number of pregnancies and have inherent difficulties in distinguishing between the impact of maternal and paternal age. New methods for measuring genetic and chromosomal defects in human sperm provide more direct approaches in identifying paternal risk factors (Wyrobek et al., 2005a,b) and growing evidence links sperm DNA damage with the risks of developmental defects and mutations in the offspring, including childhood cancer and infertility (Aitken et al., 2003). Advancing male age has been associated with increased frequencies of certain genetic and chromosomal defects in sperm (Crow, 2000, Shi and Martin, 2000; Tiemann-Boege et al., 2002, Bosch et al., 2003, Sloter et al., 2004), but there remains unexplained differences in age dependencies among the major categories of sperm damage (Sloter et al., 2004).

Older men seem to produce more sperm with mutations associated with achondroplasia and Apert syndrome (Tiemann-Boege et al., 2002, Glaser et al., 2003; Wyrobek et al., 2006) and with certain types of sperm DNA damage measured by the Comet and the sperm chromatin structure assay (SCSA) analyses (Morris, 2002; Singh et al., 2003; Wyrobek et al., 2006), but not more aneuploid sperm (Wyrobek et al., 2006). Moskovtsev et al., (2006) demonstrated that the rate of sperm with fragmented DNA doubled in 45 years and older men compared to those less than 30 years old. Increased necrosis, DNA damage and apoptosis while rapid progression and total motility declined with advancing male age beginning as early as 35 years of age (Siddighi et al., 2007). Plastira et al., (2007) demonstrated that increased age in infertile patients was 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.

**Obesity and Nutritional factors**

High caloric foods, sedentary work, no exercise, easy transportation along with increasing use of modern technologies that reduce the need for physical activity can explain the increasing prevalence of obesity around the world. Obesity is a growing concern worldwide. The prevalence of obesity has risen dramatically in developed countries over the past 2 to 3 decades (Oken and Gillman, 2003). Obesity has reached epidemic proportions in the United States with more than 20% of adults defined as clinically obese and an additional 30% defined as overweight (Heindel,
Obesity is a condition in which excess body fat has accumulated and has been associated with an increased risk of many serious illnesses such as cardiovascular diseases, diabetes mellitus and some types of cancer (Visscher and Seidell, 2001). It is gradually recognizing that obesity is one of the causes of subfertility.

Obesity has been associated with reproductive disorders in women, including menstrual abnormality, infertility, miscarriage and reduced success of assisted reproduction (Norman and Clark, 1998; Pasquali et al., 2003). Its role in male reproduction has also been documented in recent years. A significant reduction in sperm concentration and motility was observed in the obese group than in males with BMI < 30 kg/m². Further, in the obese group, sperm count continuously decreased with aging. In addition, men presenting with a BMI > 25 kg/m² have fewer chromatin-intact, normal-motile sperm cells per ejaculate (Koloszar et al., 2005, Kort et al., 2006). Obesity was also linked with disturbances of penile hemodynamics and found to be an independent clinical factor for vasculogenic erectile dysfunction (Zohdy et al., 2007). But in another study the incidence of erectile dysfunction did not vary across BMI categories when corrected for potential contributing factors (Hammoud et al., 2008). Chavarro et al., (2010) observed significant differences between body weight and reproductive hormone levels but only obesity was associated with increased sperm DNA damage and only the most obese men (BMI ≥ 35 kg/m²) were having lower sperm count. They further suggested that differences in reproductive hormone levels due to increased body weight do not necessarily lead to impaired reproductive potential in men.

The exact mechanism of obesity-mediated effect on reproduction is not fully understood. However, overweight and obese men were found to have a markedly changed sex hormone profile along with reduced semen quality. Serum testosterone, SHBG and inhibin B decreased while free androgen index and estradiol increased with increasing BMI (Jensen et al., 2004; Osuna et al., 2008; Aggerholm et al., 2008). Earlier Strain et al., (1982) and Haffner et al., (1993) also observed that the plasma levels of SHBG, total testosterone, free testosterone and FSH were lowered in obese men compared to non-obese men. Decline of androgen levels (Coviello et al., 2004; Jarow and Zirkin, 2005; Osuna et al., 2006; Aggerholm et al., 2008), increased estrogen levels (Goyal et al., 2003) and suppression of the hypothalamic-pituitary-
testicular axis (Giagulli et al., 1994) have been suggested as potential aetiologies of altered spermatogenesis in obese males (Hammoud et al., 2006) based on experimental and clinical studies. Infertility in overweight/obese males has also been explained by leptin insensitivity, which may be due to decreased hypothalamic Kiss 1 expression, a potential regulator of GnRH/LH/FSH release, thus providing a bridge between metabolic regulation and fertility (Teerds et al., 2011).

In addition to obesity, some dietary habits of people from different ethnic background may have some effect on reproductive health (Chavarro et al., 2008). They suggested that higher intake of soy foods and soy isoflavones is associated with lower sperm concentration. Thus, consumption of certain food items might have some risk associated with reproductive dysfunction. However, more studies are needed on this aspect.

**Stress**

Emotional and physical changes and environmental components may lead to stress. Infertility itself is the most stressful event for the person diagnosed with this condition. In general, stress affects biological systems leading to various impairments and might affect reproductive health. In many instances, stress has a subtle and less influence. Podolska and Bidzan (2011) mentioned that psychological causes are often the primary factors but sometimes they are secondary derivatives of the therapeutic process and a wide scope of factors must be considered to attempt psychological analysis of patients treated for infertility. Negro-Vilar (1993) in an overview mentioned that chronic or severe stress in animals or humans was associated with decrease in sperm count, motility and morphology. In couples suffering from involuntary childlessness, a higher frequency of male sexual disturbances expressed as erectile dysfunction, ejaculatory disorders, loss of libido and a decrease in the frequency of intercourse were observed (Lenzi et al., 2003). Fecundity of men experiencing the stress of a family member's death was found to be temporarily diminished (Fenster et al., 1997). Mental stress caused by final exams negatively affected semen quality during stress period compared to the non-stress period (Eskiocak et al., 2005). Physical stress leads to low testosterone levels due to a reduction in LH pulse frequency (Aono et al., 1972). A few investigators found no or very less adverse effects on reproduction. Psychological job strain does not seem to affect male reproductive function (Fenster et al., 1997; Hjollund et al., 2004). A
prospective study showed small or nonexistent effect of a man's daily life psychological stress on his semen quality. Moreover, no consistent association between stress and serum concentration of LH, FSH, inhibin B, testosterone, or estradiol was found (Hjollund et al., 2004). However, the data available points some adverse effect of stress on male reproductive function or as an additional risk factor for subfertility and this might depend upon the types of stress.

Heat

Temperature influences the development of germ cells as well as reproductive cycle of living beings. Nature has kept the scrotum outside the body cavity so that the temperature of the testes remains lower than that of the body temperature. Even moderate or physiological elevation in scrotal skin temperature is associated with a substantially reduced sperm concentration, which results in a poor semen quality (Hjollund et al., 2000). Lahdetie (1995) reported that active sperm production is dependent on an environment that is 4°C lower than the normal body temperature. Wang et al., (1997) reported that elevation of testicular temperature by 1°C above the base line depresses spermatogenesis by 14% and thereby decreases sperm output. They also mentioned that exposure to high temperature results in modification of sperm morphology. The mean value of sperms with abnormal morphology rises from 30 to 60% within 6-8 months of exposure to high temperature. They explained that elevated testiculooepididymal temperature decreases the synthesis of sperm membrane coating protein, which in turn results in the elevation of morphologically abnormal sperms in the ejaculate (Wang et al., 1997).

Recently, Dada et al., (2001, 2003) suggested that exposure to high temperature causes deterioration in sperm morphology and impairs motility as well as sperm production that has resulted into a deleterious effect on male fertility. Heat, either due to endogenous (such as high fevers) or exogenous stimuli, decreases sperm concentration, impairs motility and reduces the number of morphologically normal sperm (Saikhun et al., 1996; Carlsen et al., 2003). The data on occupational health exposure and male fertility was reviewed by Thonneau et al., (1998). They mentioned that the rise of testicular temperature induced by cryptorchidism, extreme heat in summer, body fever, tight clothing, sauna or exposure to high temperature during occupational exposure can cause impairments in spermatogenesis. The toxic effect of wet hyperthermia on semen quality may be reversible in some infertile men.
observed by Shefi et al., (2007) Further, Zorgniotti and Mac Leod (1973) have reported an improvement of sperm alterations in men wearing a cooling device inducing a chronic hypothermia of the testis.

There are few reports on reproductive health of workers occupationally exposed to high temperature. Figa-Talmanca et al., (1992) mentioned a higher prevalence of pathologic sperm profile among the exposed subjects of ceramic industry compared to control. Bonde (1990) studied metal arc alloyed steel welders with a moderate exposure to radiant heat but without substantial exposure to welding fumes toxicants, experienced a reversible decrease in semen quality. Sperm morphology also deteriorated during six weeks of exposure and increased after a break in the exposure (Bonde, 1990). Kumar et al., (2003) also mentioned that welding work might have some adverse effects on sperm motility, morphology and physiologic function even though sperm concentration was in the normal range. Further, based on experimental studies, it is reported that elevation in abdominal temperature increases the risk of apoptosis in spermatogenic cells (Shikone et al., 1994, Yin et al., 1997). These data suggests the possible role of temperature on the deterioration of male reproductive function.

Drugs

Chronic medication can play a significant role in the pathogenesis of male reproductive health. It is known that some of the drugs/compounds may reach to the seminal plasma. There are evidence that many drugs enter the male genitourinary tract by an ion-trapping process. Lipid solubility and the degree of ionisation of the drug, which depend on the pH of plasma and seminal fluid, are important factors in this process (Pichini et al., 1994). Major groups of drugs that may affect male sexual function include drugs of abuse, central nervous system depressants, antihypertensives, anticholinergics, psychotherapeutics agents (Wilson, 1991). Narcotic drugs exert their primary effect on the hypothalamic-pituitary axis and their secondary effects on the gonads and accessory sex organs. Narcotics decrease gonadotropin secretion and stimulate prolactin secretion, both of which are inhibitory to male sexual function (Smith, 1982). Marijuana, most widely used psychoactive cannabis drug, the primary effect is at the level of the hypothalamus, with subsequent effects on gonadotropins and testosterone (Smith, 1982) and is reported as a gonadal toxin (Fody and Walker, 1985). Infertile couples with habituation of
marijuana showed greater number of leukocytes in their seminal fluid without any effect on sperm count, motility or percentage of oval sperm (Saleh et al., 2002a). Further, delta-9-tetrahydrocannabinol (THC) a recreational cannabis drug reduced the percentage of progressive sperm motility and acrosome reactions in vitro (Whan et al., 2006).

A significant negative correlation was also found between the duration of khat (Catha edulis) consumption, another drug of abuse and semen parameters. Deformed heads showed aberrated nuclei with immature nuclear chromatin and polymorphic intranuclear inclusions; these were associated with acrosomal defects. Persistent cytoplasmic droplets were also frequently observed (El-Shoura et al., 1995). Bracken et al., (1990) mentioned that high prevalence of cocaine use among male population was associated with subfertility with a decrease in sperm count and motility. Yelian et al., (1994) demonstrated human spermatozoa acutely exposed to high concentrations of cocaine had decreased two motion kinematics, straight line velocity and linearity of sperm but had no significant effects on sperm motility and fertilizing capability. In another study, exposure of males to cocaine does not decrease viability and motility but has been linked to abnormal development of their offspring as the sperm may act as a vector to transport cocaine into an ovum (Yazigi et al., 1991; Cone et al., 1996)

Among heroin addicts, an elevation in circulating total thyroxine, triiodothyronine and prolactin level (Chan et al., 1979) while depletion in serum concentrations of testosterone, FSH and cortisol were observed (Malik et al., 1992; Rasheed and Tareen, 1995). Semen analyses of heroin addicts and from the dual heroin-methadone users were abnormal. In all cases asthenospermia was one of the abnormalities whereas 24% showed teratospermia and hypospermia and 17% showed oligozoospermia. Such seminal pathology, might be an early indication of heroin toxicity to the male reproductive tract (Ragni et al., 1988). The available data on various drugs of abuse suggests that they might have adverse effect on semen quality.

**Radiation/Electromagnetic waves**

Radiation can be classified as ionizing or non-ionizing radiation. It is an established fact that ionizing radiation affects both male as well as female
reproduction. Non-ionizing radiation refers to any type of electromagnetic radiation i.e., near ultraviolet, visible light, infrared, microwave, radio waves, low frequency (radio-frequency) and static fields. There has been growing public concern on the effects of electromagnetic radiation (EMR) on human health including possible association with increased risk of cancer, effects on cellular DNA and reproduction. A number of experimental studies showed that electromagnetic waves have a wide range of damaging effects on the male reproductive system and sperm parameters. However, similar studies are limited in humans (Derias et al., 2006). Use of cell phones is reported to be associated with deterioration in semen quality by decreasing the sperm count, motility, viability and normal morphology as the duration of daily exposure to cell phones increases (Wdowiak et al., 2007, Agarwal et al., 2008). Very recently, Gutschi et al., (2011) showed that cell phone use negatively affects sperm quality in 2110 men attending the infertility clinic. EMR emitted by cellular phone significantly reduces human sperm motility (Fejes et al., 2005; Erogul et al., 2006). 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. Earlier, Agarwal et al., (2009) based on in vitro study, concluded that radio-frequency electromagnetic waves emitted from cell phone may lead to oxidative stress in human semen. Long-term EMR exposure may lead to behavioural or structural changes of the male germ cell that may be observed later in life (Erogul et al., 2006). Still more work is needed on the use of cell phone and fertility with better study design by incorporating confounding factors associated with fertility.

Military men exposed to high frequency EMR through aerials and communication equipment (self-reported) had significant linear trends with lower ratio of boys to girls at birth and higher prevalence of involuntary childlessness (Baste et al., 2008). Susa and Pavicic, (2007) reported that radio-frequency fields could interact with charged intracellular macromolecular structures and could affect the mammalian reproductive system and sperm cells whereas in a population-based studies of a wide range of radio-frequencies from occupational or residential exposures, no strong associations on birth defects, fertility, neuroblastoma in offspring and reproductive hormones were found (Jauchem, 2008).
ENVIRONMENTAL FACTORS

A huge number of environmental chemicals such as metals, pesticides, solvents etc. have the potential of causing adverse effects on the functioning of endocrine system that might results in an adverse health effects to the progeny and some of them might be harmful to the reproductive health. These chemicals and their byproducts enter into the environment due to industrial revolution.

Metals

A large number of workers are exposed to metals during their occupation. General population is also exposed to different metals and their oxides through the environment. All the living beings including human are exposed to lead to some extent due to contaminated air, water and food. Lead (Pb) and cadmium (Cd) are highly toxic metals in humans and other mammals. All the stages of spermatogenesis would be the potent target of chronic exposure to even very low doses of toxic heavy metals.

Workers are exposed to high doses of lead in various occupations. Earlier, vehicular exhaust used to be a major source of lead exposure for the general population, but nowadays due to the use of unleaded gasoline, the problem has subsided to some extent. However, leaded petrol is still in use in many of the underdeveloped areas of the world. A number of experimental studies are available on the effects of lead on various animal species of both sexes. Epidemiological and case studies are available which indicated that occupational exposures to lead have adverse effects on human reproduction. A study on workers of a newspaper printing press indicated that the average sperm counts were significantly lower and lesser proportion of them were found to be motile in the exposed subjects as compared to control. These changes were associated with the level of lead in blood (Roychowdhury et al., 1986). In a review on male reproductive toxicity of lead in animals and humans, the authors reported that human studies focused mainly on semen quality, endocrine function and birth rates in occupationally exposed subjects. These human studies showed that exposure to inorganic lead greater than 40 µg/dl in blood impaired male reproductive function by reducing sperm count, volume and density or changing sperm motility and morphology. However, no relevant effects were detected on endocrine profile (Apostoli et al., 1998). Moderate exposure to Pb
(Blood Pb < 40 μg/dl) and Cd (Blood Cd < 10 μg/l) could significantly reduce human semen quality without conclusive evidence of impairment of male reproductive endocrine function has been reported by Telisman et al., (2000). Later, Bonde et al., (2002) reported the adverse effects of lead on sperm concentration and susceptibility to acid induced denaturation of sperm chromatin are unlikely at blood lead concentrations below 45 μg/dl. Very recently, mean lead levels as high as 48.5 μg/dl have been reported among traffic police officers in Peru where leaded gasoline was used. Authors found that sperm motility and viability differed significantly between the < 40 μg/dl and ≥ 40 μg/dl categories and decrease in sperm motility and viability with increasing Pb in simple linear regression (Eibensteiner et al., 2005). In addition, on the basis of a cross sectional study among men employed at a lead smelter unit, Alexander et al., (1998) concluded that blood lead concentrations below the currently accepted workers protection criteria seem to adversely affect spermatogenesis.

Painters and construction workers occupationally exposed to lead and solvents are reported to have adverse effects on both male and female reproduction (Daniell and Vaughan 1988). Battery workers exposed to lead indicated a significant decrease in fertility (Gennart et al., 1992) and significant levels of asthenospermia and teratospermia (Lerda, 1992). Mehta and Anandkumar (1997) reported a decline in sperm count in a study of population from Bangalore, India and they have correlated this decline with the changes in various pollution indices such as suspended particulate matter, sulphur dioxide and lead. The effect of lead on semen quality was investigated in a study of 85 tollgate workers and the same number of age-matched men living in the same area. The tollgate workers were exposed to traffic pollution and in turn increased quantities of lead and they had poorer semen parameters (De Rosa et al., 2003). Seminal plasma lead levels and artificial insemination cycle fecundity are strongly and negatively correlated (Benoff et al., 2003a; b). Recently, it has been shown that the presence of lead and cadmium in the reproductive tract of men might be related to a moderate alteration of their seminal parameters (Mendiola et al., 2011). In addition to these, lead has been reported to cause oxidative cellular damage in reproductive tissues of adult male rats, which may be closely associated with the ROS production (Marchlewicz et al., 2004). Earlier, Agarwal et al., (2003) mentioned that ROS plays an essential role in the pathogenesis of many reproductive processes. In an animal study, lead increased ROS production, reduced sperm motility and sperm oocyte penetration rate and
decreased seminal antioxidants. Lead levels in the semen have been found to have a significant but weak positive correlation between degradation of DNA bases as measured by 8-OHdG (Xu et al., 2003). These heavy metals can induce OS through their capacity to interact with ROS, increasing their oxidant activity or by affecting membrane integrity (Oteiza et al., 2004).

Cadmium exposure can occur through contaminated air, water, food and might also through tobacco smoke. Cadmium can reach into the body by ingestion with food, such as fish and rice, from areas with contaminated groundwater. Food and cigarette smoke are the biggest sources of cadmium exposure for the general population (ATSDR, 1998). Effects of cadmium on the testis have been reviewed by Gunn & Gould (1970). The experimental studies available point that cadmium causes testicular necrosis in several animal species, although there is scanty data on its possible effects in humans. Semen cadmium levels have been correlated with sperm motility and curvilinear velocity (Noack-Fuller et al., 1993). Significant correlations have been reported between blood cadmium levels and volume of semen, midpiece defects and immature forms of spermatozoa (Chia et al., 1992). Xu et al., (1993) reported a significant inverse correlation between blood cadmium level and sperm density among oligospermic men and between seminal plasma cadmium and semen volume among men without known occupational exposure to cadmium. Further, higher levels of cadmium have been reported in infertile men compared to fertile men by Saaranen et al., (1987). A significant inverse correlation was found between Cd and sperm density, sperm number per ejaculate in a study carried out in China among non-smokers, which indicated that Cd in seminal plasma could affect semen quality and oxidative DNA damage in human spermatozoa (Xu et al., 2003). However, no significant correlation between seminal cadmium concentrations and conventional semen parameters or between cadmium concentration and the fertility status of the patients was observed by Keck et al., (1995).

Cadmium is also associated with deleterious effects on the gonadal function and with changes in the secretory pattern of other pituitary hormones like prolactin, adreno cortico trophic hormone (ACTH), growth hormone (GH) or thyroid stimulating hormone (TSH). The available data indicates the existence of a disruption in the regulatory mechanisms of the hypothalamic-pituitary axis by cadmium (Lafuente et al., 1999). However, Mason (1990) found no difference between cadmium-exposed
In a manufacturing plant and unexposed controls in serum testosterone, FSH and LH levels. Further, a recent report has highlighted the potential of Cd to mimic the effects of estrogen in various tissues (Henson and Chedrese, 2004). Based on the most of the data available, one can consider the reproductive toxic potential of cadmium.

In addition to lead and cadmium, other toxic metals such as chromium, mercury, arsenic, etc. had also reported to be a potent cause of deterioration to the male reproductive health.

**Trace Elements**

Trace elements are required in small concentration as essential components of biological enzyme systems or of structural portions of biologically active constituents. These trace elements include iron, iodine, fluorine, copper, zinc, chromium, cobalt, molybdenum, selenium, tin, vanadium, nickel and silicon. Some of these are toxic at high doses to reproductive health and few are essential in trace amount for various physiological functions of the body.

Copper is an essential trace element found in its elemental form and also as a component of many minerals. Copper is essential for various physiological functions and it is also required by some cellular enzymes and other biomacro molecular components for their normal function. A large number of enzymes are copper containing proteins eg. cytochrome oxidase, erythrocyte superoxide dismutase, lysyl oxidase, skin tyrosine oxidase and diamine oxidase. The levels of copper in serum vary because of interaction with other metals such as zinc, molybdenum, cadmium, iron and calcium. Several elements viz. zinc and cadmium reduce copper absorption thereby reducing the serum/plasma copper levels Exposure to copper has been shown to be linked to a decreased sperm count and to be a cause of terato- and asthenozoospermia (Lahdetie, 1995). Significant correlations between seminal copper concentrations and sperm count, progressive motility and normal morphology have been reported (Jockenhovel et al., 1990). Similarly, blood plasma copper levels were also significantly correlated with sperm motility (Wong et al., 2001). Both Zn and Cu are linked with the structure/activity center of SOD, which is closely related to sperm motility. Decreases in the Zn and Cu levels will lead to the lowering of the superoxide dismutase (SOD) activity as reported by Zhang et al., (2000). In vitro
studies have also reported the toxic effect of copper on sperm motility. Incubation with the metal caused a fall in the percentage of motile sperm, which was directly related to the surface area of copper employed and to the copper content of whole semen. These changes were accompanied by a decrease in semen zinc levels and an uptake of copper by individual sperm cells (Battersby et al., 1982). The significance of copper in seminal plasma therefore becomes a matter of interest in order to understand the role of copper in human reproduction.

Most zinc ore found naturally in the environment is in the form of zinc sulfide. Zinc compounds are widely used in industry (ATSDR, 1994). Zinc is essential for DNA replication, RNA polymerases, protein synthesis and many metabolic processes. All cell replication, protein synthesis and growth processes are partially dependent upon zinc (EHC, 2001) and included in many nutritional supplements also (Sokas, 1998). Zinc is an essential element important for growth, the nervous system and especially the immune system (Wellinghausen and Rink, 1998). Zinc is an essential trace element for humans taking role in electron transfer in many enzymatic reactions (Gul et al., 2009). The adult human body contains 1–3 g of zinc, about 0.1% of which is replenished daily (Maret and Sandstead, 2006). Zinc is an essential food element needed by the body in small amounts. Too little zinc in the diet can lead to poor health, reproductive problems and lowered ability to resist disease. Too much zinc can also be harmful to health (ATSDR, 1994). Zinc is necessary for growth, sexual maturation and reproduction. Zinc in human semen seems to play an important role in the physiology of spermatozoa. Zinc in seminal plasma stabilizes the cell membrane and nuclear chromatin of spermatozoa. It may also have an antibacterial function (Lin et al., 2000). The zinc content of the prostate gland, the seminal fluid and ejaculated sperm are very high and testicular zinc is essential for spermatogenesis (Vallee and Falchuk, 1993). Kvist et al., (1988) found a positive relation between zinc in sperm nuclei and the resistance of the chromatin to decondense when exposed to a detergent. They observed that the infertile men had lower degree of sperm chromatin stability and lower sperm zinc content than the fertile donors. Low content of nuclear zinc would impair the structural stability of the chromatin and thereby increase the vulnerability of the male genome.

In an in vitro study using spermatozoa from fertile men, a high zinc concentration in the culture medium impaired both sperm motility and sperm
penetration of ZP-free hamster oocytes (Blazak and Overstreet, 1982; Riffo et al., 1992). Seminal Zn concentration has been recently correlated with sperm count and the duration of abstinence in subfertile men whereas in normozoospermic men, high seminal Zn concentration may have an adverse effect on the ZP-induced acrosome reaction (Liu et al., 2009) Earlier, a study has shown that the Zn level in seminal fluid and serum is not associated with silent male genital tract infection (indicated by seminal leukocytes); nor is it related to semen cultures in asymptomatic individuals. Further, they observed that Zn did not influence sperm capacity to penetrate cervical mucus in vitro or in vivo and did not affect subsequent fertility (Kruse et al., 2002).

There are conflicting reports regarding the levels of zinc in seminal plasma and different semen quality parameters. Seminal zinc level has been reported to be correlated with duration of abstinence (Liu et al., 2009), volume (Meeker et al., 2008), pH (Wong et al., 2001), sperm count (Madding et al., 1986), sperm morphology (Colagar et al., 2009) as well as sperm density, motility and viability (Chia et al., 2000). Fuse et al., (1999) reported a positive correlation of zinc with sperm count, motility and plasma testosterone concentration while there was no correlation with sperm morphology. Recently, Zinc had shown to be reduced in all infertile patients (Abdella et al., 2010) Ali et al., (2007) reported that zinc might contribute to fertility through its significant effects on various semen parameters. It seems that the estimation of seminal plasma zinc may help in investigation and treatment of infertile males. However, Carreras and Mendoza (1990) reported a negative correlation of zinc with sperm motility. A study had shown that men with high total zinc intake had 50% lower frequencies of disomy X than the moderate intake group and 39% lower frequencies than the low intake group. But, no consistent associations were found between antioxidant or zinc intakes and sperm aneuploidy in the study (Young et al., 2008) Xu et al., (1993) also reported a positive correlation between concentrations of selenium and zinc in seminal plasma (SeSP and ZnSP) and sperm density in normospermic men but not in oligozoospermic men. Wong et al., (2001) demonstrated weak correlations of blood plasma zinc concentrations with sperm count, motility and abnormal sperm morphology. Zinc and magnesium concentrations in seminal plasma correlated weakly with sperm count and copper concentrations in blood plasma with motility. Mankad et al., (2007) reported that the mean α-glucosidase activity was lowest among the azoospermic group with respect to oligozoospermic and normozoospermic groups. A significant positive correlation was
observed between zinc levels and sperm count and zinc and α-glucosidase activity in seminal plasma. Dissanayake et al., (2010) showed that count, motility, viability, pH and viscosity are affected by variations of seminal plasma zinc. Another study had shown that the high molecular weight zinc binding properties is good index of sperm function rather than the total seminal plasma zinc levels (Rasheed, 2009).

Seminal Zn levels had shown to be lower in the subfertile populations as compared to fertile group (Chia et al., 2000; Ebisch et al., 2006) whereas no difference has been observed by others (Umeyama et al., 1986; Omu and Fernandes, 2001; Henkel et al., 2003). Zinc has been shown to have membrane-stabilizing and antioxidant activity and to maintain sperm viability by inhibiting DNAases (Aitken and Clarkson, 1987). Zinc appears to be a potent scavenger of excessive superoxide anions produced by defective spermatozoa and/or leukocytes in human semen after ejaculation (Plante et al., 1994; Irvine, 1996). Thus, it seems that seminal plasma, because of its high content of zinc, will exert protective, antioxidant-like activity sufficient to cope with the excessive amount of superoxide anions (Gavella and Lipovac, 1998). Colagar et al., (2009) mentioned that zinc has anti-oxidative properties and plays an important role in scavenging ROS. They concluded that poor Zn nutrition may be an important risk factor for low quality of sperm and idiopathic male infertility. However, it has been reported that extremely high levels of zinc may inhibit sperm motility (Sorenson et al., 1999) and the function of the mannose receptor on the sperm head (Lin et al., 2000). Lewis Jones et al., (1996) concluded that seminal plasma zinc is an unreliable marker of spermatogenic activity. However a large number of available findings suggest a positive role in male reproduction. Hunt et al., (1992) deduced the important role of zinc in testosterone production and its need in the spermatogenesis process. Bedwal and Bahuguna (1994) mentioned that, zinc, copper and selenium are important in reproduction of males and females. Zinc content is high in the adult testis and the prostate has a higher concentration of zinc than any other organ of the body. Zinc deficiency first impairs angiotensin converting enzyme (ACE) activity and this in turn leads to depletion of testosterone and inhibition of spermatogenesis. Further, they suggested that zinc is thought to help to extend the functional life span of the ejaculated spermatozoa.
The beneficial role of Zn in providing protection against calcium-, lead-, cobalt- and mercury-induced testicular or other organ toxicity has been reported (Miller, 1983; Saxena et al., 1989; Anderson et al., 1993; Afonne et al., 2000). Zn increases hepatocellular metallothionein (MT) and because of their high affinity for metals, MT may play a physiologic role in the absorption, storage and metabolism of important trace metals such as Zn and Cu, as well as in the detoxification of certain metals such as cadmium, mercury and chromium (Valberg and Flanagan, 1983; Cousins, 1985). Zn is essential for spermatogenesis and induction of hepatocellular metallothionein, while its pretreatment protects animals and cell cultures from the acute toxicity of Cd (Liu et al., 1990). Heavy smoking has shown to be associated with low sperm count, motility, morphology and increased seminal cadmium levels. Zn modulates the putative effect of Cd by its enhancement of T-helper 2 cytokines expression and down-regulation of T-helper 1 cytokines (Al-Bader et al., 1999).

Earlier Favier (1992) reported that the diverse effects of zinc can be explained by its multiple actions on the metabolism of androgen hormones, estrogen and progesterone, together with the prostaglandins. Zinc has also been implicated in testicular development, sperm maturation and testosterone synthesis. Both folate and zinc have antioxidant properties that counteract ROS (Ebisch et al., 2007). Onyenmechi et al., (2002) observed that chronic exposure to chromium VI produces a marked testicular toxicity, which can be prevented by concomitant zinc administration. Zinc could reduce adsorption of fluorine, with the result of decreased fluorine in the body. Thus it antagonizes fluorotic toxicity by improving the activity of CuZn-SOD and antagonizing lipid peroxidation (Xia et al., 2001). Appropriate zinc had shown to antagonize male reproductive toxicity of fluorine on molecular level by antagonizing lipid peroxidation, influencing reproduction endocrine, activity of enzyme and Fas expression (Li et al., 2006). In male reproduction zinc might be necessary for spermatogenesis and testosterone production and sperm quality. Thus, Zn is essential for sexual reproduction and development.

Owing to these, this study was planned to assess the role of lifestyle, environmental factors and semen quality by investigating oxidative stress in semen, sperm quality and sperm DNA damage with respect to lifestyle factors as well as environmental exposure. The data might be useful in advocating for adopting the healthy lifestyle in order to reduce the male reproductive health problems.