Human beings are exposed to several thousands of environmental and occupational hazards used in industrial processes, therapeutics, developmental activities or in the agriculture. The male reproductive system is very sensitive to these occupational or environmental hazards. A person can be exposed to these hazards in their workplace accidently, which may affect their reproductive function. However, usually, the effect of exposure to potential hazards on reproduction is not detected till they are interested in starting their family. Over the past two decades, the incidence of infertility has increased from 8 to 15% in industrialized countries (Dondero et al., 1991). Occupational exposure to pesticides leads to decrease in the sperm concentration (Whorton et al., 1979), morphology (Wyrobek et al., 1981), motility (Wyrobek et al., 1981; Lerda and Rizzi, 1991; Yucra et al., 2006; Miranda et al., 2013) and complete atrophy of the seminiferous epithelium (Potashnik et al., 1978). Similarly, decrease in semen volume, sperm concentration, motility and morphology was observed in the person exposed to lead (Gustafson et al., 1989; Ng et al., 1991; Apostoll et al., 1998). Altered level of serum reproductive hormones is common feature of men exposed to occupational hazards (Gustafson et al., 1989; Ng et al., 1991; Padungtod et al., 1998; Yucra et al., 2006; Miranda et al., 2013). This suggests that male reproductive system and spermatogenesis are highly sensitive to occupational and/or environmental hazards.

X-rays was discovered in 1895 by physicist Wilhelm Conrad Rontgen (Fransson, 1994; Tubiana, 1996). The news of this discovery immediately created an immense public interest and also a research in several directions. The use of X-rays on patients to investigate the skeleton and subsequently the lung and other organs was started by
physicians and physicists as early as in January 1896 six months after the discovery of X-rays (Tubiana, 1996). In 1900, some of the doctors and surgeons unwittingly overexposed themselves to X-rays ultimately showed the potential dangers of radiation exposure. According to United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) report, largest contribution to ionizing radiation exposure to men is through its diagnostic and therapeutic uses (UNSCEAR, 1982). Generally, radiation health workers are employees of hospitals and private clinics where radiation is used for diagnostic and therapeutic purpose. There are approximately 7.35 million radiation health workers worldwide including India (UNSCEAR, 2008). Thus, hospital workers are constantly exposed to very low doses of ionizing radiation (UNSCEAR, 2008) throughout their reproductive age which is one of the major concerns. The levels of radiation exposure to health worker are well within the limits and the limit of 20 mSv/year is seldom or never exceeded (Thierens et al., 1996). However, the occupational exposure to low levels of ionizing radiation has shifted the attention of researchers from the identification of harmful effects to the prevention of possible stochastic effects (Valentin, 2002; Clarke and Valentin, 2009). Interestingly, an increased risk of leukemia has been observed in the people occupationally exposed to ionizing radiation (Smith and Doll, 1981; Muirhead et al., 1999).

**Gonadal radio-sensitivity:**

The reproductive organs have the highest sensitivity to radiation which is represented by the highest weighting factor of 0.20 (ICRP, 1991; NCRP, 1993) because of presence of highly proliferating tissue compartments in the reproductive organs especially testes &
ovary (Table 1). Testes are more sensitive to ionizing radiation due to which it can be significantly functionally impaired by very low doses of radiation (Howell et al., 2005). In the 1970’s in U.S, prisoners volunteered to be subjected to X-rays of their testes, which aimed to determine the deleterious effect of ionizing radiation exposure on spermatogenesis. This study showed that a dose of 0.11 Gy leads to suppression of sperm count and radiation exposure of 3-5 Gy causes permanent sterility (Clifton and Bremner, 1983). In a study on people who worked in a clean-up of the Chernobyl nuclear disaster in Ukraine, a significant change in the sperm characteristics were observed in the individuals exposed to more than 100mSv radiation (Cheburkov and Cheburkova, 1993). Irradiation is an iatrogenic male reproductive toxin which can affect the cell especially spermatogenic cells (Vergouwen et al., 1995). Several abnormalities such as low sperm counts, increased abnormal spermatozoa, and defective sperm function has been observed in the testes following irradiation (Shen et al., 1999; Shin et al., 2009; Kim et al., 2011). Such adverse events in the testis result in several abnormalities in spermatogenesis, potentially resulting in temporary or permanent infertility.

Spermatogenesis is a complex process in which spermatogonial stem cells undergoes cellular division through mitosis and meiosis and differentiation to give rise to mature spermatozoa. In mammals, spermatogenesis is the only biological process in which meiosis occurs in the adult state and this process is known to be very sensitive to any occupational or environmental hazards including ionizing radiation (Fischbein et al., 1997). Due to the diverse nature of spermatogenesis, and the wide variety of different cell types within the testes, many potential targets exists for disruption of this process.
The major function of the testes is to produce genetically competent haploid spermatozoa which can fertilize the oocyte successfully. Approximately, 50% of the conception do not give rise to viable fetus in human (Collins, 1995), which may be because of the DNA damage in the spermatozoa (Khadem et al., 2014). The expression of the effect of ionizing radiation on the sperm DNA damage is directly proportional to the dose and duration of the radiation exposure (Adiga et al., 2010; Kumar et al., 2013) and lead to temporarily or permanently sterility in male (Bianchi, 1983; Meistrich, 1986).

**Table 1: Sensitivity of various organs to ionizing radiation**

<table>
<thead>
<tr>
<th>Tissue or Organ</th>
<th>Tissue weighing factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gonads</td>
<td>0.20</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>0.12</td>
</tr>
<tr>
<td>Colon</td>
<td>0.12</td>
</tr>
<tr>
<td>Lung</td>
<td>0.12</td>
</tr>
<tr>
<td>Stomach</td>
<td>0.12</td>
</tr>
<tr>
<td>Bladder</td>
<td>0.05</td>
</tr>
<tr>
<td>Breast</td>
<td>0.05</td>
</tr>
<tr>
<td>Liver</td>
<td>0.05</td>
</tr>
<tr>
<td>Oesophagus</td>
<td>0.05</td>
</tr>
<tr>
<td>Thyroid</td>
<td>0.05</td>
</tr>
<tr>
<td>Skin</td>
<td>0.01</td>
</tr>
<tr>
<td>Bone surface</td>
<td>0.01</td>
</tr>
<tr>
<td>Remainders</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>Whole Body Total</strong></td>
<td><strong>1.00</strong></td>
</tr>
</tbody>
</table>
Decades of animal and human research have convincingly documented that a variety of risk factors including exposure of a male to ionizing radiation can cause genomic instability (Balakrishnan and Rao, 1999; Cigarran et al., 2001; Tawn and Whitehouse, 2003), alter cellular and humoral immunity (Godekmerdan et al., 2004; Serhatlioglu et al., 2004) and even impair fertility (Wyrobek et al., 1997; Ahamadi and Ng 1999). Exposure of germ cells to ionizing radiation may have mutagenic consequences (Hoyes et al., 1994; Brinkworth, 2000). Several studies have demonstrated the detrimental effect of radiation exposure on male and female reproductive systems in animal species (Mole et al., 1966; Baker 1971; Saharan et al., 1977; Adiga et al., 2007a,b; Adiga et al., 2010). An impaired fetal development, pregnancy loss and abnormal development of somatic cell in the foetus was observed in the pregnancy derived from the testicular irradiated mice (Adiga et al., 2010; Kumar et al., 2013). Higher frequency of abnormalities was observed in the pronuclear stage embryos derived from the radiation exposed spermatozoa in vitro (Ahmadi and Ng, 1999). In addition, higher incidence of tumor was observed in the offspring derived from the paternal irradiation (Wyrobek, 1993; Friedler, 1996; Lord, 1999). However, these studies suggest that genetic damage induced by ionizing radiation may not be repaired effectively and it manifests itself in spermatozoa. It may lead to harmful effects when fertilization occurs with spermatozoa carrying these genetic damages. There is growing concern for the children of men who have been conceived after exposed to chemotherapy and radiation (Hawkins et al., 1995; Dubrova et al., 1996). Although studies have not proven a link in the human population (Byrne et al., 1998; Wakeford and Tawn, 2000; Tice et al., 2000), these evidences from animal studies
clearly suggests that their finding must be viewed with caution and further research should be undertaken to get more information.

Influence of ionizing radiation on sperm characteristics:

It is now evident that well controlled semen evaluation studies have contributed substantially to current knowledge on reproductive toxicity of many chemicals in humans (Bonde, 2010). The assessment of sperm functional characteristics is an essential parameter in the evaluation of sperm quality and in the establishments of association between sperm quality and male fertility. A decrease in ejaculate volume, sperm motility, viability and morphology was observed in the men engaged in clean-up after the Chernobyl nuclear plant disaster (Cheburakov and Cheburakova, 1993). Further, Fischbein et al., (1997) observed a decrease in the motility and percentage of progressive motility in the samples from the liquidators as compared with their controls in clean-up workers at Chernobyl in Ukraine. A higher frequency of malformations in the sperm head was also observed in the liquidator group by using quantitative ultramorphological analysis (Fischbein et al., 1997). These studies raised the major concern on the effect of radiation exposure on the sperm functional integrity.

Sperm parameter such as motility and morphology is considered as most informative parameter for the assessment of sperm quality and to establish a meaningful association between sperm quality and fertility potential in men (Bonde et al., 1998; Guzick et al., 2001). Owing to the subjectivity of conventional semen analysis techniques, Computer-aided sperm analysis (CASA) has been considered as sensitive tool in the assessment of
male factor infertility (Verstegen et al., 2002). Several studies have revealed its value in clinical (Vantman et al., 1988, 1989), epidemiologic (Eskenazi et al., 1991), and basic biologic applications (Mbizvo et al., 1990). It improves our knowledge and ability to manipulate spermatozoa based on their sperm kinematics pattern and its use gives us high precision and provision of qualitative data on sperm kinematics compared to manual methods (WHO, 1999). CASA parameters such as linearity (LIN), curvilinear velocity (VCL), and average path velocity (VAP), may serve as prognostic indicators for fertilization potential of spermatozoa. Interestingly, in vitro fertilization rate and time to conception is significantly related to concentration and movement characteristics of motile spermatozoa estimated by CASA (Barratt et al., 1993; Irvine et al., 1994; Krause 1995).

Integrity of the nucleus of human spermatozoa is essential to achieve successful fertilization and embryo development. The presence of nuclear vacuoles in human spermatozoa may alter the homogeneous nature of the chromatin and lead to DNA damage (Oliveira et al., 2010). Therefore, evaluation of the size and number of vacuoles present in sperm head is of importance in judging the fertilizing ability of spermatozoa (ICMR, 2000-2001). Several studies have shown that, sperm DNA damage is associated with incomplete/failure in chromatin condensation and nuclear weakness and it has been linked to large vacuoles in the sperm head (Boitrelle et al., 2011; Franco et al., 2012). Sperm head vacuoles are known to cause defective zona binding (Thundathil et al., 1998), affect early embryonic development and increased miscarriage rate (Berkovitz et
However, till now there is no study to find out the effect of occupational radiation exposure on sperm functional characteristics in radiation health workers.

**Ionizing radiation, sperm DNA integrity and reproductive outcome:**

Sperm DNA is an independent measure of sperm quality and considered as better prognostic and diagnostic value than semen parameters like concentration, motility and morphology (Zini et al., 2001). Analysis of sperm chromatin in occupational exposure is well established marker for genetic risk assessment in humans (Duty et al., 2003; Migliore et al., 2002). The association between DNA damage in spermatozoa with poor embryonic development in the patients undergoing medically assisted conception further confirms the significance of sperm DNA (Seli et al., 2004).

Mechanism of action of ionizing radiation exposure and their cellular effects are well established. Ionizing radiation can induce DNA damage directly by acting on nucleus or indirectly by inducing free radicals in the cell which will ultimately act on DNA. The interaction between the ionizing radiation and cellular DNA produces DNA lesions which include single strand breaks (SSBs), double strand breaks (DSBs), damage to nucleotid bases, cross links between DNA-DNA and DNA-protein and damage to the nucleotid bases (Natarajan, 1993; Chaubey et al., 2001). Fertilization, embryo quality and health of the offspring can be affected by any defect in the sperm DNA (Haines et al., 2002). Previous studies from our laboratory have observed that exposure of testicular regions of male mice resulted in sperm DNA damage in a dose-dependent manner and analysis clearly demonstrated a significant increase in the DNA damage even in
spermatozoa exposed to the lowest dose of radiation which is 2.5 Gy (Adiga et al., 2007, 2010; Kumar et al., 2013). Moreover, possible influence of sperm DNA damage on transgenerational changes in genomic instability in the pre-implantation embryos was also observed, which suggests that the sperm DNA damage can cause genomic instability in pre-implantation embryos (Adiga et al., 2010). p53 and p21 mediated checkpoint response was observed in the mouse embryo derived from the DNA damaged spermatozoa induced by ionizing radiation followed by the excessive apoptosis in the inner cell mass of the blastocyst which ultimately resulted in defective implantation and post-implantation embryonic demise (Shimura et al., 2002; Toyoshima et al., 2005; Adiga et al., 2007). In addition, a dose-dependent decline in number of litters and high incidence of post-natal death was observed in the progenies derived from DNA damaged sperm in first generation mice, (Kumar et al., 2013). Several studies have reported a decrease in the conception rate, decreased incidence of live birth (Little 1999; Parker et al., 1999; Selby 2000; Abrahamson and Tawn, 2001), abnormalities in the children of the parents exposed to ionizing radiation occupationally (Green et al., 1997; Doyle et al., 2000b) and altered reproductive health among radiographers (Shakhatreh 2001). However, there is no study on the influence of occupational radiation exposure on sperm DNA integrity in radiation health workers occupationally exposed to radiation on their workplace.

**Assessment of sperm DNA integrity/fragmentation:**

There are various techniques available for the assessment of the DNA integrity in human spermatozoa. Sperm chromatin structure assay (SCSA), single cell gel electrophoresis
(comet) assay and TdT-mediated dUTP-biotin nick end labeling (TUNEL) assay are the most commonly used techniques in epidemiological studies.

1. Sperm chromatin structure assay (SCSA): Several techniques have been established so far to examine the integrity of sperm DNA. SCSA is first described by Evenson et al. (1980), which is now extensively used for the evaluation of DNA damage in both animal and human spermatozoa (Evenson and Jost, 1994; Spano et al., 1998). It utilizes the metachromatic properties of acridine orange (AO) to distinguish between single stranded and double stranded DNA in sperm chromatin by means of low pH- or heat-denatured (red fluorescence) and native (green fluorescence) respectively. Abnormal chromatin structure is measured by flow cytometry that records the ratio of denatured DNA to native DNA. A high ratio has shown to be correlated with high sperm concentration and sperm head abnormalities. Further, it has been shown that if the percentage of cells with abnormal ratios exceeds 30-40%, then fertilization is unlikely (Evenson et al., 1999; Krishnamurthy et al., 2000; Spano et al., 2000). The variation of analysis of SCSA within a man is much lower than for the classical measures (Evenson et al., 1991; Spano et al., 1998). Several animal toxicology studies have consistently measured the high level of repeatability of SCSA measures and convincing biological dose response interpretations (Evenson et al., 1989, 1993). Thus, SCSA results can be considered to characterize DNA damage with a higher degree of certainty than the other analysis in any cross sectional, or single sample analysis of a patient.

2. Single cell gel electrophoresis (comet) assay: Single cell gel electrophoresis (comet) assay is extensively used to examine the genotoxicity of a substance in both somatic cells
and gametes (Singh et al., 1988; Aravindan et al., 1997) and also to study the radiation induced DNA damage in germ cells including spermatozoa (Morris, 2002). This has been used as a biomarker of the exposure for the evaluation of DNA damage in the molecular epidemiology studies (Betti et al., 1994; Garaj- Vrhovac and Kopjar, 1998; Piperakis et al., 1999; Maluf et al., 2001; Garaj-Vrhovac et al., 2002). This technique can be used in the study of repair kinetics and also for the evaluation of DNA damage at the level of single cells (Singh et al., 1988; Hellman et al., 1995; Olive, 1999) which includes the detection of single strand breaks (SSBs), double strand breaks (DSBs) and alkali-labile sites in the DNA. Comet assay is also used in the evaluation of DNA damage associated with apoptosis (Piperakis et al., 1999; Olive, 1999), thus considered as one of the most important techniques in epidemiological study.

3. TdT-mediated dUTP-biotin nick end labeling (TUNEL) assay: The TUNEL assay is a method for staining of apoptotic cells which is universally accepted as one of the best techniques for apoptosis detection in situ and also exhibit the biochemical hallmark of apoptosis-internucleosomal DNA fragmentation (Arend et al., 1990; Bortner et al., 1995; Wyllie, 1980). It is originally described by Gavrieli et al., (1992) and principally based on incorporation of labeled dUTP into free 3’-hydroxyl terminal in the presence of the enzyme terminal deoxynucleotidyl transferase. It can effectively detect single and double stranded DNA damage (Gavrieli et al., 1992). TUNEL assay is also useful in detecting the DNA damage in the necrotic cells induced by exposure in toxicological study (Ansari et al., 1993). Moreover, cells undergoing the repair process can also be stained by this
method (Kanoh et al., 1999). Therefore, TUNEL assay may be a useful technique for the detection of DNA damage in the apoptotic cell in the toxicological study.

**Ionizing radiation and epigenetic changes:**

Epigenetics is a study of mechanism and phenomena involved in stable genetic modification and gene expression without affecting the genotype or DNA sequence (Aguilera et al., 2010). This mechanism is very important in replacement of histone by protamine in spermatid, regulation of gene expression, DNA methylation and chromatin structure (Bird, 2002; Feinberg, 2007; Umlauf et al., 2008). These modifications are very stable but can be altered by physiological, pathological and/or environmental factors (Holliday, 2006; Rakyan and Beck, 2006; Whitelaw and Whitelaw, 2006). There are some major concerns about the transmission of epigenetic abnormalities, such as Angelman and Beckwith-Wiedmann syndromes through assisted medical conception (Gosden et al., 2003; Marques et al., 2004). These concerns have been bolstered by a report suggesting a strong association between abnormal sperm imprinting and defective spermatogenesis (Marques et al., 2004). There is growing evidence suggesting that DNA methylation is one of the epigenetic codes influencing heterochromatin remodeling (Bannister et al., 2001; Nielsen et al., 2001; Richards and Elgin, 2002).

DNA methylation, which is defined as transfer of a methyl group to the five positioned carbon of a cytosine with in CpG dinucleotides is one of the important epigenetic modifications known so far. This process takes place throughout the development and responsible for the normal gene expression in an individual. It is one of the most
important indicators of sperm head condensation and incomplete or aberrant condensation of sperm chromatin is associated with higher levels of DNA damage (Aitken and De-lullis, 2007). In addition, DNA methylation of CpG dinucleotides is known to have influences on both chromatin structure and gene expression (Paulsen and Ferguson-Smith, 2001). DNA hypermethylation is mostly responsible for the silencing of the gene and also considered as hallmarks in cancer associated from the inactivation of the tumor suppressor gene (Jones and Baylin, 2002; Esteller, 2008).

The epigenetics of an individual can be altered by exposure to environmental factors and living place and/or workplace has been shown to affect the epigenetics of adult individuals (Aguilera et al., 2010). These environmental factors may not affect the epigenetic modification always but a strong association has been established by number of studies. A study by Christensen et al., (2009) observed that atleast 24 CpG loci were hypermethylated in the pleural tissues in the asbestos exposed individuals. A reduction in the methylation level is also observed in the people exposed to some of the environmental pollutants like chromium (Shiao et al., 2005), cadmium (Takiguchi et al., 2003), and nickel (Salnikow and Costa; 2000) which may be because of inhibiting activity of DNA methyltransferases. Some of the popularly used xenobiotics like bisphenol A (Maffini et al., 2006) and vinclozolin (Anway et al., 2006) are shown to change the DNA methylation pattern. However, till now there is no study to find out the effect of low levels of occupational radiation exposure on DNA methylation pattern in the health workers.
Ionizing radiation and reactive oxygen species (ROS) & antioxidants:

The term, oxidative stress refers to an imbalance between reduced level of antioxidants and excessive production and accumulation of ROS (Sikka, 2001; Agarwal et al., 2003). ROS are highly unstable and are known to have several targets in spermatozoa such as membrane and DNA. Generation of lipid peroxides in spermatozoa at an increased level impairs the normal sperm function (Aitken and Clarkson, 1987). Presence of polyunsaturated fatty acids (PUFA) in the plasma membrane of the human spermatozoa makes it very sensitive to ROS induced damage (Aitken and Clarkson, 1987; Aitken et al., 1989). Since a mature spermatozoa has negligible or no cytoplasm, even though it contains all the major intracellular antioxidants such as glutathione, glutathione peroxidase (Alvarez and Storey, 1989) and superoxide dismutase (SOD) (Alvarez et al., 1987; Zini et al., 1993), they are present at very level (Aitken, 1994). Due to these reasons, the spermatozoa are highly susceptible to ROS-induced damages.

Semen is a mixture of spermatozoa suspended in secretions from testes and epididymis which, at the time of ejaculation, are combined with secretions from prostate, seminal vesicles and bulbourethral gland, collectively known as seminal plasma. It plays an important role in various sperm function like maturation, capacitation and fertilization. A reduction in the sperm density, motility and higher incidence of defective spermatozoa is observed in the sample containing high level of ROS in human seminal plasma (Aitken, 1989). Importance of seminal plasma in the protection of spermatozoa against ROS is well known (Iwasaki and Gagnon, 1992), only a few studies have investigated its antioxidative properties and the possible relationship between infertility and plasmatic
antioxidant defences (Lewis et al., 1995; Thiele et al., 1995). Seminal plasma contains both enzymatic antioxidants such as glutathione peroxidase, glutathione reductase, catalase, superoxide dismutase (SOD) (Alvarez et al., 1987) and non-enzymatic antioxidant molecules such as ascorbic acid, uric acid, thiols (Lewis et al., 1997). As long as spermatozoa are suspended in seminal plasma, they are protected from oxidative damage by lipid peroxidation (Kankofer et al., 2005). Seminal plasma is shown to be very effective against ROS or free radicals induced cell damage (Halliwell, 1997). Levels of Glutathione (GSH) and glutathione disulfide (GSSG), as well as activities of enzymes like glutathione peroxidase, glutathione reductase, catalase, and superoxide dismutase efficiently controls the levels of ROS production.

Antioxidants are defined as agents which protects the cell against the damaging effects of the ROS (Surai et al., 2001b) including the oxidative stress induced by (Ivaniota et al., 1998; Neyfakh et al., 1998a,b; Kumerova et al., 2000). A decrease in the antioxidant level in blood plasma was observed in the radioactively contaminated areas, such as Chernobyl in Ukraine which may be responsible for increased levels of mutation (Dubrova et al., 1996; Ellegren et al., 1997; Kovalchuk et al., 2000; Møller and Mousseau, 2001). The increase in the incidence of mutation in the radioactively contaminated areas may be indirectly due to the reduced level of antioxidants or directly by increase in the level of DNA damage. An association was observed in the decreased antioxidant defence owing to the use of antioxidants for free radical scavenging in the recovery workers exposed to the Chernobyl accident (Ivaniota et al., 1998; Neyfakh et al., 1998a; Kumerova et al., 2000). These studies clearly provided the important link
between the antioxidant defence mechanism and exposure level in the radiation exposed subjects. Moreover, whenever tissues are challenged with oxidative stress, the body tries to overcome the deleterious effect by elevating the expression of antioxidant enzymes and antioxidant molecules as an adaptive response (Limón-Pacheco et al., 2009). However, till now there is no available that links the effect of occupational radiation exposure on the level of antioxidants in plasma and its effect in terms of mutation rate and DNA damage.

Glutathione (GSH) is an abundant tripeptide antioxidant molecule present in the cytoplasm and body fluids. It directly reacts with hydrogen peroxide, superoxide-anion, hydroxyl, and alkoxy radicals by its free sulphhydryl groups (Meister and Anderson, 1983) and thus plays an important role in the protection of cell against ROS and free radicals induced damage. In addition, it is able to protect the cell efficiently against singlet oxygen induced DNA damage (Lafleur et al., 1994) by acting as a ROS scavenger and maintains the sperm motility, sperm viability and sperm morphology. It is an antioxidant which is present in the seminal plasma and protects the sperm from ROS. The scavenging property of GSH is mainly due to its function as cofactor for antioxidant enzyme glutathione peroxidase (GPX) and also its ability to react directly with ROS by donating hydrogen ion (H+) from its free sulfhydryl group. De novo synthesis of GSH is required for cell activation and proper S and G2 phase transits (Poot et al., 1995). GSH is very important for a number of enzymes by acting as a co-factor and protects the cell against damage due to the presence of the sulphydryl group (SH) (Luberda et al., 2005). It is present in reduced (GSH) and oxidized form (GSSG), which protects the cells
against ROS (Luberda et al., 2005). Reduced form is considered as active form of the GSH and contains comprises 98% of the total GSH under normal condition (Serru et al., 2001). The reduced/oxidized GSH ratio serves as an indicator of cellular damage (Luberda, 2005; Serru et al., 2001). Several studies have reported the alteration in the reduced/oxidized GSH ratio in aging, cancer, HIV replication, cardiovascular diseases and also in neuro degenerative diseases (Herzenberg et al., 1997; Asensi et al., 1999; Hernanz et al., 2000; Kleinman and Ritchie, 2000; Lang et al., 2000; Owen and Butterfield, 2010). Thus, assessment of ratio of reduced/oxidized GSH may be an important marker to assess the detrimental effect caused by any environmental or occupational hazards.

Total antioxidant capacity (TAC) is collective action of all the enzymatic and non-enzymatic antioxidants present in seminal plasma or blood plasma. It is also considered as important marker of oxidative stress in vivo (Ghiselli et al., 2000). Hence, measuring plasma TAC may help in the evaluation of physiological, environmental, and nutritional factors of the redox status in humans. Seminal plasma TAC is closely related to male fertility, and the decreased level of TAC in seminal plasma may be one of the causes of male infertility (Shi et al., 2005). Appropriate TAC provides a favorable environment for sperm swimming and decreased in TAC is associated with impaired sperm function as a result of either increased reactive oxygen species (ROS) production or insufficient antioxidant capacity (Koya et al., 2003).

Superoxide dismutase (SOD) is an enzyme that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide (Archibald and Fridovich, 1982; Fridovich, 1995;
Bertini et al., 1998) and fairly ubiquitous in aerobic organisms. Hydrogen peroxide might conceivably act as a metabolic signal under certain circumstances, possibly by oxidizing specific protein thiol groups and triggering intracellular events (Halliwell and Gutteridge, 1989). Hydrogen peroxide crosses cell membranes easily and affects the cell organelles. High concentrations of hydrogen peroxide can inactivate the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase in mammalian cells (Hyslop et al., 1988). Thus, SOD’s are important antioxidants and act fundamentally as protective agents against ROS mediated diseases such as carcinoma, inflammation and aging (Dalle-Donne et al., 2006; Valko et al., 2007). Previous studies have shown an increase in the SOD concentration in the blood plasma of the radiation exposed health workers (Durovic et al., 2008). This enzyme is also present in the testes at a relatively high concentration compared to other organs and its function is very important under the germ cell regulation (Mruk et al., 2002).

Lipid peroxidation is a process in which oxidants or free radicals attacks on polyunsaturated fatty acids (PUFA) (Yin et al., 2011). According to cellular metabolic capabilities and repair capacity, the affected cell may promote cell survival or induce cell death. Lipid peroxidation is the primary effect of ROS and is considered as an indicator of membrane polyunsaturated fatty acid oxidation (Alvarez and Storey, 1995). Malonaldehyde (MDA) is one of the most important mutagenic products of lipid peroxidation (Esterbauer et al., 1990). Sperm quality and function is negatively correlated with ROS (Benedetti et al., 2012). MDA is produced under unfavorable condition and has high affinity to react with DNA or proteins that leads to the formation of adducts
Excessive production of the MDA is associated with several pathological conditions (Merendino et al., 2003; Baskol et al., 2006; Shanmugam et al., 2008; Sanyal et al., 2009; Li et al., 2012; Garcia et al., 2013). However, evaluation of the MDA level in seminal plasma has been recognized as an important tool in the assessment of sperm reproductive capacity and functional competence in infertile men (Benedetti et al., 2012). However, till now, there is no study to find out the effect of occupational radiation exposure on the sperm functional characteristics and the seminal plasma antioxidants level in the radiation health subjects.

**Ionizing radiation induced cytogenetic changes:**

Cytogenetic analysis of peripheral blood samples are the most commonly used techniques for the evaluation of genotoxicity by occupational or environmental hazards. Blood samples are comparatively easy to obtain and relatively inexpensive culture techniques can be used to rapidly generate abundant metaphases of optimum length and quality. The peripheral blood acts as an internal control as effect of any environmental or occupational hazards influences changes in the blood. Peripheral blood is a plasma-based suspension of erythrocytes, platelets and leukocytes. Only the leukocytes are nucleated and therefore, of potential use to the cytogenetic analysis after in vitro culture. Cytogenetic alteration has been considered as potential biomarkers to identify individual at risk in time of preventive interventions (Perera and Whyatt, 1994; Hagmar et al., 1998).
The effect of ionizing radiation on the DNA damage is well known. DNA double strand breaks are one of the primary lesions, which can be easily observed in the metaphase chromosome (Natarajan, 1993; Pfeiffer et al., 2000). Chromosomal aberrations in the metaphase chromosome such as acentrics, dicentrics and aneuploidy have been considered as reliable biomarkers for the assessment of harmful effects induced by ionizing radiation exposure in humans (Lloyd, 1992; Hoffmann and Schmitz-feuerhake, 1999). Though occupational radiation exposure levels now strictly fall well within the accepted limits (Maffei et al., 2002), chromosomal abnormalities is still considered as one of the major hazards in the peripheral blood lymphocyte of radiation health workers (Barquinero et al., 1993; Bonassi et al., 1997; Rozgaj et al., 1999; Cardoso et al., 2001). Several studies have been conducted in the past on the peripheral blood lymphocytes of the hospital workers and found significant increase in DNA damage (Garaj-Vrhovac et al., 2003; Martinez et al., 2010), micronuclei incidence (Gourabi et al., 1998; Thierens et al., 2000; Maffei et al., 2002; Sari-Minomdier et al., 2007; Eken et al., 2010) acentrics, dicentrics and chromatid breaks (Lalic et al., 2001; Rozgaj et al., 2002) in these subjects. Alterations in gene expression patterns involved in DNA repair, cell cycle regulation/proliferation, and stress response was also observed in the hospital workers exposed to radiation (Fachin et al., 2009), which raises the serious concern about the long term effects of radiation exposure.

Evaluation of chromosomal aberration is major used parameters in the biological dosimetry (Garaj-Vrhovac et al., 2003; Martinez et al., 2010; Lalic et al., 2001). However, technique is time consuming and requires highly skilled personnel due to
which monitoring of large scale of population is difficult. Assessment of micronuclei (MN) in peripheral blood lymphocyte is used as an alternative option to chromosomal aberrations analysis (Fenech and Morley, 1985; Fenech et al., 1999). Basically, micronuclei is a part of whole chromosome or acentric fragments of the chromosome which is separated from the main nuclei during cell division and considered as a best reliable biomarker of exposure to genotoxic agents (Fenech, 1998). In radiation exposure studies, MN assessment has been considered a reliable biomarker for biological dosimetry (da Cruz et al., 1994; Wuttke et al., 1996). Many researchers have used this technique for the assessment of cytogenetic damage in the populations exposed to high and low level of radiation (Thierens et al., 1996; Chang et al., 1999; Tsai et al., 2001; Cardoso et al., 2001). Radiological workers have shown an elevated numbers of centromere positive MN by the fluorescence in situ hybridization technique (Thierens et al., 2000). Several studies have found significant increase in micronucleus incidence in the blood lymphocyte of the hospital workers occupationally exposed to low doses of ionizing radiation (Gourabi, Mozdarani, 1998; Thierens et al., 2000; Maffei et al., 2002; Sari-Minomdier et al., 2007; Eken et al., 2010). In contrast, none of the studies have compared the sensitivity of MN assay or chromosomal aberration assay in the peripheral blood lymphocyte of the exposed population.

Reproductive hormonal changes in occupational exposure:

The spermatogenesis is one of the complex processes which is regulated by several endocrine and paracrine signals. The release of gonadotropin releasing hormone (GnRH), from the hypothalamus is considered as master regulator of the spermatogenesis and
proliferation and survival of germ cell depends heavily on GnRH dependent mechanisms (McLachlan et al., 2002a). Mitotic and meiotic divisions require master regulators such as follicle stimulating hormone (FSH), testosterone and leutinizing hormone (LH) from the hypothalamo-pituitary-testicular (HPT) axis (Ruwanpura et al., 2010). FSH supports the spermatogonial development whereas, testosterone partly supports spermatocyte maturation (McLachlan et al., 2002a; Ruwanpura et al., 2008a,b). In addition, testosterone plays an important role in the spermiation. However, both testosterone and FSH is required for the release of spermatids from Sertoli cell (McLachlan et al., 2002a) while, LH mainly acts on the Leydig cells which synthesize the testosterone (Wahlstrom et al., 1983). Several studies on occupational hazards have reported that semen quality is associated with changes in reproductive hormones in men (Sina et al., 1975; Subhan et al., 1995; Jensen et al., 1997; Mahmoud et al., 1998; Meekar et al., 2007) Moreover, these occupational hazards reflects both primary and secondary effect on the testes and HPT axis as an altered sperm functional characteristics in lead (Gustafson et al., 1989; Ng et al., 1991) and pesticides (Yucra et al., 2006; Miranda et al., 2013) exposed subjects.

As per PUBMED literature survey, there are no reports on the effect of the occupational exposure in radiation health workers for the evaluation of sperm DNA damage and reproductive toxicity. Therefore, this study was planned to evaluate sperm functional, genetic, epigenetic and endocrine hormones in the subjects who are handling radiation sources at their workplace and its association with the level of radiation exposure as measured by the radiation dosimetry. In addition to this, this study was also planned to
evaluate the effect of occupational radiation exposure on plasma antioxidants level and its association with the DNA integrity.