Summary & Conclusion
Recent reports on deteriorating semen quality from the recent decades has led to the speculation that environmental and occupational exposure to industrial agents and/or risk factors associated with lifestyle such as obesity, smoking, caffeine, chewing, alcohol, drugs, mobiles as well as some of the dietary habits might be interfering with man's ability to produce healthy spermatozoa. The male reproductive system is vulnerable to these factors, which might be due to the fact that sensitive events take place during spermatogenesis. Owing to these, a prospective hospital based study was conducted among the 240 subjects attending the OPD of Obstetrics and Gynaecology, Civil hospital and Institute of Kidney Diseases, for infertility assessment. A detailed history was recorded on predesigned proforma, such as smoking, chewing and alcohol habits. Information regarding occupational exposure to agents that can possibly affect spermatogenesis such as exposure to metals, pesticides, dust, heat and solvents was also taken. Reproductive history including duration of active married life and details of miscarriages, abortions, etc. was noted for all the men and their partners.

Semen samples were obtained from all subjects within 3-7 days of sexual abstinence and physical parameters such as liquefaction time, pH, appearance, viscosity and volume were noted. After liquefaction, semen analysis was carried out to determine the sperm count, motility, viability and morphology. Few sperm functional parameters such as Hypo osmotic swelling test and acrosome intactness test were also performed. Semen samples were further analyzed for sperm DNA damage by neutral comet assay and the slides were scored using an image-analysis system attached to a fluorescence microscope with CASP software. The parameters in comet assay assessed were %Head DNA, %Tail DNA, tail length, comet length, tail moment and olive tail moment. Apoptosis were also done using DNA diffusion assay. Sperm aneuploidy was assessed by Fluorescent in situ hybridization. Sperm DNA/ chromatin integrity was determined by using four different tests i.e. Acridine Orange (AO) test, Aniline blue (AB), Chromomycin A3 (CA3) and Toluidine blue (TB) staining. Various enzymatic and non-enzymatic antioxidants in seminal plasma were
assessed as a biomarker for oxidative stress. Oxidative stress in seminal plasma was assessed by conducting biochemical estimation of Lipid peroxidation (LPO), Protein carbonyls (PC), catalase (CAT), super oxide dismutase (SOD), glutathione-s-transferase (GST), reduced glutathione (GSH), glutathione reductase (GRd), glutathione peroxidase (GPx), glucose-6-phosphate dehydrogenase (G6PD), thiols, ascorbic acid and protein. Biomarkers for the function of the accessory sex glands such as alpha-glucosidase for epididymal dysfunction, fructose for seminal vesicle and acid phosphatase for prostate gland functioning were estimated in the seminal plasma. Whole blood samples were digested using microwave digestion for metal i.e. Pb and Cd estimation whereas serum and seminal zinc as well as copper levels were measured using atomic absorption spectrophotometer. Measurement of reproductive (LH, FSH and testosterone) as well thyroid (T₃, T₄ and TSH) hormones were carried out in serum using ELISA.

For analysis purpose, the subjects were classified as fertile and subfertile depending on their seminal characteristics and it was observed that 47.5% subjects were fertile whereas the rest 52.5% were subfertile. Fertile subjects included subjects i.e. having normal sperm count, motility and morphology within the recommended values whereas subfertile included azoospermic, oligozoospermic, asthenozoospermic, teratozoospermic as well as the combination of these conditions. Further, results showed that 19 subjects were azoospermic, 63 were oligozoospermic while 158 had sperm count equal to or greater than the WHO recommended limit of 20 millions/ml. Analysis of sperm motility data revealed that 36.7% subjects were asthenozoospermic while 63.3% had total progressively motile sperm greater than or equal to 50%. Further, sperm morphology data showed that 29.6% subjects were teratozoospermic while 70.4% subjects were having normal morphology. About 12.9% semen samples were hyperviscous whereas 87.1% samples had normal semen viscosity. The influence of lifestyle factors such as age, body mass index, tobacco smoking/ chewing and alcohol consumption was also assessed with respect to semen quality. Attempt was made to correlate the exposure to toxic substances and lifestyle factors with semen quality, DNA/chromatin damage, oxidative stress and hormone levels. Levels of metals in blood/serum and seminal plasma were also correlated with semen quality parameters such as sperm count, progressive motility, morphology, DNA damage markers and biomarkers of accessory sex glands. The main salient findings of the study are as follows:
The general characteristics of the study subjects revealed that the mean age of the study population was 31.91 ± 0.37 years ranging from 20 to 45 years. The duration of active married life of the study group ranged from 1 to 23 years. Mean body mass index of the study group was 22.21 ± 0.26 kg/m². While categorizing the subjects into exposed to toxic substances and non-exposed according to their status of exposure to toxic metals, pesticides, solvents, heat, etc. based on their occupational history, it was observed that 36.7% of the subjects had either present or previous exposures to harmful substances at their workplace.

An evaluation of sperm parameters revealed a mean sperm count of 42.21 ± 2.01 millions/ml which ranged from 0.5 to 129 millions/ml. The sperm count, progressive motility, normal morphology and viability were significantly higher among the fertile as compared to sub fertile subjects. Hypo osmotic swelling test showed that percentage of coiled tails were higher among the fertile as compared to sub fertile subjects. Also, acrosome intactness test showed that percentage of halo formation rate as well as halo diameter were higher among the fertile with respect to sub fertile subjects.

Age was found to have significant negative correlation with sperm count and viability whereas BMI showed significant negative correlation with sperm count, viability and percentage of normal morphology. Further, the incidence of oligoasthenoteratozoospermia was significantly higher among the subjects with higher BMI.

The subjects indulged in the lifestyle habits such as tobacco smoking/chewing and/or alcohol showed higher odds ratio for subfertility indicating the higher risk of declining semen quality among the subjects with these habits.

The use of cell phone (Electromagnetic radiations), both in terms of daily use frequency as well as duration of possession showed negative effect on sperm count, total progressive motility, normal morphology and viability as compared to cell phone non-users, but the changes were statistically not significant.

Chewing mixture containing areca nut and tobacco as one of its main component, was found to have significant negative effect on fast progressive motility and...
percentage of normal morphology. Further, the percentage of oligoasthenoteratozoospermia was higher among the chewers as compared to the subjects with no habit. Among oligozoospermic subjects, total progressive motility as well as normal morphology was significantly lower among chewers as compared to non-chewers. Further chewing of areca nut and tobacco habit was correlated positively with sperm DNA damage and have a significant role in increasing the sperm DNA damage with increase in chewing frequency and duration. Chewing was also found to have positive correlation with sperm aneuploidy.

- The prevalence of asthenozoospermia and azoospermia was significantly higher among the exposed subjects as compared to non-exposed subjects. Subjects ever exposed to toxicants were found to have higher risk of subfertility.

- The oxidative stress markers such as LPO and PC were negatively correlated whereas the seminal enzymatic and non-enzymatic antioxidants level like SOD, CAT, GST, GPx, GRd, GSH, thiols and ascorbic acid were positively correlated with sperm count, progressive motility and normal morphology.

- Sperm DNA damage, assessed by comet assay was significantly higher in oligozoospermics as compared to normozoospermics. It was also significantly higher among asthenozoospermics and teratozoospermics as compared to progressively motile and morphologically normal subjects respectively. Moreover, sperm DNA damage was found to be significantly higher among the subjects exposed to only lifestyle factors as compared to the subjects non-exposed to either lifestyle or toxic factors. Lipid peroxidation was observed to have significantly positive relationship with olive tail moment, which indicates the role of oxidative stress in inducing sperm DNA damage.

- The sperm DNA/chromatin damage as assessed by acridine orange, aniline blue, toluidine blue and chromomycin A3 staining also showed the significantly positive results with respect to declining sperm count, motility and morphology. Further, the damage was higher among the subjects having lifestyle and/or toxicant exposure as compared to non-exposed subjects. But the results were statistically non-significant. All the four techniques were found to be sensitive enough to estimate sperm DNA/chromatin integrity and it can be further
concluded that the AB and TB methods are inexpensive as well as simple and in addition have the advantage of providing permanent preparations which can be observed under light microscope.

- Hormone analysis revealed that mean serum FSH and LH levels were significantly higher in azoospermics and oligozoospermics as compared to normozoospermics. While no significant difference was observed in the testosterone level among different groups on the basis of sperm count. Further, Pb levels showed significant positive correlation with serum FSH levels. Serum T3 level was found to be lowest whereas the T4 level was highest among the azoospermics as compared to oligozoospermics and normozoospermics, though the results were statistically non-significant. Moreover, serum TSH level showed non-significant alterations with respect to sperm count.

- Analysis of Pb levels in whole blood among different groups on the basis of count, motility and morphology showed that mean blood lead levels were significantly higher in azoospermics as compared to normozoospermic subjects. Similarly, the mean blood Pb levels were higher among asthenozoospermics and teratozoospermics as compared to progressively motile and morphologically normal subjects respectively. However, the difference was statistically non-significant.

- Blood Pb levels showed significant positive correlation with the percentage of cytoplasmic droplet whereas significant negative correlation with seminal fructose level. It was observed that blood Pb and Cd levels were highest among the subjects having only toxicant exposure as compared to the non-exposed subjects. Further, both blood Pb and Cd levels correlated positively with the DNA damage parameters while negatively with seminal antioxidant activity.

- Copper was measured in both serum and seminal plasma and it was observed that mean Cu level in serum was significantly higher among asthenozoospermics and teratozoospermics as compared to the subjects with normal motility and normal morphology respectively.

- Mean serum zinc level showed a lowering trend from normozoospermic subjects towards azoospermics through oligozoospermics. Serum zinc level was also
higher in the subjects having normal sperm motility as compared to asthenozoospermics. Alpha glucosidase levels were found to have significant positive correlation with seminal plasma zinc levels.

Seminal plasma Cu level was found to be highest whereas serum and seminal plasma zinc level were lowest among the subjects having both lifestyle and toxicant exposure as compared to the subjects with no exposure, though the difference were statistically non-significant.

The study clearly suggests the role of lifestyle factors and reproductive toxicants in deterioration of semen quality as well as inducing DNA damage in sperm. Free radical generation induced by various lifestyle factors and reproductive toxicants might be associated with the impairment of semen quality, which is reflected in terms of the alterations in the antioxidant enzyme system and increased sperm DNA damage. Thus, it can be inferred that the elimination of oxidative stress whereas antioxidant supplementation could be useful in the prognosis of subjects showing sperm abnormalities and thus, the functional ability could be improved thereby.