Discussion

1. Fertility status

In Section-I, the critical analysis of the data collected for 150 normal healthy couples who were investigated for fertility status shows that 56% of the couples had male factor infertility. This observation falls very close to the recent data of WHO survey in which 51% couples were found to be infertile (Puri et al., 2000).

In the present study the fertile men whose wives had well defined reproductive abnormality makes about 35% of the population. These observations are also in agreement with those of other authors that 30% infertility is reported to be due to female factor (Martin, 1994).

In Section-II, in the occupation-based environmentally exposed subjects, the highest incidence of infertility (83% infertile) among the three exposed groups in the present study was observed in heat-exposed subjects. The cause of infertility could be multi-factorial according to observations recorded, e.g., due to heat exposure, wearing tight jeans and under-wears. Heat induced reduced sperm count is a well-established fact since 1960's when Procope (1965) documented that hyperthermia impairs sperm production. The reduced semen quality, delayed conception and decreased fertility due to heat exposure have also been reported by many workers (Rachootin and Olsen, 1983, Baird and Wilcox, 1986, Chia et al., 1994 and Mur et al., 1998).

Tight-fitting jeans and underwears heat the scrotum and testes which inhibits the sperm production and also affects the sperm vitality. The testicles were designed to hang free and remain cool (below the body temperature) and anything that raises them up to body temperature like hot baths, saunas, tight
Discussion

jeans, cycling, long periods of driving 'cooks' them and reduces fertility. The highest infertility in heat-exposed group (G-5) among the three occupationally exposed groups in the present studies also showed highly positive correlation with exposure to multiple partners (Jejeebhoy, 1998).

In heat exposed group (G-5), the highest percentage of people wearing tight underwears (25%) among all groups goes well in correlation with the highest infertility rate (83%) and suggests that this factor partly may also be partly contributing along with heat, to cause infertility.

In welding workers group (G-6), the welding workers also showed 50% infertility rate. The fertility status of these workers was better than that of severely heat-exposed group (G-5). In this group of subjects the exposure was also to heat and the welding fumes of Mild Steel. The correlation of delayed conception and reduced semen quality with respect to welding has also been reported by Bonde (1990b, 1993, 1999) and Bonde et al (1990).

In contrast to the other two occupationally exposed groups (heat and welding), the exponentially high fertility status among zinc-galvanizing workers (94% fertile) may be associated with their exposure to zinc fumes irrespective of the adverse effects of other environmental insults like heat and inorganic solvents. No specific reports were found with respect to zinc exposure at work place and fertility status among men.

2. Personal history

2.1 Age

The average age of both infertile as well as fertile men ranged between 28 to 33 years in the present study which is the peak reproductive age among men.
Discussion

After reviewing the semen characteristics and mean age of all the study groups, no case of age related azoospermia and oligospermia was observed. The results of the present study go well in agreement with the previous studies of Check et al (1989), Krause and Habermann (2000) and Sharon et al (2001) that no decline in fertility potential and semen parameters are associated with increase of male age.

2.2 Smoking and Alcohol habits

2.2.1 Smoking

The adverse effects of cigarette smoking on male fertility are well established since 1973 by Briggs, and later by many other workers (Evans et al 1981, Kulikauskas et al, 1985, Rantala and Koskimies, 1987 and Stuart, 1995). The effect was also observed in case of welding workers in the present study that in combination with heat and metal fumes, such a high incidence (30%) of smoking habits might contribute to their declined sperm count, subnormal sperm vitality and high incidence of infertility (50%). The significantly high (p<0.05) prevalence of smoking habit in infertile men especially in unknown azoospermics (21%) and normospermic-infertile men (20%), as compared to normal fertile subjects (9%), further strengthens the hypothesis that smoking may not have any direct effect on sperm production but it affects a person’s fertility status.

Saaranen et al (1986), Ochedalski et al (1994) and Marilyn et al (1996), in their study have concluded that smoking is associated with lowered semen quality in terms of sperm density, sperm count and motility. Regular smoking causes 23% decrease in sperm density and it also affects the hypothalamic-pituitary-gonadal axis, which most commonly affects the estradiol and estrone levels. In males, these hormones are required for testosterone production. Smoking damages the ability of the sperms to find the egg and excessive smoking may even damage the sperm DNA.
Discussion

2.2.2 Alcohol

The hypospermatogenesis and maturation arrest in subjects with second degree testicular failure in non-obstructive azoospermic (G-1.2) and oligospermic (G-2) subjects was found significantly correlated with their habit of alcohol consumption. Male infertility is a multi-factorial problem but high frequency of alcohol consumption especially in these two groups, who were not exposed to any other environmental insult; the alcohol might have caused deleterious effect on spermatogenesis as mentioned by many authors (Graffin and Wilson, 1992, Mann and Lutwak-Mann, 1981). Alcohol is described as the direct gonado-toxin in males, and its excessive use affects the hormonal axis (Melvyn, 1999).

2.3 Exposure to multiple partners

A significantly high exposure frequency in fertile men as compared to infertile men do not make any clear-cut consensus regarding the exposure to multiple partners and the fertility status of men. But, the highest exposure frequency (16%) in the heat exposed men and the highest infertility rate (83%) among all the study groups were strongly correlated. The results of welding workers (10% exposure frequency) were also found positively correlated. Sexually transmitted diseases (STD) which may arise as a consequence of multiple unsafe sexual exposure, are on the rise globally. It has been reported that among men with demonstrable causes of infertility, one in three is probably infertile as a result of STDs (Jejeebhoy, 1998).

3. Semen Profile

3.1 Semen volume

The mean semen volume of all the infertile and fertile groups in both Section-I and Section-II did not show any statistical difference with their fertility status. The semen volume in all the study groups in the present study was found to be within the normal range of 2 to 6 ml (WHO, 1999)
The bulk of the semen volume is contributed by the accessory glands i.e. the prostate and the seminal vesicles. No well-defined studies are available to support the significance of semen volume with the fertility status of men. A certain amount of seminal fluid is required for fertility but minimal and maximal volume of seminal fluid needed for fertilization are not known (Sherins et al, 1977). However, Gopalakrishnan et al (1992b) in their retrospective study conducted to find a correlation of semen volume with semen quality with respect to fertility indicated that high and low semen volumes were associated with high incidence of spermatozoa exhibiting sub-fertile characteristics. These authors further suggested that normal semen volume is not an indicator of semen quality. This fact was also strengthened in the present study that despite the normal semen volume in most of the infertile subjects in all the study groups, they had poor semen quality in terms of other semen parameters like spermatozoal concentration, morphology, motility, vitality and fertilizing ability.

High semen volumes (up to 10 ml) as observed in some of the oligospermic subjects (G-2) may be associated with inherently high seminal fluid production and long period of abstinence (Pryor, 1981, Comhaire and Vermeulen, 1995).

Semen volume may give a rough estimate of the semen quality and may be considered as one of the qualitative parameters for infertility evaluation but is not an absolute parameter for fertility assessment. Altered semen volume, either low or high may contribute to 10 to 20% cause of infertility (Gopalakrishnan et al, 1992b).

3.2 Semen pH

The mean semen pH of the infertile and fertile subjects ranged between 7.4 and 7.9 and found to be within the normal limits (7.2 to 8.0) as prescribed by WHO (1999).
Eggert et al (1993) stressed in their study that pH is an important determinant in the sperm-mucus interactions. But, there is no good evidence that semen pH singularly affects the sperm quality (Gopalakrishnan, 1995).

3.3 Non-spermatozoal cells in semen (Leucocytes and Immature germ cells)

The non-spermatozoal cells in the semen mainly comprised of polymorphonuclear leucocytes, epithelial cells and immature germ cells.

3.3a Leucocytes

Predominantly, the neutrophils are known to be present in most human ejaculates but their excessive presence (>1 million/ml), which is known as leucocytospermia, may indicate reproductive tract infection (WHO, 1999). The leucocytospermia, if remains undetected and untreated, may lead to defective semen profile in terms of reduced sperm count, sperm motility as well as loss of sperm function as a result of oxidative stress and secretion of cytotoxic cytokins (Wolff et al, 1990, Aitkin and Clarkson, 1987 and Hill et al, 1987).

Gopalakrishnan et al (1989) postulated the high incidence of morphological deformities of spermatozoa in subjects having abnormally high pus cells in their semen. These morphological abnormalities of the spermatozoa considered as pathological in most of the infertile subjects were associated with malformation of sperm head. This could be due to the altered distribution and differential functional maturity of chromatin material in spermatozoa. Evenson et al (1980) proved by their flow cytometry study of spermatozoa that there is significant decrease in resistance of the denatured spermatozoa in the presence of high concentration of pus cells in sub-fertile subjects. The disturbance occurring at the chromatin distribution level results either in excessive nuclear material forming large, amorphous heads or deficient nuclear material forming micro-heads. These morphological changes like amorphous or micro-heads were also noticed in the present study which were reversed after successful treatment.
of infections and the response to treatment depended on the chronicity and the site of infection

Since malformed spermatozoal head is directly correlated with fertility status of men (Roger et al, 1983), it is yet to be established what kind of organelle in the head are responsible for their structural deformity in response to infections. Gopalakrishnan et al (1988) in their study also described that characteristics of spermatozoa severely alter even in men with asymptomatic bacterial infections. The highest frequency of men having abnormally high leucocytes in semen of oligo- and normospermic-infertile subjects in the present study may also be the significant contributing factor to their subnormal sperm motility, vitality and high incidence of morphologically abnormal spermatozoa as mentioned by Gopalakrishnan et al (1988, 1989). Conception could be apparently guaranteed if these men were treated for leucocytospermia.

3.3b Immature germ cells

The presence of immature germ cells observed predominantly in non-obstructive azoospermic and oligospermic-infertile subjects in non-occupationally exposed group and in the heat-exposed azoospermic subjects was in perfect correlation with their clinical examination and elevated FSH levels. The presence of immature germ cells in semen was in perfect agreement with their testicular biopsy reports, which also correspondingly showed the hypospermatogenesis and maturation arrest and confirmed that azoospermia was of non-obstructive nature.

The elevated FSH levels and significantly low values of biochemical testicular markers like lactic dehydrogenase in non-obstructive azoospermic subjects observed in this study and also that of Roy et al (2001) are no doubt the reliable markers of the spermatogenesis but seminal germ cell morphology studies are the only non-invasive dependable testicular marker to establish a definite diagnosis of azoospermia.
Discussion

The presence of immature germ cells in different stages of spermatogenesis ranging from primary spermatocytes to spermatid in semen as confirmed by Papanicolaue staining technique WHO (1999) was a clear indicator to rule out obstructive azoospermia from non-obstructive azoospermia. It was suggestive of mainly two things:

1) The azoospermia is of non-obstructive nature
2) The spermatogenic status of testis can be well recognized

Roy et al (2001) also established a similar hypothesis that immature germ cells in the semen of azoospermic or oligospermic patients is predictive of second-degree testicular failure associated with spermatogenetic disorders which can be seen as maturation arrest at any stage.

On the other hand, absence of immature germ cells in 7% of azoospemic subjects in the present study also supported the sensitivity of seminal germ cell morphology evaluation technique and established that the subjects had obstructive azoospermia. The results well matched with those of testicular biopsy reports of these subjects and that of Roy et al (2001) which showed normal spermatogenesis up to mature spermatozoa level in cases of obstructive azoospermic patients.

The correlation of immature germ cells in the semen with the fertility status was well studied by Gopalakrishnan et al (1989). They derived a hypothesis that during the late stages of spermatogenesis, certain membrane elaborations, known as membrane scrolls may be derived from nuclear envelope which indicate the formation of new membrane and impairs further stages of spermatogenesis. Gopalakrishnan et al (1989) further elaborated that these malformed spermatozoa recognized as immature germ cells contain finely granular nuclear chromatin and dense clumps of nucleolus. Reduced fertility rate
Discussion in the presence of immature germ cells has also been reported in their study that was clearly noticed in the present study as well.

### 3.4 Sperm morphology

Remarkably high percentage of morphologically abnormal spermatozoa in terms of mostly head defects in 20% and 28% of the men in oligospermic-infertile and normospermic-infertile subjects respectively was positively correlated with their fertility status and the results of the present study agree with the hypothesis (Roger et al, 1983, Gopalakrishnan et al, 1990a and Check et al, 1992) that abnormalities in the sperm head, with a consequent loss of functional nuclear chromatin would seriously reduce their fertilizing potential in men.

This functional deformity in the sperm head nuclear chromatin may often lead to loss of plasma membrane integrity of spermatozoa which fail to decondense at the time of acrosome reaction. The morphological defects in spermatozoa can be attributed to various causes at any stage in sperm development; both genetic or acquired. Gopalakrishnan et al (1992a) also emphasized the importance of sperm morphology and mentioned that at least 14% morphologically normal sperms should be required for fertilization to occur, whereas WHO (1999) established >30% as normal, standard sperm morphological criteria.

### 3.5 Sperm count

The results of the present study clearly indicate that sperm count is one of the most important parameters to decide the fertility status of men, as none of the men with less than 20 million/ml sperm count was found to be fertile. Among the total population of 150 subjects in Section-I, only 61% of the subjects including the fertile men (44%) had normal sperm count. However, 20% of the population had oligospermia and 19% men presented with azoospermia. Such a high incidence of azoospermia and oligospermia may likely be due to lifestyle, nutritional habits, environmental factors and congenital defects.
Carlsen et al (1992) have reported that over the last 50 years, the average sperm count of men have dropped by one half and the semen volume by 20%.

In the present study, the data regarding environmentally exposed groups (section-II) clearly indicates that frequent exposure to heat for longer durations (5-6 hours per day) has direct effect on spermatogenesis and the fertilizing ability of males. The markedly reduced sperm count in males exposed to high temperature (G-5) and welding fumes (G-6) were significantly correlated with each other. Dikshit et al (1987) also discovered that in industrial employees, working long hours in poorly ventilated, hot and chemically infested conditions were twice likely of having oligospermia than office workers.

On the other hand, a significantly improved sperm count in galvanizing workers may be due to inhalation of Zn fumes which seems to be positively correlated with their best fertility status among all the study groups.

3.6 Sperm Vitality and Motility

Looking at the status of spermatozoal vitality (%live sperms) and Rapid Progressive Motility (RPM) in infertile subjects, both of these vital parameters were found markedly reduced (p<0.001) in oligospermic-infertile and normospermic-infertile groups as compared to fertile men. A similar exponential decline in sperm vitality and motility was observed in heat and welding workers as shown in chapter 4, sections 3.6 and 3.7. The zinc galvanizing workers shared a comparatively better status than the other two exposed groups. The reduced semen quality in terms of sperm vitality and motility was positively correlated with infertility in the present study which strengthens the age old theory of Leeuwenhock as mentioned by Mann and Lutwak-Mann (1981) that the birth of offspring can only be guaranteed if sufficient number of spermatozoa in man are energetically motile to survive in the female tract for a certain period of time, presumably to attain their full potential. Spermatozoa have adaptability to reach...
the ovum in the female tract due to their vigorous motility of tail and the act of penetration into the ovum can be proclaimed only by alive cells (Tortora, 1983).

For fertilization, only one sperm is required but the minimum standard level for sperm vitality (at-least 75% live spermatozooa in a normal fertile man) (WHO 1999). This fact was well confirmed in the present study.

The observations made on the sperm vitality and motility in oligospermic-infertile and normospermic-infertile men as well as in the occupationally exposed groups (section-II) clearly show that the sperm count is not the only deciding factor for male fertility, rather the spermatozoa must have an adequate level of viability and rapid progressive motility to travel through the female tract and penetrate the zona pellucida which is a pre-requisite for natural conception (Mortimer, 1994). Heat related deteriorated sperm functions like sperm vitality and motility are well documented (Mur et al, 1994).

4. Hormone profile

No significant difference in testosterone levels among infertile and fertile groups in section-I and occupationally exposed groups in section-II shows that infertility in males under any conditions whether normal or under environmental stress due to exposure to heat, noxious chemicals and metal fumes do not affect their androgenic status. This also supports the fact that infertility and impotence is not synonymous (Martin, 1994).

The normal LH levels in G1.1 group of azoospermic subjects and also the absence of immature germ cells in their semen positively suggested to classify the cause of azoospermia in them probably due to ductal occlusion. The high FSH levels in this group (obstructive azoospermic) may be associated with negative feed back mechanism through inhibin on the hypothalamic-pituitary axis. On the other hand, abnormally high LH and FSH level in rest of the
azoospermic subjects (G1.2 and G1.3) significantly correlated with their hypo-spermatogenic status due to first or second-degree testicular failure. The High FSH levels due to maturation arrest at any mitotic stage in these subjects were also positively correlated with the presence of immature germ cells in the semen. In case of hypo-spermatogenesis and maturation arrest the LH and FSH levels tend to elevate due to negative feed back on the hypothalamic-pituitary-testicular control mechanism as also reviewed by Griffin and Wilson (1992).

The most probable cause of testicular defects in azoospermic and severely oligospermic men and the subsequent low sperm count, vitality and motility among infertile subjects in both environmentally exposed and unexposed groups has been detected to be multi-factorial.

A wide range of factors contributing to normal spermatogenesis and sperm metabolism other than congenital defects have been explored in this study to explain the possible causes of abnormal semen profile in infertile men as compared to normal fertile men.

A highly positive correlation has been observed between the low semen profile of infertile and occupationally exposed group of subjects with reference to the semen biochemistry, the electrolyte and trace element levels.

5. Testicular biopsy and Histopathology

The azoospermic subjects who showed high serum FSH levels and also showed immature germ cells in their semen also showed hypospermatogenesis in their histopathology sections of testicular biopsies. Biopsy studies were undertaken to confirm the cause of azoospermia whether obstructive or non-obstructive and to ascertain the reliability of the germ cell morphology studies, various biochemical markers and trace element levels in their assessment. The
correlations were of significant value in obstructive and non-obstructive azoospermic subjects as also observed by Roy et al (2001)

6. Semen Biochemistry

The seminal plasma biochemical estimations in the present study have been proved to be highly suggestive of the physiological and anatomical status of the subject's reproductive functions in different groups. The significantly low levels of all the four parameters that is Lactic dehydrogenase, fructose, α-glucosidase and citric acid in G-1.1 azoospermics as compared the normal control group (G-4) clearly indicated that these subjects had ductal occlusion.

6.1 Correlation of lactic dehydrogenase (LDH) levels with semen parameters

Significantly low levels of lactic dehydrogenase (LDH) in non-obstructive azoospermics due to testicular failure (G-1.2), in unknown azoospermics (G-1.3) and oligospermics (G-2) as compared to that of normospermic subjects (G-3 and G-4) and on the other hand highest levels of LDH in zinc-galvanizing workers (G-7), who had highest sperm count among all the study groups clearly indicates that lactic dehydrogenase activity in semen is positively correlated with sperm count. This fact has long been established by Szeinberg et al (1966) when they discovered that this enzyme was completely undetectable in damaged germinal epithelium which resulted into delayed or defective spermatogenesis or complete spermatogenic arrest. Blackshaw et al (1973) also discovered suppressed formation of LDH in heat-damaged male gonads. In the present study the LDH level in heat-exposed men was found to be comparatively lower than that of control group (G-5) but the difference was not statistically significant.

The correlation of seminal plasma LDH levels with sperm quality, in terms of motility and fertilizing ability was also discussed by Burgos et al (1979), Gavella and Cvitkovic (1985), Casano et al (1991), Verma et al (1993),
Orlando et al (1988, 1994), Noguera et al (1994), Aydin et al (1997) and Laudat et al (1997). This sperm specific unique mitochondrial enzyme appears in the pachytene stage in primary spermatocytes and these LDH laden mitochondria are carried through all the stages of gametogenesis till final mature sperm is formed. This mitochondrial enzyme is released during glycolysis of fructose for the production of energy required for sperm integrity, motility and sperm functions (Mann and Lutwak-Mann, 1981). In the present study, the correlation of LDH levels with sperm vitality was clearly noticed in oligospermic (G-2) and normospermic-infertile (G-3) subjects in section-I and heat-exposed (G-5) and welding workers (G-6) in section-II. A significantly positive correlation with sperm motility was found only in heat-exposed workers (G-5).
6.2 Correlation of Fructose levels with semen parameters

The seminal plasma fructose did not show any significant correlation with sperm production in this study as also mentioned by Mann and Lutwak-Mann (1981) that fructose is a seminal vesicle marker. Rajalaxmi et al (1989) also depicted in their study that fructose levels in normospermic-infertile and proven fertile men were similar. The significantly low levels of fructose in obstructive azoospermic (G-1.1) and unknown azoospermic (1.3) may be due to ductal occlusion and in case of heat-exposed group (G-5), extremely low fructose levels may be correlated with exhaustive use of ATP and high metabolic rate due to high temperature.

In semen, the main source of ATP production for spermatozoal survival and metabolic processes like motility and fertilizing ability is provided mainly by anaerobic fructolysis (Mann and Lutwak-Mann, 1981).
A highly positive correlation of fructose levels with sperm vitality and motility was observed in the present study as the asthenozoospermic subjects in most of the study groups i.e. normospermic-infertile, heat-exposed workers, welding workers and zinc-galvanizing workers (G-3, G-5, G-6 and G-7) showed significantly low fructose levels. However, the fructose levels in oligo- and normospermic-infertile subjects with high percentage of dead spermatozoa was significantly low. Similar findings were reported in the recent studies of Suominen (2001) and Gonzels and Villena (1997, 2001).
6.3 Correlation of neutral α-glucosidase levels with semen parameters

The estimation of α-glucosidase in seminal plasma is one of the important laboratory diagnosis tests prescribed by WHO (1999) to differentiate obstructive azoospermia from non-obstructive azoospermia as its activity is reported significantly reduced in obstructive azoospermia (Trembley et al, 1982, Guerin et al, 1990, Yeung et al, 1990 and Yeung and Cooper, 1994). Similar findings that significantly reduced neutral α-glucosidase levels in obstructive azoospermic (G-1.1) group in the present study strongly recommended that estimations of neutral α-glucosidase levels in azoospermic patients be carried out before going for expansive and invasive procedures to rule out obstructive azoospermia.

The physiological role of α-glucosidase in spermatozoal metabolic functions and epididymal sperm maturation was established by Kalla et al (1997). Remarkably reduced enzyme activity in oligospermic (G-2) and heat-exposed (G-5) subjects signified either the subnormal functional potential of spermatozoa at the epididymal level or obstructive oligospermia in some cases as reported in chapter 4 section 5, which was positively correlated with markedly reduced sperm motility and high percentage of dead spermatozoa in this group. Kalla et al (1997) also discovered that the enzyme activity was maximum in caput region and decreases progressively in corpus and cauda epididymis, which was positively correlated with sperm motility. Ali et al (1994) also suggested the correlation of α-glucosidase with fertilizing potential of spermatozoa.

6.4 Correlation of citric acid level with semen parameters

The exact metabolic role of seminal plasma citric acid is yet to be established but its levels in the seminal fluid help in the assessment of ventral lobe prostatic functions. Dondero et al (1972) described that seminal citric acid levels reflect an individual’s androgenic status. Boursnell and Noble (1975) and Karagiannidis (1976) reported that citric acid in seminal plasma is a chelator of Ca and Zn and it may be helping in Zn absorption in spermatozoa. In the present
study, no definite consensus could be arrived at regarding the role of citric acid in sperm metabolism. Significantly reduced levels in obstructive azoospermic (G-1.1), unknown azoospermic (1.3) and oligospermic (G-2) could be correlated with ductal occlusion.

7. Electrolyte levels

7.1 Correlation of Electrolyte levels with Sperm Count

A significantly high serum levels of Na in non-obstructive azoospermic (G-1.2) and both Na and K in oligospermic (G-2) men as compared to the normal control men (G-4) may lead to hypothesize that hypernatremia alone or in combination with hyperkalemia due to any cause in the body may be responsible for hypospermatogenesis and maturation arrest in these subjects. This fact was further strengthened when zinc galvanizing workers showed significantly low serum K and Na levels as compared to those of control group (G-4) and their sperm counts were also positively correlated with their serum electrolyte levels.

The results clearly suggest that serum electrolyte levels can be used as one of the differential diagnostic criteria to rule out the cause of testicular failure. Further studies are required to strengthen this hypothesis, as the role of electrolytes in spermatogenic development is still unexplored.

On further investigations, the seminal plasma K levels in non-obstructive azoospermic group (G-1.2) were found significantly low in this study. These reports suggest that along with testicular failure, the seminal vesicles of these subjects may also be underdeveloped as Mann (1964) reviewed that under normal physiological conditions, the vesicular fluid contains high K and low Na level and reverse correlation exists in the prostatic fluid. The relatively low fructose and normal citric acid levels in these subjects was positively correlated with their low seminal plasma K levels.
7.2 Correlation of Electrolyte levels with sperm vitality

Relatively normal seminal plasma K levels and significantly high Na levels in the in heat-exposed (G-5) and welding workers (G-6) and on the other hand significantly high spermatozoal K and Na levels in oligo-infertile (G-2), heat-exposed (G-5) and welding workers (G-6) clearly shows that K/Na ratio in these subjects was greatly disturbed. The disturbance might have been due to dysfunction of Mg\textsuperscript{2+}-dependent Na\textsuperscript{+}-K\textsuperscript{+} pump. The presence of membrane bound enzymes and substrate specific binding sites was discussed by Gordon and Dandekar (1977). They further reported that Na\textsuperscript{+}-K\textsuperscript{+}ATPase along with balancing of Na\textsuperscript{+}-K\textsuperscript{+} exchange pump was also involved in spermatozoal head-to-head association which were facilitated by Mg, Mn and Ca ions. Relatively excess of intracellular Mg\textsuperscript{2+} inhibits the Na\textsuperscript{+}-K\textsuperscript{+}ATPase activity.

In the present study, a very high intracellular K and Na in oligospermic-infertile (G-2), heat-exposed (G-5) and welding workers (G-6) may be eventually an after-effect of exceptionally high Mg inside the spermatozoa. High intracellular K and Na gives rise to hyper-polarization of spermatozoa. Excessive influx of cations cause the spermatozoa to swell up and finally the plasmalemma bursts and they become dead. Abnormally high percentage of dead cells in these groups was positively correlated with intracellular high electrolyte levels. Apparently high levels of K and Na in seminal plasma may be due to the release of intracellular contents from the dead spermatozoa into the seminal plasma.

7.3 Correlation of Electrolyte levels with sperm motility

In normal motile spermatozoa the intracellular K is higher than extracellular and reverse is true for Na as in any other cells of the body and this normal K/Na gradient is controlled by Na\textsuperscript{+}-K\textsuperscript{+} pump (Quinn and White, 1967). The correlation of abnormally high spermatozoal K with low sperm motility has long been postulate by many workers (Sheth and Rao, 1962, Mann, 1964).
Discussion

In the present study also a highly positive correlation between elevated spermatozoal K and significantly reduced sperm motility in 90% of the oligospermic, 70% of heat-exposed, and 62% of welding workers were noticed.

The above-mentioned electrolyte imbalance due to heat exposure and environmental pollutants has a very simple and well-established physiological basis. With the effect of high temperature, the membrane permeability is known to increase, as heat increases the net diffusion. The membrane proteins get configurationally changed and the pores of leak channels are widened which allow free permeability of K and Na first at the blood-testes barrier, and then at the seminal plasma - spermatozoal membrane barrier.

Extremely high levels of both potassium and sodium inside the spermatozoa indicate that both the leak channels and the Na\(^+\)-K\(^+\) pump might have been so affected, that membrane potential would have also been altered. The high influx of Na inside the cells leads to depolarization of the spermatozoal membrane and the spermatozoa remain in the depolarized state as the net intracellular K level is also manifold high as compared to extracellular, due to increased permeability through leak channels (Guyton and Hall, 2000 and Ganong, 2002). There is a net positive charge inside the spermatozoa and they become hyperpolarized and nonfunctional, which immobilizes them and reduces their fertilizing ability.

After continuous sheer stress of electrolyte imbalance, the spermatozoa become dead as their membrane fails to undergo re-polarization after the abnormally high influx of both Na and K. The Na\(^+\)-K\(^+\) pump becomes incapable of balancing such a high degree of electrolyte imbalance. An exorbitant amount of ATP is used up in this process that the cells become deficient of ATP required for sperm motility. That is why, in all the three occupationally exposed groups in Section-II (heat-exposed workers, welding workers and the zinc galvanizing
The electrolyte status of galvanizing workers (G-7) was different from all other study groups. The observations made with reference to that group clearly suggest that correspondingly low levels of potassium and sodium in serum and optimally higher levels in the seminal plasma and spermatozoa (except for lower spermatozoal Na) in these workers has brought drastic improvements at the spermatogenic level in terms of better sperm output and fertilizing ability of the spermatozoa. These findings strengthen the fact that in this group, 30 out of 32 men were fertile. The high potassium and low sodium inside the spermatozoa clearly shows their normal and intact integrity in this group.

8. Trace element levels

To establish a cumulative role of trace elements (Zn, Mg, Ca, Cu, Fe, Mn, S, P, As, Cr, Al, Ni, Mo, Pb, B, Hg, Co and Cd) in male fertility with respect to their semen profile is yet an incomplete task. In the present study, an attempt has been made to recognize a possible role of each trace element with respect to its concentration in the serum, seminal plasma and spermatozoa. None of the previous studies give a complete picture of their metabolic significance with respect to their serum, seminal plasma and spermatozoa levels. Many scientific teams have estimated these levels singularly or in groups in only serum, in only semen, in only spermatozoa, in serum and spermatozoa or in serum and seminal plasma, with respect to fertility status of men as reviewed in chapter 2. Looking at the available data of various scientific groups, no clear-cut correlation can be made with regard to the range of concentrations of all the above mentioned trace elements separately in serum, seminal plasma and spermatozoa separately in an average fertile male. The present study was designed to develop a comprehensive approach to find such a correlation and to understand their metabolic importance in male fertility regulation.
Discussion

The results of the present study clearly indicate that Zn was found approximately 75-100 times greater in seminal plasma as compared to serum and spermatozoa. This observation seems to be in true correlation with the long established facts that prostatic Zn concentration is maximum of all the body tissues (Mann, 1964). The Ca in seminal plasma, which is chiefly contributed by the prostatic secretion was found to be three times greater than the blood plasma (Mann, 1964 and Quinn et al, 1965).

8.1 Correlation of Trace element levels with sperm count, vitality and motility

8.1.1 Zinc

The analysis of data on zinc levels in serum, seminal plasma and spermatozoa in different infertile and fertile groups in section-I and also in occupationally exposed groups in section-II clearly indicates that there is no significant correlation between serum zinc levels and the spermatogenic status as its levels shared a same range in both obstructive and non-obstructive azoospermia as well as in the normospermic-infertile and normal fertile groups. The serum zinc levels in heat-exposed and even in the zinc galvanizing workers were also in the same range as that of the control group. The role of serum zinc levels with respect to semen quality is not well studied but in a recent study Lin et al (2000) and Kruse et al (2002) also showed similar results that they found no significant association of seminal plasma and serum zinc levels with the semen quality. No correlation of serum zinc levels with sperm motility and sperm vitality was observed in the present study. It can be emphasized on the basis of present results and also the previous findings of Lin et al (2000) and Kruse et al (2002) that routine serum zinc estimation do not give any dependable inference for infertility evaluation in males. Significantly high serum zinc levels were detected only in oligospermic-infertile and welding workers as compared to that of control group.
In oligospermic-infertile subjects the serum levels of Zn, Mg, Ca, and also that Cu and Fe were found to be significantly higher than the normal fertile subjects which shows that these subjects might have been associated with some underlying pathology as their serum Na and K levels were also high. Such a high serum electrolyte and trace element levels might have caused overall low semen profile such as suppression of spermatogenesis, subnormal rapid progressive sperm motility, less than normal percentage of viable spermatozoa in their semen and abnormally high population of morphologically abnormal spermatozoa. This high serum profile of electrolytes and other trace elements (Zn, Mg, Ca, Cu and Fe) in oligospermic-infertile group is also positively correlated with high prevalence of immature germ cells in their semen. It may be suggestive of the hypothesis that high serum levels of trace elements and electrolytes affect the blood-testis barrier and cause both proliferative and maturation arrest during spermatogenesis. No significant correlation between high serum zinc levels and leucocytospermia (leucocytes >1 million/ml in semen) was observed in the present study as also mentioned by Kruse et al (2002) and Waltraud et al (2002).

The seminal plasma and spermatozoal zinc levels showed a remarkable positive correlation with spermatogenic status, sperm vitality and the sperm motility. In seminal plasma significantly low zinc levels detected in non-obstructive azoospermic subjects and remarkably high levels in zinc galvanizing workers in the present study clearly show that zinc is an essential component for sperm production and its deficiency could cause as severe effects as azoospermia. The seminal plasma zinc levels were not significantly correlated with spermatozoal vitality.

Pandey et al (1983) and Sorensen, et al, (1999a) observed the inter-correlations between Zn, Mg and Ca. Pandey et al (1983) also found that the levels of Zn Mg and Ca in whole semen were significantly subnormal in 82% of the infertile men. They also mentioned that Zn deficiency has been related to defective germinal epithelium in testes, atrophic seminiferous tubules, lack of
spermatid maturation, azoospermia and low gonadotropin-androgen levels. In a recent study conducted by Wong et al. (2002), Zn has been reported to be one of the most essential element in spermatogenesis which acts as co-factor of metalloenzymes involved in DNA transcription, expression of steroid receptors and protein synthesis. In another study, Chia et al. (2000) postulated that seminal plasma Zn levels were significantly correlated with sperm density (p<0001) and Zn contribute towards fertility through positive effect on spermatogenesis.

Correlation of Zn, Mg and Ca was also noticed in the present study with reference to sperm motility as the concentration of these three elements in seminal plasma were found to be significantly low in astheno-oligospermic-infertile group of subjects (G-2).

Seminal plasma is a vital fluid for the function of spermatozoa in the natural process of mating apart from being a transport medium. The disturbances in the composition of the accessory sex gland secretions and even the coordination of sequence in which the glands empty their contents could hamper sperm motility and their survival (Gopalakrishnan et al., 1990b). Zinc in semen originates from prostatic glandular epithelium and its receptors are located on the spermatozoa. A positive correlation of seminal plasma zinc levels with spermatozoal motility was also discussed by Gopalakrishnan et al. (1990b), Sorensen et al. (1997, 1998).

Herrmann (1971) reported the presence of zinc-binding proteins of prostatic origin which facilitate transfer of Zn to human spermatozoa. Stegmayr et al. (1982) and Stegmayr and Ronquist (1982) discovered prostasomes in seminal plasma either free or attached to spermatozoa. These organelle stimulate forward progressive motility of spermatozoa through Zn, Mg and Ca dependent ATPase present in prostasomes. Stankovic and Mikac-Devic (1976), Caldamone et al. (1979), and Umeyama et al. (1986) have also shown highly positive correlation between Zn, Mg and Ca in seminal plasma. They discovered that
forward progressive motility got inhibited or stimulated depending upon their concentrations.

Carpino et al (1998) discovered seminal zinc fractions are bound to high molecular weight proteins (HMW) in the seminal plasma. They discovered that increased unbound seminal zinc contributes to decreased sperm motility. In oligo-asthenozoospermic subjects the decreased motility is correlated with high zinc uptake by spermatozoa and subsequently low seminal plasma levels. The high intra-spermatozoal zinc also reduces the membrane functionality and increases the dead sperm percentage in the semen. Similar findings were also detected in the present study when zinc levels in oligospermic-infertile group (G-2) in spermatozoa were highest among all the study groups and found to be ten fold higher than the normal fertile subjects. Such a high zinc levels in spermatozoa in oligospermic-infertile group were proved to be toxic in terms of sperm motility, viability and their fertilizing ability.

Another point of view regarding reduced sperm motility with high spermatozoal zinc content was discussed by Henkel et al (1999) as they studied that comparatively low flagellar zinc contents are required in the outer dense fibers (ODFs) of flagella to achieve stiffening of sperm flagella during forward progressive movement. The stiffening is achieved by formation of disulphide (-S-S-) bridges, which are a prerequisite for the generation of sperm motility. High spermatozoal zinc levels as observed in oligospermic-infertile group in this study may be correlated with low sperm motility due to lack of flagellar stiffening.

Zinc is an essential spermatozoal constituent which is required in adequate concentration for maintaining normal sperm functions. Bedwal and Bahuguna (1994) discovered that some Zn-metalloenzymes responsible for sperm motility are present in the spermatozoa, which become dysfunctional with Zn deficiency. As a matter of fact, no case of spermatozoal zinc deficiency was noticed in any of the groups in this study. Significantly high zinc levels in zinc
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galvanizing workers as compared to normal fertile group in seminal plasma and in spermatozoa has shown dramatic improvements in their semen profile as their average sperm count was higher than the control group and their spermatozoa showed considerably good rapid progressive motility. The average percentage of viable spermatozoa was also within the normal range. This shows that significantly improved zinc status in spermatozoa (up to five times higher than the normal control group) was specifically due to zinc inhalation during the process of galvanizing and is beneficial in terms of fertility as zinc galvanizing group showed the highest fertility rate among all the study groups (93%).

Recently much debate is going on regarding the beneficial role of zinc on sperm production and functions (Sorensen et al, 1999b). The most essential function of Zn for fertilization has been reported as its contribution in chromatin de-condensation at the time of acrosome reaction and human spermatozoa are reported to accumulate more zinc in the head from prostatic fluid upon ejaculation (Kvist 1980a, 1980b, 1980c, Kvist et al,1980; Kvist and Eliasson, 1980 and Kvist et al, 1987). High zinc levels, as found in galvanizing workers (G-7) in the present study, are known to be protective against oxidative stress to spermatozoa and prevents premature acrosome reaction, thus giving membrane stability to the spermatozoa (Suzuki et al,1985, Gavella and Lipovac, 1998 and Riffo et al, 1992). Further, Gavella et al (1995, 1999) also postulated the protective role of zinc. It inhibits superoxide anion production and superoxide dismutase (SOD) activity in spermatozoa. This activity is extremely important in certain oxidative events occurring after ejaculation into the female tract and also some modulatory role in spermatozoal functions required for fertilization.

High spermatozoal zinc levels observed in heat-exposed and welding workers were due to different reason. Heat-exposed subjects showed four times higher spermatozoal zinc whereas welding workers had almost two times zinc concentration in their spermatozoa as compared to the normal control group. These elevated levels in heat-exposed and welding workers were due to overall
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increased sperm cell membrane permeability on account of hyperthermia in both the groups. These groups of workers showed exceptionally high electrolyte levels as well as other trace elements like Mg, Ca, Fe, S, P and Al.

It can be reviewed from the present results that exposure to high temperature does not alter the normal physiology of an individual as serum levels of all the trace elements were found to be within the normal limits. Most of the seminal plasma trace elements were also normal that shows normal prostatic functions in these men.

However, abnormally high spermatozoal levels of almost all the trace elements in heat-exposed subjects strengthens the belief that spermatozoa are most sensitive to high temperature. Boursnell and Noble (1975) mentioned that Zn, which represents a typical secretory product of the male accessory fluids is firmly associated with spermatozoa. Most of the zinc taken up by spermatozoa is highly temperature-dependent. In apparently ‘cooked’ testes at high scrotal temperatures the membrane permeability of spermatozoa is highly increased which results into high influx of ions. Extremely high concentrations especially of Zn, Ca, Mg and Mn along with K cause markedly reduced sperm motility, decreased fertilizing ability, increased number of dead cells and high percentage of morphologically abnormal dead cell, mostly with head defects.

Correlation of high spermatozoa zinc levels and correspondingly high percentage of dead spermatozoa was significantly observed in the present study. Similar findings were reported by Lindholmer and Elisson (1972) when they discovered high spermatozoal Zn and Mg in subjects with high percentage of dead and immotile spermatozoa.

Relatively high spermatozoal levels of Zn and almost all the trace elements in subjects with less than normal percentage of viable spermatozoa
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(sperm vitality <75%) in infertile groups of both unexposed as well as exposed groups is clearly indicative of mainly five things:

i) defective Na⁺-K⁺ ATPase pump
ii) increased membrane permeability through leak channels
iii) de-polarization of the spermatozoal membrane
iv) reduced fertilizing ability of the spermatozoa
v) infertility.

The presence of abnormally high dead spermatozoa in semen of oligospermic-infertile, normospermic infertile and heat-exposed groups could be due to multiple reasons like oxidative stress due to infections (Wolff et al, 1990; Aitkin and Clarkson, 1987 and Hill et al, 1987), environmental stress due to heat and pollutants (Mur et al, 1998), and nutritional deficiencies (Steven, 2000).

Gopalakrishnan et al (1990b) discussed the importance of surface membrane properties and membrane integrity of spermatozoa in the process of normal gamete fusion. Any process, which leads to increased life span of spermatozoa, will enhance the possibility of conception. They also discussed that zinc has a definite role in improving sperm membrane integrity. That is why any functional disturbance in the prostate gland secretion may lead to infertility due to reduced fertilizing ability of spermatozoa.

Carpino et al (1998) hypothesized that oligoasthenozoospermic subjects who had higher intra-spermatozoal zinc levels showed reduced functional integrity of their sperm membrane. This fact was strengthened by the present study when similar findings were attributed to oligoasthenozoospermic group. Further investigations are required to find a definite correlation between zinc levels and spermatozoal integrity and viability.
8.1.2 Magnesium (Mg)

Serum magnesium (Mg) levels also showed no definite correlation with sperm count, vitality and forward progressive motility in most of the study groups except that in oligospermic-infertile group where the levels were significantly high. On the other hand in unknown azoospermic and zinc galvanizing group (average sperm count 131.25 million/ml) the levels were found significantly lower than the normal fertile group (G-4). So, in the present study, no true conclusion could be drawn from the serum magnesium levels about the cause of infertility. Also, no studies are available regarding correlation of serum Mg levels and semen profile with regard to fertility status in males. With reference to the present results it can be postulated that serum Mg levels can not be taken as the diagnostic tool for evaluation of male infertility.

Similarly, the seminal plasma Mg levels also showed no correlation with sperm count and spermatozoal vitality except in oligo-asthenospermic subjects where the seminal plasma Mg was found to be significantly lower and reverse was true for the spermatozoal concentration which was found to be remarkably higher in these subjects as compared to that of subjects with normal progressive sperm motility.

Spermatozoal Mg concentration in heat-exposed and welding workers was also found to be extremely high; in heat exposed, approximately 10 fold and in welding workers approximately 2 fold increase was observed. This was probably due to the obvious reason that exposure to heat increases the membrane permeability of spermatozoa.

Over the years there has been much debate on the role of Mg in sperm motility. Its effectiveness and functionality has been discussed mainly in combination with Zn and Ca by many workers (Lindholmer and Eliasson, 1972, Stegmayr et al 1982, Pandey et al 1983, Adamopoulos and Deliyiannis 1983, Saaranen et al, 1989b and Sorensen et al, 1999a). Abou-Shakra et al (1989)
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detected no significant correlation of seminal plasma Zn and Mg levels with either spermatozoal concentration or motility. The similar findings were attributed in the present study. Stegmayr et al (1982) discovered that Mg, Ca and Zn in the seminal plasma exert stimulatory or inhibitory effects on sperm progressive motility depending on their concentrations.

Contrary to the present findings Papadimas et al (1983) and Umeyama et al (1986) found a highly positive correlation between zinc and Mg concentration in the whole semen in the different study groups (normal fertile, normospermic-infertile, oligospermic, severely oligospermic and azoospermic) that their concentration decreased gradually in proportion to sperm density. Their studies also suggested synergistic action between Mg and Zn.

The correlation of low seminal plasma Mg and low sperm motility could be explained on the basis that Mg is an important cation associated with many enzyme systems like Na^+-K^+ ATPase and adenylate cyclase especially involved in carbohydrate metabolism. This enzyme is particularly required in the phosphorylation of protein kinase and c-AMP-dependent ATP release required for sperm motility. The energy for sperm motility is derived from anaerobic glycolysis of fructose for which the substrate is provided by the seminal vesicles (Mann and Lutwak-Mann, 1981, Pandey et al, 1983). Low seminal plasma Mg in oligoasthenospermic subjects as noticed in this study, may be correlated with low enzyme activity responsible for fructolysis. Stegmayr et al (1982) discovered some secretory granules of prostatic origin in the seminal plasma (prostasomes) which may exert a direct regulatory role in spermatozoal motility. The Mg and Ca dependent Na^+-K^+ ATPase is reported to be present in the membrane of these granules, which is competitively inhibited by Zn. The high spermatozoal concentration of Mg in oligospermic group is possibly due to altered membrane permeability of spermatozoa due to defective Na^+-K^+ pump under low Na^+-K^+ ATPase activity.
8.1.3 Calcium (Ca)

No correlation of serum Ca concentration was observed with sperm count, vitality and rapid progressive motility. Only in oligospermic-infertile the serum Ca levels were significantly high and in zinc galvanizing group the levels were significantly low. These groups showed the similar trend for serum electrolytes (K and Na) and for Zn, Mg, Ca and Fe in oligospermic-infertile group and for K, Na, Mg and Ca for zinc galvanizing group. These findings only weakly suggest that high serum electrolytes, Zn, Mg, Ca and Fe in the body severely affect the spermatogenesis and their comparatively low levels improve the sperm output. No such reports are available to support this fact and certainly more extensive studies are required in this direction.

The seminal plasma Ca concentration also showed no significant correlation with sperm count and vitality in the present study. These findings precisely matched with those of earlier studies (Umeyama et al 1986, Abou-Shakra et al, 1989).

The Ca in seminal plasma, in addition to controlling acrosome reaction, sperm capacitation and polarity reversal has been actively involved in spermatozoal motility. Forrester and Bradley (1980) discussed the presence of some Calmodulin like Ca-binding proteins in the spermatozoa. The formation of Ca-Calmodulin complex activates a series of enzyme systems which are concerned with phosphorylation of proteins which further regulates the formation of c-AMP and generation of energy for sperm motility. In the present study, the low seminal plasma Ca levels were apparently due to increased influx into the spermatozoa as discussed earlier with electrolyte imbalance. Many workers have associated the low seminal plasma and high spermatozoal Ca with suppressed sperm motility (Yanagimachi, 1982, Arver, 1982; Pandey et al, 1983 and Prien et al, 1990).
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No reports are available regarding the correlation of seminal plasma and spermatozoal Ca levels with sperm vitality aspect. The present results were analyzed in this direction and it was discovered that seminal plasma Ca concentration did not show any correlation with percentage of dead cells in the semen, rather spermatozoal Ca contents were found to be almost double in the subjects with less than 75% live spermatozoa as compared to those of subjects with normal sperm vitality (>75% live) in oligospermic-infertile group. In this study group, the high spermatozoal Ca has been found in combination with that of high electrolytes, Zn and Mg. It is still to be established whether these elements affect the spermatozoal vitality singularly or as a group.

8.1.4 Copper (Cu)

Copper (Cu), because of its high reactivity is known to be highly toxic. Under normal conditions, the ingested copper is absorbed from the stomach and upper part of the small intestine. Then it passes to the blood stream and is loosely bound to the albumin and passes to the liver. The liver concentrates the copper first in the cytosol, then in mitochondria and finally in the nuclei depending upon the dose and the length of time of ingestion. Then Cu reappears in the blood in the form of ceruloplasmin, a Cu containing protein which has a ferridoxase activity. The exact function of this protein in Cu metabolism is not fully understood but there is some evidence that this protein help in Cu exchange during oxidative activity (Hill, 1977). In a separate study, Loewit (1971) and Holland and White (1980) also identified Cu as one of the toxic elements toxic to male reproductive system especially spermatozoa.

The toxic effects of high Cu levels have been clearly demonstrated in the present study also. The high serum and seminal plasma Cu levels as estimated in normo-asthenospermic subjects and significantly high serum concentrations in non-obstructive and unknown azoosperms and also in oligospermic-infertile groups showed deleterious effects on sperm production and markedly reduced rapid progressive sperm motility. Smith et al (1983), Abou-Shakra et al (1989)...
and Jockenhovel et al (1990) found that excessive concentrations of Cu reduce the oxidative processes and glucose consumption, which reduces or abolishes the spermatozoal motility. The adverse effects of high Cu concentration on sperm motility and viability were also demonstrated in vitro by Roblero et al (1996) where they found that high Cu concentration not only significantly reduced the sperm motility (to 50%), but also reduced their fertilizing ability and sperm-oocyte interactions without altering their capacity to migrate through the female genital tract. They further investigated that membrane fusion of gametes and the cytoplasmic mechanisms for chromatin decondensation were greatly affected at high Cu concentration.

The seminal plasma Cu content did show any significant difference with respect to sperm count and vitality in the present study as also reported in the studies of Smith et al (1983) and Abou-Shakra et al (1989). But Umeyama et al (1986), showed conflicting results in their multi-element study on whole semen conducted in infertile and fertile men that Cu concentrations in infertile subjects was much higher than that of fertile subjects.

Contrary to its toxic effects, the beneficial role of Cu has also been discussed by many scientists. The Cu content of spermatozoa of all the study groups in the present study was below the detection limits of the ICAP-AES instrument (<1ng/ml). But reports are available that in spermatozoa, Cu is an essential component of numerous metalloenzymes and metalloprotein systems, of which the cytochrome-c-oxidase and superoxide dismutase are the well recognized enzymes (Mann and Lutwak-mann 1981, Jockenhovel et al, 1990 and Bedwal and Bahuguna, 1994). Jockenhovel et al (1990) further mentioned that Cu is contained in the superoxide dismutase (SOD) and this enzyme protects human spermatozoa against peroxidative damage of cellular enzymes and structures. In infertile men especially with leucocytospermia, there is high production of reactive oxygen species (ROS). The adequate concentrations of Cu in the SOD plays an important role in the removal of superoxide anions from
the spermatozoal cells and also stabilizes the nuclear chromatin of human spermatozoa by formation of –S-S- cross links from thiol groups on adjacent structural proteins and nucleoprotein chains. So within normal limits, Cu plays a protective role in maintaining spermatozoal integrity and vitality and also protects the spermatozoa from oxidative stress generated due to infections. Only at high levels it is proved to be toxic. Further studies are required to formulate the normal range upto which it has a protective role.

8.1.5 Iron (Fe)

The high serum iron content was estimated only in oligospermic-infertile among the unexposed infertile groups. Serum iron levels were high in welding and zinc galvanizing group obviously due to exposure and active inhalation during their working process. In oligospermic-infertile group, high serum Fe level has been estimated in combination with K, Na, Zn, Mg, Ca and Cu. No clear inference could be drawn whether high level of Fe alone or in combination of other trace elements has suppressing effect on spermatogenesis.

In contrast to high serum levels in oligospermic-infertile group, the seminal plasma Fe in azoospermic subjects (non-obstructive and unknown) in unexposed section and in welding and zinc-galvanizing workers in occupationally exposed groups has shown significantly reduced levels. From such findings no inference can be made regarding the role of seminal plasma Fe levels and their correlation with the sperm count and fertility status in men as non-obstructive azoospermic and the zinc galvanizing groups are at the two extreme ends in terms of sperm count and fertility in the present study. Their seminal plasma Fe levels were very closely associated with each other (5.64±1.60 μg/ml for non-obstructive azoospermic and 5.84±2.19 μg/ml for zinc-galvanizing group). The role of seminal concentration of Fe in male infertility is not fully explored. Only limited reports are available which do not give any conclusive inference. The present results well matched with the multi-element studies of Abou-Shakra et al (1989) conducted in seminal plasma of infertile and fertile subjects. But the findings of
Umeyama et al (1986) in whole semen gave opposite picture regarding Fe that its concentrations were found higher in infertile men as compared to normal fertile men. The correlation of iron concentration with fertility was discussed by Nikolaev et al (1998) as they discovered that iron and non-hemic spermoplasmic ferroproteins were involved in ejaculate thinning, maintaining semen viscosity and sperm pH. Significantly low seminal plasma iron levels in normo-asthenospermic subjects in the present study may be correlated with low semen pH in some of the subjects, which may be correlated with reduced sperm motility. No correlation of seminal plasma iron was observed with spermatozoal vitality. The exact role of iron in sperm motility and vitality needs further studies.

The high spermatozoal iron content in oligospermic-infertile group could be due to altered membrane permeability on account of electrolyte imbalance and defective Na⁺-K⁺ pump which caused high influx of iron along with other elements, whereas in environmentally exposed groups (Heat, welding and zinc-galvanizing) it could be due to mainly two reasons:

i) The common factor in all the three groups that elevated scrotal temperatures due to their working discipline might have affected spermatozoal sensitivity and their membrane permeability, leading to influx of major elements and the electrolytes.

ii) In welding and zinc-galvanizing group, due to inhalation of mild steel fumes which is a mixture of iron, manganese, carbon, sulphur and phosphorus, the Fe levels in serum, seminal plasma as well as in spermatozoa were found to be significantly high as compared to those of control group but they were not as high as found in oligospermic-infertile group.

The reprotoxic or protective role of high or low spermatozoal iron contents has not been fully explored as yet. From the available information regarding iron metabolism, it is known to be an important component of certain enzyme...
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systems present inside the mitochondria cytochrome-C-reductase. Acetyl CoA dehydrogenase and NADH-reductase (Chatterjee and Rana Shinde, 2002). Out of these enzymes, cytochrome-C-reductase and and cytochrome-c-oxidase have been detected in spermatozoa (Mann and Lutwak-Mann, 1981). In the activity of these enzymes, free Fe which catalyses the conversion of oxygen free radicals (O’₂) and superoxide free radicals to hydroxy free radical (OH’) is known to be toxic, whereas the bound iron which is generally bound to ferritin is known to be non-toxic. On the basis of this information, high spermatozoal iron content as detected in oligospermic-infertile group might have had highly toxic effects on the spermatozoa reducing sperm motility. When found within normal limits as in the zinc-galvanizing workers might have had beneficial effects. More extensive studies are required to formulate a definite hypothesis in this direction.

8.1.6 Manganese (Mn)

No correlation of sperm count, vitality and rapid progressive motility was detected with respect to serum and seminal plasma concentrations of Mn in all the infertile groups of non-occupationally exposed groups. The results were in close agreement with that of Umeyama et al (1983) and Abou-Shakra et al (1989) as they discovered in their multi-element analysis that Mn levels in seminal plasma showed no significant effect on sperm count but it may have influence on spermatozoal motility or fertilizing ability.

Only the welding workers showed significantly higher serum and seminal plasma levels of Mn. Such high Mn levels in these workers were attributed to inhalation of mild steel fumes during the process of welding in which Mn constituted one of the principle components. The results of the present study shared a common consensus with that of Wu et al (1996) where they also found significantly higher semen concentrations of Mn, Cu, Cr, Ni and Fe in electric welding workers. As a consequence of these high trace element levels, Wu and his co-workers concluded direct toxic effects of Mn on sperm production and found reduced sperm count and also reduced percentage of motile and viable
spermatozoa in their subjects. Similar findings were also observed in the present study as the Zn, Fe, Mn, P, Cr, Ni, and Pb in serum and Fe, Mn, P, Cr and Al in seminal plasma were found considerably high in welding workers.

The spermatozoal Mn contents were below detection limits of ICAP-AES instrument in all the study groups and also no reports are available regarding spermatozoal Mn levels with respect to infertility evaluation.

8.1.7 Sulphur (S)

The serum and seminal plasma sulphur (S) levels showed no significant correlation with the sperm count and vitality. In oligospermic-infertile subjects with rapid progressive sperm motility less than 25% group the seminal plasma S levels were found to be significantly low. Reverse was true in heat-exposed and zinc-galvanizing group. Significantly high seminal plasma S levels were detected in heat-exposed and zinc-galvanizing group. The spermatozoal S levels were found to be maximum in oligospermic-infertile group, followed by heat-exposed and welding group which were significantly higher than those of the normal control group. Battersby and Chandler (1977) in their X-ray microanalysis study on localization of trace elements in different regions of spermatozoa and their possible role in spermatozoal motility discovered that S along with Na, K, Mg, Ca, Cu and Zn was specifically concentrated in the mid piece region of spermatozoa and P was found in the nucleus. The S concentration showed positive correlation with that of Mg and P levels in their study. Similar correlation was detected in the present study.

8.1.8 Phosphorus (P)

The serum phosphorus (P) levels in different infertile and normal fertile groups was not significantly correlated with sperm count. The high serum level detected in welding workers was probably due to the high S content in the welding fumes, which got actively absorbed in the blood, seminal plasma and the spermatozoa through the blood-testis barrier. In contrast, the significantly low
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serum P concentration in zinc-galvanizing workers as compared to that of normal control group as well as that of heat-exposed and the welding group may suggest that low P contents in the blood has stimulatory effect on spermatogenesis but not on spermatozoal functions. This fact was clearly noticed in oligospermic-infertile and heat-exposed subjects with subnormal spermatozoal motility (<25% rapid progressive motility). No reports are available regarding serum levels of P and its correlation with male infertility. Further studies are required to strengthen the present findings.

Adamopoulos and Deliyiannis (1983) discovered that seminal plasma P was higher in asthenospermic but lower in azoospermic subjects. Similar correlation was observed in the seminal plasma levels of oligospermic-infertile asthenospermic subjects but no significant difference was observed in the azoospermic and normospermic levels.

Battersby and Chandler (1977) discovered that P in semen is mainly contained in the spermatozoa where it is concentrated in the nucleus and the acrosome. A highly positive correlation of P was reported in their study with Mg and Ca in spermatozoa with regard to sperm motility. The results of the present study well matched with these reported findings as the spermatozoal P level along with Mg and Ca in oligospermic-infertile asthenospermic subjects was detected to be significantly high.

8.1.9 Arsenic (As)

A well known toxic element that is Arsenic (As) was reported to be present in the human ejaculate along with essential trace elements required for various sperm functions. Oster and Prellwitz (1985) estimated the arsenic content in ejaculates of men in West Germany to be in the same range as in the blood i.e. less than 5μg/l. A similar range of arsenic was detected in the present study. The main source of arsenic is drinking water and the food. High serum and semen
arsenic contents were reported in men who take more of sea-food like crabs (Schramm and Oster, 1987).

No correlation of serum and seminal plasma arsenic concentration was detected in any of the infertile groups with respect to sperm count, vitality and motility. Only zinc-galvanizing workers showed a positive correlation of arsenic concentration that significantly low serum and seminal plasma levels in these workers were associated with improved sperm count.

Schramm and Oster (1987) discussed the toxic effect of arsenic that in high doses it can be even fatal. Sub-lethal doses of inorganic arsenic may be carcinogenic and teratogenic. It is also known to affect the RNA and DNA synthesis that possibly influence the sperm development. In their study, the spermatozoal levels of arsenic were below detection limits but in the present set up the spermatozoal arsenic levels were detectable in all the groups except in normospermic-infertile and the zinc-galvanizing groups (the values were <1ng/ml). The spermatozoal arsenic values were significantly high in oligospermic infertile and the heat-exposed group. Both these groups had sub-normal sperm concentration, which strengthens the fact that high arsenic contents is toxic and interfere with the spermatozoal development as postulated by Schramm and Oster (1987).

No correlation of spermatozoal vitality and rapid progressive motility was observed with serum, seminal plasma and spermatozoal arsenic concentration in any of the study groups and there have been no investigative reports available regarding any essential functions of human life being controlled by arsenic or its being essential for sperm development and its functions.
8.1.10 Chromium (Cr)

The role of Cr in male fertility regulation has not been studied extensively. Only limited reports are available where its levels are assessed in infertile and fertile groups of subjects. In a multi-element study of Abou-Shakra et al (1989) in seminal plasma, the Cr levels were not found to be significantly different in normospermic-infertile, oligospermic-infertile, severely oligospermic-infertile and azoospermic subjects. The present findings in the similar groups also showed no significant correlation in serum as well as seminal plasma Cr levels. Only the welding workers group, who were exposed to Cr through welding fumes, had significantly higher serum and seminal plasma Cr levels. Similar findings were reported by Bonde (1993) in the welding workers where he mentioned that Cr accumulates in the testes following long-term exposure to welding fumes. He also noticed decline in semen volume, liquefaction time, sperm count and percentage of motile and viable spermatozoa in response to such toxic burden of high seminal plasma Cr and Mn depending on the time and the number of years of exposure. The spermatozoal Cr content was below detection limits of the instrument (ICAP-AES).

8.1.11 Aluminium (Al)

The serum as well as seminal plasma Al level was not found to be significantly different in azoospermic, oligospermic-infertile and normospermic-infertile groups as compared to the normal fertile subjects in section-I. Similar findings were reported in the studies of Abou-Shakra et al (1989) as they also noticed no significant difference in seminal plasma Al levels in the similar group of subjects. But highest spermatozoal Al levels in oligospermic-infertile and also significantly high levels in normospermic-infertile group showed that abnormally high spermatozoal Al may not be correlated with sperm count but it may be one of the contributing factors in causing infertility in these subjects.

In occupationally exposed groups (section-II), it has been noticed that exposure to heat does not significantly affect the Al status in serum and in
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Seminal plasma but in spermatozoa there is highly elevated level probably due to increased permeability of spermatozoal membrane on account of high temperature. Such increased influx of Al into spermatozoa was also observed in case of welding and galvanizing workers as compared to the normal fertile subjects. The welding workers showed significantly low seminal plasma Al levels probably due to its high influx in the spermatozoa. Only in zinc galvanizing group the Al levels were detected significantly high in all the three samples (serum, seminal plasma and spermatozoa), which could be as a consequence of exposure to galvanizing fumes.

A highly positive correlation of spermatozoal Al levels with infertility has also been observed in the studies of Umeyama et al (1986) in whole semen where they noticed high Al levels in normospermic-infertile group. But conflicting results were presented by Mur et al (1998) in their fertility analysis studies conducted on aluminium industry workers as they found better birth rates in the exposed group as compared to the control (unexposed) group, despite the presence of another major risk factor (exposure to heat) in those workers. More detailed studies are required to formulate a definite hypothesis whether Al is involved in fertility regulation or its high spermatozoal levels suppress the fertilizing ability of spermatozoa.

8.1.12 Nickel (Ni)

The role of Ni in human reproduction is not fully understood but it is known to be required in trace amounts for growth and reproduction. Its minimum daily requirement is approximately 20 μg per day. In lower animals its deficiency causes impaired growth and reproduction and also causes anaemia, but no such reports are available in human beings (Chatterjee and Rana Shinde 2002). In the present study opposite trend was observed in the unexposed section as serum Ni levels in oligospermic-infertile group was found to be significantly higher than that of normal fertile group.
Discussion

On the other hand, welding and zinc-galvanizing workers also showed high serum Ni levels which show no correlation with their fertility status. In seminal plasma also, non-obstructive azoospermic subjects had significantly high Ni concentrations as compared to control group. In contrast, the zinc-galvanizing group, which had the highest fertility rate among all the study groups, also had a similar high seminal plasma Ni range as that of azoospermic. The results of Umeyama et al (1986) are also conflicting as they found higher semen Ni levels in infertile subjects as compared to fertile subjects. The spermatozoal Ni levels were found to be below detection limits (<1ng/ml) in the present study. From the previous data available and the present results, no inference can be drowned regarding Ni levels in serum or in seminal plasma and its possible role in reproduction.

8.1.13 Molybdenum (Mo)

The main source of Molybdenum (Mo) is cereals and dry legumes, which supply more than 50 µg of Mo per day (Chatterjee and Rana Shinde, 2002). There are limited studies regarding the role of Mo with respect to fertility. Umeyama et al (1986) in their multi-element study in the whole semen noticed that levels of Mo along with Al were higher in the normospermic-infertile group as compared to that of proven fertile group. No such correlation was observed in the present study both in serum as well as in seminal plasma. The spermatozoal Mo levels were found below detection limit in this study. Abou-Shakra et al (1989) found even seminal plasma Mo levels below detection limits and this correlation of Mo levels with fertility could not be established.

8.1.14 Lead (Pb)

Lead is a well-known repro-toxin. There are number of reasons by which lead can reduce male fertility. There is a weak perception that exposure to lead is associated with delayed conception and decreased fertility rate. Alterations in sperm chromatin stability and epigenetic effects are few of the most probable mechanisms which can cause infertility (Sallman, 2001). Apostoli et al (1998)
also postulated that more than 40 µg/dl in the blood may impair reproductive functions by reducing sperm count, semen volume and density, and bring about alterations in the sperm motility and morphology. In yet another study Telisman et al (2000) also concluded that even moderate exposure to Pb (blood Pb <400 µg/l) or Cd (blood Cd <10 µg/l) can significantly reduce human semen quality in terms of reduced sperm count, viability and total motile spermatozoa.

In the present study, mean serum Pb levels in the unexposed infertile groups were lower than the toxic range as given by Apostoli et al (1998) and Telisman et al (2000) and no such evidence of lead related infertility was noticed as the mean serum lead levels in azoospermic, oligospermic-infertile, normospermic-infertile and in the fertile groups were not significantly different.

In the occupationally exposed section, welding workers had significantly higher serum Pb levels (0.66±0.71 µg/ml) as compared to control group (0.11±0.27 µg/ml). In these workers, high serum lead level may be one of the contributory factors in lower semen profile and low fertility rate.

There is epidemiological evidence that exposure to industrial metals and aerosols may reduce fertility in term of delayed conception, and reduced sperm quality (Saaranen et al 1989b, Bonde 1993, Spinelli et al 1997). The severity of the problem is correlated with both exposure dose and length of exposure (Bonde, 1990b).

Leinders et al (1992) reported that both Pb and Cd in high concentrations interfere with the ion permeation through K+ and Ca++ channels present in the spermatozoal membrane. The acrosome exocytosis induced by sperm-zona binding is required for sperm penetration through zona pellucida which requires sequential action of sperm head delayed (outward) rectifier voltage-gated potassium (K+) channels (VGKC) and L-type voltage dependent Ca++ ion channels (L-VDCC) (Benoff, 1999).
Discussion

The α-subunit of VGKC consists of six transmembrane segments, S1 to S6 (Catterall, 1995). The transmembrane voltage sensor is S4 and the segments S5 to S6 contain the ion conducting pores and binding sites for channel blockers and metal ions like Pb and Cd (Shieh and Kirsch, 1994, Catterall, 1995, Christie, 1995, Doyle, et al, 1998). Multiple L-VDC α-1 isoforms (e.g. α-1A, α-1B, α-1C, α-1D and α-1E) are generated by alternate splicing of the primary transcript from each gene. The α-1C subunit forms the pore of the L-VDCC, which is expressed in human testis and in spermatozoa (Goodwin et al 1999 a, b). This subunit contains the binding sites for various pharmacological agents and ions, which cause reversible infertile state (Benoff et al, 1994).

In the present investigation neither any changes in the levels of Pb in seminal plasma and spermatozoa have been detected, nor any significant changes in the sperm physiology have been found. The observations of Leinders et al (1992) are suggestive of the fact that higher levels of Pb in the semen or spermatozoa may be causing ion channels mediated damage to the spermatozoa. The levels of Pb detected in the welding workers seems to be much less than those subjects in which Pb levels in serum was found to be much higher (Benoff et al, 2000).

Seminal plasma and spermatozoa lead levels did not show any positive correlation with spermatozoal count and their functional abilities in terms of motility and vitality in the present study. Similar results were reported by Umeyama et al (1986) and Abou-Shakra et al (1989).

No correlation of serum, seminal plasma and spermatozoal Pb levels was observed with respect to sperm vitality and motility in this study but reports are available that high blood Pb levels are associated with asthenozoospermia (Chia et al, 1992).
8.1.15 Boron (B)

No correlation of serum, seminal plasma and spermatozoal boron (B) concentration was observed with respect to sperm count, vitality and motility and this element and no studies are available regarding this element in connection with male infertility.

8.1.16 Mercury (Hg)

Reports on the effect of mercury on spermatogenesis and sperm function are few. In the studies of Chia et al (1992), no correlation of blood Hg level with oligospermia, teratozoospermia and asthenozoospermia was noticed. In a recent study undertaken by Choy et al (2002) in Hong Kong in sub-fertile males the levels of Hg in blood and seminal fluid were estimated. They detected a positive correlation between high seminal fluid Hg levels with abnormal sperm morphology (head and mid piece defects) and reduced straight line velocity of spermatozoa. Blood mercury levels were significantly higher than those in semen, which suggests a functional blood-testis barrier to mercury. Neither the overall percentage of motile spermatozoa nor the sperm concentration were correlated with Hg concentrations. They further concluded that mercury may be taken as spermatotoxicant as it impairs fertility potential. Previous in vitro studies of Mohamed et al (1986) have revealed that possible binding sites for mercury in spermatozoa are the sulfahydryl groups in the spermatozoal membrane in head, mid piece and tail region. Subsequently, sperm membrane permeability, mitochondrial functional integrity, DNA synthesis and the microtubule sliding assembly of the sperm motor apparatus are the possible target sites of mercury toxicity. (Lee and Dixon 1975, Vogel et al 1985, Ernst et al 1991a and Liu et al 1995). In addition, the supporting cells such as Sertoli and Leydig cells in the testis, and the cells in the epididymis and the seminal vesicles are also reported to be targets of mercury toxicity (Ernst et al, 1991b, Li, 1995).

In the present study, the mercury levels were below detection limits in serum, seminal plasma and in spermatozoa. In Hong Kong population the high...
mercury detected in blood as well as in seminal fluid might have been due to dietary consumption of sea-food (Choy et al, 2002).

8.1.17 Cobalt (Co)
No reports are available regarding the role of Co in male fertility regulation. This element is present in ultra trace amount in the body that its levels were undetectable in serum, seminal plasma and spermatozoa. Abou-Shakra et al (1989) also reported the similar finding in their multi-element analysis that Co could not be detected in seminal plasma because of its level less than 1ng/ml as estimated by ICAP-AES.

8.1.18 Cadmium (Cd)
Cadmium is another reprotoxic element which along with Pb was found to be significantly high in semen of infertile men as compared to that of fertile men in the studies of Umeyama et al (1986). In another study Smith et al (1983) and Saaranen et al (1989) found high seminal plasma Cd levels in heavy smokers (smoking >20 cigarettes /day) as compared to non-smokers. Chia et al (1992) also detected the adverse effect of Cd along with Pb, Hg, Cu and Zn when they found significantly poor semen profile in subjects with high blood Cd concentration.

In the present study, Cd was less than 1ng/ml and found undetected in serum, seminal plasma and spermatozoa. Similar findings were made by Abou-Shakra et al (1989) as seminal plasma Cd was undetected in their studies too.

On the basis of above-mentioned discussion regarding the trace element status in different groups of subjects, an overall picture can be drawn about the correlation of trace element levels with male fertility status. Further studies are certainly required to support the present findings regarding some of the trace elements as the available data had too many voids to explain the metabolic role.
Discussion

of each trace element with respect to infertility. Moreover, this is the first study in which concentration of 20 trace elements including the electrolytes has been done separately in serum, seminal plasma and spermatozoa and their correlation has been discussed with respect to fertility status of men in two different sections of the population (occupationally exposed and non-occupationally exposed to environmental insults).

From the present study it can be concluded that trace element estimation in serum, seminal plasma and spermatozoa can be used as one of the dependable investigative tool in infertility diagnosis but only after the normal range of each trace element is established in seminal plasma and spermatozoa separately for the ejaculated semen, epididymal semen and the testicular semen. Probably the field remains still open for further exploration of details of trace element status of seminal elements in different regions of the reproductive tract to pin point the exact site of defect and make a differential diagnosis of unexplained infertility.