Summary

The present investigations embodies studies on the semen profile including enzyme markers present in the semen, histopathology of the testes, hormone profile and trace element analysis in both normal and infertile subjects. The electrolytes and trace element analysis were performed in serum, seminal plasma and spermatozoa of both fertile and infertile subjects.

Investigations have been carried out in two sections. Section-I included 200 subjects from general population, working in normal environmental conditions and in section-II, 97 subjects participated who were exposed to occupational hazards of heat, welding and zinc galvanizing. After taking into consideration the exclusion criteria of sexual dysfunction, 150 subjects in section-I and 76 subjects in section-II were finally enrolled for the present investigations.

In section-I, out of 150 subjects, 84 (56%) subjects were infertile and 66 (44%) were fertile. In section-II, Out of 76 occupationally exposed subjects, 24 subjects were exposed to heat (17% fertile and 83% infertile), 20 subjects were working in welding units (50% fertile and 50% infertile) and 32 subjects were working in the zinc galvanizing units (94% fertile and 6% infertile). These subjects had been exposed to their respective places of work for 2-5 years approximately 6 hours per day. All the subjects in section-I and section-II were between the age group of 20 to 60 years and the average age among all the groups was not significantly different. The minimum mean age was 27.8 years and maximum mean age was 33.8 years.

A detailed history and physical examination as per WHO protocol was followed for all subjects in both the sections.
The subjects in section-I were divided into four groups on the basis of semen characteristics. Group-I (G-I) included azoospermic subjects, which was further divided on the basis of clinical examination and histopathological studies of testicular biopsies into obstructive azoospermics (G-1.1), non-obstructive azoospermics (G-1.2) and unknown azoospermics (G-1.3) where cause of azoospermia could not be established.

The group-2 (G-2) and group-3 (G-3) included oligospermic and normospermic-infertile subjects respectively, whereas group-4 (G-4) included normal fertile subjects. The group-4 subjects served as normal control for all the investigations in this study.

Each semen sample was subjected to evaluations according to WHO analysis protocol, which included parameters like semen viscosity, liquefaction time, semen volume and pH. The spermatozoal parameters included sperm concentration, morphology, vitality and motility. A quantitative estimation of presence of non-spermatozoal cells in the semen, if any, was made in each case. Hormonal and testicular histopathological investigations were made in selected subjects. Biochemical estimations of lactic dehydrogenase (LDH), fructose, neutral alpha-glucosidase and citric acid were made in the seminal plasma of both fertile and infertile subjects in both section-I and section-II.

Electrolytes (potassium and sodium) and trace elements like zinc (Zn) magnesium, (Mg) calcium, (Ca), copper, (Cu) iron, (Fe) sulphur, (S) phosphorus, (P) manganese, (Mn) aluminum, (Al) arsenic, (As) chromium, (Cr) molybdenum, (Mo) nickel, (Ni) lead, (Pb) boron, (B) mercury, (Hg) cobalt (Co) and cadmium (Cd) were estimated in the serum, seminal plasma and spermatozoa of infertile and fertile subjects in section-I and section-II. Inductively Coupled Argon Plasma-Atomic Emission Spectrometer (ICAP-AES) was used to estimate the electrolytes and trace elements in all the samples.
Summary

On the basis of physical and clinical examinations, semen analysis, hormone profile and histopathological studies of the testes, the obstructive azoospermia was found in 10 (7%) of the subjects, non-obstructive azoospermia due to testicular failure was detected in 5 (3%) of subjects and in 14 (9%) of the subjects the cause of azoospermia remained unknown in group- (G1.1,1.2 and1.3) In group-2 (G-2), 30 (20%) subjects were oligospermic, with their mean sperm count less than 20 million/ml and 25 (17%) subjects were normospermic (G-3). These normospermic-infertile subjects had subnormal semen profile. The fertile group (G-4) showed all the semen parameters within normal limits.

The average semen pH, liquefaction time and semen viscosity did not show any significant difference among the infertile and fertile groups in section-I and also in section-II. The results obtained are in agreement with the standard values recommended by WHO (1999).

The most prominent non-spermatozoal cells in the seminal plasma were pus cells. In section-I, the prevalence of pus cells was significantly high in non-obstructive azoospermic, unknown azoospermic oligospermic and normospermic-infertile subjects. In section-II, the pus cells were observed only in heat-exposed subjects, whereas in zinc galvanizing workers and the welding workers, the pus cells were either absent or were less than (<1 million/ml). The presence of abnormally high pus cells (>1 million/ml as specified by WHO) was positively correlated to high levels of dead spermatozoa in their semen (<75% live spermatozoa). The high levels of pus cells in infertile groups in section-I was positively correlated with their infertility status and on the other hand the absence of pus cells in zinc galvanizing and normal control group was positively correlated with their fertility status.

The other major category of non-spermatozoal cells found in the semen was that of immature germ cells. In the non-obstructive azoospermic (2 subjects), unknown azoospermic (4 subjects) and oligospermic groups (3 subjects), the presence of immature germ cells significantly indicates their poor spermatogenic
status. The germ cells were found to be absent in normospermic-infertile and normal fertile group in section-I, and in welding and zinc galvanizing workers in section-II. Only the heat-exposed group showed immature germ cells in their semen.

Morphologically abnormal spermatozoa were observed to be significantly high (>30%) in oligospermic and normospermic-infertile subjects in section-I and only in heat-exposed subjects in section-II. The morphological abnormality included mainly head defects like double heads, swollen heads and pin heads in these groups. Such abnormal spermatozoa were absent in normal fertile (control) group, welding workers group and in zinc galvanizing group in section-II. The presence of such high abnormal spermatozoa was positively correlated with their infertility status.

The average sperm count in the fertile subjects (Group 4) was 114.21±56.87 million/ml. On the other hand, in oligospermic group (G-2), the average sperm count was 11.22±7.11 million/ml. None of the oligospermic subjects were fertile. In normospermic-infertile group (G-3) the mean sperm count was 76.86±42.59 million/ml. In section-II also the average sperm count in heat-exposed and welding groups were 37.0±28.64 million/ml and 57.5±23.97 million/ml respectively. The zinc-galvanizing group had an average sperm count of 131.25±27.17 million/ml.

The average sperm vitality (% live spermatozoa) in the normal fertile group was 88%, which was in agreement of WHO specifications (>=75% live spermatozoa / ejaculate). The mean sperm vitality in infertile subjects in section-I was found to be 46.8% in oligospermic-infertile group and 46% in normospermic-infertile groups. In section-II, the mean sperm vitality in heat-exposed and the welding groups was 56% and 60% respectively, which was also subnormal. The zinc-galvanizing group had borderline sperm vitality of 74%.
Summary

All four types of sperm motility patterns (a - rapid progressive motility, 
b - slow progressive motility, c - non-progressive motility and d - immotile) were 
analyzed among all the groups as specified by WHO and the rapid progressive 
motility (RPM) in more than 25% of spermatozoa was taken as normal. In normal 
fertile group, the average sperm RPM was 73%, which was in agreement with 
WHO criteria. In oligospermic and normospermic-infertile groups in section-I, the 
mean RPM was 25% and 28% respectively, which was significantly lower than 
that of the fertile group. The mean rapid progressive sperm motility in section-II 
was 30% in heat-exposed group, 32.5% in welding group and 55% in zinc 
galvanizing group.

The mean serum testosterone levels ranged between 3.87 ng/ml and 5.65 
ng/ml in the infertile and fertile groups in section-I and between 3.55 ng/ml to 
4.74 ng/ml in section-II. These data are in agreement with the known values of 
serum testosterone (3-12 ng/ml).

The serum FSH and LH levels in oligospermic-infertile group were 2.14 
mlU/ml and 5.2mlU/ml, in normospermic-infertile group were 3.9 mlU/ml and 6.6 
mlU/ml, and in normal fertile group were 4.33 mlU/ml and 5.4 mlU/ml, 
respectively. These values were within the normal range (1.0 - 8.0 mlU/ml for 
FSH and 1.08 - 8.3 mlU/ml for LH). Elevated FSH levels were seen in obstructive 
azoospermic (7.98 mlU/ml) and non-obstructive azoospermic (7.80 mlU/ml) 
subjects as compared to the control group. In obstructive azoospermic group, the 
LH levels were normal (4.96 mlU/ml). The normal LH and testosterone levels in 
this group indicated their normal Leydig cell functions.

The azoospermia and severe oligospermia whether obstructive or non- 
obstructive was further confirmed by histopathological studies of testicular 
biopsies. The normal testicular functions were attributed in 7% of the 
azoospermic subjects who showed normal spermatogenesis in their 
histopathological sections. These reports corroborated with their normal LH and 
testosterone levels, absence of immature germ cells in their semen and normal
palpable testes. In another group of azoospermic subjects (3%) the histopathological reports showed maturation arrest which confirmed that azoospermia in these subjects was due to testicular failure. Oligospermia due to testicular failure was confirmed in 4 out of 5 subjects who participated in histopathological studies.

The mean seminal plasma LDH levels ranged between 2781.8 U/L (unknown azoospermic group) to 5030.6 U/L (normal fertile group) in section-I. The LDH levels showed a positive correlation with sperm count, as the levels were significantly low in non-obstructive azoospermic (2982.0 U/L) and oligospermic (2908.0 U/L) groups as compared to normospermic-infertile (4736.5 U/L) and normal fertile group. The LDH levels in obstructive azoospermic group were 3186.0 U/L. In section-II, the LDH levels were 4200.9 U/L (heat-exposed group), 3833.5 U/L (welding group) and 6022.5 U/L (zinc galvanizing group). These levels also showed positive correlation with sperm vitality as the levels were found to be low in subjects with subnormal sperm vitality (<75% live spermatozoa) in oligospermic and normospermic-infertile subjects in section-I. No correlation of LDH levels was observed with rapid progressive sperm motility.

The mean fructose level in the normal fertile group was 10.61 μmol/ml (45.62 μmol/ejaculate) which was in agreement with the WHO specified normal levels (13 μmol or more/ejaculate). The fructose levels were found to be significantly low in obstructive azoospermic group (2.39 μmol/ml or 10.27 μmol/ejaculate) in section-I. In oligospermic-infertile and normospermic-infertile groups, the mean fructose levels were found to be 6.34 μmol/ml (22.19 μmol/ejaculate) and 6.57 μmol/ml 24.96 μmol/ejaculate), respectively, which were not significantly different from the control group. In occupationally exposed groups in section-II, the levels were found to be low in heat-exposed group as compared to the control group i.e. 2.93 μmol/ml (12.8 μmol/ejaculate) but in welding workers and zinc galvanizing groups the fructose levels were 10.28 μmol/ml (45.23 μmol/ejaculate) and 9.05 μmol/ml 32.58 μmol/ejaculate), respectively, which were also not significantly different from the control group. In
oligospermic-infertile and normospermic-infertile groups in section-I the fructose
levels were significantly low in subjects with low sperm vitality (<75% live
spermatozoa) as compared to the control group. A similar decline in fructose
level was observed in subjects with low sperm motility (rapid progressive motility
<25%) in normospermic-infertile subjects in section-I.

Lower fructose levels were also observed in subjects with low sperm
vitality (<75% live spermatozoa) and low sperm motility (<25% rapid progressive
sperm motility) in welding workers group and zinc-galvanizing group in section-II.
In heat-exposed group, the fructose levels did not show any significant difference
with sperm motility and sperm vitality.

The mean value of neutral α-glucosidase activity, in the normal fertile
group was 16.32 mU/ml (70.18 mU/ejaculate). The enzyme activity was
significantly low in 8.87±8.71 mU/ml (38.14 mU/ejaculate) and 8.99±6.66 mU/ml
(31.46 mU/ejaculate), respectively obstructive azoospermic and oligospermic
groups respectively. In non-obstructive azoospermic, unknown azoospermic and
normospermic-infertile groups in section-I, the α-glucosidase activity was within
the normal range. In section-II, in heat-exposed and welding workers groups, the
α-glucosidase activity was within the normal range. The zinc-galvanizing group
showed significantly high enzyme activity (29.29 mU/ml) as compared to the
control group (16.32 mU/ml). The levels did not show any significant difference
with sperm vitality and rapid progressive sperm motility except in heat-exposed
group in section-II, the enzyme activity was significantly low in subjects with low
sperm vitality (8.42±5.76 mU/ml) and in subjects with less than 25% rapid
progressive sperm motility (8.71±6.15 mU/ml) as compared to the control group
(16.32 mU/ml).

The mean citric acid levels in the normal fertile group were 15.42 μmol/ml
(66.30 μmol/ejaculate), which were in close approximation with the normal values
recommended by WHO (52 μmol or more per ejaculate). The citric acid levels
were found to be significantly low in obstructive azoospermic (10.82 μmol/ml or
46.52 μmol/ejaculate), unknown azoospermic (9.50±8.71 μmol/ml or 41.8
µmol/ejaculate) and in oligospermic groups (11.37 µmol/ml or 39.79 µmol/ejaculate) but in normospermic-infertile group, no significant difference was observed as compared to control group in section-I. In the occupationally exposed groups (section-II) the mean citric acid values were 13.58 µmol/ml (59.75 µmol/ejaculate) in heat-exposed group, 14.13 µmol/ml (62.17 µmol/ejaculate) in welding workers group and 16.60 µmol/ml (73.04 µmol/ejaculate) in zinc galvanizing group. The citric acid levels in all the three exposed groups were within the normal range. The citric acid levels did not show any correlation with sperm vitality and sperm motility.

The levels of electrolytes (K, Na) and trace elements (Zn, Mg, Ca, Cu, Fe, Mn, S, P, As, Cr, Al, Ni, Mo, Pb and B in serum, seminal plasma and spermatozoa were within detection limits of the Inductively Coupled Argon Plasma-Atomic Emission Spectrometer (ICAP-AES). The levels of electrolytes and trace elements in serum, seminal plasma and spermatozoa of normal fertile group (G-4) as estimated by ICAP-AES were taken as reference levels for comparison with the levels in infertile groups in section-I and occupationally exposed groups in section-II.

The levels of Hg, Co and Cd were below the detection limits (<1ng/ml) by ICP-AES in all the three samples (serum, seminal plasma and spermatozoa) of all the subjects in section-I and section-II.

The mean values of serum electrolytes, K and Na, of the normal fertile men were 188.03±140.02µg/ml and 2325.6±563.9µg/ml respectively. The serum trace element levels in normal fertile group in this study were Zn(1.47±0.36µg/ml), Mg(23.03±5.91µg/ml), Ca(98.44±37.69µg/ml), Cu(0.90±0.26µg/ml), Fe(6.01±1.73µg/ml), Mn(0.11±0.14µg/ml), S(904.29±227.56µg/ml), P(186.39±93.63 µg/ml), As(0.46±0.50 µg/ml), Cr(0.06±0.09 µg/ml), Al(7.22±2.56 µg/ml), Ni(0.05±0.07 µg/ml), Mo(0.32±0.39 µg/ml), Pb(0.11±0.27 µg/ml) and B(1.75±1.24 µg/ml).
Summary

The obstructive azoospermic (G-1.1, 1.2 &1.3) and normospermic-infertile (G-3) groups in section-I did not show any significant variation in the serum electrolyte and trace element levels as compared to the normal fertile group (G-4). In non-obstructive azoospermic group, the serum levels of Na and Cu were higher but K, Zn, Mg, Ca, Fe, Mn, S, P, As, Cr, Al, Ni, Mo, Pb and B did not show any significant variation with the control group.

In oligospermic-infertile group (G-2), the serum levels of Na, Zn, Mg, Ca, Cu, Fe and Ni were significantly higher than that the control of group but K, Mn, S, P, As, Cr, Al, Mo, Pb and B did not show any significant variation with the control group (G-4).

Subjects of heat-exposed group in section-II showed no significant variation in serum electrolyte and trace element levels. The welding workers group had higher serum Zn, Fe, Mn, P, Cr, Ni and Pb levels but K, Na, Mg, Ca, Cu, S, As, Al, Mo, and B levels in this group did normospermic show any significant difference with the control group, the Hg, Co and Cd were below detection limit. In zinc galvanizing group the serum levels of K, Na, Mg, Ca, S, P, As, Mo, were significantly low and Fe, Al and Ni levels were high as compared to the control group. The levels of Zn, Cu, Mn, Cr, Pb and B were not significantly different in this group from the control group.

The mean seminal plasma electrolyte levels estimated in the normal fertile group were K (829.84±274.06 μg/ml) and Na(1680.7±413.6 μg/ml), and the mean trace element levels were Zn (111.63±65.32 μg/ml), Mg (57.33±50.84 μg/ml), Ca (241.54±102.8 μg/ml), Cu (0.33±0.60 μg/ml), Fe (10.82±12.41 μg/ml), Mn (0.13±0.23 μg/ml), S (171.14±49.48 μg/ml), P(572.15±337.75 μg/ml), As (0.50±0.50 μg/ml), Cr (0.04±0.06 μg/ml), Al (7.44±3.40 μg/ml), Ni (0.05±0.11 μg/ml), Mo (0.05±0.13 μg/ml), Pb (0.07±0.23 μg/ml) and B (1.01±0.89 μg/ml).

In section-I, in obstructive azoospermic and normospermic-infertile groups in which the testicular functions and the spermatogenic status were normal, the
seminal plasma electrolytes and trace element concentrations did not show any significant variation from the normal fertile control group.

In the non-obstructive azoospermic group, in which the azoosperma was due to hypospermatogenesis, the seminal plasma K was lower but Na levels were in the same range as compared to the control group. In this group seminal plasma Zn and Fe were low and Ni was found to be higher than the control group. There was no significant variation in the levels of Mg, Ca, Cu, Mn, S, P, As, Cr, Al, Mo, Pb and B in non-obstructive azoospermic group.

In oligospermic-infertile group, electrolytes (K and Na) and the trace elements levels in seminal plasma were not significantly different from that of the control group except that in this group the seminal plasma Fe was significantly higher and P was lower in subjects with low sperm vitality (<75% live spermatozoa). Zn, Mg, Ca, S and P were significantly lower in subjects with low sperm motility (<25% rapid progressive sperm motility). The seminal plasma levels of electrolytes (K and Na) and other trace elements like Cu, Mn, As, Cr, Al, Ni, Mo, Pb and B did not show any significant difference with sperm count, vitality and motility in oligospermic-infertile group as compared to the control group.

The seminal plasma K in section-II did not show any difference among the three occupationally exposed groups (heat-exposed, welding workers and zinc-galvanizing groups) but Na levels were significantly higher in all the three exposed groups as compared to the control group.

In heat-exposed group, the seminal plasma trace elements levels of Zn, Mg, Ca, Fe, Mn, P, As, Cr, Al, Ni, Mo, Pb and B were not significantly different as compared to the control group. Only the S levels were estimated as significantly high especially in subjects having low sperm motility (<25% rapid progressive motility) in this group.

The seminal plasma levels of Fe and Al in welding workers group were low but Mn, P and Cr were significantly higher than the control group. The levels
Summary

of Zn, Mg, Ca, Cu, S, As, Ni, Mo, Pb and B did not show any significant difference with the control group.

In zinc galvanizing group, the seminal plasma levels of Zn, S, P, Cr, Al, Ni, Pb and B were higher but Mg, Fe and As were found to be significantly low as compared to the control group. The levels of Ca, Cu, Mn and Mo did not show any significant difference with the control group.

The levels of Cu, Mn, Cr, Ni, Mo, Hg, Co and Cd in spermatozoa were below detection limits (<1ng/ml) in all the infertile, fertile and the occupationally exposed groups, whereas in normospermic-infertile the spermatozoal levels of As, Pb and B were also undetectable. The spermatozoa levels of electrolytes and trace elements (expressed as µg/million spermatozoa) in the normal fertile group were K(0.95±1.27), Na(1.81±2.10), Zn(0.12±0.15), Mg(1.09±1.40), Ca(0.73±0.99), Fe(0.03±0.07), S(0.40±0.35), P(12.73±22.18), As(0.01±0.05), Al(0.04±0.08), Pb(0.01±0.04) and B(0.003±0.02).

In section-I, in oligospermic-infertile group, The spermatozoal levels of both K (7.19±4.64 µg/million spermatozoa) and Na ((13.91±12.44 µg/million spermatozoa) were approximately 10 fold high as compared to the control group (K, 0.95±1.27 µg/million spermatozoa and Na, 1.81±2.10 µg/million spermatozoa). The levels of Zn, Mg, Ca, Fe, S, P, As and Al were also significantly higher but Pb and B did not show any significant difference as compared to the control group. The levels of Cu, Mn, Cr, Ni, Mo, Hg, Co and Cd were below detection limit (<1ng/ml). The levels of spermatozoal Mg, Ca, P and Al were found to be especially high in subjects with subnormal sperm motility (<25% rapid progressive motility) moreover Zn, Mg and Ca was also higher in subjects with low sperm vitality (<75% live spermatozoa) in this group.

In normospermic-infertile group the spermatozoal levels of K, Na, Zn, Mg, Ca, Fe, S, P, As, Pb and B did not show any significant difference, only Al was detected higher with respect to the control group. The levels of Cu, Mn, Cr, Ni, Mo, Hg, Co and Cd were below detection limit (<1ng/ml) in spermatozoa in this
group. No correlation of spermatozoal electrolytes and trace element levels was detected with respect to sperm vitality and sperm motility in this group.

In section-II, the spermatozoal electrolytes (K and Na) in all the three occupationally exposed groups (heat-exposed, welding and zinc galvanizing groups) were also significantly high as compared to the control group. The heat-exposed group shared approximately the same electrolyte status as that of the oligospermic group.

In heat-exposed group the spermatozoal levels of Zn, Mg, Ca, Fe, S, P, As and Al were higher but Pb and B did not show any significant difference as compared to the control group. The levels of Cu, Mn, Cr, Ni and Mo were below detection limit (<1ng/ml) in spermatozoa in this group.

In welding group the spermatozoal levels of Zn, Mg, Fe, S, P and Al were higher but Ca, As, Pb and B did not show any significant difference as compared to the control group. The levels of Cu, Mn, Cr, Ni and Mo were below detection limit (<1ng/ml) in spermatozoa in this group.

In zinc galvanizing group the spermatozoal level of Zn (0.67±0.60 μg/million spermatozoa) was higher than that of control group (0.12±0.15 μg/million spermatozoa) but it was much lower than that of oligospermic-infertile group (1.12±1.67 μg/million spermatozoa) in section-I. The levels of other trace elements like Ca, Fe and Al were also found to significantly high but spermatozoal Mg, S and P, were in the same range as the control group. The levels of Cu, Mn, Cr, Ni and Mo, As, Pb and B were below detection limit (<1ng/ml) in spermatozoa in this group.

The present study concludes that there could be a variety of causes that could bring about changes in the ionic distribution between seminal plasma and spermatozoa. These may result into irreversible loss of sperm motility and sperm integrity.
Summary

It can be safely concluded from the results of this study that K, Na, Zn, Mg, Ca, Mn, Cu, Fe, S, P, As and Al are essential elements for sperm production and their fertilizing potential. These elements may independently or jointly, affect the physiological integrity of spermatozoa. Abnormally high concentrations of these elements have adverse effects on sperm motility and vitality which eventually affects the fertilizing ability of spermatozoa. The high spermatozoal contents of Zn, Mg, Ca, Cu and Fe in particular have been proved to be toxic. These elements work within narrow normal limits. It is essential to maintain the normal levels of electrolytes and essential trace elements in the body, both in the blood as well in the semen to assure fertility to the man.