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Infertility

It is extremely important to understand that infertility is a highly distressing condition, which carries with it a burden of social stigma and a sense of personal failure. It deprives a couple of unique relationship of personal happiness, feeling of parenthood and old age security; and inflicts devastating trauma on both the partners. A large percentage of women feel that infertility is the worst thing that could happen to them and feel themselves responsible and guilty even when the cause of infertility may be in the male.

Even today, the approach to an infertile couple starts with an evaluation of female partner and to its extremity; the investigations begin and end with her alone. The woman is subjected to painful and expensive procedures like laparoscopy, hysterosalphigiography and sonographic evaluations; and it is not uncommon for a man to divorce, not one but several wives under the mistaken belief that she alone is responsible.

Recently, Puri et al (2000) have reviewed that infertility though not fatal, is a serious socio-physiological issue. In most developing countries, infertility is not a personal problem for the couple, but parents, relatives, neighbors and probably the entire community around the infertile couple feels anxious and concerned. So, looking at the magnitude of the problem, an urgent action is required, particularly in majority of the cases where infertility is avoidable. There is very little evidence on the levels or patterns of infertility in India and South Asia. The recent National Family Health Survey has estimated childlessness as 2.4% among currently married women over 40 years in India (IIPS, 1994,1995, Jejeebhoy, 1998).

Due to lack of proper epidemiological data on reproductive disorders and infertility in our country, it requires serious attention towards the development of scientific investigations. The help of properly trained and
experienced clinical and para-clinical specialists who have expertise both in male and female problems is required. There is an urgent need to develop an appropriate algorithm for systematic investigation and management or guidance of infertile couples at different levels. It needs standardization of laboratory tests that would be economically cheaper, feasible and easily accessible, still scientifically valid and dependable through appropriate studies (Roy, 1996).

It is believed that a thorough evaluation of factors contributing to fertility, usually a probable diagnosis can be made in 85-90% of couples; and in 50 to 60% of the couples, pregnancy will be achieved with appropriate treatment; without applications of advanced reproductive technologies (ART) such as in vitro fertilization (IVF). In the first visit of infertility assessment of a couple, participation of both partners is ideal which can enable the clinician to understand the dynamics of their relationship and their individual acceptance of the concept of infertility. General information regarding reproduction and timing of events may assist the couple in correcting coital frequency and other factors contributing to infertility taken as myths due to ignorance. Pregnancy will occur even without treatment in about 15-20% of couples, which are diagnosed as infertile (Martin, 1994).

Under normal circumstances, medical evaluation of both partners be undertaken simultaneously, and it makes more sense, however, to start with the male partner whose initial evaluation may be performed rapidly and non-invasively.

Fertility in males

For the normal reproductive functions and to be fertile, a person must be anatomically and physiologically normal. It comprises three main broad areas; spermatogenesis which simply means the production of spermatozoa, the performance of male sexual act and the regulation of male reproductive
functions by various hormones, more particularly, the Hypothalamic-Pituitary-Gonadal axis (Guyton and Hall, 2000).

Testes

The testes develop high on the embryo’s posterior abdominal wall and usually descend into the scrotum at 32 weeks. The condition of undescended testes (uni- or bi-lateral) is known as cryptorchidism. The testes, normally measure 5 cm (2 inch) in length and 2.5 cm (1 inch) in diameter (Tortora, 1983). Testicular exocrine activity is associated with seminiferous tubules, tubuli recti, rete testis and efferent ducts, which involves formation of testicular semen (a suspension of spermatozoa in testicular plasma) which is ultimately released into the epididymis. On the other hand, testicular endocrine activity involves the synthesis of steroid hormones, mostly the testosterone (Mann and Lutwak-Mann, 1981).

Each testis contains around 900 tightly coiled seminiferous tubules that contain two types of cells - germ cells and Sertoli cells. At the time of puberty (12-14 years of age), spermatozoa are produced from the germ cell by the process of spermatogenesis undergoing various mitotic and meiotic divisions. Between the seminiferous tubules are clusters of interstitial cells of Leydig. These cells secrete the testosterone (Seeley et al, 1989).

Sertoli cells are large cells, which extend from the periphery to the lumen of the seminiferous tubules. Their main function is to nourish the germ cells, formation of blood – testis barrier, and secretion of androgen binding protein (ABP), inhibin and Mullerian Inhibiting Substance (MIS). Sertoli cells also contain aromatase, an enzyme which converts androgens into estrogen. FSH and testosterone are essential for onset and maintenance of spermatogenesis, whereas, spermiogenesis is estrogen dependent (Guyton and Hall, 2000, Ganong, 2002).

Spermatozoa

In man, spermatogenesis takes 74 days. Spermatozoa are produced at the rate of 300 million per day, and once ejaculated have a life expectancy
of about 48 hours within the female reproductive tract. According to one estimate every time a man breathes, he produces 1000 spermatozoa in a normal fertile male. Spermatozoa have the adaptability for reaching a female ovum due to the vigorous motility of their tail and penetration into the ovum as sperm acrosome contains various proteolytic enzymes (Tortora, 1983).

Epididymis

Along the dorso-lateral border of each testis lies a tightly coiled tubule known as ductus epididymis, which measures about 6 meter in length and 1 mm in diameter. Functionally, it is divided into three regions, head, body and tail, or caput, corpus and cauda epididymis. Their functions are, concentration, maturation and storage, respectively. In man, the sperms stay in the epididymis from 18 hours to 10 days to complete their maturation. The post testicular sperm maturation involves morphological, biochemical, biophysical and metabolic changes. Physiologically, the spermatozoa develop motility and become capable of fertilization. Morphologically, there are changes in sperm surface and alterations in spermatozoa’s electrophoretic properties and their agglutination by lectins indicating modifications to cell surface glycoproteins. Finally, nuclear chromatin condensation and stabilization occurs in the epididymis. Within the epididymis, the spermatozoa appear to be immotile until they come in contact with either oxygen or a glycolysable substrate. Probably, because of this dormant state, they survive longer and retain their fertilizing ability in the epididymis than in any other part of the male or female reproductive tract. The complete deficiency of fructose and high potassium/sodium ratio may be considered as the cause of immobility of epididymal spermatozoa especially in cauda region. Biochemically, the organic components such as glycercylphosphorylcholine and carnitine and a variety of highly active enzymes like neutral alpha-glucosidase are very important functional components of epididymal plasma (Mortimer, 1994).

Vas Deferens

The spermatozoa enter the vas deferens upon leaving the cauda epididymis. The human vas is a tube 35-45 cm long and 0.5 mm wide in
internal diameter, which extends as far as the ejaculatory duct through which the contents of the vas and seminal vesicles are jointly channeled into the urethra. The spermatozoa are propelled outside by smooth muscle contractions which are controlled by oxytocin release from hypothalamus and sympathetico-adrenal system. Any condition, such as varicocele or spermatocele which partially or completely compresses the lumen of the vas, can lead to severe oligospermia (sperm count < 10 million/ml) or azoospermia (no spermatozoa), causing infertility in the man. In certain cases like cystic fibrosis, there is congenital absence of single or both vas deferens which leads to sterility (Mann and Lutwak-Mann, 1981). The vasa deferentia are not a physiological site of sperm storage and contain only 2% of total spermatozoa in the male tract (Mortimer, 1994).

**Ejaculatory Ducts and Urethra**

After their production in the seminiferous tubule, spermatozoa leave the testes through the efferent ductules and pass through a series of ducts to reach the exterior of the body during ejaculation. The ejaculatory ducts about 2.5 cm long, project into the prostate gland and end by opening into the urethra, which is about 20 cm long and extends from the urinary bladder to the distal end of the penis (Seeley et al, 1989).

**Accessory Sexual Glands**

A pair of seminal vesicles, a single prostate and a pair of bulbo-urthral glands pour their contents into the male genital tract to form the semen. Their exact physiological role in male fertility is yet to be explored but secretions of accessory glands form an integral composition of the semen and their dysfunction is often associated with impaired fertility (Mortimer, 1994).

**Semen**

The human semen ejaculate is a thick, viscous fluid containing spermatozoa suspended in secretions from testes and accessory glands. Normal semen volume ranges between 1.5 to 5 ml. Inadequate volume i.e. less than 1.3 ml may be due to retrograde ejaculation secondary to trauma to
lumber sympathetic plexus or by surgery that damages bladder neck (Howards, 1995).

A normal semen is opalescent–cream-white in colour, liquefies within 60 minutes at room temperature and it is alkaline in nature with pH ranges between 7.2 to 8.0 Average Sperm count is between 50 to 100 million/ml with less than 20% abnormal forms (WHO, 1992).

In the semen, spermatozoa and testicular fluid contribute only 5%, the seminal vesicles produce about 60 to70% of the fluid, the prostate gland contributes approximately 20 to 30%, and rest of the 5% is secreted from the bulb-urethral gland just before ejaculation for the lubrication of urethra and neutralization of any acidic urinary residue in the urethra before ejaculation (Seeley et al, 1989; Mortimer, 1994 and Guyton and Hall, 2000).

**Sperm Motility**

**ATP-controlled mechanism of sperm motility**

The progressive movement of free-swimming spermatozoa is described as undulating bending waves propagated backwards along the flagellum, which develops a thrust to enable spermatozoa to propel themselves in the forward direction. Mammalian spermatozoa generate helical or other types of three dimensional bending wave patterns. Brokaw (1980) studied that these flagellar waves are due to interaction of two flagellar proteins, tubulin and dynein, which are ATP-controlled (Mann and Lutwak-Mann, 1981)

**Cyclic-AMP dependent Sperm activity**

Gray et al (1971) described the presence of cyclic AMP in spermatozoa. This second messenger (cyclic AMP) is synthesized by the action of Mg^{+2}-dependent adenylate cyclase from ATP. In human spermatozoa the enzyme gets activated by Mn^{+2} ions and is insensitive to hormones. The primary effect of c-AMP is to increase Ca^{+2} transport across
the plasmalemma which increases the intracellular Ca level. Secondly, the c-AMP dependent protein kinase activity brings about phosphorylation of proteins by ATP. This effect has been demonstrated in human spermatozoa; that some of the proteins undergoing phosphorylation were located in sperm membranes and partly control the function of tubulin in spermatozoa. It has been emphasized that c-AMP acts directly on the plasma membrane and thereby regulates the exchange of ions across the cell membrane.

Biochemical markers of Testes and Accessory Gland Functions


Testicular and Epididymal enzymes

Testis-Specific Lactic Dehydrogenase

Markert and Moller (1959) first discovered that lactate dehydrogenase occurs in its five isoforms in mammalian somatic tissues; LDH-1 to LDH-5. Within a few years, it was established by many workers that testis and spermatozoa differ basically from somatic tissues and contain yet another variant of LDH which is commonly known as LDH-X (Blanco and Zinkham, 1963, Blanco et al, 1975, Goldberg, 1963 & 1977). This isozyme LDH-X was contained in unique and specific mitochondria in the primary spermatocytes and these mitochondria are carried throughout all the stages of gametogenesis to the final maturation of spermatozoa (Domenech et al, 1972).

Later, Hintz and Goldberg, (1977) also strongly mentioned in their study that lactate dehydrogenase is a sperm specific enzyme and is a useful biochemical marker which acts like a fingerprint of a particular step in spermatogenesis. Within the germ cells, LDH-X is present largely in the
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mitochondria, but partly in the cytosol also. This LDH-X which is a C4-homotetramer is composed entirely of one type of subunit i.e. C. So the enzyme is more correctly designated as LDH-C4.

In normal mammalian testis, LDH-C4 is specifically associated with the formation of pachytene primary spermatocytes, as it has been found to be absent in immature testis and reported to appear only during puberty (Blackshow and Elkington, 1970; Goldberg and Hawtrey, 1967).

It has been found that this enzyme is completely undetectable in damaged germinal epithelium, which results to delayed or defective spermatogenesis or even complete spermatogenenic arrest (Szeinberg et al, 1966).

Blackshaw et al (1973) have seen a suppressed formation of LDH by heat damage to the male gonads. Later, Mann and Lutwak-Mann (1981) reviewed that permeability of spermatozoa under sustained physical or chemical damage to the membranes in the plasmalemma, acrosome or in the mitochondrial region is greatly increased. Irrespective of the cause, such spermatozoa release their intracellular constituents including enzymes such as LDH, glutamic-oxaloacitic transaminase, hyaluronidase and acid phosphatase. That is why LDH-X (or LDH-C4) acts as a perfect biochemical marker specific to spermatozoa. Moreover, Virji (1985) demonstrated that incubation of the semen at 37°C up to 6 hours did not alter the LDH-C4 activity in seminal plasma.

Gavella and Cvitkovic (1985) reported that LDH-X is specifically required for some specialized functions like sperm motility and for metabolic requirements of mature sperms for their fertilizing ability. Complete absence of LDH-X is also significantly associated with defect beyond spermatocytes in spermetogenic cycle.

Keltimlidis et al (1989) in their study on infertile men found that LDH-X was detected in men with sperm concentration greater than 16 million/ml and
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there was a positive correlation between LDH-X concentration and the sperm count but no such correlation was observed with % sperm motility. It has become a scientific consensus by now that LDH-X is reliable marker for both germinal activity and spermatozoal quality with respect to total sperm count and mobile sperm count (Verma et al, 1993; Orlando et al 1988, 1994; Noguera et al, 1994; Aydin et al, 1997 and Laudat et al, 1997).

Epididymis

It has been reviewed by Mann (1964) that as early as 1913, Tournade and Merland discovered for the first time that epididymis not only acts as a repository for spermatozoa but also has a secretory function together with prostate and seminal vesicles to form semen. However, the main function of epididymis remains ripening and life-prolonging influence on spermatozoa.

Alpha-glucosidase

Sheth and Rao (1962) first reported the alpha-glucosidase activity in seminal plasma of man, bull, rabbit and fowl. Later, Grandmount et al (1983) reported that both acid and neutral alpha-glucosidase are present in the reproductive organs of male rats.

Based on the studies available from different laboratories, it has been established that acid alpha-glucosidase is mainly contributed by the testis and the prostate, whereas the neutral alpha-glucosidase is only contributed by the epididymis, which contains the highest level of activity of the reproductive organs (Grandmont et al, 1983; Paquin et al 1984; Besancon et al, 1985; Jouhiainen and Vanha-Pertulla, 1985; Cooper et al, 1988 and Yeung et al, 1990).

Kalla et al (1997) investigated the physiological role of neutral alpha-glucosidase in sperm metabolism. It was also discovered that the activity of this enzyme is maximum in the caput region with progressive decrease in the corpus and cauda regions of epididymis. In their experimental set-up on rats, Kalla and his co-workers also noticed a clear-cut positive correlation of alpha-glucosidase with the testicular hormones, especially the testosterone.
On the basis of experimental and clinical data available over the past few years, it has been hypothesized that neutral alpha-glucosidase is an important epididymal marker. The enzyme has been reported to play a vital role in metabolic activity of spermatozoa (Yeung and Cooper, 1994). Its activity is significantly reduced in obstructive azoospermia (Trembley et al, 1982; Guerin et al, 1990, Yeung et al, 1990 and Yeung and Cooper, 1994). A correlation of semen alpha-glucosidase activity with fertilizing ability of spermatozoa has also been suggested by Ali et al (1994).

**Accessory gland markers**

**Seminal vesicles**

A pair of glandular sacs or tubes of approximately 5-6 cm length arise from the vas deferens and lie on the dorsal face of the bladder. A yellowish, viscous, alkaline secretion from the seminal vesicle, which is commonly known as vesicular fluid, is rich in fructose. Other major constituents of the vesicular fluid are ascorbic acid, inorganic phosphorus, potassium and prostaglandins (Mann and Lutwak-Mann, 1981).

**Fructose**

Huggins and Johnson (1933) were the first to observe the reducing sugars in human semen, which are derived from the secretions of seminal vesicles and are absent in prostatic fluid. Later, Mann (1946) identified the seminal sugar as fructose. It was shown that bulk of the seminal fructose is secreted by the seminal vesicles. It has long been reviewed that survival and motility of spermatozoa in an anaerobic atmosphere in the seminal plasma and the female reproductive tract is maintained by the energy provided by anaerobic fructolysis whereas, aerobically the effect of fructose is less striking and oxygen alone can induce endogenous respiration and by itself provokes sperm motility as in epididymal spermatozoa.

A positive correlation between the rate of anaerobic fructolysis and the degree of motility existed in human semen (Peterson and Freund, 1976).
Recently also the correlation of low seminal fructose in asthenozoospermic subjects has been noticed by many workers (Jones and Connor, 2000, Suominen, 2001, Gonzales and Villena, 1997, 2001).

Linder and Mann (1960) showed in their study that testicular testosterone content was significantly correlated with the weight of the seminal vesicles and their fructose and citric acid contents. Apart from testosterone, several other factors like size and storage capacity of the accessory glands, frequency of ejaculation and sperm to seminal plasma ratio also influence the secretion of fructose and citric acid.

Fructose is the specific biochemical marker of the seminal vesicles to assess their secretory function reliability. In congenital absence of vas deferens, there will be agenesis of seminal vesicles. In such cases of excretory azoospermia, seminal fructose will be nil whereas, in secretory azoospermia, due to defective spermatogenesis and excretory azoospermia due to blockage at the epididymal level, the seminal fructose will be normal (Mortimer, 1994).

Prostate

The prostate is a tubulo-alveolar gland about the size of a chestnut in the normal adult man which surrounds the urethra immediately beneath the neck of the bladder. The main mass of the gland is made up of two prominent lateral lobes with round borders. The posterior surface is triangular and flattened and usually has a longitudinal depression in the midline (Mann and Lutwak-Mann, 1981).

Huggins and Webster (1948) showed a peculiar functional duality in the anterior and posterior regions of the prostate. The human prostatic secretion is usually colourless fluid, slightly acidic (pH = 6.5) and found completely devoid of reducing sugars. It is rich in Zn, acid phosphatase and citric acid. Other important constituents of the secretion are spermine, cholesterol, phospholipids and fibrinolysins (Mann, 1964 and Ganong, 2002).
Citric acid

Citrate in semen was first discovered by Schersten (1929 and 1936). Later, Huggins and Neale (1942) confirmed that human prostatic secretion is the main source of seminal citric acid. Huggins (1947) re-assured that ventral prostate is rich in acid phosphatase and citric acid and the analysis of these two constituents provides a most convenient assessment of the functional status of human prostate. The exact mechanism of citric acid biosynthesis and its metabolic role is still obscure but Dondero et al (1972) postulated that its level in human seminal plasma reflects an individual’s androgenic state. They found a positive correlation between blood plasma testosterone and citric acid levels.

Recently, Roy et al (2001) have emphasized that fructose along with seminal germ cell morphology and other biochemical markers like glycerylphosphoryl choline (GPC) and acid phosphatase are very valuable and dependable non-invasive markers to differentiate obstructive azoospermia from non-obstructive.

Hormone profile and male fertility status

Bruno et al (1986) reported that serum testosterone levels were lower in infertile group and also postulated that serum FSH and LH dropped significantly (p<0.001) when sperm count was less than 5 million/ml. Both the gonadotrophins were tightly tuned with sperm count and the spermatogenic potential. Garcia et al (1992) in their study of differential diagnosis of human azoospermia observed that FSH level was found to be higher in secretory azoospermia where spermatogenesis was deteriorated.

The physiological role of testosterone and gonadotrophins (FSH and LH) in male reproduction has been extensively reviewed by many authors (Graffin and Wilson, 1992)
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Electrolyte levels in semen and male fertility

Potassium (K) and Sodium (Na)

The role of electrolytes in semen has been discussed by Battersby and Chandler (1977). Potassium to sodium ratio (K/Na) or potassium concentration in semen may be an important factor in sperm motility. It has long been established by Sheth and Rao (1962), that high potassium concentration in male reproductive tract is responsible for the immotility of epididymal sperms.

The male accessory secretions have generally high K/Na ratio than most other body fluids but the epididymal seminal plasma exhibits the highest ratio. The spermatozoa (241/173) also have higher ratio than the seminal plasma (161/290) mg/100 ml. A potassium shift may also be as an important factor in initiating the motility and metabolism of spermatozoa after the completion of their epididymal passage and ripening process. The reciprocal relationship between Na and K in the sperm cells and seminal plasma has been observed (Mann, 1964).

The potassium concentration in vesicular fluid is much higher (20 mM) as compared to the sodium concentration in man. In contrast to seminal vesicles, the reverse is true in prostatic fluid which is rich in sodium (156 m equiv./L) as compared to potassium ions (30 m equiv./L). Other ions present in the prostatic fluid are citrate, chloride, bicarbonate and phosphate. The relative rates of sperm penetration by inorganic ions is in the order of NO₃⁻ > I⁻ >Br⁻ and > Cl⁻. Cations penetrate the sperm membrane much more slowly than anions. In whole semen, motile spermatozoa efficiently keep up ionic gradients across the plasma membrane; K inside the sperms is higher than outside; and reverse is true for Na (Quinn and White, 1967). This K and Na gradient is actively controlled by ouabain-sensitive Na/K pump as for any other cells of the body. This ionic gradient builds an unevenly distributed negative electric charge across the spermatozoal plasma membrane.
Gordon and Dandekar (1977) also discussed the presence of membrane-bound enzymes and substrate-specific binding sites in spermatozoa. Sperm plasmalemma contains Na⁺-K⁺ ATPase like membranes of other cells. This enzyme, which is activated by Mg⁺ ions and inhibited by ouabain along with balancing the Na/K exchange pump, is also involved in head-to-head associations between spermatozoa which are facilitated by Mg, Mn and Ca ions. Kobayashi, et al, (1978) reported that relatively excessive Mg⁺² ions inhibits Na⁺-K⁺ ATPase activity.

**Ionic and metabolic exchange reactions between seminal plasma and spermatozoa**

There is variety of causes, which bring about changes in the ionic distribution between seminal plasma and spermatozoa, which may result into irreversible loss of sperm motility. These include calcium shift towards inside of spermatozoa, efflux of potassium and magnesium from and influx of sodium into the spermatozoa. These alterations are different from the transfer of potassium and sodium at the time of fructolysis during normal metabolism of semen under aerobic and anaerobic condition (Quinn and White, 1966).

Spermatozoal respiration is associated with ionic exchange, particularly the Ca⁺² ions. In epididymal spermatozoa which are exposed to relatively hypotonic conditions, the spermatozoal respiration is increased with increased Ca levels due to energy – linked uptake of Ca by the spermatozoal mitochondria (Storey and Keyhani, 1974).

**Trace Elements**

As early as 1920s, the concentrations of various trace elements in human tissues were estimated using atomic emission spectrometry. Later, Tipton and Stewart (1970) considered it necessary to supplement emission spectrometry with other more appropriate methods, such as colorimetry and flame photometry for major elements and atomic absorption spectrophotometry for trace elements.
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Few years later, the concept of Inductively Coupled Plasma – Mass Emission Spectrometry (ICP-MES) was introduced when it was discovered that this method had improved sensitivity over atomic absorption spectrometry and relatively small sample size was sufficient. The results obtained were in satisfactory accuracy of determination when compared with atomic absorption spectrometry. This advanced technique has an added advantage that it can perform simultaneous multi-element analysis of both biological and non-biological materials (Dahlquist and Knoll, 1978).

Trace Elements in Semen and male fertility

With the advent of modern technology and increasing interest in the field of andrology, a group of scientific community has already agreed with the belief that a new approach is certainly required to reach some acceptable diagnosis of approximately 15 to 20% unexplained male infertility. A new era of understanding started when many workers in different corners of the world shared a common understanding that trace metals and minerals though required in small quantities have a decisive role in male fertility indeed, as for any other vital function of the body. Their presence in blood, whole semen, seminal fluid and even in spermatozoa has been well studied globally (Battersby and Chandler, 1977; Stegmayer et al, 1982; Umeyama et al, 1986 and Abou-Shakra et al, 1989).


Today, even the reports are available that oral supplementation of some essential trace metals for reproduction like zinc and selenium have
significantly improved the semen profile of infertile patients, especially those with idiopathic oligospermia (Tikkiwal et al, 1987, Wong et al, 2002).

Battersby and Chandler (1977) first studied the structural localization of trace elements in spermatozoa. They found that Na, Mg, S, K, Ca, Cu, and Zn were especially concentrated in mid piece region while P was found in high levels in the nucleus and acrosome, but no good correlation was found between sub-cellular levels of trace elements and sperm motility.

Even after such an extensive research in the field of andrology towards the role of trace elements in male fertility regulation, voids still exist to find a clear-cut correlation between semen profile and fertility status. An appropriate range of their concentration in seminal plasma as well as in spermatozoa is still to be established within which a particular trace element is beneficial and above which concentration it behaves as repro-toxic especially in human beings.

In view of the above-mentioned importance of trace elements in male reproductive functions, the role of each element was reviewed separately and an effort made to reach a consensus of understanding the metabolic requirement of these elements in human fertility regulation.

Zinc

Bernard and Vladesco (1921) were the first to observe zinc in human prostate and measured 9.4 and 11.3 mg/g of fresh tissue and 49.1 and 53.1mg/100g zinc of dry weight. Mawson and Fischer (1951) estimated the zinc content in different tissues in rat and compared it with genital organs.

Mawson and Fischer (1951) and Danscher and Rebbe (1974) found that posterio-lateral lobe of prostate was the richest source of zinc than any other organ in the body except bone. Epididymis also contained considerable amount of zinc whereas in the seminal vesicles, it was towards the lower limit; the mean zinc content of human prostate is 62.9 mg/100 gm. (dry weight).
Prostasomes, the organelle of prostatic origin, have been reported to be present in the seminal plasma free or attached to spermatozoa. These organelle have been found to stimulate forward progressive motility through Zn$^{+2}$-, Mg$^{+2}$- and Ca$^{+2}$- dependent ATPase present in those prostasomes (Ronquist et al, 1978a; Stegmayer et al, 1982 and Stegmayer and Ronquist, 1982).

Zinc represents a typical secretory product of the male accessory fluids, which associates firmly with spermatozoa. Most of the zinc taken up by the spermatozoa from the seminal plasma is highly temperature-dependent. About 50% of total zinc present in the fresh seminal plasma was absorbed by spermatozoa when semen was cooled at 4°C and left for 30 minutes, but half of that amount was absorbed at 20 to 25°C. A similar effect was observed when buffered citrate solution was added to a washed sperm suspension (Boursnell and Noble, 1975). This effect suggests that citric acid may help in zinc absorption in spermatozoa. Most of the zinc, which spermatozoa remove from seminal plasma, comes from low molecular weight zinc ligands and once taken up, zinc is not released from the sperm cells by heating the semen.

Herrmann, (1975) reported the presence of zinc-binding proteins of prostatic origin for facilitation of zinc transfer to human spermatozoa from the seminal plasma.

Janick et al (1971) believed that there was no difference in motility of vasal vs. ejaculated sperms in spite of large differences in their zinc contents. The class of ejaculates with highest fertilizability (with >10 million/ml count and >40% sperm motility) had significantly higher zinc levels in seminal plasma; sperm motility and seminal plasma zinc levels were significantly higher in this class than other categories i.e. oligospermic and less than 40% motility, oligospermic and less than 40% motility, normospermic and less than 40% motility.

The level of Zn and Mg in human spermatozoa was studied and the values range (0.46 – 19.65) for Zn (mean 5.5) μg/100 million sperms and
(0.20 - 12.63) for Mg (mean 3.7) μg/100 million sperms. The highest Zn and Mg levels were found in sperms from specimens with low acid phosphatase activity in seminal plasma and with high percentage of dead and immotile sperms (Lindholmer and Eliasson, 1972).

The mean semen zinc levels in fertile volunteers and post-vasectomized patients were not significantly different but difference was observed in patients with prostatitis and the trend was found towards increasing zinc levels with increasing fertilizing ability (Marmar et al, 1975).

Stankovic and Mikac-Devis (1976) discovered that copper and zinc are antagonistically related to each other in numerous processes. Zn showed positive correlation with sperm count showing lowest levels in azoospermic patients. This suggested that oligospermia might be associated with zinc deficiency. Copper in semen was found in µgms and it did not change with the spermatozoal activity.

Danscher et al (1978) showed adverse effect of high Zn level in seminal plasma with depressed motility while Stankovic and Mikac-Devic (1976) and Caldamone et al (1979) have shown the opposite effects on sperm motility.

Papadimas, et al (1983) and Umeyama et al (1986) have shown high positive correlation between zinc, magnesium and calcium in seminal plasma. The progressive motility is inhibited or stimulated depending on their concentration.

Caldomone et al (1979) and Skandhan et al (1985) have found that Zn concentration in semen decreases with number and activity of spermatozoa. The reports on the role of Zn on sperm motility are conflicting according to different researchers. No correlation of seminal zinc concentration to either sperm density or motility in normo-, oligo-, or azoospermic males has been reported by Abou-Shakra et al (1989).
Studies of Lewis-Jones et al (1996) also showed no statistically significant correlation of Zn concentration and sperm motility; when fructose concentration was negatively correlated with motile sperm concentration.

Carreras and Mendoza (1990) in their study of seminal plasma zinc levels in fertile and infertile men have found higher zinc levels in asthenozoospermic men as compared to normo-, oligoastheno- (p<0.001), oligo- and azoospermic men (p<0.01). A significant negative correlation was observed in all the groups between percent forms showing normal progressive motility and zinc concentration in seminal plasma. Although zinc is required in seminal plasma for normal functionality, yet excessive high levels of this ion may be related to defective motility in asthenospermic samples.

Bedwal and Bahuguna (1994) have reviewed the possible role of Zinc, Copper and Selenium in reproduction and assimilated the explanations for molecular basis of their physiological functions as sperm motility and fertilizing ability. They have discovered that some of the important enzymes of spermatozoa are Zn-metalloenzymes and they become dysfunctional with Zn deficiency.

Saaranen et al (1989b) had earlier reported that Se and Zn can protect the testes against deleterious effects of Cd, Zn protects by competitive binding with metallo-enzymes or due to scavenger function of Zn -induced metallothioneins.

Sorensen (1999) has discussed in detail the physiological and pathological role of zinc in the male reproduction. In his work on "Localization and functional aspects of zinc ions on male reproduction", it has been reported that zinc plays an important role in testicle differentiation, synthesis and activity of testosterone, spermatogenesis, chromatin stability, motility of spermatozoa and acrosome reaction.
The exact localization of zinc ions in the rat Leydig cells, Sertoli cells and developing sperm cells was detected by him using autometallography. The exchange of zinc ions from spermatozoa to epididymal cells has been suggested which play an important role in the maturation of sperm cells (Srivastava, et al., 1983; Stoltenberg et al, 1997 and Zalewski et al ,1996).

Over the years, there has been much debate on the role of zinc in sperm functions (Sorensen et al, 1999a). Autometalloigraphical studies of the semen smears show that the sperm head accumulates manifold higher concentrations of zinc than seminal plasma (Stoltenberg et al, 1997).

It has also been reported that prostatic-derived zinc is an essential element for chromatin stability and the ability of chromatin de-condensation at the time of acrosome reaction (Kvist et al , 1980, Kvist and Elliason, 1980, Kvist,1982; Kvist and Bjorndahl,1985, Kvist et al, 1987). It has been further reported that ejaculated sperm nuclei contained more zinc than vasal and epididymal spermatozoa which suggests that human spermatozoa accumulate zinc from the prostatic fluid upon ejaculation (Bjorndahl and Kwist, 1982,1985, Bjorndahl et al, 1986 and Kvist et al 1985).

Suzuki et al (1985), Gavella et al (1999) and Riffo et al (1992) observed in their studies that high zinc level is protective and offer antioxidant like behavior against oxidative damage to spermatozoa and also depresses acrosome reaction and premature acrosome reaction Premature acrosome reaction of spermatozoa is prevented by alterations of potassium permeability and inhibition of the entry of ions through sperm membrane.

Gavella et al (1999) in their further in-vitro studies have again emphasized that zinc has some inhibitory role on both superoxide anions production and superoxide dismutase (SOD) activity in human spermatozoa. This activity may have certain important zinc related mechanisms involved in oxidative events occurring after ejaculation and also some modulatory roles on germ cell functions.
Carpino et al (1998) have observed that seminal zinc fractions are bound to high molecular weight (HMW) proteins in the seminal plasma. In asthenozoospermic patients, there are normal seminal zinc levels as compared to control (fertile) subjects. On further division of asthenozoospermic patients into normo- and oligo- asthenozoospermics, the results show that high zinc contents were seen in spermatozoa of oligoasthenospermic as compared to normoasthenozoospermic patients and the control group. This data suggests that increased unbound (free) seminal zinc could contribute to the decreased sperm motility in normoasthenozoospermic and oligoasthenospermic patients. In oligoasthenospermics, further decrease in motility may be due to major zinc uptake by spermatozoa. The higher intra-sperm zinc content in these patients could be the cause of their low sperm membrane functionality. It has also been seen that there is a positive correlation between high seminal plasma zinc levels and asthenospermic males and an adequate seminal plasma content of zinc is required for normal sperm functioning (Carreras et al, 1990 and Fuse et al, 1999).

Henkel et al (1999) studied the zinc levels in different constituents of semen to find a definite correlation of zinc with sperm motility. The mean seminal plasma and whole ejaculate zinc concentrations were 144.3 and 146.9 mg/l, respectively and these concentrations were correlated with sperm motility. The sperm head contained only 6.7% of the total spermatozoal content. The zinc concentration of flagella was negatively correlated with sperm motility and velocity. The flagellar zinc is mainly located in outer dense fibers (ODFs) and there is structural and chemical modification of the elements during epididymal sperm maturation including zinc elimination. A low zinc content of ODFs is required after epididymal transit to achieve stiffened ODFs. Stiffening of ODFs takes place by formation of disulphide (-S-S-) bridges, which is an important physiological step for generation of sperm motility, especially the progressive motility.

Sorensen et al (1999b) took up further study of sperm cell motility in human spermatozoa to understand the exact role of zinc ions by subjecting
the sperms to intracellular and extracellular chelating agents—Diethldithiocarbamate (DEDTC) and Ethyline diamine tetra acitic acid (EDTA). It was observed that even very small concentrations (0.01 mM) of DEDTC immobilized the sperm cells within 80 minutes while EDTA showed no depressing effect. Rather at concentrations of 0 and 0.5 mM EDTA enhanced the straight velocity of spermatozoa (effect was not found at high concentrations). This study suggests that intracellular mitochondrial zinc ions play an important role in motility while the loosely bound or free zinc ions in the seminal plasma exert only a secondary role on human sperm cell motility. The role of these chelating agents on rat and dog epididymal spermatozoal motility was also studied earlier by Saito et al (1967).

Now, there is one school of thought about the role of zinc saying that there is no significant difference in plasma Zn levels in infertile and fertile men (Smith et al, 1983). There is no consistent relationship between plasma (13.5±2.1) and seminal plasma (1.65±1.25) µmol/l Zn levels. Seminal plasma Zn levels did not differ convincingly between fertile and infertile men but there is a clear-cut positive correlation between sperm density and seminal plasma Zn levels in fertile control subjects (p=0.002) men with count more than 20 million/ml had significantly higher seminal plasma Zn levels vis a vis oligospermic (p=0.01); but this relationship is not consistent within infertile men. No significant associations were found between concentration of Zn, Cu, Cd and Pb in blood and between Zn and Cu in seminal plasma. Another group of workers also report that there is no significant correlation between Zn concentration in seminal fluid and sperm density and sperm motility. They say that there is no full understanding of Zn with male infertility (Madding et al, 1986; Abou-Shakra et al, 1989 and Smith et al, 1993).

Lewis-Jones et al (1996) and Robak-Cholubek et al (1998) found no correlation of seminal plasma zinc concentration with sperm density and sperm motility and concluded that zinc is an unreliable marker of spermatogenesis.
Lin et al (2000) in their study concluded that zinc at extremely high concentrations (10 to 100 times the normal range), may be anti-bacterial, may inhibit sperm motility and may inhibit the mannose receptors on the sperm head but within the normal limits zinc has no significant correlation with spermatozoal percent motility, rapid progressive motility, average sperm velocity, curvilinear velocity and amplitude of lateral head displacement and also there is no correlation with conventional semen parameters either. In a recent study it has been observed that seminal plasma and serum zinc levels are not significantly related with male genital tract infections indicated by the presence of seminal leucocytes and also not related with local antisperm-antibody levels of IgG and IgA and semen cultures (Waltraud et al, 2002). (High serum zinc levels were defined as >=1.5 mg/l). Further more, the lack of association of seminal plasma and serum zinc levels with semen quality parameters indicates that routine zinc estimation during infertility evaluation is not recommended (Lin et al, 2000 and Kruse et al, 2002).

Another view is that seminal plasma zinc concentrations are significantly correlated with sperm density (p<0.0001), motility (p< 0.0001) and vitality (p< 0.0001). So zinc can contribute towards fertility through its positive effect on spermatogenesis; the means of seminal plasma and serum zinc concentrations were significantly lower in infertile vis a vis fertile males, (Chia et al, 2000). This fact is further strengthened by administrating oral supplementation of folic acid and zinc sulfate to sub-fertile males with idiopathic asthenozoospermia or oligospermia; also as studied earlier by Hartoma (1977) and Tikkiwal et al (1987). The results have shown improved sperm count in sub-fertile male. Mohan et al (1997) also got significantly lower concentrations of seminal plasma zinc in infertile men and also their serum zinc levels were in significant correlation. zinc is essential in spermatogenesis as a cofactor of metalloenzymes involved in DNA transcription, expression of steroid receptors and protein synthesis.

Further studies are required to establish a fact that functional deficiencies in folate and zinc are a risk factor for male factor sub-fertility (Wong et al, 2002).
Magnesium (Mg)

Many workers have studied the role of Mg in semen metabolism in association with Zn and Ca (Lindholmer and Eliasson, 1972, Sorensen et al, 1999a). It has been discovered that Mg and Ca concentrations are correlated with acid phosphatase and fructose levels. The level of Zn and Mg in human spermatozoa was studied and their values range between (0.46 – 19.65), (mean 5.5) µg/100 million sperms for Zn and (0.20 - 12.63), (mean 3.7) µg/100 million sperms for Mg. The highest Zn and Mg levels were found in spermatozoa from specimens with low acid phosphatase activity in seminal plasma and with high percentage of dead and immotile sperms. High Mg concentration was found in spermatozoa from specimens with more than 50% abnormal cells. No correlation was found in spermatozoa level of Zn and Mg with sperm density, progressive motility or oxygen consumption and not even with fructose concentration in seminal plasma (Lindholmer and Eliasson, 1972). Magnesium (Mg) concentration was found to be higher in spermatozoa than in seminal plasma (Mann and Lutwak-Mann, 1981).

Relation of Zn and Mg concentration with motility

The spermatozoal Zn and Mg concentration was not significantly different in groups with motility less than 30%, 31-59% and more than 60% motility, but the ratio of Zn(sperms)/Zn(Seminal plasma) and that of Mg(sperms)/Mg(Seminal plasma) was significantly higher in groups with 31-59% motile sperms as compared to other two groups (p<0.05 and 0.025, respectively). There was no correlation between Zn (sperms), Zn (Seminal plasma), Mg (sperms), and Mg (Seminal plasma) with the numeric values of progressive motility (Lindholmer and Eliasson, 1972).

Relation of Zn and Mg concentration with sperm viability

In the group with greater than 30% dead sperms Zn (sperms) was significantly higher than other groups with less number of dead cells (p<0.05) but it was not true for Mg (sperms). There was no correlation between percentage of dead sperms and ratios of Zn(sperms) / Mg(sperms), Zn(sperms) / Zn(semenalplasma), or Mg(sperms ) / Mg(semenal plasma) (Lindholmer and Eliasson, 1972).
Relation of Zn and Mg concentration with sperm morphology

Mg (sperms) and the ratio Mg (sperms) / Mg(seminal plasma) was found to be higher in groups with >50% abnormal sperms vis a vis less number of abnormal spermatozoa. These high values could not be correlated with any special defects in spermatozoa e.g. head, mid-piece and tail defects. No such correlation was observed for Zn(sperms) (Lindholmer and Eliasson, 1972).

Mean Mg concentration was lower in normospermic, asthenospermic and oligospermic men with higher leucocyte content, but in vasectomized men Mg levels were similar to those of the normospermics. Mg concentration in asthenospermics and oligospermics was lower vis a vis normospermic and it is in correlation with Ca concentration. Mg is negatively correlated to pH (Stegmayr et al, 1982, Adamopoulos and Deliyiannis, 1983).

Umeyama et al (1986) and Sorensen et al (1999a) discovered that Mg and Zn levels decreased gradually in proportion to sperm density and their concentration was the highest in normospermic-infertile (g-2) group as compared to fertile (g-1), oligospermic-infertile (g-3), severely oligospermic — infertile (g-4) and azoospermic (g-5) groups.

Pandey et al (1983) studied the Mg, Ca and Zn levels in the whole semen of infertile men. Among the infertile men, 82% showed subnormal quantities of one or more of these elements. So these elements, jointly or independently, control the physiological integrity of spermatozoa outside the male genitalia. Zn deficiency has been associated with defective germinal epithelium of testes, atrophic seminiferous tubules, lack of spermatid maturation, azoospermia and low gonadotrophin-androgen levels in lower animals.

Calcium (Ca)

Calcium is another mineral which is taken up by spermatozoa from the male accessory secretions. A very high concentration of total Ca level has been marked in both seminal plasma and the spermatozoa in man. The Ca in
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Seminal plasma, which is chiefly contributed by the prostatic secretion was found to be three times greater than that of blood plasma (Mann, 1964 and Quinn et al, 1965).

At low concentrations (1mM) the Ca uptake is inhibited by Calcium ionophores, but at high concentrations, the ionophores stimulate the Ca uptake. The physiological role of calcium in acrosome reaction, sperm capacitation and polarity reversal have long been established. (McGrady and Nelson, 1974 and Storey, 1975).

The calcium in spermatozoa and seminal plasma exists either in the ionized form (free), complexed with small chelators such as citric acid or in the protein-bound form. The seminal plasma contains less ionized calcium than the spermatozoa, whereas the protein-bound calcium occurs almost equally in spermatozoa as well as in seminal plasma (Karagiamidis, 1976).

Brooks and Siegel, (1973) extracted calcium-binding proteins in spermatozoa and Lukac et al (1976) in seminal plasma. Forrester and Bradley (1980) described that one of the Ca-binding protein resembles Calmodulin. The Ca-Calmodulin complex binds and activates certain specific enzymes, which are concerned with phosphorylation of proteins. Intracellular calcium concentration as well as that of cyclic AMP is reported to be regulated by Calmodulin.

Stegmayer et al (1982) estimated the Ca, Mg and Zn in organelle (granules and vesicles) of prostatic origin which are reported to be present in human seminal plasma. Yanagimachi & Usui (1974), Babcock et al (1978), Nelson (1978) and Singh et al (1978) reported that these organelle contain certain metals in different concentrations where Ca, Mg, and Zn are decreasing order. The exact function of these secretory granules is not yet established but Ca\(^{2+}\) ions are known to play an important role in acrosome reaction and control of sperm motility. These organelle may function as selective regulators of Ca ion concentration in microenvironment of sperm cells through Mg\(^{2+}\) and Ca\(^{2+}\)- dependent ATPase. Zn also probably affects cell...
membranes and exerts an antagonistic effect on both Mg^{2+} and Ca^{2+}
dependent ATPase system and through endogenous protein kinase activity in
the membranes of secretory granules and vesicles (Ronquist et al 1978 a, b).
Similarly, a targeted release of Ca^{2+} from these organelles may take place as
a result of phosphorylation of membrane proteins (Stegmayer et al, 1982).

Adamopoulos and Deliyiannis (1983) found that Ca level in
normospermic (249±62), oligospermic (195±11), azoospermic (285±157) and
vasectomized (250±50) patients was significantly different (p-value <0.01)
with respect to normospermic patients.

Umeyama et al (1986) studied fourteen trace elements namely Al, Fe,
Ca, Cr, Cd, Cu, Mg, Pb, Zn, Ni, Sr, Mn, Mo, and Sn, in whole semen of
Japanese men in the age group of 26 to 48 years in infertile and known fertile
men. These trace elements act jointly or independently in human metabolism.
In their study, Ca concentration was found to be the highest followed by then
comes Zn and Mg. The mean Ca levels in 5 groups namely (g-1) fertile
(245±79) mg/l, (g-2) normospermic-infertile (330±172) mg/l,
(g-3) oligospermic-infertile (249±94) mg/l, (g-4) severely oligospermic-infertile
(269±115) mg/l and (g-5) azoospermic (213±73) mg/l showed no significant
difference. Many trace elements like Fe, Al, Cd, Cu, Mg, Mn, Mo, Sn and Zn
were found to be significantly higher in normozoospermic-infertile as
compared to fertile men whereas, Ca, Cr, Mg, Pb, Sr, and Zn were almost the
same in both fertile and infertile men. The concentration of Zn decreased
gradually with decreasing number of spermatozoa.

Abou-Shakra et al (1989), also conducted multi-elemental analysis
namely, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Pb, Ru, Se, V, and Zn in
semenal plasma with Inductively Coupled Plasma-source Mass Spectrometry
(ICP-MS). Most of the trace elements showed no significant correlation in the
four groups of infertile patients i.e. normospermic- infertile, oligospermic,
severely oligospermic and azoospermics. Ca levels of normospermic (167
±44), oligospermic (202±58), severely oligospermic (179±50) and
azoospermic (195±63) µg/ml were detected in seminal plasma. The large
difference of Ca levels found in the values of Abou-Shakra et al (1989) in seminal plasma and those of Umeyama et al (1986) in whole semen might be due to the fact that sperms are stored in epididymis which is known to be rich in Ca ions. This vital element has been reported to regulate several properties of sperms. High concentration of Ca is reported to suppress sperm motility (Arver, 1982). A study conducted on the Mg, Ca and Zn levels in whole semen of infertile men shows that excess as well as deficiency of Ca and Mg is associated with abnormal neuro-chemical transport (Pandey et al, 1983).

Prien et al (1990) found no significant difference between seminal total Ca concentrations regardless of sperm motility. However, men with hypomotility showed significantly (p<0.001) low Ca concentration vis a vis normal motility.

Logoglu et al (1997) estimated total Ca concentration in seminal fluid of fertile and infertile men and presented conflicting data as compared to the results of Umeyama et al (1986), Abou-Shakra et al (1989) and Prien et al (1990). Normospermic-infertile group showed significantly (p<0.05) lower Ca levels (113±15.2) µgm/ml vis a vis fertile group (152±9.9) µgm/ml. The lower Ca levels in oligozoospermic-infertile men (137±14.5) µgm/ml showed no significant difference with fertile men. In the control group, no significant correlation existed between seminal Ca and sperm density and percent motility. The study strongly suggests that seminal Ca levels should be estimated in normospermic-infertile men who are also asthenospermic.

It has long been known (Zanaveld et al, 1989) that extracellular Ca is required for successful fertilization. To initiate acrosome reaction and sperm-egg interaction, Ca²⁺ ion influx is required. Calcium is also involved in sperm motility (Fraser, 1977, Yanagimachi, 1982, Yanagimachi and Usui, 1974 and Feng et al, 1988).

However, Arver (1982) showed that high concentration of Ca suppresses sperm motility. So, on the basis of all the data available, it may be concluded that seminal Ca may be involved in sperm motility, this effect being
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stimulatory or inhibitory depending on its concentration. Fraser (1977,1987) reported that the optimal seminal Calcium is required to promote sperm motility at all steps leading to successful fertilization. Sorensen et al, (1999a) also observed the similar inter-correlations between Mg, Ca and Zn.

Copper (Cu)

Skandhan (1992) reviewed that the effect of copper on spermatozoa was studied as early as 1856 by Quaterphages. Its role in sperms is not very clear but it appears to be involved in sperm mobility and it may also act on the pituitary receptors to control the release of LH.

Stankovic and Mikac-Devis (1976) in their study on zinc and copper levels in whole semen to find a correlation with fertility in men, found that copper and zinc are antagonistically related to each other in numerous processes. Copper in semen was found in µgm amounts and it did not change with the activity of semen.

Battersby and Chandler (1977) studied the correlation between elemental concentrations namely Na, Mg, S, K, Ca, Cu, P and Zn and motility using X-ray microanalysis of human sperm cells. In the semen donors of having motility ranging from 0% to 85% showed that concentration of these elements did not show any strong correlation with sperm motility except that copper was positively correlated with motility when high- and low-fertility groups were compared. From the study, it was concluded that sub-cellular element concentration is not a major factor in determining sperm motility in human semen.

Smith et al (1983) estimated the Zn, Cu, Cd, and Pb levels by atomic absorption in fertile and infertile men to find a possible role of these elements with regard to fertility. It has been observed that infertile men had higher plasma Cu levels (16.1±2.8) than proven fertile men (14.5±2.9) µmol/l (p <0.01) (~1.5 µmol mean difference) (p=0.007). Seminal plasma Cu did not differ significantly between infertile (1.65±1.21) and fertile men (1.64±1.21)
μmol/l and no significant relation was observed in Cu levels in seminal plasma and plasma.

Umeyama et al (1986) divided the subjects into five groups namely G-1 (proven fertile), G-2 (normospermic-infertile), G-3 (oligospermic-infertile), G-4 (severely oligospermic-infertile) and G-5 (azoospermic). They observed conflicting results and found that semen copper concentrations of infertile men were much higher than those of fertile men in the multi-element study conducted in fertile and infertile men. The values of semen Cu in the five infertile groups (G-2 to G-5) were 0.034±0.07 mg/l, 0.106±0.16 mg/l, 0.067±0.08 mg/l, 0.056±0.08 mg/l and 0.069±0.19 mg/l respectively and in the fertile group (G-1) was 0.074±0.14 mg/l.

The results of Abou-Shakra et al (1989) are in good agreement with that of Smith et al (1983) as Cu concentrations measured by ICP-MS in seminal plasma of normospermic (0.17±0.06 μg/ml), oligospermic (0.16±0.0758 μg/ml), severely oligospermic (0.18±0.07 μg/ml) and azoospermic (0.17±0.04 μg/ml) were much lower as measured earlier by Umeyama et al (1986) but not found to be significantly different among the different groups.

Later, Jockenhovel et al (1990) also found that semen copper concentration of infertile (194.99±5.70)μg/l and fertile men (183.39±14.37)μg/l, studied with atomic absorption spectrometer did not differ significantly. However, a significant correlation existed with sperm concentration (p<0.001), percentage of progressive motility (p<0.005) and normal morphology (p<0.005). Since, the highest correlation was found with sperm count, it is possible that copper might have leaked out from the spermatozoa. Copper is an essential trace element and an important component of many enzyme systems.

Jockenhovel et al (1990) further explains that superoxide dismutase (SOD) contains 1.85 g-atoms of copper and is present in human spermatozoa. This enzyme plays an important role in protecting human
spermatozoa against peroxidative damage of cellular enzymes and structures. As the human sperm’s plasma membrane contains large amounts of unsaturated fatty acids and is sensitive to lipid peroxidation which can eventually result in cell damage through permeabilization of sperm plasma membrane. It has been reported that production of reactive oxygen species (ROS) by spermatozoa is increased in infertile men. The presence of SOD in human seminal plasma and sperms and the fact that copper is an essential part of superoxide- removing systems, gives evidence to the hypothesis that copper concentrations in the semen could have some positive correlation with the quality of the ejaculate.

Further evidence for the protective role of copper for spermatozoa is added by the fact that it stabilizes the nuclear chromatin of the human sperms by the formation of \(-S-S-\) cross links from thiol groups on adjacent structural proteins and nucleoprotein chains. During passage through the epididymis, the sulpha-hydral groups of sperm heads and tails are oxidized to disulphide bonds (Bedford et al 1973). Thus copper might be involved in epididymal maturation.

Sakandhan (1992) further studied the role of copper on spermatozoa and found that in seminal plasma, the level of Cu falls in azoospermic and rises in oligospermic and asthenospermics patients.

Bedwal and Bahuguna (1994) have reported that copper is an important component of numerous metalloenzymes and metalloproteins and its lack leads to defects in cardiovascular, nervous, skeletal, immune and reproductive systems. Of these enzymes, cytochrome–c-oxidase and superoxide dismutase have been extensively studied.

Different authors have reported the toxic effects of Cu since a very long time. In male reproductive system, copper has been identified as one of the elements toxic to spermatozoa (Loewit, 1971; Holland and White, 1980). The significantly different plasma copper level may have some pathological relevance although its relationship to male fertility is still not fully understood.
as there is no significant difference in seminal plasma copper concentration in fertile and infertile men (Smith et al, 1983, Abou-Shakra et al, 1989, and Jockenhovel et al, 1990).

The excessive concentrations of Cu reduce the oxidative processes and glucose consumption, which reduces or abolishes the spermatozoal motility.

Roblero et al (1996) also studied the role of copper ions on motility, viability, acrosome reaction and fertilizing ability of human spermatozoa in vitro. The results show that Cu at concentration similar to that released from an Intra Uterine Device (IUD) affects the fertilizing ability of human gametes in vitro and interferes with the sperm-oocyte interaction leading to impairment of fertilization. This property is exploited to use Cu for male contraception in animal system (Kapur et al, 1984).

Iron (Fe)

Mann and Lutwak-Mann (1981) reported that iron (Fe) partly occurs loosely bound in lectoferrin. Umeyama et al (1986) found significantly higher contents of Iron (Fe) in normospermic-infertile subjects as compared to the fertile subjects.

Later, Abou-Shakra et al. (1989), in their multi-element analysis found that Fe levels in seminal plasma of normospermic (0.19±0.07 μg/ml, oligospermic (0.21±0.09 μg/ml), severely oligospermic (0.20±0.12 μg/ml) and azoospermic (0.18±0.13μg/ml) has no significant correlation with male infertility. It was discovered that iron and non-hemic spermoplasm ferroproteins present in human semen are involved in male reproduction. Iron participates in ejaculate thinning, formation of viscosity, maintenance of sperm pH and it has important role in improving fertilizing ability of spermatozoa (Nikolaev et al, 1998).
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Manganese (Mn)

Abou-Shakra et al (1989) in their multi-element analysis found that Mn levels in seminal plasma (µg/ml) of normospermic (0.017±0.006), oligospermic (0.024±0.008), severely oligospermic (0.020±0.008) and azoospermic (0.020±0.009); are significantly different in normospermic and oligospermic men (p>0.05) whereas normospermics show the lowest values in all the four groups which shows that there is no significant effect of Mn on sperm count but may have influence on their motility or fertilizing ability.

Sulphur (S) and Phosphorus (P)

Sulphur and phosphorus are both likely to be stable elements incorporated into the sperm nucleus during spermiogenesis. It was later found that Na, Mg, S, K, Ca, Cu, and Zn were especially concentrated in mid piece region while P was found in high levels in the nucleus and acrosome (Battersby and Chandler, 1976).

Adamopoulos and Deliyiannis (1983) found that Phosphorus (P) content in azoospermic (320±24µg/ml) and vasectomized (501±80 µg/ml) subjects was significantly less (p<0.05) than that of normospermic (512±97 µg/ml) and oligospermic (516±172 µg/ml) subjects.

Arsenic (As)

Arsenic content in blood was found to be less than 5 µg/l and spermatozoa did not contain any measurable concentration of As within the detection limits by Atomic Absorption Spectrometer (Oster and Prellwitz, 1985; Schramm and Oster, 1987).

Arsenic (As) may have a possible influence on sperm development as it has been reported to affect RNA and DNA synthesis and DNA repair. It is reported to be present in seminal fluid and its concentrations are in accordance with blood (Schramm and Oster, 1987).
Cadmium (Cd)

The cadmium is a known reproductive toxicant. Parizek (1956) was the first to report the spermatogenic arrest on account of cadmium administration in experimental animals. He also observed that damage could be prevented by simultaneous administration of zinc salts.

Johnson (1977) and Zielinska-Psuja et al (1979) hypothesized that antispermatogenic effect of cadmium on seminiferous epithelium was partly due to ischemia and necrotic changes arising due to interference of cadmium with testicular vascularization and breakdown of blood-testis barrier (Aoki and Hoffer, 1978).

Umeyama et al (1986) declared that Cd and Pb have been proved to be harmful to fertility as Cd was significantly higher in infertile vis a vis fertile group (p<0.005).

The serum as well as seminal plasma Cd level was significantly (p<0.01) higher in heavy smokers (>20 cigarettes/day) than in nonsmokers (Smith et al 1983; Saaranen et al 1989a).

No correlation between semen quality and seminal plasma or serum Cd was observed. The reported Cd levels in subjects with more than 40 million sperm count were 0.24±0.21 μgm/l in seminal plasma and 0.27±0.11 μgm/l in serum; whereas with less than 40 million count were 0.22±0.17 μgm/l and 0.31±0.09 μgm/l; with sperm motility greater than 50% were 0.18±0.19 μgm/l and 0.28±0.10 μgm/l and sperm motility less than 50% were 0.29±0.20 and 0.29±0.11 μgm/l, respectively in seminal plasma and serum. The mean seminal plasma Cd level in proven fertile men was 0.17±0.23 μgm/l and in infertile men was 0.26±0.22 μgm/l. The seminal plasma Cd level has no correlation with Zn and Se which themselves showed high positive correlation to each other (Saaranen et al, 1989a).

Chia et al (1992) estimated the blood concentration of cadmium, lead, mercury, copper and zinc and their correlation with semen volume, total
sperm count, sperm viability, progressive motility and sperm morphology was also observed. The asthenozoospermic-infertile men showed significantly higher blood Cd concentrations vis a vis normospermics and also significant correlation existed between semen volume, mid-piece defects and immature germ cells. High blood Cd concentration may have an effect on spermatogenesis. Benoff et al (1997) studied the role of Cd in varicocele-associated infertility.

**Chromium (Cr)**

Abou-Shakra et al (1989) studied Cr levels in seminal plasma (μg/ml) of normospermics (0.32±0.17), oligospermic (0.55±0.29), severely oligospermic (0.51±0.32) and azoospermic (0.41±0.20). No significant correlation has been found in Cr level with sperm count but the values were lowest in normospermic–infertile males.

Chromium (Cr) shows spermiotoxic effect on human testis along with Ni and Mn in workers exposed to welding fumes. These metals are strong mutagens and carcinogens depending upon the period of exposure (Bonde, 1990)

Li et al (2001) also showed that in Cr exposed male workers and normal controls, there was no significant difference in semen volume, liquefaction time, LH level and not even in their serum and seminal plasma levels.

**Mercury (Hg)**

Chia et al (1992) reviewed the reports on the effect of mercury on spermatogenesis are few. No significant difference was observed in the mean blood concentration of Hg, Cu and Zn in oligospermics, teratozoospermics and asthenozoospermics.
Aluminium (Al), Nickel (Ni), Molybdenum (Mo) and Cobalt (Co)

Abou-Shakra et al (1989) reported that Cd, Co, Mo, Ni and Se were below the detection limits (<1ng/ml). So no evaluation of results had been undertaken.

Umeyama et al (1986) noticed that semen Ni had a lower concentration in infertile than in fertile men. Mo and Al levels studied in 5 groups namely fertile (g-1); normospermic-infertile (g-2); oligospermic-infertile (g-3), severely oligospermic–infertile (g-4) and azoospermic (g-5) showed highest concentration in normospermic-infertile men. This suggests that these elements may be independently or jointly responsible for the physiological integrity of the spermatozoa in semen. Al, Cd, Cu, Mg, Mn, Mo, and Zn had significantly higher concentration in normospermic-infertile than fertile men (g-1).

Lead (Pb)

Lead is a known environmental pollutant which exerts a direct chemosterilizing effect on the testes and have a mutagenic effect. High incidence of fetal deaths and congenital abnormalities were reported in offspring of the women whose husbands were involved in pottery glazing and were exposed to lead (Mann and Lutwak-Mann, 1981).

Abou-Shakra et al (1989) found that Pb levels in seminal plasma (µg/ml) of normospermic (0.008±0.011), oligospermic (0.012±0.012), severely oligospermic (0.006±0.004) and azoospermic (0.010±0.013) has no direct involvement in male infertility.

In rats and other rodents, it was indicated that more than 30-40 µg/dl lead concentration in blood is associated with impaired spermatogenesis and reduced androgen concentrations; whereas, human studies reveal that exposure to inorganic lead >40 µg/dl in blood may lead to impaired reproductive functions by reducing sperm count, volume and density, or changing sperm motility and morphology, but no effects were detected on endocrine profile (Apostoli et al, 1998).
Recently, Sallmen (2001) also hypothesized that lead is a reproductive toxicant. The studies only weakly suggest that males exposed to lead are associated with delayed conception. The findings of time-to-pregnancy and fertility rate studies are contradictory. There are a number of reasons by which exposure to lead may reduce male infertility. On the basis of animal studies, alterations in sperm chromatin stability or epigenetic effects may be the most probable mechanisms involved at low exposure level.

Telisman et al (2000) studied the role of biomarkers like lead, cadmium, zinc and copper on semen quality and reproductive endocrine functions. The estimations of blood lead, blood cadmium, seminal plasma zinc and serum copper were done in industrial workers of 20-43 years age group. The results indicate that there is a Pb-related decrease in sperm density, in counts of total, motile and viable spermatozoa and in parameters of prostate secretory function (seminal fluid Zn, acid phosphatase and citric acid in seminal fluid). There is increase in abnormal sperm head morphology, serum testosterone and estradiol levels. There was significant (p<0.05) correlation between blood Cd, semen Zn, semen Cu and smoking habits, alcohol consumption and age on reproductive functions. Blood Cd (BCd) contributed to decreased sperm motility, serum testosterone and abnormal sperm morphology. No significant Pb- or Cd-related influence was found on seminal plasma LDH and fructose level. There were significant correlations between blood and seminal plasma Pb and Cd levels. Even moderate exposures to Pb(BPb<400µg/l) or Cd (<10µg/l) can significantly reduce human semen quality without conclusive evidence of impairment of male reproductive endocrine function.

Jockenhovel et al (1990) studied the lead and copper status in fertile and infertile men. No significant correlation was found in semen concentration of lead with sperm concentration, progressive motility and morphology. This has been observed by others too (Saaranen et al 1989 and Stachel et al, 1989). The mean semen lead concentration of infertile men (11.18±0.62) µg/l were significantly higher than fertile men (5.61±0.53)µg/l (p<0.005). Low dose exposure to lead through environment does not cause gross abnormalities of
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sperm production and function but men chronically exposed to lead as part of their occupation show subtle impairment of sperm functions and decreased chromatin stability.

Umeyama et al (1986) also observed Cd and Pb harmful to fertility. In their study, Cd was significantly higher in infertile vis a vis fertile group (p<0.005) whereas, semen Pb levels have no significant correlation in these groups. Chia et al (1992) found high blood Pb and Cd concentrations in asthenozoospermics as compared to other groups.

Occupation-based Environmental Exposures and male fertility status

Heat

The adverse effects of heat on male fertility have been observed in humans for more than 20 years (Brun and Clavert, 1977). They reported changes in spermatogenesis. Exposure to heat in a Finnish sauna bath of 12 married medical students resulted in a lowered sperm count followed by rapid recovery (Procope, 1965). The role of hyperthermia on the testicles has also been put forward as an explanation of hypo-fertility (Zorgniotti et al, 1982).

Rachootin and Olsen (1983) and Baird and Wilcox (1986) clearly showed the relationship between occupational exposure and decreased male fertility which was also associated with delayed conception in some of the workers. Chia et al (1994) observed oligospermia in heat-exposed workers.

Fertility evaluation of aluminium industry workers was done to study the influence of heat and static magnetic field and it was observed that semen quality was significantly reduced in the exposed workers (Mur et al, 1998)

Electric welding

A study conducted on 637 Danish metal workers, electricians and welders showed that there is significant increased rate of delayed conception
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(Bonde, 1990a, Bonde et al, 1990). Welding work is associated with increased risk of reduced semen quality.

A non-significantly increased risk of sperm abnormalities was encountered in welding workers. The welders are exposed to welding fumes, whose levels and qualitative composition show great variation depending upon welding material, methods, posture and other circumstances. The content of Mn, Ni and Cr in welding fumes may represent a hazard for the spermatogenesis as these metals, after inhalation, are distributed in the tissues including the testes. Mn causes testisatrophia whereas, Ni and Cr are strong mutagens and carcinogens. So, it is possible that these metals alone or in combination have spermiotoxic effects on human testis.

In welding workers, other exposure is heat, which is a potential hazard (Bonde, 1990a). Later he reviewed the risk of male subfecundity due to metal welding in Danish population. The studies of semen quality, infertility, fertility, and adverse pregnancy outcome and childhood malignancies have been covered with reference to his previous studies (Bonde, 1993).

Wu et al (1996) in a study conducted on 211 occupationally exposed men to manganese and electric welding fumes with 99 controls, observed that semen concentrations of Mn, Cu, Cr, Ni and Fe in welding exposed group were significantly higher and also the liquefaction time was higher than that of controls. The semen profile of welding workers that is, semen volume, sperm count, viable sperm count and percentage of motile sperms was significantly lower in exposed workers vis a vis the controls. Semen quality and sex hormones with reference to metal welding was studied in Danish workers who were first time pregnancy planners. Their median sperm count was 56 millions/ml and there was no statistical significant correlation between their morphology, sperm motility or sex hormones (testosterone, FSH and LH). This negative correlation may not apply to populations exposed to high welding fumes and welders exposed to heat hazards (Hjollund et al, 1998).
Zinc galvanizing furnace workers

There are not many reports on the effect of exposure particularly to zinc fumes on the metal industry workers and their fertility status. The data available regarding workplace exposure to large variety of organic solvents, metal fumes and dusts or a combination of these exposures reveal that such exposures are associated with reduced semen quality (Tielimans et al, 1999; Kenkel et al, 2001).

No report is available regarding the fertility status of workers exposed to zinc fumes alone or in combination with aromatic solvents in galvanizing industry.

Seeing the data of different scientists, it has become desirable to undertake a comprehensive study to analyze the concentrations of various trace elements; enzymes and hormones and a few additional biochemical markers (fructose and citric acid) to build a possible correlation to explain male infertility. In today’s clinical set-up at-least, at the level of Tertiary Health Centers (THCs) and the Human Reproductive Research Centers (HRRCs), further research can be carried out to make the trace element profile along with other advanced male infertility tests as a diagnostic tool.