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## MINERAL NUTRIENTS AND MALE FERTILITY

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### ABSTRACT

Semen is a complex mixture containing a variety of organic and inorganic compounds. While the production and functions of semen are well understood, studies focused on the macro- and micronutrients necessary for male fertility are constantly appearing with new information. Chemical elements play a crucial role in male reproduction, as an unbalance in their amounts may lead to defective spermatogenesis, reduced *libido*, and consequently, male fertility impairment. Dietary and feeding supplementation has the ability to increase male reproductive performance which is why the effects of minerals in diet cannot be ignored. This review will provide recent information to a better understanding of the positive as well as negative roles of selected macro- and micronutrients on male fertility.

**Keywords:** Minerals, semen, spermatozoa, fertility, macronutrients, micronutrients

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## INTRODUCTION

Male fertility is a complex feature composed of various physiological processes including growth and development of the reproductive system from birth to adolescence, spermatogenesis, ejaculation and mating behavior (including *libido* and *coitus*). Optimal semen quality requires all these physiological processes to be coordinated and ideally interconnected (Cupps, 1991). Semen quality varies qualitatively and quantitatively with age, illness, sexual activity, and diet of the organism, with many studies being focused on the biochemistry of semen. Biochemical contributions to male fertility also include natural environmental factors, different anthropogenic activities as well as other sources. Minerals represent an extensive group of ecophysiological importance among these sources (Marzec-Wróblewska et al., 2012).

In relation to their role in male reproduction, chemical elements may be essentially divided in the following three groups:

1. Essential minerals, with high concentrations and crucial functions present in semen: Na, K, Cl, Ca, Mg, P, S (Marzec-Wróblewska et al., 2012).
2. Trace elements, which are critical to maintain proper functions of biomolecules, but are required in relatively low amounts, as their elevated concentrations may have a toxic impact on the sperm development, structure or function: Fe, Cu, Mn, Se, Zn, Co, I, Mo (Massányi et al., 2004; 2005).
3. Heavy metals, which do not have any detectable biological roles in ejaculates. On the contrary, many authors have reported heavy metal-associated detrimental effects on semen quality and fertility rates either by a direct impact on the testicular function (Lukáč et al., 2007) or mediated via hormonal imbalances (Kňazická et al., 2013) or toxicant-induced oxidative stress (Tvrđá et al., 2013). Male fertility-associated toxicity has been observed especially in the case of Pb, Hg, Cd, As and Al.

Chemical nutrients are shown to have an indirect but crucial effect on male reproduction, as an unbalance in their amounts may lead to a defective spermatogenesis, structural or functional sperm abnormalities, reduced *libido*, and consequently, impairment of male fertility. Dietary and feeding supplementation, as well as environmental characteristics have the ability to modulate male reproductive performance, which is why the presence of chemical elements in the male reproductive system and ejaculate cannot be ignored. This review will provide complex information to understand the participation of important dietary and environmental chemical elements on male reproductive features, processes and functions.

## SODIUM (Na) AND POTASSIUM (K)

Both cations are considered to be two essential macronutrients found in large amount in animal cells.

Na is defined as an extracellular element while K is intracellular in nature, and it has been suggested that seminal ionic equilibrium and osmotic pressure are primarily maintained by these two ions (Hawk et al., 1964). Their presence in semen is reported to be responsible for the maintenance of osmolarity and activity of spermatozoa (Ahmad and Chaudhry, 1980).

According to Cragle et al. (1958), K is highly concentrated within spermatozoa when compared to the seminal plasma, while Na is found excluded from the cells to some extent. At the same time, the significance of K within the sperm cells, as well as the exclusion of Na from spermatozoa in relation to storage quality and fertilizing capacity, is not fully understood yet. However, it is generally believed that both the presence of K as well as Na exclusion may be the result of metabolic activity and substrate utilization by the sperm cell (Sorensen and Anderson, 1956).

While the K concentration within spermatozoa fluctuates much less than the plasma K, Na amounts within spermatozoa vary as directly and greatly as the corresponding plasma Na. The accessory sexual glands of certain farm animals accumulate K to a level much higher than that found in the blood plasma. On the other hand, the increase in K is matched by a decrease in Na to a lower level when compared to the blood plasma. It is suggested that energy is expended by the accessory glands to accumulate K and Ca in the seminal fluid, and that Na is displaced by these two minerals, maintaining a constant osmotic pressure (Cragle et al., 1958).

At the same time, K has been defined as important in volume regulation processes vital for spermatozoa, which are based on spermatozoa achieving a stable volume crucial for their surveillance in the male and female genital tract. K ion channels have additional important functions in the sperm physiology, serving as means of communication between the spermatozoon and its environment. Additionally, they play vital roles in the regulation of sperm motility, chemotaxis and the acrosome reaction (Darszon et al., 1999; Barfield et al., 2005), and in situations when spermatozoa encounter a hypo-osmotic challenge upon ejaculation into the female tract (Yeung and Cooper, 2008). Furthermore, K ions may be an indication of sperm plasma membrane integrity (Asadpour, 2012).

Low Na levels have been linked to general infertility and embryonic mortality in several farm animals (Dittman, 2008). On the other hand, high levels of Na and K ions were associated with low percentages of motile sperm, and such semen was considered to be of lower quality in the study by Asadpour (2012), also suggesting that Na and K generally establish the osmotic balance, and seminal

plasma osmolarity ultimately plays an important role in the activation of the sperm cell. Inversely, **Zamiri and Khodaei (2005)** showed that low levels of Na and K were associated with high percentage of motile sperm. Furthermore, **Cragle et al. (1958)** as well as **Sheth and Rao (1962)** found that oxygen uptake, glycolysis and fructolysis could be inhibited by K, indicating that this element may adversely affect spermatozoa motility, which was additionally confirmed by **Cevik et al. (2007)**. Moreover, **Ford (2001)** and **Griveau et al. (1994)** concluded that at low pH the K ion pairs with the superoxide causing a significant increase in lipid peroxidation (LPO) and free radical formation, both of which are inversely correlated with sperm motility and seminal antioxidant status.

#### CALCIUM (Ca)

Calcium is required in many physiological processes as a regulator in all living cells, including spermatozoa. Most of the intracellular Ca is found bound to proteins in the cell membrane, mitochondria and nucleus, which is why the concentration of Ca in the intracellular fluid is considerably reduced (**Kaplan et al., 2002; Eghbali et al., 2010a**).

Spermatozoa are highly differentiated cells with the plasma membrane being the major cellular component, involved in diverse and complex functioning of the sperm cell to achieve fertilization. Many of these functional processes are made effective by the transport of ions across the plasma membrane through ion channels, with various types of Ca channels being the most studied in the sperm behavior (**Publicover et al., 2007; Yeung and Cooper, 2008**).

Ca is a part of the second messenger system involved in many cell functions. It is needed for the disruption of the mitochondria membrane by allowing protein kinases to stimulate the side-chain cleavage of cholesterol, which is the first step in steroidogenesis located in the Leydig cells (**Cupps, 1991; Eghbali et al., 2010a**).

According to **Semczuk and Kurpisz (2006)**, Ca helps to maintain the osmolar balance and takes part in nutrient transfer. It is necessary for the last stage of capacitation, and the following acrosome reaction, as well as a hyperactive motility of spermatozoa. Experiments have shown that addition of ionophore A23187, which actively transports the Ca ions from the extracellular to the intracellular space, induces the acrosome reaction (**Aitken et al., 1993a**). However, it was proved that the initiation of acrosome reaction with the ionophore is possible with the presence of Ca ions in the extracellular space only (**De Jonge, 1994**). At the same time, exposure of capacitated spermatozoa to periovulation follicular fluid or progesterone caused a Ca influx from the extracellular space with a subsequent initiation of the acrosome reaction (**De Jonge, 1999**).

Furthermore **Alvarez et al. (2012)** showed that once within the female reproductive tract, and as a consequence of attractants released by the oocyte, Ca concentration rises changing the flagellum's beating pattern, spurring the sperm to turn. Additionally, the rate of the Ca increase dictates how sharply the sperm turns, whereas the path of the subsequent run depends on the steepness of the Ca decline (**Alvarez et al., 2012**). Also, as recorded by **Swann (1990)**, at fertilization, spermatozoa activate eggs by causing transient increases in the intracellular free Ca concentration.

The exact impact of Ca on the sperm cell motility has not been completely elucidated, however significant and positive correlation between Ca and sperm motility was observed in the study of **Eghbali et al., (2010a)**, explained by a direct involvement of Ca in the regulation of cellular energy production and nutrient transport, essential for spermatozoa when reaching the *locus* of fertilization. Moreover it was noticed that the addition of Ca with calsemin to isolated ram caudal spermatozoa caused a stimulation of flagellar beat activity (**Bradley and Forrester, 1982**). **Prien et al. (1990)** compared sperm motility, velocity, and progressive movement with total and ionized Ca in patients with normal and decreased sperm motility. No difference in total Ca was found, but a statistically significant decrease in seminal ionized Ca was found in men with a decreased motility. **Kaya et al. (2002)** concluded that increasing ejaculation frequency may decrease the sperm motility due to a reduction of Ca in the seminal plasma.

Moreover as shown by **Eghbali et al., (2010a)**, Ca present in the seminal plasma of buffalo bulls plays an important role in preserving spermatozoa motility and viability, as well as antioxidant status by protecting the sperm cells from oxidative damage.

On the other hand, negative correlation between the Ca content in the seminal plasma and spermatozoa motility was found in bovine semen (**Machal et al., 2002**). **Magnus et al. (1990a)** found no association between ionized Ca concentrations and the proportion of spermatozoa displaying progressive movement. **Arver and Sjöberg (1982)** reported low ionized Ca to be associated with more and better progressive motile spermatozoa. Results of **Asadpour (2012)** showed that high levels of Ca were associated with lower percentage of motile sperm in rams. **Garcia and Graham (1989)** showed that a reverse proportional correlation existed between the Ca content and the motility of seminal cells.

#### MAGNESIUM (Mg)

Mg is the second most prevalent intracellular cation, which is essentially involved in the metabolic activity of the cell. Most of the element is bound to proteins and negatively charged molecules, 80% of the cytosolic Mg may be found within ATP. The nucleus, mitochondria and endoplasmic reticulum contain significant amounts of Mg. Transport of Mg across the cell membrane is regulated by a specific transport system (**Eghbali et al., 2010a; Burtis et al., 2013**).

Intracellular Mg is involved in the activity of different hormone receptor complexes located in the cell membrane (**Cupps, 1991**) and is an important cofactor of more than 300 enzymes active in various catalytic reactions. Furthermore, Mg plays a significant role in the energy metabolism and nucleic acid synthesis.

**Wang et al. (2005)** suggest that Mg located in the seminal plasma is related to prostatic secretions. This statement is supported by **Stegmayr et al. (1982)** reporting that the human seminal plasma contains secretory granules and vesicles of prostatic origin, which might have a regulatory effect on the sperm motility by modulating the concentration of essential cations in their environment. Membranes of these organelles contain Mg and Ca-dependent ATPase competitively inhibited by Zn (**Ronquist et al., 1987a;b**), which explains positive associations between Zn and Mg detected by **Umeyama et al. (1986)** and **Sorensen et al. (1999)**.

Additionally, Mg was proved to be involved in the osmolar balance and nutrient transfer. The presence of Mg is necessary for capacitation, hyperactivation and acrosome reaction of spermatozoa (**Semczuk and Kurpisz, 2006**).

However, the relationship between Mg and sperm quality is still unclear. Some experimental data show that the Mg amount in the seminal plasma increases with sperm concentration but has no significant relationship with sperm motility (**Wong et al., 2001**). On the other hand, positive effects of Mg on the motility, morphology and concentration of spermatozoa were reported by **Marzec-Wróblewska et al. (2012)**. Similar results were obtained by **Abdul-Rasheed (2010)** and **Eghbali et al. (2010a)** showing a significant decrease in the infertile seminal plasma Mg levels and indicating that magnesium may be a good criterion for prostate function and sperm quality. Also, seminal plasma Mg correlated significantly and positively with sperm motility and morphology. **Kaludin and Dimitrova (1986)** found a direct proportional correlation between Mg content and ram spermatozoa motility. Meanwhile, **Kaya et al. (2002)** by increasing ejaculation frequency observed a reduction in Mg content of seminal plasma in parallel with a decrease in sperm motility and concentration, as well as seminal volume.

On the contrary, according to **Wang et al. (2005)**, the Mg concentration in the seminal plasma of a poor spermatid quality group was quite close to that of a normal group. **Garcia and Graham (1989)** observed that solutions containing Mg provided significantly less protection to bovine sperm cells during freezing and thawing.

Nonetheless, Mg has been proved to be beneficial for the antioxidant capacity of semen. **Eghbali et al. (2010a)** showed that the Mg content in the seminal plasma was highly positively associated with the total antioxidant status of semen. As shown by **Chandra et al. (2013)**, Mg intake decreased LPO and increased the activity superoxide dismutase (SOD) as well as catalase (CAT) in rat testicular tissue. Furthermore, strong and significant associations between Mg and glutathione (GSH) were found in the study by **Townsend et al. (2003)** proving that GSH is Mg dependent, as the glutathione synthetase requires Mg cations for activation.

#### CHLORINE (Cl)

Chlorine is the dominant anion present in the body and serves as an integral low molecular weight intracellular osmolyte related to the osmolarity of extracellular fluids and determination of cell volume. The cellular volume essentially serves to re-establish the osmolyte equilibrium across the cell membrane, as water loss accompanies the loss of osmolytes, hence hypotonic swelling is halted and reversed (**Al-Habori, 1994; O'Neill, 1999**). The net efflux of the osmolytes including Cl derives from a swelling-induced activation of specific transport pathways. In the case of most animal cell types, including spermatozoa, K and Cl leave the cell by a parallel activation of several volume-sensitive K and anion channels, however organic osmolyte efflux can also take place through a volume-sensitive anion channel, commonly known as chloride channel. Several types of Cl channels may be detected in a sperm cell, performing various functions and being activated by different mechanisms, e.g. by intracellular Ca, cyclic AMP, transmembrane voltage, or cell swelling (**Al-Habori, 1994**).

With respect to spermatozoa, Cl channels have been detected in mouse (**Espinosa et al., 1998**) and boar semen (**Melendrez and Meizel, 1996**); there is further evidence that similar channels participate in the mouse, boar and human acrosome reaction (**Petrunkina et al., 2004**).

On the other hand, Cl present in culture media for spermatozoa or seminal plasma at the time of ejaculation may have a controversial impact on spermatozoa viability, as shown by **Sharma and Ludwick (1976)**. While low elevations of Cl concentration had a positive effect on the bovine spermatozoa motility

parameters during a prolonged time, high concentrations had an instant negative effect on the sperm survival rate.

## IODINE (I)

Iodine is referred to as a trace mineral, since the amount necessary to maintain normal body conditions is very small. Most of I is concentrated in the thyroid gland (Barakat, 2004). It is an essential element that is necessary for normal thyroid function, as I from dietary sources is used in the synthesis of thyroxine (T4) and triiodothyroxine (T3) (Sanchez, 1995). The presence of a functioning thyroid is a crucial prerequisite for a normal growth and maturation. The mechanisms by which the thyroid hormones regulate metamorphic changes are not completely understood. Presumably, the hormones may act as a trigger for genetically determined sequence of changes in various cells. Within these changes it appears that T4 and T3 act precisely and locally on the cells whose development is being directed (Kaltenback, 1966).

The results by Crissman et al. (2000) indicate that neonatal I deficiency may significantly increase the spermatogenic function, including testes weights, Sertoli cells and round spermatids, but without any significant impact on the sperm motility, morphology or testicular histopathology.

It appears that the basic thyroid hormone-mediated control of the testicular development is applicable to humans as well as other animal species. In humans, macroorchidism and very high sperm counts were associated with congenital hypothyroidism (Bruder et al., 1995; Castro-Magana et al., 1988). Furthermore, neonatal hyperthyroidism in rats, caused by daily injections of T3 resulted in smaller testes and prematurely truncating Sertoli cell proliferation (Van Haaster et al., 1993), suggesting that the controlling effect of the thyroid hormones on the testis development may be continuous through the subnormal, normal, and supernormal range of hormone levels. Therefore, a measurable decline in the spermatogenic capacity of human populations caused by juvenile I status appears to be quite plausible (Crissman et al., 2000).

On the other hand, administration of potassium iodine to bulls improved the ejaculate volume (Simirnov, 1972). Darwish et al. (1974) cited that feeding I during the summer period increased the ejaculate volume of Friesian bulls. Likewise, Simirnov (1972) and Sanchez (1995) noticed that in regions where I deficiency occurs, bull fertility may be affected due to a decreased libido, ejaculate volume, sperm motility and sperm cell concentration. Moreover, Reddi and Raj (1986) recorded that in experimental conditions of hypothyroidism, male goats exhibited a dramatic loss of libido, low sperm viability and increased morphologically abnormal spermatozoa. After the end of the treatment the male sexual behavior as well as the values for semen characters returned to normal. Groppe et al. (1983) concluded that the impact of I supplementation on the testicular growth was only detected in very young animals.

On the other hand, Barakat (2004) recorded a significant increase in the individual motility of bulls supplemented with I. At the same time, I supplementation had no effect on the sperm viability or abnormalities, being in accordance with El-Wishy et al. (1967). Also, it was concluded that KI supplementation to bulls had a beneficial effect on the semen quality and quantity, initial fructose concentration, as well as the endocrinological output of male hormones together with a positive relationship on the growth and maturation (Barakat, 2004).

## PHOSPHORUS (P)

Phosphorus is one of the most important minerals in animal nutrition, with 80% of the element found in the bones and teeth and the remainder located in body fluids and soft tissue. P plays a key metabolic role and has more physiological functions than any other mineral (Marzec-Wróblewska et al., 2012).

The chemical nature of P compounds in mammalian semen, and their distribution between spermatozoa and seminal plasma, has been the subject of relatively few studies in the past. Generally, P plays a major role in the maintenance of osmotic pressure, buffer capacity and acid-base balance. Phosphorylation is responsible for glycolysis and direct oxidation of carbohydrates, excretion, transport of lipids, exchange of amino acids, etc. P is also a component of a large number of co-enzymes. The element forms part of the structure of nucleic acids, which are carriers of genetic information, regulate protein biosynthesis and immunity (Marzec-Wróblewska et al., 2012).

A well-established P biomolecule is ATP, which was first isolated from ram sperm by Mann (1945a). Other nucleotides that have been reported to occur in sperm are ADP and AMP, which, together with ATP are defined as universal accumulators and donors of energy (Newton and Rothschild, 1961). The occurrence of guanine and cytosine nucleotides has been inferred from their presence in acid hydrolysates from bovine and buffalo sperm (Abraham and Bhargava, 1962). Furthermore, nicotinamide coenzymes extracted from bull and rabbit sperm have been noted by Bistocchi et al. (1968).

Nevertheless, according to Brooks (1970) it is difficult to detect the exact amount of P and P-derived molecules due to the presence of powerful phosphatases and nucleotidases in the seminal plasma, capable of rapidly metabolizing organic phosphorus compounds. Not only do phosphatases occur within the sperm, but large quantities of these enzymes have been detected in the seminal plasma too (Brooks, 1970).

The presence of glycerylphosphorylcholine in mammalian semen was demonstrated by Dawson et al. (1957), who found that this molecule was derived from the epididymal secretion of bulls and boars. Further evidence that this compound is not of spermatozoal origin was indicated by its absence in washed ram sperm preparations and their inability to liberate this compound during incubation. Glycerylphosphorylcholine has been reported to occur in bull and ram semen by Seamark et al. (1968) and, like glycerylphosphorylcholine, it appears to be originated in the accessory sex organs as demonstrated in the rat and stallion. According to Brooks (1970), the absence of UDP-glucose and glucose 1-phosphate is not surprising, since mammalian spermatozoa are known to have only a negligible content of glycogen (Mann and Rottenberg, 1966).

Yanagisawa et al. (1968) found the guanine nucleotides bound to tubulin, a protein constituting the microtubules of the sperm flagellum. Their data served as evidence for the presence of a nucleoside diphosphokinase that catalyses the transfer of the terminal phosphate from ATP to GDP, postulating that GTP serves to contract the tubulin molecules. Several studies (Mann, 1945b; Lundquist, 1949) on semen metabolism have indicated the important role of P-containing enzymes in sperm glycolysis. Furthermore, glycolysis is an important source of sperm energy and appears to be the preferential source when a broad spectrum of suitable substrates is available (Lundquist, 1949; Flerchinger and Erb, 1955).

Moreover, many enzymes have been identified in the semen of several species with indication of considerable interspecies variation (Mann, 1974). MacLeod (1939) and Lundquist (1949) observed seminal enzymes capable of dephosphorylating phosphate esters.

Although semen of the bull is not particularly high in alkaline phosphatase and even lower in acid phosphatase (Flerchinger and Erb, 1955) as compared with the other species, it is a particularly rich source of 5-nucleotidase (Mann, 1974). Washed sperm suspensions utilize glycolyzable sugars in preference to phospholipids (Lardy and Phillips, 1941). At the same time, phospholipids provide a source of energy in sugar-free suspensions under aerobic conditions (Lardy and Phillips, 1941).

P is required for the maintenance of glycolysis and motility (Flerchinger and Erb, 1955), though it was suggested that P-containing diluents are associated with motility inhibition even when the peroxide accumulation is prevented with catalase (Bishop and Salisbury, 1965). Inversely, the P concentration detected in the seminal plasma of active bulls by Machal et al. (2002) was positively correlated with all quality as well as quantity characteristics of bovine semen. According to Arrata et al. (1978), there was a significant correlation between motility, progression, and the glycerylphosphorylcholine (GPC) ratio. Poor motility and progression in the specimens were accompanied by low GPC ratios regardless of the sperm counts. Furthermore, Hula et al. (1993) performed a phospholipid analysis of whole ejaculates from 12 healthy and 35 infertile subjects. It was shown that inorganic phosphorus of total phospholipids decreased in ejaculates of men with secretory infertility. Lyso-phosphatidyl choline (LPC) was not detected in ejaculates of men with relative infertility. Additionally, the amount of lyso-phosphatidyl ethanolamine (lyso-PE) and sphingomyelin decreased in ejaculates of persons associated with infertility.

## SULFUR (S)

Sulfur is a naturally occurring mineral that is a key constituent of several nutrients considered essential to human and animal health. S-dependent nutrients include the amino acids methionine, cysteine and taurine, as well as the vitamins biotin and thiamin. Because a wide array of foods, particularly high-protein foods contain S, deficiencies of the mineral are generally rare (Marzec-Wróblewska et al., 2012).

Studies by Lehninger (1966) and Tumenbaevish et al. (2012) have shown that some thiol compounds have a positive effect on the viability of semen. It is also established that the sulphhydryl (SH-) groups are involved in conjugation reactions of oxidative phosphorylation (Prokoptsov et al., 1974) and play a role in mitochondrial membrane permeability (Lehninger, 1966).

It is known that one of the causes of sperm cell damage during cryopreservation is the oxidation of SH-groups with the formation of disulfide (-SS-) bonds, leading to protein denaturation. Tumenbaevish et al. (2012) revealed that the S-containing compounds increased mobility and survivability of the absolute rate of cryopreserved spermatozoa. The best performance was obtained with unithiol, dithiothreitol and mercaptoethanol. Additionally, the S-containing compounds were able to prevent acrosome damage, peeling and swelling, ruptures of the plasma membrane and acrosomal reduction. The S-containing compounds with the exception of cysteine had also a protective effect on the safety and activity of the enzyme lactate dehydrogenase in spermatozoa. Study of cytochrome oxidase activity showed that all the S compounds reduced the activity of the enzyme involved in the terminal part of the respiratory chain, which is associated with switching the breathing mostly on aerobic glycolysis. Moreover unithiol, dithiothreitol and mercaptoethanol had a significant protective effect on the safety of the conjugacy respiration and phosphorylation. The S compounds contributed to the suppression of LPO, reducing the intensity of accumulation of toxic peroxides, stabilizing thiol enzymes and increasing the viability of gametes after cryopreservation (Tumenbaevish et al., 2012).

Experiments conducted by **Moroz et al. (1999)** with mixed bull sperm showed that thiols and dithiols (glutathione, unithiol, cysteine) stabilized the redox transitions of pyridine nucleotides and flavoproteins of bull sperm, protecting the regulatory mechanisms of energy metabolism from cold injury.

On the contrary, sulfur dioxide (SO<sub>2</sub>) is a well-known toxic pollutant to which humans and animals may be exposed (**Cape et al., 2003**). A decline of the seminal quality after exposure to seasonal air pollution consisting primarily of SO<sub>2</sub> has been reported in the study by **Selevan et al. (2000)**. According to **Zhang et al. (2006)** spermatogenesis was affected in the testes of male rats after SO<sub>2</sub> administration, as demonstrated by structural and functional changes of the testicular tissue together with disturbances in the hypothalamic-pituitary-testicular axis, as the testosterone produced by the Leydig cells plays an important role in spermatogenesis (**Holstein, 2003**). According to the authors, each checkpoint response induced by SO<sub>2</sub> could be interfering with spermatogenesis disturbing normal testosterone levels, thereby reducing sperm motility.

## ZINC (Zn)

Zinc is an element crucial for the membrane and nuclear chromatin stability as well as for the mechanical properties of accessory fibers, tail morphology, and sperm motility (**Caldamone et al., 1979**). Extensive evidence suggests that seminal Zn has an important role in the physiologic functions of the sperm cell and that its reduced levels result in low seminal quality and subsequent chances of fertilization (**Caldamone et al., 1979; Colagar et al., 2009**).

Several factors are associated with seminal Zn concentration. Inflammatory conditions considerably influence the secretory function of the prostate (**Kruse et al., 2002**), which may result in an impaired turnover and a decreased secretion of Zn. Accumulation of toxic heavy metals in testicular tissues may also reduce the Zn amount in semen (**Ebisch et al., 2006; Akinloye and Orowojolu, 2006**). Many investigators have shown that chronic prostatitis is associated with a drop of the Zn content within the prostatic fluid (**Wong et al., 2001**). Frequent ejaculation is another possible factor that can reduce the seminal plasma Zn levels (**King et al., 2000**). Low Zn content of semen may generally affect the semen quality in different ways. Some mechanisms include reduced antioxidant capacity (**Prasad et al., 2004**) or counteracting the effects of other heavy metals (**Batra et al., 2001**).

Zn is present both in spermatozoa and in seminal plasma, with a concentration considerably higher than in the other body fluids (**Marzec-Wróblewska et al., 2012**). The total content of Zn in mammalian semen is high and has been found to be critical for spermatogenesis (**Colagar et al., 2009**). **Calvin et al. (1975)** state that Zn is concentrated especially in the tail region of the sperm cell.

Zn in immature spermatozoa is mainly located in outer dense fibres of the flagellum, where it is bound to the sulphhydryl groups of cysteine. The majority of its content is reduced during epididymal sperm maturation, which leads to an increased stabilization of the outer dense fibre proteins by oxidation of sulphhydryl groups to disulphide bridges. This stabilization of outer dense fibre proteins seems to be an essential step for the generation of sperm motility, especially progressive motility (**Henkel et al., 1999**).

Furthermore, Zn is involved in the control of sperm motility through its association with ATP during contractions and its regulative effects on the phospholipid energy reserves (**Barber et al., 2001**).

Zn is a vital component of enzymes involved in steroidogenesis. Additionally, it has been shown that Zn may act indirectly through the pituitary to regulate the gonadotropic hormones (**Hurley and Doane, 1989**).

In case of a Zn deficiency, research has shown that the amounts of Zn found in the testis, epididymis, and dorsolateral prostate are reduced drastically (**Millar et al., 1958**).

As shown by **Colagar et al. (2009)**, fertile subjects displayed significantly higher levels of Zn in their seminal plasma than the infertile groups. **Dissanayake et al. (2010)** state that pathozoospermia is associated with low-seminal plasma Zn levels. Moreover it was noticed that the seminal fluid with higher percentage of motile spermatozoa contains plasma with higher Zn concentration (**Wong et al., 2001**).

Lower concentrations of Zn occur in asthenozoospermic and oligoasthenozoospermic patients (**Zhao and Xiong, 2005**), while men with low Zn levels in the blood serum are more exposed to the risk of asthenozoospermia (**Yuyan et al., 2008**).

Some authors have observed high concentration of Zn to be associated with enhanced sperm parameters, including sperm count (**Edorh et al., 2003; Mankad et al., 2006**), motility (**Fuse et al., 1999**) and normal morphology (**Chia et al., 2000; Edorh et al., 2003**). **Zhao and Xiong (2005)** noticed a positive relationship between poor production of sperm and sperm motility with a lower content of Zn in the seminal plasma of infertile subjects. **Deng et al. (2005)** reported that Zn treatment had a positive effect on sperm motility, and supplementation of Zn proved to be an effective method for the treatment of infertile males with chronic prostatitis. According to **Kumar et al. (2006)**, experiments with Zn-supplement-fed bulls led to a higher semen volume, sperm concentration, percentage of live sperm and motility.

A direct connection between Zn and sperm morphology was also suggested (**Massányi et al., 2005**), based on an inverse relation between the percentage of broken flagellum and a decreased Zn content (**Massányi et al., 2004**).

Many studies however could not find any significant association between the total Zn in seminal plasma and sperm quality (**Bakalczuk et al. 1994; Lin et al., 2000; Wong et al., 2001**). According to **Sorensen et al. (1999)**, high Zn concentration exhibited inhibitory effects on the progressive motility of spermatozoa, but not on the percentage of motile spermatozoa, whereas **Danscher et al. (1978)** reported high Zn concentration to be associated with poor motility of sperm.

**Bakst and Richards (1985)** demonstrated that adding ZnSO<sub>4</sub> *in vitro* to turkey semen suppressed the sperm O<sub>2</sub> uptake, but did not affect the general male fertility. Therefore, Zn may have functioned as a metabolic inhibitor on turkey semen, thereby decreasing sperm motility and prolonging survivability in the sperm storage tubules (**Barber et al., 2005**).

Evidence suggests that Zn has antioxidant effects, as one of the consequences of Zn deficiency may be an increase in oxidative damage induced by reactive oxygen species (ROS) (**Zago et al., 2001**). Zn is an essential component of the Cu/Zn SOD, which has potent antioxidant effects on the sperm function (**Ebisch et al., 2007**). Zn, through its competition with Cu and Fe for the membrane binding sites, reduces the potential for the formation of the hydroxyl radicals via redox cycling (**Zago et al., 2001**). High levels of seminal ROS may decrease the effective concentration of seminal Zn (**Powell, 2000**). Several studies support this hypothesis, noting that a decrease in the Zn concentration may lead to an increase in oxidation of DNA, proteins, and lipids, causing the loss of spermatozoa membrane integrity and that Zn has an important role in the inhibition of seminal oxidative stress (**Oteiza et al., 1995; Powell, 2000; Marzec-Wróblewska et al., 2012**).

## SELENIUM (Se)

Selenium is an essential trace element occurring in organic and inorganic forms (**Moslemi and Tavanbakhsh, 2011**). Se appears in higher concentration in semen than in the seminal plasma and affects the sperm concentration as well as the percentage of normally formed sperm (**Hawkes and Turek, 2001; Akinloye et al., 2005**). Seminal plasma Se presumably originates from epithelial secretions of the accessory sex glands (prostate gland, seminal vesicles, and epididymis) (**Hawkes and Turek, 2001**). Inorganic Se was found to be better and more easily metabolized as well as incorporated than the organic form (**López et al., 2011**).

Se is generally associated with amino acids, especially cysteine (selenocysteine) and methionine (selenomethionine) (**Marzec-Wróblewska et al., 2012**). Selenodeiodinase enzymes (types I, II, and III iodothyronine deiodinases) control the metabolism of thyroid hormones, which in turn are essential for normal development (**Defranca et al., 1995**) and function (**Latchoumycandane et al., 1997**) of rat testes. In humans, adult hyperthyroidism has been associated with increased luteinizing hormone (LH) and follicle-stimulating hormone (FSH) responses to exogenous gonadotropin-releasing hormone, increased sex hormone-binding globulin, and an increase in *libido*, whereas adult hypothyroidism has been associated with testicular resistance to gonadotropins, decreased testosterone and sex hormone binding globulin, diminished *libido* and impotence (**Jannini et al., 1995**).

Se deficiency has been linked to reproductive problems in rats, mice, chickens, pigs, sheep, and cattle (**Combs and Combs, 1986**), as Se is required for normal testicular development and spermatogenesis in rats (**Behne et al., 1996**), mice, and pigs (**Combs and Combs, 1986**). Furthermore, selenoproteins participate in the maintenance of the sperm structure integrity (**Moslemi and Tavanbakhsh, 2011**).

Se, in the form of selenocysteine, represents the catalytic center in the active sites of at least 9 human enzymes, including 4 glutathione peroxidase antioxidant enzymes (**Mills, 1959; Chu et al., 1996**). In rat and human testes and seminal vesicles, Se is converted to phospholipid hydroperoxide glutathione peroxidase (PHGPx), which is present as a soluble peroxidase in the spermatids but persists in mature spermatozoa as an enzymatically inactive, oxidatively cross-linked, insoluble protein. In the midpiece of mature spermatozoa, PHGPx protein represents at least 50% of the capsule material that embeds the helix of mitochondria (**Roveri et al., 1992; Lei et al., 1997; Ursini et al., 1999**).

On the other hand, Se itself is also an antioxidant, as increasing Se concentrations encourage the antioxidant GSH-Px activity, thus decreasing ROS and leading to increased male fertility (**Irvine, 1996**). Se could also protect against oxidative DNA damage in somatic cells (**Haegle et al., 1994; Xua et al., 2003**). Additionally, GSH-Px protects cellular membranes and lipid-containing organelles from peroxidative damage by inhibition and destruction of endogenous peroxides. GSH-Px catalyzes the breakdown of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and certain organic hydroperoxides released by GSH during the process of redox cycling. GSH is vital to sperm antioxidant defenses and has exhibited positive effects on sperm viability (**Irvine, 1996**). Se and GSH are essential for the formation of the GSH-Px, as deficiencies of either substance can lead to instability of the spermatozoa mid-piece, resulting in defective motility (**Ursini et al., 1999**).

Moreover, Se can immobilize the toxically active excess of metals that accumulate mostly in parenchymal organs (e.g. Hg, Pb, Ag, Ta); however, this

may appear disadvantageous for the general metabolism (Pasternak and Floriańczyk, 1995; Kabata-Pendias and Mukherjee, 2007).

For many years, reports have shown that Se deficiencies may cause an impaired male fertility in cattle, boars, rats, and mice (Xua et al., 2003). Although it is difficult to deplete testes of Se because of the organ's affinity for the element, sperm from second and third generation Se-deficient rats were largely immotile and showed a high incidence of sperm midpiece defects due to disorganization of the mitochondrial helix (Spallholz et al., 1981). Serum Se was reported to be low in men with oligospermia and azospermia (Krsnjavi et al., 1992). Se-deficient cattle exhibited reproductive disorders, including weak or silent periods, poor fertilization (Corah and Ives, 1992), reduced sperm (Olson, 1995) and uterine motility (Smith and Akinbamijo, 2000). Saarenen et al. (1989) also noticed correlations between low sperm Se content and abnormal morphology as well as motility of bovine spermatozoa. Moreover, Se administration to subfertile patients induced a statistically significant rise in sperm motility (Foote, 1999) and improved the reproductive performance in sheep and mice (Shen et al., 1999).

Se added directly to spermatozoa *in vitro* may also alter the sperm function. Incubation of ram spermatozoa with selenite, selenocysteine and selenomethionine significantly improved the sperm motility and oxygen consumption (Alabi et al., 1985). Furthermore, Se supplementation increased the motility of spermatozoa cultured in the presence of hexavalent chromium (Ramamoorthi et al., 2008).

Conversely, reports have shown that the male reproductive system is quite sensitive to excessive levels of Se. Ingestion of 2 to 4 ppm of Se for 5 weeks by rats caused a dose-dependent reduction in testicular and *cauda epididymis* as well as body weights. The same experiment showed that the sperm concentration, motility, and percentage of live spermatozoa decreased, with an immediate increase in the percentage of atypical sperm (Kaur and Parshad, 1994). At the same time, several reports associate the high seminal plasma Se with impaired sperm motility in humans as well as in animals (Takasaki et al., 1987; Hansen and Deguchi, 1996). Inversely, Wirth et al. (2007) have not found any Se impact on any of the examined quality parameters of human spermatozoa. This is consistent with observations from rat studies in which the Se content of testes was unchanged by dietary Se deficiency or excess (Behne et al., 1996). As shown by Shalini and Bansal (2008) both excess and deficiency of Se in the diet caused a reduction of murine sperm concentration and motility. Supplementation of human and rat diet with Se has also been reported to cause a decrease in sperm motility (Kaur and Parshad, 1994; Hawkes and Turek, 2001). Marin-Guzman et al. (2000) observed a decline in boar sperm motility when 0.3 to 0.9 ppm of Se was added to the semen samples. According to Scott et al. (2008), human sperm motility appeared to be the greatest at a Se concentration of 50-69 ng/mL, however when the Se level was less than 36 ng/mL, the risk of male infertility was higher. At the same time, levels above 80 ng/mL were associated with abortions or miscarriage.

## COPPER (Cu)

Copper is an important microelement playing numerous roles in a variety of physiological and regulatory processes (Dobrzanski et al., 1996). It is involved as an integral part of enzymes active in redox processes (ferroxidases, tyrosinase, lxyoxidase) (Massanyi et al., 2003) and cellular respiration (cytochrome-oxidase). Besides, Cu is associated with the cardiac function, bone formation, connective tissue development, neurotransmitter biosynthesis, peptide hormone maturation, pigmentation of tissues, as well as myelination of the spinal cord (Agarwal et al., 1990; Georgopoulos et al., 2001).

Cu has direct effects on Fe absorption and metabolism and thus it indirectly affects the haemoglobin biosynthesis (Georgopoulos et al., 2001). It is strongly bioaccumulated (Andreji et al., 2006), especially within the liver and reproductive organs. Its deficiency or toxicity may lead to physiological abnormalities with the Cu concentration in the body being connected to its concentration in food and environment (Georgopoulos et al., 2001).

Within the organism, Cu is usually bound in ionic forms. It is pointed out that abnormal levels of this metal may affect spermatogenesis with respect to the sperm production, maturation and fertilizing capacity (Wong et al., 2001; Cheah and Yang, 2011).

Cu appears to be involved in spermatozoa mobility and it may also act on the pituitary receptors which control the release of LH. In the seminal fluid, the Cu concentration appears to fall in cases of azospermia and to increase in oligo- and asthenozoospermia (Pleban and Mei, 1983; Skandhan, 1992). A positive correlation between the Cu concentration in the seminal plasma and sperm motility observed by Eghbali et al. (2008) was in agreement with the reports by Skandhan (1992) and Massanyi et al. (2005). Also, a weak but positive correlation between blood Cu and sperm motility was detected by Wong et al. (2001). A positive effect of Cu on the sperm concentration and count was also noticed. Men with sperm concentrations above 40 million/ml showed a higher Cu seminal concentration than men with azospermia (Jackenhovel et al., 1999). Positive correlations were noticed between the Cu concentration in blood, sperm count in the ejaculate, spermatozoa with progressive motility, as well as between the Cu concentration in the seminal plasma and the volume of ejaculate, spermatozoa motility and progressive motility (Machal et al., 2002). Inversely,

no significant differences in the effect of Cu concentration were found in teratospermic and normozoospermic men (Kwenang et al., 1987). Pant and Srivastava (2003) reported no significant difference in the Cu levels among different infertile categories. They did however observe a positive correlation between Cu and fructose in oligoasthenospermic and azospermic men, respectively.

Cu is necessary for many enzymes including the cytosolic dimeric Cu/Zn SOD, a dominant antioxidant preventing the deleterious effects of ROS on spermatozoa (Semczuk and Kurpisz, 2006).

Cu within a normal physiologic range is essential for the male fertility, yet its high level is detrimental to sperm morphology (Cheah and Yang, 2011). Toxic effects of Cu on semen has often been reported. Cu reduces the oxidative processes and glucose consumption, which in turn reduces or abolishes spermatozoa motility (Skandhan, 1992; Pesch et al., 2006).

Excessive Cu can inhibit the oxidation and zymolysis of spermatozoa, and thus compromise their activity. Furthermore, Cu is reported to be able to kill spermatozoa directly, as well as to inhibit their mobility and nidation of the zygote (Wang et al., 2005).

In boar semen, Cu can inhibit enzymes with functional sulfidryl groups, bind to and affect the conformation of nucleic acids, disrupt pathways of oxidative phosphorylation, although the precise response depends upon the individual properties of the metal (Massanyi et al., 2003). High concentration of Cu usually has a harmful consequence on the reproductive system, which is connected to the structure of the testes as well as the spermatozoa function (Machal et al., 2002).

Toxic effects of Cu on the seminal plasma are manifested in the decrease of motile spermatozoa and an increase of sperm malformations (Massanyi et al., 2005). Maynard et al. (1975) reported that the contact of spermatozoa with copper ions (Cu<sup>2+</sup>) is probably responsible for their decreased motility. Cu chelation complexes suppress spermatogenesis (Oster and Salgo, 1979) and their high concentrations have a toxic effect on the spermatozoa motility (White and Rainbow, 1985; Viarengo et al., 1996).

Dhami et al. (1994) pointed out the controversial *in vitro* impact of Cu on spermatozoa motility. Roychoudhury and Massanyi (2008) demonstrated the negative influence of CuSO<sub>4</sub> on the rabbit semen motility and subsequently confirmed changes in male reproductive functions. Experiments by Knazicka et al. (2012) resulted in similar observations confirming that Cu is a toxic element on bovine spermatozoa motility at high doses. Roychoudhury et al. (2010) found that doses above 3.70 µg/mL CuSO<sub>4</sub> have a negative effect in relation to the spermatozoa motility, morphology and membrane integrity. Sinohara et al. (2005) recorded significant correlations among the Cu content in semen and spermatozoa concentration, semen volume and abnormal morphology. Incubation with the metal caused a fall in the percentage of motile spermatozoa, which was directly related to the surface area of Cu at employed and to the Cu amount in whole semen. Low concentrations of copper ions caused a less significant fall in spermatozoa motility, although the metal was generally more toxic than Zn or cadmium ions.

According to Rebrel et al. (1996) motility, viability and acrosome reaction in spermatozoa incubated for 5 h were significantly affected by Cu at a concentration of 100 µg/mL, but not at lower concentrations. Moreover, incubation of spermatozoa in a Cu-containing solution caused a fall in the Mg concentration in the nucleus and acrosome regions (Battersby et al., 1982) and a decrease of the Na content in the head and mid-piece, as well as a decrease of the K and Zn amount in the head while augmenting the Cu level (Maynard et al., 1975). Cu in combination with Zn decreased the quantity of glucose utilized by spermatozoa and the quantity of glucose oxidized, causing the accumulation of lactate (Holland and White, 1980). Vrzulová et al. (1995) also observed a negative impact of Cu on the microscopic structure of the testes. Additionally, the accumulation of Cu by spermatozoa caused a significant decrease in the protein level and increase in the catalase activity (Marzec-Wróblewska et al., 2012).

## IRON (Fe)

Iron is an essential component of a group of heme proteins active in oxygen transport or as enzymes within the redox system. A small amount of Fe is present in several nonheme metalloenzymes (Kaplan et al., 2002). Furthermore, it is needed for cyclo-oxygenases, cytochromes, many hydroxylase/oxidase enzymes, ribonucleotide reductase, aconitase, succinate dehydrogenase, catalase, and many others (Aydemmir et al., 2006). The major complexes coordinating Fe with the cell are heme and heme-containing proteins, hemosiderin and ferritin (Sarafanov et al., 2008).

Regulation of the Fe content of the body is achieved by controlling Fe uptake in the gut (Aydemmir et al., 2006). In vertebrates, Fe is transported within the organism between the sites of absorption, storage and utilization essentially by transferrin which can bind up two atoms of Fe reversibly and very tightly (Dorea, 2000; Nevo and Nelson, 2006; Wallander et al., 2006).

Fe is indispensable for life, as Fe proteins are essential for oxygen and electron transport, cell respiration, DNA synthesis, energetic reactions, and nitrogen fixation (Nevo and Nelson, 2006; Wallander et al., 2006). Fe participates in the processes of oxygenation and reduction, entering into the composition of many enzymes and metalloprotein compounds. The general function of Fe in the cells is protection against toxic products of oxygenation reactions. Both, absorption

and metabolic function of Fe are linked to other chemical elements. A particular antagonistic activity is exerted by Cd, Mn, Pb and Zn. Interactions with Cu are complex and frequently synergetic during their cooperation in the oxidation and reduction processes (Marzec-Wróblewska et al., 2012).

Testicular Fe represents the accumulation of ferritin, where Fe is safely stored within the Leydig cells. Little is known about ferritin, the Fe storage protein (Carreau et al., 1994), even though it has been identified in the testes (Mazur and Shorr; 1950). Fe (due to its cellular toxicity in ionic form) is bound to transferrin as a transport protein. The Fe-transferrin complex is internalized through a transferrin membrane receptor and, after internalization, it dissociates Fe to cellular Fe storage proteins until further use (Harrison and Arosio; 1996). Studies on the Fe transport into the testes via the transferrin receptor have focused primarily on the Sertoli cell function (Toebosch et al., 1987), but the Leydig cell through its storage of Fe via ferritin may also play a role in the Fe homeostasis and may be a primary source of Fe for the Sertoli transport to developing sperm. Storage of Fe in Leydig cells also provides an extra layer of protection to germinal cells and still maintains easy availability of Fe to the Sertoli and germ cells. The demands for Fe are the greatest during sperm production (Wise et al., 2003).

Fe deficiency reduces the activity of iron-containing and iron-dependent enzymes (Mudron et al., 1996), with a subsequent impact on the overall fertility status, as well as normal growth and development of the foetus (Dorea, 2000). In general, semen contains a certain amount of Fe, as its physiological level is required for a normal spermatozoa production. According to Eghbali et al. (2010b), the total Fe content of the buffalo seminal plasma was highly associated with sperm motility and viability. They came to the conclusion, that the Fe content within the seminal plasma is important for the preservation of sperm motility and viability after ejaculation, and its presence will help spermatozoa to maintain their functions. Disproportionate levels of divalent ferrous iron ( $\text{Fe}^{2+}$ ) reduce the testicular size (Lucesoli et al., 1999). According to Kňazická et al. (2012) lower concentrations of  $\text{FeSO}_4$  ( $\leq 250 \mu\text{mol}/\text{dm}^3$ ) sustained the spermatozoa motility and energy metabolism, which are key factors supporting the spermatozoa function. Additionally, the authors found that iron at low concentrations ( $\leq 62.50 \mu\text{mol}/\text{dm}^3$ ) increased the overall percentage of motile spermatozoa.

Although Fe and its compounds are primarily not toxic for animals and humans, its overload can bear negatively on the organism (Perera et al., 2002). Disturbances in the regulative absorption mechanism can appear due to pathological conditions or prolonged intake of high Fe doses. In these cases Fe is bound in the form of ferric phosphate (haemosiderin) or into proteins, and is distributed into the liver (Semczuk and Kurpisz, 2006; Kabata-Pendias and Mukherjee, 2007). Its toxicity may be connected to the catalysis of many deleterious reactions in the cells and tissues (Reilly, 2004). High doses of Fe could affect a wide range of mechanisms (Defrere et al., 2008), lead to tissue damage (Reilly, 2004) or lesions (Defrere et al., 2008).

Increased Fe concentration can bear negatively on the morphology and DNA integrity of spermatozoa (Perrera et al., 2002; Massányi et al., 2004). Significant differences in the Fe concentration between sperm of severely teratospermic subjects were reported in contrast to no differences in normozoospermic subjects (Kwenang et al., 1987).

Fe administration to rats may result in testicular atrophy, morphological changes in the testes, impaired spermatogenesis, epididymal lesions and impaired reproductive performance (Crawford, 1995; Whittaker et al., 1997). The mechanism(s) involved in the production of testicular changes by Fe is not fully understood. Fe accumulation is associated with either acute or chronic Fe overload leading to a subtle Fe increase in the testes, subsequently associated with oxidative damage to lipids, proteins and DNA (Lucesoli and Fraga, 1995; Lucesoli et al., 1999).

Furthermore, as serum ferritin is highly correlated with the presence of hypogonadism (Papadimas et al., 1996), excessive Fe is destructive to the testicular function and spermatogenesis (Merker et al., 1996; Lucesoli et al., 1999), and smaller testes and reduced sperm production may be related to the elevated Fe concentrations.

Fe overload may increase oxidative stress in testes and epididymal sperm possibly causing infertility (Huang et al., 2001). High  $\text{FeSO}_4$  doses ( $> 200 \mu\text{mol}/\text{dm}^3$ ) *in vitro* decreased all the motility parameters of bovine spermatozoa in relation to time, however without any cytotoxic effect on the mitochondrial complex, with a potential toxicity reflected in other molecular pathways (Kňazická et al., 2012).

Moreover, Comaschi et al. (1989) and Aitken et al. (1993b) demonstrated that ferrous ions may catalyse the breakdown of pre-existing lipid hydroperoxides in spermatozoa with a subsequent propagation of LPO chain reactions through the generation of peroxy and alkoxy radicals. Also, in the presence of ROS, Fe can be released from binding proteins, inducing oxidative stress and ascorbate oxidation (Boyer and McCleary, 1987; Halliwell and Gutteridge, 1990). Elevated Fe concentrations might indicate an increase of the ROS generation, as suggested by Aydemir et al. (2006), according to who, the serum Fe concentrations were positively correlated with spermatozoa malondialdehyde (MDA) and abnormal morphology.

## MANGANESE (Mn)

Manganese is an ubiquitous transition metal found naturally in the environment. It is also released into the air from mining and manufacturing operations and from combustion of gasoline additives. Human exposure to ambient levels of Mn is universal and occurs mainly via air and dust exposures (Wirth et al., 2007).

Mn is required for ubiquitous enzymatic reactions. It has a unique redox chemistry, with several accessible oxidizing states (Armstrong, 2008). High intracellular Mn levels protect against oxidative damage in various organisms (Reddi et al., 2009). ROS scavenging activity of Mn related to a rapid quenching of peroxy radicals has been demonstrated in several studies proving that Mn may protect from Fe induced oxidative stress (Srizaki et al., 1998). It could reduce the ferrous-ascorbate mediated LPO in placental membranes (Anand et al., 2001) leading to an increase of the Fe level, providing a direct evidence towards Fe-mediated LPO (Chen et al., 2006).

Antioxidant and protective effects of Mn against LPO have been studied in various biological systems (Srizaki et al., 1998; Campanella et al., 2005; Chen et al., 2006). High intracellular Mn provides protection against oxidative damage through currently unknown pathways and recently it has been found that for protective reasons the metal is provided by the Nramp transporters (Reddi et al., 2009).

Poranen et al. (2008) clarified that the structural flexibility caused by Mn is also important for the enzymatic dynamics, as Mn is required for RNA polymerization. It activates several polymerases at low concentrations, but inhibits them at higher concentrations.

Mn is an essential element for humans in small quantities, although its overexposure may be toxic to reproductive health (Lapointe et al., 1996; Anderson et al., 2007; Liu et al., 2009).

The absence of Mn may lead to the inhibition of enzymatic systems required for sperm motility. Lafond et al. (1988) reported lower Mn levels in the seminal plasma from men with lower sperm density. Among 52 male partners of infertile couples, normospermic infertile patients had higher serum Mn levels compared to those with oligospermia or azoospermia (Adejuwon et al., 1996), suggesting a potential role of Mn in the evaluation of infertile males.

Cheema et al. (2009) and Lapointe et al. (1996) observed beneficial effects of 0.1 mM  $\text{MnCl}_2$  for the maintenance of sperm motility without detrimental effects on the *mucus* penetration or fertilizing ability and hypothesized that Mn could have an effect on the sperm adenylate cyclase activity leading to an increased Ca concentration and motility. Furthermore, Mn supplementation resulted in a significant improvement of the post-thaw motility and hypoosmic swelling of frozen cattle bull spermatozoa in a dose dependent manner. Similarly, addition of Mn to human washed spermatozoa resulted in a stimulation of progressive motility in a time and dose dependent manner (Magnus et al., 1990b). Moreover, positive effects of Mn have been studied on buffalo and cattle bull spermatozoa incubated with lipid peroxidation catalysts (Singh et al., 1989; Bilaspuri and Bansal, 2008).

Mn proved to be efficient against formaldehyde toxicity-induced spermatozoa abnormalities and oxidative stress (Zhou et al., 2006; Tajadini et al., 2013). Eybl and Kotyzova (2010) showed that Mn pre-treatment in acute cadmium intoxication significantly protects the testes against oxidative damage *in vivo*.

According to Cheema et al. (2009) the supplementation of Mn could reduce the level of MDA production significantly in cooled and frozen-thawed spermatozoa, but to a maximum level on addition of 200  $\mu\text{M}$  of Mn. An *in vitro* experiment using semen from healthy males found that Mn supplementation improved the total thiol and reduced GSH levels under normal and oxidative stress conditions (Bansal and Kaur, 2009). Other studies using semen samples obtained from normozoospermic donors found that a specific superoxide scavenger, named  $\text{Mn}^{3+}$  tetrakis (1-methyl-4-pyridyl) porphyrin, could attenuate the effects of superoxide on sperm motility parameters (Aboua et al., 2009).

In contrast, results of animal experiments and studies of human occupational Mn exposure have indicated that exposure to high levels of the metal might impair male fertility. One study reported that high Mn levels were associated with erectile dysfunction, however involving workers with severe sickness induced by occupational exposure to Mn (Guiying, 2000). The damaging effects of Mn on male reproductive function have been mainly examined in occupationally-exposed men, and showed prolonged time to semen liquefaction and decreased sperm motility among workers in contact with Mn, and decreasing percentage of motile sperm with increasing duration of employment as a miner (Yue and Fuming, 1997). In workers who had been in contact with Mn for more than 10 years, the activities of protective enzymes such as CAT were lower than in controls, suggesting that high Mn levels might result in a decreased activity of antioxidant enzymes, resulting in subsequent oxidative damage (Gao et al., 2006).

In a study by Wirth et al. (2007) high Mn levels were associated with an increased risk of low sperm motility and low sperm concentration. There are thus conflicting results regarding the effects of Mn on semen quality in infertile men, and more studies are needed to explore the dose threshold values for infertility. Furthermore, occupational studies found that chronic high Mn levels in male workers resulted in impotence (Penalver, 1955), decreased birth rates (Lauwerys et al., 1985) and decreased semen parameters (Penalver, 1955). Elbetieha et al. (1997; 2011) postulated that ingestion of high doses of  $\text{MnCl}_2$  by

male and female mice had adverse effects on fertility and reproduction. Moreover,  $MnSO_4$  adversely affected the semen quality index and sperm viability in broiler breed semen *in vitro* (Barber et al., 2005).

Huang et al. (2001) showed that 500 ppm Mn significantly inhibited the sperm motility but with no accompanying change in seminal MDA levels. According to the results by Li et al. (2012), the negative impact of high Mn levels on sperm viability, progressive motility, and morphology were more obvious than the beneficial effects. Furthermore, animal and *in vitro* experiments indicate that high Mn exposure decreases sperm motility and concentration (Huang et al., 2001; Ponnappakkam et al., 2003) possibly via membrane LPO (Yiin et al., 1996).

#### MOLYBDENIUM (Mo)

Molybdenum is an ubiquitous trace element found in food, drinking water and is present in multivitamin/multimineral supplements (Vyskocil and Viau, 1999). It is used in manufacturers of electric and electronic parts, glass, ceramic, lubricants, dyes, catalysts and pigments (Padney and Singh, 2002).

Mo is an essential, trace and micronutrient element, which plays an important role in animal physiology (Schroeder et al., 1962; Pennington and Jones, 1987). It is a constituent of at least three mammalian metaloflavoproteins (xanthine oxidase, aldehyde oxidase and sulphite oxidase) as well as nitrate reductase (Schroeder et al., 1962; Padney and Singh, 2002), all of which are involved in protein synthesis, metabolism of fats and carbohydrates, detoxification of preservatives and sulfites, as well as the mobilization and utilization of Fe in the body, with subsequent direct effects on biological processes controlling growth and reproductive performance (Vyskocil and Viau, 1999; Padney and Singh, 2002). Mo is known to act as an anticarcinogen (Luo et al., 1983).

However, the reproductive toxicity of Mo has been postulated in several animal studies. A decreased male fertility was observed in rats exposed to high levels of Mo for 13 weeks (Vyskocil and Viau, 1999; Padney and Singh (2002). In a recent study of catfish from polluted waters, Yamaguchi et al. (2007) found significant inverse associations between the tissue Mo concentration and gonadosomatic index. According to Meeker et al. (2008), the Mo content in human blood plasma was negatively correlated with total motile sperm and with normal sperm morphology. Furthermore, Mo in pair with Cu were evaluated as significant risk factors in the final model for sperm morphology. Padney and Singh (2002) observed no mortality in rats exposed orally to sodium molybdate, indicating no acute toxicity of Mo. However, the decrease in body organ weight gain profile (testes, epididymides, seminal vesicles and prostate gland) of rats might be due to cellular loss during the histopathological changes.

Moreover, decreased activities of testicular enzymes related to the germinal epithelium and Sertoli cells indicate damage to these particular cell types by different doses of Mo in a dose dependent manner (Pandey et al., 1999). These observations are well supported with histopathological examinations indicating degeneration of seminiferous tubules, disturbed spermatogenesis, increase in intertubular spaces and either few or a complete absence of spermatozoa (Saxena et al., 1990; Pandey et al., 1999). Furthermore, it has been concluded that the Sertoli cell damage may be responsible for the germ cell degeneration (Srivastava et al., 1990; 1992). A significant reduction in total epididymal sperm count and sperm motility, with different doses of sodium molybdate, may be due to direct reprotoxic effects of Mo. The increased percentage of morphological abnormalities, observed in different regions of spermatozoa by Sobti and Gill (1989) following sodium molybdate exposure, may be due to the toxic potential of this heavy metal (Pandey and Srivastava, 2000). According to Working et al. (1985a) male rats exposed to Mo were able to impregnate unexposed females but comparatively in lower number. The recorded decrease in male fertility has been attributed to a direct cytotoxic action on the testes resulting in an increase in sperm abnormalities.

Animal studies also suggest interactions between Mo and other minerals, especially Cu. Mo has a chelating effect on Cu and has been associated with impaired Cu use in animal studies (Lyubimov et al., 2004); also, individuals who are deficient in Cu intake or have a Cu metabolism dysfunction may be at increased risk for Mo toxicity (Meeker et al., 2008). In a study of rams, Van Niekerk and Van Niekerk (1989) found a lower semen volume and sperm concentration, motility and morphology in a Cu-deficient group, created through supplementation with Mo and sulfate, compared with a control group that was given additional Cu supplementation. The semen quality returned to normal when the Cu deficiency was reversed. Interestingly, dietary Cu supplementation prevented the adverse effects on sperm at the same high Mo dose levels (Lyubimov et al., 2004).

#### COBALT (Co)

Exposure to cobalt can occur through inhalation, oral or dermal routes. Mammals, including humans, are exposed to natural sources of Co present in food, water and air. In addition to naturally occurring environmental forms, Co compounds may also be present in certain occupational settings and in some consumer products (Hidiroglou, 1979).

Co in the chemically distinct form of Vitamin B12 is essential for the organism. While humans require Vitamin B12 directly, mammals including domestic farm

ruminants require the bioavailable Co ion for reproductive health. It is common in veterinary and agricultural practice to provide Co salt supplements to ensure a sufficient source of bioavailable Co for animal health. Dietary doses of Co that are either deficient or over-exposure have been reported to have harmful effects. Co deficiency is associated with "wasting disease" in farm ruminants where animals fail to thrive and their reproductive output is significantly decreased (Kennedy et al., 1996). At the same time, over-exposures to water-soluble Co salts (thus the Co ion) have been associated with reproductive toxicity.

Previous studies have indicated that exposure to Co might cause adverse effects on the male reproductive system (Pedigo et al., 1988). In studying the impact of Co on male rat reproduction Hoey (1966) observed testicular necrosis of both the seminiferous tubules and testicular interstitial tissue after a subchronic exposure to Co via daily injections of 0.40 mmole/kg body weight over a 30 day period. Testicular atrophy was demonstrated after chronic oral exposure of male rats to Co (20 mg/kg body weight) for 69 days (Nation et al., 1983). Chronic Co ingestion (20 mg/kg body weight) caused depletion of live sperm and produced toxic effects on the germinal epithelium (Corrier et al., 1985). Pedigo et al. (1988) showed that chronic exposure to Co dramatically affected the male mice fertility in a time-and dose-dependent manner, while acute administration had minimal effects. Likewise, continuous exposure of male mice to Co (400 ppm) in drinking water resulted in a reproducible, sequential pattern of seminiferous tubule degeneration (Anderson et al., 1992). Inhalation of the soluble cobalt sulfate caused reduced sperm motility in mice, and at a higher concentration, the number of abnormal sperm was increased, while the testicular and epididymal weights were decreased (Anderson et al., 1992).

Occupational exposure to various Co compounds is of concern because of their genotoxic (Madzhariva et al., 2010), mutagenic (Jensen and Tüchsen, 1990) and carcinogenic (Lison et al., 2001) effects.

Elbetieha et al. (2008) studied the effects of cobalt chloride ( $CoCl_2$ ) on the fertility of adult male Swiss mice. General male fertility, as well as the number of pregnant females was significantly decreased in the exposed mice. Also, the number of viable fetuses was decreased in females impregnated by exposed males at the three concentrations. Absolute epididymal and testicular weights as well as epididymal and sperm counts together with daily sperm production were significantly decreased in males that ingested  $CoCl_2$ . Histological evaluation of the testes revealed several abnormalities including hypertrophy of the interstitial Leydig cells, congested blood vessels, degeneration of the spermatogonial cells and necrosis of both the seminiferous tubules and the interstitial tissue. In rats  $CoCl_2$  complexed with histidine, lysine, glycylglycine, EDTA, casein, or glycine, being absorbed less than free  $CoCl_2$ .

Lukac et al. (2007) and Elbetieha et al. (2008) observed a sloughing of germ and Sertoli cells, as well as a shrinkage of the seminiferous tubules after Co administration. Formation of empty spaces within the epithelium was also observed. Further analysis revealed a significant decrease in the relative volume of seminiferous epithelium in Co treated animals, whereas the relative volume of interstitium was significantly increased, probably as a consequence of increased Leydig cell volume that could predicting elevated testosterone levels (Pavlova et al., 2012). Pedigo et al. (1988) presumed that Co interferes with local regulatory mechanisms in the testosterone synthesis.

Besides a possible indirect effect on spermatogenesis is explained by a readily Co crossing of the blood-testis barrier. Subsequently, a direct cytotoxic effect of Co on spermatogenic and Sertoli cells is possible (Corrier et al., 1985). Mollenhauer et al. (1985) suggested that the testicular degeneration was not a primary response to Co but the testes become hypoxic due to both the blockage of veins and arteries by red blood cells and to the changes in permeability caused by thickening of basal lamina.

These findings suggest that the effects of Co depend on the type of compound used and on stability of its complex. Time duration and age of the experimental animals are also important (Pavlova et al., 2012).

#### CHROMIUM (Cr)

Chromium is a naturally occurring elements found in rocks, plants, volcanic dust and gases. Cr in the organism is distributed rather regularly in each tissue and is necessary for its normal development (Marzec-Wróblewska et al., 2012).

In humans and animals trivalent Cr ( $Cr^{+3}$ ) is an essential nutrient playing an important role in glucose, fat and protein metabolism (Kumar et al., 2005).  $Cr^{+3}$  is an important component of enzymes and stimulates their mutual activity. Trivalent Cr is also postulated to be involved in maintaining the structural integrity of nucleic acids (Anderson and Mertz, 1977). The interaction between  $Cr^{+3}$  and dichromates greatly reduced the amount of nucleic acids extractable from tissues with trichloroacetic acid. This effect was specific for chromates only and was not observed with other compounds (Hermann and Speck, 1954).  $Cr^{+3}$  also protects RNA against heat denaturation indicating that this metal may be involved in maintaining the tertiary structure of nucleic acids (Fuwa et al., 1960). Cr deficiency occurs rarely (Kabata-Pendias and Mukherjee, 2007). Sperm cells being rich in nucleic acids might be affected by low levels of dietary Cr. According to Anderson and Polansky (1981), male rats raised on a low  $Cr^{+3}$  diet had decreased sperm counts and fertility compared to the Cr-supplemented controls. Additionally, the frequency of conception was low.

The toxicity of Cr depends on the oxidation state, hexavalent chromium ( $\text{Cr}^{+6}$ ) being more toxic than the trivalent form. In addition,  $\text{Cr}^{+6}$  is the more readily absorbed by both inhalation and oral routes (Assem and Zhu, 2007).

Chronic exposure to  $\text{Cr}^{+3}$  resulted in weight loss, anaemia, liver dysfunction and renal failure. Nevertheless,  $\text{Cr}^{+3}$  is not considered to be mutagenic in most cellular systems and there is no firm evidence that *in vivo* it is mutagenic or carcinogenic to humans or experimental animals. Moreover, studies have not shown  $\text{Cr}^{+3}$  to be carcinogenic. There is not sufficient evidence to suggest that  $\text{Cr}^{+3}$  compounds could be reproductive or developmental toxicants (Assem and Zhu, 2007) even though according to Al-Hamood et al. (1998), fertility was reduced in male offspring exposed to potassium dichromate via their mother during gestational and lactational periods. Body weights and weights of testes, seminal vesicles and preputial glands were reduced in trivalent-exposed male offspring. Furthermore, body, seminal vesicles and preputial gland weights were significantly reduced in males exposed to  $\text{Cr}^{+3}$  via drinking water for 12 weeks, whereas the testicular weight was significantly increased in males exposed to these compound (Elbetieha and Al-Hamood, 1997).

On the other hand,  $\text{Cr}^{+6}$  is considered to be a serious toxicant, exposure to which is reported to cause pulmonary carcinoma, dermatitis, hepatotoxicity, nephrotoxicity and gastrotoxicity in humans and laboratory animals (Foglietta et al., 1998; Assem and Zhu, 2007).  $\text{Cr}^{+6}$  compounds are positive in the majority of *in-vitro* mutagenicity tests reported and may cause chromosomal aberrations and sister chromatid exchanges in humans. Furthermore,  $\text{Cr}^{+6}$  has been classified as a human carcinogen by the inhalation route of exposure and potassium dichromate may be toxic to the reproductive system and the developing foetus (Assem and Zhu, 2007).

In the experiments with monkeys (*Macaca radiata* Geoffrey),  $\text{Cr}^{+6}$  given in drinking water for six months caused a reduction of sperm concentration and sperm forward motility (Subramanian et al., 2006). Additionally, laboratory mice injected with  $\text{CrO}_3$  displayed increased a variety of sperm abnormalities (Acharya et al., 2006). Simultaneously, decreased sperm counts and percentages of motile sperm (Li et al., 2001) as well as increased percentages of morphologically abnormal spermatozoa were found in men occupationally exposed to  $\text{Cr}^{+6}$  (Kumar et al., 2005). At the same time Danadevi et al. (2003) recorded negative concentrations between the blood Cr content in males exposed to  $\text{Cr}^{+6}$  and rapid linear sperm motility and concentration. Semen abnormalities correlated with the number of years of exposure to welding fumes containing  $\text{Cr}^{+6}$ . Furthermore, Li et al. (2001) studying the fertility status of male workers occupationally exposed to hexavalent  $\text{Cr}^{+6}$  recorded that the sperm motility decreased, as well as lactate dehydrogenase (LDH), and lactate dehydrogenase C4 isoenzyme (LDH-x) in the seminal plasma. FSH was higher than the control. On the other hand, there were no significant differences in semen volume, semen liquefaction time, luteinizing hormone (LH) level in serum, and Cr concentration in both serum and seminal plasma between the exposed workers and the control group. Additionally, feeding  $\text{Cr}^{+6}$  to rats caused a visible disruption in germ cell arrangement near the walls of the seminiferous tubules. The diameters of seminiferous tubules in exposed rats were smaller.

Male welding of stainless steel is associated with an increased risk of spontaneous abortions. Mutagenic effects of  $\text{Cr}^{+6}$  has been found previously in both somatic and germ cells, and the findings could be due to mutations in the male genome (Hjollund et al., 2000). The results suggested that damage to convoluted seminiferous tubule epithelium, reduction of spermatozoa formation and increase in prevalence of teratospermia could be caused by exposure to certain concentration of  $\text{Cr}^{+6}$  (Li et al., 1999). On the other hand, according to Hjollund et al. (2005), no increased risk of spontaneous abortion was found in IVF treated women, who became pregnant by men exposed to welding of any sort. Also, Hjollund et al. (1998) and Bonde and Ernst (1992) recorded no statistically significant differences attributable to welding in proportions of morphologically normal sperm, sperm motility assessed by computer-aided sperm analysis, or sex hormones (testosterone, FSH or LH).

Experiments by Aruldas et al. (2005) and Subramanian et al. (2006) performed on monkeys (*Macaca radiata*) that received drinking water containing  $\text{Cr}^{+6}$ , a negative Cr impact on spermatogenesis mediated by induction of oxidative stress was observed. Cr treatment also disrupted spermatogenesis, leading to accumulation of prematurely released spermatocytes and spermatids in the lumen of seminiferous tubules. Granulation of chromatin and vacuolation between the acrosomal cap and manchette microtubules of elongated spermatids and in the Golgi area of round spermatids were observed (Pereira et al., 2005). The specific activities of antioxidant enzymes (SOD, CAT, GPx, glutathione reductase and glucose-6-phosphate dehydrogenase) as well as non-enzymatic antioxidants (GSH, vitamins A, C and E) decreased, whilst the testicular concentration of  $\text{H}_2\text{O}_2$  and hydroxyl radicals increased. Induction of oxidative stress in the experiments with mice receiving  $\text{Cr}^{+6}$  was also detected (Pereira et al., 2005). In men occupationally exposed to  $\text{Cr}^{+6}$ , decreases of Zn concentration in the sperm cells and increases of blood FSH were observed (Li et al., 2001).

Acharya et al. (2006) suggested that  $\text{CrO}_3$  exposure suppressed antioxidant enzymes and ascorbic acid with a concomitant increase in the level of LPO to adversely affect testicular function. Supplementation of vitamin C and vitamin E could partially prevent the incidence of abnormal sperm population and increased the sperm count. Vitamin C happened to be more effective in ameliorating germ cells from degeneration and from mutation to abnormal sperm. Possible

antioxidative role of both the vitamins have been studied for significant decrease in LPO associated with marked elevation in sperm count level and significant decrease in the percentage of abnormal sperm formation in  $\text{CrO}_3$ -treated mice.

Administration of  $\text{Cr}^{+6}$  in adult rats daily for 15 days produced significant increases in the blood and testicular chromium levels. Although no light microscopic pathologic changes or alterations in epididymal sperm counts and motility were observed, lanthanum perfusion in treated rats revealed leakage of Sertoli-cell tight junctions under electron microscopy. A few tubules showed marked ultracellular alterations in the form of vacuolization of cytoplasm and degeneration of mitochondria in the epithelial cells. Late stage spermatids were the most affected germ cells. The mitochondrial sheath of the midpiece was vacuolated, incomplete, swollen, or broken in places. The observed alterations may result in the disruption of normal testicular physiology leading to reproductive impairment after chromium exposure (Murthy et al., 1991).

## CONCLUSION

Male reproduction and fertility include processes which require strict and rigid conditions to produce mature and healthy spermatozoa. One of the requirements needed to be fulfilled is the abundance of chemical nutrients which are crucial for spermatogenesis, promotion of spermatozoa motility and quality, as well as for the Sertoli and Leydig cell development. In addition, some nutritional elements are involved during capacitation, hyperactivation, acrosome reaction and oocyte fusion.

Molecular mechanisms of many chemical elements involved in male fertility are however still unclear. The deleterious impact of excessive amounts of trace minerals are still not completely and clearly known. Researchers should focus on experimenting and finding out the critical concentrations and mechanisms of these elements in enhancing or ceasing the sperm production and viability. Further studies should be performed, focusing also on the complex relationships between chemical elements, enzymatic and nonenzymatic mechanisms, as well as other contributing proteins and/or biomolecules.

At the same time, assessment of the total seminal concentration of minerals may be a useful tool to determine the sperm fertilization potential. Also, evaluation of their concentrations in semen of infertile individuals is recommended. Furthermore, it is crucial to define a standard and optimal concentration of dietary nutrients needed to reach an optimal testicular growth, spermatogenesis and semen quality. Thus, additional investigations on adequate quantities of trace minerals for mammalian fertility could lay a strong base for further approaches on exploring the best combination of chemical elements with an appropriate dosage as a part of the nutritional prevention or therapy of male infertility.

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