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Manganese: A Potent Antioxidant in Semen

Review Article

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ABSTRACT

Antioxidant are the agents which dispose / scavenge the reactive oxygen species, minimize the oxidative stress / lipid peroxidation and ultimately improve the fertilizing potential of the spermatozoa. Currently, many antioxidants are under investigation and manganese is one of them in reducing the oxidative stress both in male and female. The antioxidative action of manganese (Mn^{2+}) on various biological systems has been studied. The high concentration of Mn^{2+} may be harmful in certain cases, but, its lower doses are effective. Mn^{2+} in very small amount (μM) affects human health and its deficiency may cause symptoms such as impaired or depressed reproductive functions. It is a cofactor of some antioxidant enzymes such as superoxide dismutase (Mn-SOD), pseudo-catalase and photosynthetic oxygen evolving center. It also facilitates the sperm capacitation and acrosome reaction. This article reviews the detrimental effects of Mn^{2+} on male fertility and alterations in physiological functions of spermatozoa on *in vitro* supplementation of Mn^{2+}. I have also provided information on the role of Mn^{2} + in other system of the body which may be applied to the future research in the field of reproductive biology.

KEY WORDS antioxidant, reproductive, semen.

INTRODUCTION

Mammalian spermatozoal membranes are rich in polyunsaturated fatty acids (PUFAs) and are sensitive to oxygen induced damage mediated by lipid peroxidation and thus are sensitive to reactive oxygen species, attack which results in oxidative stress (Sikka, 1996). Oxidative stress can be measured by many methods; one of them is assessment of lipid peroxidation (Buege and Steven, 1978). Oxidative stress decreases the fertility potential and has deleterious effects on the physiology of spermatozoa (Agarwal *et al.* 2008).

The term oxidative stress is generally applied when oxidants outnumber the antioxidants (Du Plessis *et al.* 2008). The imbalance between the production of reactive oxygen substance (ROS) and a biological systems ability to readily detoxify the reactive intermediates or easily repair the resulting damage is known as oxidative stress (Agarwal *et al.* 2003). The main destructive aspects of oxidative stress are the production of ROS which includes peroxyl radicals, alkoxyl radicals, and organic hydroperoxides (Sharma and Agarwal, 1996).

Oxidative stress results in decreased sperm motility presumably by a rapid loss of intracellular ATP, leading to axonemal damage, loss of membrane fluidity and integrity, decreased sperm viability and increased mid-piece sperm morphological defects with deleterious effects on sperm capacitation and acrosome reaction (Sikka, 1996; O' Flaherty *et al.* 1997).

Freezing and thawing of sperm samples is routinely performed in cattle breeding industries in order to perform Artificial Insemination. These procedures are known to produce ROS in sperm samples. As the sperm plasma membrane is one of the key structures affected by cryopreserva-

tion (Agarwal *et al*. 2004). Sperm cryopreservation and thawing is associated with increased ROS production and decreased antioxidant level (Said *et al*. 2005).

Spermatozoa are protected by various antioxidants and antioxidant enzymes in the seminal plasma or spermatozoa itself (Kim and Parthasarathy, 1998). Antioxidants are the agents which break the oxidative chain reaction, thereby reduce the oxidative stress (Miller and Slebodzinska, 1993; Kumar and Mahmood, 2001). This article reviews the importance of manganese as an antioxidant. In addition, the significant alterations in physiological functions of the spermatozoa on *in vitro* supplementation of Mn^{2+} will also be reviewed.

Manganese has the following properties

Mn2^{+} as a cofactor of many antioxidant enzymes

Trace elements are essential for the functions of various enzymes and other proteins (Lukac *et al*. 2009). Manganese is an essential metal acts as a cofactor for many enzymes, and, therefore, plays important biological functions (Prestifilippo *et al*. 2008). It is an element of great importance in the life cycle of plants and animals (Campanella *et al*. 2005).

It plays an essential role as an activator/cofactor of various enzymatic systems such as superoxide dismutase (SOD), pseudo-catalase and photosynthetic oxygen evolving center (Coassin *et al*. 1992). It may modulate the lipid hydroperoxide levels produced under physiological conditions in conjugation with glutathione peroxidase (Luberda, 2005). Mn^{2+} may stimulate the enzymes of glutathione cycle and affect the total thiols (TSH), glutathione reduced (GSH), glutathione oxidized (GSSG) contents in human (Bansal and Anand, 2009) and bull spermatozoa (Bansal, 2006).

Mn^{2+} stimulates adenylate cyclase enzyme activity

Mn^{2+} as an allosteric regulator of adenylyl cyclase was first proposed by the late Evaneer (14). Among many metal ions such as Co^{2+}, Cd^{2+}, Zn^{2+}, Mg^{2+}, Ca^{2+}; manganese stimulates the activity of sperm adenylate cyclase enzyme to maximum extent (Braun, 1975). In rhesus monkey (*Macaca mulatta*), Mn^{2+} over the range of 0.5 -20 millimolar stimulates adenylate cyclase activity 2 to 50 folds (Edmund *et al*. 1971).

Manganese is a potent stimulator of adenylate cyclase activity in the sperm cells and cyclic adenosine monophosphate (cAMP) concentration are correlated with motility in the same cells (Lapointe *et al*. 1996). Mann and Mann (1981) and Eddy and O'Brien (1993) have described that energy transduction within sperm tail is made possible by the molecular diffusion of mitochondrial adenosine triphosphate (ATP) along the flagellum for rhythmic flagellar movements. This energy, according to these workers is generated along the flagellum by a mechanic-chemical process coupled to enzymatic dephosphorylation of ATP.

$$ATP \xrightarrow[Mg^{2}+,\ 25\ ^{\circ}C,\ pH\ 7]{ATPase} ADP + Pi + 7\ kcal$$

However, this ATP dephosphorylation is not entirely irreversible. Some of the dephosphorylated ATP can be resynthesized as a result of axonemal adenylate kinase activity.

$$2\ ADP \xrightarrow[Stimulation\ by\ Mn^{2+}]{Adenylate\ kinase} ATP + AMP$$

Manganese as a quencher of oxidative stress / lipid peroxidation (LPO)

The antioxdative action of Mn^{2+} on various peroxidizing systems (sperms, neurons) has been studied (Bansal and Anand, 2009). It inhibits LPO produced by a free radical producing system but not produced by single oxygen (Bansal and Anand, 2009). It has also been assigned as a chain breaking antioxidant as it is able to quench peroxyl radicals (Coassin *et al*. 1992). Mn^{2+} showed free radical scavenging capacity, exhibiting relative rate constant ratio respectively of 0.513 and 0.287. Chain breaking antioxidant capacity of Mn^{2+} seems to be related to the rapid quenching of the peroxyl radicals according to the reaction (Coassin *et al*. 1992):

$$ROO + Mn^{2+} + H^{+} \longrightarrow ROOH + Mn^{3+}$$

It decreases the production of thiobarbituric acid reactive substances. This may be due to its capacity to quench the superoxide anions and hydroxyl radicals and also due to its chain breaking capacity (Anand and Kanwar, 2001). In various organisms, high intracellular manganese provides protection against oxidative damage through unknown pathways (Reddi *et al*. 2009). The efficacy of manganese as an antioxidant has been drastically reduced in cells that hyper accumulates phosphate (Reddi *et al*. 2009). It is well known that Mn^{2+} is a potent inhibitor of *in vitro* LPO in a variety of systems, while it also exerts a superoxide radical scavenging action, which may account for the inhibition of LPO. Direct inhibition by Mn^{2+} appears to be a factor in reducing *in vivo* LPO. However, the inhibitory mechanism of Mn^{2+} in LPO has not been fully elucidated (Tampo and Yonaha, 1992). Shukla and Chandra (1981) reported that 1 µ*M* Mn^{2+} significantly inhibited malondialdehyde (MDA) production in LPO of brain homogenates, which may con-

tain a trace amount of endogenous iron.Mn^{2+} inhibits iron supported LPO even at low concentrations of Mn^{2+}. It competes with iron at anionic oxygen of phosphate groups in phospholipids to inhibit LPO (Tampo and Yonaha, 1992).

Some authors proposed that Mn^{2+} might be forming a complex with unsaturated lipids making them more resistant to attack by peroxides (Cavallini *et al*. 1984). Mn^{2+} inhibits the free radical chain which follows the formation of hydroperoxides and that lead to the formation of MDA. Mn^{2+} is able to form complexes with O_2^- and OH giving rise to complexes like MnO_2^{2+} and $Mn(OH)^{2+}$. It has been reported that Mn^{2+} is able to scavenge the superoxide anion; while hexaquo Mn II is a poor scavenger of O_2^-. The interaction of Mn^{2+} with the reported free radical species supports the hypothesis of general antioxidant action that might occur through the reduction of lipid free radicals (RO. and ROO.) making them unable to carry on the process of LPO (Cavallini *et al*. 1984). Manganese exerted better antioxidant results than zinc (Zn) and nickel (Ni) to reduce ferrous ascorbate induced and / or nicotine induced LPO (Arabi, 2005). Manganese has also proved to be the best antioxidant in reducing the ferrous ascorbate induced LPO in human placental membranes (Anand and Kanwar, 2001) and in bull spermatozoa (Bansal and Bilaspuri, 2008). Mn^{2+} protects membrane from peroxidative damage produced by the superoxide radicals (O_2^-).

Role of manganese by alterations in glutathione cycle

Bansal (2006) suggested that Mn^{2+} supplementation to bull sperm alters the -SH contents (TSH, GSH, GSSG) of bull sperm under induced oxidative stress conditions, but this contents (-SH) are required for its normal functioning.

Manganese affects the lipid and phospholipid contents

Under oxidative stress conditions, -PUFAs-of sperm membrane get converted to lipid peroxides which in turn make the membrane more fusogenic and fragile (Guraya, 1999). Thus, membrane integrity and flexibility decreases which ultimately leads to leakage of lipids and phospholipids from the membrane and thereby decreasing their contents.Mn^{2+} supplementation inhibits LPO, thus increasing the membrane integrity and viability which are required for storage of lipids and phospholipids. Mn^{2+} supplementation to the sperm reduced the leakage of lipids and phospholipid contents under normal and induced oxidative stress conditions (Bansal, 2006).

Effect of Manganese on semen

Role of manganese in enhancement in sperm motility

Manganese stimulates the progressive motility of human washed sperm in a time and dose dependent manner (Magnus *et al*. 1990). A maintained response has been best seen with doses 0.2 -1 m*M*. Effects of Mn^{2+} on sperm motility may be mediated through a common cation binding site on the adenylate cyclase. A hypothetical model was proposed by Bansal and Bilaspuri (2010) for the role of Mn^{2+} in enhancing the sperm motility. It has been suggested that Mn^{2+}supplementation stimulates adenylate cyclase (membrane bound enzyme) activity in sperm, which in turn enhances the level of cyclic adenosine monophosphate (cAMP; Tash and Means, 1983; Magnus *et al*. 1990). This increase in concentration of cAMP through a cascade of events phosphorylated the axonemal proteins, which are involved in sperm movement.

Therefore, the increase in motility in response to Mn^{2+} supplementation may have been mediated through a signal transduction pathway.

Role of manganese in enhancement in sperm viability

Larsen (1994) found that high concentration of Mn^{2+} is related to cell death. Bilaspuri and Bansal (2008) suggested that supplementation of 60 µ*M* Mn^{2+} to the bull sperm permits the rise in intracellular calcium ($Ca^{2+}{}_i$) level without decreasing their viability. Further, Mn^{2+} has beneficial effects on sperm survival during capacitation and acrosome reaction (Bansal, 2006). It is suggested that anti-oxidative property of Mn^{2+} stabilizes the plasma membrane, thereby maintaining the membrane integrity and viability (Bilaspuri and Bansal, 2008).

Manganese facilitates the sperm capacitation and acrosome reaction

Bilaspuri and Bansal (2008) suggested that supplementation of Mn^{2+} to the bull sperm enhances the percentage of acrosome reaction by decreasing the oxidative stress. As manganese inhibits LPO both *in vitro* (Tam and McCay, 1970) and *in vivo* (Shukla and Chandra, 1981), therefore, it has been suggested that its antioxidative property stabilizes the plasma membrane integrity and viability (Bansal, 2006). According to Bilaspuri and Bansal (2008) hypothetical model, calmodulin or calmodulin like proteins loosely bind to the plasma membrane and / or Ca^{2+} or Mg^{2+} ATPase. These bindings enhance the extrusion of intracellular calcium ($Ca^{2+}{}_i$) and interfere with the capacitation and acrosome reaction processes. However, Mn^{2+} supplementation stimulates the calamodulin removal from its receptors, thereby enhancing the $Ca^{2+}{}_i$ level. As more and more $Ca^{2+}{}_i$ depositing leads to vesiculation of acrosome, it causes the fusion of outer acrosomal membrane with the plasma membrane, thus resulting in acrosome reaction. Extracellular addition of Mn^{2+} ions also enhance the level of cAMP by stimulating Ca^{2+} or Mg^{2+} ATPase which lead to the activation of calcium channel openings, thereby, depositing more Ca^{2+}_i. Thus, Mn^{2+} promotes the acrosome reaction.

Manganese prevents the loss from freezing / thawing

Mn^{2+} protects the sperm from the loss of freezing and thawing procedures as it is able to enter the cell more easily and help sperm to maintain or recover appropriate ion balance and thus suffer less from the freezing and thawing procedures (Lapointe *et al*. 1996**).**

CONCLUSION

Measurement of oxidative stress in not a routine clinical practice. Therefore, it is need to evaluate it without the use of sophisticated equipment e.g. chemiluminescence. Also, it is important to find the optimum dose of Mn^{2+} which is useful in the treatment of male infertility and better functioning of other reproductive processes. It is concluded that Manganese may be used as a potent antioxidant /additive to sperm samples to be used for assisted reproductive techniques (ART) like IVF and/or ICSI by prolonging the viability and quality of semen.

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