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ORIGINAL RESEARCH

# The potential effects and interactions of oxidative stress and trace minerals on fresh and frozen semen in bulls – a review

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Reproduction is one of the most important factors determining successful cattle farming systems. Management practices, such as nutritional supplementation, can influence the reproductive performance of cattle. The objective of this literature review is to determine the potential value of injectable trace mineral administration on fresh and cryopreserved semen quality of bulls. A search of keywords related to the topic was performed on published articles and textbooks. The search was narrowed to the 40 most relevant references.

Several studies have demonstrated a positive association between trace mineral supplementation and bull semen quality. Moderate amounts of reactive oxygen species (ROS) play an important role in normal spermatogenesis, but oxidative stress (OS), as experienced with adverse environmental conditions or disease, can contribute to idiopathic male infertility by negatively impacting spermatogenesis. Trace minerals such as selenium, copper, zinc, and manganese have been demonstrated to have antioxidant effects in mammals. Due to the complexity of oral ingested trace mineral bioavailability, injectable trace mineral supplementation prior to physiological periods with known deficiencies or increased requirement can benefit the animal.

The potential benefits of injectable trace mineral supplementation to minimise oxidative damage to spermatogenesis in breeding bulls need further investigation. Positive results from such studies can lead to the implementation of injectable trace mineral supplementation strategies prior to the breeding season to minimise the detrimental effects of OS and can improve semen quality.

**Keywords:** trace minerals, reactive oxygen species, oxidative stress, antioxidants, semen quality, cryopreservation

## Introduction

Various factors influence the reproductive ability of bulls, but semen quality (concentration, percentage motile and morphology of spermatozoa) is still one of the most important parameters to be evaluated when determining the breeding soundness of bulls.

Trace minerals are inorganic compounds that are needed in small amounts (parts per million) on a daily basis because they act as co-enzymes in various metabolic processes (Arthington & Havenga 2012) to maintain normal physiological and metabolic functions (Yatoo et al. 2013) as indicated with oral supplementation of copper (Cu), iron (Fe), magnesium, selenium (Se), and zinc (Zn) in reproduction and oxidative stress (OS) studies (Lehmkuhler 2010).

Various studies have shown that bioavailability of oral trace mineral supplementation is influenced by various factors (environmental, chemical, and management) supporting the recommendation of additional injectable trace mineral supplementation 90 days prior to breeding (Hill et al. 2015).

## Trace mineral intake and loss

Cattle gain most of their daily trace minerals from their diets, of which the trace mineral availability is influenced by factors such as soil type, pH, rainfall and diet composition (level of fertilisation, fibre digestibility, stem-leaf ratio and sulphur-containing amino acids) as well as by antagonistic reactions with calcium (Ca), Fe, molybdenum (Mo) and sulphur (S) (Mehdi & Dufrasne 2016) which occur mainly in the rumen and negatively affect their absorption and bioavailability (Arthington 2007).

Prior to the breeding season, bulls can be exposed to several stress-associated conditions that negatively influence their trace mineral status (Orr et al. 1990) as demonstrated in an increased loss of Cu and Zn in urine due to vaccination.

Injectable trace minerals have been commercially available for many years, but recently much more attention has been paid to their scientific application and assessment of efficacy. The main advantage of injectable trace mineral supplementation is the targeted delivery of a known amount of trace minerals, without the negative effect of antagonism in the digestion, to an individual animal before critical physiological periods.

## Oxidative stress

Reactive oxygen species (ROS) are produced by living organisms as a result of normal cellular processes (sperm production), inflammatory events (disease conditions), stressful events (mating) and environmental factors (heat stress) (Birben et al. 2012). In the absence of endogenous defence mechanisms, excessive levels of ROS lead to an imbalance between oxidants and antioxidants (Celi 2011), resulting in a condition called OS (Bansal & Bilaspuri 2011).

OS results from increased exposure to or the production of oxidants but can also result from increased turnover or low dietary intake of antioxidants (Celi 2011). Most studies related to OS involved mastitis, pneumonia, retained foetal membranes, metabolic diseases, and bull infertility.

Disturbances in the homeostasis of Zn, Se, manganese (Mn) and Cu can cause inflammatory changes (Chih-Hung et al. 2013) and OS due to their role in metabolic processes as antioxidants (Lehmkuhler 2010) to keep ROS in balance (Arthington & Havenga 2012).

DNA damage can develop due to OS that is present in the animal (Jeeva et al. 2015) and these high concentrations of ROS lead to adverse modifications to all cellular components, altering their function, which is the root of physiological and pathological diseases (Jeeva et al. 2015). This DNA damage, in the form of point mutations, can also damage sperm thereby negatively affecting male fertility (Spears & Weiss 2008) during conditions such as subclinical infection, resulting in defective spermatogenesis and the presence of abnormal spermatozoa in semen (Bansal & Bilaspuri 2011).

### *Endogenous sources of reactive oxygen species production*

Normal cellular metabolism can lead to the production of ROS from molecular oxygen as well as during the process of phagocytosis and destruction of microorganisms by neutrophils and can be divided into (Birben et al. 2012):

- Free radicals – molecules containing unpaired electrons (Agarwal et al. 2014) that are able to cause DNA mutations (Sanocka & Kurpisz 2004);
- Non-radicals – unpaired electrons that are shared by two free radicals (Birben et al. 2012).

### *Exogenous sources of reactive oxygen species production*

External factors (smoke, dust, heavy metals, and excessive environmental temperature variations) cause the release of inflammatory mediators and accumulation of neutrophils and macrophages resulting in excessive ROS production (Birben et al. 2012). Other exogenous sources like low levels of Zn or Se, which act as a cofactor for antioxidant enzymes, such as glutathione peroxidase (GSH-Px), can inhibit their action leading to OS (Birben et al. 2012).

### *Point mutation*

Point mutation, that can be caused by ROS (Agarwal et al. 2014), is a genetic mutation that can change the entire process of cellular reproduction and usually takes place during DNA replication (Biology Dictionary 2019). Changing the sequence (inserted or deleted) of amino acids in proteins may change the entire peptide, thereby changing the entire protein, resulting in a decrease or complete loss of its function. These changes can happen as a result of point mutations in both single and double stranded DNA resulting in a variety of effects ranging from benign to catastrophic, with regard to protein production, composition and function (Birben et al. 2012). Point mutations are known to cause nuclear abnormalities in spermatozoa, due to the sensitivity of the spermatogenesis process to OS (Agarwal et al. 2014).

There are two types of DNA base changes, namely base substitutions and base additions or deletions (Griffiths et al. 2000), that can be caused as a result of oxidative damage to cells in the presence of metal ions such as iron ($Fe^{2+}$), copper ($Cu^{2+}$) and nickel ($Ni^{2+}$) (Birben et al. 2012) by ROS.

### *Antioxidants*

The animal is equipped with different enzymatic and non-enzymatic antioxidant defence systems (Birben et al. 2012) by which free radicals are removed to counterbalance the impact of OS.

#### *Enzymatic antioxidants include:*

**Superoxide dismutase (SOD)**, a metalloenzyme influenced by Fe, Cu, Zn and Mn (Birben et al. 2012) act as a powerful first line of antioxidant in cells against ROS. One of its main actions includes to act as catalyst in the dismutating of superoxide anion ($^{\bullet}O_2$) to hydrogen peroxide ($H_2O_2$) and oxygen ($O_2$). SOD requires a metal cofactor for its activity and various forms of SOD exist, including Fe-SOD, Cu, Zn-SOD, and Mn-SOD, based on the required metal ion (Ighodaro et al. 2018). Of all SOD antioxidant activity, Cu, Zn-SOD is regarded as the most impactful.

**GSH-Px**, which is an important intracellular (Ighodaro et al. 2018) or extracellular antioxidant enzyme in mammals (Birben et al. 2012), depending on substrate specificity. It is Se-dependent (Shen et al 1999) and also responsible for the breakdown of hydrogen peroxide to water and oxygen (Ighodaro et al. 2018).

**Catalase**, which is present in cytoplasm (Jeeva et al. 2015) and using either Fe or Mn (Ighodaro et al. 2018) as cofactor also acts as a catalyst in the metabolism of hydrogen peroxide, a free radical, into water and oxygen and by this completing the proses of detoxification initiated by SOD (Ighodaro et al. 2018).

#### *Non-enzymatic antioxidants represented by dietary supplements or synthetic antioxidants (Bansal & Bilaspuri 2011) include:*

**Vitamin C (ascorbic acid)**, which is a water-soluble vitamin that provides intracellular and extracellular antioxidant protection.

**Vitamin E (α-Tocopherol)**, which is a lipid-soluble vitamin and predominantly a membrane-bound antioxidant in cells, a major chain-breaking antioxidant, reacting directly against free radicals (Birben et al. 2012).

**β-Carotene**, which is a pigment found in plants that react with and neutralise superoxide.

**Glutathione (GSH)**, which protects cell membranes (Birben et al. 2012) and is found abundantly in all cells.

## Reactive oxygen species in the male reproductive system

Previous research confirmed cellular generation of controlled quantities (low to moderate concentrations) of ROS by spermatozoa metabolism in mammalian species including bulls (Aitken et al. 2012) and proved to be essential for maturation, capacitation and the acrosome reaction of spermatozoa (Cheema et al. 2009). The regulation of ROS within suitable physiological levels is critical for cell viability, organ function, adequate sperm functionality and early embryo development (Bansal & Bilaspuri 2011) as demonstrated by a survey indicating that 40% of men from the USA with fertility problems suffer from elevated levels of ROS in their seminal plasma (Agarwal et al. 2014).

In bovine semen, the four major ROS that adversely affect sperm motility and impair fertilisation and embryo development (Rahman et al. 2012) are generated by dead spermatozoa, leukocytes (particularly neutrophils and macrophages) and immature spermatozoa (Bansal & Bilaspuri 2011), and are classified (Bansal & Bilaspuri 2008) as follows:

- Superoxide anion ($^{\bullet}O_2^-$)
- Hydroxyl radical ($OH^{\bullet}$)
- Hydrogen peroxide ($H_2O_2$)
- Hypochlorite radical ($OHCl^{\bullet}$)

Male infertility due to poor sperm motility, which is in most cases a more important reason for low fertility than low sperm concentration (Bansal & Bilaspuri 2008), can result from OS (Agarwal et al. 2014) as gametes are susceptible to ROS involved in the peroxidation of lipids, DNA damage (point mutations), and the formation of protein modifications resulting in electron leakage from the spermatozoa mitochondria with lower sperm motility, DNA integrity and vitality (Aitken et al. 2012) which also influences the female reproductive tract's immune response to sperm antigens (Aitken et al. 2012).

Antioxidants, control of OS and improvement of sperm motility could support male fertility (Bansal & Bilaspuri 2008) and reproduction efficacy.

### *Effect of reactive oxygen species on membrane lipids*

One of the main differences in mammals between their somatic cells and spermatozoa plasma membranes is the high levels of polyunsaturated fatty acids (PUFA) that are present in the spermatozoa membranes and which are accountable for the flexibility and functional ability of spermatozoa (Sanocka & Kurpisz 2004). These high levels of PUFA in cell membranes result in gonadal cells and spermatozoa, exposed to elevated levels of ROS, to be susceptible to lipid peroxidation (LPO) and free radical attacks (Bansal & Bilaspuri 2011). This LPO cascade (Tvrdá et al. 2013) is an obstructive process resulting in the accumulation of lipid peroxides in the spermatozoa membrane (Bansal & Bilaspuri 2011), and which can overwhelm the antioxidative defence mechanism (Henkel 2011) resulting in OS. The exposed spermatozoa are unable to repair the oxidative damage (Saleh & Agarwal 2002), because of the absence of cytoplasmic enzyme repair systems and the overloading of the antioxidant capacity of the seminal plasma (Agarwal et al. 2014) resulting in lower sperm motility due to axonal damage, decreased spermatozoa viability and increased mitochondrial spermatozoa defects (Bansal & Bilaspuri 2011).

Excessive ROS generation (of which the threshold levels for interference of sperm ability is unknown), overwhelms the protective mechanisms and initiates lipid and/or protein layer changes in the plasma membrane of sperm. These membrane changes lead to remodelling of DNA, interrupting spermatogenesis and sperm metabolism and resulting in damaged sperm motility and initiation of sperm apoptosis (Bansal & Bilaspuri 2011) as well as the inability to interact with the zona pellucida (Sanocka & Kurpisz 2004).

### *Reduction of oxidative stress of spermatozoa*

Different antioxidant mechanisms are responsible for the regulation of ROS production and protection of spermatozoa against OS. These intra seminal plasma non-enzymatic and enzymatic antioxidant defence mechanisms (Henkel 2011) include Vitamin E, as mentioned previously. Trace minerals (Cu, Zn Mn and Se) are important role players in regulating ROS with manganese ($Mn^{2+}$), decreasing OS (Bansal & Bilaspuri 2008) and strengthening sperm motility, viability, capacitation, and acrosome reaction.

Cu and Zn dependent SOD together with catalase protects spermatozoa against OS by protecting them against spontaneous $O_2$ toxicity. The Se-sensitive GSHPx supports antioxidant activity with a decrease in LPO, improved acrosome integrity and sperm morphology (Bansal & Bilaspuri 2011).

### *Cryopreservation and oxidative stress*

Cryopreservation of bull semen, a procedure routinely done for artificial insemination and improvement of herd genetics across the world, results in excessive ROS production in semen due to the exposure of semen to temperature variations and atmospheric oxygen. These exposures cause alterations in cell water volume, and, leaving spermatozoa susceptible to LPO of the plasma membrane due to the high levels of PUFA, result in excessive levels of ROS in post-thaw semen with associated detrimental effects on sperm motility, viability, membrane integrity, antioxidant status and fertility (Bansal & Bilaspuri 2011). These sperm damages lead to negative effects on capacitation, the acrosome reaction, penetration into the zona pellucida, fertilisation, and, through these mechanisms, decrease successful fertilisation (Cheema et al. 2009).

Previous research using freezing extenders (Bucak et al. 2010) indicated that supplementation of semen extenders with antioxidants has a defensive effect on the plasma membrane of frozen bovine sperm, maintaining both metabolic activity and cellular viability (Cheema et al. 2009), and resulting in a lower percentage of sperm cells with acrosome and total sperm deformities.

From the above, it transpires that a fine balance (not too high, and not too low) of ROS in semen is required to maintain spermatozoa function, and that trace minerals can play an important role in maintaining that balance.

## Trace mineral assessment, bioavailability, and antagonism

Four of the 17 identified essential trace minerals for cattle, Cu, Zn, Mn, and Se have been nominated by the National Research Council (NRC) as potentially deficient in grazing cattle. (Yatoo et al. 2013).

The demand for trace minerals is less than 100 parts per million in the diet and is affected by various aspects, including the physiological state of the animal and bioavailability of the supplied minerals (Spears 2014). The determining of trace mineral levels in ruminants is mostly requested due to performance that is below expectation (Kincaid 2000) with marginal deficiencies that can reduce several functions, including reproduction (Spears 2014). Whether these deficiencies are primary or secondary (Arthington 2003) they are ultimately assessed by evaluating the clinical and performance response (dose response) of animals to trace mineral supplementation (Kincaid 2000).

**Primary mineral deficiencies** are the result of the utilisation of feeds obtained by nature and without any artificial supplementation and that are naturally low in one or more of the minerals (Arthington 2003)

**Secondary mineral deficiencies** are more frequently found and result from the ingestion of mineral antagonists that inhibit the normal metabolism of another mineral (Arthington 2003). These antagonists can be ingested through feed or water.

## Trace mineral supplementation

The goal of any trace mineral supplementation programme is to provide adequate but nontoxic amounts of each trace mineral that is required by the animal on a daily basis irrespective of physiological stage, and is influenced by:

### *Bioavailability*

Bioavailability may be defined as the proportion of an ingested mineral, irrespective of the presence of antagonists in the daily diet (Spears 2014), the chemical form, animal species, physiological state, previous nutrition, or route of supplementation (Ledoux & Shannon 2005), that is absorbed and carried to its site of action and transformed to the physiologically active form (Ledoux & Shannon 2005) which can be utilised by the animal.

The major trace mineral antagonists in ruminant nutrition that are commonly found in feedstuffs include Fe which is abundantly found in earth and antagonised Cu, Zn and Mn; Ca, S and Mo which affects Zn bioavailability (Ledoux & Shannon 2005); S, which is found naturally in nearly all feedstuffs and decreases the absorption of Cu and Se (Arthington 2003); and once again, Mo which can impact Cu nutrition and form part of the important interactions between Cu and S (Spears 2014).

### *Orally supplemented trace minerals*

Orally supplemented trace minerals are essential on a daily basis, but intake can be influenced by various factors, including palatability; animal dominance; feeding trough (bunk) space; level of antagonism; soil, water and roughage quality; financial cost; and the chemical form (inorganic or organic) of the supplement. Inorganic trace mineral sources are usually the cheapest but are characterised by higher levels of antagonism and lower bioavailability while organic trace minerals sources are trace mineral complexes or trace minerals chelated to organic molecules like amino acids or polysaccharides which resist antagonistic interactions and increase bioavailability (Spears 2014).

### *Injectable trace mineral supplementation*

Oral trace mineral supplements must always be available to the animals but achieving adequate mineral levels by consumption of natural diet and oral supplement is difficult due to the above mentioned reasons, and the implementation of a "top up" injectable trace mineral supplementation prior to critical physiological periods is advised because it could improve the animal's mineral status rapidly due to bypassing digestion and associated interferences by other minerals in the gastrointestinal tract, thus having a higher absorption coefficient (Preedy et al. 2017).

## The importance of trace minerals in male reproduction

The importance of trace minerals in animal nutrition has been studied by different professional disciplines due to previous research indicating their importance in various metabolic functions, production, and reproduction. Although the macro- and micro-minerals (Zn, Cu, Mn, Se, Fe and Co), incorporated in mammalian seminal plasma and spermatozoa are required in narrow limits, research indicated that they have a direct effect on spermatozoa quality (Massányi et al. 2004) and positive effects were reported on sperm cell motility, morphology, particularly for Zn, Mg and Se, as they are fundamental cofactors for a range of bioactive molecules and interference to their absorption and bioavailability may have a destructive effect on sperm viability and morphology, preservation and fertilisation capacity of spermatozoa (Tvrdá et al. 2013).

### *Selenium*

Se acts as a stimulus of different antioxidative enzymes and is responsible as cofactor of the GSH-Px system regulating extra- and intra-cellular peroxidases. The supplementation of Zn together with Se results in additional antioxidative defence action compared to the supplementation of individual elements (Ghorbani et al. 2018).

Besides the other functions of Se, it is essential for the production of the following selenoproteins in the testis:

- phospholipid hydroperoxide glutathione peroxidase x (PHGP x);
- phospholipid hydroperoxide glutathione peroxidase 4 (PHGP 4); and
- selenoprotein V.

Most of the selenium found in the testis is associated with PHGP x, a structural protein enhancing sperm motility and is necessary for chromatin condensation and spermatozoa head formation and PHGP 4 (Lehmkuhler 2010). Se improves testicular and semen glutathione peroxidase activity (Kumar et al. 2013) protecting cells from oxidative stress (Lehmkuhler 2010) and free

radical accumulation proceeding to damaged cell membranes and reduce sperm motility (Kumar et al. 2013).

Se is critical to spermatogenesis and in bulls the epididymis and testis retained the greatest amount of Se 23 days post-injection, with peak accumulation of Se reached in semen 40 days post-injection of an organic Se source while among the accessory glands the prostate and seminal vesicles contain the highest levels (National Research Council [US] 1983).

### *Manganese*

Mn enhances bull sperm motility and viability, facilitates capacitation and the acrosome reaction, and improves sperm survival after cryopreservation due to its effect on the glutathione content of sperm and its effect on the lipid and phospholipid content of spermatozoa membranes resulting in reduced ROS generation by spermatozoa (Lehmkuhler 2010) as well as OS in spermatozoa chilled to 4 $^{0}$C and frozen-thawed semen (Cheema et al. 2009). Mn increased membrane integrity and viability, which are required to prevent leakage of lipids under normal and induced oxidative stress conditions (Bansal 2013).

### *Zinc*

Zn acts as cofactor of metallo-enzymes, like superoxide dismutase which contains Zn and Cu and is present in seminal plasma as well as spermatozoa (Tvrdá et al. 2013) where it acts as an antioxidant, dismutating superoxide radicals, minimising lipid peroxidation and stabilising lysosome membranes (Kumar et al. 2013).

Within spermatozoa, Zn is concentrated in the tail fibres (Blom & Wolstrup 1976) resulting in a positive effect on spermatozoa motility parameters and a deficiency of Zn is associated with spermatogenic failure, with inadequate maturation of spermatozoa to obtain motility (Henkel et al. 2003). In crossbred bulls, after six months of oral supplementation with organic as well as inorganic sources of Zn, increases in semen volume, sperm concentration, percentage motility, and percentage live sperm were observed compared to negative control animals (Lehmkuhler 2010).

Furthermore, Zn deficiency has been associated with several reproduction disorders, including retarded testicular growth, delayed puberty, degenerative changes of the seminiferous tubules, and abnormal numbers and abnormal morphology of Leydig cells, which negatively affects testosterone production and reduces the storage of Zn in the testis, epididymis, and prostate (Massányi et al. 2003).

### *Copper*

Cu deficiency contributes to the most common nutritional problems existing today. In large parts of Africa, Fe is excessively available, which, according to previous research, may impact negatively on Cu absorption due to antagonistic reactions aggravating the condition. Cu deficiencies are associated with impeded development, spermatozoa viability and morphology defects (Tvrdá et al. 2013).

Cu is essential for proper activation of the Cu/Zn superoxide dismutase enzyme leading to increased numbers of normal and healthy sperm cells which is supported by previous research that indicated a positive association between seminal plasma Cu and superoxide dismutase (Tvrdá et al. 2013).

## Conclusion

It is concluded that the potential protective effects of injectable trace mineral supplementation against oxidative damage to spermatogenesis in breeding bulls warrants further investigation.

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