

Review Article

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## Vitamin E and Glutathion as Antioxidant in Liquid Preservation of Semen: A Review

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### ABSTRACT

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For improvement of genetic program in animal, artificial insemination (AI) with liquid or frozen semen and quality of semen plays a major role for it. Sperm cells have a high content of polyunsaturated fatty acids in their membranes, and they lack a significant cytoplasmic component containing antioxidants. Therefore, they are susceptible to lipid peroxidation by the action of the reactive oxygen species (ROS). It results in the inhibition of both sperm ATP production and sperm movement, particularly forward progression. The addition of vitamin E and glutathione, as primary antioxidants to the semen extender prevented the loss of sperm motility by inhibition of lipid peroxidation caused by ROS in chilled boar spermatozoa.

### Introduction

Spermatozoal membrane is rich in polyunsaturated fatty acid (PUFA) and is easily damaged by reactive oxygen species (ROS) or membrane lipid peroxide ion (Silva, 2006). The equilibrium between the amount of ROS produced and scavenged is related with the gamete cell stability and damage. Reactive oxygen species reduce the sperm freezing potential, dysfunction the sperm by lipid peroxidation and affect the cell

membrane integrity. These changes finally alter the cytological parameters of semen making them incompetent to fertilize with the ova (Budai *et al.*, 2014). The ROS can be neutralized by antioxidants which serve as defense mechanism against the lipid peroxidation of semen and help in maintaining sperm motility and viability.

Their addition into semen extenders have been reported to improve semen quality in animals Bucak and Ateşşahin (2008).

### **Liquid preservation of semen**

Use of liquid semen cooled to room temperature, to intermediate temperatures (+16-20°C) or chilled (+5°C) dominates in some livestock species. During liquid preservation of semen, damage to sperm cells occurs during the transportation, handling of semen, concentration of extender and reactive oxygen species etc. Addition of antioxidants into semen extenders have been reported to improve semen quality in boar (Satorre *et al.*, 2007), bull (Bilodeau *et al.*, 2001), stallion (Almeida and Ball, 2005), dog (Michael *et al.*, 2007), cat (Thuwanut *et al.*, 2010) and goat (Sinha *et al.*, 1996).

### **Oxidative stress and reactive oxygen species**

Oxidative stress is a condition associated with an increased rate of cellular damage induced by oxygen and oxygen derived oxidants commonly known as ROS. Oxidative stress is associated with lipid peroxidation of the sperm outer membrane which leads to loss of sperm motility (Urata *et al.*, 2001) decreased fertilization capacity (Aitken, 1994) and increased chromatin damage (Aitken and Krausz, 2001). ROS represent a broad category of molecules that indicate the collection of radicals (hydroxyl ion, superoxide, nitric oxide, peroxy, etc.) and non-radicals (ozone, single oxygen, lipid peroxides, hydrogen peroxide) and oxygen derivatives (Agarwal and Prabakaran, 2005).

Mammalian spermatozoa are rich in poly unsaturated fatty acids that make them very susceptible to production of ROS (free radical), induced peroxide damage (Sikka *et al.*, 1982). The production of ROS by sperm is a normal physiological process, but an imbalance between ROS generation and scavenging activity is detrimental to the sperm and associated with male infertility

(Sikka, 1996). ROS induces peroxidation of critical thiol group in protein which alters the structure and function of spermatozoa and make them susceptible to macrophage attack, (Alvarez and Storey, 1989; Sharma and Agarwal, 1996).

Spermatozoa are capable of generating controlled low amount of endogenous ROS that play significant role in inducing sperm capacitation, acrosomal reaction and acquisition of sperm fertilizing ability (Sikka, 1996). But the high level of ROS endangered the sperm motility, viability and function by interacting with membrane lipids, protein and nuclear, mitochondrial DNA (Aitken and Clarkson, 1987). Involvement of seminal leukocytes with the reactive oxygen species, alter the mitochondrial membrane potential lead to damage of DNA in the human spermatozoa (Lobascio *et al.*, 2015). Mitochondrial permeability transition increases reactive oxygen species production and induces DNA fragmentation in human spermatozoa (Treulen *et al.*, 2015). Hydrogen peroxide appears to be the primary ROS responsible for sperm membrane damage (Alvarez and Storey, 1989). It is believed that sperm viability is reduced upto 50% after the freeze-thawing process (Watson, 1995; Kalthur *et al.*, 2011).

### **Types of antioxidants used in preservation of semen**

An antioxidant that reduces oxidative stress and improves sperm motility could be useful in the management of male infertility (Bansal and Bilaspuri, 2008). Various antioxidants and antioxidant enzymes have been used to prevent oxidative damage to spermatozoa (Kim and Parthasarathy, 1998). Non enzymatic antioxidants are also known as synthetic antioxidants that include vitamins such as vitamin C, vitamin E and minerals like zinc, taurine, hypotaurine, and

glutathione can reduce oxidative stress in female reproduction (Agarwal *et al.*, 2005).

### **Vitamin E as an antioxidant**

Cryoprotectants and thawing procedure are associated with significant reduction in sperm motility induced by ROS and that effect can be avoided by adding vitamin E at different doses to the extender at rates like 5 milli mole/ 2.5 milli mole and 10 milli mole concentration (Sharma *et al.*, 1996). Vitamin E is believed to be the primary component of the antioxidant system of spermatozoa (Surai *et al.*, 1998). *In vitro* studies shown that vitamin E is a potent chain breaking antioxidant, that scavenge intermediate peroxy and alkoxy radicals in protecting spermatozoa against endogenous oxidative membrane damage and it appears to have a dose dependant protective effect (Agrawal *et al.*, 2005). The concentration of vitamin E also reduces the MDA by 10-20 percent. Cerolini *et al.*, (2000) used vitamin E as a potent chain breaking antioxidant for prevention of oxidative damage on boar sperm cell membrane in semen maintained in liquid state. The  $\alpha$ -tocopherol concentration may be conditioning the cryopreserved boar sperm functionality. The addition of antioxidants could be useful to reduce oxidative stress, thus improving the functionality of cryopreserved boar spermatozoa. Kalthur *et al.*, (2011) supplemented vitamin E @ 5 mM and observed significant improvement in the post-thaw motility and DNA integrity in normozoospermic and asthenozoospermic semen samples.

### **Glutathione as an antioxidant**

The spermatozoa are readily undergoing lipid peroxidation, particularly in the presence of oxygen. Addition of antioxidant like glutathione (GSH) might have beneficial

effects like sperm motility in frozen bull semen. GSH plays important roles as protective agents against ROS-induced damages in many cell types (Halliwell and Gutteridge, 1998). GSH protects the spermatozoa from oxidative damage by inhibiting the lipid peroxidation process (Foote *et al.*, 2002). GSH also helps in maintaining the integrity of normal acrosome (Sinha *et al.*, 1996) and stabilizes the plasma lemma of spermatozoa and increases motility (El-kon and Darwish, 2011). It has been reported that, addition of antioxidant such as GSH to equine semen (Aurnich *et al.*, 1997; Baumber *et al.*, 2003) has shown to protect sperm against the harmful effects of ROS.

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