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ANTIOXIDATIVE ROLES OF ASCORBIC ACID AND TOCOPHEROL IN SEMEN PRESERVATION - A REVIEW

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ABSTRACT

Cryopreservation of semen induces osmotic stress resulting in increased reactive oxygen species (ROS). Sperm cells are potential targets by ROS because of the high saturation of lipid bilayer in the plasma membrane. In order to neutralise the detrimental effects of ROS to spermatozoa, antioxidants are added to extenders before storage. The addition of antioxidants to semen extenders improved semen quality in animals. This review highlights the applications of ascorbic acid and tocopherol used in different research efforts to improve the quality of spermatozoa after storage. it focuses on efforts to improve our current understanding of the mechanism by which the antioxidants enhance their protective effects on stored spermatozoa, and prolong the lifespan of the spermatozoa.

Keywords: Ascorbic acid, Tocopherol, Semen, Preservation, Stress

INTRODUCTION

An important aspect of assisted reproductive technology in animals is artificial insemination, which usually involves the use of cryopreserved semen. Cryopreservation of sperm is an applicable technique in infertility management, but it may influence the post-thaw qualities of sperm, including morphology, motility, viability and DNA integrity (Amidi *et al*., 2016). Colas *et al*., (2009) showed that detrimental changes occurring in sperm cells after the process of freezing and thawing are due to adverse changes in sperm cell membrane lipid composition and DNA damage (Zribi *et al*., 2010). The high saturation of lipid bilayer in the plasma membrane of sperm cells make them potential targets of reactive oxygen species (ROS) which ultimately induce damage to sperm cells. Osmotic stress, cold shock, intracellular ice-crystal formation, increased production of ROS species and a reduction in antioxidant defence systems or a combination of these may be the mechanism underlining cryodamage to sperm cells (Chatterjee and Gagnon 2001; Wang *et al*., 1997; Bilodeau *et al*., 2000). The equilibrium between the amount of ROS produced and scavenged is related with the gamete cell stability and damage. The ROS reduce the sperm cell freezing potential, dysfunction by lipid peroxidation and the sperm cell membrane integrity. These factors alter the cytological parameters of sperm impairing their ability to fertilize the ova (Budai *et al*., 2014). Sarangi *et al*., (2018) suggested that spermatozoa of the mammalian species are highly sensitive to oxidative damage due to the presence of high lipid bilayer of plasma membrane. Osmotic stress is induced during cryopreservation, thus bringing about increased generation of ROS. Ball, (2008)

showed that osmotic stress is induced by changes in cell volume, resulting from the movement of water and solutes across the sperm plasma membrane, which induce increased generation of ROS. Amidi *et al*., (2016) reported that the primary manifestations of ROS-induced damage to sperm cells is an oxidative attack of bis-allylic methylene groups of sperm phospholipid-bound polyunsaturated fatty acids (PUFAs), leading to lipid peroxidation (LPO). The ROS has positive influence as physiologic amounts of hydrogen peroxide (H_2O_2), superoxide anions (O^{2-}) and hydroxyl radicals (OH), have a vital and critical role in sperm functions including sperm capacitation, acrosome reaction, and binding to zona pellucida (Agarwal *et al*., 2006). In order to neutralise the detrimental effects of ROS, spermatozoa and seminal plasma possess a number of antioxidant systems that scavenge ROS and prevent internal cellular damage (Gadea *et al*., 2011). Antioxidants are the main defence factors against oxidative stress induced by ROS (Silva *et al*., 2011). Prevention of oxidative stress is not feasible, but measures may be adopted to prevent oxidative stress to the sperm by adding antioxidants. The addition of antioxidants into semen extenders improve semen quality in animals (Bucak *et al*., 2008). There are two types of antioxidants: enzymatic antioxidants and non-enzymatic antioxidants (Kefer *et al*., 2009). Enzymatic antioxidants include; catalase, glutathione peroxidase (GPx), superoxide dismutase (SOD) and glutathione reductase (GR), which all partake in sperm natural antioxidant defence system (Silva *et al*., 2011; Partyka *et al*., 2012). Non-enzymatic antioxidants or dietary supplements, include reduced glutathione (GSH), urate, ascorbic acid, vitamin E (tocopherol), carotenoids (b-carotene), ubiquinones, taurine and hypotaurine, selenium and zinc (Alvarez and Storey 1989; Therond *et al*., 1996). This article reviews the impact of antioxidants in reducing oxidative stress on spermatozoa functions, and aims to increase the understanding of the roles of ascorbic acid and tocopherol as antioxidants in sperm protection.

Vitamin C (Ascorbic Acid)

Vitamin C (Ascorbic acid) is a water-soluble antioxidant having minimal toxicity and high ability to scavenge ROS. It combats lipid peroxidation, neutralises the effects of H_2O_2 on DNA and aids in recycling inactive vitamin E (Niki 1987; Sierens *et al*., 2002). The concentration of ascorbic acid in seminal plasma has been linked with low sperm counts, reduced motility, increased numbers of abnormal sperm and agglutination in humans (Sierens *et al*., 2002). Ascorbic acid occurs in the seminal vesicles, where it is the major antioxidant present in seminal plasma of fertile males (Lewis *et al*., 1995). Vitamin C has a 10-fold higher concentration in seminal plasma than in the serum (Jacob et al., 1992). It has a more powerful antioxidant action when peroxyradicals are present in the aqueous phase (Frei *et al*., 1989) than in the lipid membrane (Doba *et al*., 1985). The seminal ascorbic acid levels correlate positively with the percentage of morphologically normal spermatozoa (Thiele *et al*., 1995), and negatively with DNA fragmentation index (Song *et al*., 2006). Ascorbic acid is rapidly oxidised into inactive form called dehydroascorbate, when exposed to a high oxidative environment (Linster and Van Schaftingen, 2007). This phenomenon makes it difficult for ascorbic acid to maintain its antioxidant activities, when spermatozoa are exposed to high oxidative environments. This led to the synthesis of an Ascorbic acid by Yamamoto *et al*. (1990) and showed that the acid is of high resistance to thermal and oxidative degradation in neutral solutions and non-reducing conditions called ascorbic acid 2-O-a-glucoside (AA-2G). Addition of AA-2G to the freezing extender improves the post-thaw quality of boar sperm by protecting the spermatozoa against DNA damage and lipid peroxidation during cryopreservation (Yoshimoto *et al*., 2008). Ascorbic acid is also present in the epididymal fluid and seminal plasma of several species (Chinoy,

1972). It is vital in the protection of spermatozoa from oxidative radicals (Buettner, 1993) and maintaining the genetic integrity of sperm cells by preventing oxidative damage to sperm DNA (Fraga *et al*., 1991). As the endogenous antioxidative capacity of semen is compromised during dilution and/or storage of semen (Maxwell and Salamon, 1993), there is a probability that longer storage period may reduce the antioxidant ability and the viability of the semen. Sajeda (2016) reported, in a study conducted on semen quality of Holstein bulls, that the addition of combinations of ascorbic acid with either catalase or reduced glutathione to Tris extender could be an advantageous tool to improve the post-cryopreservation of semen quality. Thuwanuta *et al*., (2011), reported an improvement in ram sperm when ascorbic acid is added to vitamins C to semen diluents. In a study by Sajeda *et al*., (2016), sperm motility and viability from Awassi ram could be improved by diluting with Tris diluent, containing ascorbic acid. The author also observed that addition of ascorbic acid not only improves viability but it also protects acrosome and membrane integrity, with sperm motility and viability of Awassi ram semen protected for 120 h of preservation at 5 ^{o}C. The findings of Hughes *et al*. (1998) on human semen; Maia *et al*. (2010) on ram semen and Singh et al. (1996) on buffalo semen also supported this observation. Studies conducted using human subjects showed that ascorbic acid concentrations of 0.02-0.6 mM adversely affect sperm motility (Donnelly *et al*., 1999a). Higher concentrations of ascorbic acid (2.5 mM) is harmful to sperm motility in frozen-thawed bull semen (Beconi *et al*., 1993). Ascorbic acid as an antioxidant, is required as a cofactor for at least eight enzymes (Halliwell and Gutteridge, 1999). In contrast, in the presence of transition metals, ascorbic acid functions as a pro-oxidant by donating an electron that reduces such ions into forms, which binds with oxygen molecules to form oxygen radicals (Azawi and Hussien, 2013). This leads to some detrimental effects on cells. Previous study revealed that vitamin C at 2.5 mM and 0.02–0.6 mM in bovine and human sperm, respectively, exert deleterious effects on sperm motility of frozen-thawed bull semen and human normozoospermic and asthenozoospermic samples (Donnelly *et al*., 1999b). Conversely, addition of addition of ascorbic acid to frozen spermatozoa of bovine at a concentration of 5 mM is protective against lipid peroxidation (Beconi et al., 1993). Ascorbic acid as an antioxidant is efficient in protecting ram spermatozoa viability and acrosomal integrity as a result of its capability of neutralising H_2O_2, produced in a hydrophilic environment (Azawi and Hussien, 2013). In camels, supplementation of the semen extender with ascorbic acid at a level of 0.5 g LG1 significantly increases sperm motility, livability percentages, percentages of curled spermatozoa and decreases sperm abnormality (El-Harairy *et al*., 2016). When ascorbic acid is used as antioxidant supplement in the semen extender for camels, it has beneficial effects on membrane integrity, function and morphology of the epididymal spermatozoa. (El-Harairy *et al*., 2016).

Vitamin E (Tocopherol)

It is a fat-soluble organic compound that is found in cell membranes. It protects sperm membranes form the effects of ROS-induced damage. Its mechanism of action is does so by breaking the covalent links between ROS and fatty-acid side chains in membrane lipids (Jeong *et al*., 2009). It also functions in preventing lipid peroxidation by scavenging free hydroxyl radicals and superoxide (Walczak-Jedrzejowsk *et al*., 2013). The concentration of tocopherol in spermatozoa

correlates with the percentage of motile spermatozoa and it is independent of the concentration of α-tocopherol in seminal plasma (Liang *et al*., 2015). beneficial effects of vitamin E have been confirmed to be based on its ability to enhance the antioxidant activities of vitamin E on semen quality

(Zubair *et al*., 2015). One of the beneficial effects of vitamin E is improvment of fertilisation rate, which was discovered by Hsu *et al*. (1998). The author postulated that it might be due to a reduction in lipid peroxidation of ram sperm. The mechanism by which vitamin E inhibits lipid peroxidation in sperm membrane is by eliminating peroxyl (ROO^-), alkoxyl (RO^-) and other radicals. In humans, Askasi *et al*. (1994) reported that vitamin E is more effective than vitamin C in improving motility of post- thawed spermatozoa. Asides from improving preservation qualities of stored semen, supplementation of vitamin E at a concentration of 5 mM to semen extender increases conception rate in assisted-reproductive technology cycles (Kalthur *et al*., 2011). In the presence of vitamin E, MDA concentration is reduced by 10-20% (Sarangi *et al*., 2018) In Ghezel rams, vitamin E improves viability, motility of spermatozoa pre- and post-cryopreservation (AminiPour *et al*., 2013). It improves hypo-osmotic swelling scores and the post-thaw motility (Askari *et al*., 1994). In bovine spermatozoa, vitamin E protects the plasma membrane integrity during deep freezing and in liquid boar semen maintained at 19^{o}C (Cerolini *et al*., 2000). Normozoospermic and asthenozoospermic semen samples obtained from boars, significantly improve when supplemented with vitamin E at a concentration of 5 mM and have significantly improved Post-thaw DNA integrity and motility (Kalthur *et al*., 2011). In Albino male mice, vitamin E decreases the defective heads of sperms (Rizzo et al., 2010). Conversely, in rams Upreti *et al*., (1997) reported that addition of vitamin E to semen extender has no effects on semen quality. The strongest lipid-soluble compound of vitamin E is alpha-tocopherol which functions to neutralise lipid radicals (Amidi *et al*., 2016). Thus vitamin E is one of the main membrane protection agents against free ROS and lipid peroxidation (Jeong *et al*., 2009; Sharma and Agarwal, 1996), and it is capable of protecting cells from ROS damage both in *vivo* and in *vitro* (Wu et al., 1990). Zubair *et al*. (2015) showed that vitamin E decreases lipid peroxidation and ROS production, which accelerates metabolism and ultimate cellular death. The addition of vitamin E to ram semen stored at 15ºC impaires the maintenance of sperm motility (Uperti *et al*., 1997), which is different from the report by Kheradmand *et al*. (2006), that vitamin E, stored at 15ºC has a protective effect on the motility and membrane integrity of ram semen. The possible reason for the discrepancy may be due to the temperature at which the semen was stored by Uperti *et al*. (1997), which and 5^{o}C by Kheradmand *et al*., (2006). In a study on goats by Penitente Filho *et al*. (2011), addition of vitamin E to semen extender influences sperm motility and integrity of sperm membrane.

CONCLUDING REMARKS

This review highlights the importance of ascorbic acid and vitamin E as non-enzymatic antioxidants in semen preservation in different species of animals. The antioxidants scavenge and prevent the formation of ROS. This mechanism of action is involved in protecting sperm cells from oxidative stress and also membrane damage. Although the antioxidants are beneficial in semen preservation, they may be harmful to sperm cells at certain concentrations. Further studies are required to evaluate the effects of combination of the antioxidants during semen storage or preservation and their resultant effects on conception rates in domestic animals.

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