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EFFECTS OF BIOLOGICAL ACTIVE SUBSTANCES TO THE SPERMATOZOA QUALITY

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ABSTRACT

The aim of this short review is to investigate the effects of biological active substances namely vitamin C and E, Quercetin, Tannic acid, Lycopene, Resveratrol, and Curcumin to viability and cryopreservation of spermatozoa; as well as the sensitivity of spermatozoa to reactive oxygen species and the strategies how to avoid oxidative stress (OS) by using naturally occurring antioxidants mentioned above. The oxidative stress, which has been associated with male infertility and many degenerative diseases, can be reduced by antioxidants via breaking the oxidative chain reactions. Lycopene is one the most highly efficient antioxidant and free radical scavenger and has protective effect on spermatozoa. *Trans*-Resveratrol also has positive effects on the production of spermatozoa. Consequently, one of the most prominent compounds "curcumin" which has widely been studied for its anti-inflammatory, anti-angiogenic, antioxidant, wound healing and anti-cancer effects; as well as showed energy-promoting and protective effects on the testicular tissue and spermatogenesis.

Keywords: Spermatozoa, oxidative stress, antioxidants, vitamins, lycopene, resveratrol, curcumin

INTRODUCTION

Insemination of farm and domestic animals is the most widespread biotechnological methods of reproduction. Artificial insemination techniques depend on the availability of high quality semen, whether fresh, diluted and stored, or frozen. Improving the quality of insemination doses are undoubtedly one of the most critical success factors of insemination. The investigation and handling of semen is considered a key and essential step for assessing fertility and the successful use of semen.

Currently used and approved procedures for the preparation of insemination of farm animals use doses of biologically and chemically difficult identifiable components. The components, which are being used to extend and protect the semen in cryopreservation and/or against any possible damage, are much complex and difficult to identify. The usage of such diluents with insemination will protect the farm animals from biological risk and diseases.

One of the most important achievements in dairy farming after the introduction of artificial insemination is the cryopreservation of bull semen, which has enabled the worldwide distribution and use of desired genetic lines at a reasonable cost **(Manjunath *et al.* 2002)**. However, it is reported that viability or fertility of frozen thawed semen is lower in buffalo compared to cattle **(Andrabi *et al.*, 2008)**.

Bansal and Bilaspuri (2010) reported that one of the most dangerous factors affecting the semen quality is Reactive Oxygen Species (ROS) **(Bansal and Bilaspuri, 2010)**. Studies have shown that antioxidants protect spermatozoa from ROS producing abnormal spermatozoa, scavenge ROS produced by leukocytes, prevent DNA fragmentation, improve semen quality in smokers, reduce cryodamage to spermatozoa, block premature sperm maturation and stimulate spermatozoa and improve assisted reproductive techniques outcome **(Agarwal *et al.* 2008)**. On the other hand the decreased activities of antioxidant enzymes are associated with various types of cancer such as prostate cancer, bladder cancer, breast cancer, hepatic cancer, multiple myeloma **(Khan *et al.* 2013)**. The synthetic antioxidants are one of the best solutions; however, the naturally occurring substances are preferable due its chemical diversity, structural complexity, availability, intrinsic biologic activity or lack of substantial toxic effects **(Tvrdá *et al.*, 2015a)**. Thus, this review work is done to investigate the effect of biological active substances, such as vitamin C and E, Quercetin, Tannic acid, Lycopene, Resveratrol and Curcumin, to the viability and cryopreservation of spermatozoa.

OXIDATIVE STRESS (OS)

The term oxidative stress is used when the oxidants outnumber antioxidants **(Bansal and Bilaspuri, 2010)**. Oxidative stress can be defined as the imbalance between the productions of reactive oxygen species (ROS) and a biological systems ability to readily detoxify the reactive intermediates or easily repair the resulting damage **(Bansal and Bilaspuri, 2010)**. The main destructive aspect of OS is the production of ROS which contains free radicals and peroxides **(Bansal and Bilaspuri, 2010)**. The production of ROS is a normal physiological process of spermatozoa however the imbalance between ROS and antioxidants, which cannot scavenge the exceeded ROS, is harmful to spermatozoa and can cause male infertility **(Bansal and Bilaspuri, 2010)**. Beside affecting male infertility some studies showed that oxidative stress has been associated with many degenerative diseases such as atherosclerosis, cancer, trauma, stroke, asthma, hyperoxia, arthritis, age pigments, dermatitis, cataractogenesis, retinal damage, hepatitis and aging **(Pandey and Rizvi 2010)**. OS has been reported to be one the most harmful factors of affecting the semen quality (Bansal and Bilaspuri, 2010). Sperms are highly sensitive to OS especially to lipid peroxidation because of their high concentration of unsaturated fatty acid in the plasma membrane **(Hekimoğlu *et al.*, 2009)**. In fact, in order to maintain the normal sperm function fatty acids are essential requirements for the male germ cell **(Hekimoğlu et al., 2009)**. OS affects spermatozoa mainly in three ways namely membrane lipid peroxidation, DNA damage and induction of apoptosis **(Durairajanayagam et al., 2014)**.

Mammalian spermatozoa represents a number of cell types which produce ROS such as hydrogen peroxide (H2O2), the superoxide anion (O_2•−), the hydroxyl radical (OH•), and hypochlorite radical (OHCl•) under aerobic conditions. ROS can combine with other molecule because of its highly reactive nature and can cause functional changes and structural damage **(Agarwal *et al.* 2008)**.

Agarwal reported that there are two ways of the generation of the reaction oxygen species: one nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system at the level of the sperm plasma membrane and the other NADPH-dependent oxidoreductase (diphorase) at the mitochondrial level **(Agarwal *et al.* 2008)**. In another study **Aitken and Koppers (2011)** stated that OS could arise due to excess exposure to ROS and/or may be due to male tract puts in place to protect the spermatozoa from free radical attack. Spermatozoa are susceptible to OS due to its possession of unsaturated fatty acid which is the main target for oxidation as well as the DNA presence in the sperm nucleus and

mitochondria **(Aitken and Koppers, 2011)**. These cells have low level of cytoplasmic antioxidant enzymes such as glutathione peroxidase (GPx) catalase and superoxide dismutase. However the cytoplasm is mainly restricted to midpiece, there is very little that the spermatozoon can protact the larger area of plasma membrane overlaying the sperm head and tail **(Aitken and Koppers, 2011)**, as well as there is very less chance to protect DNA rather than to compact it with protamines **(Aitken and Koppers, 2011)**.

OS targets all cellular components including lipids, proteins, nucleic acids, and sugars **(Agarwal *et al.* 2008)**. The OS damage depends on nature and amount of ROS produced, the duration of ROS exposure as well as extra-cellular factors such as temperature, oxygen tension and the composition of the surrounding environment (e.g. ions, proteins, and ROS scavengers) **(Agarwal *et al.* 2008)**. Increasing amount of ROS is inversely correlated to the sperm motility. Lipid peroxidation status of the spermatozoa caused the loss of motility as incubated over the night and the evidence could be the dilution of antioxidants (a-tocopherol) to revive sperm motility both in vivo and in vitro **(Agarwal *et al.* 2008)**. ROS can also cause apoptosis in the sperm **(Agarwal *et al.* 2008)**.

ANTIOXIDANTS

Semen cryopreservation is one of the most important procedures in the livestock industry. However, sperm cryopreservation and thawing is associated with increased ROS production and decreased antioxidant level **(Bansal and Bilaspuri, 2010)**, in fact sperm plasma membrane is one the key structures affected by cryopreservation **(Bansal and Bilaspuri, 2010)**. Recent studies show that the addition of antioxidants to cryopreservation extenders has positive effect and improve semen parameters such as sperm motility, membrane integrity after thawing **(Bansal and Bilaspuri, 2010)**. Furthermore, the addition of antioxidants during the IVF procedures impaired sperm quality, normal pronuclear formation, and embryo development to the blastocyst stage **(Bansal and Bilaspuri, 2010)**. Moreover, antioxidants protects spermatozoa from ROS produced by leukocytes, prevent DNA fragmentation, improve semen quality, reduce cryodamage to spermatozoa, block premature sperm maturation and provide an overall stimulation to the sperm cells **(Agarwal *et al.*, 2007; Tvrda *et al.*, 2013)**.

One of the primary components of the antioxidant system of spermatozoa and the major membrane protectants against ROS and lipid peroxidation (LPO) is Vitamin E **(Yousef *et al.*, 2003)**. It is one of the main chain-breaking antioxidants that can deoxidize the free radicals such as peroxyl and alkoxyl (ROO•) generated during ferrous ascorbate-induced LPO **(Sinclair, 2000; Bansal and Bilaspuri, 2010)** and forms a stable complex such as tocopheroxyl radical **(Tvrda *et al.*, 2013)**. It mainly scavenges superoxide, hydrogen peroxide, and hydroxyl radicals in the sperm membranes **(Agarwal et al, 2008)**. Due to its lipid solubility, it's located in the first line of defense against the peroxidation of polyunsaturated fatty acids in the cellular membrane phospholipids **(Horton et al., 2002)**. **Tvrda et al. (2013)** showed that vitamin E increased the motility from 0 to 24 hours of incubation, as well as suggested that it prevents the rapid loss of motility which normally occurs during the incubation of spermatozoa and maintains the motility under oxidative stress conditions, thus, improves the percentage of motile and viable spermatozoa under *in vitro* conditions **(Tvrda *et al.*, 2013)**. **Agarwal et al. (2004)** suggested that the supplementation of vitamin E (10 mmol/l) along with other extender in cryopreservation maintains sperm motility more efficiently than cryoprotectants alone. **Aitken et al. (1989)** and **Verma and Kanwar (1998)** showed that vitamin E (800 µmol/l, 10 mmol/l) proved to be protective against lipid peroxidation **(Aitken et al., 1989; Verma and Kanwar, 1998; Agarwal et al., 2004)**. How the vitamin E protects the spermatozoa could be by promoting sperm membrane integrity and increases the protection of the sperm cells against the formation of the superoxide radical, which supposed to be the most aggressive ROS **(Tvrda *et al.*, 2013)**, that has also been observed on humans by **Verma and Kanwar (1999)** and **Slebodzinska et al. (1995)** on boars.

Vitamin C (ascorbic acid) is also one of the most important chain-breaking antioxidant, which neutralizes hydroxyl, superoxide, and hydrogen peroxide radicals and prevents sperm agglutination **(Agarwal *et al,* 2008)**. It is found in seminal plasma with the concentrations 10 times higher than serum **(Dawson *et al.*, 1987; Jacob *et al.*, 1992; Agarwal *et al.*, 2010).** As well as prevents lipid peroxidation, recycles vitamin E and protects against DNA damage induced by hydrogen peroxide radical **(Agarwal et al, 2008)**. At low concentration vitamin C may act as oxidant and at high concentration it acts as an antioxidant **(Affranchino *et al.*, 1991; Breininger *et al.*, 2005; Andrabi *et al.*, 2008)**. However, higher concentration of vitamin C (>20 µmol/l) has no protective effect against H_2O_2- induced peroxidative damage of motility, in fact it increase the damage in both normozoospermic and asthenozoospermic patients **(Donnelly *et al.*, 1999; Agarwal *et al.*, 2004)**. A study has also demonstrated that 10 mmol/l of vitamin C with TEST yolk buffer failed to reduce the loss of motility in cryopreserved semen samples **(Askari et al., 1994; Agarwal et al., 2004)**. Vitamin E at concentrations of 40 and 60 µmol/l had also showed similar results **(Donnelly et al., 1999; Agarwal et al., 2004)**. However **Andrabi et al. (2008)** reported that vitamin E (1 mg/ml) with the tris-citric acid improved the quality of frozen-thawed buffalo spermatozoa **(Beconi *et al.*, 1993; Andrabi *et al.*, 2008)**. The addition of vitamin C (300 and 600 µmol/l) and vitamin E (40 and 60 µmol/l) to sperm preparation medium significantly reduced (P < 0.005) H2O2- induced ROS in all concentrations **(Donnelly et al., 1999b, 2000; Agarwal et al., 2004)**. **Donnelly et al. (1999)** reported that sperm suspension incubated at 300 and 600 µmol/l of vitamin C provided complete protection against H_2O_2 induced DNA damage (P < 0.005), similar dose dependent results were achieved by vitamin E. Thus, both vitamin E and vitamin C protected normozoospermic and asthenozoospermic samples from DNA damage induced by H_2O_2 **(Donnelly *et al.*, 1999b; Agarwal *et al.*, 2004)**. Consiqently the addition of vitamin C might reduce endogenous oxidative DNA damage, decreasing the risk of genetic defects, particularly in populations with low vitamin C levels, such as smokers **(Agarwal *et al.*, 2010)**.

Another class of naturally occurring polyphenolic compounds "flavonoids", which are present in photosynthesising cells **(Saito, 1974; *Salunkhe et al.*, 1982; Boots *et al.*, 2008)**. Flavonoids have also been reported to be beneficial for various diseases such as cancer, stroke, and cardiovascular diseases **(Kumar and Pandey, 2013)**. The therapeutic effect of flavonoids could be due to their strong antioxidative properties **(Kumar and Pandey, 2013)**, and are suggested to be good candidates for antioxidant therapy **(Boots *et al.*, 2008)**. Within the flavonoid family, quercetin (3,30,40,5,7-pentahydroxylflavone) an excellent in vitro antioxidant **(Boots et al., 2008)**, and is the most potent scavenger of ROS, each O_2•− **(Hanasaki et al., 1994; Cushnie and Lamb, 2005)**, and RNS like NO_2•− (van Acker *et al.*, 1995; Haenen and Bast, 1999) and $ONOO_2$•− **(Haenen *et al.*, 1997; Heijnen *et al.*, 2001; Boots *et al.*, 2008)**. The antioxidant capacities of quercetin could be attributed to the presence of two antioxidant pharmacophores within the molecule which have the optimal configuration for free radical scavenging **(Heijnen *et al.*, 2002; Boots *et al,* 2008)**. In addition, quercetin is known *in vitro* to possess anti-inflammatory capacities **(Read,1995; Orsolic *et al.*, 2004)**, anti-fibrotic **(Lee *et al.*, 2003)**, anti-coagulative **(Bucki et al., 2003)**, anti-bacterial **(Cushnie and Lamb, 2005)**, anti-atherogenic **(de Whalley *et al.*,1990; Perez-Vizcaino *et al.*, 2006)**, anti-hypertensive **(Duarte *et al.*, 2001; Perez-Vizcaino *et al.*, 2006)** and anti-proliferative properties **(Kuo, 1996; Orsolic *et al.*, 2004; Orsolic et al., 2004; Gulati *et al.*, 2006; Boots *et al.*, 2008)**. Quercetin was proposed as a cryoprotective agent (28), based on the observation that QUE administration was able to preserve the spermatozoa survival, DNA integrity and postthaw functionality **(Tvrda *et al,* 2014)**.

The antioxidant activity of phenolic compounds is mainly attributed to their redox properties that allow them to act as reducing agents, hydrogen donors and quenchers of singlet oxygen. Furthermore, they might also possess metal chelation properties **(Rice-Evans, 1995; Liyana-Pathirana and Shahidi, 2006; Gulcin *et al.*, 2010)**. Tannic acid – also called tannis – is a water-soluble polyphenol that is found in several beverages including red wine, beer, coffee, black tea, green tea, and many fruits such as grapes, pears, bananas, as well as sorghum, black-eyed peas, lentils and chocolate **(Chung et al., 1998a; King and Young, 1999; Gulcin et al., 2010)**. Tannic acid has antioxidant **(Lopes et al., 1999; Ferguson, 2001; Wu et al., 2004; Andrade et al., 2005)**, antimutagenic **(Ferguson, 2001; Horikawa *et al.*, 1994; Chen and Chung, 2000)** and anticarcinogenic properties **(Horikawa *et al.*, 1994; Athar *et al.*, 1989; Gali *et al.*, 1992; Nepka *et al.*, 1999; Gulcin *et al.*, 2010)**. However, in the presence of copper ions, tannic acid acts either as a prooxidant, promoting DNA damage **(Ferguson, 2001; Khan and Hadi, 1998; Khan *et al.*, 2000)**, or as an antioxidant, suppressing hydroxyl radical formation **(Andrade *et al.*, 2005; Gulcin *et al.*, 2010)**. It was reported that the hydrophobic ''core'' and hydrophilic ''shell'' features might be responsible for its antioxidant action of tannic acid **(Isenburg *et al.*, 2006; Gulcin *et al.*, 2010)**.

LYCOPENE

Recent studies have shown that lycopene has highly efficient antioxidant and free radical scavenging capability **(Turk et al. 2007)**. Lycopene is from the carotenoids family which is naturally found in fruits and in vegetable and gives the plants bright yellow, orange and red colors **(Durairajanayagam *et al,* 2014)**. Carotenoids are one of the most important components for human diet because of their antioxidants properties beside sources of vitamin A and the only way humans take them in is by consumption of vegetable and fruits **(Durairajanayagam *et al,* 2014)**. Lycopene is a red pigmented unsaturated linear carotenoid with the molecular weight of 536.85 Da, that contains 11 conjugated and 2 non-conjugated double bonds **(Durairajanayagam *et al,* 2014)**. Thus it contains many electrons which can be donated to free radicals that results their neutralization **(Durairajanayagam *et al,* 2014)**. Hence lycopene play the role of an antioxidant by reducing ROS production and the oxidative stress less severe, therefore prevents oxidative damage to lipids, proteins and DNA **(Durairajanayagam *et al,* 2014)**. Lycopene is considered one of the most potent singlet oxygen quencher of its family **(Durairajanayagam *et al,* 2014)**, therefore it's twice more effective then β-carotene and 10 times more effective then α-tocopherol **(Durairajanayagam *et al,* 2014)**. Lycopene is also known for scavenge other free radicals such as hydrogen peroxide, nitrogen dioxide and hydroxyl radicals **(Durairajanayagam *et al,* 2014)**.

Lycopene could also act as nonoxidative way by exerting its effects through aiding in gap junction communication, modulating gene expression, regulating the cell cycle and enhancing the immune system **(Durairajanayagam *et al,***

2014). **Durairajanayagam et al. (2014)** have also reported that lycopene beside biomarkers of oxidative stress, improve sperm parameters such as sperm count and concentration, motility, viability and morphology. **Hekimoğlu et al. (2009)** has also suggested that the protective effect of lycopene against ischemia/reperfusion induced loss of sperm function could be because of its antioxidant properties.

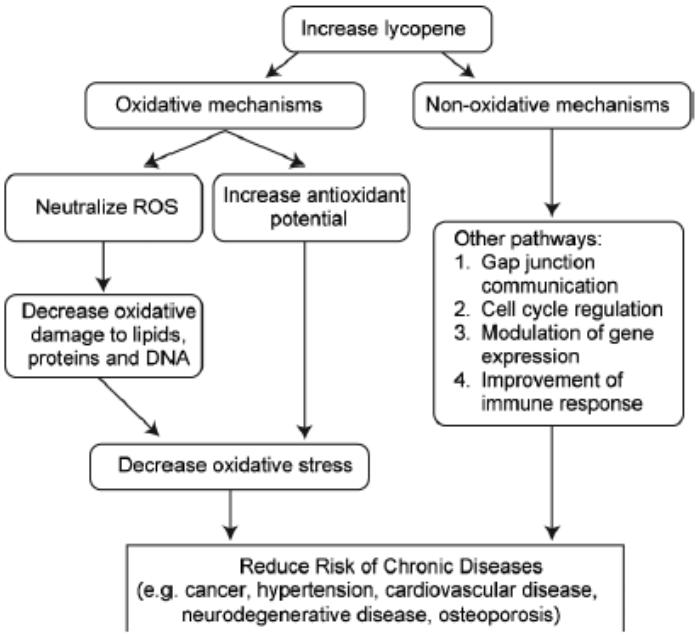

Figure 1 General mechanisms of action of lycopene. The proposed mechanisms of action of lycopene which scavenge reactive oxygen species and consequently reduce the oxidative stress **(Durairajanayagam *et al,* 2014)**.

Lycopene has protective effect on each: testicular toxicity, spermiotoxicity, cardiotoxicity, hepatotoxicity and nephrotoxicity **(Krishnamoorthy *et al.*, 2013)**. As well as lycopene could protect Leydig cellular StAR protein and steroidogenic enzyme expression and confirmed its activity against Polychlorinated biphenyls **(Krishnamoorthy *et al.*, 2013)**. Lycopene improved almost all Cyclosporine A-induced damages in the structure of testis and the other deteriorated histopathological findings that could be as result of lycopne's ability to react with free oxygen metabolites **(Turk *et al.*, 2007)**. Cyclosporine A is a fungal peptide which is mostly used in the transplant surgery as an immunosuppressant to prevent the rejection reactions **(Turk *et al.*, 2007)**.

RESVERATROL

Resveratrol (3,4',5-trihydroxystilbene) is a phytoalexin which is mostly found in dietary sources such as gramps, plums and peanuts. When it is extracted by methanol, it is an off white powder with the molecular weight of 228 **(Aggarwal et al., 2004)**. Reveratrol exists as *cis*- and *trans*-isomers. If the *trans*-isomer is protected from high pH and light, it is more stable and is **preferred (Aggarwal et al., 2004; Alarcon de la Lastra and Villegas, 2007)**. Several studies have identified resveratrol as a beneficial agent each in cardio- and vascular-protection, an anti-aging in treating age-related human diseases, neuro-protector and one the foremost a cancer chemo-protector **(Aggarwal et al., 2004; Alarcon de la Lastra and Villegas, 2007; Pandey and Rizvi 2010)**. Majority of the scientific evidences of benefits of resveratrol are based on *in vitro* studies in which both *cis* and *trans*-isomers have been tested. However, *trans*-resveratrol is predominantly consumed with food **(Alarcon de la Lastra and Villegas, 2007)**. Resveratrol inhibited the Fe^{2+}-catalyzed lipid hydroperoxide-dependent peroxidation of sonicated phosphatidylcholine liposomes more efficiently than either the hydrophilic analogue of vitamin E, Trolox, or vitamin C ascorbate **(Tadolini *et al.*, 2000)**.

Belguendouz et al. discovered that resveratrol through chelating and free radical scavenging mechanisms protected lowdensity lipoprotein (LDL) against peroxidative degradation **(Belguendouz *et al.*, 1997)**.

Juan et al., **2005** was the first who found out the enhancement in spermatozoa production in rats by daily oral administration of *trans*-resveratrol **(Juan *et al.*, 2005)**. Trans-Resveratrol is an effective scavenger of hydroxyl, superoxide, and metal-induced radicals, having antioxidant abilities in cells producing ROS and as well as shows a protective effect against lipid peroxidation in cell membranes and DNA damage caused by ROS **(Juan et al., 2005)**. **Cao and Li (2004)** reported the mechanism of the protective effects of resveratrol in various cardiovascular disorders, who showed that a number of endogenous antioxidants such as SOD, CAT, glutathione reductase (GR), glutathione S-transferase (GST), and NAD(P) H:quinone oxidoreductase 1 (NQO1) in cultured cardiomyocytes can be induced by low micromolar concentrations of resveratrol.

CURCUMIN

Curcumin a vibrant yellow spice, (diferuloylmethane)—(1,7-bis (4-hydroxy- 3-methoxyphenyl)-1,6-hepadiene-3,5-dione) found in the spice turmeric, from the rhizomes of the plant *curcuma longa linn* from Zingiberaceae family **(Tvrdá E. et al., 2015b)**, which has been used worldwide for the treatment of various types of inflammatory conditions and diseases for centuries **(Ammon and Wahl, 1991; Maheshwari et al. 2006; Sandur et al., 2007)** and is an agent that is non-toxic to humans and was proved to be extremely safe even at very high doses **(Kunnumakkara et al., 2007; Anand *et al.*, 2007)**. It has been reported that curcumin has variety of pharmacological activities such as anti-inflammatory **(Srimal and Dhawan, 1973; Satoskar *et al.*, 1986)**, anti-**cancer (Kuttan *et al.*, 1985; Maheshwari *et al.*, 2006)**, anti-**oxidant (Sharma, 1976; Toda *et al.*, 1985; Maheshwari *et al.*, 2006)**, wound healing **(Sidhu *et al.*, 1998; Maheshwari *et al.*, 2006)** and anti-microbial effects **(Negi *et al.*, 1999; Maheshwari *et al.*, 2006)**. **Araujo and Leon (2001)** mentioned that turmeric by maintaining the activities of antioxidant enzymes higher reduce the lipid peroxidation **(Araujo and Leon, 2001)**. Maheshwari reported that curcumin shows strong antioxidant activity in comparison to vitamins C and **E (Maheshwari et al. 2006)**. The proactive and therapeutic effects of curcumin are mainly attributed to its antioxidant property **(Maheshwari et al. 2006)**. It is a potent scavenger of several reactive oxygen species such as superoxide anion radicals, hydroxyl radicals, lipid peroxidation and so on **(Maheshwari et al. 2006)**.

Studies both *in vivo* and *in vitro* suggest the involvement of curcumin in energy-promoting and protective effects of curcumin on the testicular tissue, spermatogenesis and oxidative balance of the sperm cell **(Tvrdá E. *et al.*, 2015b)**. In addition, male toxicity caused by various environmental and physiological factors has been reversed by curcumin **(Tvrdá E. *et al.*, 2015b)**. **Sandur et al. (2007)** reported that curcumin is more potent antioxidant then α-tocopherol.

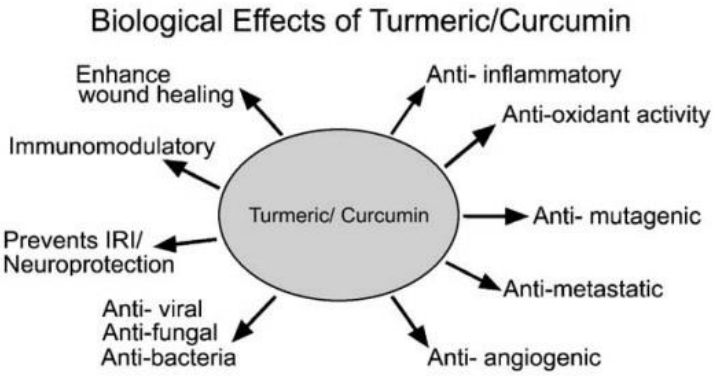

Figure 2 Schematic showing multiple biological activities of turmeric/curcumin **(Maheshwari *et al.*, 2006)**.

El Wakf et al. (2011) concluded that curcumin reduced nitrate reproductive toxicity, by normalizing sperm number, weights of sex organs, male sex hormones and other reproductive disorders and therefore enhances male fertility **(El Wakf *et al.*, 2011)**. In another study from our department various doses of curcumin (from 50 to 10 μM/L) increased the percentage of motile and viable spermatozoa; however it reduced and prevented the intracellular overproduction of free radicals within the sperm mitochondrial membrane **(Tvrdá E. *et al.*, 2015b)**.

CONCLUSION

Spermatozoa are protected by various antioxidants and enzymes – with antioxidant quality – in the seminal plasma or in spermatozoa itself to prevent oxidative damage. In general, antioxidants are compounds and reaction which dispose, scavenge, and suppress the formation of ROS, or oppose their actions. Many studies suggest that resveratrol exerts antioxidant activity and is a potent inhibitor of ROS. Antioxidants break the oxidative chain reaction, thus reduce the oxidative stress. Studies have therefore been shown that the addition of cryopreservation extenders with antioxidants have provided a cryoprotective effect on bull, ram, goat, boar, canine, and human sperm quality, thus improving semen parameters, for example, sperm motility, membrane integrity after thawing. Consequently, the addition of stated active substances could be of scientific importance for long term preservation of spermatozoa storage before further andrological experiments and clinical procedures, such as artificial insemination or in vitro fertilization techniques.

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