Research Article **CODEN: AJPCFF** **ISSN: 2321 – 0915**

Asian Journal of Phytomedicine and Clinical Research

Journal home page: www.ajpcrjournal.com

IMPACT OF OXIDATIVE STRESS AND ANTIOXIDANT THERAPIES ON MALE REPRODUCTIVE HEALTH

Dharmendra Arya[1], Kiran Bhagour[1], R. S. Gupta[*1]

[1*]Reproductive Physiology Lab, Centre of Advanced Studies, Department of Zoology, University of Rajasthan, Jaipur, Rajasthan, India.

ABSTRACT

The male factor is a major contributory factor to infertility. Oxidative stress is the important cause of male infertility which is oxygen-derived radicals, generated constantly as a main part of normal aerobic life. These radicals are formed in mitochondria as oxygen is reduced along the electron transport chain and collectively known as reactive oxygen species (ROS). It is a powerful mechanism causing sperm damage, deformity and finally leads to male infertility. Antioxidants suppress the action of these reactive oxygen species in a regulatory manner but in several conditions *viz.*, aging, physical injury, chronic disease and toxin exposure, these ROS production increases and causes oxidative stress which leads to cellular damage. The review article enlightens the physiological and pathological role of ROS in normal sperm function and to explore the role of antioxidant therapies in management of ROS causes infertility.

KEYWORDS

Reactive Oxygen Species, Antioxidants, Infertility and Oxidative Stress.

Author for Correspondence:

Gupta R S,

Reproductive Physiology Section,

Department of Zoology,

University of Rajasthan, Jaipur, India.

Email: gupta_rs@hotmail.com

INTRODUCTION

At present time infertility is a major clinical health issue, affecting medically and psychosocially to couples. Male infertility is a foremost factor in infertile couples. There are several cases which lead infertility such as varicocoele, cryptorchidism, obstructive lesions, cystic fibrosis, trauma and tumours. Of the many causes of male infertility, Oxidative stress (OS) is also considered as one of the cause for male infertility.

Oxygen is essential to sustain life for all aerobic forms. ROS are reactive oxidizing agents' contain free radicals. Mainly reported ROS having a potent

role in reproductive biology includes the superoxide anion radical, the hydroxyl radical, the peroxyl radical, subclass of free radicals derived from nitrogen, which includes nitric oxide, peroxynitrite, nitroxyl anion, peroxynitrous acid, hydrogen peroxide, peroxyl radical and hypochlorite ion. In several complications such as chronic disease states, aging, toxin exposure, physical injury, and exposure to many types of foods, the level of these ROS get increases and causes oxidative stress which leads to cellular damage. OS has been defined as an imbalance between the Reactive Oxygen Species (ROS) generation and antioxidant scavenging activities[1].

Many antioxidant therapies have been used to improve sperm quality. Treatment includes varied compounds, *viz.*, carnitines, kallikrein, phosphatidylcholine, pentoxifylline and vitamins A, C and E[2,3]. This research area has received a great impulse and the importance of ROS generation and lipid per oxidation has been underlined as a mechanism that damages mammalian spermatozoa[4,5]. This article briefly enumerates the pathophysiological effects of ROS generation on the male reproductive system and the possible ways of preventing and minimizing oxidative stress with the goal of achieving positive results in infertile males and review the pathways of sperm OS and antioxidant defense to better understand which conditions are at risk of disequilibrium and which antioxidant therapies can lead to a real improvement of human sperm quality *in vitro* and *in vivo*.

Reactive Oxygen Species (ROS) and Oxidative Stress (Figure No.1)

Oxygen is most important element for all aerobic life. Oxygen is used into all biological oxidation mechanisms. ROS are formed as necessary intermediates of metal catalyzed oxidation reactions. Most reactive oxygen species are generated as by-products during mitochondrial electron transport. Atomic oxygen has two unpaired electrons in separate orbits in its outer electron shell and this electron structure makes it more susceptible for radical formation. The continuously reduction of oxygen through the addition of electrons leads to the formation of ROS. These ROS have a large category of molecules such as radical (superoxide; hydroxyl ions; nitric oxide; peroxyl*etc*.) and non-radical (hydrogen peroxide; lipid peroxide; ozone; singlet oxygen)[6]. The production of ROS by spermatozoa has been suggested to occur through two ways: Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system at the level of the sperm plasma membrane and NADPH-dependent oxidoreductase (diphorase) at the mitochondrial level[7,8]. The principal ROS produced by spermatozoa seems to be the superoxide anion radical, which generates hydrogen peroxide, spontaneously or following the activity of superoxide dismutase[9]. The excessive production of ROS leads to oxidative stress in cells and associated with several complication such as cell damage, DNA destruction and cell membrane damage *etc*. Scientific evidence supported the role of oxidative stress (OS) as a causative factor in many human degenerative processes, diseases, syndromes and ageing processes[10,11].

Physiological role of ROS on Reproductive health

The physiological levels of reactive oxygen species are necessary to maintain normal sperm cell function, but in limited quantity[12]. The balance between the amounts of ROS produced and the amounts of antioxidant scavenged at any moment governs whether a given sperm function will be stimulated or inhibited[13]. Minimum amount of ROS is requiring for sperm motility, acrosomal reaction and capacitation as well for fertilization[14,15]. The low levels of ROS are associated with sperm high motility[16,17]. Co-incubation of spermatozoa with low concentrations of hydrogen peroxide stimulates sperm capacitation, hyper activation, acrosome reaction and oocyte fusion.[16,18,19]. ROS also have been implicated in sperm-oocyte interaction[20]. Lipid per oxidation caused by low levels of ROS leads to modification of the plasma membrane, thus facilitating sperm-oocyte adhesion[18].

Pathological role of ROS on Reproductive health

ROS damage the mitochondrial membrane and the damaged mitochondrial membrane causes an increase in ROS production[21]. WHO concluded that oxidative stress occurs even in patients with a very

low seminal leukocyte count (between 0 and1x 10^6 /ml) and a rise in ROS occurs with an increase in leukocyte count and presence of any leukocytes is associated with oxidative stress may impair infertility[22]. Lipid per oxidation present in sperm plasma membrane in the form of polyunsaturated fatty acids (PUFA). ROS attacks PUFA in the cell membrane, leading toa cascade of chemical reactions called lipid per oxidation. The reactions proceed through three main steps- initiation, propagation and termination[12,16,23,24]. Malondialdehyde is the byproduct of lipid per oxidation, used in various biochemical assays to monitor the degree of per oxidative damage sustained by spermatozoa[12,25].

Effect on Sperm DNA

Sperm chromatin protects the genetic integrity and facilitates the transfer of the paternal genome through the male and female reproductive tracts. Tight packaging of sperm DNA and the antioxidants in seminal plasma protects sperm DNA from oxidative stress[26]. Despite this tight DNA packaging and the seminal plasma protection from oxidative damage, many correlations have also been observed between ROS generation and DNA alteration[26-30]. The exposure of spermatozoa to iatrogenically induced ROS significantly increases DNA fragmentation, modification of all bases, and production of base-free sites, deletions, frame shifts, DNA cross-links and chromosomal rearrangements[31,32]. Indeed, ROS have been shown to induce the DNA protein cross-linking in chromatin[33-35]. Significant positive correlation with DNA fragmentation, high frequency of DNA single and double-strand breaks[30,32,36,37]. And oxidative DNA base changes[38,39]. DNA fragmentation seems to be inversely correlated with sperm count, morphology, motility and fertilization rate[35,40-42]. ROS also causes gene mutations, resulting in decreased semen quality[43,44]. Other mechanisms such as denaturation and DNA base-pair oxidation also may be involved[39]. A common byproduct of DNA oxidation, 8-hydroxy-2-deoxyguanosine has been considered a key biomarker of this oxidative DNA damage[45]. DNA damage in the Y chromosome also can cause gene deletion in the Y chromosome of the offspring, leading to infertility[42].

Effect on Sperm Motility

Increased ROS production reduces the sperm motility[1,46], spermatozoa-oocyte fusion[14,47]and acrosome activity[48]. It has been reported that more than half (55%) of the oligozoospermic patients who display a spermatozoa-oocyte penetration rate lower than 25% have an elevated ROS production[49]. Furthermore, spermatozoa of oligozoospermic patients have been confirmed as a very important source of ROS[50]. A strong correlation between sperm function, including motility and the percentage of ROS-producing spermatozoa has been reported[51]. Some studies reported midpiece abnormalities[52,53] and some others showed that ROS-induced motility decrease is associated with a growth of lipid per oxidation measured as malondialdehyde (MDA)[54-56] and DNA modifications[55]. It has also been postulated that OS could be a cause for hyper-viscosity of seminal plasma in infertile males[57]. Increased ROS levels also have been correlated with decreased sperm motility[58-60]. Glucose-6-phosphate dehydrogenase (G-6-PD) in sperms, used to fuel the ROS generation by NADPH oxidase[61]. Another study involved a series of interrelated events resulting in a decrease in axonemal protein phosphorylation and sperm immobilization, associated with a reduction in membrane fluidity that is necessary for sperm-oocyte fusion[62].

Apoptosis

The Apoptotic process referred to a sequential biochemical and structural changes in response to tissue damage and it helps in elimination of abnormal spermatozoa[63]. Increase levels of ROS disturb the inner and outer mitochondrial membranes as well as stimulate releasing of the cytochrome-c protein and induced the caspases and apoptosis. In patients with male factor infertility, increased sperm damage by ROS was shown to correlate with higher levels of cytochrome c and caspase9 and 3 indicative of apoptosis[64].

Antioxidant mechanism against Oxidative stress (Fig-2)

An antioxidant is a molecule that reduces the oxidative stress in cells and protects them from damage. Seminal fluid have enzymatic antioxidants such as superoxide dismutase (SOD), catalase and glutathione peroxidase/ glutathione reductase as well as non-enzymatic antioxidants *viz.*, vitamin A, vitamin E, ascorbate, urate, pyruvate, glutathione, albumin, ubiquitol, taurine, and hypotaurine. There are several studies claimed that these antioxidants avoided ROS producing abnormal spermatozoa, scavenge ROS produced by leukocytes, prevent DNA fragmentation, reduce cryo-damage to spermatozoa, block premature sperm maturation and stimulate spermatozoa. Some experimental and clinical summary of role of antioxidant against ROS causes oxidative stress in reproductive system are described below:-

Superoxide dismutase (SOD)

SOD defends spermatozoa against spontaneous O_2 toxicity and LPO. SOD and catalase also eliminate O_2 produced by NADPH oxidase in neutrophils and play an important role in reducing LPO activity and hence protecting spermatozoa against oxidative damage. Human seminal plasma contains an amount of SOD which is responsible for high motility of spermatozoa via inhibits the lipid per oxidation in the xanthine oxidase system[65]. Another study suggested that SOD may protect rabbit spermatozoa from LPO induce oxidative stress[66].

Catalase

It was found that the activity of catalase is measured lower in as the no zoo spermic infertile subjects when compared to normal zoo spermic subjects and it protects spermatozoa from O_2induced toxicity[67].

Glutathione (GSH) and Glutathione peroxidase (GPx)

GSH and GPX are the main agents that can eradicate the hydrogen peroxide generated. The sulphydryl group (SH) of glutathione confers its protective action against oxidative damage[68]. In mouse and human sperm cells, glutathione peroxidase removes hydroperoxide and protects sperm from peroxidation[69]. This scavenging activity of GSH reduce the effects of oxidative stress in sperm cells, which could result in lipid per oxidation of plasma lemma, irreversible loss of motility, leakage of intracellular enzymes and damage of the chromatin[70]. SOD and GPx combined together, play an important role against the damaging effects of superoxide anion radical and hydrogen peroxide[65].

Vitamin E and Vitamin C

Vitamin E and Vitamin Care major membrane protective factor against reactive oxygen species (ROS) and lipid per oxidation (LPO). They both are showing a chain-breaking activity in membrane. The bull semen was treated with Vitamin E (2.5 mM) in the presence of oxidative stress showed a significant increase in sperm motility and viability and reduced the level of LPO[71]. The oral administration of vitamin E (400 mg) and selenium (225 µg) in men for the period of 3 months showed reduction in malondialdehyde (MDA) concentration and an improvement in sperm motility[72]. In addition, vitamin C (100 mg/kgb.wt.) showed an inhibition of PCB-induced ROS generation in adult male albino rats[73].

Coenzyme Q-10

It is present in the mid part of spermatozoa and act as energy promoting factors[74]. They are also showed a protective activity counter to per oxidative damage and recycle vitamin E and prevent its pro-oxidant activity.[23]The dose of coenzyme Q-10 (200 mg/day) was given to idiopathic as the no zoo spermic subjects exhibit an improvement in sperm parameters[75].

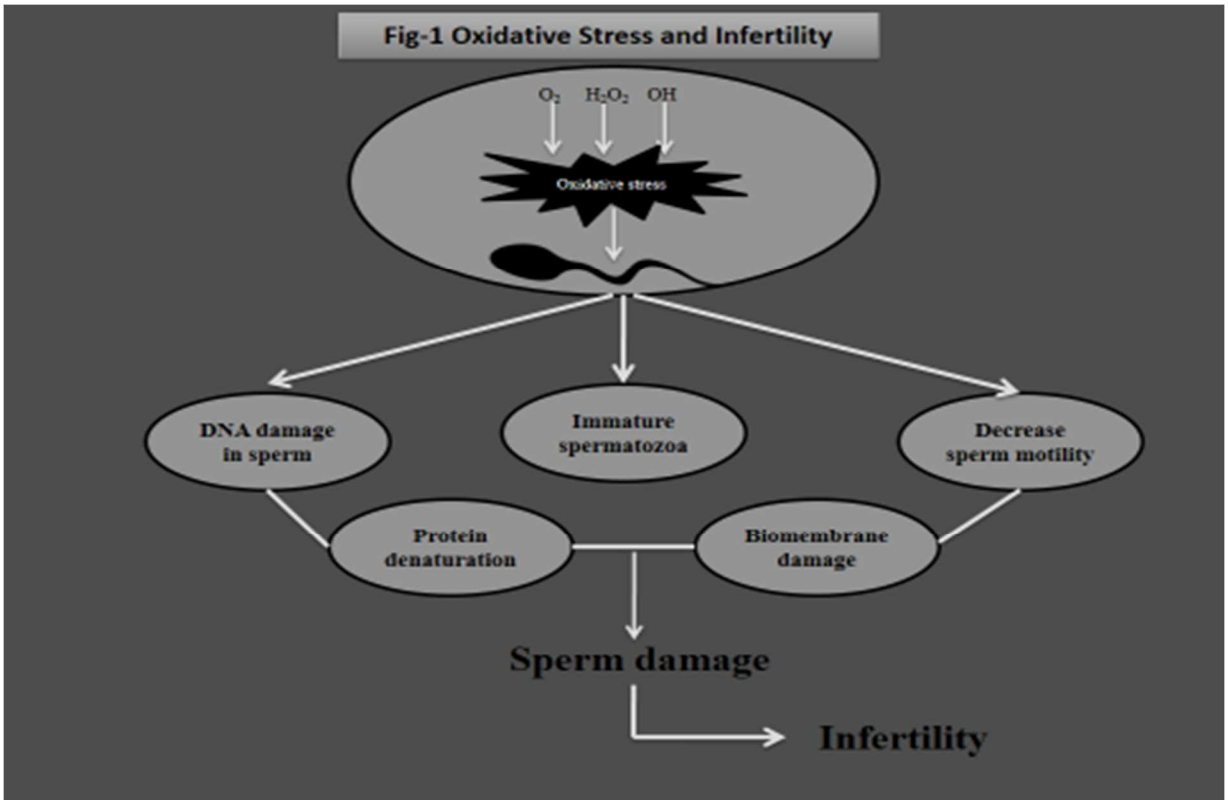
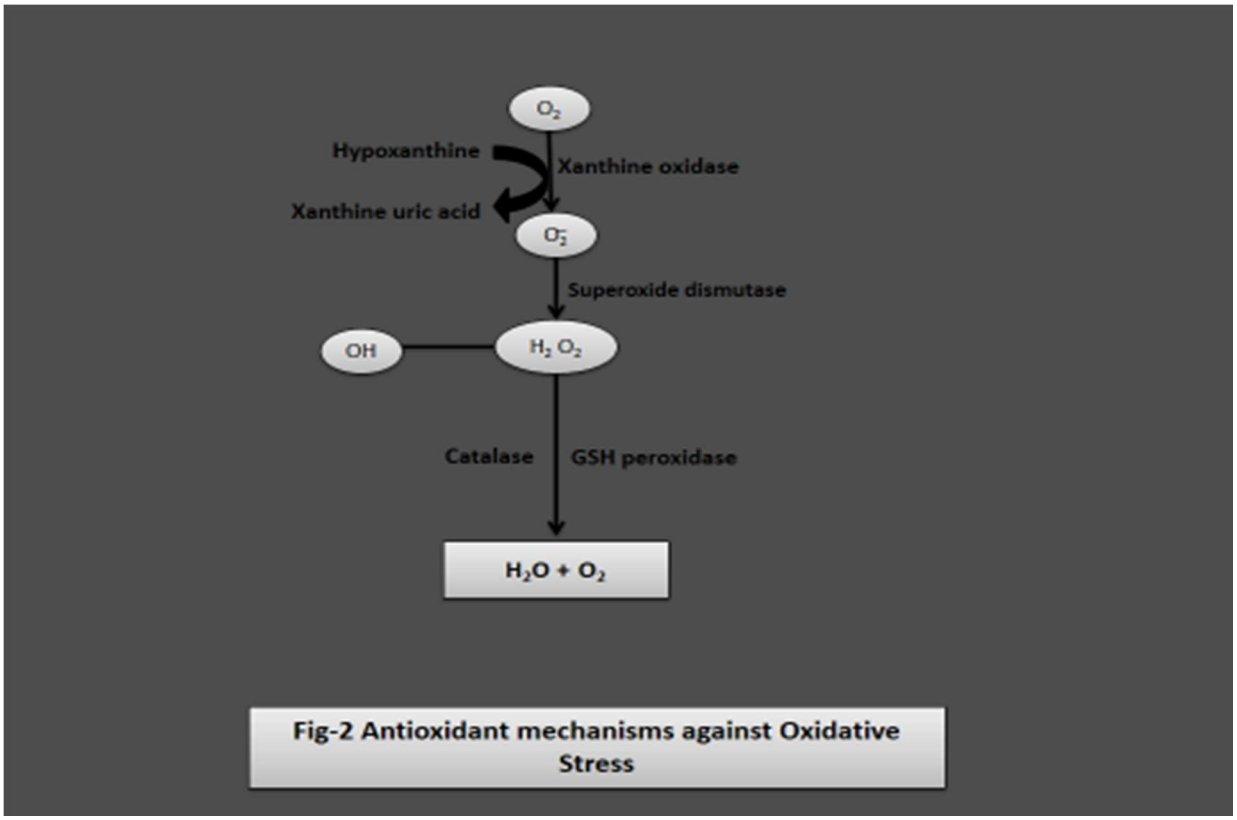


Fig-1 Oxidative Stress and Infertility
O_2 H_2O_2 OH
Oxidative stress
DNA damage in sperm
Immature spermatozoa
Decrease sperm motility
Protein denaturation
Biomembrane damage
Sperm damage
Infertility


O_2
Hypoxanthine
Xanthine oxidase
Xanthine uric acid
O_2^-
Superoxide dismutase
OH
H_2O_2
Catalase
GSH peroxidase
$H_2O + O_2$
Fig-2 Antioxidant mechanisms against Oxidative Stress

CONCLUSION

In the last decade, a number of studies suggested the toxic effect of oxidative stress on male reproductive health. ROS generated oxygen toxicity is an inherent challenge to mankind. Spermatozoa and oocytes exhibit an inherent capacity to generate limited amount of ROS for the fertilization but in many cases these ROS production increases and causes oxidative stress which leads to cellular damage. A variety of defense mechanisms encompassing antioxidant enzymes i.e., SOD, catalase, GSH, peroxidase, reductase, vitamins and ubiquinol are available to balance risks from ROS and improve sperm quality. The review article may provide suitable information to newly researchers about pathophysiological role of ROS on male reproductive health as well as the antioxidants mechanisms against oxidative stress induced infertility.

ACKNOWLEDGEMENT

The authors wish to express their sincere gratitude to Reproductive Physiology Lab, Centre of Advanced Studies, Department of Zoology, University of Rajasthan Jaipur, Rajasthan, India for providing necessary facilities to carry out this research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPBY

1. Lector C. Oxidative stress and role of antioxidants in normal and abnormal sperm function, *Front Biosci*, 1(1), 1996, 78-86.
2. Mann T, Lutwak-Mann C. Male Reproductive Function and Semen, *Berlin: Springer*, 1981.
3. Lanzafame F, Chapman M G, Guglielmino A, Gearon C M, Forman R G. Pharmacological stimulation of sperm motility, *Hum Reprod*, 9(2), 1994, 192-9.
4. Jones R, Mann T. Lipid peroxides in spermatozoa; formation, role of plasmalogen, and physiological significance, Proceedings of the Royal Society of London B: *Biological Sciences*, 193(1113), 1976, 317-33.
5. Saleh R, Ramadan A, Agarwal A. Oxidative stress and male infertility: from research bench to clinical practice, *J Androl*, 23(6), 2002, 737-52.
6. Agarwal A, Prabakaran S A. Mechanism, measurement, and prevention of oxidative stress in male reproductive physiology, *Indian J Exp Biol*, 43(11), 2005, 963-74.
7. Aitken R J, Buckingham D, West K, Wu F C, Zikopoulos K, Richardson D W. Differential contribution of leucocytes and spermatozoa to the generation of reactive oxygen species in the ejaculates of oligozoospermic patients and fertile donors, *J Reprod Fertil*, 94(2), 1992, 451-62.
8. Gavella M, Lipovac V. NADH-dependent oxidoreductase (diaphorase) activity and isozyme pattern of sperm in infertile men, *Arch Androl*, 28(2), 1992, 135-41.
9. Alvarez J G, Touchstone J C, Blasco L, Storey B T. Spontaneous lipid peroxidation and production of hydrogen peroxide and superoxide in human spermatozoa, Superoxide dismutase as major enzyme protectant against oxygen toxicity, *J Androl*, 8(5), 1987, 338-48.
10. Cutler R G. Antioxidants and aging, *Am J Clin Nutr*, 53(1), 1991, 373S-79S.
11. Davies K J. Oxidative stress: the paradox of aerobic life, *Biochem Soc Symp*, 6, 1995, 1-31.
12. Aitken R J, Fisher H. Reactive oxygen species generation and human spermatozoa: the balance of benefit and risk, *BioEssays*, 16(4), 1994, 259-67.
13. Sharma R K, Agarwal A. Role of reactive oxygen species in male infertility, *Uro*, 48(6), 1996, 835-50.
14. Griveau J F, Lannou D. Reactive oxygen species and human spermatozoa: physiology and pathology, *Int J Androl*, 20(2), 1997, 61-9.
15. Agarwal A, Nallella K P, Allamaneni S S, Said T M. Role of antioxidants in treatment

of male infertility: an overview of the literature, *Reprod Biomed Online*, 8(16), 2004, 616-27.
16. Aitken R J. Free radicals, lipid per oxidation and sperm function, *Reprod Fertil Dev*, 7(4), 1995, 659-68.
17. Visconti P E, Bailey J L, Moore G D, Pan D, Olds-Clarke P, Kopf G S. Capacitation of mouse spermatozoa, I Correlation between the capacitation state and protein tyrosine phosphorylation, *Devel*, 121(4), 1995, 1129-37.
18. Kodama H, Kuribayashi Y, Gagnon C. Effect of sperm lipid per oxidation on fertilization, *J Androl*, 17(2), 1996, 151-57.
19. De Lamirande E, Gagnon C. Human sperm hyper activation and capacitation as parts of an oxidative process, *Free Radic Biol Med*, 14(2), 1993, 157-66.
20. Agarwal A, Prabhakaran S A, Sikka S C. Clinical relevance of oxidative stress in patients with male factor infertility: evidence-based analysis, *AUA Update*, 26, 2007, 1-12.
21. Evenson D P, Darzynkiewicz Z, Melamed M R. Simultaneous measurement by flow cytometry of sperm cell viability and mitochondrial membrane potential related to cell motility, *J Histochem Cytochem*, 30(3), 1982, 279-80.
22. World Health Organization (WHO). WHO laboratory manual for the examination of human serum and sperm-cervical mucus interaction, Cambridge, UK: University Press, 4th edition, 1999.
23. Alvarez J G, Storey B T. Differential incorporation of fatty acids into and per oxidative loss of fatty acids from phospholipids of human spermatozoa, *Mol Reprod Dev*, 42(3), 1995, 334-46.
24. Griveau J F, Dumont E, Renard P, Callegari J P, Le Lannou D. Reactive oxygen species, lipid per oxidation and enzymatic defence systems in human spermatozoa, *J Reprod Fertil*, 103(1), 1995, 17-26.
25. Aitken R J, Clarkson J S, Fishel S. Generation of reactive oxygen species, lipid peroxidation, and human sperm function, *BiolReprod*, 41(1), 1989, 183-97.
26. Twigg J, Irvine D S, Houston P, Fulton N, Michael L, Aitken R J. Iatrogenic DNA damage induced in human spermatozoa during sperm preparation: protective significance of seminal plasma, *Mol Hum Reprod*, 4(5), 1998, 439-45.
27. Lee A G, East M J, Fround R J. Are essential fatty acids essential for membrane function? *Prog Lipid Res*, 25(1-4), 1986, 41-46.
28. Manicardi G C, Tombacco A, Bizzaro D, Bianchi U, Bianchi P G, Sakkas D. DNA strand breaks in ejaculated human spermatozoa: comparison of susceptibility to the nick translation and terminal transferase assays, *Histochem J*, 30(1), 1998, 33-39.
29. Zalata A A, Christophe A B, Depuydt C E, Schoonjans F, Comhaire F H. The fatty acid composition of phospholipids of spermatozoa from infertile patients, *Mol Hum Reprod*, 41(2), 1998, 111-18.
30. Liang R, Senturker S, Shi X, Bal W, Dizdarogluand M, Kasprzak K S. Effects of Ni (II) and Cu (II) on DNA interaction with the N-terminal sequence of human protamine P2: enhancement of binding and mediation of oxidative DNA strand scission and base damage, *Carcinogenesis*, 20(5), 1999, 893-8.
31. Aitken R J, Gordon E, Harkiss D, Twigg J P, Milne P, Jennings Z, Irvine D S. Relative impact of oxidative stress on the functional competence and genomic integrity of human spermatozoa, *BiolReprod*, 59(5), 1998, 1037-46.
32. Barroso G, Morshedi M, Oehringer S. Analysis of DNA fragmentation, plasma membrane translocation of phosphatidylserine and oxidative stress in human spermatozoa, *Hum Reprod*, 15(6), 2000, 1338-44.

33. Nackerdien Z, Rao G, Cacciuttolo M A, Gajewski E, Dizdaroglu M. Chemical nature of DNA-protein cross-links produced in mammalian chromatin by hydrogen peroxide in the presence of iron or copper ions, *Biochem*, 30(20), 1991, 4873-9.
34. Oliski R, Nackerdien Z, Dizdaroglu M. DNA protein cross linking between thymine and tyrosine in chromatin of gamma irradiated and hydrogen peroxide treated cultured human cells, *Arch Biochem Biophys*, 297(1), 1992, 139-143.
35. Twigg J P, Irvine D S, Aitken R J. Oxidative damage to DNA in human spermatozoa does not preclude pronucleus formation at intracytoplasmic sperm injection, *Hum Reprod*, 13(7), 1998, 1864-71.
36. Dizdaroglu M. Oxidative damage to DNA in mammalian chromatin, *Mutat Res*, 275(3-6), 1992, 331-42.
37. Chiu S M, Xue L Y, Friedman L R, Oleinick N L. Differential dependence on chromatin structure for copper and iron ion induction of DNA double strand breaks, *Biochem*, 34(8), 1995, 2653-61.
38. Hughes C M, Lewis S E, McKelvey-Martin V J, Thompson W. A comparison of baseline and induced DNA damage in human spermatozoa from fertile and infertile men, using a modified comet assay, *Mol Hum Reprod*, 2(8), 1996, 613-9.
39. Kodama H, Yamaguchi R, Fukuda J, Kasai H, Tanaka T. Increased oxidative deoxyribonucleic acid damage in the spermatozoa of infertile male patients, *FertilSteril*, 68(3), 1997, 519-24.
40. Sun J G, Jurisicova A, Casper R F. Deletion of deoxyribonucleic acid fragmentation in human sperm: correlation with fertilization in vitro, *Biol Reprod*, 56(3), 1997, 602-7.
41. Shen H, Ong C. Detection of oxidative DNA damage in human sperm and its association with sperm function and male infertility, *Free RadicBiol Med*, 28(4), 2000, 529-36.
42. Aitken R J, Krausz C. Oxidative stress, DNA damage and the Y chromosome, *Reprod*, 122(4), 2001, 497-506.
43. Spiropoulos J, Turnbull D M, Chinnery P F. Can mitochondrial DNA mutations cause sperm dysfunction? *Mol Hum Reprod*, 8(8), 2002, 719-21.
44. Sharma R K, Said T, Agarwal A. Sperm DNA damage and its clinical relevance in assessing reproductive outcome, *Asian J Androl*, 6(2), 2004, 139-48.
45. Helbock H J, Beckman K B, Shigenaga M K, Walter P B, Woodall A A, Yeo H C *et al*. DNA oxidation matters: the HPLC-electrochemical detection assay of 8-oxo-deoxyguanosine and 8-oxo-guanine, *Proc Natl Acad Sci*, 95(1), 1998, 288-93.
46. De Lamirande E, Gagnon C. Reactive oxygen species and human spermatozoa, II. Depletion of adenosine triphosphate plays an important role in the inhibition of sperm motility, *J Androl*, 13(5), 1992, 379-86.
47. Aitken R J, Irvine D S, Wu F C. Prospective analysis of sperm oocyte fusion and reactive oxygen species generation as criteria for the diagnosis of infertility, *Am J Obstet Gynecol*, 164(2), 1991, 542-51.
48. Zalata A A, Ahmed A H, Allamaneni S S, Comhaire F H, Agarwal A. Relationship between acrosin activity of human spermatozoa and oxidative stress, *Asian J Androl*, 6(4), 2004, 313-18.
49. Aitken R J, Clarkson J S, Hargreave T B, Irvine D S, Wu F C. Analysis of the relationship between defective sperm function and the generation of reactive oxygen species in cases of oligozoospermia, *J Androl*, 10(3), 1989, 214-20.
50. Zalata A, Hafez T, Comhaire F. Evaluation of the role of reactive oxygen species in male infertility, *Hum Reprod*, 10(6), 1995, 1444-51.
51. Gil-Guzman E, Ollero M, Lopez M C *et al.* Differential production of reactive oxygen species by subset of human spermatozoa at

different stages of maturation, *Hum Reprod*, 16(9), 2001, 1922-30.
52. Rao B, Soufir J C, Martin M, David G. Lipid peroxidation in human spermatozoa as relatd to midpiece abnormalities and motility, *Gamete Res*, 24(2), 1989, 127-34.
53. Kim J G, Parthasarathy S. Oxidation and the spermatozoa, *Seminars in Reproductive Endocrinology*, 16(2), 1998, 235-39.
54. Suleiman S A, Ali M E, Zaki Z M *et al.* Lipid peroxidation and human sperm motility: protective role of vitamin E, *J Androl*, 17(5), 1996, 530-37.
55. Chen C S, Wei Y H, Chao H T, Pan R L. Hydroxyl radical-induced decline in motility and increase in lipid peroxidation and DNA modification in human sperm, *IUBMB Life*, 43(2), 1997, 291-303.
56. Hsieh Y Y, Chang C C, Lin C S. Seminal malondialdehyde concentration but not glutathione peroxidase activity is negatively correlated with seminal concentration and motility, *Int J Biol Sci*, 2(1), 2006, 23-29.
57. Aydemir B, Onaran I, Kiziler A R, Alici B, Akyolcu M C. The influence of oxidative damage on viscosity of seminal fluid in infertile men, *J Androl*, 29(1), 2008, 41-46.
58. Lenzi A, Lombardo F, Gandini L, Alfano P, Dondero F. Computer assisted sperm motility analysis at the moment of induced pregnancy during gonadotropin treatment for hypogonadotropichypogonadism, *J Endocrinol Invest*, 16, 1993, 683-86.
59. Agarwal A, Ikemoto I, Loughlin K R. Relationship of sperm parameters with levels of reactive oxygen species in semen specimens, *J Urol*, 152(1), 1994, 107-10.
60. Armstrong J S, Rajasekaran M, Chamulitrat W, Gatti P, Hellstrom W J, Sikka S C. Characterization of reactive oxygen species induced effects on human spermatozoa movement and energy metabolism, *Free Radic Biol Med*, 26(7-8), 1999, 869-80.
61. Aitken R J, Fisher H M, Fulton N, Gomez E, Knox W, Lewis B, Irvine S. Reactive oxygen species generation by human spermatozoa is induced by exogenous NADPH and inhibited by the flavoprotein inhibitors diphenyleneiodonium and quinacrine, *Mol Reprod Devel*, 47(4), 1997, 468-82.
62. Lamirande E, Gagnon C. Reactive oxygen species and human spermatozoa, *J Androl*, 13(5), 1992, 379-86.
63. Sakkas D, Mariethoz E, Manicardi G, Bizzaro D, Bianchi P G, Bianchi U. Origin of DNA damage in ejaculated human spermatozoa, *Rev Reprod*, 4(1), 1999, 31-37.
64. Wang X, Sharma R K, Sikka S C, Thomas A J, Falcone T, Agarwal A. Oxidative stress is associated with increased apoptosis leading to spermatozoa DNA damage in patients with male factor infertility, *FertilSteril*, 80(3), 2003, 531-35.
65. Nissen H P, Kreysel H W. Superoxide dismutase in human semen, *KlinWochenschr*, 61(1), 1983, 63-65.
66. Alvarez, J G, Storey B T. Role of superoxide dismutase in protecting rabbit spermatozoa, *Biol Reprod*, 28(5), 1983, 1129-36.
67. Jeulin C, Soufir J C, Weber P, Laval-Martin D and Calvayrac R. Catalase activity in human spermatozoa and seminal plasma, *Gamete Res*, 24(2), 1989, 185-96.
68. Luberda Z. The role of glutathione in mammalian gametes, *Reprod Biol*, 5(1), 2005, 5-17.
69. Alvarez J G, Storey B T. Role of glutathione peroxidase in protecting mammalian spermatozoa from loss of motility caused by spontaneous lipid per oxidation, *Gamete Res*, 23(1), 1989, 77-90.
70. Luberda Z. Present conception regarding the effect on reactive oxygen species on functions of mammalian spermatozoa, *Postępy Biologii Komórki*, 28, 2001, 309-16.
71. Bansal A K, Bilaspuri G S. Antioxidant effect of vitamin E on motility, viability and lipid peroxidation of cattle spermatozoa under oxidative stress, *Anim Sci Pap Rep*, 27(1), 2009, 5-14.

72. Keskes-Ammar L, Feki-Chakroun N, Rebai T, Sahnoun Z, Ghozzi H, Hammami S, Bahloul A. Sperm oxidative stress and the effect of an oral vitamin E and selenium supplement on semen quality in infertile men, Arch Androl, 49(2), 2003, 83-94.
73. Krishnamoorthy G, Venkataraman P, Arunkumar A, Vignesh R C, Aruldhas M M, Arunakaran J. Ameliorative effect of vitamins (α-tocopherol and ascorbic acid) on PCB (Aroclor 1254) induced oxidative stress in rat epididymal sperm, *Reprod Toxico*, 23(2), 2007, 239-45.
74. Lewin A, Lavon H. The effect of coenzyme Q10 on sperm motility and function, *Mol Aspects Med*, 18 (Suppl), 1997, S213-19.
75. Balercia G, Buldreghini E, Vignini A, Tiano L, Paggi F, Amoroso S, Littarru G. Coenzyme Q 10 treatment in infertile men with idiopathic asthenozoospermia: a placebo-controlled, double-blind randomized trial, *Fertil Steril*, 91(5), 2009, 1785-92.

Please cite this article in press as: Gupta R S *et al.* Impact of oxidative stress and antioxidant therapies on male reproductive health, *Asian Journal of Phytomedicine and Clinical Research,* 4(4), 2016, 159-168.