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ORIGINAL ARTICLE

Effect of Oxidative Stress on Animal Reproduction

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Abstract

In India domestic animals are always in a state of hyperthermia due to atmospheric condition, their own morphology and poor managemental approach by farmers. Hyperthermia keeps animal in a state of negative energy balance and oxidative stress (OS). Different oxygen and nitrogen compounds called reactive species produced during intermediate steps of metabolism, act as powerful oxidants. These oxidants cause OS that leads to number of reproductive problems and apoptosis of early embryos proving animal as infertile. OS cause alteration in cellular components (such as lipids, proteins and nucleic acids) of oocytes and embryos that leads to apoptosis. OS also regulates expression pattern of developmental genes in oocytes and embryos. Antioxidants (enzymatic and non enzymatic) can improve reproductive problems by reducing OS.

Key words: Oxidative stress, Animal reproduction, Embryo implantation, Antioxidant and fertility.

1. Introduction

Several factors affect reproductive performance of animals, most important is the atmospheric condition. All domestic animals are always in a state of hyperthermia due to atmospheric condition, their own morphology and poor managemental approach by farmers. Hyperthermia keeps animal in a state of negative energy balance and oxidative stress (OS) that cause number of reproductive problems and apoptosis of early embryos leads to infertility. The presence of oxidant and antioxidant systems in various reproductive tissues has brought a great interest to know the effect of OS on reproduction. Heat stress (hyperthermia) cause increase in intracellular reactive oxygen species (ROS) and decrease in glutathione (GSH) level leads to OS (Sakatani *et al.,* 2004). OS results by imbalance between the unstable metabolites of oxygen named as ROS and their normal scavengers antioxidants. OS cause several pregnancy related disorders, defective embryo development leading to pregnancy loss and also affect almost all organs of body (Aruoma *et al.,* 2006). OS is also attributed to cell membrane damage, DNA damage, modulate gene expression and finally leads to apoptosis. Therefore it is necessary to ameliorate the state of OS to reduce reproductive problems. It has already been reported long before that apoptosis is strongly correlated with OS due to high levels of ROS in embryos. Early embryonic development are adversely affected by ROS like Super oxide anion (O_2^-), Hydrogen peroxide (H_2O_2) and Hydroxyl ion (OH^-) which are originated from embryo metabolism and the surrounded environment (Agarwal *et al.,* 2012).

2. What is Oxidative Stress

Different oxygen and nitrogen compounds act as reactive molecules causing OS to cells. ROS are oxygen derived molecules produced during intermediate steps of oxygen metabolism which act as powerful oxidants. They are superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-) etc. There are some nitrogen compounds like nitric oxide (NO) called reactive nitrogen species (RNS) which acts as radicals and contribute to OS. Hydroxyl radical is considered to be most toxic of all ROS since it is very reactive with all kinds of biological macromolecules. From RNS, nitric oxide (NO) is an abundant reactive radical that acts as an important oxidative element in a large variety of diverse physiological processes. Aerobic metabolism generates ROS which are highly reactive due to unpaired valence shell electrons and capable of initiating an uncontrolled cascade of chain reactions. ROS have the ability to react with any molecule and modify it, resulting in structural and functional alterations (Sharma and Agarwal, 1996). Controlled production of such compounds play role in physiological reproductive processes such as hormone signaling, oocyte maturation, folliculogenesis, tubal function, ovarian steroidogenesis, and germ cell functions etc. Under normal physiological conditions,

ROS and antioxidants maintain a stable ratio. Shift towards ROS give rise to OS. In other way it can be explained as when elevation of ROS exceeds the body's antioxidant defence mechanism, OS results. The ROS can be radicals or non-radicals and the antioxidants are enzymes or other substances like catalase, superoxide dismutase, glutathione peroxidase, ascorbate, tocopherol etc (Sharma *et al.,* 2004). Normally, OS is regulated by antioxidants, which have the ability to scavenge and neutralize free radicals. However, higher level of ROS when crossed antioxidant capacity, leads to OS. The high energy electrons of ROS are capable of modulating gene expression and transcription factors, with the ability to modify and damage DNA (Sikka, 2004).

3. Oxidants and Cellular Targets

Oxidants are compounds which are capable of oxidizing target molecules. These can act by removal of a hydrogen atom, removal an electron or the addition of oxygen. Oxidants can be divided into various groups, depending on their chemical nature or reactivity. Oxidants are mainly radicals and non-radicals. Radicals are compound contain an unpaired electron in their outer orbital, that renders the molecule unstable. Whereas non-radical compounds are highly reactive without being radicals. Radical group includes hydroxyl (OH^-), nitric oxide (NO^-) and superoxide (O_2^-) and non-radical oxidants include peroxynitrite ($ONOO^-$), hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl) (Lykkesfeldt and Svendsen, 2007). All living cells under aerobic conditions are continuously exposed to large numbers of oxidants derived from various endogenous and exogenous sources (Halliwell and Gutteridge, 1999). Endogenous oxidants are signalling molecules those are involved in the control of major cascades, such as apoptosis and inflammation. Increased oxidant production resulted in OS that cause several pregnancy related disorders, defective embryo development leading to pregnancy loss and also affect almost all organs of body (Aruoma *et al.,* 2006). OS is also attributed to cell membrane damage, DNA damage, modulate gene expression and finally leads to apoptosis. Therefore it is necessary to ameliorate the state of OS to reduce reproductive problems.

4. Effect of Oxidative Stress on Reproduction

Successful implantation of embryo in uterus requires good quality and endometrium receptive embryo. Interference with any of these two prerequisites leads to implantation failure. OS can jeopardize embryonic health and endometrial receptivity. The effect of OS on reproduction is amplified because of lack of physiological defence mechanisms due to number of ROS that play role. OS is etiology of infertility. Intracellular homeostasis is maintained through a balance between pro-oxidants compounds and antioxidants. Antioxidants have the ability to oppose the effects of pro-oxidants by hindering ROS production, scavenging ROS and repairing cell damage caused by ROS. Antioxidants are of two types as non-enzymatic and enzymatic. Non-enzymatic antioxidants consist of vitamin C, taurine, hypotaurine, cysteamine and glutathione, whereas enzymatic antioxidants include superoxide dismutase, catalase and glutathione peroxidise. Within the body, enzymatic antioxidants protect the gametes and embryos during fertilization and pregnancy from OS that induce pathological changes. In vitro maturation, fertilization and culture (IVMFC) procedures lack these natural antioxidants and therefore expose gametes and embryos to a level of OS higher than that experienced in *in vivo*. Free radicals are thought to act as a determinant in reproductive outcome due to their effects on oocytes, sperm and embryos in their follicular fluid, tubal fluid and peritoneal fluid microenvironments. Sperm damage induced by OS includes membrane and DNA damage leading to necrozoospermia, asthenozoospermia and DNA fragmentation (Sharma *et al.,* 2004). OS markers in follicular fluid such as lipid peroxidation, total antioxidant capacity and superoxide dismutase activity are strongly correlated with oocyte fertilization and pregnancy rates where as 8-hydroxy-2'deoxyguanosine an OS marker in granulosa cells displays a negative correlation with embryo quality following IVF (Agarwal *et al.,* 2012). Report tells some level of oxidants are needed for physiological regulation of processes such as capacitation and acrosome reaction but if these levels increase above a critical threshold, there is structural and functional damage, which lead to motility loss, premature acrosomal reaction, lipid peroxidation, DNA damage and apoptosis (Hsu *et al.,* 1999).

5. Oxidative Stress and Oocyte and Embryo Development

Like other living aerobic cells, the embryo and oocyte are major sources of ROS because they use oxygen to produce energy through mitochondrial oxidative phosphorylation. They produce ROS by several pathways, namely oxidative phosphorylation, NADPH oxidase and xanthine oxidase systems (Guerin *et al.,* 2001). *In vitro* setup can never mimic the exact physiological conditions of *in vivo* system. Multiple factors putting effect on IVMFC set up to increase OS that leads to suboptimal outcome. Oocyte defense mechanism against ROS varies according to its own

developmental stage. The integrity of the antioxidant defenses within the different stages of oocyte development may contribute significantly to the overall quality of the oocytes. ROS is produced either intra cellularly from gametes or extra cellularly from environment i.e. micromilieu. Oocytes and embryos contribute in ROS levels because of their metabolism. The external environment that surrounds the IVMFC procedure also plays an important role in the development of OS. The reduction of *in vitro* embryo development in higher oxygen concentration is due to OS with the generation of ROS. Therefore most important external factor that affects viability of oocyte and embryo is oxygen concentration. Generated ROS cause DNA damage which is significantly increase in *in vitro* cultured embryos as compare to *in vivo* derived embryos (Goto *et al.,* 1993*).* Studies have demonstrated that there is more delay or arrest of *in vitro* embryo development as compared to *in vivo* development. Main important factor contribute to this difference is oxygen concentration. Oxygen concentration in uterine environment is 2-8% as compare to high atmospheric concentration of 20%. Detrimental effect of oxygen concentration on embryo development has already been studied in number of species (Takahasi, 2012). ROS such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and the highly toxic hydroxyl free radical (OH^-) are produced continuously in mitochondria because of the leakage of high energy electrons along the electron transport chain. Though mitochondrial proteins and lipids are damaged but mitochondrial DNA (mtDNA) is a major target for oxidative attack because of its location near the inner mitochondrial membrane sites where oxidants are formed, as well as its lack both protective histones and DNA repair activity. It is already reflected that increased ROS levels affect cell membranes, DNA, and mitochondria. Effects of ROS on sperm cause abnormal DNA that leads to produce poor quality embryo. Oocyte maturation and embryo development are also affected by increased ROS or decreased antioxidant defenses. ROS causing OS hamper the activity of the enzymes of energy generation within the embryo. Increased levels of ROS can inactivate glyceraldehyde-3 phosphate dehydrogenase and thus reduce adenosine triphosphate (ATP) generation (Halliwell, 1989). The developmental competence of the oocyte is believed to be of utmost complexity. Evidence exists that OS may be one of the factors that control follicular atresia, initial recruitment of the primordial follicular cohort, subsequent growth of follicles, and selection and dominance of the follicle for ovulation.

6. Oxidative Stress and Gene Expression in Oocyte and Embryo

Synthesis of messenger RNA (mRNA) from template DNA is called gene transcription and occurs in the cell nucleus. After modification, mRNA is transported to the cytoplasm, where it is translated into a protein. Gene transcription is controlled by various transcription factors. Translocation of a transcription factor to the cell nucleus and subsequent binding to a target genes results in protein synthesis (Macdonald *et al.,* 2003). Number of transcription factors altered gene activation, one is nuclear factor κB (Nfkb) which is crucial for normal function of immune system and it can be activated in cells by a hydrogen peroxide (Barnes, 1997). Gene expression during oocyte maturation, fertilization and early embryo development until zygotic gene activation is regulated mainly by translational activation of maternally derived mRNAs. This process requires the presence of a Poly A binding protein. The transcriptional silencing that begins with oocyte maturation persists during the initial mitotic division of the embryonic cells. Activation of zygotic transcription also called zygotic gene activation (ZGA). In mouse and human ZGA occur at the two cell and four to eight cell stages respectively. The major intracellular regulator of redox homeostasis is GSH. NFκB has been implicated in the upregulation of the expression of the rate limiting enzyme for GSH synthesis. OS exerts toxic effects by altering cellular molecules such as lipids, proteins and nucleic acids. This can lead to an increase in membrane permeability, loss of membrane integrity, enzyme inactivation, structural damage to DNA, mitochondrial alterations, adenosine triphosphate depletion and apoptosis (Aruoma *et al.,* 2006). Environmental condition highly affects expression pattern of genes in oocytes and embryos. There is difference in gene expression pattern in oocyte and embryos developed *in vivo* and *in vitro* (Balasubramaniam *et al.,* 2007). Oocyte and embryo themselves are major source of ROS, as oxygen metabolism fulfils their inherent energy needs. *In vitro* system has more oxygen tension than *in vivo* system. So *in vitro* culture with high oxygen tension (20%) as in atmosphere influence generation of OS and affect gene expression as compared to *in vitro* culture with low oxygen tension (5%) (Harvey, 2007). From our previous observations it was found that Connexin 43 (Cx43) is expressed from immature oocytes to morula stage produced *in-vitro* but not expressed in blastocysts stage and Poly A polymerase (PAP) is expressed in all stages of developing embryos starting from immature oocytes to blastocysts stage (Mishra *et al.,* 2010) but other study explains that there is expression of Cx43 in blastocyst stage in *in-vivo* derived embryos (Wrenzycki *et al.,* 1996). Therefore it has been concluded that culture condition hinder the expression of Cx43 in blastocyst and it may be due to OS. The relative mRNA

expression of OS related genes like mitochondrial Mn-superoxide dismutase (MnSOD), cytosolic Cu/Zn superoxide dismutase (Cu/ZnSOD), gamma-glutamyl-cysteine transferase, glutathione peroxidase and sarcosine oxidase was evaluated in oocytes at different stages of development. It was found that irrespective of the oocyte stage, transcripts of all five enzymes were found. Interestingly, the cytoplasmic Cu/ZnSOD transcripts were expressed in significantly higher levels in *in-vitro* matured oocytes. Whereas mitochondrial MnSOD was expressed in higher levels in oocytes derived from smaller follicles, suggesting that mitochondrial defenses vary according to the stage of oocyte development (Lonergan *et al.,* 2003). The activities of the enzymes (SOD, glutathione peroxidase and catalase) were significantly lower in denuded oocytes compared with cumulus-intact oocytes. Therefore, these antioxidant enzymes could scavenge ROS during IVM. The expression of many genes can be up regulated or down regulated by ROS. The antioxidant enzymes like SOD, catalase and GPX are stimulated by OS (Guerin *et al.,* 2001). Expression of Cu, Zn-SOD at all stages of maturation suggested that this enzyme plays a crucial role in protecting embryos against OS. Expression pattern of different antioxidant genes in different species reflect the variations in the ability of embryos developed *in-vivo* or *in-vitro*. Unfavourable embryo culture results alteration in antioxidant enzyme gene expression.

7. Approach to Reduce Oxidative Stress

To optimise *in-vitro* embryo production, OS must be controlled during culture. Oocytes and embryos must be protected by reducing oxygen concentration in environment, addition of free radical scavengers to culture media, use of antioxidants (enzymetic and non-enzymetic) can improve embryo production by reducing OS. Enzymatic antioxidants like superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase etc and non-enzymatic antioxidants include vitamins (vitamin C and E), minerals, chelating agents, glutathione, cysteamine, taurine, pyruvate etc protect cells from oxidative damage and improve embryo production (Guerin *et al.,* 2001). The most effective antioxidant in OS is dependent on the specific molecules causing the stress. The low molecular weight thiol, glutathione, and sulfhydryls are primary participants in cellular antioxidant systems. Glutathione is abundant in cytoplasm, nuclei and mitochondria and is major soluble antioxidant in these cell compartments. *In-vitro* studies showed that ascorbic acid inhibited replication of bacteria and prevent hydrogen peroxide injury to cultured microvascular endothelial cells (Armour *et al.,* 2001). Low vitamin E concentration is associated with evidence of OS. Vitamin E derivatives have been shown to inhibit NFκB activation *in-vitro*. Vitamin E has been shown to be protective for OS in number of animal studies (Goode, 1993). Synthetic antioxidant agents like dimethyl sulphoxide (DMSO) is a potent antioxidant agent that regulates transcription factor activation and inhibit hepatic NFκB activation and intracellular adhesion molecule 1 (ICAM-1) gene expression (Chang *et al.,* 1999). Pyrrolidine dithiocarbamate (PDTC) is a water-soluble, low molecular weight substance which has antioxidant properties. It has been shown to inhibit NFκB activation. There are number factors which can be modified to control OS and discussed briefly below:

7.1 Temperature

As discussed in introduction higher temperature (hyperthermia) increases production of ROS. Therefore optimum temperature should be maintained to reduce OS for better embryo development and gene expression.

7.2 Use of Anti-oxidants

Culture media generate ROS depending on their composition. Metallic ions such as Fe_2^- and Cu_2^- present in culture media and media additives accelerate ROS generation. Serum albumin and serum which are commonly added to culture media cause an increase in H_2O_2 production. Several antioxidants, such as ascorbic acid, urate, isoflavones, taurine, hypotaurine, genistein, α tocopherol and free radical scavengers (L-Carnitine, ergothioneine etc.) can be used as culture media supplements to reduce the risk of OS and subsequent DNA damage (Sikka, 2004). Addition of thiol compound, ethylene diamine tetraacetic acid as chelating agent can also overcome the developmental arrest (Guerin *et al.,* 2001).

7.3 Oxygen Concentration

Studies have shown that embryos cultured in an environment with atmospheric oxygen concentration and exposed to increased levels of hydrogen peroxide radicals resulted significantly more DNA fragmentation compared with embryos grown in an environment of low oxygen tension. Reports of successful blastocyst development with embryos cultured in a low oxygen tension environment imply that the oxygen concentration in *in-vitro* plays an important role in IVMFC procedures (Harvey, 2007). Higher oxygen tension above 5% had detrimental effects on development of fertilized oocytes because high oxygen tension activates various oxidase enzyme systems in the cells and helps to ROS generation within them. Therefore a low O_2 concentration during *in-vitro* culture of embryos decreases the H_2O_2 content and

therefore reduces DNA fragmentation and improves developmental competence

7.4 Light Source

Visible light acts as an exogenous source of OS which promotes ROS production, causing cellular damage through the oxidation of DNA bases and DNA strand breaks. Smaller amounts of short wavelength visible light are advantageous as they expose oocytes and embryos to lower levels of OS than cool white light (Takenaka *et al.,* 2007). These results suggest that minimizing the exposure of oocytes, zygotes and embryos to visible light and near UV light will serve to better mimic *in-vivo* conditions, thereby yielding more successful IVMFC outcomes. Transient exposure to visible light is sufficient to cause an increase in H_2O_2 in embryos (Goto *et al.,* 1993).

8. Antioxidants and Fertility

Due to presence of so many internal and external sources of ROS that contribute to OS, it is essential to explore ways to neutralize the potentially negative effects of OS on successful outcomes. Antioxidants are defined as any substance that, when present at low concentrations compared to that of an oxidizable substrate, significantly delays or inhibits oxidation of that substrate (Halliwell and Gutteridge, 1989). Antioxidants have the ability to oppose the effects of pro-oxidants by hindering ROS production, scavenging ROS and repairing cell damage caused by ROS by donating electrons to oxidants, thus making them harmless to cellular macromolecules. The antioxidants thereby become radicals themselves, but these are far more stable and are not capable of inducing cellular damage. Scavenging of the ROS by various antioxidants has been proposed to lead to a better environment for the pre-implanted embryos. Water-soluble antioxidants may function as a first line of antioxidants to scavenge excess of reactive oxygen species in plasma, whereas lipid soluble antioxidants such as tocopherol and carotene scavenge reactive oxygen species affecting the membrane lipids (Mikhail *et al.,* 1994). Antioxidants are of two types as non-enzymatic and enzymatic. Non-enzymatic antioxidants consist of vitamin C, taurine, hypotaurine, cysteamine and glutathione, whereas enzymatic antioxidants include superoxide dismutase, catalase, and glutathione peroxidase (Sharma *et al.,* 2004).

8.1 Non-Enzymatic Antioxidants

The non-enzymatic antioxidants consist of dietary supplements and synthetic antioxidants such as vitamin C, GSH, taurine, hypotaurine, vitamin E, Zn, selenium (Se), betacarotene, carotene and carnitine etc.

8.1.1 Vitamin E

In vitro studies show that vitamin E is a major antioxidant in the sperm membrane and it appears to have a dose-dependent protective effect. Cryopreservation and thawing procedures are associated with a significant reduction in sperm motility induced by OS, and these effects can be avoided by adding vitamin E to cryoprotectants. Vitamin E and selenium supplementation lead to a significant decrease in MDA concentrations and improved sperm motility (Keskes-Ammar *et al.,* 2003). Significant amount of vitamin E is present in ovary and follicular fluid and it inhibits NADPH oxidase mediated generation of superoxide anion. Vitamin E protects membrane against OS (Pascoe *et al.,* 1987).

8.1.2 Vitamin C

Vitamin C is a known redox catalyst that can reduce and neutralize ROS. Vitamin C is another important antioxidant contributing up to 65% of the antioxidant capacity of the seminal plasma. Dietary supplementation of vitamin C protects sperm from endogenous oxidative DNA damage, thereby decreasing the risk of genetic defects, particularly in populations with low vitamin C levels (Abel *et al.,* 1982). Vitamin C and vitamin E, has been shown to have beneficial effects in preventing luteal phase deficiency and resultant increased pregnancy rate.

8.1.3 Glutathione

Glutathione is the most abundant non-thiol protein in mammalian cells. Glutathione deficiency can lead to instability of the mid-piece, resulting in defective motility. It protects plasma membrane from lipid peroxidation, scavenges superoxide and prevents O_2 formation that led to significant improvement in sperm quality (Irvine, 1996). GSH appears to be the main non-enzymatic defence system against ROS in embryos. GSH plays a role in reducing the environment in oocytes and embryos, and is also the substrate of glutathione peroxidase (GPX), the main antioxidant enzyme. The concentration of GSH in embryos is highly correlated to early development (Takahashi *et al.,* 1993). DNA damage has been observed in the embryos when GSH synthesis is inhibited.

8.1.4 L-cysteine

L-cysteine is a precursor of glutathione that improves sperm motility and reduces ROS-induced DNA damage (Oeda *et al.,* 1997). Cysteine and cysteamine (CSH) increase the GSH content of the oocyte. Cysteamine acts as a scavenger and is an antioxidant essential for the maintenance of high GSH levels. Furthermore, CSH can be converted to hypotaurine, another antioxidant. Hypotaurine

neutralize hydroxyl radicals and acts as important antioxidant for gamets and embryos. CSH enhance the development of oocytes that has been matured and fertilized *in-vitro*.

8.1.5 Carotenoids

Carotenoids play an important role in protecting the cells and organisms by scavenging the superoxide radicals (Sies and Stahl, 1995).

8.1.6 Carnitine

Carnitine promotes membrane stability and plays an important role in sperm maturation and development. Carnitine improves embryo survivability by protecting them from apoptosis (Agarwal *et al.,* 2004).

8.2 Enzymatic Antioxidants

Enzymatic antioxidants neutralize excess ROS and prevent damage to cell structures. Endogenous antioxidants enzymes include superoxide dismutase **(**SOD), catalase, glutathione peroxidase (GPx) and glutathione oxidase.

8.2.1 Superoxide Dismutase (SOD)

The enzyme SOD exists as three isoenzymes SOD1, SOD2 and SOD3. SOD1 contains Cu and zinc (Zn) as metal co-factors and is located in the cytosol. SOD2 is a mitochondrial isoform containing manganese (Mn), and SOD3 encodes the extracellular form. SOD scavenges both extracellular and intracellular superoxide anion and prevents lipid peroxidation of the plasma membrane. In order to act against H_2O_2, it must be conjugated with catalase or glutathione peroxidase. SOD also prevents premature hyperactivation and capacitation induced by superoxide radicals before ejaculation. Transcripts coding of SODs are present at all stages of maturation and developmental stages of embryos suggesting this enzyme plays a crucial role protecting embryos against OS.

8.2.2 Glutathione Peroxidase (GPX) and Glutathione Reductase (GRD)

This enzymes acts an excellent protection against lipid peroxidation of plasma membrane of spermatozoa. It scavenges lipid peroxides thereby arresting the progressive chain reaction of lipid peroxidation. It also scavenges H_2O_2, which is responsible for the initiation of lipid peroxidation. Glutathione reductase (GRD) stimulates the reduction of glutathione disulfide (GSSG) to reduced glutathione (GSH). This ensures a steady supply of the reductive substrate NADPH to GPX. GSH/glutathione peroxidase is the main reducing agents in the body and act as scavenging antioxidants in the epididymis and testes. Their modification of the spermatozoa membrane confers protection on the lipid constituents, thus preserving sperm viability and motility. Deficiency of GPX leads to abnormal embryo development and finally resulted in apoptotic cell death and produce lethal phenotype.

9. Conclusion

Oxidative stress results by imbalance between the unstable metabolites of oxygen named as ROS and their normal scavengers antioxidants. OS cause several pregnancy related disorders, defective embryo development leading to pregnancy loss and also affect almost all organs of body. OS is also attributed to cell membrane damage, DNA damage, modulate gene expression and finally leads to apoptosis. Therefore it is necessary to ameliorate the state of OS to reduce reproductive problems.

References

Abel BJ, Carswell G and Elton R (1982). Randomised trial of clomiphene citrate treatment and vitamin C for male infertility. *British Journal of Urology*, 54(6): 780-784.

Agarwal A, Aponte-Mellado A, Premkumar BJ, Shaman A and Gupta S (2012). The effects of oxidative stress on female reproduction: a review. *Reproductive Biology and Endocrinology*, 10: 49-80.

Agarwal A, Nallella KP, Allamaneni SS and Said TM (2004). Role of antioxidants in treatment of male infertility: an overview of the literature. *Reproductive Biomedicine Online*, 8(6): 616-627.

Armour J, Tyml K, Lidington D and Wilson JX (2001). Ascorbate prevents microvascular dysfunction in the skeletal muscle of the septic rat. *Journal of Applied Physiology*, 90: 795-803.

Aruoma OI, Sunb B, Fujiib H, Neergheenc VS, Bahorunc T, Kangd KS and Sung MK (2006). Low molecular proanthocyanidin dietary biofactor Oligonol: Its modulation of oxidative stress, bioefficacy, neuroprotection, food application and chemoprevention potentials. *Bio Factors,* 27: 245-265.

Balasubramaniam S, Son WJ, Kumar BM, Ock SA, Yoo JG, Im GS, Choe SY and Rho GJ (2007). Expression pattern of oxygen and stress responsive gene transcript at various developmental stages of *in vitro* and *in vivo* preimplantation bovine embryos. *Theriogenology,* 68: 265-275.

Barnes PJ (1997). Nuclear factor-κB. *The International Journal of Biochemistry and Cell Biology,* 29: 867-870.

Chang CK, Llanes S and Schumer W (1999). Inhibitory effect of dimethyl sulfoxide on nuclear factor-κB activation and intercellular adhesion molecule 1 gene expression in septic rats. *Journal of Surgical Research,* 82: 294-299.

Goode HF and Webster NR (1993). Free radicals and antioxidants in sepsis. *Critical Care Medicine*, 21: 1770-1776.

Goto Y, Noda Y, Mori T and Nakan M (1993). Increased generation of reactive species in embryo cultured *in-vitro. Free Radical Biology and Medicine,* 15: 69-75.

Guerin P, Mouatassim SEl and Menezo Y (2001). Oxidative stress and protection against reactive oxygen species in the pre-implantation embryo and its surroundings. *Human Reproduction Update*, 7: 175-189.

Halliwell B (1989). Free radicals, reactive oxygen species and human disease: a critical evaluation with special reference to atherosclerosis. *British Journal of Experimental Pathology*, 70: 737-757.

Halliwell B and Gutteridge JMC (1999). Free Radicals in Biology and Medicine (3rd Ed). *Oxford University Press, New York.*

Halliwell B and Gutteridge JMC (1989). The chemistry of oxygen radicals and other derived species. In: Halliwell B and Gutteridge JMC (Eds), Free Radicals in Biology and Medicine (2nd Edn). *Clarendon Press, Oxford*. Pages 22-85.

Harvey AJ (2007). The role of oxygen in ruminant preimplantation embryo development and metabolism. *Animal Reproduction Science*, 98: 113-128.

Hsu PC, Hsu CC and Guo YL (1999). Hydrogen peroxide induces premature acrosome reaction in rat sperm and reduces their penetration of the zona pellucid. *Toxicology*, 139: 93-101.

Irvine DS (1996). Glutathione as a treatment for male infertility. *Reviews of Reproduction*, 1(1): 6-12.

Keskes-Ammar L, Feki-Chakroun N, Rebai T, Sahnoun Z, Ghozzi H, Hammami S, et al. (2003). Sperm oxidative stress and the effect of an oral vitamin E and selenium supplement on semen quality in infertile men. *Archives of Andrology*, 49(2): 83-94.

Lonergan P, Gutierrez-Adan A, Rizos D, Pintado B, de la Fuente J and Boland MP (2003). Relative messenger RNA abundance in bovine oocytes collected *in vitro* or *in vivo* before and 20 hours after the preovulatory luteinizing hormone surge. *Molecular Reproduction and Development,* 66: 297-305.

Lykkesfeldt J and Svendsen O (2007). Oxidants and antioxidants in disease: Oxidative stress in farm animals. *The Veterinary Journal,* 173: 502-511.

Macdonald J, Galley HF and Webster NR (2003). Oxidative stress and gene expression in sepsis. *British Journal of Anaesthesia,* 90 (2): 221-232.

Mikhail MS, Anyaegbunam A, Garfinkel D, Palan PR, Basu J and Romney SL (1994). Preeclampsia and antioxidant nutrients: decreased plasma levels of reduced ascorbic acid, alpha-tocopherol, and beta-carotene in women with preeclampsia. *American Journal of Obstetrics and Gynecology*, 171(1): 150-157.

Mishra A, Sharma GT and Sai Kumar G (2010). Expression profile of Connexin 43(Cx43) and Poly A Polymerase (PAP) genes in buffalo *(Bubalus bubalis)* embryos produced *in vitro. Journal of Applied Animal Research*, 38: 29-32.

Oeda T, Henkel R, Ohmori H and Schill WB (1997). Scavenging effect of N-acetyl-L-cysteine against reactive oxygen species in human semen: a possible therapeutic modality for male factor infertility? *Andrologia,* 29(3): 125-131.

Pascoe GA, Fariss MW, Olafsdottir K and Reed DJ (1987). A role of vitamin E in protection against cell injury: maintenance of intracellular glutathione precursors and biosynthesis. *European Journal of Biochemistry*, 166: 241-247.

Sakatani M, Kobayashi S and Takahashi M (2004). Effects of heat shock on in vitro development and intracellular oxidative state of bovine preimplantation embryos. *Molecular Reproduction and Development*, 67: 77-82.

Sharma RK and Agarwal A (1996). Role of reactive oxygen species in male infertility. *Urology,* 48: 835-850.

Sharma RK, Said T and Agarwal A (2004). Sperm DNA damage and its clinical relevance in assessing reproductive outcome. *Asian Journal of Andrology,* 6(2): 139-148.

Sies H and Stahl W (1995). Vitamins E and C, beta-carotene, and other carotenoids as antioxidants. *The American Journal of Clinical Nutrition*, 62(6): 1315S-1321S.

Sikka SC (2004). Role of oxidative stress and antioxidants in andrology and assisted reproductive technology. *Journal of Andrology*, 25: 5-18.

Takahashi M (2012). Oxidative stress and redox regulation on *in vitro* development of mammalian embryos. *Journal of Reproduction and Development*, 58(1): 1-9.

Takenaka M, Horiuchi T and Yanagimachi R (2007). Effects of light on development of mammalian zygotes. *Proceedings of the National Academy of Sciences, USA,* 104(36): 14289-14293.

Wrenzycki C, Herrmann D, Carnwath JW and Niemann H (1996). Expression of the gap junction gene connexin43 (Cx43) in preimplantation bovine embryos derived *in vitro* and *in vivo. Journal of Reproduction and Fertility*, 108: 17-24.