Chapter

Antioxidant and Infertility

Huda Mahmood Shakir

Abstract

Unexplained sub-fertility is commonly identified if couples fail to conceive after 1 yr. of everyday unprotected sexual intercourse even though investigations for ovulation, tubal patency and semen evaluation are ordinary. Many previous studies had shown that oxidative stress plays an important role in human fertility. Free radicals are neutralized by an elaborate antioxidant defense system. In a healthy body, pro-oxidants and antioxidants maintain a ratio and a shift in this ratio towards pro-oxidants gives rise to oxidative stress. There are two types of antioxidants in the human body: enzymatic and non-enzymatic antioxidants. Under normal conditions, antioxidants convert ROS to H_2O to prevent overproduction of ROS. All cells in the human body are capable of synthesizing glutathione specially the liver. Free radicals appear to have a physiological role in female reproductive system in many different processes such as: oocyte maturation, fertilization, luteal regression, endometrial shedding and progesterone production by the corpus luteum. Protection from ROS is afforded by scavengers present in both male and female reproductive tract fluids, as well as in seminal plasma elevated concentrations of ROS in these environments may have detrimental effects on the spermatozoa, oocytes, sperm oocyte interaction and embryos both in the Fallopian tube and the peritoneal cavity; therefore oxidative stress modulates a host of reproductive pathologies affecting natural fertility in a woman's life.

Keywords: ROS, reactive oxygen species, OS, oxidative stress, NO, nitric oxide, NO_2, nitrogen dioxide, MDA, malondiadehyde

1. Introduction

Reproductive failure is a significant public health concern. Infertility, carries significant personal, societal and financial consequences. One of the most important and underappreciated reproductive health problems in developing countries is the high rate of infertility and childlessness.

Causes of infertility can be found in about 90% of cases, about 10% of patients do not know why they can not conceive this is called unexplained infertility [1].

Unexplained infertility is a diagnosis of exclusion, when the standard investigation of both the female and male partner has ruled out other infertility diagnosis.

A couple is considered to have unexplained infertility if the woman ovulated and had a normal and hysterosalpingogram, and the man a normal semen analysis. Critical factors to be considered in evaluating and managing unexplained infertility are the duration of infertility and female age [2].

In case of unexplained infertility, any form of treatment is speculated.

A period of three years of unexplained infertility is generally accepted as minimum duration before active intervention is considered.

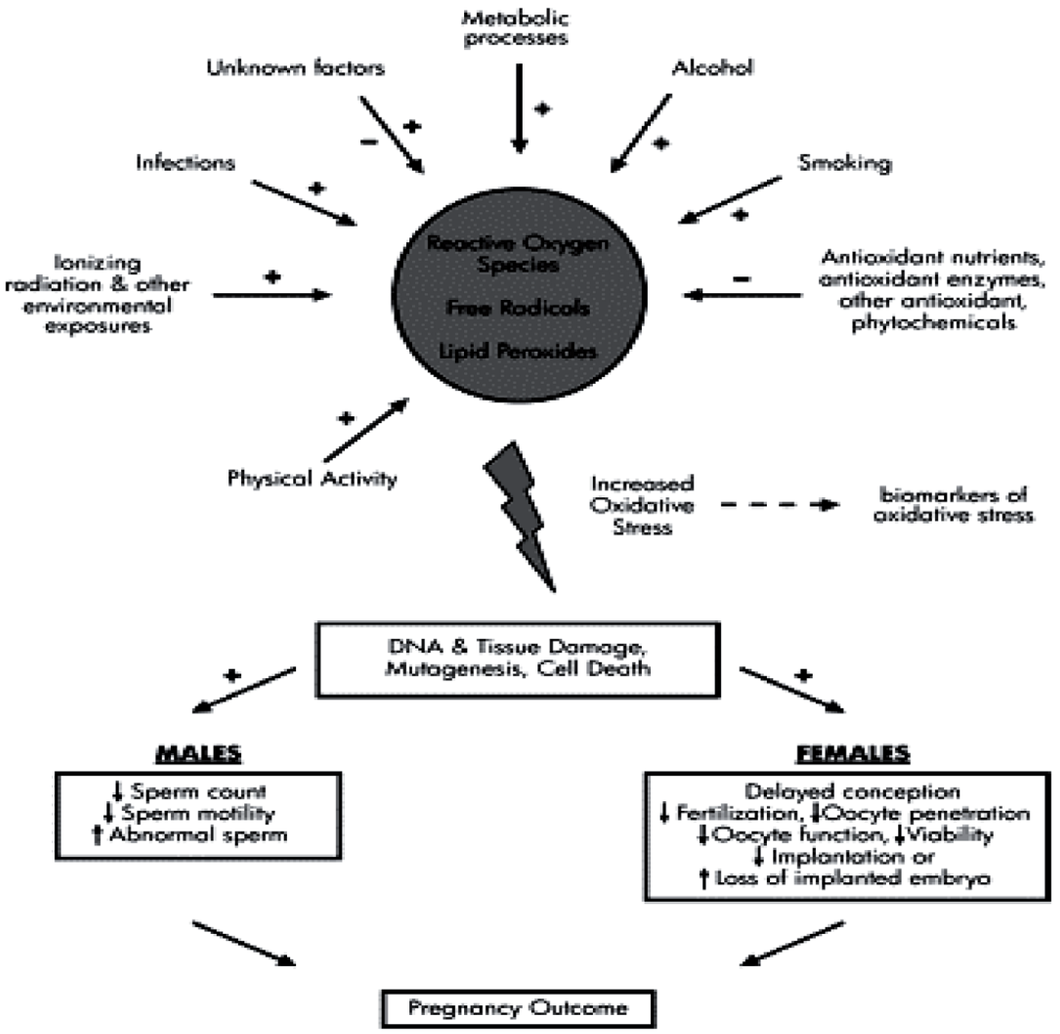

Figure 1.
Role of oxidative stress in fertility.

Empirical treatment with clomiphene,intrauterine insemination are used in treatment of unexplained infertility, if failed, invitro fertilization is considered.

Because female ovary is the source of oocytes and regulating hormones, free radicals in the gynecologic environment is likely to be an important mediator of conception. Recently there is a growing evidence of possible role of highly reactive products of oxygen, termed free radicals, in infertility [3].

A free radical is any atom (e.g. oxygen, nitrogen) with at least one unpaired electron in the outermost [4]. Free radicals are neutralized by an elaborate antioxidant defense system. In a healthy body, pro-oxidants and antioxidants maintain a ratio and a shift in this ratio towards pro-oxidants gives rise to oxidative stress [3].

In this case free radical species which are unstable and highly reactive, will become stable by acquiring electrons from nucleic acids, lipids, proteins, carbohydrates or any nearby molecule causing a cascade of chain reactions resulting in cellular damage and diseas [5]. Because free radicals are unstable, and difficult to measure, traditional indices of oxidative stress include downstream markers of oxidative damage to macromolecules such as lipids, proteins and DNA. Oxidative stress is also indirectly assessed by estimating capacity for antioxidant defense in serum, or other body fluids. Such measures include assessment of enzymatic antioxidant activity and individual assessment of circulating non-enzymatic antioxidant levels [6].

Successful initiation of pregnancy requires the ovulation of a mature oocyte, production of competent sperm, proximity of sperm and oocyte in the reproductive

tract, fertilization of the oocyte, transport of the conceptus into the uterus, and implantation of the embryo into a properly prepared, healthy endometrium. A dysfunction in any one of these complex biological steps can cause infertility [7].

Free radicals can affect the female fertility potential in number of ways which can contribute negatively to a number of reproductive processes including folliculogenesis, oocyte maturation, sperm DNA damage, necrozoospermia, asthenospermia, endometriosis [8]. High levels of ROS play an important role in the etiology of male and female infertility. It has been proposed that the imbalance between antioxidants and ROS, favoring the latter, is responsible for increased OS levels that induce infertility [9]. Fissore *et al* [10] have found that OS is associated with maternal aging and postovulatory aging of the ova. The Role of oxidative stress in fertility is shown in **Figure 1**.

The role of Oxidative Stress in female reproductive diseases and infertility is under intense investigations [8]. Whenever there is imbalance in the levels of ROS and antioxidants, damage can occur to oocytes and embryos through various pathological mechanisms [11].

2. Role of free radicals in unexplained infertility

2.1 Free radicals

A free radical is any atom (e.g. oxygen, nitrogen) with at least one unpaired electron in the outermost shell [4].

Free radical atoms are unstable and highly reactive. They become stable by acquiring electrons from nucleic acids, lipids, proteins, carbohydrates or any nearby molecule causing a cascade of chain reactions resulting in cellular damage and disease [5]. The terms free radical and ROS are commonly used in an interchangeable manner, despite the fact that not all ROS are free radicals [12]. For example, hydrogen peroxide (H_2O_2) is considered a ROS but it is not a free radical since it does not contain unpaired electrons. In addition, there is a sub-class of free radicals derived from nitrogen [13].

There must be a balance between oxidants and antioxidants, generation of an excess of free radical results in oxidative stress [14].

2.2 Types of free radical species

There are two major types of free radical species: reactive oxygen species (ROS) and reactive nitrogen species (NOS).

2.3 Reactive oxygen species (ROS)

The Oxygen centered free radicals are class of powerful oxidants in the human body. The most common ROS include: the superoxide anion (O_2^-), the hydroxyl radical ($OH^{\cdot}$), singlet oxygen ($1O_2$), and a number of related species, such as hydrogen peroxide (H_2O_2), that do not themselves contain unpaired electrons but are often involved in the generation of free radicals [15].

2.4 Reactive nitrogen species

The two common examples of reactive nitrogen species are nitric oxide (NO) and nitrogen dioxide (NO_2). Nitric oxide is a highly reactive free radical results in cells and tissues damage [16].

2.5 Sources of free radicals in human body

The main four endogenous normal sources appear to account for most of the oxidants produced by cells; are aerobic respiration in mitochondria, Phagocyte, Peroxisomes and Cytochrome P450 enzymes. Exogenous sources may significantly increase the large endogenous oxidant load which include: Iron and copper salts [17]; smoking and alcohol; normal diets (fried food, caffien) [18]; radiation, sunlight; pollution and xenobiotics (Drugs,pesticides, anesthetics,andindustrial solvents) [19].

2.6 Pathological damage of free radicals

Interaction of free radicals with other compounds results in a chain reactions of oxidation and reduction which ultimately can lead to cellulardamage [6].

Oxidation of DNA molecules, for example, can result in mutation where as oxidation of protein can result in protein cross-linking and loss function [20].

Reactive oxygen species can attack polyunsaturated fatty acids of the cell membrane leading to a chain of chemical reactions called lipid peroxidation and this will lead to decrease structural fluidity of these compounds, thus resulting in loss of integrity of cellular membranes [6]. It is possible to measure the extent of peroxidative damage by estimatingthe stable end products of lipid peroxidation such as MDA (malondiadehyde) [3].

2.7 Types of antioxidants

There are two types of antioxidants in the human body: enzymatic and non-enzymatic antioxidants [11]:

2.7.1 Enzymatic antioxidants

Enzymatic antioxidants are also known as natural antioxidants. They are mainly composed of:

- Superoxide dismutase.
- Catalase.
- Glutathione peroxidase.

Antioxidant enzymes may act in a coordinate manner to defend living tissue from oxidant [21].

Superoxide dismutase is a protein dimer,destroys the free radical superoxide by converting it to peroxide [22].

Catalase is a hemoprotein enzyme of the oxidoreductase class that catalyzes the convertion of hydrogen peroxide to water and oxygen, active H_2O_2 [23].

Glutathione peroxidase, is a tetramer protein containing selenium, and uses glutathione as a co-substrate. Glutathione peroxidase is a cytosolic enzyme and also eliminates H_2O_2; but, in comparison to catalase, has a wider range of substrates including lipid peroxides. Glutathione peroxidase primarily functions to detoxify low levels of H_2O_2 in the cell [24].

2.7.2 Non-enzymatic antioxidants

Non-enzymatic antioxidants are also known as synthetic antioxidants or dietary supplements. The body's complex antioxidant system is influenced by dietary intake

of antioxidant vitamins and minerals such as vitamin C, vitamin E, selenium, zinc, glutathione and beta carotene [11].

2.7.2.1 Vitamin C (Ascorbic acid)

Vitamin C is a water soluble vitamin found in many fruit and vegetable.

Vitamin C is required for optimal functions of number of enzymes;deficiency cause scurvy and poor wound repair. It is also considered a chain breaking antioxidant that stops the propagation of the peroxidative process. Vitamin C also helps recycle oxidized vitamin E and glutathione, It is an unstable,easily oxidized acid and can be destroyed by oxygen, alkaline and high temperature. Humans cannot synthesize vitamin C, so they take it from exogenous supplement or diet found in many fruit and vegetable [25].

Tocopherol and Glutathione (GSH),also rely on vitamin C for regeneration back to their active isoforms. The relationship between vitamin C and Glutathione is unique. Vitamin C reduces GSH back to the active form. Once reduced, Glutathione will regenerate vitamin C from its oxidized state. Vitamin C protects the DNA of the cells from the damage caused by free radicals and mutagens. It prevents harmful genetic alterations within cells [26, 27].

2.7.2.2 Vitamin E

Vitamin E is the collective name for a set of at least eight related tocopherols and tocotrienols compounds with similar biological antioxidants activity. Vitamin E is the first line of defense against lipid peroxidation. Moreover, it plays a very important function in lending red blood cells flexibility as they make their way through the arterial network and helps prolong the life of erythrocytes, immune function, and has positive effects in the fertility [28].

Vitamin C regenerates Vitamin E and Vitamin C is,in turn by regenerated glutathione [29].

2.8 Antioxidant defense system

Organisms have developed efficient protective mechanisms against excessive accumulation of free radicals called antioxidants [30].

Free radicals are neutralized by an elaborate antioxidant defense system. In a healthy body, pro-oxidants and antioxidants maintain a ratio and a shift in this ratio towards pro-oxidants gives rise to oxidative stress. Whenever ROS levels become pathologically elevated, antioxidants begin to work and help minimize the oxidative damage, repair it or prevent it [31]. An antioxidant can be defined as any substance that, when present at low concentration compared to those of an oxidizable substance, significantly delays or prevents the oxidation of that substrate [32].

Under normal conditions, antioxidants convert ROS to H_2O to prevent overproduction of ROS.

The different possible mechanisms by which antioxidants may offer protection against free radical damage include:

- Prevention of formation of free radicals.

- Interception of free radicals by scavenging the reactive metabolites and converting them to less reactive molecules.

- Facilitating the repair of damage caused by free radicals.

- Providing a favorable environment for effective functioning of other antioxidants [33].

2.9 Oxidative stress in female reproduction

- The presence of oxidant and antioxidant systems in various reproductive tissues has evoked great interest in the role of OS in human reproduction. The role of ROS and antioxidants in relation to female reproductive function has, in contrast, received relatively little attention [8].
- Oxidative stress influences the entire reproductive span of women's life and even thereafter (i.e. menopause).

- Free radicals appear to have a physiological role in female reproductive system in many different processes such as: oocyte maturation, fertilization, luteal regression, endometrial shedding and progesterone production by the corpus luteum [34]. Protection from ROS is afforded by scavengers present in both male and female reproductive tract fluids, as well as in seminal plasma [35].

2.9.1 Free radicals, antioxidants, and reproductive processes in women

The production of a viable oocyte is modulated by a complex interaction of endocrine, paracrine and autocrine factors, leading to follicular maturation, granulosa cell maturation, ovulation and luteinization [36]. Elevated concentrations of ROS in these environments may have detrimental effects on the spermatozoa, oocytes, sperm oocyte interaction and embryos both in the Fallopian tube and the peritoneal cavity;therefore oxidative stress modulates a host of reproductive pathologies affecting natural fertility in a woman's life [37]. Free radicals plays a role in the physiology of ovarian function [36]. They may have a regulatory role in oocyte maturation, folliculogenesis, ovarian steroidogenesis and luteolysis. There is a delicate balance between ROS and antioxidant enzymes in the ovarian tissues [11]. Vitamin C deficiency characteristically produces ovarian atrophy and extensive follicular atresia [36]. Glutathione has been identified as critical for oocyte maturation and formation of the male sperm pronucleus (PN) [4]

- Glutathione in mature oocytes is thought to be a highly relevant biochemical marker for the viability of mammalian oocytes [38]. Hence, Follicular ROS initiate apoptosis; whereas follicular Glutathione, in addition to FSH, protect against apoptosis in cultured pre-ovulatory rat follicles [39]. The secreted Glutathione would protect oocytes against excessively produced ROS that occurs during the ovulation, thus maintaining fertilization potency [40].

- [41] observe that integrity of the antioxidant defenses within the different stages of oocyte development may contribute significantly to the overall quality of the oocytes. One consequence of an excess of ROS in the ovary may be plasma membrane damage of the oocytes. The significance of such damage for female fertility, however, is unknown.

- Glutathione peroxidase may also maintain low levels of free radicals inside the follicle and thus play an important role in gametogenesis and fertilization [11]. Glutathione is considered the major source of redox potential in the oocyte [14].

- Oocytes are also rich in glutathione reductase [24].

- Reactive oxygen species are produced during luteal regression [28]. Because the corpus luteum produces much of the progesterone; ROS are produced as a byproduct [28]. The detoxification of the produced ROS by GSH in conjunction with antioxidative enzymes would be particularly important for the corpus luteum and surrounding cells (**Figure 2**) [42].

Reactive oxygen species may act as important mediators in hormone signaling, ovarian steroidogenesis and germ cell function [43].

Oxidative stress may affect theca-interstitial cells by inducing their proliferation and growth. Higher doses of Oxidative stress inhibited the proliferation of the theca-interstitial cells while antioxidants stimulate the release of gonadotrophins from the adenohypophysis [44].

Cells involved in steroidogenesis such as theca cells, granulosa lutein cells, and hilus cells show stronger oxidative enzyme activity [45]. Over-exposure of the ovary to H_2O_2 causes the LH receptor to uncouple from adenyle cyclase, thereby impairing protein synthesis and cholesterol utilization by mitochondria [28].

Data suggest that vitamin C has defined functions in hormone secretion, gamete protection, and gonadal tissue remodeling [26]. Steroidogenesis appears to be ascorbate-dependent [27] and the reduced concentrations of antioxidants often coincide with poor fertilization success rates [46].

Reactive oxygen species therefore play a role in the formation of the corpus luteum and steroidogenesis. Imbalance in redox leading to luteal regression that results in lack of luteal support to pregnancy [37].

Endogenous NO system exists in the fallopian tubes [36]. NO has a relaxing effect on smooth muscles and it has similar effects on tubular contractility. Deficiency of NO may lead to tubal motility dysfunction, resulting in retention of the ovum,

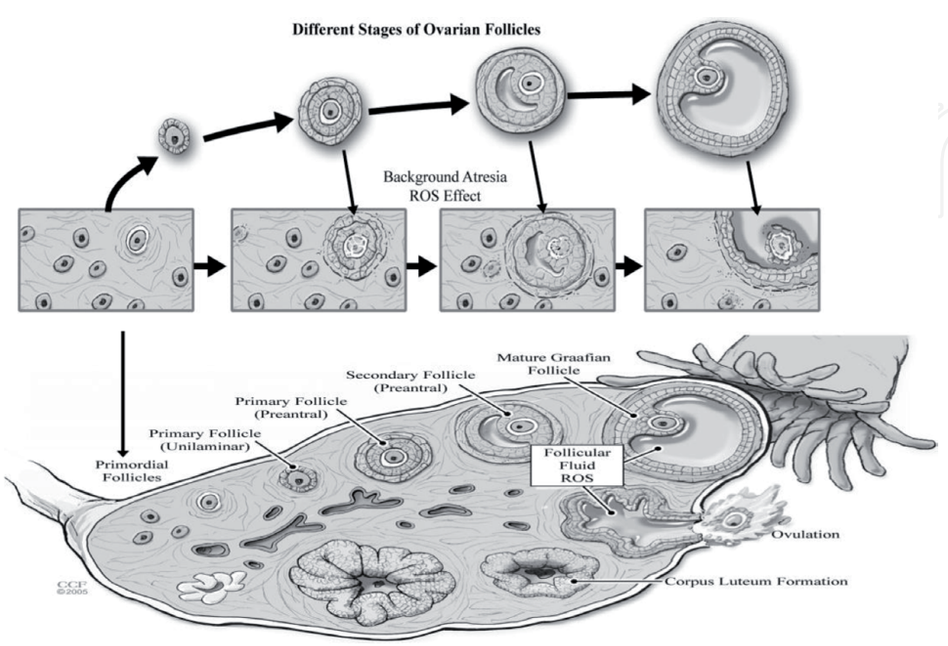

Figure 2.
Reactive oxygen species in folliculogenesis in women ovary [43].

delayed sperm transport and infertility. Increased NO levels in the fallopian tubes are cytotoxic to the invading microbes and also may be toxic to spermatozoa [11].

In male, ROS cause infertility by two principal mechanisms:

- Reactive oxygen species damage the sperm membrane which, in turn, reduces the sperm's motility and ability to fuse with the oocyte.

- Damage sperm DNA, compromising the paternal genomic contribution to the embryo [13].

The percentage of sperm with DNA damage is negatively correlated with the fertilization rate [47]. Oocytes can repair DNA damage to some extent, but when the damage is severe, embryo death and miscarriages can occur.

The inability of sperm to fuse with an oocyte appears to be due to the effects of ROS on the sperm membrane. As a result of lipid peroxidation process, spermatozoa are unable to initiate the necessary biochemical reactions associated with acrosome reaction, zona pellucida binding and oocyte penetration [29].

In addition, ROS brings about changes in the endometrium that prepare it for implantation [36]. Nitric oxide functions as an important vasodilator, neurotransmitter, regulator of implantation [8] and may also contribute as an anti-platelet agent during implantation [48].

3. The effect of some factors on free radicals levels

3.1 Body weight

The effects of body weight and weight change on OS have only recently been investigated. More studies on this topic are needed, since both inadequate and excessive energy intakes have been associated with reduced fertility among women [49].

Research has focused on the effects of energy intake on hormonal patterns and menstrual cycles, ovulatory dysfunction and later age at menarche have been associated with both low and high body mass index (BMI, calculated as kg/m^2), energy intake and high levels of physical activity [50].

3.2 Age

It has been suggested that the age-related decline in fertility is modulated by OS. Moreover, there is an age related decline in the number and quality of follicles in females [51].

Several studies have investigated if there is an age-associated increase in the generation of oxidants by mitochondria [11]. Free radical activity of human follicular fluid increases with age [10] as does apoptosis (programmed cell death) of human granulosa and cumulus cells [9]. Carbone,*et al*. [52] find that a reduction in the expression of glutathione and CAT activity is demonstrated in older women compared with young controls. Yeh et al. [53] show alterations in antioxidant defense with age. It is hypothesized that diminished antioxidant status may induce apoptosis during luteal regression and lead to decreased progesterone synthesis.

3.3 Smoking

Smoking is known to decrease fertility in women [51], likely through an increase in OS. A history of smoking is associated with high levels of oxidative stress [36].

The oxides of nitrogen (NO) in cigarette smoke damage macromolecules and deplete antioxidant. This is likely to contribute significantly to the pathology of smoking [11].

Dietary intakes of smokers; however, are different from non-smokers, confounding this relationship [11]. It is found that intrafollicular exposure to cigarette smoking metabolites was associated with a significant increase in follicular lipid peroxidation and decrease in the local antioxidative potential [3]. Smoking significantly reduced glutathione peroxidase concentration in the follicular fluid [24]. Consequently, OS imbalance may be responsible for impaired folliculogenesis in female smokers [43].

4. Overcoming OS in female infertility

Oxidative stress can be overcome by reducing generation of ROS or increasing the amounts of antioxidants available [11]. So prior to the treatment of female infertility, ROS levels should be assessed. By estimating ROS levels, it may be possible to identify the causes of infertility, especially in cases of idiopathic infertility [43]. It is important to identify the source of increased ROS generation [3]. Patients with history of smoking should be advised to stop smoking. In addition, Any exposure to drugs, toxic substances and radiation should be checked and patients should be advised to stop exposure to them.

Infections of the reproductive tract should be treated with appropriate antibiotics [11]. Initially, specific therapeutic options directed against the etiological cause of raised ROS should be tried [3]. After treating the primary cause, patients can be advised to take antioxidant supplementation. Antioxidants can be started directly when a specific etiology cannot be identified (unexplained infertility) [43]. Considerable interest has been generated in the use of antioxidants to overcome the adverse and pathological results of OS. Some studies that used nutritional supplements and antioxidants, like vitamin C supplementation to protect against ROS and OS. However, there is a lack of consensus on the type and dosage of antioxidants to be used [3]. In vivo antioxidants may be helpful in smoker infertile women [24]. A study, impact of a nutritional supplement, containing vitamin E, iron, zinc and selenium, is examined. The patients receiving the supplement experienced a significant increase in ovulation rates and pregnancy rates compared with the placebo group [28]. Indirect evidence of the importance of OS and its control with antioxidant intake is provided by studies that have shown that preconceptional multivitamin supplementation may enhance fertility, perhaps by increasing menstrual cycle regularity [44] or via prevention of ovulatory disorders [46]. In general, when supplemental vitamins C and E are given to older mice, the age-associated reduction in ovulation is partially prevented [51]. There is sufficient evidence to hypothesize that diet, particularly its constituent antioxidants, and OS may influence the timing and maintenance of a viable pregnancy [4].

5. Conclusion

- Assessment of OS as a cause of unexplained female infertility must be carried out to discriminate OS infertility from other causes of infertility.
- There is inverse significant relationship between GSH level with age and duration of infertility increment in patients with unexplained infertility.
- There is an inverse significant relationship between Vitamine C and age increment in patients with unexplained infertility.

IntechOpen

Author details

Huda Mahmood Shakir
Department of Obstetrics and Gynaecology, Ibn Sina University for Medical and Pharmaceuticals Sciences, Iraq

*Address all correspondence to: hudaalfatal2010@gmail.com

IntechOpen

References

[1] Siristatidis ,C. and Bhattacharya,S. (2007). Unexplained infertility: does it really exist? Does it matter?. Human Reproduction J., 22(8)2084-2087.

[2] Smith,S.; Pfiefer, S.M. and Collins, J. (2003). Diagnosis and management of female infertility. J.A.M.A. ,290:17.

[3] Agarwal, A. and Allamaneni, S.S.(2004).Role of free radicals in female reproductive diseases and assisted reproduction.Reproductive BioMedicine Online, 9:338-347.

[4] Ruder,E.; Hartman' J.; Blumberg ,J. and Goldman ,M.(2008). Oxidative stress and antioxidants: exposure and impact on female fertility. Human Reproduction and Embryology J.

[5] Van Langendonckt, A.; Casanas-Roux, F.; Donnez, J.(2002). Oxidative stress and peritoneal endometriosis. Fertil. Steril. J.,77:861-870.

[6] Janiki,Dj.(2008).oxidative stress. pittsburgh mind body center.

[7] Goldman, M.B.; Missmer, S.A. and Barberi ,R.L. (2000). Infertility. In:Women and Helath . San Diego: Academic Press., 196-214.

[8] Guerin, P.; El Mouatassim, S. and Menezo, Y. (2001). Oxidative stress and protection against reactive oxygen species in the pre-implantation embryo and its surroundings. Hum. Reprod. Update, 7:175-189.

[9] Savita, S. M.; Anitha, S. K.; Chaya ,S. D. and Bharati,A . (2009). Oxidative stress-mediated essential polyunsaturated fatty acid alterations in female infertility .Human Fertility,12 (1)28-33.

[10] Fissore, R. A.; Kurokawa, M.;Knott, J.; Zhang, M. and Smyth, J.(2002). Mechanisms underlying oocyte activation and postovulatory ageing. Reproduction J., 124:745-754.

[11] Agarwal, A., Gupta, S. and Sharma, R.(2005). Oxidative stress and its implications in female infertility - a clinician's perspective. Reprod. Biomed. Online,11(5): 641-650

[12] Cheeseman ,K.H. and Slater, T.F. (1993). An introduction to free radical biochemistry. Med Bull , 49:481-493.

[13] Tremellen, J. (2008). Oxidative stress and male infertility—a clinical perspective .Hum. Reprod. J. , 14(3):243-258.

[14] Fujii, J.; Iuchi, Y. and Okada, F.(2005). Fundamental roles of reactive oxygen species and protective mechanisms in the female reproductive system. Reprod. Biol. Endocrinol., 3:43-53.

[15] Christophersen, A. G.; Jun, H.; Jŕgensen, K., and Skibsted, L. H. (1991). Photobleaching of astaxanthin and canthaxanthin: quantum-yields dependence of solvent, temperature, and wavelength of irradiation in relation to packageing and storage of carotenoid pigmented salmonoids. Z. Lebensm. Unters. Forsch., 192:433-439

[16] Dong, M.; Shi, Y. and Cheng, Q. (2001). Increased nitric oxide in peritoneal fluid from women with idiopathic infertility and endometriosis. J Reprod Med, 46:887-891.

[17] Lauffer, R. B. (1992). Iron and Human Disease. CRC, Boca Raton, FL.

[18] Ames, B. N.; Profet, M. and Gold, L. S. (1990). Proc. Natl. Acad. Sci. USA ,87: 7777-7781.

[19] Bruce, N. Mark, K. and Tory, M. (1993). Oxidants, antioxidants, and the degenerative diseases of aging(cancer/ mutation/endogenous DNA adducts/

oxygen radicals). Proc. Natl. Acad. Sci. USA, 90: 7915-7922.

[20] Bergamini, C.M.; Gambetti, S.; Dondi, A. and Cervellati, C.(2004). Oxygen, reactive oxygen species and tissue damage. Curr. Pharm. Des.,10: 1611-1626.

[21] Quinlan, T.; Spivack, S. and Mossman, B.T (1994). Regulation of Antioxidant Enzymes in Lung after Oxidant Injury. Environmental Health Perspectives, 102(2):77-81.

[22] Benov, L. and Fridovich, I. (1998). Growth in iron-enriched medium partially compensates Escherichia coli for the lack of manganese and iron superoxide dismutase. Bio.l Chem. J., 273: 10313-10316.

[23] Brioukhanov, A.; Netrusov, A. and Eggen, R. (2006). The catalase and superoxide dismutase genes are transcriptionally up-regulated upon oxidative stress in the strictly anaerobic archaeon Methanosarcina barkeri. Microbiology, 152: 1671-1677.

[24] Paszkowski, T.; Traub, A.I.; Robinson, S.Y. and McMaster, D. (1995). Selenium dependent glutathione peroxidase activity in human follicular fluid. Clin. Chim. Acta., 236: 173-180

[25] Eidan, B.; almukhtar, N.; Altemimmi, H. (2009), relashionship betwwen cervical and blood free radicals concentration in unexplained infertility.

[26] Franceschi, R.T. (1992). The role of ascorbic acid in mesenchymal differentiation. Nutr. Rev. , 50:65-70

[27] Tsuji, M.; Ito, Y.; Terada, N. and Mori H.(1989). Ovarian aromatase activity in scorbutic mutantrats unable to synthesize ascorbic acid. Acta. Endocrinol. (Copenh) , 121:595-602.

[28] Behrman, H.R.; Kodaman, P.H.; Preston, S.L. and Gao, S.(2001). Oxidative stress and the ovary. J. of the Society for Gynecol. Investigation, 8: S40-42.

[29] Griveau, J.F and Le Lannou, D. (1997). Reactive oxygen species and human spermatozoa: physiology and pathology. Int. J. Androl., 20:61-69.

[30] Ebisch, I.M.; Thomas, C.M.; Peters, W.H.; Braat, D.D. and Steegers-Theunissen, R.P. (2007). The importance of folate, zinc and antioxidants in the pathogenesis and prevention of subfertility. Hum. Reprod. Update, 13:163-174.

[31] Zini, A.; Garrels, K. and Phang, D. (2000). Antioxidant activity in the semen of fertile and infertile men. Urology J.,55:922-6.

[32] Lunec, J. (1990). Review Article, Ann Clin. Biochem., 27:173.

[33] Battino, M.; Ferreiro, M.S.; Galalrdo, I. ; Newman, H.N. and Bullon, P.(2002).The antioxidant capacity of saliva. J. Clin. Periodontol., 29:189-194.

[34] Sugino, N.; Nakata, M. and Kashida, A.(2000). Decreased superoxide dismutase expression and increased concentration of lipid peroxide and prostaglandin F2” in the deciduas of failed pregnancy. Mol. Hum. Reprod., 6(7): 642-647

[35] Urner, F.; and Sakkas, D. (2005). Involvement of the pentose phosphate pathway and redox regulation in fertilization in the mouse. Mol. Reprod. Dev., 70,494-503.

[36] Agarwal, A.; Saleh, R.A. and Bedaiwy, M.A.(2003).Role of reactive oxygen species in the pathophysiology of human reproduction. Fertil. Steril. J., 79: 829-843.

[37] Agarwal, A; Gupta, S.; Malhotra, N.; Sharma, D. and Chandra, A. (2009). Oxidative Stress and its Role in Female

Infertility and Assisted Reproduction: Clinical Implications. International Journal of Fertil. and Steril., 2(4): 147-164.

[38] Zuelke, K.A.; Jeffay, S.C.; Zucker, R.M. and Perreault, S.D.(2003). Glutathione (GSH) concentrations vary with the cell cycle in maturing hamster oocytes, zygotes, and pre-implantation stage embryos. Mol. Reprod. Dev., 64:106-112.

[39] Turton. M. and Luderer. U. (2006). Opposing Effects of Glutathione Depletion and FSH on Reactive Oxygen Species and Apoptosis in Cultured Preovulatory Rat Follicles. Endocrinology, 147:1224-1236.

[40] Ikeda, S.; Kitagawa, M.; Imai, H. and Yamada, M.(2005). The roles of vitamin A for cytoplasmic maturation of bovine oocytes. J. Reprod. Dev., 51:23-35.

[41] Goto, T.; Jones, G.M.; Lolatgis, N.; Pera, M.F.; Trounson ,A.O. and Monk M.(2002).Identification and characterisation of known and novel transcripts expressed during the final stages of human oocyte maturation. Mol. Reprod .Dev., 62:13-28.

[42] Tatemoto, H.; Sakuraim N. and Muto, N.(2000). Protection of porcine oocytes against apoptotic cell death caused by oxidative stress during In vitro maturation: role of cumulus cells. Biology of Reproduction ,63 805-810.

[43] Agarwal, A.; Gupta, S.; Sekhon, L. and Shaha,R.(2008).Redox Considerations in Female Reproductive Function and Assisted Reproduction: From Molecular Mechanisms to Health Implications. Antioxidants and redox signaling J., 10(8):1376-1396

[44] Duleba, A.J.; Foyouzi, N. and Karaca, M.(2004). Proliferation of ovarian theca-interstitial cells is modulated by antioxidants and oxidative stress. Human Reproduction, 19:1519-1524.

[45] Cohen, G.; Dembiec, D. and Marcus, J. (1970). Measurement of catalase activity. Analyt.Biochem., 34 : 30-38.

[46] Paszkowski, T.; Clarke, R. and Hornstein, M. (2002). Smoking induces oxidative stress inside the Graafianfollicle. J. Obstet. Gynecol. Reprod. Biol.,17(4):921-925

[47] Sun, J.G.; Jurisicova, A. and Casper, R.F.(1997). Detection of deoxyribonucleic acid fragmentation in human sperm: correlation with fertilization in vitro.Biol. Reprod., 56:602-607

[48] Cameron, I.T. and Campbell, S. (1998). Nitric oxide in the endometrium. European Society of Hum. Reprod. and Embryology, 4(5):565.

[49] Allaire, C.(2006). Endometriosis and infertility. Reprod. Med. J., 51:164-168.

[50] De Souza, M.J. and Williams, .I. (2004). Physiological Aspects and Clinical Sequelae of Energy Deficiency and Hypoestrogenism in Exercising Women. Human Reproduction Update, 10(5):433-448.

[51] Tarin, J.; Ten, J.; Vendrell, F.J.; de Oliveira, M.N. and Cano, A. (1998). Effects of maternal ageing and dietary antioxidant supplementation on ovulation, fertilisation and embryo development in vitro in the mouse. Reprod. Nutr. Dev. , 38:499-508.

[52] Carbone, M.C.; Tatone, C. and Monache, S.(2003). Antioxidant enzymatic defences in human follicular fluid: characterization and age-dependent changes. Hum. Reprod., 9:639-643.

[53] Yeh, J.; Bowman, M.J.; Browne, R.W. and Chen, N. 2005. Reproductive aging results in a reconfigured ovarian antioxidant defense profile in rats. Fertil. Steril. J.; 84(2):1109-1113.