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Review Article

Impact of Oxidative stress on Infertility, with emphasis on infertility management strategies

Abstract

Oxidative stress (OS) is a state of imbalance between pro-oxidants (Reactive Oxygen species and Reactive Nitrogen species) and antioxidant defences. It plays a pivotal role in infertility issues (paternal as well as maternal). As such different reproductive diseases such as Polycystic Ovary Syndrome (PCOS), Endometriosis, unexplained infertility, Impairment of Spermatozoa, Sperm Dysfunction and Sperm DNA Fragmentation (SDF), are caused, which destroys the process of reproduction. Several pregnancy complications such as miscarriage, recurrent respiratory loss (RPL), preeclampsia and intrauterine growth restriction (IUGR), are also associated to OS. It is well established that factors such as obesity, malnutrition, life style factors – smoking, alcohol and recreational drugs, environmental and occupational exposures – pesticides and endocrine disrupting chemicals, EDCs, hampers the reproductive process by setting up OS. Thus, as an infertility management strategy, procedures such as antioxidant supplementation and Assisted Reproductive Techniques (ART), are to be undertaken. There is also a wide scope for research in the field of EDCs linked to oxidative stress, which can be a hope to further elucidate the path of reproduction.

Abbreviations

OS: Oxidative Stress; ROS: Reactive Oxygen Species; PCOS: Polycystic Ovary Syndrome; SDF: Sperm DNA Fragmentation; RPL: Recurrent Respiratory Loss; IUGR: Intrauterine Growth Restriction; EDCs: Endocrine Disrupting Chemicals; ART: Assisted Reproductive Techniques; IVF: In Vitro Fertilization; ICSI: Intracytoplasmic Sperm Injection; IUI: Intrauterine Insemination; MDA: Malondialdehyde; PBBs: Polychlorinated Biphenyls; BPA: Bisphenol A.

Introduction

Oxidative stress is the root for many disease conditions such as Infertility. It is caused due to an imbalance between pro-oxidants and antioxidants [1]. This imbalance is created due to enhanced levels of ROS (Reactive Oxygen Species) and / or RNS (Reactive Nitrogen Species) in plasma and blood of patients. In addition, there is often a decreased level of antioxidant mechanism, thereby deteriorating the balance ratio.

As oxidative stress is one of the major causes for infertility, excessive generation of ROS causes an environment unsuitable for normal female physiological reactions [1]. This leads to reproductive diseases such as PCOS, Endometriosis and unexplained infertility in women [2]. Pregnancy complications

such as miscarriage, recurrent respiratory loss (RPL), preeclampsia and intrauterine growth restriction, IUGR, are all linked to oxidative stress [3].

Not only maternal, but paternal reproductive disease conditions are also due to oxidative stress. In this review, some of them such as Impairment of Spermatozoa, Sperm Dysfunction, Sperm DNA Fragmentation (SDF), are discussed briefly.

Various factors are also responsible for infertility, such as obesity, malnutrition, life style factors – smoking, alcohol and recreational drugs [4], environmental and occupational exposures – pesticides and endocrine disrupting chemicals, EDCs [5-7]. These factors are linked to oxidative stress.

This problem of infertility due to oxidative stress or other reasons, can be solved by Antioxidant supplementation such as glutathione and/ or Assisted Reproductive Techniques, ART [2], finally to bring a smile on the couple's face. Measurement of oxidation-reduction potential by MiOXSYS® system, is yet another approach to solve the problem of oxidative stress [8].

Major complications due to oxidative stress, pertaining to infertility:

1. **Male Infertility** – i) Impairment of Spermatozoa

ii) Sperm Dysfunction

iii) Sperm DNA Fragmentation (SDF)

2. Female Infertility – i) Polycystic Ovary Syndrome (PCOS)

ii) Endometriosis

3. Pregnancy complications – i) The Placenta

ii) Miscarriage

iii) Recurrent pregnancy loss

iv) Preeclampsia

Male Infertility

i) **Impairment of Spermatozoa in humans:** Impaired sperms are one of the major causes of infertility. It has been documented that the nucleohistone compartment of sperms which contains histone-bound DNA sequences like telomeres and promoters of genes for embryonic development, are often at a high risk of oxidative stress, leading to infertility [8,9]. Also, the relative decrease in protamine 2 levels, in the ratio of protamine1: protamine2, at the level of m-RNA and protein, also results in infertility. Again, gene mutations encoding protamines lead to structural changes in sperm chromatin structure, thereby leading to infertility [10,11].

ii) **Sperm dysfunction :** The mechanism of sperm dysfunction is related to the oxidative stress, which actually causes distortion of the sperm DNA integrity, by damaging proteins and lipids present in sperm cell plasma membrane. This has a negative effect on sperm cell membrane fluidity and permeability, leading to infertility [12]. Also, it has been established that sperm motility gets reduced as ROS becomes directly proportional to the level of lipid peroxidation [13]. The sperm dysfunction is also related to the lipid peroxidation cascade activation, initiated by ROS-mediated oxidation of lipids in sperm cell membrane. Figure 1, explains the same.

iii) **Sperm DNA Fragmentation (SDF):** According to the researchers, SDF is mediated by free-radicals [14,15]. Again, the mechanisms underlying SDF include single-strand and double-strand breaks, DNA fragmentation, the introduction of abasic sites, purine, pyrimidine and deoxyribose modifications and DNA crosslinking, which can result in arrest or induction of gene transcription, induction of signal transduction pathways, accelerated telomeric DNA attrition, replication errors, genomic instability and GC to TA transversions [16-18]. As such mechanisms are also known to be the causes of carcinogenesis, these may explain a link between infertility and cancer. Also, male infertility is closely associated with ROS-induced DNA damage, which in turn accelerates the germ-cell apoptosis process, leading to decline in sperm count [19]. Figure 2 explains the same.

Female Infertility

i) **Polycystic Ovary Syndrome (PCOS):** The word “polycystic” means “many cysts. PCOS is said to develop when within the

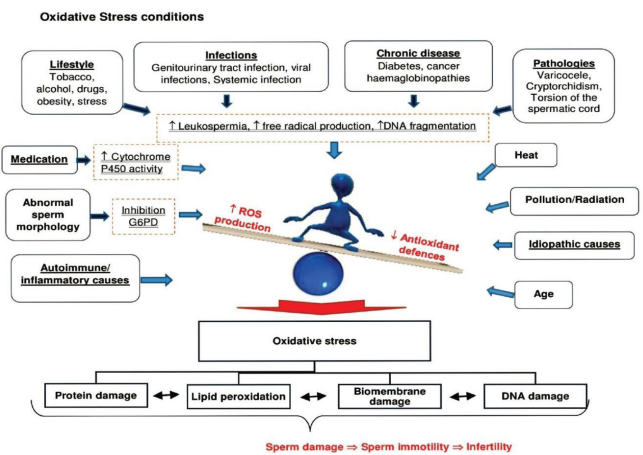


Figure 1: Oxidative stress leading to infertility [61].

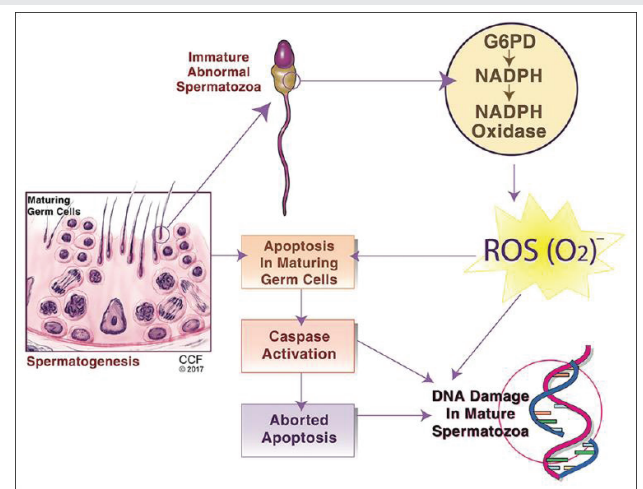


Figure 2: Germ cell Apoptosis process during spermatogenesis [62].

ovaries, numerous minute fluid-filled sacs appear. Each of these sacs is a follicle which holds an immature egg. These eggs do not develop to complete the ovulation process. Again, due to this lack of ovulation, there is a change in the hormonal levels of the female body. For eg. estrogen and progesterone levels get reduced while androgen levels are higher. The menstrual cycle in the females therefore, gets disturbed due to hyperandrogenism. So, women with fewer periods than normal, often lead to PCOS. Figure 3 explains the same.

Several disease conditions arise due to pathogenesis of PCOS: Hyperinsulinemia, reproductive aberration, hyperandrogenism, oligomenorrhea, amenorrhea, obesity complications, acanthosis nigricans, insulin resistance and genetic factor complications [20]. 6-10% of premenopausal women suffer from PCOS [21].

Oxidative stress (OS) and PCOS

Oxidative stress leads to PCOS. It is well established, that ROS (Reactive Oxygen Species) is more in PCOS patients, especially for insulin resistance patients [22]. Also, a decrease in GSH content and GSH to GSSG ratio in leukocytes suggests a deterioration of mitochondrial function and an increase in

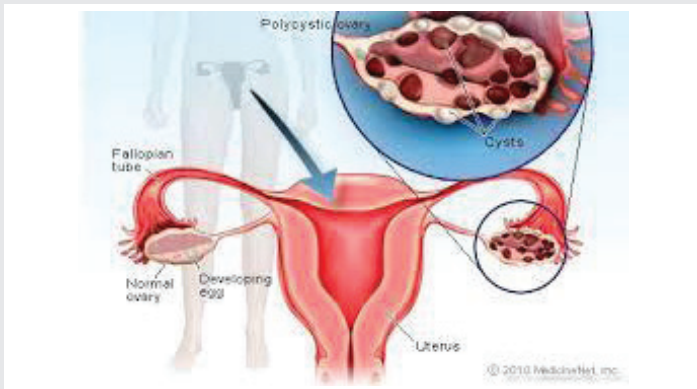


Figure 3: Polycystic Ovary Syndrome (PCOS) [63].

oxidative stress leading to PCOS. As Protein carbonyl (PC) is an important biomarker of oxidative stress, it is observed that PC content is higher in PCOS patients compared to control. Further, PC content is known to have a positive correlation with fasting insulin, suggesting a strong association between insulin resistance and protein oxidation in PCOS. Malonal dialdehyde or MDA (produced during decomposition of polyunsaturated fatty acids) is one of the biomarkers of OS. It is seen that blood MDA levels are significantly ($P= 0.01$) higher in young, lean, non-IR PCOS patients, in comparison to the control [23]. Again, SOD (Superoxide dismutase) levels are higher in PCOS patients, as compared to control. As IL-6 (Interleukin-6) and TNF- α (Tumor necrosis factor alpha) are pro-inflammatory markers of oxidative stress, it is well established that IL-6 is more in PCOS patients and shoots up in the case of PCOS-IR patients [22]. TNF- α , on the other hand is higher in both PCOS-IR as well as PCOS-non IR patients.

ii) **Endometriosis:** Endometriosis occurs when the tissue that forms the **uterine lining** (the lining of the womb) is present on various organs within the body. Endometriosis is usually found in the lower abdomen, or pelvis, but can also appear elsewhere in the body. Endometriosis is explained by Figure 4.

Women with endometriosis often suffer from lower abdominal pain, pain with periods, or pain with sexual intercourse, and may report having a hard time getting pregnant. However, some women with Endometriosis, may be asymptomatic.

Endometriosis and Fertility

Between 20 and 40% of women with infertility, normally suffer from endometriosis. Endometriosis seems to impair fertility in 2 ways: firstly, by causing deformation in the fallopian tubes so that they are unable to pick up the egg after ovulation, and secondly, by causing inflammations that in turn causes impairment of the functioning of the ovary, egg, fallopian tubes or uterus. Figure 5 shows the endometriosis tissue on the bladder wall and left ovary.

Pregnancy complications

i) **The Placenta:** In cases of incomplete placentation, oxidative stress occurs at an excessive level and leads to an

elevated release of placental debris and vesicles into the maternal circulation. These extracellular vesicles carry active molecules (proteins of microRNA) generated by stressed placental cells. Once in the blood, they meet maternal endothelial cells and potentially transfer their contents, leading to transcriptome alterations and inflammation. Eventually, the endothelium of maternal organs is affected along with the deterioration of syncytiotrophoblast [24]. Figure 6 explains the effect of oxidative stress on placental function and pathological events in pregnancy.

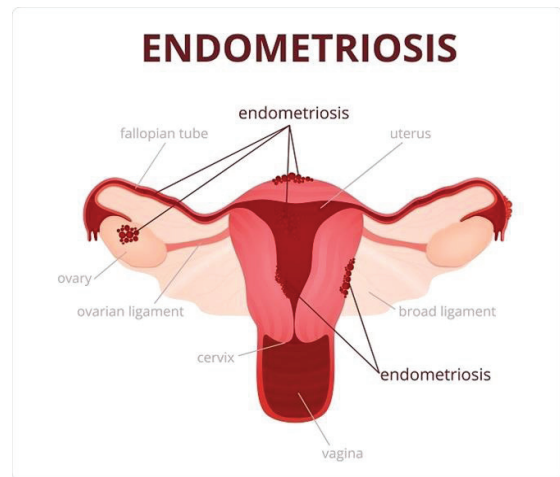


Figure 4: Endometriosis [64].

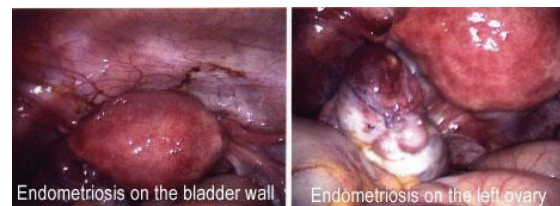


Figure 5: Shows endometriosis tissue on the bladder wall and left ovary. (Source : obGyn.net) [65].

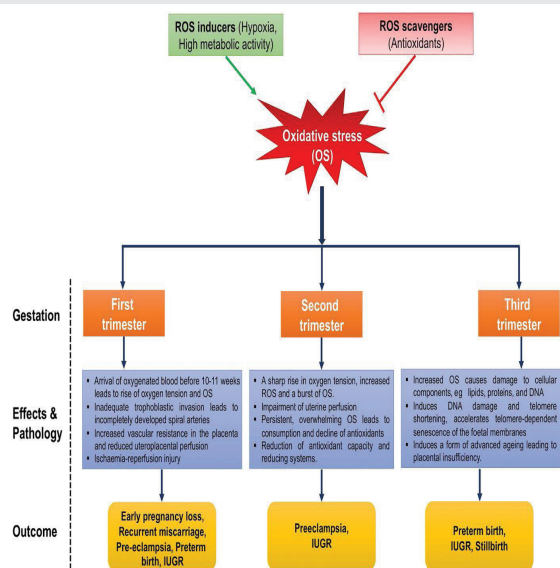


Figure 6: Effect of oxidative stress on placental function and pathological events in pregnancy [66].

ii) **Miscarriage or Spontaneous Abortion:** Miscarriage or spontaneous abortion is the unintentional termination of pregnancy when the fetal weight is < 500g. Placental oxidative stress is one of the major cause of miscarriage. Due to premature intraplacental circulation at 8 to 9 weeks of pregnancy, instead of 10–12 weeks of gestation, high oxidative stress is set up [25,26] which distorts the syncytiotrophoblast layer of the placenta, thereby causing impairment of the placenta [27]. Apoptosis of the placental tissues may also occur due to oxidative stress- induced inflammatory processes. Hence, due to this degeneration of the placenta, miscarriage often occurs [2]. Figure 7 depicts the types of spontaneous abortion.

iii) **Recurrent Pregnancy Loss (RPL):** Recurrent pregnancy loss refers to more than three consecutive pregnancy losses. The reason behind this situation is the enhancement of Natural Killer cells (NK cells) in placental blood circulation, thereby increasing pre-implantation angiogenesis. This in turn causes premature intra-placental maternal circulation, leading to very high oxidative stress [28]. It has been well established, that in patients with RPL, there is an enhancement of lipid peroxides and GSH in plasma [29], in addition to the reduction in levels of vitamin E and β-carotene, indicating an elevated level of oxidative stress [30]. Another study, reveals that there is significantly decreased levels of antioxidant enzymes like GPx, SOD and catalase, in addition to increased MDA levels, in patients with RPL, again indicating augmented oxidative stress [31]. Figure 8, explains the etiology of Recurrent Pregnancy loss, that is, Primary versus Secondary.

iv) **Preeclampsia:** Preeclampsia refers to a complex multisystem disorder that is characterized by high blood pressure pregnancy complication. This is a leading cause of maternal and fetal morbidity, accounting to 3% to 14% of pregnancies worldwide [32, 33]. In this context, Placental ischemia /hypoxia plays a very important role in augmenting the oxidative stress, which in turn leads to endothelial cell dysfunction [33, 34] and systemic vasoconstriction [35]. Also, it is seen that the oxidative stress has contributed to the apoptosis of villous trophoblasts, in patients with Preeclampsia [2]. Figure 9 explains Preeclampsia and Placental Abruption, diagrammatically.

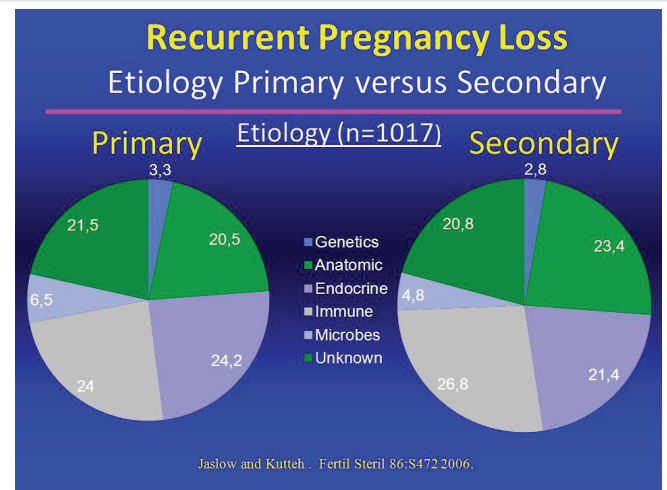


Figure 8: Etiology of Recurrent Pregnancy Loss – Primary versus Secondary [68].

Factors governing Infertility, associated with Oxidative Stress :

1. Age
2. Body weight – i) Obesity
- ii) Underweight
3. Lifestyle factors – i) Cigarette
- ii) Alcohol usage
- iii) Intake of Recreational drugs
4. Environmental and occupational exposures –
- i) Pesticides
- ii) Endocrine Disrupting Chemicals (EDCs)

Figures 10,11, explain the various factors leading to female and male infertility.

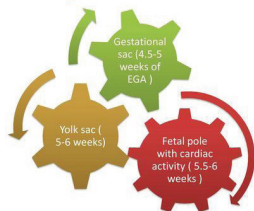
1. **Age:** Age is a vital factor for infertility, as fertility declines with advancing mother’s age. Paternal age is also important in terms of fertility, as retrogression in gamete quality, increased levels of oxidative DNA damage (ODD) and decline in semen quality. Hence, sperm DNA is often exposed to oxidative stress because of advancement in paternal age [9,36].

2. Body weight

i) **Obesity:** The pathogenesis of obesity is closely associated to the excessive generation of ROS, thereby setting up of Oxidative stress [32]. Hence, overweight can complicate pregnancy issues, both in terms of maternal as well as fetal health. Also, dysfunctional hormonal system and irregular menses, are known to be closely associated with overweight or obesity [38].

ii) **Underweight:** Malnourishment in reproductive females is associated to deterioration of endothelium- dependent vasodilation, triggering oxidative stress [39].

Transvaginal ultrasound evaluation timeline :



Types of spontaneous abortion :

	Cervix open	Cervix closed
Products passed	Incomplete abortion	Complete abortion
Products not passed	Inevitable abortion	Missed abortion

1st Trimester

The most common cause of SAB is chromosomal abnormalities. Which increases with the increasing maternal age.

2nd Trimester

Mostly caused by maternal systemic disease, abnormal placentation, or other anatomic considerations.

Figure 7: Types of Spontaneous abortion [67].

3. Lifestyle Factors

i) **Cigarette smoking:** It has been well established that infertility, pregnancy complications, damage to the developing embryo, higher rates of fetal loss, decreased fetal growth, preterm birth and miscarriage, are all the results of maternal smoking [2,40,41]. As cigarette smoking is composed of many toxic chemicals and pro-oxidants, it triggers the production of ROS, setting up oxidative stress in follicular microenvironment [42].

ii) **Alcohol usage:** Alcohol consumption gives rise to the production of metabolites like acetyl and methyl radicals, responsible for ROS generation. Regular alcohol consumption, triggers lipid peroxidation and lowers SOD antioxidant activity along with GSH levels, thereby elevating the level of ROS in plasma of pregnant mother. Hence, maternal alcohol usage can thus result in Intrauterine Growth Restriction (IUGR), low birth weight, increased risk of congenital disorder, early pregnancy loss and spontaneous abortion (miscarriage) [2,4].

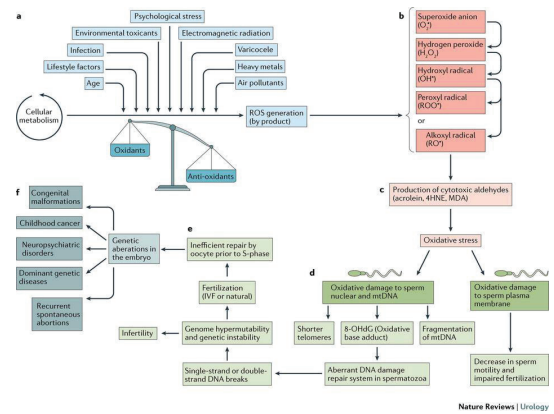


Figure 11: Explains male infertility due to various factors [71].

iii) **Recreational Drug usage:** The active constituent of Marijuana (Recreational drug) that is Cannabinoids, produce free radicals which can alter central as well as peripheral nervous system functioning [43]. Another important constituent of the drug which causes psychological impacts in smokers is called delta-9-tetrahydrocannabinol (THC). This THC is known to induce breakage in DNA strands (Chan et al. 1998), being associated with ROS generation [44-46].

Cocaine, another important addictive drug, triggers lipid peroxidation, through several metabolites, thereby producing oxidative stress and lipid peroxy radicals [47]. While formaldehyde (oxidative metabolites of cocaine) generates ROS, Norcocaine another oxidative metabolite of cocaine causes oxidative stress by depleting GSH stores [4]. Also, ROS induces apoptosis [48,49].

4. Environmental and Occupational exposures

i) **Pesticides:** It has been well documented that the spouses of agricultural male workers who are in direct contact with pesticide chemicals like DDT (Organochlorine pesticides), are victims of miscarriage or spontaneous abortion [50].

Again, polychlorinated biphenyls or PCBs (pesticide) are known to enhance free radical generation by setting up oxidative stress, through induction of endothelial dysfunction [51] and cell membrane destruction [2]. Interestingly, PCB exposure causes suppression of Vitamin E levels [51].

Organophosphate pesticides on the other hand, causes depletion of GSH along with increase in ROS, triggering oxidative stress [52-54]. Figure 12, explains the role of pesticides in infertility.

ii) **Endocrine Disrupting Chemicals (EDCs):** Endocrine Disrupting Chemicals (EDCs) like the phthalates acts as an anti-androgenic compound, deteriorating the male reproductive system and is associated with the setting up of the oxidative stress [55]. Also, it has been documented, that there is an association between urinary phthalate metabolites and polyvinyl chloride workers [55]. After taking into consideration, the age, smoking status and coffee consumption, it has been observed that the urinary phthalate metabolites causes sperm

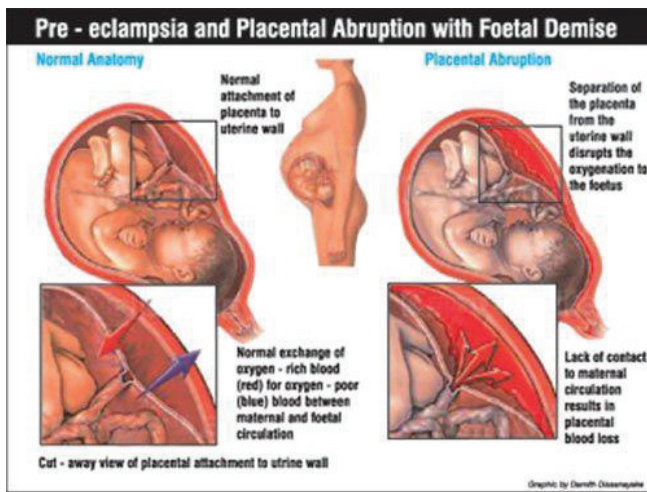


Figure 9: Preeclampsia and placental abruption with foetal demise [69].

Oxidative Stress

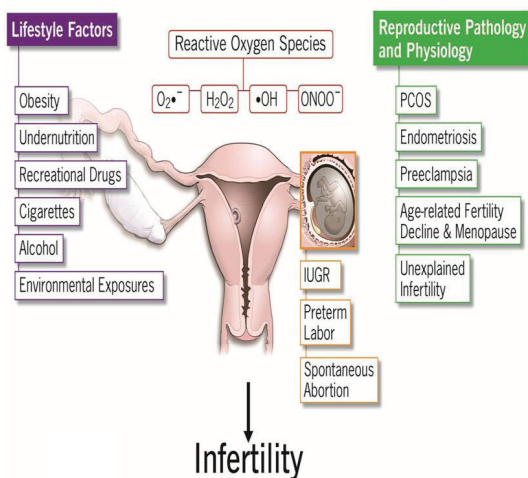


Figure 10: Explains female infertility due to various factors [70].

apoptosis and ROS generation. Limited evidence suggests that BPA (Bisphenol A) sets up oxidative stress, which in turn impairs the male reproductive capacity. Figure 13, explains the role of EDCs in infertility.

Therapeutic solutions to Infertility

- i) Antioxidant Supplementation
- ii) Assisted Reproductive Techniques
- i) **Antioxidant Supplementation**

As oxidative stress is one of the main causes of infertility,

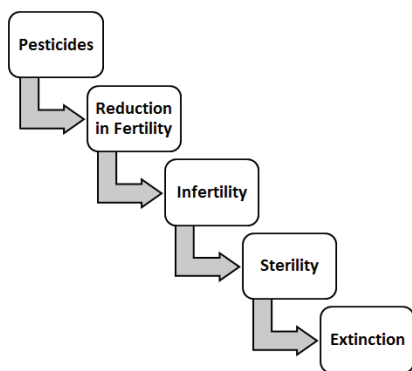


Figure 12: Explains role of pesticides in infertility [72].

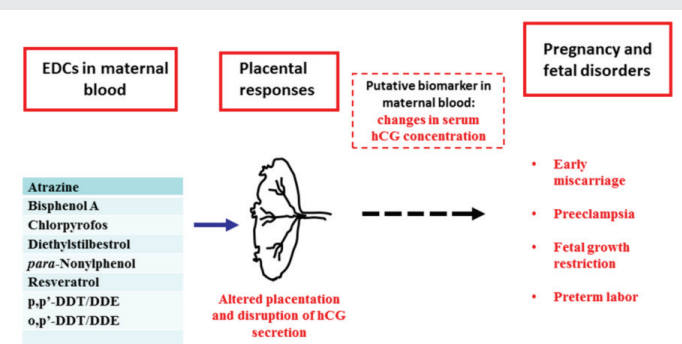


Figure 13: Explains the role of EDCs in infertility [73].

antioxidant supplementation is required to scavenge the free radicals, thereby creating a balance between ROS and antioxidants. Antioxidants such as vitamin C, vitamin E, selenium, zinc and glutathione are important for male infertility [56,57]. Table 1, depicts the antioxidants, their characteristics and effects on semen parameters.

Glutathione – the principal antioxidant for male infertility

Glutathione prevents male infertility by scavenging lipid peroxides in the plasma membrane of spermatozoa, thereby reducing the oxidative stress. It also scavenges hydrogen peroxide, thereby arresting lipid peroxidation and reducing oxidative stress [20].

Glutathione – the principal antioxidant for female infertility

During folliculogenesis, Glutathione shields eggs from oxidative stress [20]. Researches, in this context have thus proved that oocytes with elevated levels of intracellular glutathione produce healthier and stronger embryos [58]. Figure 14, explains the antioxidant supplementation important for female fertility.

Glutathione – impact on autoimmune issues

Glutathione is indeed a boon for those with immunological miscarriages or if the body is rejecting one’s mate’s sperm [20].

ii) Assisted Reproductive techniques

Assisted Reproductive techniques are the treatments of choice for the infertile men or women. In cases of endometriosis, tubal factor infertility, male factor infertility and unexplained infertility, these techniques can bring a new born baby to the affected couple [59].

These techniques include intrauterine insemination, IVF and intracytoplasmic sperm injection (ICSI). The same have been explained by Figures 15-17.

Conclusion

As oxidative stress is one of the major causes of infertility (Figure 18), couples especially must include antioxidants in

Table 1: Antioxidants, their characteristics and effects on semen parameters.

Antioxidants	Characteristics	Impact on semen parameters
Vitamin E	Major chain – breaking antioxidant in sperm plasma membrane	Preserves and restores sperm motility and morphology by suppressing lipid peroxidative damage to sperm plasma membrane.
Vitamin C	Principal hydrophilic antioxidant in seminal plasma, contributes to 65% of chain-breaking antioxidant capacity	Increases sperm motility in a dosedependent manner High doses can damage chromatin structures by disrupting disulphide bridges and therefore increasing the cellular density of staining from 17.5-21.5% following a 3mM dose
Carnitine	Water-soluble antioxidant primarily derived from human diet ,found at high concentrations in the epididymis	Has a key role in sperm maturation and metabolism, decreases fatty acid oxidation by enhancing the cellular energetics of mitochondria , protects from ROS-induced damage to sperm DNA and to the sperm plasma membrane, improves sperm motility and count
Glutathione	Most abundant reducing agent in the body and protects proteins, lipids and nucleic acid against oxidative damage	Glutathione administration increases forward progressive sperm motility in infertile men Oral glutathione has poor bioavailability
CoQ10	Abundant in the sperm midpiece and has important antioxidant and metabolic functions	Involved in energy generation in spermatozoa Incubation of human spermatozoa of infertile men with appropriate doses (50 mM) of CoQ10 significantly improves sperm motility as well as function

diet, refrain themselves from bad habits such as smoking, alcohol, recreational drugs and perform yoga and meditation daily, to ensure a happy family life with children. Further research work on Endocrine Disrupting Chemicals (EDCs) and endometriosis [60] need to be carried out, in order to elucidate the path of human fertility. Advancement in science and technology have also gifted techniques such as Assisted Reproductive Techniques (ART), which aims to bring a smile on the couple's face.

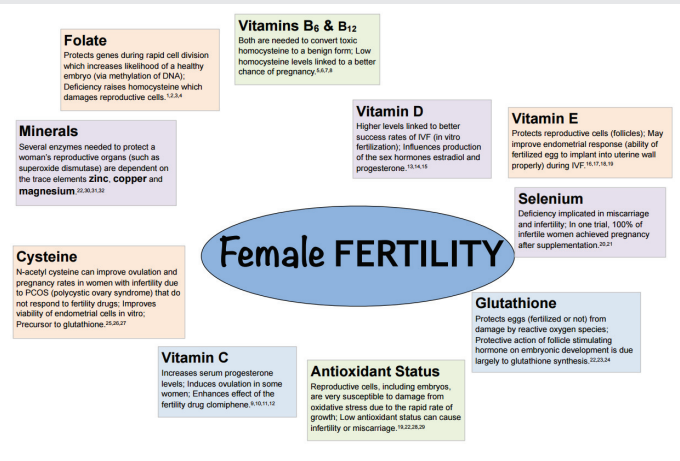


Figure 14: Antioxidant supplementation important for female fertility [74].

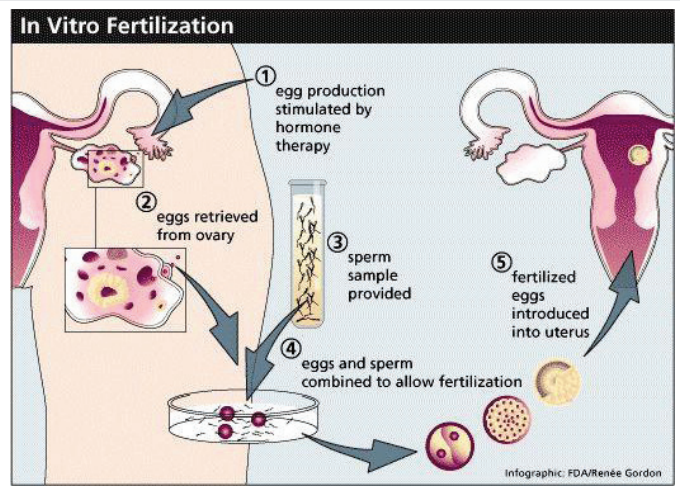


Figure 15: In vitro Fertilization [75].

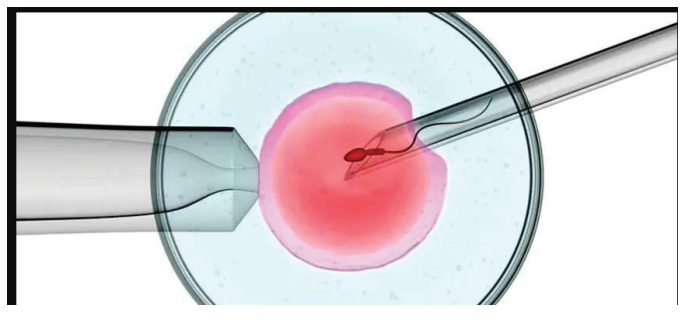


Figure 16: Intracytoplasmic sperm injection (ICSI) [76].

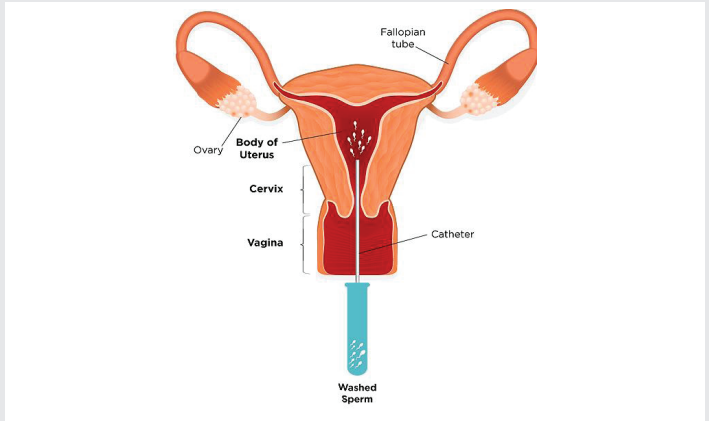


Figure 17: Intrauterine Insemination [77].

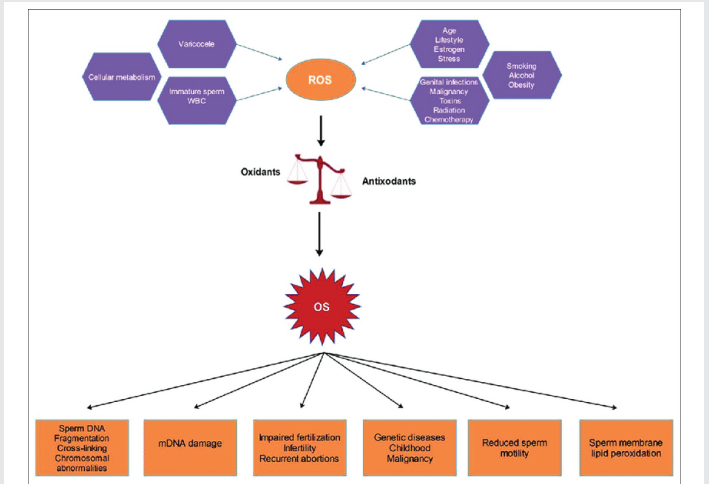


Figure 18: Sources of reactive oxygen species in the body and their pathological consequences on semen, fertility and health. ROS = Reactive oxygen species, OS = Oxidative stress [78].

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