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Review Article

# Seminal Oxidative Stress Biochemistry in Male Infertile Patients–An Overview

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**ABSTRACT**

The problem of infertility affects approximately 15 percent of couples in their reproductive years. Even after frequent, unprotected sexual activities for a year, or more, they are not able to conceive. Male infertility is a factor in more than a third of these couples. The cause of male infertility is low sperm production, abnormal sperm function, or blockages preventing sperm from being delivered. Infertility in men can be caused by illnesses, injuries, chronic health problems, lifestyle choices, and other factors. When you can't conceive a child, it can be stressful, and frustrating, but there are several treatments available for male infertility. Sometimes signs and symptoms can be attributed to underlying problems. This includes an inherited disorder, hormonal imbalance, dilated veins around the testes, or a condition that prevents the passage of sperm. Male infertility is predominantly caused by varicocele, infection, abnormal ejaculation, antibodies that attack sperm, tumours, undescended testicles, hormonal imbalance, defects in the tubes that carry sperm, chromosome abnormalities, sexual dysfunction, and celiac disease. The oxidative biochemistry in sperm are addressed in this study.

**Keywords:** Reactive oxygen species, Sperm quality, Free radical, Non-systematic review, Intrinsic, and extrinsic factors.

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## INTRODUCTION

Infertility is a global health issue. It is estimated that worldwide, 60 to 80 million couples struggle with infertility each year, of which approximately 15–20 million live in India alone.[1,2] According to a large study of more than 8,500 couples by the World Health Organisation (WHO), the male factor contributes to infertility by 51.2%. Infertility is defined as failure to conceive after one year of regular unprotected intercourse with the same partner. As per WHO study, the incidence of men infertility in India is about 10–15%. Male infertility contributes to 50% of the total infertile cases.[3] Over 90% of male infertility complaints are due to low sperm counts, low sperm quality, or both. Sperm count below 20 million per millilitre is called Oligospermia (low sperm count). Sperm motility problems and irregular sperm morphology are examples of poor sperm quality. Seminal analysis is a critical component in making a definite diagnosis of male infertility. This test evaluates sperm count, motility, and morphology. Reactive oxygen species (ROS), which are defined as oxygen ions, free radicals, and peroxides, induce infertility in two ways. First, ROS damage the sperm membrane, reducing motility, and the ability of the sperm to fuse with the egg. Second, the ROS directly degrade the sperm deoxyribonucleic acid (DNA), jeopardising the embryo's family genomic impact. Despite the widespread link between poor sperm quality and oxidative stress, men are rarely checked for or treated for this problem. Instead, 'mechanical' therapies such as *in vitro* fertilisation–intracytoplasmic sperm injection (IVF–ICSI), or intrauterine insemination (IUI) are typically provided. This is less than ideal because neither IVF–ICSI nor IUI therapy directly alleviates oxidative damage to sperm DNA. Furthermore, immediate treatment of oxidative stress may enable spontaneous conception, preserving expensive medical resources.[4–6] The prevalence for male infertility is exactly not known, but estimates suggest that the 10–year comparison of sperm quality, and quantity (2000–2001 to 2010–2011), the percentage of semen ejaculation, which is considered less than normal (below 4 ml), increased from 34% to 65% and the most suitable ejaculation volume (more than 4 ml) went down from 15% to 3%. The study from India [7] stating that, declining

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semen quality among 7,770 South Indian infertile men and it was confirmed that 30.31 % of decline in sperm count, sperm morphology, and motility was reduced by 51.25 % and 22.92 % respectively. But, these confirmations are failing to project the reason for drop in percentage of semen ejaculation, and quality in infertile men. A similar study in Calcutta,[8] included the semen analysis of 3,729 men presenting with infertility problems in two distinct decades between 1981–1985 and 2000–2006. The result confirmed that, decline in the sperm motility parameters, and seminal volume in the present decade. A decline was seen only in sperm motility with increasing age in both decades. The exact reason for the decline in semen quality is still not clear. There are few studies from western countries have demonstrated a link in relation to the oxidative stress in semen and which is created by leukocytes in seminal plasma. In India, there is no studies which demonstrates the association between the oxidative stress, and semen quality in the larger population. This be a huge lacuna or unfilled space in male infertility research in India and also in other countries.

## MATERIALS AND METHODS

It's a non-systematic narrative review conducted with the aim to describe the specific problem or cause for decline in sperm quality. The literature search was done on Cochrane Library, Springer, Elsevier titles, Scopus, and Science Direct. The boolean search strategy were adopted and the keywords used were oxidative stress, male infertility, sperm quality, spermatozoa, ROS, intrinsic, and extrinsic factors. Additionally, specific words were also searched. Abstracts in the research paper were assessed, based that the full–text publications were saved, and reviewed. Case study, case review, books, and monographs were excluded. Editorials, letter to communication, and opinions were included in this review work. Several governments, organisation, and associated websites were examined for relevant information. No segregation of the collected literatures like national and international works. The literature was not categorised according to a certain time period. The majority of the literature was arranged in a simple narrative format.

### Oxidative Stress Biochemistry

Males may have two primary ROS spawning systems: A putative nicotinamide adenine dinucleotide phosphate oxidase (NADH) at the sperm membrane and in mitochondrial level low sperm diphorase (mitochondrial NADH–dependent oxidoreductase) reaction.[9] Mitochondria is the major energy source for sperms and ROS are principally produced in bovine sperm by dead spermatozoa via an aromatic amino acid oxidase–catalysed process.[10] The two primary sources of ROS in sperm are leukocytes and immature spermatozoa.[11] Excessive ROS generation by leukocytes, particularly neutrophils, and macrophages, has been linked to sperm abnormalities.[12] The absence of endogenous defence mechanisms and second exposure of gametes and embryos to various manipulation techniques as well as environment that can contribute to the formation of oxidative stress are two important dynamics supporting ROS build up *in vitro*.[13] ROS levels may occasionally fluctuate within a fertile person, but they have no effect on sperm concentration, or motility. This might be because healthy people have enough antioxidant defence systems. Variations in the ROS levels might be caused by transitory subclinical infection and transient spermatogenesis defects such as cytoplasm retention or the periodic presence of aberrant spermatozoa in sperm.[14]

ROS formation is a normal biological process in the body, but an imbalance between ROS and antioxidant scavenging activity is detrimental to sperm quality, and results in male infertility.[15] ROS produced by spermatozoa have an important role in normal physiological activities such as sperm capacitation, acrosome response, fertilizations ability maintenance, and stability of the mitochondrial capsule in the mid-piece in bovine.[16] Controlled ROS production may act as signalling molecules (second messengers) in a variety of cell types; they are crucial mediators of sperm activities. Evidence has been revealed that superoxide anion ($\bullet O^2$), in particular, is essential for the late stages of embryo development, such as two germ cell layers, and the egg cylinder.[17] Although there is a substantial negative association between ROS and IVF rate,[18] regulated formation of ROS has been demonstrated to be needed for the development of capacitation and hyper activation,[19] the two sperm processes required for fertilizations. *In vivo* physiological quantities of ROS are implicated in sperm membrane fluidity, fertilising ability, and acrosome response.[20] Maintaining an appropriate ROS level is thus critical for proper sperm functioning. ROS have a negative impact on the sperm plasma membrane, DNA, and physiological processes, lowering the quality of spermatozoa. The axosome and related thick fibres of the mid-piece within sperm are coated by mitochondria, which create energy from adenosine triphosphate (ATP) depleted intracellular reserves.[21] Elevated ROS inhibits movement and fertilisation capability. Further, leukocytospermia is a condition where there's an upsurge in leukocyte infiltration in the sperm, which is related with a large number of defective sperm morphology, reduced sperm motility, and increased DNA damage. When compared to males without varicocele, individuals with varicocele had higher seminal lipid peroxidation.[22] A balance of ROS and antioxidants is required for optimum sperm activity and fertilisation. ROS causes spermatozoa to produce cyclic adenosine monophosphate (cAMP), which inhibits tyrosine phosphatase, resulting in tyrosine phosphorylation. $H_2O_2$ specifically induces capacitation via tyrosine phosphorylation, which initiates a cell signalling cascade. Capacitation needs ROS but can also be blocked by catalase (CAT).[23] High amounts of ROS have been shown to stimulate the acrosome reaction, whereas catalase, or superoxide dismutase (SOD) suppresses the acrosome response 3. ROS–modulated tyrosine phosphorylation appears to be the mechanism causing the acrosome response.[24] ROS

can potentially have an impact on motility. When spermatozoa are exposed to ROS, hyperactivation occurs.[25] The flagellum becomes hyper activated as a result of ROS–mediated tyrosine phosphorylation. Tyrosine phosphorylation also promotes sperm–oocyte fusion by increasing sperm membrane binding to the zona pellucida ZP–3 protein, promoting sperm–oocyte fusion.[19]

## DISCUSSION

Though generation of ROS is essential for reproductive function as they act as signalling molecule, oxidative stress (OS) is found to have some detrimental effects over fertility. Redox Imbalance occurs as a result of increased ROS along with decreased antioxidant defence, leading to reduced sperm motility, and sperm DNA damage. There are large amounts of unsaturated fatty acids in the cell membrane of spermatozoa making it more susceptible to the deleterious effects of ROS promoting the peroxidation of lipids which causes increased in intracellular oxidative burden.[26–29] Also, ROS induces cyclic adenosine monophosphate (cAMP) in spermatozoa that inhibits tyrosine phosphatase leading to tyrosine phosphorylation.[23] In particular, $H_2O_2$ stimulates capacitation via tyrosine phosphorylation triggering a cell signalling cascade. Capacitation not only requires ROS, but it can be inhibited by catalase (CAT). It has been described that high levels of ROS promote the acrosome reaction, whereas the presence of CAT, or superoxide dismutase (SOD) inhibits the acrosome reaction. The mechanism of inducing the acrosome reaction appears to be ROS–modulated tyrosine phosphorylation. Motility can also be affected by ROS. Hyper activation is increased when spermatozoa are exposed to ROS.[25] ROS–mediated tyrosine phosphorylation in the flagellum causes hyper activation.[19] Tyrosine phosphorylation also augments sperm membrane binding to the zona pellucida ZP–3 protein, promoting sperm–oocyte fusion. ATP is formed as a result of oxidative phosphorylation that is enzymatically controlled within mitochondria. As a result of this enzymatic reduction, free radicals are formed due to unpaired electrons. The addition of one electron to dioxygen leads to the formation of primary ROS is called the superoxide anion radical which is then converted to secondary ROS directly/indirectly as hydroxyl/peroxyl/hydrogen peroxide (the same applies to nitrogen derivative).[5,30,31] Semen analysis has revealed that leukocytes with neutral predominance and sperm are the two principal sources of free radical's producers.[12] There is a positive correlation between seminal leukocyte number and ROS production.[32,33] Inverse correlations between sperm maturation (teratozoospermia) and NADPH production.[34,24] A study in animal models shows that expose to pesticides and phthalates will not only induce oxidative stress also can lead to the disruption of hypothalamic–pituitary gonadal axis leading to inhibition of gametogenesis and steroidogenesis.[35,36] Smoking can trigger the recruitment of leukocytes to the genital tract leading to the presence of elevated ROS in the seminal fluid.[37,38] Consumption of excess alcohol promotes ROS production and interferes with body's antioxidant defence mechanism. Acetaldehyde is a by–product of ethanol interacts with proteins and lipids leading to ROS production and a decrease in antioxidants.[39]

## CONCLUSION

The reactive oxygen species in sperm can cause DNA damage while also interfering with physiological processes and high ROS levels limit the sperm motility. Males with high levels of ROS who receive assisted reproductive technology therapy are at a higher risk of fertilisation failure or failing to produce healthy babies. Oxidative stress in semen is a serious health issue was not studied clearly to understand its mechanism and incidence. The outcome of future therapeutic trials with antioxidants target and individuals with moderate to severe sperm oxidative stress may aid the physicians to increase the fertility potential in male infertile patients.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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