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ANALYTICAL STUDY ON OXIDATIVE STRESS AND SPERM DYSFUNCTION USING MICRODELETION ANALYSIS

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ABSTRACT

Reactive oxygen species (ROS), which are unavoidable byproducts of oxygen metabolism, are harmful compounds with the potential to have positive impacts through controlling important cell signaling cascades. The most frequent cause of infertility is defective sperm function, which up until recently proved challenging to diagnose and treat. By producing peroxides and free radicals that harm all cell constituents, including proteins, lipids, and DNA, disturbances in the normal redox state of cells can have harmful effects. However, occasionally, a little portion of a chromosome is lost during this process, leading to a microdeletion. Due to the possible negative consequences of high levels of reactive oxygen species (ROS) on sperm quantity, motility, quality, and function, including damage to sperm nuclear DNA, oxidative stress (OS) in the reproductive tract is now a genuine phenomenon and cause for concern. The maturation of sperm depends on ROS.

KEYWORDS Oxidative Stress; Sperm; DNA; Male Infertility and Reactive Oxygen Species (Ros),

INTRODUCTION

Reactive oxygen species (ROS), which are unavoidable byproducts of oxygen metabolism, are harmful compounds with the potential to have positive impacts through controlling important cell signalingcascades. ROS control intracellular signaling cascades at normal physiological levels, controlling crucial physiological processes include sperm maturation, hyperactivation, capacitation, acrosome response, and fertilization. However, issues arise when the ROS concentration goes above the physiological limit. Carbohydrates, nucleic acids, proteins, and lipids are just a few of the cellular constituents that are adversely affected by this redox potential imbalance. The most frequent cause of infertility is defective sperm function, which up until recently proved challenging to diagnose and treat. This challenge was exacerbated in part by our limited knowledge of the elements influencing both normal and aberrant sperm function. Failure to conceive after at least 12 months of unprotected sexual activity is referred to as infertility. Although the reason and severity of infertility can vary depending on a person's geographic location and socio-economic position, it is a condition that affects people from all walks of life worldwide. Eight to ten percent of couples in the reproductive age range seek medical attention, usually after two years of unsuccessful attempts to conceive. According to the WHO between 60 and 80 million couples worldwide have infertility each year, of which 15 to 20 million possibly live in India alone.

Oxidative stress is a result of an imbalance between a biological system's ability to quickly detoxify the reactive intermediates or to repair the damage that results from the systemic manifestation of reactive oxygen species (ROS). By producing peroxides and free radicals that harm all cell constituents, including proteins, lipids, and DNA, disturbances in the normal redox state of cells can have harmful effects. Base damage and DNA strand breakage are results of oxidative stress brought on by oxidative metabolism. The ROS produced, such as O2, OH and H2O2 are what mostly cause indirect base damage (hydrogen peroxide). Additionally, in redox signaling, some reactive oxidative species serve as messengers for cells. Thus, oxidative stress can result in changes to the regular functioning of cellular signaling pathways.

Male infertility is brought on by a number of things, from faulty sperm function to psychological or behavioral issues. The most obvious factor contributing to male infertility that can be identified visually and physiologically is sperm malfunction. Following more research, the terms "immotile cilia syndrome" and "primary ciliary dyskinesia" were proposed (PCD).When a section of a chromosome is missing, an anomaly known as a microdeletion results. In actuality, it means exactly what it sounds like: deletion; micro (small) (taken away). Almost all of our cells include chromosomes with DNA; we receive 23 from each parent for a total of 46; you may recall this from high school biology. Your body breaks down these DNA strands as you reproduce your cells by dividing them during your lifetime in order to make them easier to use. However, occasionally, a very small portion of a chromosome gets cut during this process, creating a microdeletion. The amount and location of a microdeletion will determine how it affects your baby's health and development. While some microdeletions have no negative effects at all, others might result in intellectual disability, issues with motor abilities, or miscarriage.

LITERATURE REVIEW

Natasha Irrera et.al (2017) Oxidative stress is a phenomenon brought on by an imbalance between a biological system's capacity to detoxify these reactive byproducts and the creation and buildup of oxygen reactive species (ROS) in cells and tissues. Although ROS are normally produced as by-products of oxygen metabolism and can play a variety of physiological roles, including cell signaling, environmental stressors like UV, ionizing radiation, pollutants, and heavy metals, as well as xenobiotics like antiblastic drugs all contribute to significantly increased ROS production, which creates the imbalance that results in cell and tissue damage (oxidative stress). Vitamin E, flavonoids, and polyphenols are a few antioxidants that have been studied in recent years for their potential or claimed benefits against oxidative stress.

MeghriKaterji (2017) In intracellular cell signaling and homeostasis, reactive oxygen species (ROS), which are frequent by-products of typical aerobic cellular metabolism, serve crucial physiological roles. Antioxidant defense mechanisms are present in the human body to control the quantities of these free radicals and preserve healthy physiological function. However, oxidative stress (OS) develops when the body's capacity to quickly detoxify ROS is exceeded. Overproduction of free radicals under OS circumstances results in oxidative damage to proteins, lipids, and nucleic acids, which significantly impairs cell health and aids in the development of diseases like cancer. Therefore, OS biomarkers can be used as crucial instruments in determining

the severity of the disease in humans. In the following review, we go over various methods for assessing OS in clinical samples.

Ahmed T. Alahmar (2019) According to current research, male infertility, decreased sperm motility, sperm DNA damage, higher risk of repeated abortions, and hereditary illnesses are all linked to oxidative stress (OS). The results of an analysis of published articles from the years 2000 to 2018 in the PubMed, Medline, Google Scholar, and Cochrane review databases were focused on the physiological and pathological effects of reactive oxygen species (ROS), sperm DNA damage, OS tests, and the relationship between OS and male infertility, pregnancy, and assisted reproductive technique outcomes. The production of ROS is necessary for reproductive health, whereas OS harms conception, pregnancy, and the genetic makeup of the offspring. Furthermore, there is disagreement regarding the best OS test, the kind and length of antioxidant treatment, as well as the patient population that should be the focus.

Roberto Gualtieri et.al (2021) A number of signaling pathways depend on reactive oxygen species (ROS), which are produced at low levels during mitochondrial respiration. When the production of ROS outpaces a cell's capacity to scavenge antioxidants, oxidative stress (OS) results, which causes cell damage. Essential functional traits like motility, capacitation, the acrosome reaction, hyperactivation, and sperm-oocyte fusion are regulated by physiological ROS generation in spermatozoa. According to a substantial body of research, spermatozoa that experience OS during in vitro modification methods in human- and animal-assisted reproduction are more frequently linked to iatrogenic ROS formation and eventually have their sperm function impaired. Studies in animal models provide sufficient proof of the negative effects of sperm OS in vitro and defective fertilization and embryo development, even though a direct correlation between sperm OS and human assisted reproductive techniques.

ROLE OF OXIDATIVE STRESS IN SPERM DNA DAMAGE AS RELATED TO MALE INFERTILITY

The fluidity of the sperm plasma membrane and the integrity of the DNA in the sperm nucleus are both negatively impacted by the overproduction of ROS in the reproductive tract. DNA strand breakage, chromatin crosslinking, and base modifications can all result from oxidative damage to DNA bases. DNA damage brought on by oxidative stress results in pro-mutagenic transformation, which in its most severe form degrades the germ line's quality and inhibits conception. Fertilization is possible when there is less oxidative damage, but the oocyte must first repair any DNA strand breaks before the first cleavage begins. DNA damage in the germ line is mediated via apoptosis and OS (Figure-1). Due to its genetic makeup and inability to repair double-stranded DNA deletions, the Y chromosome is particularly susceptible to DNA damage. Infertile males, especially those with defective seminal parameters, have a higher fraction of sperm DNA damage than fertile healthy men with normal seminal parameters almost always do. Idiopathic infertile males may exhibit typical seminal patterns.

The fact that the most effective ART methods for treating male factor infertility have significant sperm DNA damage is really concerning. In order to lower the possibility of introducing

spermatozoa with strand breaks during ICSI, it is always preferable to use spermatozoa with normal morphology. This isn't always the case because it has been shown that traditional sperm characteristics including sperm count, motility, and morphology aren't necessarily related to the presence of DNA damage. Furthermore, this has important therapeutic ramifications since employing spermatozoa with DNA damage for in vitro fertilization may result in paternal transmission of defective genetic material, which would be harmful for fetal development. These results imply that estimating the proportion of DNA-damaged spermatozoa in fertile and infertile men may be significant, and that methods for identifying and choosing spermatozoa with intact DNA during IVF/ICSI procedures will need to be developed.

Infertile men with varicoceles have recently been found to have much higher amounts of DNA damage in their sperm. The discovery of increased seminal OS in varicoceles patients may suggest that OS plays a significant role in the etiology of sperm DNA damage in these patients. A small number of research have looked at potential therapies to lessen sperm DNA damage, even though Zina et al. showed that varicocelectomy can improve human sperm DNA integrity in infertile males with clinical varicoceles. Avoiding gonadotropins (smoking, medications), as well as hyperthermia (hot tubs, saunas), has been suggested as a possible treatment for sperm DNA damage. According to studies, oral antioxidants given over a relatively short time period can lessen sperm DNA damage. These suggestions, however, are based on limited, uncontrolled research, and no treatment for aberrant DNA integrity has yet been demonstrated to provide positive clinical outcomes.

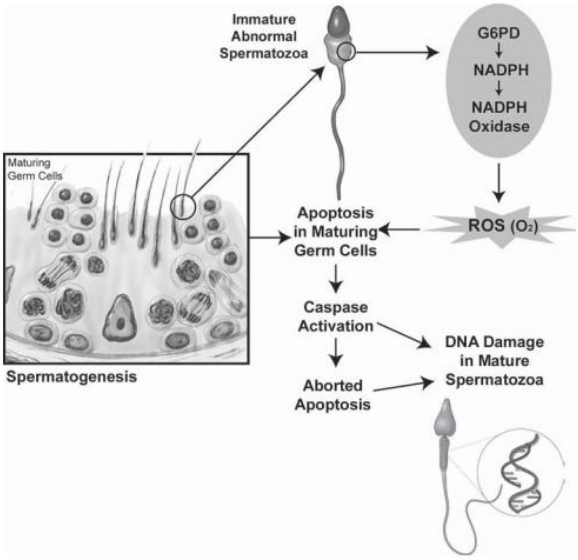

Figure 1 – Mechanistic pathway showing sperm DNA damage due to oxidative stress.

REACTIVE OXYGEN SPECIES AND SEMINAL OXIDATIVE STRESS

Small levels of ROS produced by spermatozoa are essential for numerous physiological functions of sperm, including capacitation, hyperactivation, and sperm-oocyte fusion. However, to keep only a tiny quantity required for regular cell activity, ROS must be continuously inactivated. As a result of the unique structural makeup of semen, excessive ROS production can harm spermatozoa. The main source of antioxidants is the cytoplasm that the spermatozoa release throughout the maturation phase. Remaining cytoplasm creates a cytoplasmic droplet in the middle of the sperm when this process is halted. It is believed that the spermatozoa harboring cytoplasmic droplets are immature and functionally flawed. High concentrations of certain cytoplasmic enzymes (G6PDH, SOD), which are also a generator of ROS, can be found in the remaining cytoplasm. Reduced antioxidant defense is the outcome of cytoplasm deficiency. High ROS and poor sperm quality are related with this process.

Leukocytes, epithelial cells, mature and immature spermatozoa, round cells from various phases of the spermatogenic process, and other cell types can all be found in human ejaculate. These include defective spermatozoa that continuously create free radicals and peroxidase-positive leukocytes. Due to the high concentration of polyunsaturated fatty acids (PUFA) in their plasma membranes, which are easily subjected to lipid peroxidation by ROS, spermatozoa are also particularly vulnerable to the harm brought on by excessive ROS. In sperm, there are primarily two methods for producing ROS. One is the NADH-dependent oxidoreductase system at the level of the mitochondria, and the other is the nicotinamide adenine dinucleotide-dependent oxidase system at the level of the sperm plasma membrane. Immature spermatozoa exhibit a substantial positive association with ROS generation, which is inversely connected with sperm quality. Furthermore, it has been observed that the quantity of mature spermatozoa with DNA damage increases as the quantity of immature spermatozoa increases in the human ejaculate. (Figure2).

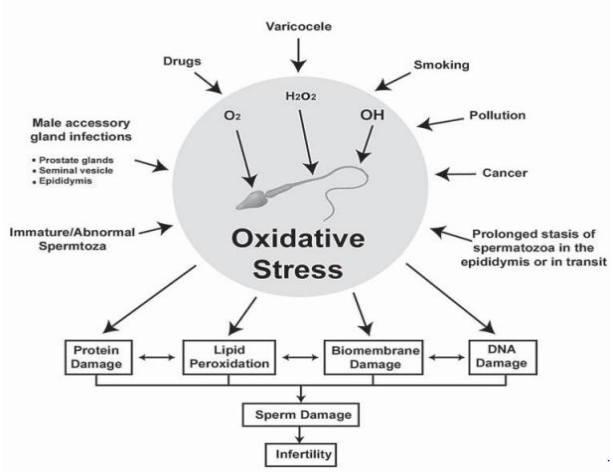

Figure 2 – Association of increasing reactive oxygen species (ROS) production with infertility.

OXIDATIVE STRESS AND EFFECT ON SPERM MOTILITY

When excessive, seminal ROS levels have the potential to be hazardous for both sperm quality and function. Reduced sperm motility, faulty acrosome reactions, and lower fertility have all been linked to increased seminal ROS generation. The kind, volume, and length of ROS exposure all affect how sperm cells behave as a result of ROS damage. The quantities of molecular components including ions, proteins, and ROS scavengers as well as environmental variables like oxygen tension and temperature can affect how much damage is caused by ROS. Low hydrogen peroxide concentrations do not affect sperm motility, but they do inhibit human sperm competence during oocyte fusion, according to Aitken et al. It's possible that ROS levels are not high enough to impact the typical seminal parameters, but they can lead to errors in other fertilization-related procedures, like sperm-oocyte contact. These results point to a possible reason for the idiopathic infertility that some patients can experience even with normal semen characteristics. A chain of events leading to decreased motility includes lipid peroxidation (LPO) of the sperm plasma membrane, which has an impact on the phosphorylation of the axonemal protein and results in sperm immobility.

Effects of ROS on different sperm functions

Physiological functions

As was previously indicated, greater ROS concentrations can negatively impact the quality of semen, which ultimately leads to infertility. Nonetheless, sperm capacitation, hyperactivation, the acrosome response, and sperm-oocyte fusion are all physiological processes that require low and controlled quantities of ROS (Figure 3).

Maturation

The epididymis is where spermatozoa maturation takes place, and it is characterized by changes to the cell membrane, reorganization of surface proteins, as well as nuclear and enzymatic remodeling. Cellular signal transduction systems that control this crucial stage of sperm formation are altered by ROS concentrations. The mammalian spermatozoon's chromosomal DNA is tightly packed because its histones have been replaced by protamine’s of lesser size. To ensure chromatin integrity, protamine cysteine residues form inter- and intramolecular desulphated linkages. ROS may aid in the creation of desulphated bonds, ensuring chromatin stability and safeguarding DNA from harm. In order to protect mitochondria from proteolytic degradation, peroxides may also help with the proper construction of the "mitochondrial capsule," which is made up of a protein network rich in disulfide bonds.

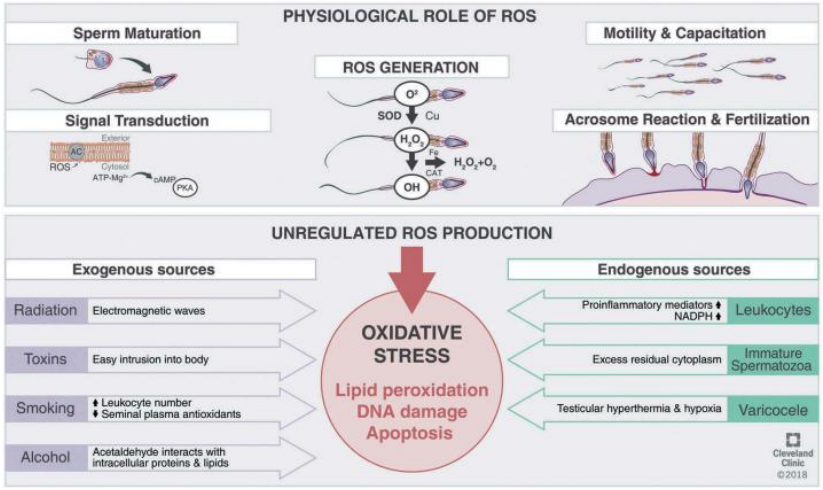

Figure 3. OS in male reproduction.

ROS as signal transducers

ROS support sperm function during several physiological phases including maturation, activation, capacitation, and acrosome reaction because of their tiny size, ubiquitous nature, and brief half-life. The redox control of cysteine residues may be the underlying mechanism of action. The redox states of the thiol groups control the activity of the enzymes. ROS causes the creation of cyclic adenosine monophosphate (cAMP) inside of the cell, which in turn triggers the activation of protein kinase A (PKA) molecules. Depending on the level of spermatozoa maturation, PKA mediates the activation of a variety of downstream pathways.

Motility and hyperactivation

A specific type of sperm motility called hyperactivation is characterized by non-linear motility, high amplitude, enhanced and asymmetric flagellar movement, and increased side-to-side sperm head displacement. It's regarded as a component of capacitation and is necessary for fertilization and effective sperm penetration of the zona pellucida. The hyperactivation pathways in spermatozoa are positively impacted by ROS. The inflow of Ca2+ and HCO3 -, along with the alkalinization of the cytosol and inactivation of an ATP-dependent Ca2+-regulatory channel (plasma membrane Ca2+-ATPase, PMCA), trigger the process of capacitation and hyperactivation. Adenylate cyclase is activated by calcium ions and ROS, specifically O2 -, to produce cAMP. NADPH oxidase is activated by cAMP through PKA activation, which increases the production of ROS. PKA can also activate protein tyrosine kinase by phosphorylating serine and tyrosine residues (PTK). PTK therefore causes tyrosine residues in the fibrous sheath surrounding the axoneme and the cytoskeleton of the sperm flagellum to become phosphorylated. By stimulating PTK and inhibiting phosphoserinephosphatase, which results in the dephosphorylation of tyrosine residues, ROS, particularly H2O2, increase tyrosine phosphorylation. Increased tyrosine phosphorylation is likely the last stage in the hyperactivation mechanism. The main ROS component to this ameliorating impact has been shown to be O2.

Capacitation

Capacitation is the final functional stage of spermatozoa maturation required for the sperm to be capable of fertilizing an ovum. According to the established molecular mechanism, ROS promotes capacitation by increasing intracellular cAMP levels, which then stimulate downstream PKA, which phosphorylates proteins that resemble MEK (extracellular signal-regulated kinase), threonine-glutamate-tyrosine, and fibrous sheath proteins. These signaling cascades cause the sperm to be fully capacitated, preparing it for the acrosome response.

Acrosome reaction

The hyperactivated spermatozoon must traverse the cumulus oophorous, attach to the oocyte's zona pellucida, and release exocytotic proteolytic enzymes to produce a pore in the extracellular matrix in order to assure fertilization. Through the phosphorylation of tyrosine proteins, Ca2+ influx, and an intracellular increase in cAMP and PKA, these acrosome events allow the spermatozoon to penetrate and unite with the egg. Through a variety of mechanisms, including the phosphorylation of three important plasma membrane proteins, ROS has been found to promote activities on the zona pellucida of the spermatozoon.

Sperm–oocyte fusion

After supporting the metabolic cascades of spermatozoa capacitation and acrosome reactions, ROS appear to improve membrane fluidity necessary for effective sperm-oocyte union. In order for PLA2 to cleave the secondary fatty acid from the membrane's phospholipid diglycerols and improve the fluidity of the membrane, ROS inhibits the activity of protein tyrosine phosphatase throughout capacitation.

Pathological functions

Pathological abnormalities develop in essential biomolecules including proteins, nucleic acids, lipids, and sugars (Figure 3) when the highly reactive ROS overwhelm the antioxidant defense mechanisms and disrupt the homeostatic balance between ROS formation and antioxidant activity. Lipid peroxidation (LPO) The plasma membrane of the sperm cell contains significant quantities of lipids, primarily PUFAs with unconjugated double bonds between their methylene groups. The hydrogen is extremely vulnerable to oxidative degradation because the hydrogen-methyl carbon link is weakened by the double bond close to the methylene group. Almost 60% of the membrane's fatty acids are lost during LPO which reduces the fluidity of the membrane, increases non-specific permeability to ions, and inhibits the activity of membrane receptors and enzymes as the intracellular ROS levels rise uncontrollably. Thus, LPO is an autocatalytic, self-replicating chemical reaction that results in aberrant fertilization. The mechanism of this oxidative damage may replicate through three main stages, namely: initiation, propagation, and termination.

Initiation involves the removal of hydrogen atoms from carbon-carbon double bonds, which ignites free radicals. These free radicals then produce lipid radicals, and the latter react with oxygen to produce peroxyl radicals. When metals like copper and iron are present, these peroxyl radicals may once more remove hydrogen atoms from the lipids, advancing the autocatalytic reaction cycle. As oxidative damage spreads, generated radicals interact with consecutive lipids to produce lethal aldehydes as a result of the breakdown of hydroperoxide. In this propagation stage, the synthesis of peroxyl and alkyl radicals proceeds in a cyclical fashion until a stable end product, malondialdehyde (MDA), is generated and the reaction chain reaches its terminus. MDA is a crucial biochemical marker for assessing and tracking the extent of peroxidative damage to spermatozoa as a result. Another LPO byproduct is 4-hydroxynonenal, which is hydrophilic and can seriously impair spermatozoa's proteomic and genomic functions.

DNA damage

Due to enhanced DNA fragmentation, chromatin cross-linking, base-pair (bp) alterations, and chromosomal microdeletions, ROS have a number of harmful effects on sperm nuclear DNA. Because LPO and, more significantly, mutations in the mitochondrial DNA (mtDNA) prevent energy from being generated, ROS are also to blame for decreased sperm motility. Reduced ATP synthesis and increased intracellular ROS production are caused by damage to at least one of the 13 genes that encode for the electron transport chain transporter system in the mitochondria. Adenine and pyridine nucleotides may be deleted by LPO or a thiol group in the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) may be oxidized by ROS to impair sperm motility.

Apoptosis

Cytochrome C can be released when ROS damage the inner and outer mitochondrial membranes. The apoptotic caspases are subsequently activated by this cytochrome C. Because infertile men have high amounts of cytochrome C in their seminal plasma, a sign of severe mitochondrial damage, this process of ROS inducing apoptosis in spermatozoa is visible in infertile men.

Evaluation of seminal OS

In 30-80% of males with unexplained infertility, ROS-mediated damage to sperm is evidently a key contributory factor. Analysis of increased ROS levels in infertile males is therefore quite rational. Despite its enormous importance, consideration of ROS measurement as a crucial component of male infertility assessments is hampered by factors including the discomfort of ROS screening, its high cost, and the lack of a generally agreed efficient analysis approach. In order to measure ROS and OS in the semen of infertile men, more than 30 different assays have been described in the literature (Table 1).

Routine semen analysis

Asthenozoospermia is possibly the best diagnostic for OS, and the routine study of semen characteristics (sperm count, morphology, and motility) enables doctors to make an almost

flawless diagnosis of OS. Seminal plasma's hyper viscosity is accompanied by an increase in MDA and a decline in antioxidant status. Moreover, increased seminal plasma viscosity and strong ROS generation are linked to Ureaplasmaurealyticum infection in the semen.Leucocytospermia, a previously mentioned well-known source of excessive ROS production, may be present if there are a large number of round cells found. However, further tests, such as the peroxidase test, seminal elastase measurement, or cluster of differentiation 45 (CD45, a transmembrane glycoprotein found on the cell surface) antibody staining, should be performed to make sure that the round cells are not immature spermatozoa. Anomaly spermatozoa are characterized by cytoplasmic droplets and disrupted sperm morphology, which cause excessive ROS generation. Last but not least, OS has been associated with poor sperm membrane integrity, which can be determined by the hypo-osmotic swelling test (HOST).

Table 1. OS testing methods

Testing method	Explanation
Routine semen analysis	Parameters of routine semen analysis can suggest the presence of OS, these include: Asthenozoospermia Abnormal morphology and retained cytoplasmic droplets Poor sperm membrane integrity Hyperviscosity Leucocytospermia
ROS measurement by chemiluminescence	The procedure involves a luminometer and a chemiluminescent probe such as luminal (5-amino-2,3,-dihydro-1,4-phthalazinedione). The free radicals contained in the semen sample, produce a light signal reacting with luminal, which is converted by the luminometer to an electric signal (photon).
TAC measurement by chemiluminescence	Luminol is also used for the measurement of the TAC within the seminal plasma, which is quantified against a vitamin E analogue 'Trolox' (a water-soluble tocopherol analogue).
LPO markers	The most commonly measured decay product is MDA, which is mediated by TBA assay where MDA combines with TBA producing a 1:2 adduct, a coloured substance measured by fluorometry or spectrophotometry
Seminal ORP	Also known as the redox potential, this is a measure of the potential for electrons to move from one chemical species to another. The MiOXSYS measures the balance between oxidants and reductants in seminal fluid.

MDA, malondialdehyde; MiOXSYS, Male Infertility Oxidative System; ORP, oxidation–reduction potential; OS, oxidative stress; ROS, reactive oxygen species; TAC, total antioxidant capacity; TBA, thiobarbituric acid.

CONCLUSION

ROS control intracellular signaling cascades at normal physiological levels, controlling crucial physiological processes include sperm maturation, hyperactivation, capacitation, acrosome response, and fertilization. Although the reason and severity of infertility can vary depending on a person's geographic location and socio-economic position, it is a condition that affects people from all walks of life worldwide. Base damage and DNA strand breakage are results of oxidative stress brought on by oxidative metabolism. Particularly, sperm motility is directly impacted by flagellar abnormalities, which frequently result in unsuccessful fertilization. While some microdeletions have no negative effects at all, others might result in intellectual disability, issues with motor abilities, or miscarriage. DNA strand breakage, chromatin crosslinking, and base modifications can all result from oxidative damage to DNA bases. DNA damage from oxidative stress leads to pro-mutagenic changes, and in its most severe form, these changes can have an impact on the quality of the germ line and impede fertilization.

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