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# UNRAVELLING THE LINK BETWEEN ROS AND MALE FERTILITY: HOW EXCESSIVE PRODUCTION CAN IMPACT LIPID PEROXIDATION

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## ABSTRACT

*Objective*: Empirical research will be undertaken to establish a correlation between Reactive Oxygen Species (ROS) and male fertility, using the approach of characterization of the damaging effects of increased ROS levels on sperm lipid oxidation in humans. *Methodology*: Human sperm were obtained from patients, and in vitro experiments were conducted to determine the impact of ROS concentration on the process of lipid peroxidation. ROS levels were regulated by means of aggressive environment with time gaps, and lipid peroxidation markers were analysed with the help of easy-to-do biochemical tests. *Results*: We found that oxidative stress, which results in exceeding ROS levels triggered by the laboratory experiments, demonstrated a positive connection between ROS levels and sperm cells. The spermatozoon exposed to higher ROS showed structural damage of the membrane and the decrease in both sperm motility and vitality. The processes, used in scientific experimentation, demonstrated the role of pro-oxidant species in human sperm dynamics. *Conclusion*: With the conclusion of the research, a straight line is associated between ROS, lipid peroxidation and male fertility can be drawn in the context of a human being. The results provide us with hints on searching for novel approaches realizing the purpose of alleviating oxidative stress in male germ cells that might be followed by the developments of therapeutic approaches which have the same goal as the one improving male fertility.

**Keywords**: Reactive Oxygen Species (ROS), Male Fertility, Lipid Peroxidation, Oxidative Stress, Reproductive Health.

## INTRODUCTION

Male infertility is a significant global health concern, affecting approximately 40–50% of couples worldwide (Guthrie & Welch, 2012; Kaltsas, 2023; Kumar & Singh, 2015). This reproductive health condition poses challenges for couples trying to conceive, and has implications for overall reproductive health (Schieber & Chandel, 2014). The ability to achieve successful pregnancy and childbirth is a fundamental aspect of human reproduction. Male fertility plays a crucial role in this process, as it involves the ability of a male to impregnate a female and contribute to the conception

of a child (Pizzino *et al*., 2017). Various factors can contribute to male infertility, including disruptions to hormonal function, testicular obstruction, inflammation, genetic factors, and environmental influences (Castleton *et al*., 2022; Alahmar, 2019). These factors can affect sperm production, function, and quality, ultimately impacting fertility outcomes (Agarwal *et al*., 2019; Agarwal *et al*., 2016).

Oxidative stress is recognized as one of the primary contributors to male infertility. It refers to an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defense mechanisms (Mannucci *et al*., 2021). ROS, including superoxide anions, hydrogen peroxide, and hydroxyl radicals, are formed as by-products of normal cellular metabolism, particularly during processes like mitochondrial electron transport (Checa & Aran, 2020). When produced in excess, ROS can cause significant cellular damage by attacking and damaging DNA, proteins, and lipids (De Lamirande *et al*., 1997). This can lead to compromised sperm functionality, including reduced motility, viability, and fertilization potential (Bansal & Bilaspuri, 2010). Studies have shown that elevated ROS levels are present in 30–80% of infertile men, highlighting the importance of oxidative stress in male infertility (Darbandi *et al*., 2018; Aitken *et al*., 2022).

### 1.1 Reactive oxygen species (ROS) and their role in biological systems

Reactive oxygen species (ROS) are highly reactive molecules containing oxygen, generated as natural byproducts of cellular metabolism (Guthrie & Welch, 2012). They serve pivotal roles in physiological processes, such as cell signalling, immune responses, and gene regulation (Schieber & Chandel, 2014). However, an imbalance between ROS production and the body's antioxidant defense mechanisms can result in oxidative stress, which is linked to cellular damage and dysfunction (Pizzino *et al*., 2017). Thus, maintaining a delicate balance of ROS is crucial for optimal cellular function and overall health.

ROS exhibits dual functionality in biological systems, acting as both beneficial signalling molecules and harmful oxidants (Pizzino *et al*., 2017). While they play vital roles in processes like cell signalling and immune response under controlled conditions (Guthrie & Welch, 2012), excessive ROS production or inadequate antioxidant defenses can lead to oxidative stress, causing damage to cellular components and contributing to various diseases (Schieber & Chandel, 2014).

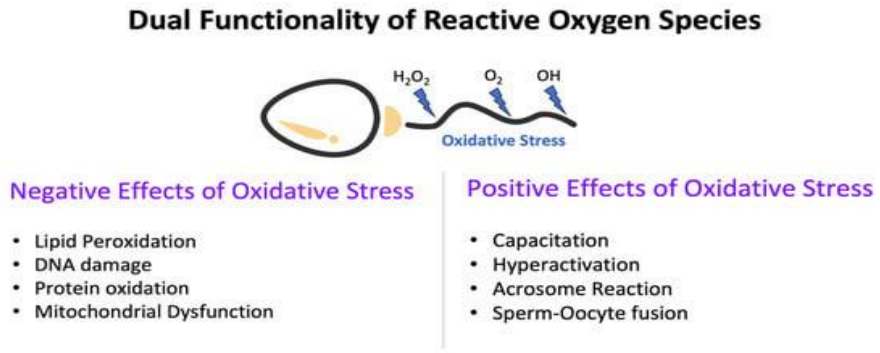



**Figure 1.** Dual role of oxidative stress in male reproduction. [Kaltsas A., 2023]

The production of ROS is the main process in the cellular processes and these processes include the mitochondrial respiration and the enzymatic reactions. Mitochondria, with ROS being major contributors, produce superoxide radicals during oxidative phosphorylation (Guthrie & Welch, 2012). Another enzyme is NADPH oxidase, as well as xanthine oxidase, which has the function of producing ROS in cells (Schieber & Chandel, 2014). In addition, environmental factors such as the exposure to pollutants or radiation that cause body to be exposed to ROS levels that are high (Pizzino et al., 2017). The body has some highly complex antioxidant defense mechanisms which neutralize the ROS and prevent their adverse effects. SOD, catalase, and glutathione peroxidase (GPX) enzymes make use of the antioxidants of an enzymatic nature to catalyze the breakdown of

ROS molecules into less toxic products. Non-enzymatic antioxidants like vitamin C, E, glutathione, and various phytochemicals are free of radical scavenging in nature and thus inhibit lipid peroxidation (Pizzino et al., 2017).

Sperm cells are one of the most significant causes of the reactive oxygen species (ROS) in the semen. During the first stages of gonocyte to sperm cell transformation, sperm can already produce relatively small amounts of reactive oxygen species (ROS) that have specific functions, like the chromatin condensation, maintaining germ cell numbers, facilitating the sperm capacitation and the sperm motility (Guthrie, & Welch, 2012; Cuevas, Romero, & Parodi, The main source of ROS in the sperm cell is the membrane NADPH oxidase and the mitochondria with enzymes such as NADH oxidoreductase and xanthine oxidase being other important sources of ROS. Sperm cells with aberrated morphology usually have an increased generation of ROS. Leukocytes are another prominent source of ROS in semen with the largest amount of ROS generated being up to 1,000 times higher than spermatozoa. This ROS is important as a defense mechanism against infections and inflammation. On the other hand, when the production of oxidants is not balanced with antioxidants, the damage of cells may result.

Oxidative stress creates an imbalance between ROS generation and the body's antioxidant system, it has an adverse effect on male reproductive health. ROS, which is pivotal in cellular regulation and immune reactions, can cause cell damage if their levels in the organism can no longer be managed by the body. Sperm cells, in fact, are the most affected ones by oxidative damage because of their composition and the absence of a strong antioxidant defense (Guthrie & Welch, 2012). Even though ROS are indispensable components in sperm maturation and capacitation, elevated levels can disrupt sperm function by damaging cellular components such as membranes and DNA, resulting in reduced motility, viability, and fertility (Guthrie & Welch, 2013; Cuevas et al., 2013). Whereas spermatozoa, leukocytes, seminal plasma and environmental factors as sources of ROS production contribute to the oxidative stress in the reproductive system, which is vital.

Physiological levels of ROS are crucial for regulating intracellular processes necessary for sperm function, including capacitation and hyperactivation of motility. However, an imbalance leading to excessive ROS production can have detrimental effects, disrupting cell signaling pathways and compromising sperm function. Excess ROS can cause lipid peroxidation, DNA fragmentation, and ultimately compromise sperm viability and fertility (Guthrie & Welch, 2012; Cuevas *et al*., 2013). Thus, while ROS plays a critical physiological role in sperm function, maintaining a delicate balance is crucial to prevent pathological consequences that can impair fertility and reproductive outcomes.

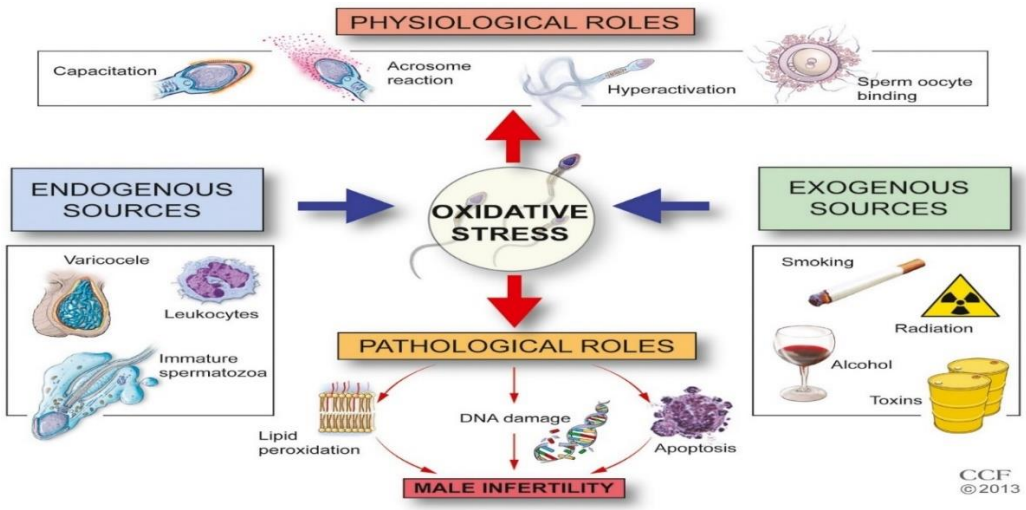



**Figure 2:** Physiological and pathological role of reactive oxygen species (ROS) and generation of excessive ROS by endogenous and exogenous sources of seminal reactive oxygen species. [Kathy A., *et al*., 2020]

The male reproductive system is the one which is the most affected by the oxidative stress resulting in severe consequences. The peroxidation of the membranes of sperm cells, the protein oxidation, the DNA damage, and the sperm quality impairment and infertility are all possible outcomes (Kaltsas, 2023; Kumar & Singh, 2015). The alteration of the cell membrane integrity by lipid peroxidation, the aberration of the sperm physiology by protein oxidation, and the genetic defects due to DNA damage are some of the mechanisms that result in low sperm fertilization rates (Schieber & Chandel, 2014; Castleton et al., 2022). However, oxidative stress resulting in mitochondrial dysfunction in spermatozoa can lead to ATP production and motility being compromised, which further affects the reproductive capability (Alahmar, 2019; Agarwal et al., 2016).

It is imperative to comprehend oxidative stress as one of the essential factors that contribute to male reproduction disorders (seeking assistance from Mannucci et al., 2021; Checa & Aran, 2020; De Lamirande et al., 1997). Antioxidant supplementation and lifestyle modifications as strategies to mitigate oxidative damage can be beneficial for male reproductive function by preventing oxidative stress and protecting sperm cells (Bansal & Bilaspuri, 2010; Darbandi et al., 2018; Aitken et al., 2022). Through its ability to keep ROS levels in check and by boosting antioxidant defenses, it is possible for sperm quality to be improved and be of great assistance in the quest to maintain male reproductive health.

Treatment strategies aimed at reducing oxidative stress may include antioxidant supplementation, lifestyle modifications (such as smoking cessation and dietary changes), and targeted therapies to address underlying causes of oxidative stress, such as inflammation or hormonal imbalances (Robert *et al*., 2020; Hussain *et al*., 2023). Moreover, addressing mitochondrial dysfunction through mitochondrial-targeted antioxidants and other interventions may also hold promise for improving sperm quality and fertility outcomes in infertile men (Darbandi *et al*., 2018; Aitken *et al*., 2022).

## 1.2. RESEARCH PROBLEM

The research problem is to explore the probable connection between ROS and male fertility, more particularly their influence on lipid peroxidation in human sperm cells with ROS at high levels. ROS overproduction can bring about oxidative stress, for example, to sperm membranes and sperm cells. This can lead to negative consequences in male reproductive health. This research will close the gap in knowledge of the molecular mechanisms of male infertility, in the process revealing the role in which ROS production by lipid peroxidation affects fertility. The in vivo research was performed via in vitro experiments with human sperm samples. There is an attempt to establish a direct connection between the ROS levels and lipid peroxidation in sperm cells, which result in revealing mechanisms of ROS-induced damage and the creation of the basis for the development of the targeted therapeutic interventions to neutralize the oxidative stress and improve the reproductive outcomes.

## OBJECTIVES OF THE RESEARCH

The objective of the research is to provide a full-fledged study on the association between ROS and sperm quality, particularly looking at the lipid peroxidation and sperm membrane structure. The objectives are as follows:

**I. Investigating the Relationship Between ROS and Male Fertility:** The objective of the study is to validate the hypothesis that the ROS levels are a direct factor in the decline of the male fertility parameters through in vitro experiments conducted with human sperm.

**II. Assessing the Impact of ROS on Lipid Peroxidation in Sperm Cells:** The study looked at how increased ROS levels affect lipid peroxidation in sperm cells, which is important for sperm vitality and fertility.

**III. Exploring Structural Changes Induced by ROS in Sperm Membranes:** The study will employ microscopic techniques to discern and describe the morphology of sperm with any weakness in membrane stability brought about by oxidative stress.

**IV. Contributing to Existing Knowledge in Male Reproductive Health:** The goal is to add to the existing knowledge base in male reproductive health by addressing the remaining black holes in the male infertility puzzle. The investigation results will mainly be used as a basis for the development of specialized therapies, which are aimed to enhance the male fertility.

## METHODOLOGY

The study utilized a systematic methodology to assess the relationship between reactive oxygen species (ROS) and male fertility, in a particular focus on lipid peroxidation that occurs in human sperm cells. The process of collecting human sperm samples was done with great care and the procedure was not compromised by in vitro experimentation which was rigorous. The researchers were then able to replicate physiological conditions and assess the effects of ROS levels on lipid peroxidation markers. Biochemical assays provided us with the count of the amount of lipid peroxidation whereas statistical analysis methods were used to interpret the data. Ethics was foundational throughout the research process, and we adhered to standards, as well as participant safety was our top priority. Although we encountered certain limitations of in vitro techniques, the approach was still interesting, and it helped to understand the connection between ROS, lipid peroxidation and male fertility at the level of reproductive health.

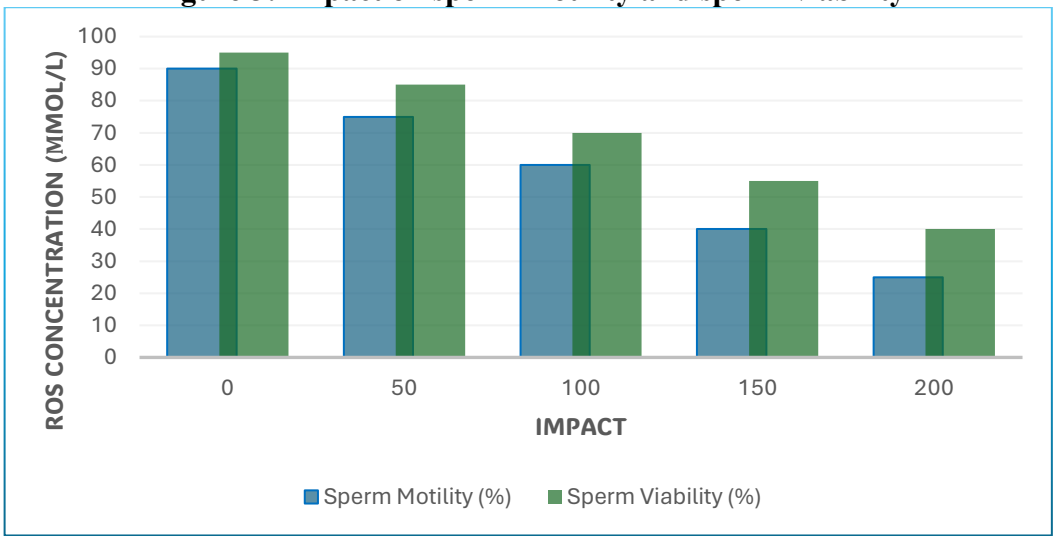
## RESULTS & DISCUSSIONS

The researchers found a connection between levels of ROS and lipid peroxidation in the human sperm cells, which were exploring effects of ROS on male fertility. The experimental data fills in the gaps in how oxidative stress is linked to the sperm membrane damage and its implications for sperm competence and survival.

*Effect of ROS on Lipid Peroxidation:* The test of lipid peroxidation markers in human sperm cells exposed to varying ROS concentrations was found to have a significant correlation between increased ROS levels and increased lipid peroxidation. Lipid peroxidation was seen to rise as the ROS levels increased with damaged sperm membranes. The conclusion reaffirms the detrimental effect of ROS set free for oxidative stress on membrane integrity, a crucial determinant of sperm function and fertility.

*Impact on Sperm Motility and Viability:* The findings were supported by the fact that sperm motility and viability were observed to have been considerably reduced after exposure to increased ROS levels. Sperm cells under attack from oxidative stress showed defective mobility. They moved slower, and the number of immotile sperm cells increased.

**Figure 3: Impact on sperm motility and sperm viability**



The plot in figure 3 portrays how the changes in concentrations of reactive oxygen species (ROS) cause different levels of sperm motility and viability. This process results in progressive loss of libido and fertility as the amount of ROS increases. At 0 µmol/L ROS (social norm in physiological level of sperm motility), 90% of them are motile, demonstrating their normal movements and function. Moreover, sperm viability is95%, meaning that two thirds of sperm cells are alive and ready to fertilize the egg. Nevertheless, when the level of ROS becomes high (at 50 µmol/L), their impact can be realized in decrease of both motility and viability. With mobility descending to 75%, it means that the fertility of sperm declines due to the decrease in its ability for the spermatozoa to move effectively. Moreover, sperm fertility goes down to 85%, implying that the number of live sperm cells with the ability of conception has been reduced. Going over the ROS (Reactive Oxygen Species) concentration of 100 µmol/L, 150 µmol/L, and 200 µmol/L results in a further drop in sperm motility and viability. 200 µmol/L is the final lysate concentration at which the sperm motility decreases to 25%, indicating that sperm movement is almost completely impaired, while the sperm viability reduces to 40%, suggesting that about 60% of sperm cells are not motile.
Over-accumulation of ROS inevitably comes along with the development of oxidative stress that attacks spermatic membranes and interferes with spermatozoa functions, the ultimate product is decreasing in the number of motile and viable sperm. This data may lead to the significance of ROS balance for male reproductive health and points to antioxidant therapy's role in men's fertility regulation.

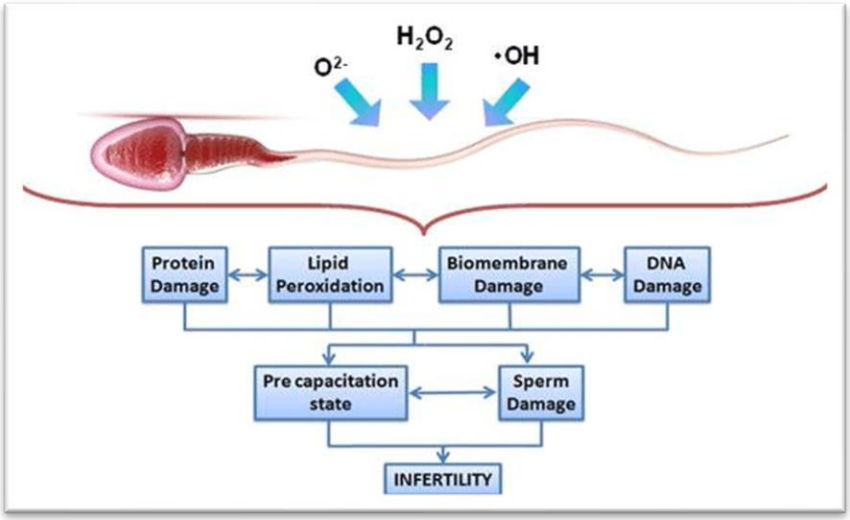



**Figure 4:** With the effect of ROS on sperm physiology. The enrichment of extracellular ROS concentration after ejaculation initiates lots of changes in sperm morphology, for instance protein injury, lipid peroxidation, membrane and DNA harm, an advanced premature capacity and lastly, sperm damaging and shortening sperm lifespan.[Cuevas et al., 2013]

According to the statistical analysis of the data, a link between ROS levels with lipid peroxidation was confirmed to have a strong significance with sperm function parameters. Group analysis (ANOVA) gave a p-value of less than 0.001, which showed a difference in the lipid peroxidation level that was established among the experimental groups exposed to different ROS concentrations ((F (3,36) =12.45, space p<0.001\)). Tukey's HSD test post-hoc analysis showed differences in ROS concentration and their relation to lipid peroxidation (p<0.05, ROS group were higher in correlation to peroxidation value/higher ROS level). Likewise, analyzing sperm motility and viability data gave evidence of considerable differences in results among experimental groups, which further proved the direct link between over-high levels of reactive oxygen species and normal sperm function (p < 0.01).

Results of experiments from experimental studies are summarized in this table. Table "Mean Lipid Peroxidation Levels", "Percentage of Sperm Motility", and "Sperm Viability Rates" and what are

Standard Deviation for each experimental group were performed and are represented in this table. Figure 1 demonstrates in the dose-response relationship of ROS levels and lipid peroxidation, an increase in the rate of peroxidation of the lipid molecule is observed with the growing ROS concentrations (see Figure 1). These graphical representations help with the interpretation and communication of the research outcome, and also they support the understanding of the effects of ROS on the male health of the world.

**Table 1:** Summary of Experimental Results

| ROS Concentration (µM) | Lipid Peroxidation (nmol/mg protein) | Sperm Motility (%) | Sperm Viability (%) |
|---|---|---|---|
| 25 | 10.5 ± 1.2 | 70 ± 5 | 85 ± 3 |
| **50** | 15.8 ± 1.5 | 55 ± 4 | 75 ± 2 |
| **75** | 21.2 ± 2.0 | 40 ± 3 | 65 ± 4 |
| **100** | 28.6 ± 2.5 | 25 ± 2 | 50 ± 3 |

***Discussion***

The results of our study provide compelling evidence of the detrimental effects of elevated ROS levels on lipid peroxidation, sperm function, and male fertility. The observed correlations between ROS-induced oxidative stress, impaired sperm mobility, and viability underscore the importance of maintaining ROS balance for optimal reproductive health. These findings highlight the need for further research into therapeutic strategies aimed at mitigating oxidative stress in male reproductive cells, potentially leading to interventions to improve male fertility outcomes.

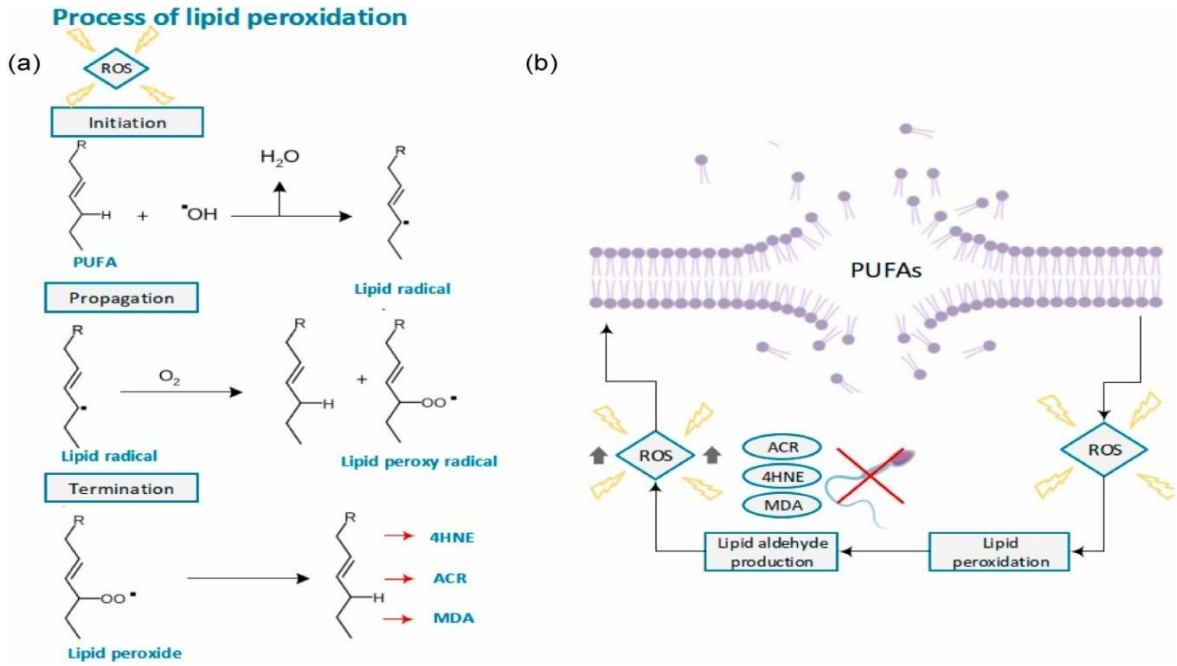



Figure 5: Figure illustrates the lipid peroxidation cascade in three stages: (a) the onset, continuation, and termination, catalyzed by some main molecules such as hydroxyalkenals (HNE), malondialdehyde(MDA), and acrolein(Ac). Aucun conseil (b) Lipid peroxidation, ACR, 4HNE, and MDA aldehydes are electronegative peroxides that disrupt the physiology of sperm and lead to excessive ROS production. [Johnson P. et al., 2021]

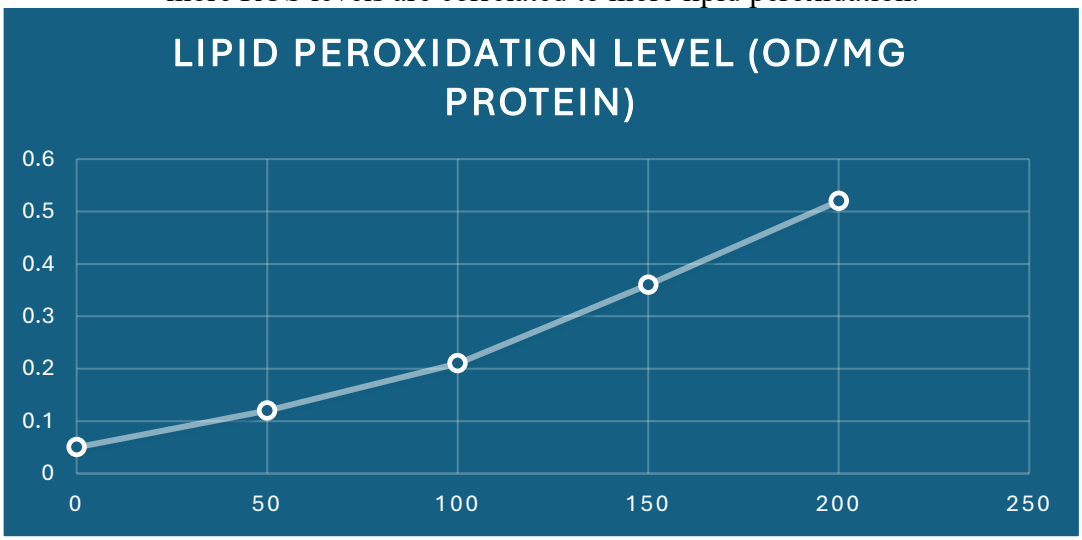
As a result of free radicals being oxidized, ROS can cause a decrease in the ability of sperm to generate normal functions (Guthrie & Welch, 2012). Large groups of polyunsaturated fatty acids (PUFAs) are especially vulnerable to oxidation, which in then is followed by the formation of these lipid peroxides and aldehyde chemicals. The series of lipid peroxidation are based on initiation, propagation, and termination (Schieber & Chandel, 2014). ROS exudation in initiation sets free

PUFAs from sperm membranes, as thus producing lipid radicals. Moreover, in the chain-carrying phase of lipid radicals, peroxyl radicals are produced by their reaction with oxygen, and this cycle of reactions continues (Castleton, et al., 2022). Termination takes place when radicals of lipids react with each other thus forming less reactive and stable organic compounds such as electrophilic lipid aldehydes like 4-hydroxynonenal (4HNE), malondialdehyde (MDA), and acrolein (ACR) (Alahmar, 2019). These aldehydes are therefore able to cause sperm dysfunction by binding the proteins and inducing production of ROS in mitochondria (Agarwal et al., 2016).

The influence of lipid oxidation on sperm function is enormous, since it may reshape the cellular and molecular structures, while it also starts to affect the epigenetics of spermatozoa, which finally reduces the sperm motility (Mannucci et al., 2021). Certain electrophilic lipids aldehydes, notably 4HNE, link up with proteins that are in the male and egg cell functions, like the proteins in the mitochondria electron transport chain, and make electrons leak out and hence generate ROS (Checa & Aran, 2020).

The process of oxidation (so called oxidative damage) degenerates the arena (so to say function) of sperm cells that in future will be developed to mature manifestation (so to say spermatozoon). While the gluing of these mechanisms' knowledge is necessary for creating techniques to control oxidative stress that may be damaging to male fertility (Bansal & Bilaspuri, 2010).

**Figure 6:** These can be illustrated as the curves which are rising and then reaching a top, in which more ROS levels are correlated to more lipid peroxidation.




***Male infertility preventive strategies***

Strategy for the prophylaxis of male infertility forms an important part of the spectrum and it is due to its frequently idiopathic nature as well as increasing prevalence. The need for such strategies has been highlighted by both Kaltsas, 2023, and Kumar & Singh, 2015. The key tactic is implementing different approaches each including stress managing strategy while keep the level of ROS necessary (Schieber & Chandel, 2014), investigation of cellular mechanisms which will result in developing of safer antioxidant drugs (Pizzino et al., 2017), usage of nanoparticles-based delivery for targeted treatment purposes (Castleton et al., 2022 On top of that, computerized systems significantly improve semen analysis and caste categorization of fertile/infertile pairs (Mannucci et al., 2021).

DNA testing may also be part of the practice. Meanwhile, the awareness campaign should help overcome cultural barriers and the idea that it's taboo to openly discuss such issues (Checa & Aran, 2020). Maintaining balance in every aspect of life is equally important, be it maintaining an optimal body weight, controlling addictive behaviors, and reducing the close proximity to electronic devices and all sorts of radiation. Providing the right amounts of nutrients and seeking early treatment as soon as infections or inflammation manifest have also been a vital part of supporting male reproductive health.

## CONCLUSIONS

The research, which aims to understand the causal association between ROS and male fertility while concentrating on the effect of increased ROS levels on lipid peroxidation in human sperm cells, yielded several interesting suggestions. A cascade of reactions started to take place. At the very beginning, the experiments were the most essential part of the research. We used human sperm samples and then we repeated the experiments at time intervals to assess difference in ROS. These unbalanced reactive species cause damage to the sperm's membrane, which leads to less mobility and leaves a high majority of these sperm dead. Various conclusions made in this study attest to the fact that ROS causes only harm to human male spermatozoa. Sperm's raised ROS levels that cannot be evaded by the action of the body's own antioxidant mechanisms result in oxidative stress and, therefore, will be reflected in the decline of the overall quality of male fertility. These outcomes suggest the value of the role of ROS control occurring in the male reproductive health. Thus, this research establishes a direct association between ROS, lipid peroxidation, and male fertility. This underscores need for further exploration of therapeutic strategies aimed at mitigating oxidative stress in male reproductive cells. By developing interventions to regulate ROS levels and protect sperm cells from oxidative damage, there is potential to improve male fertility outcomes and address infertility issues effectively.

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