PSM
Microbiology

Review Article **2023 | Volume 8 | Issue 3 | 68-81**

Open Access

Article Information

Received: November 20, 2023

Accepted: November 26, 2023

Published: November 30, 2023

Keywords
Male,
Infertility,
iNOS,
Diagnoses,
Therapy.

Authors' Contribution
AJA conceived and designed the study; wrote, and revised the paper.

How to cite
Alkhatib, A.J., 2023. Increased Expression of Inducible Nitric Oxide Synthase (iNOS) in Sperms as an Indicator of Male Infertility: New Medical Hypothesis. PSM Microbiol., 8(3): 68-81.

***Correspondence**
Ahed J Alkhatib
Email:
ajalkhatib@just.edu.jo

Possible submissions
Submit your article

Increased Expression of Inducible Nitric Oxide Synthase (iNOS) in Sperms as an Indicator of Male Infertility: New Medical Hypothesis

Ahed J Alkhatib[1,2,3]*

[1]Department of Legal Medicine, Toxicology and Forensic Medicine, Jordan University of Science and Technology, Irbid, Jordan.
[2]International Mariinskaya Academy, Department of Medicine and Critical Care, Department of Philosophy, Academician Secretary of the Department of Sociology, Irbid, Jordan.
[3]Cypress International Institute University, Texas, USA.

Abstract:
The number of men who are unable to father children is on the rise all around the world. The evaluation of neurological and inflammatory characteristics of the male reproductive system was the primary objective of this particular investigation. In addition to this, we desired to impart our knowledge to others employing the inducible nitric oxide synthase synthesis in sperm. Nitric oxide (NO) has been shown to play beneficial functions in a variety of aspects of male reproduction. On the other hand, NO that is produced by iNOS has the potential to have negative effects and may play a role in male infertility.

Scan QR code to visit this journal.

©2023 PSM Journals. This work at PSM Microbiology; ISSN (Online): 2518-3834, is an open-access article distributed under the terms and conditions of the Creative Commons Attribution-Non-commercial 4.0 International (CC BY-NC 4.0) licence. To view a copy of this licence, visit https://creativecommons.org/licenses/by-nc/4.0/.

INTRODUCTION

A serious worldwide health problem, infertility is defined as the failure to conceive after at least a year of frequent, unprotected sexual activity. More importantly, it is a rare medical ailment because two people are affected rather than just one. A couple's path to motherhood is not always as straightforward as it is made up to be, especially in today's society. Presumably, the male factor accounts for around 50% of all infertility cases, which affects 7% of all males worldwide (Krausz and Riera-Escamilla, 2018).

Clinical infertility is defined as the inability of a couple to conceive after 12 months of unprotected sexual activity that does not result in a pregnancy (Evers, 2002). This definition applies to couples who have tried to conceive but have been unsuccessful. There have been reports that the global male fertility rate is on the decline (Sengupta *et al.,* 2017), and the World Health Organization (WHO) indicates that there are between 45 and 80 million infertile couples across the globe (Agarwal *et al.,* 2015). In addition, there are claims that the global female fertility rate is on the rise (Bold and Swinburne, 2022). It is estimated that one couple out of every seven in the United Kingdom has trouble conceiving (NICE, 2017a). It is also believed that male factor infertility accounts for 40–50% of all cases of infertility (Kumar and Singh, 2015). It is estimated that 15% of all cases of infertility in men can be attributable to a low sperm count (NICE, 2017a). This number is derived from estimates made by several researchers. Infertility is almost always brought on by an inadequate number of viable sperm. Infertility can have a variety of root reasons, including but not limited to poor sperm shape, reduced testosterone levels, genetics, weaker motility, decreased DNA stability, and medical conditions (NHS, 2023a).

Genetic factors in male infertility

Even while it is obvious that genetic factors contribute to male infertility, diagnostically useful genes are conspicuously lacking, and at least 40% of cases are categorized as idiopathic (Tüttelmann *et al.,* 2018; Oud *et al.,* 2019). Although the precise processes are still unclear, the decline in semen quality over the previous fifty years suggests the impact of changed lifestyles and exposure to dangerous environmental factors (Levine *et al.,* 2023). The ability to simultaneously investigate many genes and find new biomarkers is provided by next-generation sequencing (NGS) technologies in the modern era of genomics (Kyrgiafini and Mamuris, 2023).

Amor and Hammadeh (2022) carried out a comprehensive research investigation to determine the role that mitochondrial polymorphisms play in male infertility. The researchers point out that mtDNA mutations have recently been related to a broad variety of illnesses, including diabetes mellitus type 2, multiple cancers, and other conditions; nevertheless, the link between mtDNA mutations and male infertility is still largely unknown at this moment. They describe a huge number of variants in their review. The majority of these variants have an influence on the production of energy and the performance of the machinery involved in mitochondrial oxidative phosphorylation (OxPhos), which ultimately leads to a reduction in sperm motility. Additionally, they draw attention to specific occurrences of paternal inheritance of mtDNA, a condition that might have significant consequences when non-normozoospermic males utilize assisted reproductive technologies to conceive and give birth to children. Kaltsas et al. (2023) discussed the concern of how aging fathers impact child health or fertility in the contribution that they made to their special issue. This is a significant subject, especially because, over the past several years, a rising number of malformations have been observed in pregnancies carried by couples in the middle years of their lives. The authors review the information that is currently available and demonstrate that there is a link between paternal age and declining sperm quality in addition to impaired testicular function. This conclusion is reached by demonstrating that there is a

correlation between paternal age and decreasing sperm quality. In addition, it would appear that a woman's fertility levels begin to fall after she reaches the age of 35; however, very little research has been done on this subject. Genetic and epigenetic alterations in sperm, such as telomere length and chromosomal abnormalities, are associated with older fathers and may be the reason for decreased sperm quality. An increased risk of infertility may be the root reason for this drop in sperm quality. When taken together, all of these characteristics can even make it less likely that a pregnancy can be successfully carried to term with the assistance of assisted reproductive technology. Children whose fathers are older than usual have a greater probability of acquiring health problems and disorders such as autism and schizophrenia. This is the last point to consider, but it is an important one. After taking into consideration all of these aspects, the authors conclude that there is an increasing need for married couples to receive aid and consultation during the years in which they are having children.

In addition, six original papers provide new information on several issues relating to male infertility. These contributions are presented below. Two of these papers are concerned with studies that involved participants from the human population. An intriguing investigation was carried out by Punjabi et al. (2023) in males who were classified as fertile (F), subfertile normozoospermic (SN), or subfertile non-normozoospermic (SN-N). The objective of the research was to determine the degree of genomic instability that was present in each of the three groups by employing a variety of tests, such as chromatin maturity, sperm DNA fragmentation (SDF), and sperm aneuploidy. Unpredictably, even normozoospermic males whose sperm parameters are higher than the lower standard standards established by the WHO might nevertheless have spermatozoa with DNA damage in them. According to this discovery, the lower reference limits lack sufficient sensitivity to accurately estimate the possibility of becoming pregnant. To be more specific, the findings of this study revealed that there was not a statistically significant difference that could be detected between any of the three SDF groups, as well as the quantity of sperm with aneuploidy did not significantly differ between the SN and SN-N groups. When compared to the F group, the SN group displayed significantly higher levels of chromatin hyperstability and significantly lower levels of chromatin decondensation than the F group. These fascinating results highlight the need for continued research aimed at either enhancing the sensitivity and accuracy of semen analysis or at identifying additional parameters that may be incorporated into future guidelines from the World Health Organisation to more accurately identify men with reproductive issues. Both of these goals are important, but the former is more pressing at this time. Therefore, it has been proposed that genomic variability may be an independent trait of sperm quality, capable of detecting difficulties that sperm analysis alone is not capable of detecting. Sperm analysis alone is not capable of detecting these problems. Researchers Kyrgiafini et al. (2022) analyzed the genomes of individuals who had been diagnosed with teratozoospermia by using an approach called whole-genome sequencing. Next, they conducted a comparison of the genomes of men with this condition to those of men with normozoospermia, which allowed them to identify several genetic variations and genes that may be involved in the pathogenesis of this disorder. These results were presented in a paper that was published in the journal Science. To be more specific, when the variants were prioritized and the bioinformatics analysis was performed, they discovered high-impact variants in about sixty genes. Others of these genes are being related to teratozoospermia for the very first time, and several other genes have previously been linked to male infertility. This paves the way for future research to be carried out in this area and offers up new avenues of inquiry. Remarkably, pathway enrichment analysis reveals that the extracellular matrix (ECM) may play a role in the course of teratozoospermia. This is notable since it suggests that the ECM may be involved. This

finding is important to notice. Even though a large amount of research has been conducted in the past documenting how significant of a part ECM plays in the process of spermatogenesis, there have not been any studies that precisely link teratozoospermia with the extracellular matrix. In conclusion, the findings of this study highlight the significance of utilizing whole-genome sequencing to reveal genetic variants that would be missed by other methods. These findings were found in the context of a study that looked at the effects of a drug on a patient's immune system.

Numerous chromosomal abnormalities, including microdeletions on the Y chromosome, gene mutations, and single nucleotide polymorphisms (SNPs), separately, have been related to an elevated risk of male infertility (Shi *et al.,* 2011).

Although significant work has been done in the past to identify genes connected to male infertility, there is a critical lack of data on the armament of SNPs. Nevertheless, they are already accessible. Men are only hemizygous for X-linked genes since they only have one copy of the X chromosome, making it more likely that these genes will quickly develop in response to selective pressure. This has led to the conclusion that genes on the X chromosome are strongly linked to male infertility (Ghadirkhomi *et al.,* 2022).

One of these genes with some type of connection to the testis or the sperm is the ubiquitin-specific protease 26 (USP26) gene. Xq26.2 is the location of USP26's single exon, which is important for the spermatogenesis process. The removal of histones, control of protein turnover during meiosis, prevention of germ cell apoptosis, and aid in the proliferation and differentiation of spermatogonial stem cells during mitosis are all responsibilities of this exon (Lahav-Baratz *et al.,* 2017). To date, scientists have found more than twenty different polymorphisms within the USP26 gene. Several of these mutations, including a cluster haplotype (370-371insACA, 494T>C, and 1423C>T), are reportedly directly linked to male infertility, according to a study done by Zhang and his colleagues (Zhang *et al.,* 2015).

Epigenetics and male infertility

In recent years, there has been an increasing amount of scholarly research focused on examining the role of epigenetics in spermatogenesis and its potential impact on male infertility (Nasri *et al.,* 2017; McSwiggin and O'Doherty, 2018). The concept of "epigenetics" comprises the various mechanisms involved in regulating gene expression, which occur independently of any changes to the underlying DNA sequence. The mechanisms encompassed in this context are DNA methylation, posttranslational changes of histones, and control mediated by microRNAs (miRNAs) (Stomper *et al.,* 2021). Throughout spermatogenesis, a series of discernible epigenomic and epigenetic modifications take place, culminating in the formation of completely mature spermatozoa possessing unique and specialized attributes. The alterations described facilitate significant restructuring of the chromatin configuration inside the spermatozoa (Jenkins and Carrell, 2011). Therefore, it can be shown that the process of spermatogenesis is highly vulnerable to epigenetic alterations. The occurrence of deviations in the DNA methylation process during spermatogenesis possesses the capacity to elicit abnormal expression of particular genes, hence potentially leading to infertility (Aston *et al.,* 2012). The exact cause of many epigenetic abnormalities that result in male reproductive failure is not yet fully understood. However, it is possible that a significant number of cases of unexplained infertility in men could be linked to DNA methylation mechanisms (Rotondo *et al.,* 2021).

Infertility assessment

An examination of the patient's physical health as well as an examination of the testicles are both required phases in the process of identifying whether or not a man is fertile (Khatun *et al.,* 2018). When conducting an examination of sperm, it is common practice to take into account the following aspects: the

amount of ejaculate that was produced, the number of sperm, the mobility of the sperm, and their morphology (Vasan, 2011). It has been shown that DNA fragmentation considerably raises the risk of miscarriage, but it is not commonly evaluated as part of fertility exams or included as part of the routine study of semen (McQueen *et al.,* 2019).

The World Health Organization (WHO) published the parameters for the evaluation of sperm in laboratory management for the evaluation of human sperm (WHO, 2010). Men were not given access to a technology that could determine whether or not they were infertile until 1980 when the first laboratory handbook for the examination of human sperm was released by the World Health Organization (WHO, 2021). In 2010, the levels of the parameters that are used to identify infertility had significant decreases from the previous year. The sperm count dropped by a significant amount, going from 20 million/mL to 15 million/mL (WHO, 2010).

According to the research that Fisch and Goluboff (1996) conducted, the total number of sperms might vary greatly from one geographic region to another. The procedure of creating global specifications that have not employed a cohort from all over the world encounters difficulties as a result of this issue. As a result of this, the reference numbers might not be relative or indicative of male fertility in a lot of different parts of the world, nor might they be typical of ethnic minority populations that reside in Western countries. In addition to this, it's possible that there might not be typical populations of ethnic minorities who live in Western countries. It is projected that a minimum of 15% of couples who are infertile will now be labeled as falling within normal limits, which will result in a large reduction in the number of couples who are sent for treatment (Murray *et al.,* 2015). This will lead to a considerable reduction in the number of couples who can conceive naturally as a result of this change. The sixth edition of the WHO Laboratory Handbook for the assessment of human sperm contains concerns about the upper and lower bounds of sperm parameters (WHO, 2021) that were addressed by the researchers who conducted the study. This guide is applied in the analysis of human sperm to conclude. The researchers wanted to stress that the 5th edition was the first publication to contain a complete data set to specify the sperm count, concentration, motility, and morphology centiles (WHO, 2021). They did this by pointing out that it was the first publication to contain the publication. They made note of the fact that this was the situation because the fifth edition was the first publication to include the publication. However, the small amount of data collected highlights how important it is to carry out high-quality research on sperm analysis in developing countries.

The National Health Service in the United Kingdom (NHS, 2023b) recommends that a couple seek an appointment with their primary care physician (NICE, 2017b) if they have been trying to conceive for one year but have been unsuccessful. In addition, the National Health Service (NHS, 2023b) recommends that those who have been trying to conceive for at least a year but have been unsuccessful should have their fertility checked. At this phase, information is given on lifestyle choices that may be changed to increase the likelihood of getting pregnant, such as drinking alcohol, smoking, using drugs recreationally, being obese, taking coffee, and wearing tight pants (NICE, 2017b). Some of these activities include drinking alcohol, using recreational drugs, smoking, and consuming alcohol. Some examples of these practices are smoking cigarettes, drinking alcoholic beverages, engaging in recreational drug use, and consuming alcohol. It would appear that hardly a single Western nation has issued food and lifestyle recommendations for men before conception (Shawe *et al.,* 2014).

Though experts in the healthcare field seldom offer more detailed dietary or lifestyle recommendations, the data that is available reveals that there are significant changes in sperm parameters as a result of food and supplementation (Salas-Huetos *et al.,* 2017).

The subject is still considered taboo (Hanna and Gough, 2020), even though male-factor infertility is growing more prevalent in today's society. In addition, the male experience of infertility is an area that has had little research done on it (Hanna and Gough, 2016). As a consequence of this, increased information ought to contribute to helping improve the therapy that is given to people who fall into this category. Recent research has indicated that infertile males have lower levels of self-confidence and self-esteem, in addition to diminished sexual performance (Jamil *et al.,* 2020). This is in comparison to fertile guys, who have higher levels of each of these factors. In addition, Rossi et al. (2016) found that most males using fertility therapies were under the impression that diet, coffee, artificial sweeteners, or medicines did not influence fertility. This was the case even though all of these potential issues were looked at. In addition, it was discovered that the beliefs of respondents were predisposed both by their physicians (84%) and the Internet (71%). This underscores the need for a comprehensive knowledge of the possibility that tailored lifestyle and dietary therapy could be used as supplements for male infertility therapy. In addition to this, there is evidence that males themselves believe that the guilt associated with infertility is worse for them than it is for women (Arya and Dibb, 2016). This is despite the fact that women are more likely to experience infertility. As a consequence of this, the goal of this project is to perform a literature review on the psychological and social implications of male infertility, while also assessing various nutritional and lifestyle therapies to enhance the quality of nutrition advice supplied to infertile and sub-fertile men.

The role of oxidative stress in male infertility

The findings of several researches indicated that there is a close correlation between male infertility and oxidative stress (OS) (Mancini et al., 1994; Sharma and Agarwal, 1996; Conte *et al.,* 1999). Varicocele, inflammation, metabolic disorders, endogenous or exogenous medications, and radiofrequency are among the risk factors for infertility that correlate with OS (Aitken and Drevet, 2020), but it can also be the lone reason that leads to unexplained infertility if it is explored closely enough. In light of this, a situation of this kind is known as male-oxidative-stress-related infertility (MOSI) (Agarwal *et al.,* 2019). The administration of antioxidants has been normal in treatment as a result of the prevalence of a specific mechanism. Despite the fact that this process often does not require a first assessment of the actual presence of OS damage, this technique has become standard because of the ubiquity of this particular mechanism. An approach such as this one may be wrong for determining the physiological role and/or the pathological repercussions of oxidants, which are occasionally referred to as reactive oxygen species (ROS) (Sharma *et al.,* 2019).

Specifically, ROS activates non-receptor tyrosine kinases as well as other serine/threonine kinases, inactivates protein tyrosine phosphatases, and regulates nuclear transcription factors. They also play a role in the signaling of cytokines and growth factors (Valko *et al.,* 2007). ROS are also involved in the regulation of nuclear transcription factors (MacLeod, 1943), H_2O_2, which is a famous example of this role, plays an important part regardless of its relatively low concentration and exceedingly sluggish reactivity with redox-regulated proteins. This is because it has a very sluggish reaction rate with redox-regulated proteins, in contrast to thiol peroxidases, which can deactivate it. The discovery that the very reactive cellular protein thiol peroxidase peroxiredoxin-2 (Prx2) may function as an H_2O_2 receptor and enhance transmission by forming a redox relay with the transcription factor STAT3 has resolved this seeming paradox (Sobotta *et al.,* 2015).

These cellular mechanisms are shared by a variety of different physiological processes, including the functioning of cardiomyocyte cells (Griendling *et al.,* 2000), the regulation of vascular tone (Ignarro *et al.,* 2002), the detection

of variations in oxygen content (Acker and Xue, 1995), cell adhesion (Albelda *et al.,* 1994), the immune response (Hamuro *et al.,* 1999), and, ultimately, the cell lifecycle, due to their involvement in survival and apoptosis (Hengartner, 2000). Therefore, it should not come as a surprise that the management of ROS generation and its repercussions is essential for a cell that is fully developed into spermatozoa. To be able to "travel" safely outside of the body, through the female genital canal, and eventually to fertilize the oocyte, spermatozoa need to undergo a lengthy maturation process in the testis and the epididymis. During this time, they must also acquire the structural arrangements necessary to carry out this process (Mancini *et al.,* 2023).

Because of these intricate mechanisms, a clear and accurate image has not yet been obtained. This is even though significant progress has been made. The purpose of this review is therefore to provide a summary of the following topics: the physiologically beneficial role of reactive oxygen species (ROS), the one-of-a-kind structure of spermatozoa that makes them susceptible to oxidative stress (OS), and, assessing seminal plasma's total antioxidant capacity (TAC) as a biological indicator of male infertility (Mancini *et al.,* 2023).

When the body's free radical defense mechanisms and antioxidant defense mechanisms are not in harmony with one another, this can lead to oxidative stress (OS). Antioxidants, both enzymatic and non-enzymatic, are normally responsible for scavenging free radical species and protecting the organism from excessive exposure to oxidative stress. Antioxidants can be divided into two categories: enzymatic and non-enzymatic. For instance, it has been established that the antioxidant vitamin E increases the fusing of spermatozoa with the egg, which results in an improvement in zona pellucida binding which is essential for successful fertilization. Another antioxidant that helps prevent damage to the plasma membrane of sperm that is produced by radicals is albumin. This antioxidant is useful through extensive research. On the other hand, there are certain conditions under which a lack of antioxidants might bring about oxidative stress (Choudhary *et al.,* 2010).

Cancer, diabetes, varicocele, rheumatoid arthritis, Acquired Immunodeficiency Syndrome (AIDS), inflammation, and liver damage are just some of the conditions that have been associated with OS (Agarwal *et al.,* 2008; Makker *et al.,* 2009; Ramya *et al.,* 2011). It has also been demonstrated that OS has several adverse effects on several aspects of the reproductive system. In addition to oxygen radicals such as the hydroxyl radical, superoxide radical, and hydrogen peroxide, reactive oxygen species, often known collectively as reactive nitrogen species (RNS), make up a subclass of nitrogen-containing compounds. Reactive nitrogen species is what is meant to be abbreviated as RNS. Examples of reactive nitrogen species (RNS) include nitric oxide, peroxynitrite anion, nitroxyl ion, and compounds that contain nitrosyl. Exposure to excessive amounts of the molecule, which adds to nitrosative stress, has the potential to have pathological effects on the male reproductive system (Sikka, 2001; Choudhary *et al.,* 2010; Pacher *et al.,* 2007; Ramya *et al.,* 2011). This is the case even though RNS is important for a range of physiological functions. Some studies (Baker *et al.,* 2005) have pointed the finger at RNS as the agent responsible for generating low sperm activity and sperm's capacity to fertilize eggs as the agent responsible for it. This article's goal, in contrast to the countless other studies that have focused almost entirely on oxidative stress and ROS, is to study the influence of RNS on male infertility. It will be possible to construct potential anti-oxidant remedies for illnesses that are connected with nitrosative stress after all of the knowledge that is now accessible on RNS has been brought together. Utilizing the treatments discussed above in conjunction with developments in assisted reproductive technology (Makker *et al.,* 2009) can lead to positive outcomes for infertile couples.

The formation of nitric oxide

The amino acid L-arginine is transformed into nitric oxide (NO) by the enzyme known as nitric oxide synthase (NOS). The result is the synthesis of nitrogen monoxide (NO), in addition to a byproduct known as L-citrulline, which requires oxygen and several cofactors such as nicotinamide adenine dinucleotide phosphate (NADPH), flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD), calmodulin, and calcium (Rosselli *et al.,* 1998; Sikka, 2001; Choudhary *et al.,* 2010). There are three different forms of NOS, and each of them exerts its influence through protein-protein interactions. 1) Endothelial NOS, also known as eNOS; 2) inducible NOS, also known as iNOS; and 3) neuronal NOS, also known as nNOS.

Endothelium NOS (eNOS): Endothelial cells are known to contain this particular kind of NOS. Each isoform has a reductase domain in its structure, and within that domain is a chemical known as tetrahydrobiopterin (BH4). This molecule is necessary to carry out an efficient synthesis of NO (Rosselli *et al.,* 1998). Testis neuronal nitric oxide synthase (TnNOS) has recently been discovered to have an important role in the production of NO (Turker *et al.,* 2004; Yan *et al.,* 2005). This conclusion elicited a level of astonishment from those who examined it. It has been found that TnNOS is only present in the Leydig cells of the testis, which raises the hypothesis that it is engaged in the process of steroidogenesis. This discovery was made possible by the fact that TnNOS can only be found in the testis. Endogenous nitric oxide synthase (eNOS) and inducible nitric oxide synthase (inducible NOS) have both been shown to be structurally connected with several different proteins. These proteins include occludin, actin, alpha-tubulin, and vimentin, which provide some insight into the role that these enzymes play in the regulation of tight junctions in the testis. In addition, studies have demonstrated that both eNOS and iNOS play a part in the process of apoptosis that takes place in germ cells. This is evidenced by the fact that both are produced by germ cells. This is shown by the fact that eNOS has been associated with degenerating germ cell lines, whilst iNOS has been associated with preserving the number of germ cells in the seminiferous epithelium. This demonstrates that iNOS is more important than eNOS. The existence of this evidence demonstrates that eNOS is the agent responsible for this process. Studies have shown that when iNOS is considered on its own, it stimulates the necrosis of germ cells by functioning as a partial mediator of alpha-fodrin proteolysis. This is the mechanism that iNOS uses to accomplish this task.

The Importance of Nitrogen Oxide (NO) in the Control of Male Reproductive Tissues

NOS is extensively distributed in spermatocytes, Leydig cells, Sertoli cells, the immature sperm head, vascular endothelial cells, the neural plexus in the adventitia of arterioles, and smooth muscle cells (Luo *et al.,* 2021). This research implies that NO/NOS can control testosterone secretion, preserve the tension in testicular arterioles, and impact sperm formation. Furthermore, NOS expression in interstitial cells and blood arteries was demonstrated by an in vitro culture of seminiferous tubules or interstitial cells, providing further evidence that NO can be created by the testis itself (O'Bryan *et al.,* 2000). In addition to this, the concentration of NO is another factor that can be used to ascertain the level of sperm. The process of nitric oxide-cyclic guanosine monophosphate (NO-CGMP) activation in testicular cells provides evidence that this route may be involved in the control of testicular processes such as the production of steroids and spermatogenesis. The injections of 5-hydroxytryptamine and L-dopamine at 8-hour intervals dramatically reduced the levels of NO and nitrate-nitrite in reproductive tissues and inhibited the activity of reproductive tissues, whereas injecting the same substances at intervals of 12 hours produced the opposite results (Kumar and Chaturvedi, 2007). Nonetheless, after reinjecting SNP at 8-hour intervals, the concentration of nitric acid-nitrite in the reproductive tissues rose; however, this was

not the case after injecting L-NAME at 12-hour intervals. This suggests that the absence of alterations in activity in vivo may have an impact on the reproductive tissues' operation (Luo *et al.,* 2021). In the group of people who had unilateral testicular torsion, the contralateral testis had elevated levels of NO content, NOS activity, and pathological scores, according to the findings of several scientific experiments. These levels of increase were present to varied degrees. In addition, the researchers who experimented found that the content of NO had a positive link with the pathological score. This indicates that the NO concentration is directly related to the degree to which the tissue was injured. The injury was probably brought on by the production of additional poisonous ONOO-, which was brought about by the interaction of NO with oxygen-free radicals on the torsional side. This was brought about by the process described above. It's possible that the testicular tissue on the opposite side was harmed as a result of this. Alternately, it may work on the testicular tissue on the contralateral side by building up proinflammatory transmitters of NO, causing excessive dilatation of blood vessels, and setting off apoptosis (Yang *et al.,* 2005). In addition, the damage described above may simultaneously stimulate further NOS activation, which may subsequently synthesize NO and inflict an even higher amount of harm. This lends credence to the hypothesis that the effect of NO on reproductive organs is proportional to the amount of exposure.

NO can act as a barrier to the generation of testosterone in the body. As a consequence of this, the impact of testosterone on the endocrine system may be controlled by NO through a system that is known as the paracrine system. It has been demonstrated that the amount of testosterone present in males affects the expression of the nNOS gene. Furthermore, the two-concentration theory of NO postulates that low concentrations of NO are associated with the development of cancer, whilst high concentrations of NO are supposed to guard against the disease. Both of these hypotheses are based on the observation that NO can be found in both high and low concentrations (Luo *et al.,* 2021). In the ongoing research on prostate cancer, the researchers have shifted their focus from the epithelial cells that react to androgens to NO therapy, the milieu surrounding the tumor, and inflammation (Luo *et al.,* 2021). Despite the fact that prostate cancer is a hormone-driven form of malignancy, this continues to be the case. It has been shown through research that nitric oxide (NO) can lessen the activation of androgen receptors. This could put a stop to the expansion of the prostate, but at the same time, low levels of NO could select prostate cells that are resistant to circumcision, leading to a more aggressive cancer phenotype. At high enough levels, nitro stress, which is produced whenever there is an excess production of NO, can aid in the prevention of prostate cancer (Soni *et al.,* 2020).

It is essential to be aware that there is a significant role for nitrogen oxide (NO) in the biology of the penis, particularly in terms of the erection that is produced by the penis. According to the findings of several different research, NO is the chemical that is responsible for the relaxation of muscular smooth muscle, which is the primary component of an erection. In this mechanism, each NO synthase subtype is responsible for a different but equally essential function (Luo *et al.,* 2021). It has been shown that inhibiting iNOS can cause penile oxidative stress, which in turn shows that iNOS may help the defensive mechanism of aberrant wound healing and fibrosis (Burnett and Musicki, 2005). nNOS is responsible for the beginning of the erectile response, eNOS is responsible for promoting the maximum erectile response, and suppression of iNOS may result in penile oxidative stress (Luo *et al.,* 2021).

Azoospermia, commonly known as the absence of spermatozoa in the ejaculate, is a common cause of male infertility. This condition can also be caused by other factors. Inducible nitric oxide synthase, also known as iNOS, is a type of nitric oxide synthase (NOS) that does not require calcium to function and can be found in the testis. The process of spermatogenesis, as well

as the demise of Sertoli and germ cells, are both affected by its presence. Twenty idiopathically infertile males who presented with nonobstructive azoospermia took part in this research as participants. Each of these males had a series of treatments designed to collect sperm from their testicles. Tissue extracts were dissected, and samples of fluid were examined, to ascertain whether or not spermatozoa were present. The histologic analysis of the samples that contained spermatozoa revealed that the seminiferous tubules were healthy and that they were lined with Sertoli cells as well as cells that were capable of making sperm. Additionally, the seminiferous tubules contained cells that were capable of producing sperm. On the other hand, in the samples that did not include spermatozoa, the diameter of the seminiferous tubules was discovered to be on the smaller side, and Sertoli-cell-only syndrome was observed in the majority of the tubules. This suggests that the absence of spermatozoa may be indicative of a genetic condition. Within the group that contained spermatozoa, the expression of iNOS was found to be extremely low in Sertoli cells, as well as in germ cells and Leydig cells. In the group that did not have any spermatozoa present, the immunostaining was quite strong in the Sertoli and Leydig cells. The results of the histologic study were supported by the findings of the electron microscopy examination. The fact that the azoospermic human testis is devoid of any germ cells and demonstrates high levels of iNOS expression in the Sertoli and Leydig cells demonstrates, in conclusion, that iNOS is an essential component in the process of spermatogenesis (Coştur *et al.,* 2012).

Our experience

We conducted a study to investigate the exposure of perchloroethylene among dry cleaning workers and to compare the effect of this exposure on the expression of iNOS in the control group and dry cleaning group using immunohistochemistry. The results showed that the level of iNOS in the study group increased significantly ($p < 0.001$). Taken together, the current study presented a new plausible mechanism to explain the deficiency in sperm quality by increasing the expression of iNOS in perchloroethylene-exposed groups.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Acker, H., Xue, D.H., 1995. Mechanisms of O_2 sensing in the carotid body in comparison with other O_2 sensing cells. News in Physiol. Sci., 10: 211–216.

Agarwal, A., Makker, K., Sharma, R., 2008. Clinical relevance of oxidative stress in male factor infertility: An update. Am. J. Reprod. Immunol., 59(1): 2-11.

Agarwal, A., Mulgund, A., Hamada, A., Chyatte, M.R., 2015. A Unique View on Male Infertility around the Globe. Reprod. Biol. Endocrinol., 13(1): 37.

Agarwal, A., Parekh, N., Panner Selvam, M.K., Henkel, R., Shah, R., Homa, S.T., at al. 2019. Male Oxidative Stress Infertility (MOSI): Proposed Terminology and Clinical Practice Guidelines for Management of Idiopathic Male Infertility. World J. Mens. Health., 37(3): 296–312.

Aitken, R.J., Drevet, J.R., 2020. The importance of oxidative stress in determining the functionality of mammalian spermatozoa: A two-edged sword. Antioxid., 9(1): 11.

Albelda, S.M., Smith, C.W., Ward, P.A. 1994. Adhesion molecules and inflammatory injuries. FASEB J., 8(8): 504–512.

Amor, H., Hammadeh, M.E., 2022. A Systematic Review of the Impact of Mitochondrial Variations on Male Infertility. Genes., 13: 1182.

Arya, S.T., Dibb, B., 2016. The experience of infertility treatment: The male perspective. Hum. Fertil., 19(4): 242–248.

Aston, K.I., Punj, V., Liu, L., Carrell, D.T., 2012. Genome-Wide Sperm Deoxyribonucleic Acid Methylation Is Altered in Some Men with Abnormal Chromatin Packaging or Poor in Vitro Fertilization Embryogenesis. Fertil. Steril., 97(2): 285–292.

Baker, M.A., Aitken, R.J., 2005. Reactive oxygen species in spermatozoa: Methods for monitoring and significance for the origins of genetic disease and infertility. Reprod. Biol. Endocrinol., 3: 1-9.

Bold, J., Swinburne, D., 2022. Pre-Conceptual Guidelines for Men: A Review of Male Infertility Experience, including Nutrition and Lifestyle Factors. Diet., 1(3): 164-181.

Burnett, A.L., Musicki, B., 2005. The nitric oxide signaling pathway in the penis. Curr. Pharm. Des., 11(30): 3987-3994.

Choudhary, R., Chawala, V.K., Soni, N.D., Kumar, J., Vyas, R.K., 2010. Oxidative stress and role of antioxidants in male infertility. Pak. J. Physiol., 6(1): 54-59.

Conte, G., Milardi, D., De Marinis, L., Mancini, A., 1999. Reactive oxygen species in male infertility. Review of literature and personal observations. Panminerva Med., 41: 45–53.

Coştur, P., Filiz, S., Gonca, S., Çulha, M., Gülecen, T., Solakoğlu, S., et al. 2012. Expression of inducible nitric oxide synthase (iNOS) in the azoospermic human testis. Andrologia., 44(S1): 654–660.

Evers, J.L., 2002. Female Subfertility. The Lancet., 360(9327): 151–159.

Fisch, H., Goluboff, E.T., 1996. Geographic Variations in Sperm Counts: A Potential Cause of Bias in Studies of Semen Quality. Fertil. Steril., 65(6): 1044–1046.

Ghadirkhomi, E., Angaji, S.A., Khosravi, M., Mashayekh, M.R., 2022. Correlation of Novel Single Nucleotide Polymorphisms of USP26, TEX15, and TNP2 Genes with Male Infertility in North West of Iran. Int. J. Fertil. Steril., 16(1): 10–16.

Griendling, K.K., Sorescu, D., Lasse'gue, B., Ushio-Fukai, M., 2000. Modulation of protein kinase activity and gene expression by reactive oxygen species and their role in vascular physiology and pathophysiology. Arterioscler. Thromb. Vasc. Biol., 20(10): 2175–2183.

Hamuro, J., Murata, Y., Suzuki, M., Takatsuki, F., Suga, T., 1999. The triggering and healing of tumor stromal inflammatory reactions regulated by oxidative and reductive macrophages. Gann. Monogr. Canc. Res., 48: 153–164.

Hanna, E., Gough, B., 2016. A qualitative analysis of men's forum posts. Health, 20(4): 363–382.

Hanna, E., Gough, B., 2020. The social construction of male infertility: A qualitative questionnaire study of men with a male factor infertility diagnosis. Sociol. Health Illn., 42(3): 465–480.

Hengartner, M.O., 2000. The biochemistry of apoptosis. Nat., 407(6805): 770–776.

Ignarro, L.J., Sisodia, M., Trinh, K., Bedrood, S., Wu, G.Y., Wei, L.H., Buga, G.M., 2002. Nebivolol inhibits vascular smooth muscle cell proliferation by mechanisms involving nitric oxide but not cyclic GMP. Nitric Oxide., 7(2): 83–90.

Jamil, S., Shoaib, M., Aziz, W., Ather, M.H., 2020. Does male factor infertility impact on self-esteem and sexual relationship? Andrologia., 52(2): e13460.

Jenkins, T.G., Carrell, D.T., 2011. The Paternal Epigenome and Embryogenesis: Poising Mechanisms for Development. Asian J. Androl., 13(1): 76–80.

Kaltsas, A., Moustakli, E., Zikopoulos, A., Georgiou, I., Dimitriadis, F., Symeonidis, E. N., et al. 2023. Impact of Advanced Paternal Age on Fertility and Risks of Genetic Disorders in Offspring. Genes., 14: 486.

Khatun, A., Rahman, M.S., Pang, M.G., 2018. Clinical Assessment of Male Fertility. Obstet. Gynecol. Sci., 61(2): 179–191.

Krausz, C., Riera-Escamilla, A., 2018. Genetics of Male Infertility. Nature Rev. Urol., 15: 369–384.

Kumar, N., Singh, A.K., 2015. Trends of Male Factor Infertility, an Important Cause of Infertility: A Review of Literature. J. Human Reprod. Sci., 8(4): 191–196.

Kumar, P., Chaturvedi, C.M., 2008. Correlation of Nitric Oxide (NO) activity and gonadal function in Japanese Quail, *Coturnix coturnix* Japonica following temporal Phase relation of serotonergic and dopaminergic oscillations. Anim. Reprod. Sci., 106(1-2):48–64.

Kyrgiafini, M.A., Mamuris, Z., 2023. Male Infertility: From Genes to Genomes. Genes., 14(5): 959.

Kyrgiafini, M.A., Giannoulis, T., Chatziparasidou, A., Christoforidis, N., Mamuris, Z., 2022. Whole-Genome Profile of Greek Patients with Teratozoospermia: Identification of Candidate Variants and Genes. Genes., 13: 1606.

Lahav-Baratz, S., Kravtsova-Ivantsiv, Y., Golan, S., Ciechanover, A., 2017. The Testis-Specific USP26 Is a Deubiquitinating Enzyme of the Ubiquitin Ligase Mdm2. Biochem. Biophys. Res. Commun., 482(1): 106–111.

Levine, H., Jørgensen, N., Martino-Andrade, A., Mendiola, J., Weksler-Derri, D., Jolles, M., et al. 2023. Temporal Trends in Sperm Count: A Systematic Review and Meta-Regression Analysis of Samples Collected Globally in the 20th and 21st Centuries. Hum. Reprod. Update., 29: 157–176.

Luo, Y., Zhu, Y., Basang, W., Wang, X., Li, C., Zhou, X., 2021. Roles of Nitric Oxide in the Regulation of Reproduction: A Review. Front. Endocrinol., 12: 752410.

MacLeod, J., 1943. The role of oxygen in the metabolism and motility of human spermatozoa. Am. J. Physiol., 138(3): 512–518.

Makker, K., Agarwal, A., Sharma, R., 2009. Oxidative stress and male infertility. In. J. Med. Res., 129: 357-367.

Mancini, A., Conte, G., De Marinis, L., Hallgass, M.E., Pozza, D., Oradei, A., Littarru, G.P., 1994. Coenzyme Q10 Levels in Human Seminal Fluid: Diagnostic and Clinical Implications. Mol. Aspects Med., 15: s249–s255.

Mancini, A., Oliva, A., Vergani, E., Festa, R., Silvestrini, A., 2023. The Dual Role of Oxidants in Male (In) fertility: Every ROSe Has a Thorn. Int. J. Mol. Sci., 24(5): 4994.

McQueen, D.B., Zhang, J., Jared, C., Robins, J.C., 2019. Sperm DNA Fragmentation and Recurrent Pregnancy Loss: A Systematic Review and Meta-Analysis. Fertil. Steril., 112(2): 54–60.e3.

McSwiggin, H.M., O'Doherty, A.M., 2018. Epigenetic Reprogramming during Spermatogenesis and Male Factor Infertility. Reprod., 156(1): 9–21.

Murray, K.S., James, A., McGeady, J.B., Reed, M.L., Kuang, W.W., Nangia, A.K., 2012. The Effect of the New 2010 World Health Organization Criteria for Semen Analyses on Male Infertility. Fertil. Steril., 98(3): 1428–1431.

Nasri, F., Gharesi-Fard, B., Namavar Jahromi, B., Farazi-fard, M.A., Banaei, M., Davari, M., Pouladfar, G., 2017. Sperm DNA Methylation of H19 Imprinted Gene and

Male Infertility. Andrologia., 49(7): e12766.

National Institute for Health and Clinical Excellence (NICE). 2017a. Fertility Problems: Assessment and Treatment. Retrieved from https://www.nice.org.uk/guidance/cg156/chapter/Recommendations#ftn.footnote_1

National Institute for Health and Clinical Excellence (NICE). 2017b. Fertility Problems: Assessment and Treatment. Retrieved from https://www.nice.org.uk/guidance/cg156/ifp/chapter/Treatments-for-men

NHS. 2023a. Infertility Overview. Retrieved from https://www.nhs.uk/conditions/infertility/

NHS. 2023b. Diagnosis: Infertility. Retrieved from https://www.nhs.uk/conditions/infertility/diagnosis/

O'Bryan, M.K., Schlatt, S., Gerdprasert, O., Phillips, D.J., de Kretser, D.M., Hedger, M.P., 2000. Inducible nitric oxide synthase in the rat testis: Evidence for potential roles in both normal function and inflammation-mediated infertility. Biol. Reprod., 63(5): 1285–1293.

Oud, M.S., Volozonoka, L., Smits, R.M., Vissers, L.E.L.M., Ramos, L., Veltman, J.A., 2019. A Systematic Review and Standardized Clinical Validity Assessment of Male Infertility Genes. Hum. Reprod., 34: 932–941.

Pacher, P., Beckman, J.S., Liaudet, L., 2007. Nitric oxide and peroxynitrite in health and disease. Physiol. Rev., 87(1): 315-424.

Punjabi, U., Goovaerts, I., Peeters, K., De Neubourg, D., 2023. Semen Thresholds of Normality Established by the WHO Do Not Reveal Genome Instability—A Potential Occult Male Factor. Genes., 14: 239.

Ramya, T., Misro, M.M., Sinha, D., Nandan, D., Mithal, S., 2011. Altered levels of seminal nitric oxide, nitric oxide synthase, and enzymatic antioxidants and their association with sperm function in infertile subjects. Fertil. Steril., 95(1): 135-140.

Rosselli, M., Keller, P.J., Dubey, R.K., 1998. Role of nitric oxide in the biology, physiology, and pathophysiology of reproduction. Hum. Reprod. Update., 4(1): 3-24.

Rossi, B.V., Bressler, L.H., Correia, K.F., Lipskind, S., Hornstein, M.D., Missmer, S.A., 2016. Lifestyle and in vitro fertilization: What do patients believe? Fertil. Res. Pract., 2: 11.

Rotondo, J.C., Lanzillotti, C., Mazziotta, C., Tognon, M., Martini, F., 2021. Epigenetics of Male Infertility: The Role of DNA Methylation. Front. Cell Dev. Biol., 9: 689624.

Salas-Huetos, A., Bulló, M., Salas-Salvadó, J., 2017. Dietary patterns, foods and nutrients in male fertility parameters and fecundability: A systematic review of observational studies. Hum. Reprod. Update., 23(4): 371–389.

Sengupta, P., Dutta, S., Krajewska-Kulak, E., 2017. The Disappearing Sperms: Analysis of Reports Published Between 1980 and 2015. Am. J. Mens. Health., 11(5): 1279–1304.

Sharma, P., Ghanghas, P., Kaushal, N., Kaur, J., Kaur, P., 2019. Epigenetics and oxidative stress: A twin-edged sword in spermatogenesis. Andrologia., 51(6): e13432.

Sharma, R.K., Agarwal, A., 1996. Role of Reactive Oxygen Species in Male Infertility. Urol., 48(6): 835–850.

Shawe, J., Delbaere, I., Ekstrand, M., Hegaard, H.K., Larsson, M., Mastroiacovo, P., Tydén, T., 2014. Preconception care

policy, guidelines, recommendations and services across six European countries: Belgium (Flanders), Denmark, Italy, the Netherlands, Sweden, and the United Kingdom. Eur. J. Contracept. Reprod. Health Care., 20(2): 77–87.

Shi, Y.C., Wei, L., Cui, Y.X., Shang, X.J., Wang, H.Y., Xia, X.Y., et al. 2011. Association between Ubiquitin-Specific Protease USP26 Polymorphism and Male Infertility in Chinese Men. Clin. Chem. Lab. Med., 49(3): 545–549.

Sikka, S.C., 2001. Relative impact of oxidative stress on male reproductive function. Curr. Medic. Chem., 8(7): 851-862.

Sobotta, M.C., Liou, W., Stocker, S., Talwar, D., Oehler, M., Ruppert, T., et al. 2015. Peroxiredoxin-2 and STAT3 form a redox relay for H_2O_2 signaling. Nat. Chem. Biol., 11(1): 64–70.

Soni, Y., Softness, K., Arora, H., Ramasamy, R., 2020. The Yin Yang Role of Nitric Oxide in Prostate Cancer. Am. J. Mens Health., 14(1): 1557988320903191.

Stomper, J., Rotondo, J.C., Greve, G., Lübbert, M., 2021. Hypomethylating agents (HMA) for the treatment of acute myeloid leukemia and myelodysplastic syndromes: mechanisms of resistance and novel HMA-based therapies. Leukemia., 35: 1873–1889.

Turker, K.I., Erdoğru, T., Gülkesen, H., Sezer, C., Usta, M., Ciftçioğlu, A., Baykara, M., 2004. The potential role of inducible nitric oxide synthase (iNOS) activity in the testicular dysfunction associated with varicocele: An experimental study. Int. Urol. Nephrol., 36(1): 67-72.

Tüttelmann, F., Ruckert, C., Röpke, A., 2018. Disorders of Spermatogenesis: Perspectives for Novel Genetic Diagnostics after 20 Years of Unchanged Routine. Med. Genet., 30: 12–20.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T.D., Mazur, M., Telser, J., 2007. Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol., 39(1): 44–84.

Vasan, S.S., 2011. Semen Analysis and Sperm Function Tests: How Much to Test? In. J. Urol., 27(1): 41–48.

World Health Organization. (2010). WHO Laboratory Manual for the Examination and Processing of Human Semen (5th ed.). Geneva, Switzerland.

World Health Organization. (2021). WHO Laboratory Manual for the Examination and Processing of Human Semen (6th ed.). Geneva, Switzerland.

Yan, H.H., Cheng, C.Y., 2005. Blood-testis barrier dynamics are regulated by an engagement/disengagement mechanism between tight and adherens junctions via peripheral adaptors. Proc. Natl. Acad. Sci. USA., 102(35): 11722-11727.

Yang, J.Z., Ajonuma, L.C., Rowlands, D.K., Tsang, L.L., Ho, L.S., Lam, S.Y., et al. 2005. The role of inducible nitric oxide synthase in gamete interaction and fertilization: A comparative study on knockout mice of three NOS isoforms. Cell Biol. Int., 29(9): 785–791.

Zhang, W., Liu, T., Mi, Y.J., Yue, L.D., Wang, J. M., Liu, D.W., et al. 2015. Evidence from Enzymatic and Meta-Analyses Does Not Support a Direct Association between USP26 Gene Variants and Male Infertility. Androl., 3(2): 271–279.