Available online at www.garmian.edu.krd

Passer Journal

Passer 5 (Issue 1) (2023) 72-77

http://passer.garmian.edu.krd/

Effect of Lipid Peroxidation on Sperm Motility in Asthenozospermia Infertile Men

Aryan Hameed Fatah[1*], Ayad Faeq Palani[2], Baxtyar Kamal Aziz[3]

[1]*Pharmaceutical chemistry, College of Medical and Applied Sciences, Charmo University, Kurdistan Region, Iraq.*
[2]*Department of Chemistry, College of Science, University of Garmian, Kalar, Kurdistan region, Iraq.*
[3]*Applied Chemistry, College of Medical and Applied Sciences, Charmo University, Kurdistan region, Iraq.*

Received 05 November 2022; revised 03 February 2023;
accepted 04 February 2023; available online 13 February 2023

DOI: 10.24271/PSR.2023.368563.1178

ABSTRACT

Sperm motility is necessary for regular fertilization because spermatozoa must travel far in the female genitaltract to defuse and fertilize the oocyte. Low sperm motility, also known as as thenozoospermia (AZ), is a prevalent cause of infertility in men. AZ is defined as a low percentage of forwarding (progressive motility), fewer than 30%, or a lack of sperm motility in examined total ejaculation. Reduced sperm motility may result from an unhealthy lifestyle, obesity, environmental pollution, oxidative stress, smoking, alcohol intake, or may be idiopathic. Oxidative may damage different sperm components like DNA, proteins, or lipids.This study aims to determine a possible relationship between semen antioxidants, lipid peroxidation, and sperm motility parameters in asthenozoospermic men.Twenty-three healthy fertile males and 57 AZ infertile men were included in this study. Samples were obtained after 3-4 days of abstinence from sexual intercourse. Exclusion criteria included medication use, smoking, drinking alcohol, chronic illness(such as diabetes mellitus, hypertension, etc.), varicocele and high viscid samples.

The results revealed a remarkable decrease in superoxide dismutase activity and a high increase in semen plasma malondialdehyde in the asthenozoospermic group. The decrease in SOD activity leads to an increase in superoxide anionlevel ($O2^{\bullet-}$), which induces lipid peroxidation in the spermatozoa membrane.Lipid peroxidation frequently affects spermatozoa motility by affecting sperm membrane and fluidity and followed by mitochondrial defect and a concomitant decrease in energy generation.

Keywords: Sperm motility, Asthenozoospermia, SOD, MDA.

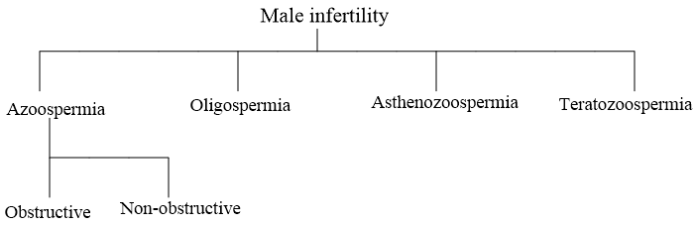
1. Introduction

Most people have plans for their future that include having children, and parenthood is the one of the adulthood goals that are most commonly wanted, not every couple who wants to get pregnant will be successful. After engaging in regular, unprotected sexual activity for 12 months or longer, couples are said to be infertile if they are unable to conceive a child or unable to terminate a pregnancy[1,2]. Globally, 10-15% of all the world scouples suffer from infertility, and according to the most recent World Health Organization (WHO) estimates, approximately 50–80 million people worldwide experience infertility[1]. The issue has been identified by the WHO as a global problem of public health[3]. In the past, people believed that women were the only individuals responsible for infertility, but current research has shown that men are equally responsible[4].

* Corresponding author
E-mail address: aryan.fatah@charmouniversity.org (Instructor).
Peer-reviewed under the responsibility of the University of Garmian.

There are two types of infertility: primary and secondary. Couples with primary infertility are individuals who have never given birth before. While in secondary infertility couples had a chance to have at least one baby and failed to reproduce[5], in all cases, men are responsible for nearly 50 % of infertility cases[4].

Numerous causes of sperm dysfunction are implicated in male infertility.Azoospermia, asthenozoospermia, oligospermia, and teratozoospermia are different types of male infertility classified by the WHO based on the quality of the sperm[6]. As shown in Scheme 1 and Table 1.



Scheme 1: Male infertility classification based on sperm quality.

Table 1: Cut-off values of sperm parameters publishedby the (WHO, 2020).

Parameter	WHO 2020	classification based on the cut-off value
Volume	1.4	Hypospermia
Sperm concentration	$15* 10^6$	Oligozoospermia
Progressive Motility	%30	Asthenozoospermia
Morphology	%4 normally formed	Teratozoospermia

Fertility is significantly influenced by the quality of the semen; according to " pregnancy outcome" research, men's fertilization ability begins to deteriorate when sperm count decreases to below 15×10^6/ml[7].

Sperm motility is necessary for regular fertilization because spermatozoa must travel far to connect with and fertilize the egg[8]. Asthenozoospermia (AZS) which is one of the main factors contributing to male infertility, is characterized by an abnormal ratio of forwarding motile sperm [grade A + B sperm velocity 50% and A 25%, according to the newest WHO guidelines], or a lack of motile spermatozoa in freshly examined ejaculation[9]. Low sperm motility could result froman unhealthy lifestyle[10], obesity[11–13], environmental pollution[14,15], oxidative stress[16], smoking, alcohol or may be idiopathic[17].

An imbalance between oxidants and antioxidants in favor of the oxidants is referred to as oxidative stress(OS)[18], or pro-oxidant and antioxidant species are not in a balanced state[19].

Oxidants are highly reactive oxygen or nitrogen species (ROS and RNS) atoms or molecules that can arise when oxygen reacts with specific compounds and have one or more free electrons in their outer orbital[20]. ROS could be generated due to mitochondrial generation, enzymatic reaction, radiation, and environmental toxins[21].

Antioxidants are chemicals that stop the oxidation of other molecules from affecting cells[22]. They could classify as enzymatic molecules (catalase, superoxide dismutase, glutathione peroxidase, etc.) and non-enzymatic molecules(minerals, vitamins, polyphenols, etc.)[23].

Due to their weak cell repair mechanisms and insufficient antioxidant defenses due to having very little cytoplasmic material, gametes are significantly harmed by OS, since their sperm membrane contains a lot of polyunsaturated fatty acids (PUFA), they are vulnerable to lipid peroxidation[24].

PUFAs are essential regulators of sperm membrane fluidity, and ATP outflow, which hinders flagellar motion[24]. Hence the peroxidation of sperm lipids may affect energy metabolism and reduce sperm motility.

The aim of this study was to find a biochemical cause of low sperm motility through the estimation of oxidative stress status in the seminal plasma of Asthenozoospermic infertile men and find the effect of oxidative stress on sperm membrane through the estimation of lipid peroxidation in sperm cellular membrane. In addition, the study aimed to find a possible relation between antioxidant levels in human semen and different sperm motility parameters.

2. Material and Method

A total of 80 samples were enrolled to complete this study, 23 semen samples from healthy fertile males and 57 samples from patients with asthenozoospermia who visited thefertility clinics to seek for treatment of infertility. Semen samples have been obtained by masturbation performed in the laboratory after 3-4days of abstinence from sexual intercourse. Samples were incubated at (37º C) for 60 minutes. Exclusion criteria included medication use, smoking, drinking alcohol, chronic illness(such as diabetes mellitus, hypertension, etc.), varicocele, and high viscid samples.

Semen analysis was carried out under WHO 2020 guidelines[25]. Sperm examinations involve assessing sperm concentration, different motility parameters, and morphology, usingCASA-based autoanalyzer. After that, seminal plasma was separated by centrifugation of the samples for 10 minutesat 4000 rpm. The separated seminal plasma was stored in a deep freezer (-80 °C) in 3 tubes for further biochemical analysis.

Malondialdehyde was estimated by mixing 200 microliter of frozen seminal plasma with TBA(thiobarbituric acid) in a hot weak acid mediumfor 60 minutes, the pink color read at 532 nm, and MDA concentration was calculated and expressed as µmole/l[26].TAC was estimated bythe addition of 100 µL of a semen sample to 1 mL of TPTZ(2.4.6-tripyridyl-s-trazine), and $FeCl_3.6H_2O$ inpH 3.6 of acetate buffer solution and incubation of the mixture in 37°C, the blue color read at 593 nm and TAC concentration calculated against $FeSO_4.7H_2O$ calibration curve and results expressed as Mmole/L[27]. Catalase was determined by the goth method(50 µL ofsemen sample mixed with H_2O_2 and ammonium molybdate in potassium-sodium buffer 7.4 medium, the yellow color read at 405 nm, and catalase concentration was calculated and expressed as KU/L[28]. Dismutase enzyme was assessed by an enzyme-linked immunosorbent assay (ELISA) kit (Bioassay, China). The kit principle was based on the bounding of SOD in the sample with human SOD antibody that plate has been pre-coated with, biotinylated antibody was added to bind with non-bind SOD in the sample binds with streptavidin-HRP. Excess streptavidin-HRP has washed away by washing with buffer. The color developed by adding the substrate solution, and the reaction was stopped by the addition of an acidic medium to the reaction mixture. The concentration of the unknown samples was determined by reading the absorbance at 450 nm and comparing it with a series of standard solutions.

3. Statistical analysis

Results are presented as Mean ±SEM, and an independent sample t-test was used tofind significant differences between both groups. Data was analyzed statistically using MedCalc statistical program v. 18.2.1; MedCalc Software bvba. Data were examined for normality by using Kolmogorov–Smirnov test. Differences between non-parametric values were analyzed by Mann-Whitney and considered significant at p-value ≤ 0.05. The correlation between different parameters has been calculated usin gthe

Pearson correlation coefficient. *r* value > 0.25 is considered weak, while > 0.5 is considered a medium, and > 0.75 is considered a strong correlation.

Table 2: Mean ± SEM of basic semen parameters of fertile and infertile groups.

Semen Analysis	Infertile Men	Fertile Men	P
Variables	(n=57)	n(23)	value
Concentration (million/ml)	26.61±3.47	58.63±7.45	< 0.01
Total sperm count(million/ejaculate)	71±8.26	214.69±31.34	< 0.01
Total motile sperm (million/ejaculate)	17.1±2.49	137.76±21.33	< 0.01
% Total motile sperm	25.02±1.31	63.69±2.78	< 0.01
% prog.Motility	17.46±1.11	48.47±2.91	< 0.01
% prog. motility(A)	8.27±0.85	30.23±2.92	< 0.01
% prog. motility (B)	9.51±0.58	17.62±1.26	< 0.01
% non-prog.motility	6.47±0.45	14.23±1.39	< 0.01
% immotile	74.73±1.31	36.3±2.78	< 0.01
%Normal morphological sperm	35.94±2.24	44.39±1.17	< 0.05
Progressive velocity	23.13±1.03	29±1.73	< 0.01
path velocity	26.54±0.99	32.51±1.73	< 0.01
Track velocity	38.05±1.25	44.45±1.97	< 0.01

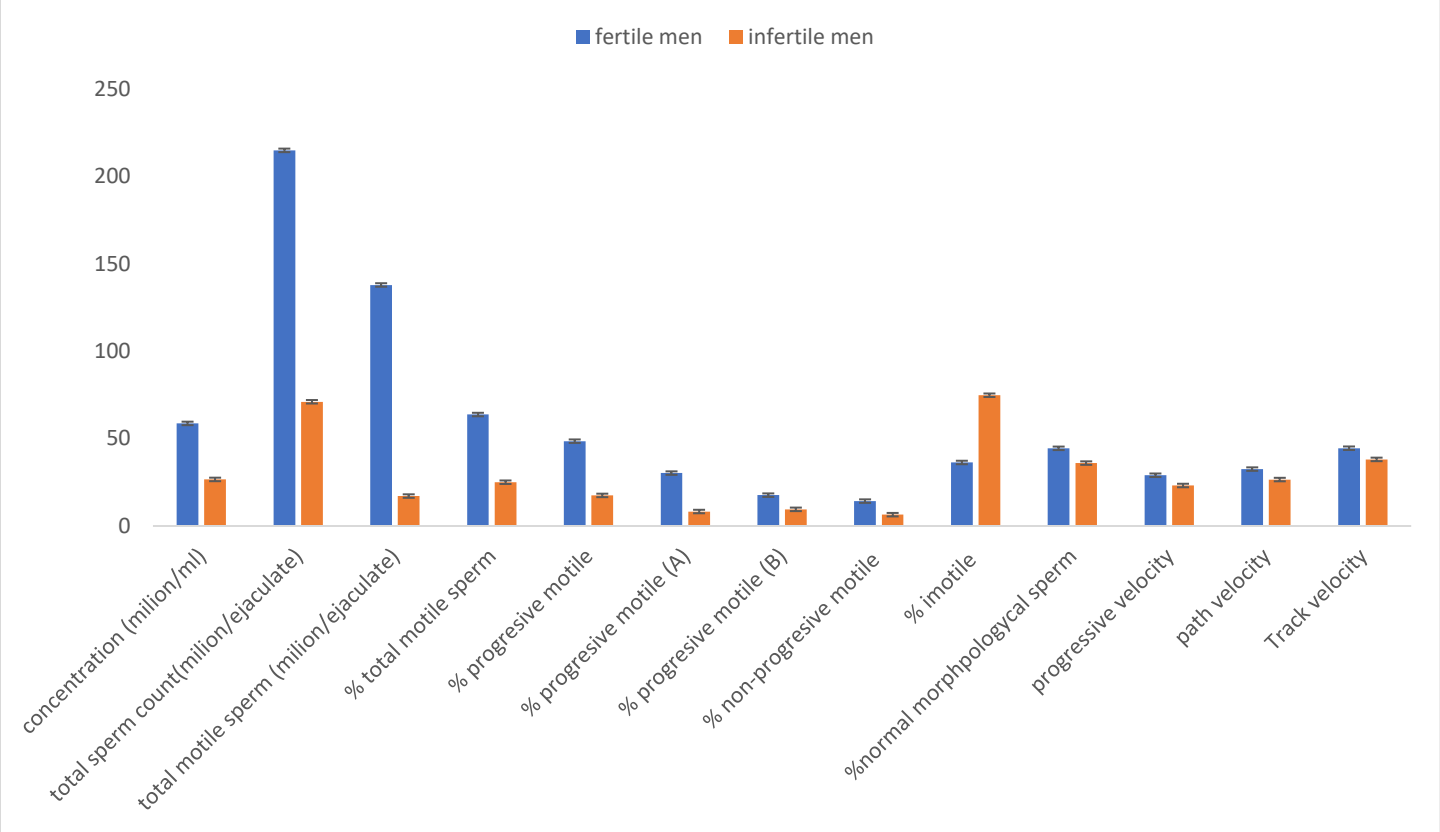


Figure 1: Basic semen parameters of infertile and fertile groups.

Seminal plasma biochemical parameters are shown in table 3 and figure2.The groups have a significant decrease ($p< 0.01$) inSOD activity of the infertile group (62.86±8.76 U/ ml) compared to the fertile group (111.97±18.49U/ ml), and a significant increase in MDA concentration in infertile men$p< 0.01$ (17.95±1.50 nmol) as compared to fertile group (10.68±1.57 nmol/ml).

Table 3: Semen plasma biochemical variables.

Semen Plasma Biochemical	Infertile Men	Fertile Men	P
variables	(n=57) Mean ± SEM	n(23) Mean ± SEM	value
catalase KU/l	11.32±1.01	12.03±1.07	n.s
SOD U/ml	62.86±8.76	111.97±18.49	< 0.01
TAC µM	811.18±39.19	785.9±51.6	ns
MDA nmol/ ml	17.95±1.5	10.68±1.57	< 0.01

SOD = superoxide dismutase, TAC = total antioxidant, MDA = malondialdehyde

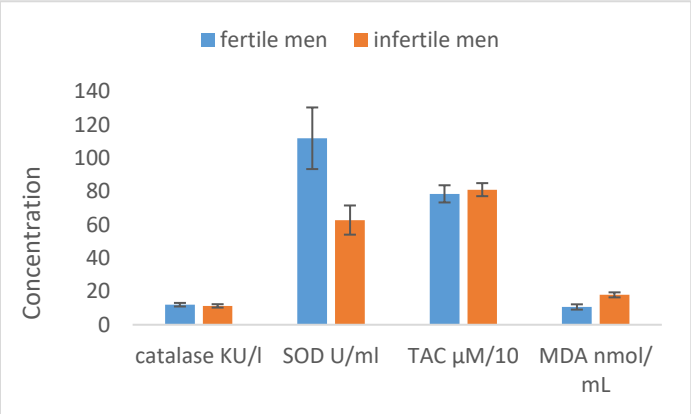


Figure 2: Mean ± SEM differences in seminal plasma biochemical variables between infertile and fertile groups.

Low sperm concentration and motility have positively correlated with semen SOD activity and negatively correlated with MDA, as shown in Table 4.

Table 4: Pearson correlation between motility variables and biochemical parameters.

Parameters	r-Value	P Value
%total motile sperm –MDA	-0.25	< 0.05
totalmotile sperm count -MDA	-0.26	< 0.05
%progresive motility - MDA	-0.35	< 0.01
%progresive motility A -MDA	-0.29	< 0.05
sperm concentration-MDA	-0.26	< 0.05
total sperm count –MDA	-0.26	< 0.05
path velocity – MDA	-0.36	< 0.01
progressive velocity – MDA	-0.33	< 0.01
Track velocity – MDA	-0.34	< 0.01
%immotile sperm-MDA	0.28	< 0.05
Sperm concentration – SOD	0.35	< 0.05
total motile sperm count -SOD	0.31	< 0.05
%immotile sperm-SOD	-0.38	< 0.01
%progressive motility-SOD	0.37	< 0.05
%total motile sperm-SOD	0.33	< 0.05
%non-progresive motile sperm-SOD	0.41	< 0.01
% progressively motile sperm B-SOD	0.36	< 0.01
total sperm count-SOD	0.46	< 0.01

4. Discussion

Sufficient progressing motile sperm are required for healthy ovum fertilization[29].The transfer of the spermatozoa to the location of fertilization within the far positions of the female genital tract requires progressive sperm mobility[30]. ASZ Infertile men have low sperm motility, and low progressive motility compared to fertile control (Table 2, Figure 1). Values of different groups can be used as borderlines in the diagnosis of male fertility based on sperm motility function.

Seminal plasma provides a suitable environment for spermatozoa to swim and protect the spermatozoa from different harmful attacks. Due to their weak cell repair mechanisms and insufficient antioxidant defenses as a result of having very little cytoplasmic material, spermatozoa depend on seminal plasma to protect against oxidative attack[24]. This is one of the characteristics that distinguishes spermatozoa from other organisms in its vulnerability to oxidative damage[31]. The distribution of the balance between ROS progression and antioxidant defense in semen may be linked to lower sperm fertilizing potentials, which results in spermatozoal dysfunction[31] Oxidative stress also contributes to low sperm quality by decreasing sperm vitality, motility, and sperm penetration ability and increase sperm DNA and membrane damage[32]. High amounts of ROS can also lead to morphological defects, increased sperm membrane permeability, and decreased fertility[33].

The Low antioxidant protection shield makes sperm particularly vulnerable to OS. Because the plasma membrane is made of a percentage of unsaturated fatty acids, male sperm are highly vulnerable to lipid peroxidation[24, 34]. The complete antioxidant defense mechanism of the spermatozoa, which is made up of both enzyme- and non-enzyme-based antioxidants, serves to protect it from harm[35].

The study result showed a significant decrease in SOD in infertile men(Table 3, figure 2) whensuperoxide dismutase (SOD) is an enzymatic antioxidant by converting superoxide radical anion ($O_2^{\bullet-}$) to H_2O_2, enzymes protect spermatozoa against oxidative degradation[23]. It plays a significant role in balancing between generation and degradation of ROS.

$$2O_2^{\bullet-} + 2H^+ \longrightarrow H_2O_2 + O_2$$

Also, data in table 4 showed a positive correlation between SOD and sperm concentration($r = 0.35$, $p< 0.05$), total sperm count($r=0.46$, $p< 0.05$), total motile sperm count($r=0.30$, $p< 0.05$), %total active motile sperm($r=0.33$, $p< 0.05$), % progressive motility($r=0.37$, $p< 0.05$), % non-progressive active sperm($r=0.41$, $p< 0.01$), % progressively active sperm B($r=0.35$, $p< 0.01$), and showed negative correlation with %immotile sperm($r=-0.37$, $p< 0.01$), these data emphasize the protective role of SOD in preventing sperm lipid peroxidation and improving sperm motility by protecting sperm cell from attacked free radicals.

Decreasing SOD activity leads to the accumulation of superoxide radical anion, which is the primary inducer of lipid peroxidation[36]. Data showed a significant increase of MDA level in the semen of infertile men (17.95±1.5 nmol/ml) compared to standard fertile men's control (10.68±1.57) (Table 3, Figure 2).

By catabolizing phospholipids and releasing PUFAs, the peroxidation of spermatozoa membrane lipids decreases the fluidity and permeability of the sperm membrane, which can have subsequent impacts such as inactivating membrane enzymes and receptors[37].

Loss of sperm membrane integrity is directly correlated with reduced sperm motility[38]. The peroxidation of unsaturated fatty acidsoccurs in membranes surrounding the spermatozoa mitochondria in addition to the plasma membrane, secondary lipid peroxidation (LPO) can begin in mitochondrial membranes as a result of increased mitochondrial production of ROS generation and the subsequent spread of the peroxidation of the sperm plasma membrane. Parallel to the sperm plasma membrane, the fluidity of the mitochondrial membranes is altered by LPO inside these structures, which upregulates the leakage of the charged particles through the mitochondrial inner membrane[39]. Such a circumstance results in the decrease of the membrane potential of the mitochondria impairs the effectiveness of mitochondrial ATP synthesis and triggers electron diffusion through themitochondrial membrane and eventually production of more mitochondrial ROS[39, 40]. Sperm cells depend on mitochondria to function properly to provide the energy required for adequate motility[39]. Loss of mitochondrial function and a concurrent defect in energy production necessary to enable normal mobility, have been classified as common etiology for sperm dysfunction andreduced sperm motility[42].

Also, data showed that MDA negatively impacts sperm activity and different motility parameters,% total motile sperm, which indicates deterioration in sperm movement parameters ($r =-0.25$, $p< 0.05$), total motile sperm count ($r =-0.26$, $p< 0.05$), % progressive motility ($r = -0.350$, $p< 0.01$), % progresive motility A ($r = -0.28$, $p< 0.05$) and sperm velocity(path velocity $r= -0.36$, $p< 0.01$), (progressive velocity $r= -0.33$, $p< 0.01$), (track velocity $r= -0.34$, $p< 0.01$).

These parameters are related to sperm progressive motility, which is an essential parameter in the determination of sperm egg-fertilization ability, a decrease of these parameters as a response to the increase of MDA will lead to loss of sperm fertilization capability.

A potential limitation of the present study was the sample size, a higher sample size will give more reliable results that can be statistically analyzed.

Further directions: the power of this research was to explain the role of oxidative stress in infertile men in the Kurdistan region, which has scarce research in this field. The value of this research is giving clinicians new examination tools on male infertility to understand the natural causes behind male infertility. The use of MDA as a tool along with the SFA test may help in the diagnosis of the causes of low sperm motility.

However, to investigate the role of lipid peroxidation on male fertility, further experiments are recommended to explain the role of lipid peroxidation on sperm morphology and sperm DNA integrity which both are essential factors in male infertility.Also, a study of the protective role of different antioxidants in contracting free radicals and oxidative stress situationsis recommended in further clinical studies.

Conclusion

It is concluded that low seminal plasma antioxidant activity directly impacts sperm basic parameters,especially sperm motility. The resulting OS stated that low antioxidant levels has a and may be considered a significant cause of loss of sperm function and vitality. Finally, semen antioxidants assayis a helpful tool for the diagnosis of male infertility due to sperm dysfunction.

Conflict of interests

None

References

1. E. Babakhanzadeh, M. Nazari, S. Ghasemifar, and A. Khodadadian, “Some of the factors involved in male infertility: A prospective review,” *Int. J. Gen. Med.*, vol. 13, pp. 29–41, 2020, doi: 10.2147/IJGM.S241099.
2. P. Committee and A. Society, “Definitions of infertility and recurrent pregnancy loss: a committee opinion,” *Fertil. Steril.*, vol. 113, no. 3, pp. 533–535, 2020, doi: 10.1016/j.fertnstert.2019.11.025.
3. J. Boivin, L. Bunting, J. A. Collins, and K. G. Nygren, “Reply: International estimates on infertility prevalence and treatment seeking: Potential need and demand for medical care,” *Hum. Reprod.*, vol. 24, no. 9, pp. 2380–2383, 2009, doi: 10.1093/humrep/dep218.
4. K. A. Turner *et al.*, “Male Infertility is a Women’s Health Issue-Research and Clinical Evaluation of Male Infertility Is Needed,” *Cells*, vol. 9, no. 4, pp. 1–14, 2020, doi: 10.3390/cells9040990.

5. U. Larsen, "Research on infertility: Which definition should we use?," *Fertil. Steril.*, vol. 83, no. 4, pp. 846–852, 2005, doi: 10.1016/j.fertnstert.2004.11.033.
6. Y. Daneshmandpour *et al.*, "MicroRNAs association with azoospermia, oligospermia, asthenozoospermia, and teratozoospermia: a systematic review," *J. Assist. Reprod. Genet.*, vol. 37, no. 4, pp. 763–775, 2020, doi: 10.1007/s10815-019-01674-9.
7. H. E. Virtanen, N. Jørgensen, and J. Toppari, "Semen quality in the 21 st century," *Nat. Rev. Urol.*, vol. 14, no. 2, pp. 120–130, 2017, doi: 10.1038/nrurol.2016.261.
8. S. M. Curi *et al.*, "Asthenozoospermia: Analysis of a large population," *Arch. Androl.*, vol. 49, no. 5, pp. 343–349, 2003, doi: 10.1080/01485010390219656.
9. Q. Yu, Q. Zhou, Q. Wei, J. Li, C. Feng, and X. Mao, "SEMG1 may be the candidate gene for idiopathic asthenozoospermia," *Andrologia*, vol. 46, no. 2, pp. 158–166, 2014, doi: 10.1111/and.12064.
10. T. Mehrannia, "The effect of cigarette smoking on semen quality of infertile men," *Pakistan J. Med. Sci.*, vol. 23, no. 5, pp. 717–719, 2007.
11. I. L. Nwajiaku L.A, Mbachu I.I, "Prevalence, Clinical Pattern and Major Causes of Male Infertility in Nnewi, South East Nigeria: A Five YearReview," *AFRIMEDIC J.*, vol. 3, no. 2, pp. 16–19, 2012.
12. S. S. Du Plessis, S. Cabler, D. A. McAlister, E. Sabanegh, and A. Agarwal, "The effect of obesity on sperm disorders and male infertility," *Nat. Rev. Urol.*, vol. 7, no. 3, pp. 153–161, 2010, doi: 10.1038/nrurol.2010.6.
13. N. Stefan *et al.*, "Circulating palmitoleate strongly and independently predicts insulin sensitivity in humans," *Diabetes Care*, vol. 33, no. 2, pp. 405–407, 2010, doi: 10.2337/dc09-0544.
14. R. M. Abarca, "Effects of Cadmium, Lead, and Mercury on the Structure and Function of Reproductive Organs," *Nuevos Sist. Comun. e Inf.*, pp. 2013–2015, 2021.
15. A. Kovacik *et al.*, "Trace elements content in semen and their interactions with sperm quality and RedOx status in freshwater fish Cyprinus carpio: A correlation study," *J. Trace Elem. Med. Biol.*, vol. 50, pp. 399–407, 2018, doi: https://doi.org/10.1016/j.jtemb.2018.08.005.
16. M. A. Baker *et al.*, "Defining the mechanisms by which the reactive oxygen species by-product, 4-hydroxynonenal, affects human sperm cell function," *Biol. Reprod.*, vol. 92, no. 4, pp. 1–10, 2015, doi: 10.1095/biolreprod.114.126680.
17. R. Kumar, G. Gautam, and N. P. Gupta, "Drug Therapy for Idiopathic Male Infertility: Rationale Versus Evidence," *J. Urol.*, vol. 176, no. 4, pp. 1307–1312, 2006, doi: https://doi.org/10.1016/j.juro.2006.06.006.
18. H. Sies, "Oxidative stress: Concept and some practical aspects," *Antioxidants*, vol. 9, no. 9, pp. 1–6, 2020, doi: 10.3390/antiox9090852.
19. A. M. Pisoschi, A. Pop, F. Iordache, L. Stanca, G. Predoi, and A. I. Serban, "Oxidative stress mitigation by antioxidants - An overview on their chemistry and influences on health status," *Eur. J. Med. Chem.*, vol. 209, p. 112891, 2021, doi: 10.1016/j.ejmech.2020.112891.
20. A. Chandrasekaran, M. del P. S. Idelchik, and J. A. Melendez, "Redox control of senescence and age-related disease," *Redox Biol.*, vol. 11, pp. 91–102, 2017, doi: 10.1016/j.redox.2016.11.005.
21. I. Liguori *et al.*, "Oxidative stress, aging, and diseases," *Clin. Interv. Aging*, vol. 13, pp. 757–772, 2018, doi: 10.2147/CIA.S158513.
22. S. K. Brar, G. S. Dhillon, and C. R. Soccol, *Biotransformation of waste biomass into high value biochemicals*. 2014.
23. A. M. Pisoschi and A. Pop, "The role of antioxidants in the chemistry of oxidative stress: A review," *Eur. J. Med. Chem.*, vol. 97, pp. 55–74, 2015, doi: 10.1016/j.ejmech.2015.04.040.
24. S. Dutta, A. Majzoub, and A. Agarwal, "Oxidative stress and sperm function: A systematic review on evaluation and management," *Arab J. Urol.*, vol. 17, no. 2, pp. 87–97, 2019, doi: 10.1080/2090598X.2019.1599624.
25. World Health, *Examination and processing of human semen*, vol. Edition, V, no. 10. 2020.
26. K. Yagi, "[39] Assay for Blood Plasma or Serum," *Methods Enzymol.*, vol. 105, no. C, pp. 328–331, 1984, doi: 10.1016/S0076-6879(84)05042-4.
27. I. F. F. Benzie and J. J. Strain, "The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': The FRAP assay," *Anal. Biochem.*, vol. 239, no. 1, pp. 70–76, 1996, doi: 10.1006/abio.1996.0292.
28. L. Góth, "A simple method for determination of serum catalase activity and revision of reference range," *Clin. Chim. Acta*, vol. 196, no. 2–3, pp. 143–151, 1991, doi: 10.1016/0009-8981(91)90067-M.
29. C. L. Markert, "Fertilization of mammalian eggs by sperm injection," *J. Exp. Zool.*, vol. 228, no. 2, pp. 195–201, Nov. 1983, doi: https://doi.org/10.1002/jez.1402280205.
30. K. Nowicka-bauer and B. Nixon, "Molecular changes induced by oxidative stress that impair human sperm motility," *Antioxidants*, vol. 9, no. 2, pp. 1–22, 2020, doi: 10.3390/antiox9020134.
31. Z. G. Badade, K. More, and J. Narshetty, "Oxidative stress adversely affects spermatogenesis in male infertility," *Biomed. Res.*, vol. 22, no. 3, pp. 323–328, 2011.
32. A. Agarwal and L. H. Sekhon, "Oxidative stress and antioxidants for idiopathic oligoasthenoteratospermia: Is it justified," *Indian J. Urol.*, vol. 27, no. 1, pp. 74–85, 2011, doi: 10.4103/0970-1591.78437.
33. F. F. Pasqualotto, R. K. Sharma, H. Kobayashi, D. R. Nelson, A. J. Thomas, and A. Agarwal, "Oxidative stress in normospermic men undergoing infertility evaluation," *J. Androl.*, vol. 22, no. 2, pp. 316–322, 2001, doi: 10.1002/j.1939-4640.2001.tb02185.x.
34. A. F. Palani, "Effect of serum antioxidant levels on sperm function in infertile male," *Middle East Fertil. Soc. J.*, vol. 23, no. 1, pp. 19–22, 2018, doi: https://doi.org/10.1016/j.mefs.2017.07.006.
35. A. Agarwal and S. A. Prabakaran, "Mechanism, measurement, and prevention of oxidative stress in male reproductive physiology," *Indian J. Exp. Biol.*, vol. 43, no. 11, pp. 963–974, 2005.
36. R. J. Aitken, D. Buckingham, and D. Harkiss, "Use of a xanthine oxidase free radical generating system to investigate the cytotoxic effects of reactive oxygen species on human spermatozoa," *J. Reprod. Fertil.*, vol. 97, no. 2, pp. 441–450, 1993, doi: 10.1530/jrf.0.0970441.
37. E. G. Bromfield, R. J. Aitken, A. L. Anderson, E. A. McLaughlin, and B. Nixon, "The impact of oxidative stress on chaperone-mediated human sperm-egg interaction," *Hum. Reprod.*, vol. 30, no. 11, pp. 2597–2613, 2015, doi: 10.1093/humrep/dev214.
38. J. P. Ramirez, A. Carreras, and C. Mendoza, "Sperm plasma membrane integrity in fertile and infertile men," *Andrologia*, vol. 24, no. 3, pp. 141–144, May 1992, doi: https://doi.org/10.1111/j.1439-0272.1992.tb02627.x.
39. N. Parker, A. Vidalpuig, and M. D. Brand, "Stimulation of mitochondrial proton conductance,by hydroxynonenal requires a high membrane potential," *Biosci. Rep.*, vol. 28, no. 2, pp. 83–88, 2008, doi: 10.1042/BSR20080002.
40. W. D. Zhang *et al.*, "Oxygen free radicals and mitochondrial signaling in oligospermia and asthenospermia," *Mol. Med. Rep.*, vol. 10, no. 4, pp. 1875–1880, 2014, doi: 10.3892/mmr.2014.2428.
41. A. Amaral and J. Ramalho-Santos, "Assessment of mitochondrial potential: Implications for the correct monitoring of human sperm function," *Int. J. Androl.*, vol. 33, no. 1, pp. 180–186, 2010, doi: 10.1111/j.1365-2605.2009.00987.x.
42. K. Nowicka-Bauer *et al.*, "Sperm mitochondrial dysfunction and oxidative stress as possible reasons for isolated asthenozoospermia," *J. Physiol. Pharmacol.*, vol. 69, no. 3, pp. 403–417, 2018, doi: 10.26402/jpp.2018.3.05.