Current Research in Health Sciences, 4(2):77–82, July-December: 2026.
DOI: https://doi.org/10.58613/crhs4210

Current Research in Health Sciences
ISSN (Online): 2584-1084

Journal homepage: https://www.gpub.org/journal-details.php?journal-id=47



Research Article

Assessment of miR-125a gene expression in Asthenozoospermia and unexplained infertility compared with control group

Dhamya Kadhim Sarhan [1*]

[1] *Department of Biology, Faculty of Science, University of Kufa, Iraq.*
[*] *Corresponding author: dhamyak.eleiwi@uokufa.edu.iq*

Article Info

Keywords: *Asthenozoospermia, Unexplained, 8-OHdG, miR-125a mRNA.*

Received: *20.09.2026;*
Accepted: *04.11.2026;*
Published: *13.11.2026*

© *2026 by the author's. The terms and conditions of the Creative Commons Attribution (CC BY) license apply to this open access article.*

Abstract

Background: Male infertility can be caused by a variety of factors, including chromosomal abnormalities, genetic abnormalities, viral infections, varicocele, ejaculatory duct blockage, radiation, chemotherapy, and erectile dysfunction. **Aim:** Evaluate semen parameters, oxidative stress biomarkers, and miR-125a gene expression in men with asthenozoospermia and unexplained infertility compared with fertile men. Semen samples were collected from infertile patients and fertile controls, and standard semen analysis was performed to assess sperm count, motility, and morphology. In addition, levels of oxidative stress markers, including malondialdehyde (MDA) and 8-hydroxy-2′-deoxyguanosine (8-OHdG), were measured, and the expression of the miR-125a gene in sperm DNA was analyzed. **Methods:** Patients with Asthenozoospermia and unexplained male infertility who visited the reproductive center at Al-Sadr Hospital in the Najaf Governorate, as well as a control group, had their semen samples taken. The patients with infertility were 36.91±1.2 years old on average. **Results:** Demonstrated a significant reduction in sperm count, progressive motility, and normal morphology in infertile men compared with the control group. Furthermore, infertile men showed significantly elevated levels of MDA and 8-OHdG, indicating increased oxidative stress and sperm DNA damage. Altered expression of the miR-125a gene was also observed in infertile men, suggesting a potential role in the regulation of sperm motility and male reproductive function. **Conclusion:** The findings of this study highlight the important relationship between oxidative stress, sperm quality, and gene expression in male infertility. These factors may serve as potential biomarkers for the diagnosis and understanding of the molecular mechanisms underlying male reproductive disorders.

1. Introduction

A male fertility factor is seen in only 30% of infertile couples and a female one in 35% [1]. Causes of male infertility include genetic abnormalities, aneuploidy, viral diseases, ejaculatory duct obstruction, varicocele, radiation and chemotherapy as well as erectile dysfunction [2]. If a patient has less than 32% motile sperm they are diagnosed as having asthenozoospermia. Low sperm motility is the main cause of reduction in male fertility [3], because only healthy sperm with robust progressive movement can go through cervical mucus, into female reproductive canal and eventually access Fallopian tubes.

Low sperm motility molecular mechanism is generally unknown, but a major one contributor to that is the generation of reactive oxygen species (ROS), which directly reduce sperm motility by increasing malondialdehyde (MDA) concentration in the semen [4, 5]. A previous study showed that the evaluation levels of sperm proteins related to energy and metabolism were higher in asthenozoospermia patients

compared to those in fertile men, as was the expression levels of high motility group (HMG) proteins that are involved in motility, regulation, folding and turnover of proteins, and stress response [6].

Unexplained infertility is the inability to get pregnant with no identifiable explanation that could be discovered with standard tests. It is a diagnosis given to 15 percent of infertile couples. There are many reasons for unexplained infertility [7]. When the reason for infertility is unknown, it's called unexplained or UMI [unexplained male infertility (UMI)] [7], but new work from basic research in mice has begun to isolate genetic markers responsible for some male infertility that cannot be picked up by standard semen analysis [8].

Oxidative free radicals and ROS generated by the human body play critical biochemical roles, in vivo generation and chemoselective detection following Creighton reaction with hydrolysis of DNA to its individual bases have made hexahydroxyguanosine (8-OH-dG) the preferable candidate for study among nucleophilic modifications [9]. When the hydroxyl HO reacts with nucleobases of the DNA strand, such as guanine, it yields C8-hydroxyguanine (8-OHGua) or its nucleoside counterpart, deoxyguanosine (8-hydroxy-2-deoxyguanosine). The formation of radical adducts begins with the reaction of HO [10]. Previous studies have also linked smoking and male infertility to elevated levels of octahydroxyquanosine (8-OHdG) in sperm DNA [11].

An essential gene that plays a significant role in the aforementioned processes is mIR-125A, which codes for the large-conductance calcium-activated potassium (BK) channel, involved in regulating membrane potential and intracellular ion homeostasis; these channels have important roles in various reproductive functions, including sperm motility and maturation as well as signaling pathways necessary for fertilization to occur [12]. Therefor measurement of mIR-125A mRNA expression in semen samples or testes tissue might be beneficial for further understanding the molecular bases of diseased state of certain male infertility types. Examining the expression patterns of this gene might help clarify whether changes in its activity are linked to aberrant sperm parameters or defects in spermatogenesis [13]. The research seeks that the sperm motility study may inhibit fertilization of the ovum and could be a factor leading to decline in male infertility due to many factors such as protein and molecular level.

2. Material and methods

The study was conducted in the laboratories of faculty members at the bureau of sciences and biology department at AL-Najaf Health directorate ministry of health, Iraq along with laboratory investigation from fertility centers from AL-Saader Medical city Province of Najaf during the period 1st November/2023 to first April 2025. Semen samples were collected from patients with Asthenozoospermia, and unexplained male infertility as well as control group (Normozoospermic fertile) attending fertility center. Age of infertile patients was (32.99± 32) years and age range patient were between (21- 45) years.

All samples were taken according to WHO (2010) from patients and control used for the estimation of seminal fluid with semen specimens [14]. Biochemical test; (90) sampless were subjected Immunological method (Enzyme-Linked-Immuno-Sorbent-Assay) had been used to measure the 8-hydroxy-2′ -deoxyguanosine protein and malondialdehyde in seminal via ELISA reader (Huma Germany origin). Peripheral blood miRNAs were isolated using the TransZol microRNA extraction Mini-Kit (TransGen Biotech, China). Peripheral blood miRNAs were isolated using the TransZol microRNA extraction Mini-Kit (TransGen Biotech, China)qRT-PCR Primers for the expression level of miR-125a with their sequence were illustrated in details in Table 1.

Table 1: qRT-PCR Primers for the expression level of miR-125a with their sequence

Primer	Sequence (5′-3′)
miRNA Forward primer	5′-GGCGACAGGTGAGGTTCTT-3′
miRNA Reverse primer	5′-GCAGGGTCCGAGGTATTC-3′
U6 Forward primer	GTTTTGTAGTTTTTGGAGTTAGTGTTGTGT
U6 Reverse primer	CTCAACCTACAATCAAAAACAACACAAACA

2.1. Statistical Analysis

The well- known statistical system (Graph Pad prism ver. 5) was adopted, and the analysis of variance table one – way anova (by Tukey´s multiple comparisons test) was used for the comparison among subdivided groups in the measured parameters. The results were expressed as (Mean ± Stander Error).

3. Results

A comparison of several seminal parameters and biochemical markers was made among 20 men with asthenozoospermia, 30 infertile men with unexplained infertility (diagnosis according to World Health Organization criteria) and 41 fertile men (control group).

The sperm count in semen between all studied groups is displayed in Figure 1. The study showed a difference between patients and control group ($p < 0.05$) Men diagnosed with asthenozoospermia displayed a lower sperm count compared to healthy fertile men, and men who were classified as unexplained infertile also showed a reduced sperm count in comparison with the control group. Data are expressed as mean ± standard error. The normal morphology results are shown in Figure 2 for three groups of patients. Results indicated significantly reduced normal forms of spermatozoa in the patients than healthy individuals ($p < 0.05$). Both asthenozoospermic and idiopathic infertile men had lower frequencies of normal sperm morphology compared to fertile men.

Progressive sperm motility in each group is depicted in Figure 3. The study found that the percentage of progressive motility was significantly decreased in the asthenozoospermia group compared to control ($p < 0.05$). Men with unexplained infertility also had lower progressive motility than fertile men.

MDA levels in seminal plasma are shown in Figure 4. The data showed significantly higher levels of MDA in the infertile subjects than control ($P < 0.05$) indicating increased oxidative stress status in both asthenozoospermic and unexplained infertile men,also 8-hydroxy-2′-deoxyguanosine (8-OHdG) level in semen Results showed in Figure 5, that 8-OHdG in infertile men was significantly higher than in fertile control group ($p < 0.05$), suggesting the presence of elevated oxidative DNA damage in sperm cells. Sagging shows in Figure 6

the expression level of MiR-125a mRNA in sperm DNA across the study groups. MiR-125a gene expression divered between those with infertility versus normal subjects ($p < 0.05$) and perhaps may be a link to miR-125a being associated with male infertility .

3.1. Semen and sperm parameters

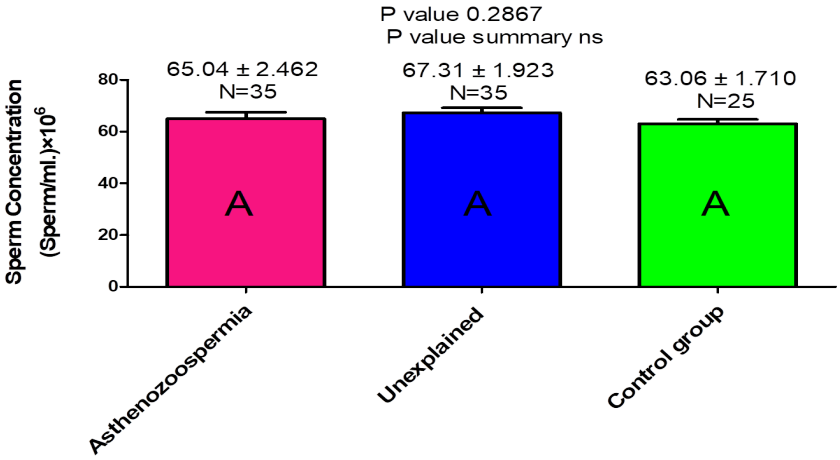


Figure 1: The comparison of sperm counts in the semen between Asthenozoospermia, Unexplained infertile men with fertile men (control). The results within the figure represent the mean ± standard error. Difference later indicates significant ($p < 0.05$)

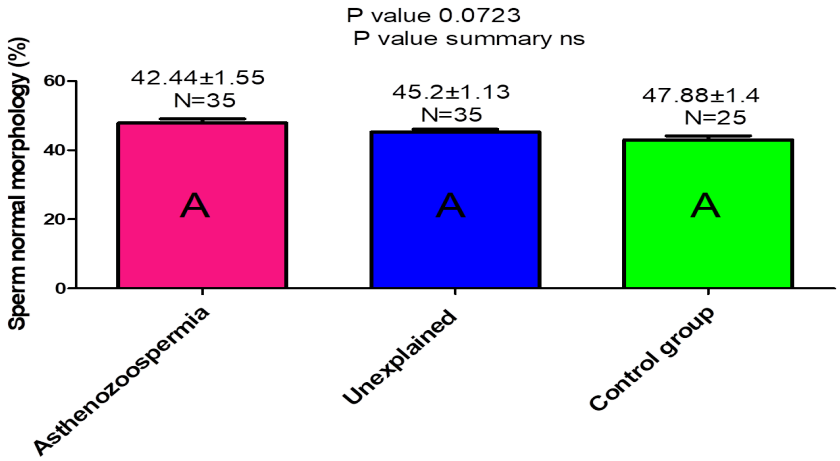


Figure 2: The comparison of Sperm normal morphology in the semen between Asthenozoospermia, Unexplained infertile men with fertile men (control). The results within the figure represent the mean ± standard error. Difference later indicates significant ($p < 0.05$)

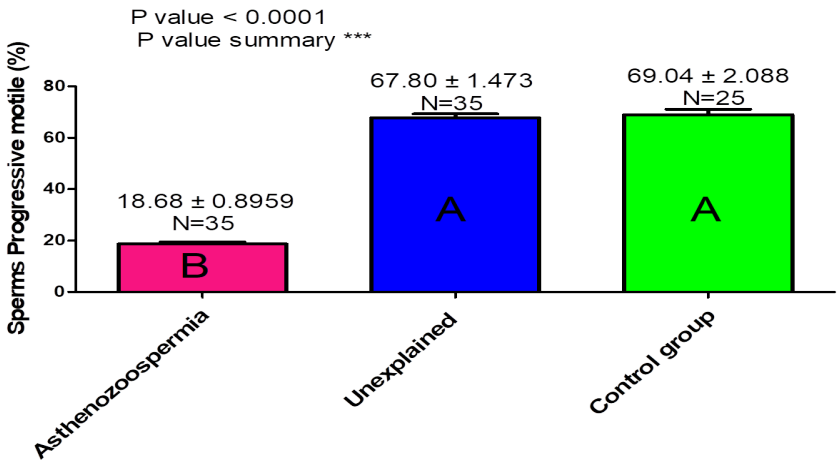


Figure 3: The comparison of Sperm Progressive motile in the semen between Asthenozoospermia, Unexplained infertile men with fertile men (control). The results within the figure represent the mean ± standard error. Difference later indicates significant ($p < 0.05$)

3.2. Malondialdehyde concentration in seminal plasma at infertility patients

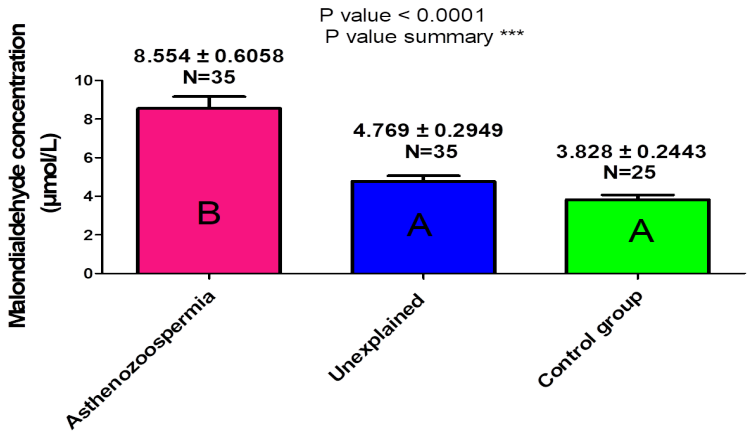


Figure 4: The comparison of malondialdehyde concentration in semen between infertile men Asthenozoospermia and unexplained men compared with control group. The results within the figure represent the mean ± standard error. Difference later indicates significant ($p < 0.05$)

3.3. 8-hydroxy-2′-deoxyguanosine protein level

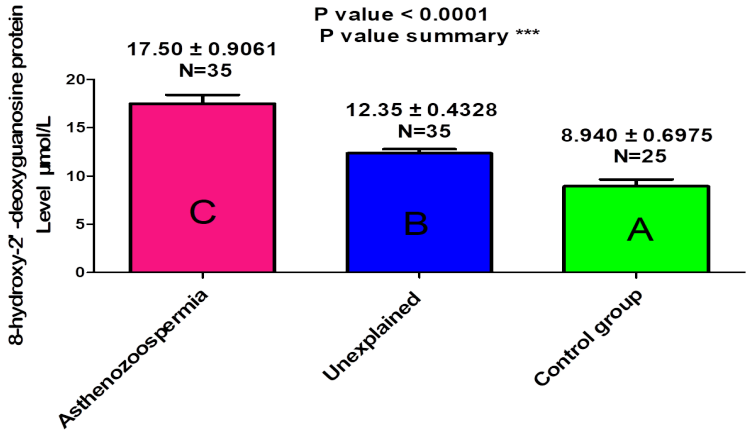


Figure 5: The comparison of 8-hydroxy-2′-deoxyguanosine protein level in semen between infertile men Asthenozoospermia and unexplained men compared with control group. The results within the figure represent the mean ± standard error. Difference later indicates significant ($p < 0.05$)

3.4. miR-125a mRNA expression Level in sperm DNA

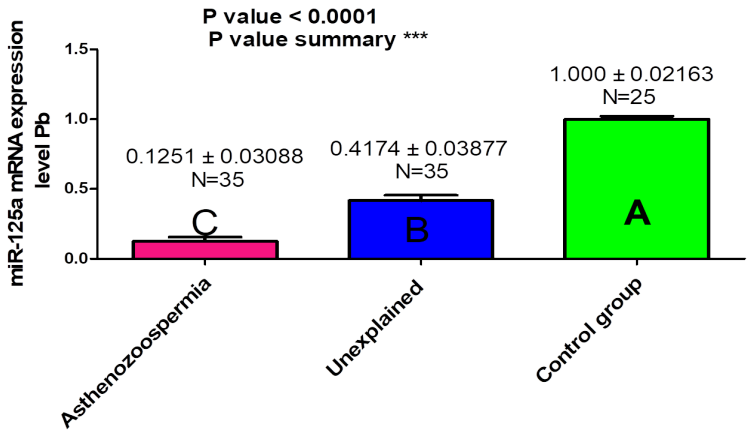


Figure 6: The comparison of miR-125a mRNA expression Level in semen between infertile men Asthenozoospermia and unexplained men compared with control group. The results within the figure represent the mean ± standard error. Difference later indicates significant ($p < 0.05$)

4. Discussion

The current study assessed seminal parameters, oxidative stress markers and expression of genes associated with semen quality in men with unexplained infertility and asthenozoospermia compared to fertile men. In conclusion, the results showed considerable changes in sperm characteristics and oxidative stress markers among infertile men that imply their relevance to male infertility.

In this study, sperm count was significantly lower in infertile men than the fertile control group. Low sperm count is one of the key features of impaired spermatogenesis and male reproductive dysfunction. The reduction noticed in the asthenozoospermic group could be associated with testicular dysfunction or augmented oxidative stress impairing sperm production and maturation [15].

The findings also indicated that infertile men have significantly more abnormal sperm morphology than the control group. Abnormal sperm morphology associated with reduced fertilization potential and poor reproductive outcomes. However, genetic or environmental factors can induce alterations at the structural level of sperm cells including oxidative stress that compromise sperm membrane and DNA integrity [16, 17]. In addition, progressive sperm motility was lower among men with asthenozoospermia and unexplained infertility. Motility, the ability of sperm to move, is critical for successful fertilization [3]. This suggests that reduced motility is related to mitochondrial dysfunction, oxidative stress, or change in the activity of ion channels which control flagellar movement [18]. Significantly higher levels of malondialdehyde (MDA) in the semen of infertile men were also evident from present findings. MDA is a known biomarker indicative of lipid peroxidation and oxidative stress. Higher MDA levels indicate oxidative stress on the sperm membrane that potentially reduces sperm motility and viability [19]. These findings are in agreement with previous studies reporting increased oxidative stress among men with impaired semen quality. Moreover, levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG), an oxidative marker of DNA damage, were significantly increased in infertile men compared with fertile controls. 8-OHdG indicates increased oxidative damage to sperm DNA that may impair sperm function, fertilization capacity and embryo development [20].

One of the significant results of this study was changes in miR-125a gene expression in sperm DNA of infertile men. miR-125a gene (encodes for large-conductance calcium-activated potassium channel) Alteration in expression of this gene may affect sperm motility and function potentially leading to male infertility [12, 21].

"Our results established how oxidative stress correlates with sperm quality and gene expression, as well as provided a major step towards the resolution of male infertility – reducing oxidative and Bernouff's stress in human sperm." The sperm function impairment in infertile men may be related to increased oxidative stress parameters, DNA damage and altered gene expression.

5. Conclusions

This study demonstrated significant alterations in semen parameters, oxidative stress biomarkers, and gene expression in infertile men compared with fertile controls. Reduced sperm count, motility, and normal morphology were observed in men with asthenozoospermia and unexplained infertility, indicating impaired sperm function. Moreover, the elevated levels of malondialdehyde (MDA) and 8-hydroxy-2′-deoxyguanosine (8-OHdG) suggest increased oxidative stress and DNA damage in sperm cells. The altered expression of the miR-125a gene may also contribute to impaired sperm motility and reproductive dysfunction. These findings highlight the important role of oxidative stress and molecular alterations in male infertility and suggest that they may serve as potential biomarkers for diagnosis and future therapeutic strategies.

Article Information

Ethical Approval: All investigations involving humans in this project were conducted with strict adherence to the principles contained in the Declaration of Helsinki of 1975 (amended in 2008) and have received approval from the relevant Ethics Committee of the Department of Biology, College of Science, University of Kufa.

Informed Consent: Written informed consent was obtained from all participants before their inclusion in the study.

Consent for Publication: Publication has been approved by the author.

Data Availability: The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: No external funding was received for this study.

Acknowledgments: The authors extend their thanks to the Najaf Health Department and to the patients for their cooperation.

AI Use Disclosure: No generative artificial intelligence tools were used in the preparation of this manuscript.

References

[1] A. Agarwal, A. Mulgund, A. Hamada, and M. R. Chyatte. A unique view on male infertility around the globe. *Reproductive Biology and Endocrinology*, 13(1):37, 26 April 2015.

[2] H. Al-Msaid and S. Aljazaeri. Study of the effect of trace elements on male fertility and its relationship to DNA damage. *Family Medicine & Primary Care Review*, 27(3):280–283, 2025. URL https://doi.org/10.5114/fmpcr.2025.153087.

[3] F. A. Garcia-Vazquez, J. Gadea, C. Matás, and W. V. Holt. Importance of sperm morphology during sperm transport and fertilization in mammals. *Asian Journal of Andrology*, 18(6):844–50, 2016. URL https://doi.org/10.4103/1008-682X.186880.

[4] R. Chianese and R. Pierantoni. Mitochondrial reactive oxygen species (ROS) production alters sperm quality. *Antioxidants*, 10(1):92, 2021. URL https://doi.org/10.3390/antiox10010092.

[5] E. de Lamirande and C. O'Flaherty. Sperm activation: role of reactive oxygen species and kinases. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics*, 1784(1):106–15, 2008. URL https://doi.org/10.1016/j.bbapap.2007.08.024.

[6] A. Asghari, S. A. Marashi, and N. Ansari-Pour. A sperm-specific proteome-scale metabolic network model identifies non-glycolytic genes for energy deficiency in asthenozoospermia. *Systems Biology in Reproductive Medicine*, 63(2):100–12, 2017. URL https://doi.org/10.1080/19396368.2016.1263367.

[7] Guideline Group on Unexplained Infertility and Romualdi D, Ata B, Bhattacharya S, Bosch E, Costello M, Gersak K, Homburg R, Mincheva M, Norman RJ, Piltonen T. Evidence-based guideline: unexplained infertility. *Human Reproduction*, 38(10):1881–90, 2023. URL https://doi.org/10.1093/humrep/dead150.

[8] A. Ray, A. Shah, A. Gudi, and R. Homburg. Unexplained infertility: an update and review of practice. *Reproductive Biomedicine Online*, 24(6):591–602, 2012. URL https://doi.org/10.1016/j.rbmo.2012.02.021.

[9] A. Valavanidis, T. Vlachogianni, and C. Fiotakis. 8-hydroxy-2′-deoxyguanosine (8-OHdG): a critical biomarker of oxidative stress and carcinogenesis. *Journal of environmental science and health Part C*, 27(2):120–39, 2009. URL https://doi.org/10.1080/10590500902885684.

[10] V. Rani, S. Asthana, M. Vadhera, U. C. Yadav, and N. Atale. Tools and Techniques to measure oxidative stress. In *InFree Radicals in Human Health and Disease*, pages 43–56. Springer India, New Delhi, 2014. URL https://doi.org/10.1007/978-81-322-2035-0_4.

[11] G. Ji, L. Yan, W. Liu, J. Qu, and A. Gu. OGG1 Ser326Cys polymorphism interacts with cigarette smoking to increase oxidative DNA damage in human sperm and the risk of male infertility. *Toxicology Letters*, 218(2):144–9, 2013. URL https://doi.org/10.1016/j.toxlet.2013.01.017.

[12] C. S. Bailey, H. J. Moldenhauer, S. M. Park, S. Keros, and A. L. Meredith. KCNMA1-linked channelopathy. *Journal of General Physiology*, 151(10):1173–89, 2019. URL https://doi.org/10.1085/jgp.201912457.

[13] H. Wang, L. L. McGoldrick, and J. J. Chung. Sperm ion channels and transporters in male fertility and infertility. *Nature Reviews Urology*, 18(1):46–66, 2021. URL https://doi.org/10.1038/s41585-020-00390-9.

[14] World Health Organization. *WHO laboratory manual for the examination and processing of human semen*. World Health Organization, 6^{th} edition, 2021.

[15] A. Asadi, R. Ghahremani, A. Abdolmaleki, and F. Rajaei. Role of sperm apoptosis and oxidative stress in male infertility: A narrative review. *International Journal of Reproductive Biomedicine*, 19(6):493, 2021. URL https://doi.org/10.18502/ijrm.v19i6.9371.

[16] H. T. Nguyen, H. N. Dang, T. T. Nguyen, T. V. Nguyen, T. C. Dang, Q. H. Nguyen, and M. T. Le. Correlations between abnormalities of morphological details and DNA fragmentation in human sperm. *Clinical and Experimental Reproductive Medicine*, 49(1):40, 2022. URL https://doi.org/10.5653/cerm.2021.04777.

[17] P. Sengupta, S. Dutta, F. F. Liew, V. Dhawan, B. Das, F. Mottola, P. Slama, L. Rocco, and S. Roychoudhury. Environmental and genetic traffic in the journey from sperm to offspring. *Biomolecules*, 13(12):1759, 2023. URL https://doi.org/10.3390/biom13121759.

[18] M. R. Miranda, V. Vestuto, O. Moltedo, M. Manfra, P. Campiglia, and G. Pepe. The ion channels involved in oxidative stress-related gastrointestinal diseases. *Oxygen*, 3(3):336–65, 2023. URL https://doi.org/10.3390/oxygen3030022.

[19] T. Yin, X. Yue, Q. Li, X. Zhou, R. Dong, J. Chen, R. Zhang, X. Wang, S. He, T. Jiang, and F. Tao. The association between the levels of oxidative stress indicators (MDA, SOD, and GSH) in seminal plasma and the risk of idiopathic oligo-asthenoteratozoospermia: does Cu or Se level alter the association? *Biological Trace Element Research*, 202(7):2941–53, 2024. URL https://doi.org/10.1007/s12011-023-03888-6.

[20] M. A. Mukheef, R. A. Ali, and H. H. Alheidery. Follicular fluid 8-Hydroxy-2-Deoxyguanosine (8-OHdG) as biomarker for oxidative stress in intracytoplasmic sperm injection. *The Journal of Medical Investigation*, 69(1):2, 2022. URL https://doi.org/10.2152/jmi.69.112.

[21] A. L. Meredith. BK Channelopathies and KCNMA1-Linked Disease Models. *Annual Review of Physiology*, 86(1):277–300, 2024. URL https://doi.org/10.1146/annurev-physiol-030323-042845.