Original Article

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Levels of 8-isoprostane and advanced oxidation protein products in relation to sperm count and motility

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ABSTRACT

Background: Oxidative stress is implicated in male infertility, with reactive oxygen species contributing to sperm dysfunction. Among oxidative stress markers, 8-isoprostanes (8-isoP) and advanced oxidation protein products (AOPP) serve as indicators of lipid peroxidation and protein oxidation, respectively. However, their relationship with specific sperm abnormalities remains unclear. **Aim**: This study investigates the oxidative stress markers 8-isoprostane (8-isoP) and advanced oxidation protein products (AOPP) across different semen profiles and explores their correlation with sperm count and motility to evaluate their potential as diagnostic biomarkers in male infertility. **Methods**: A cross-sectional study was conducted on male partners of infertile couples between May 2024 and February 2025. Subjects were categorized into four groups based on semen analysis: normozoospermia (control), oligozoospermia, asthenozoospermia, and azoospermia. Seminal plasma levels of 8-isoP and AOPP were measured using sandwich ELISA kits, and results were compared among the groups. **Results**: Levels of 8-isoprostanes (8-isoP) and advanced oxidation protein products (AOPP) were significantly elevated in the asthenozoospermia group. In the azoospermia group, AOPP levels showed a significant increase, while 8-isoP exhibited a near-significant rise compared to the control. In contrast, the oligozoospermia group showed no significant increase in either marker. **Conclusions**: Although both markers increased in asthenozoospermia and azoospermia, AOPP showed greater sensitivity, highlighting protein oxidation's role in severe sperm dysfunction. The oligospermic group showed no increase in both markers, indicating that isolated reduction in sperm count is unlikely to be caused by oxidative stress.

Keywords: Oxidative stress, 8-isoprostanes, AOPP, oligozoospermia, infertility, asthenozoospermia.

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INTRODUCTION

Infertility is a multifaceted medical condition affecting approximately 70 million couples worldwide. It is defined by the WHO as the inability to conceive after 12 months of unprotected, regular sexual intercourse.[1] Male factors contribute to nearly 50% of cases. In most of these cases, an identifiable cause can be found, including genetic causes such as Y-chromosome deletions, varicocele, infections of the male reproductive tract, presence of

antisperm antibodies, non-obstructive and obstructive azoospermia, and hypogonadism. However, despite advances in our understanding of male infertility, 15–30% of cases remain unexplained, with no clear underlying cause.[2] While conventional diagnostic approaches often rely on standard semen analysis, this assessment fails to capture the full spectrum of factors influencing male reproductive potential. In particular, the role of oxidative stress, mediated by reactive oxygen species (ROS), has emerged as a significant contributor to infertility.[3] even among individuals with normal semen parameters.[4] Reactive oxygen species, which are byproducts of cellular metabolism, play a dual role in sperm physiology. At low levels, they are essential for processes such as sperm capacitation, hyperactivation, and acrosome reaction, which are critical for fertilization.[5] However, due to the high vulnerability of spermatozoa to oxidative damage and their limited protective antioxidant system.[6] excessive ROS production can result in oxidative stress, leading to lipid peroxidation, DNA damage, and protein oxidation in spermatozoa. These alterations compromise sperm function and genetic integrity, ultimately impairing fertilization and embryonic development.[7] Despite growing recognition of oxidative stress in male infertility, the diagnostic potential of newer ROS markers remains underexplored. Emerging biomarkers, such as 8-isoprostanes (8-isoP) and advanced oxidation protein products (AOPP), offer a promising avenue for understanding the underlying mechanisms of infertility.[8,9] These markers provide insight into oxidative damage at the molecular level, potentially revealing previously unrecognized pathways contributing to impaired sperm function.[10] This research aims to assess seminal plasma levels of oxidative stress markers (8-isoprostanes and AOPP) in men with normozoospermia, oligozoospermia, asthenozoospermia, and azoospermia. It will examine their association with sperm motility and count, evaluate their potential as markers for detecting oxidative stress in semen, and clarify their role in male infertility and subfertility.[11]

MATERIALS AND METHODS

This comparative cross-sectional study was conducted at the fertility clinics of Azadi Hospital between August 10, 2024, and February 10, 2025. Men with normospermia were included in the study as a distinct control group, serving as a reference for comparison with individuals exhibiting abnormal sperm parameters. A total of 88 male participants were recruited and categorized into four groups: normozoospermia (n = 23) (age 32.4 ± 4.8 years), oligozoospermia (n = 23) (age 31.3 ± 5.9 years), asthenozoospermia (n = 24) (age 34.8 ± 5.1 years), and azoospermia (n = 18) (age 32.3 ± 5.0 years). Normozoospermia was defined as having a sperm concentration of 15 million/mL or more and progressive motility of at least 32%. Oligozoospermia was characterized by a sperm concentration of less than 15 million/mL but with normal motility. Asthenozoospermia referred to samples with progressive motility below 32%, but with normal sperm concentration. Azoospermia was defined by the complete absence of sperm in the ejaculate. Participants were included if they were male partners of couples who had experienced infertility for at least one year and were aged between 20 and 45 years, representing the typical reproductive age group. To preserve the study's focus on individual sperm abnormalities, men with active infections or febrile conditions were excluded. Additionally, samples displaying oligoasthenozoospermia, where both sperm count and motility were abnormal, were excluded to ensure that each sample reflected only a single sperm abnormality. This approach allowed for more precise comparisons across distinct sperm abnormality categories. Board approval was obtained from the regional health directorate prior to the initiation of sample collection, and both verbal and written consent were taken from each participant. Participants were instructed to observe a minimum of three days of sexual abstinence, as per WHO guidelines, to standardize semen quality across samples. Semen was collected by masturbation into sterile, wide-mouthed containers provided by the laboratory. Upon receipt, each sample was labeled with the participant's identification and collection time. The samples were then placed in an incubator at 37°C for 30 minutes to allow for liquefaction. After liquefaction, macroscopic examination of the semen assessed parameters such as volume, viscosity, color, and pH. This was followed by microscopic analysis using Computer-Assisted Sperm Analysis (CASA) technology, performed with an XSZ-N107T microscope equipped with a digital camera linked to a computer system. A trained laboratory technician prepared slides from the semen samples, which were examined under the microscope. The digital camera captured high-resolution images, and the software automatically analyzed key sperm parameters such as

concentration, motility, and morphology. The use of CASA provided objective, standardized, and reproducible results, reducing observer variability commonly associated with manual analysis.[11] Following the completion of sperm analysis, the remaining semen sample was centrifuged at 3000 rpm for 10 minutes to separate the seminal plasma from sperm cells. The supernatant, representing the seminal plasma, was carefully collected and stored at –20°C for subsequent biochemical analysis. This stored plasma was used to measure the concentrations of the oxidative stress markers 8-isoprostanes and AOPP, enabling further evaluation of their relationship with the different types of sperm abnormalities observed in the study population. Biochemical analysis Measurement of 8-Isoprostanes (8-isoP) The levels of 8-isoprostanes (8-isoP) in seminal plasma were measured using a commercially available sandwich ELISA kit (Bioassay Technology Laboratory, catalog no. E6778Hu, UK), which uses plates pre-coated with 8-iso-PGF2α antibody. According to the manufacturer, the kit accurately quantifies human 8-isoprostane (8-iso-PGF2α) in serum, plasma, cell culture supernates, ascites, tissue homogenates, and other biological fluids. The assay was performed following the provided protocol. Absorbance was read at 450 nm using an ELISA reader (Paramedical PKL), and concentrations were expressed as ng/L.

Measurement of Advanced Oxidation Protein Products (AOPP) AOPP concentrations were assessed using a commercially available ELISA kit (Bioassay Technology Laboratory, catalog no. E1266Hu, England). This is a sandwich ELISA kit that uses plates pre-coated with AOPP antibody. According to the manufacturer, the kit accurately quantifies human AOPP in serum, plasma, cell culture supernates, ascites, tissue homogenates, or other biological fluids. The assay was performed following the provided protocol. Absorbance was read at 450 nm using an ELISA reader (Paramedical PKL), and concentrations were expressed as ng/mL.

Data were entered and analyzed using SPSS 27.0 for Windows software. Descriptive statistics included frequencies, means, standard deviation, standard error of the mean (SEM), and percentages. Group differences were assessed using the Kruskal-Wallis test for multiple-group comparisons and the Mann-Whitney U test for pairwise comparisons. All hypothesis tests were two-tailed, with statistical significance set at $p < 0.05$ and a 95% confidence interval (CI).

RESULTS

Table 1 presents the mean values of sperm parameters across the four study groups. Table 2 shows the concentrations of the two markers in each group along with the p-value of each in comparison with normozoospermic controls. Figures 1 and 2 demonstrate the average concentrations of the two markers in the four study groups. AOPP levels were significantly elevated in the asthenozoospermia ($p = 0.003$) and azoospermia ($p = 0.001$) groups, while 8-isoP levels showed a significant increase only in the asthenozoospermia group ($p = 0.01$). In the azoospermia group, 8-isoP did not reach statistical significance ($p = 0.052$), although the result approached borderline significance. Neither marker showed a significant elevation in the oligozoospermia group.

Table 1: Seminal parameters in normozoospermic, oligozoospermic, asthenozoospermic, and azoospermic males

Subjects	Concentration (million/mL)	Motility (%)	Morphology (%)
Normozoospermia (n=23)	60.43 ± 5.37	74.18 ± 1.93	59.24 ± 2.20
Oligozoospermia (n=23)	5.72 ± 0.99	57.95 ± 3.53	30.18 ± 3.52
Asthenozoospermia (n=24)	45.57 ± 6.27	21.36 ± 2.61	18.71 ± 3.36
Azoospermia (n=18)	0	-	-

Data are reported as Mean ± SEM; n = number of cases.

Table 2: Seminal plasma levels of 8-isoprostane and AOPP in controls and other groups.				
Control group:	8-isoprostane (ng/L)		AOPP (ng/mL)	
Normozoospermia (n=23)	50.96 ± 2.80		3.90 ± 0.69	
Abnormal sperm parameter groups:	8-isoprostane (ng/L)	p-value	AOPP (ng/mL)	p-value
Oligozoospermia (n=23)	45.91 ± 4.64	p = 0.06	2.82 ± 0.56	p = 0.2
Asthenozoospermia (n=24)	69.50 ± 7.11	p = 0.01*	8.12 ± 1.04	p = 0.003*
Azoospermia (n=18)	58.81 ± 5.26	p = 0.52	8.15 ± 1.02	p = 0.001*
Data are reported as mean ± SEM; * indicates significant p-value (p < 0.05)				

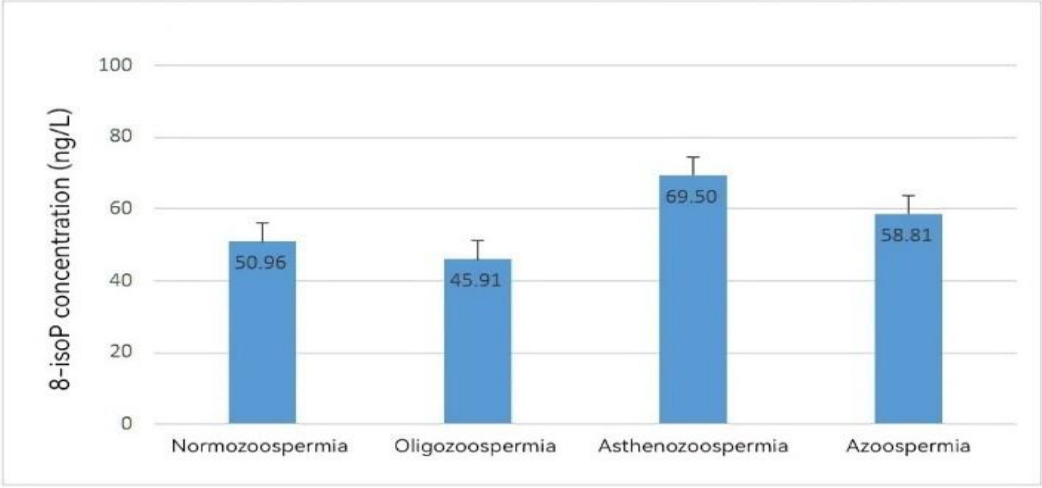

Figure 1: Average concentration of 8-isoP in the four groups (ng/L)

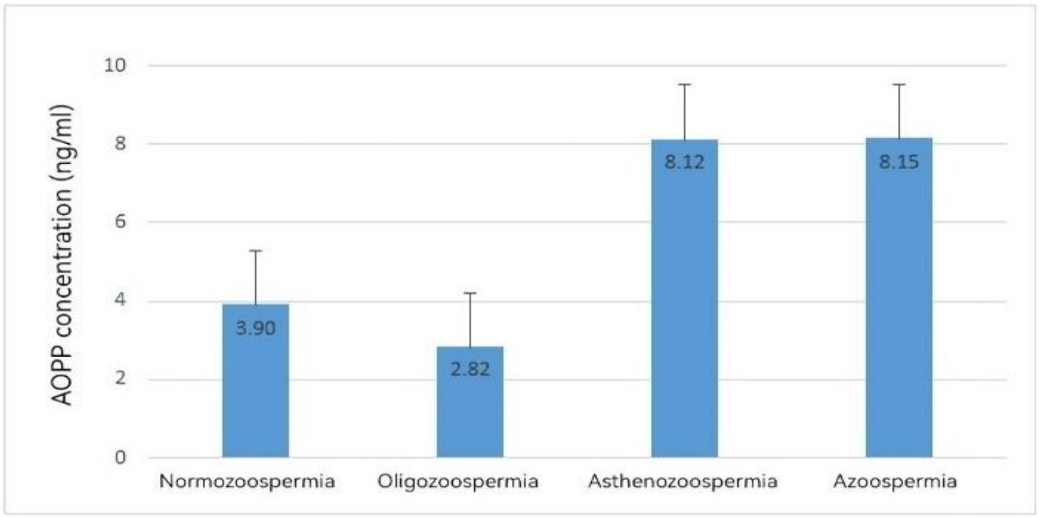

Figure 2: Average concentration of AOPP in the four groups (ng/mL)

DISCUSSION

Motility dysfunction in sperm is strongly linked to oxidative stress—a relationship well documented in the literature as Several studies have demonstrated that oxidative stress negatively affects sperm motility.[12-14] One author even suggested that impaired motility may itself serve as a biomarker of oxidative imbalance in the male reproductive system.[15] Excessive generation of reactive oxygen species (ROS), particularly under inflammatory conditions, promotes rigidity of the sperm flagellar membrane by depleting its lipid content. This leads to motility inhibition, sperm agglutination, and asthenozoospermia.[16] Additionally, ROS interfere with mitochondrial oxidative phosphorylation, causing ATP depletion and further compromising motility.[4] In our study, oxidative stress markers were significantly elevated in the asthenozoospermic group. AOPP levels increased from 3.90 ng/mL in the normozoospermic group to 8.12 ng/mL, an increase of approximately 108%. Similarly, 8-isoprostane levels rose from 50.96 to 69.50 ng/L, representing a 36% increase. The significant elevation of both markers highlights the role of oxidative stress in the pathophysiology of male infertility. However, the greater rise in AOPP suggests that protein oxidation might exert a more immediate and functionally disruptive effect on sperm motility than lipid peroxidation. 8-Isoprostanes (8-iso-PGF2α) are stable and reliable markers of lipid peroxidation, formed through free radical-induced oxidation of arachidonic acid.[17] Beyond serving as markers, 8-isoprostanes also exert detrimental biological effects.[18] and are associated with disease severity in conditions such as asthma, cardiovascular disease, chronic obstructive pulmonary disease (COPD), and neurodegenerative disorders like Alzheimer's disease.[19] On the other hand, advanced oxidation protein products (AOPPs) arise from the reaction of plasma proteins, especially albumin, with chlorinated oxidants like hypochlorous acid. This leads to structural modifications such as dityrosine cross-linking and carbonyl formation, which compromise protein function. AOPPs are increasingly recognized as key markers of oxidative stress and have been implicated in several pathological states, including multiple sclerosis and Parkinson's disease.[20] Our results align with earlier reports linking oxidative stress to sperm motility impairment. Khosrowbeygi et al. (2008) observed elevated 8-isoprostane levels and reduced antioxidant defenses in men with combined sperm abnormalities, including asthenozoospermia. In contrast to their mixed grouping, our study categorized participants into distinct groups, allowing a clearer analysis of oxidative stress effects on motility alone.[21] Abdalrahman et al. (2021) also reported a negative correlation between seminal malondialdehyde (MDA) levels and sperm motility. MDA, like 8-isoprostane, is a marker of lipid peroxidation. They further observed a reduction in total antioxidant capacity (TAC) in asthenozoospermic men. These findings support the contribution of lipid peroxidation to impaired sperm motility.[22] Demir and Ozden (2022) found elevated AOPP levels in men with oligoasthenozoospermia and oligoasthenoteratozoospermia. Although their groups had overlapping abnormalities, their findings further support the role of protein oxidation in motility dysfunction.[23] Together, these studies corroborate our conclusion that oxidative stress—especially protein oxidation—plays a central role in reduced sperm motility. Interestingly, the oligozoospermic group in our study did not show significant differences in oxidative stress markers compared to the normozoospermic group. In fact, average levels of both AOPP and 8-isoprostane were slightly lower. The relationship between oxidative stress and low sperm count remains unclear. Some evidence suggests that oxidative damage contributes to reduced sperm count via DNA fragmentation and induction of abortive apoptosis.[4] However, this is more frequently observed in combined abnormalities, as in oligoasthenozoospermia, where both count and motility are impaired. For example, Demir and Ozden (2022) reported increased AOPP, total oxidant status (TOS), and oxidative stress index (OSI) in oligoasthenozoospermic men.[23] Similarly, Agarwal et al. (2014) observed elevated ROS in oligoasthenoteratozoospermia and other combined abnormalities, reinforcing the idea that high oxidative stress may affect both motility and count.[24] A possible explanation for the apparent difference in our results is that oxidative stress likely affects sperm function in a gradual sequence. At lower levels, it primarily impairs motility by damaging the sperm membrane, which is highly vulnerable to lipid peroxidation. As oxidative stress increases, it can cause DNA fragmentation, eventually triggering apoptosis and leading to a reduction in sperm count. In the present study, the oligozoospermic group exhibited normal motility, suggesting that oxidative stress was not a major contributing factor in this group. Instead, the reduced sperm count is more likely attributable to other causes, such as genetic, hormonal, or unexplored etiological

factors. This view is supported by findings from Aitken and Baker (2020) in their study on the role of genetics and oxidative stress in male infertility. They noted that the intrinsic apoptotic pathway is closely linked to a rapid loss of motility and that any stressor initiating apoptosis in spermatozoa leads to mitochondrial ROS production and a subsequent decline in sperm function.[25] In the azoospermic group, both oxidative stress markers were elevated. AOPP levels reached statistical significance ($p = 0.001$), while 8-isoprostane levels showed a trend toward significance ($p = 0.052$). The markedly elevated AOPP levels suggest that protein oxidation plays a prominent role in azoospermia, possibly reflecting underlying inflammatory or cellular damage mechanisms. These results are consistent with previous research linking azoospermia to heightened oxidative stress. Cito et al. (2019) reported significantly elevated ROS production in white blood cells, higher lipid peroxidation markers, and reduced antioxidant capacity in men with non-obstructive azoospermia, indicating substantial oxidative damage associated with impaired spermatogenesis.[26] Similarly, Kratz et al. (2016) found that seminal AOPP levels were markedly higher in azoospermic patients than in fertile controls, while levels of the antioxidant hormone melatonin were significantly lower and inversely correlated with AOPP concentrations.[27] Together, these studies reinforce the present finding that protein oxidation, as reflected by elevated AOPP, is a prominent feature of azoospermia and may reflect both increased oxidative damage and diminished antioxidant defense. An important limitation of this study is the relatively small sample size in each subgroup, particularly in the azoospermic group, which may have limited the statistical power to detect subtle but clinically relevant differences, especially for 8-isoprostane levels that approached but did not reach significance. Additionally, this study did not assess antioxidant capacity or related defense mechanisms, which are often included in similar research to provide a more comprehensive evaluation of oxidative balance. Measuring antioxidant parameters would have considerably increased the study's cost and complexity and was therefore beyond the scope of the current work.

CONCLUSIONS

In conclusion, the consistent and significant elevation of AOPP in both asthenozoospermic and azoospermic groups underscores its value as a more sensitive marker of oxidative stress in male infertility and suggests that protein oxidation may play a particularly important role in severe sperm dysfunction. The second important takeaway from this study is that isolated oligozoospermia did not exhibit elevated oxidative stress markers, indicating that its underlying causes are more likely related to non-oxidative mechanisms. One potential clinical application of measuring AOPP in seminal plasma lies in its capacity to reflect the effectiveness of targeted interventions that aim to lower oxidative stress. Changing AOPP levels can offer practical feedback on how well patients respond to antioxidant supplementation and to strategies designed to reduce exposure to oxidative triggers, including environmental pollutants, lifestyle-related factors, and dietary habits. By monitoring these variations, clinicians may gain a clearer understanding of the impact of these measures on the seminal oxidative environment and, potentially, on overall sperm quality.

REFERENCES

1. Szamatowicz M, Szamatowicz J. Proven and unproven methods for diagnosis and treatment of infertility. Adv Med Sci. 2020;65(1):93–96.
2. Gelbaya TA, Potdar N, Jeve YB, Nardo LG. Definition and epidemiology of unexplained infertility. Obstet Gynecol Surv. 2014;69(2):109–115.
3. Agarwal A, Virk G, Ong C, du Plessis SS. Effect of oxidative stress on male reproduction. World J Mens Health. 2014;32(1):1–17.
4. Gudeloglu A, Brahmbhatt J, Parekattil S. Definitions and epidemiology of unexplained male infertility. In: Schattman GL, Esteves SC, Agarwal A, editors. Unexplained infertility: pathophysiology, evaluation, and treatment. New York: Springer; 2015. p. 15–30.
5. Evans EPP, Scholten JTM, Mzyk A, et al. Male subfertility and oxidative stress. Redox Biol. 2021;46:102071.
6. Bisht S, Faiq M, Tolahunase M, Dada R. Oxidative stress and male infertility. Nat Rev Urol. 2017;14(8):470–485.
7. Takalani NB, Monageng EM, Mohlala K, et al. Role of oxidative stress in male infertility. Reprod Fertil. 2023;4(3):e230024.
8. Janiszewska E, Kokot I, Kmieciak A, et al. Associations between seminal plasma advanced oxidation protein products and redox-related biochemical parameters in infertile men. Cells. 2022;11(22):3667.
9. Signorini C, Moretti E, Collodel G. Role of isoprostanes in human male infertility. Syst Biol Reprod Med. 2020;66(5):291–299.
10. Finelli R, Leisegang K, Kandil H, Agarwal A. Oxidative stress in the pathogenesis and management of varicocele. World J Mens Health. 2022;40(1):87–103.
11. Schubert B, Badiou M, Force A. Computer-aided sperm analysis: a key player in routine sperm assessment. Andrologia. 2019;51(10):e13417.
12. Nowicka-Bauer K, Nixon B. Molecular changes induced by oxidative stress that impair human sperm motility. Antioxidants (Basel). 2020;9(2):134.

13. Wang Y, Fu X, Li H. Mechanisms of oxidative stress-induced sperm dysfunction. Front Endocrinol (Lausanne). 2025;16:1520835.
14. Kurkowska W, Bogacz A, Janiszewska M, et al. Oxidative stress is associated with reduced sperm motility in normal semen. Am J Mens Health. 2020;14(5):1557988320939731.
15. Alahmar AT. Role of oxidative stress in male infertility: an updated review. J Hum Reprod Sci. 2019;12(1):4–18.
16. Hussain T, Kandeel M, Metwally E, et al. Harmful effects of oxidative stress on male fertility: mechanistic insights. Front Endocrinol (Lausanne). 2023;14:1070692.
17. Adgent MA, Gebretsadik T, Elaiho CR, et al. Association between prenatal F2-isoprostanes and childhood wheeze/asthma. Free Radic Biol Med. 2022;189:85–90.
18. Kaviarasan S, Muniandy S, Qvist R, Ismail IS. F2-isoprostanes as biomarkers for type 2 diabetes. J Clin Biochem Nutr. 2009;45(1):1–8.
19. Janssen LJ. Isoprostanes: roles in pulmonary pathophysiology. Am J Physiol Lung Cell Mol Physiol. 2001;280(6):L1067–L1082.
20. Liu Z, Yao X, Jiang W, et al. Advanced oxidation protein products induce neuroinflammation via MAPK–NF-κB signaling. J Neuroinflammation. 2020;17(1):90.
21. Khosrowbeygi A, Zarghami N. Seminal plasma 8-isoprostane levels and sperm quality. Indian J Clin Biochem. 2008;23(1):49–52.
22. Abd Elrahman MM, El Makawy AI, Hassanane MS, et al. Correlation between asthenozoospermia and mitochondrial DNA mutations. J Genet Eng Biotechnol. 2021;19(1):11.
23. Demir M, Ozdem S. Evaluation of total antioxidant capacity in human seminal plasma. Int J Med Biochem. 2022;5(1):54–59.
24. Agarwal A, Mulgund A, Sharma R, Sabanegh E. Mechanisms of oligozoospermia: an oxidative stress perspective. Syst Biol Reprod Med. 2014;60(4):206–216.
25. Aitken RJ, Baker MA. Genetics and oxidative stress in male infertility: a unifying hypothesis. Front Endocrinol (Lausanne). 2020;11:581838.
26. Cito G, Becatti M, Natali A, et al. Redox status assessment in non-obstructive azoospermia. Andrology. 2020;8(2):364–371.
27. Kratz EM, Piwowar A, Zeman M, et al. Advanced oxidation protein products and melatonin levels in seminal plasma. Reprod Fertil Dev. 2016;28(4):507–515.