



Investigating the relationship of seminal oxidative damage with smoking and occupations in infertile men with normal semen parameters

✉Neslihan Hekim^{a,*}, ✉Sezgin Gunes^a, ✉Oguz Ulusoy^a, ✉Sercan Ergun^a, ✉Ramazan Asci^b

^aOndokuz Mayıs University, Faculty of Medicine, Department of Medical Biology, Samsun, Türkiye

^bOndokuz Mayıs University, Faculty of Medicine, Department of Urology, Samsun, Türkiye

Abstract

ARTICLE INFO

Keywords:

Male infertility
Occupation
Oxidative damage
Smoking

Received: Nov 20, 2023

Accepted: Jan 18, 2024

Available Online: 26.01.2024

DOI:

[10.5455/annalsmedres.2023.11.310](https://doi.org/10.5455/annalsmedres.2023.11.310)

Aim: The study aimed to assess the level of seminal oxidative damage in normozoospermic infertile patients by evaluating 8-OHdG and also to analyze the relationship between oxidative damage in infertility and factors such as smoking, occupation and age.

Materials and Methods: Seminal plasma samples were obtained from raw semen samples from 13 normozoospermic infertile men among the patients who applied to Ondokuz Mayıs University Urology Clinics for fertility evaluation and 10 normozoospermic proven fertile volunteers. Oxidative damage in seminal plasma was assessed by measuring the amount of 8-OHdG by indirect enzyme-linked immunosorbent assay. The data were also evaluated in terms of the smoking status and occupation stated by the patients and controls.

Results: The fertile group had more seminal oxidative damage than the infertile group (487.33 ± 209.42 vs 283.12 ± 102.73 , $p=0.013$). Comparing the non-smoker men, the smoking group's total sperm count was decreased (84.94 ± 24.09 vs 115.80 ± 25.46 , $p=0.036$). Age, semen characteristics, daily cigarette consumption, and partner age did not differ between the infertile and control groups. Additionally, no difference was found between the smoker and non-smoker groups, and also among the occupation groups in terms of oxidative damage ($p>0.05$). No correlation was found between age, oxidative damage, smoking and semen parameters ($p>0.05$).

Conclusion: The findings of this study could indicate that the level of seminal oxidative damage alone cannot be effective in infertile men. Also, as smoking or occupation, more factors should be considered for evaluating oxidative damage in infertile men.



Copyright © 2024 The author(s) - Available online at www.annalsmedres.org. This is an Open Access article distributed under the terms of Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License.

Introduction

Reactive oxygen species (ROS) are produced naturally during metabolism as residues of oxygen and have essential roles in cell communication and homeostasis. For normal physiological processes such as spermatozoa maturing, acrosomal reaction, capacitation, hyper activation, and sperm-oocyte fusion, low quantities of ROS are necessary [1, 2]. Thus, maintaining the redox balance is critical in the male reproductive system [3]. Excessive increase in ROS amount and/or deficiency of enzymatic or non-enzymatic antioxidant mechanisms lead to oxidative stress [3]. Because of their high levels of polyunsaturated fats in their cell surfaces, lack of antioxidants as a result of their small cytoplasmic volume, and inadequate DNA repair mechanisms, spermatozoa are actually quite vulnerable to the

negative effects of ROS [4]. Guanine is the most sensitive base to oxidative stress compared to other bases and upon oxidation, it becomes 8-hydroxy-deoxyguanosine (8-OHdG), a mutagenic lesion [3]. Increasing the amount of ROS may lead to sperm DNA fragmentation [2]. 8-OHdG is also frequently used assess oxidative DNA damage in sperm [5].

Routine semen analysis is the most essential stage in evaluating male fertility. Semen analysis is carried out according to the World Health Organization (WHO) recommendations for inter-laboratory standardization and evaluated with the lower values determined by population data [6]. Along with physical examination and medical history, routine semen analysis can give a general idea of male reproductive potential. However, semen analysis alone cannot measure all the functions of spermatozoa required for fertilization [7]. Additional analyzes, including measurement of seminal oxidative damage, stand out for predicting male

*Corresponding author:

Email address: gntkurt@gmail.com (✉Neslihan Hekim)

reproductive prospects.

The objective of this research was to ascertain seminal oxidative damage in infertile men with normal semen parameters by evaluating the amount of 8-OHdG and also to analyze the possible correlation of oxidative damage with factors such as smoking, occupation and age in male infertility.

Materials and Methods

Patient and control groups and semen analysis

Ethical approval of the study was given by Ondokuz Mayıs University (OMU) Clinical Research Ethics Committee with the decision number of OMÜ KAEEK 2022/547. Normozoospermic infertile male patients who had applied to OMU Medical Faculty Urology Clinic comprised the study group. In the calculation made using t-test with GPower 3.1 program, considering the Type I error as 5% and the power of the study as 80%, and the effect size as 0.5, the number of patients required to be included in each group was calculated as at least 13. The patients were among the couples who could not achieve a spontaneous pregnancy despite unprotected sexual activity for a year. Normozoospermic fertile men with children younger than 2 years old from the same age interval formed the control group. All participants were between the ages of 18-50. The study was explained to the participants, and those who provided consent were included. Each participant completed a questionnaire including their smoking habits, infertility history, occupation, and age of their partner. Semen samples of the participants were obtained via masturbating following two to five days without having sexual intercourse. OMU Andrology Laboratory conducted routine semen analyses. According to the latest guideline of the WHO published in 2021, the minimum values are 16 million per ml for sperm concentration, 39 million per ml for total sperm count, 30 and 42% for progressive motile sperm and total motility rates, respectively, and 4% for normal morphological spermatozoa [6]. Accordingly, participants with semen parameters higher than these values were evaluated as normozoospermic. Patients with aberrations in their semen parameters, histories of urogenital infection or cancer, diagnosis of obstructive or non-obstructive azoospermia, outside the age intervals, karyotype anomalies, Y-chromosome azoospermia factor microdeletions or cystic fibrosis transmembrane regulator (*CFTR*) variations, along with female infertility issues were not included in the study.

Obtaining seminal plasma

After routine semen analysis, the samples to be included in the study were brought to OMU Medical Biology Department. Raw semen samples were placed in sterile microcentrifuge tubes and centrifuged at 2000 rpm for 10 minutes. As supernatant seminal plasma was separated by removing the sperm cells. Separated seminal plasma samples of patients and controls were aliquoted to be evaluated and kept at -80 °C until assessment of oxidative damage.

Measurement of seminal oxidative damage

Oxidative damage in seminal plasma samples was determined indirectly by enzyme-linked immunosorbent assay

(ELISA) of oxidized guanine species using a commercial kit (DNA/RNA Oxidative Damage High Sensitivity ELISA Kit, Cayman Chemical) [8]. The seminal plasma samples were centrifuged at 10000 rpm for another 10 minutes right before the analysis thus, seminal plasma was separated from residual cells. Samples were then diluted 1:20 with ELISA buffer. Analysis was performed according to the kit manufacturer's protocol [8]. Briefly, standards were prepared by serial dilutions in tubes from 1 to 8, with ELISA buffer and bulk standard. The non-specific binding and maximum binding (B0) wells of the microplate received ELISA buffer. 50 µl of samples and standards were put in the wells designated for the samples and standards on the microplate. After adding 8-OHdG-acetylcholinesterase (AChE) tracer and monoclonal antibody, the microplate was subjected to incubation at +4°C for 18 hours. The following day, the microplate was washed off to eliminate unattached substances, and the wells were then filled with Ellman's Reagent containing AChE substrate. After adding the tracer to the TA well, the microplate was kept at ambient temperature and dark for two hours. The enzymatic reaction was analyzed by reading the absorbance in a microplate spectrophotometer (Multiskan GO, Thermo Fisher Scientific, Finland) at a wavelength of 420 nm, oppositely related to the wells' free 8-OHdG concentrations. A spreadsheet from the kit supplier's (Cayman) website was used to construct the standard curve and analyze the average readings obtained (www.caymanchem.com/analysis/elisa).

Statistical analysis

Shapiro-Wilk test was performed to analyze the conformity of the data to the normal distribution. Data with a normal distribution ($p > 0.05$) were analyzed with parametric tests. In addition, data skewed from the normal curve were evaluated by non-parametric tests. All data was provided as arithmetic mean \pm standard deviation. The difference between two independent groups was evaluated with Mann Whitney-U. Correlations between semen parameters, oxidative damage, age, and smoking were analyzed by Spearman's rank correlation. All p values were calculated using the two-tailed method, and statistical significance was defined as a value below 0.05. All statistical analyzes were carried out with IBM SPSS 22.0 (IBM, Quebec, Canada).

Results

Thirteen infertile and 10 fertile men, all with normal semen parameters, were included in the study. According to the results of the questionnaires, 10 of the 23 participants were defined themselves as workers, two as teachers, three as self-employed, and the rest as guards, government officials, shopkeepers, refugees, social workers, waiters, operators and drivers. Eighteen participants stated that they used 5-15 cigarettes per day, while the remaining stated that they never smoked.

The standard curve of the absorbance from %B/B0 (%Bound/Maximum Bound) values of the serially diluted standards against the 8-OHdG concentration was obtained with a linear regression equation ($R^2 = 0.9972$). The distribution of seminal plasma oxidative damage determined ac-

Table 1. Comparison of data between two groups.

Groups	Age	Oxidative Damage	Volume (ml)	Leukocyte (10 ³ /ml)	Sperm Concentration (10 ⁶ /ml)	Total Sperm Count (10 ⁶ / ejaculate)	TPMSC	Progressive Motility (A) (%)	Non-progressive Motility (B) (%)	Immotility (%)	Motility (A+B) (%)	Normal Morphology (%)	Cigarettes/day	Partner's Age	
Infertile (n=13)	Mean	31.77	283.12	3.08	384.62	29.23	89.69	40.31	43.77	5.85	50.38	49.62	6.08	6.15	30.46
	SD	6.91	102.73	0.76	177.23	9.47	34.69	20.31	7.99	1.86	6.95	6.95	0.95	5.46	6.81
	Mean	10.04	8.92	13.15	12.38	10.50	11.54	11.00	9.85	13.15	14.04	9.96	10.42	10.27	10.42
	Rank														
Fertile (n=10)	Mean	36.80	487.33	2.80	330.00	34.50	94.20	47.30	50.80	5.00	44.20	55.80	6.90	9.00	33.90
	SD	5.55	209.42	0.59	67.49	5.87	13.45	8.15	5.73	0.00	5.73	5.73	1.52	3.16	4.86
	Mean	14.55	16.00	10.50	11.50	13.95	12.60	13.30	14.80	10.50	9.35	14.65	14.05	14.25	14.05
	Rank														
P	.113	.013	.319	.703	.225	.709	.419	.080	.112	.096	.096	.157	.129	.202	
Total (n=23)	Mean	33.96	371.91	2.96	360.87	31.52	91.65	43.35	46.83	5.48	47.70	52.30	6.43	7.39	31.96
	SD	6.72	185.50	0.69	140.58	8.38	27.12	16.27	7.81	1.44	7.04	7.04	1.27	4.74	6.17

TPMSC: Total progressive motile sperm count. Statistically significant results are given in bold.

Table 2. Comparison of data in smokers and non-smokers.

Groups	Age	Oxidative Damage	Volume (ml)	Leukocyte (10 ³ /ml)	Sperm Concentration (10 ⁶ /ml)	Total Sperm Count (10 ⁶ / ejaculate)	TPMSC	Progressive Motility (A) (%)	Non-progressive Motility (B) (%)	Immotility (%)	Motility (A+B) (%)	Normal Morphology (%)	Cigarettes/day	Partner's Age	
Ever smoker (n=18)	Mean	32.94	32.94	2.89	366.67	29.89	84.94	40.00	46.28	5.56	48.17	51.83	6.33	9.44	30.83
	SD	6.15	6.15	0.65	153.39	7.84	24.09	16.00	8.17	1.62	7.33	7.33	1.33	2.91	5.52
	Mean	11.00	11.00	11.39	12.08	10.64	10.44	10.67	11.50	11.83	12.50	11.50	11.28	14.50	10.81
	Rank														
Never smoker (n=5)	Mean	37.60	37.60	3.20	340.00	37.40	115.80	55.40	48.80	5.20	46.00	54.00	6.80	0.00	36.00
	SD	8.14	8.14	0.84	89.44	8.32	25.46	11.67	6.72	0.45	6.28	6.28	1.10	0.00	7.31
	Mean	15.60	15.60	14.20	11.70	16.90	17.60	16.80	13.80	12.60	10.20	13.80	14.60	3.00	16.30
	Rank														
P	.179	.179	.380	.891	.067	.036	.073	.498	.702	.496	.496	.280	.000	.108	
Total (n=23)	Mean	33.96	33.96	2.96	360.87	31.52	91.65	43.35	46.83	5.48	47.70	52.30	6.43	7.39	1.96
	SD	6.72	6.72	0.69	140.58	8.38	27.12	16.27	7.81	1.44	7.04	7.04	1.27	4.74	6.17

TPMSC: Total progressive motile sperm count. Statistically significant results are given in bold.

Table 3. Correlation analysis between data.

Spearman's rho	Volume (ml)	Leukocyte (10 ³ /ml)	Sperm Concentration (10 ⁶ /ml)	Total Sperm Count (10 ⁶ / ejaculate)	TPMSC	Progressive Motility (A) (%)	Non-progressive Motility (B) (%)	Immotility (%)	Motility (A+B) (%)	Normal Morphology (%)	
Age	ρ	-.059	.093	.251	.096	.103	-.030	.167	.050	-.050	.280
	ρ	.789	.674	.248	.664	.639	.890	.445	.820	.820	.195
Oxidative Damage	ρ	-.203	-.217	.254	.156	.199	.338	-.177	-.342	.342	-.019
	ρ	.353	.319	.242	.478	.363	.115	.418	.110	.110	.932
Cigarettes/day	ρ	-.161	-.269	-.191	-.258	-.188	-.026	-.019	.013	-.013	-.082
	ρ	.464	.214	.382	.235	.392	.906	.930	.953	.953	.712

ρ : Correlation Coefficient, TPMSC: Total progressive motile sperm count.

According to the standard curve, semen parameters, patient and partners' ages, and the number of cigarettes smoked daily between normozoospermic fertile and normozoospermic infertile groups are given in Table 1. There was no difference between the infertile and fertile groups in terms

of age, semen parameters, number of cigarettes per day, and the age of their partners. However, seminal oxidative damage in the fertile group was found to be higher than in the infertile group (487.33 ± 209.42 vs 283.12 ± 102.73 , $p=0.013$, Figure 1). Seminal oxidative damage, age, part-

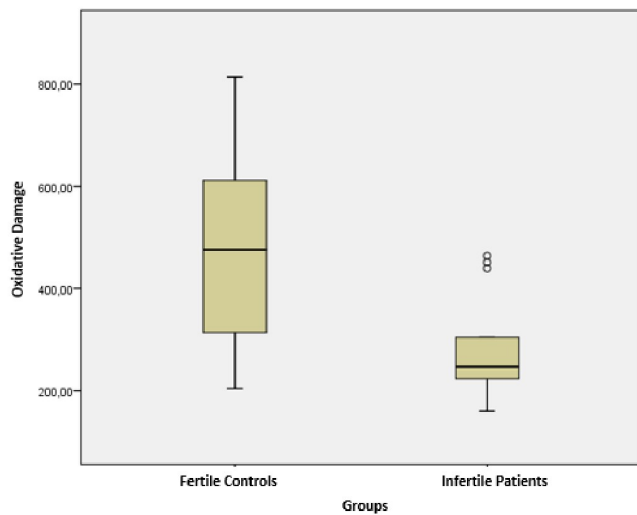


Figure 1. Oxidative damage between groups.

ner's age, and semen parameters of the smokers and non-smokers were compared (Table 2). There was no difference between the smoker and non-smoker groups in terms of oxidative damage. However, although all participants were normozoospermic, total sperm count was lower in the smoking group compared to the non-smoker group (84.94 ± 24.09 vs 115.80 ± 25.46 , $p=0.036$).

Participants were grouped as workers ($n=13$), government officials ($n=4$), shopkeepers ($n=4$), and drivers ($n=2$) in order to perform a statistical analysis of the possible effects of all participants' occupations on seminal oxidative damage. Occupational subgroups were then evaluated for oxidative damage and semen analysis (not shown). As a result, oxidative damage was determined to be the highest in the government officials group (516.33 ± 226.82) followed by workers (368.03 ± 182.64), drivers (342.89 ± 136.36) and shopkeepers (254.63 ± 120.40). However, there was no statistical significance among the occupation groups regarding oxidative damage ($p>0.05$). No correlation was found between age, oxidative damage, smoking and semen parameters ($p>0.05$) (Table 3).

Discussion

This study found that the level of seminal oxidative damage in normozoospermic infertile men was lower than in normozoospermic fertile men. No effect of smoking or occupation on seminal oxidative damage was found. Under normal physiological conditions, small amounts of ROS are necessary for the normal functions of spermatozoa. Human spermatozoa produce a certain amount of ROS during capacitation, acrosome reaction and oocyte fusion [1]. On the other hand increased generation of ROS damages cellular components, especially DNA, and prevents sperm from fertilizing the ovum [9]. Studies have shown that oxidative stress negatively effects the semen quality and fertility, especially by causing sperm DNA damage [9]. Determining the level of oxidative damage that can affect sperm and male reproductive potential may be a complex process. Lifestyle-related factors like smoking, drinking alcohol, poor diet and lack of exercise, or environmental

variables like exposure to ambient toxins and increased leukocyte count due to urogenital infections may increase the amount of seminal ROS [10]. In this study, seminal oxidative damage was found to be lower in the infertile group. Similarly, in another study conducted in our laboratory, idiopathic oligoasthenoteratozoospermic men were not different from proven fertile men in terms of seminal 8-OHdG levels [8]. In addition, in parallel with the present study, no correlation of seminal oxidative damage with semen parameters was found [8]. However, it is known that the decrease in antioxidant levels or the deterioration of their neutralizing effects may lead to seminal oxidative damage with increased ROS [1]. ROS can readily target the delicate sperm cell membrane, which may impact sperm function [9]. Since spermatozoa have insufficient room for antioxidant enzymatic activities due to DNA condensation and an absence of cytoplasm, seminal fluid is a crucial source of antioxidants in semen [9]. Enzyme-based and non-enzymatic fat- or water-soluble antioxidants are abundant in seminal plasma [4, 10]. Additionally, infertile men may use supplements containing various mixed antioxidants at different concentrations [11]. Decreasing seminal plasma antioxidant levels have been shown to have negative effects on semen parameters, sperm functions and reproductive outcomes in men [4, 11, 12]. Considering that antioxidants are readily available as food supplements, the possibility of using antioxidants comes to mind in the infertile men included in the present study. In addition, albeit it was not significantly different, the fertile group was found to have a greater mean of the daily number of cigarettes. These conditions may explain the higher levels of oxidative damage in the fertile group.

Smoking has been shown to cause oxidative stress by increasing the amount of ROS due to the free radicals it contains [13]. It is also known that smoking increases oxidative stress in seminal fluid [14]. Infertile men who smoke have been reported to be twice more likely to have seminal ROS levels than nonsmokers [14]. However, the results of studies about the impact of cigarette smoking on fertility in men have been varied. Researches revealed that the number of cigarettes smoked daily and the length of smoking may have an impact on semen parameters and reproductive outcomes [15-17]. This study found no association between cigarette smoking and oxidative damage. However, although all participants were normozoospermic, the total sperm counts were lower in smokers, similar to the previous studies. Collodel et al. reported that semen parameters of non-smoking infertile men were similar, but sperm concentration differed based on the daily cigarette consumption [15]. Nevertheless, another study reported that total sperm counts were inversely correlated to the cumulative smoking dose determined by the intensity, duration, and onset age of smoking in men [16]. In the same study, there was not a significant distinction in semen parameters between smokers and nonsmokers [16]. Similar to this, a meta-analysis of 16 studies with over 10,000 individuals found that smoking reduced number of sperm but did not affect sperm motility [17]. In our study, no difference was found between occupational groups in terms of seminal oxidative damage. In previous studies, oxidized guanine levels and oxidative stress have been reported to

increase in the spermatozoa of men working in certain occupations which they were more exposed to environmental pollutants [18]. Since DNA repair is known to occur mainly maternally after fertilization [19], the ages of the partners of the men in the two groups were also compared in our study. There was no difference in the age of their partners between the two groups. Previous studies have shown that compared to older controls; younger oocytes may neutralize the effects of sperm DNA damage, which is most likely a natural consequence of oxidative stress [19, 20].

Conclusion

Oxidative stress is accepted as one of the critical factors leading to infertility in men. However, many factors affect the formation and amount of oxidative stress. In this study, the level of oxidative damage in men with normal parameters in semen analysis considered the first stage of fertility evaluation was evaluated together with the smoking status of the patients and their occupation. On the other hand, the major limitation of the study was the small number of participants. In conclusion, this study may suggest that the level of seminal oxidative damage alone cannot be effective in infertile men and that smoking or occupation alone cannot be associated with oxidative damage. However, there is a need for larger-scale studies that also evaluate additional characteristics like obesity, alcohol usage and antioxidant use.

Conflict of interests

The authors declare that they have no relevant or material financial interests that relate to the research described in this paper.

Funding

The present study was partially carried out with the remaining parts of the materials supplied by the previous projects.

Ethical approval

This study was approved by Ethics Committee of Ondokuz Mayıs University (OMU) Clinical Research Ethics Committee (OMÜ KAEK 2022/547, 03.05.2023).

Author contribution

Research idea: NH, Design of the study: NH, Acquisition of data for the study: NH, RA, OU, Analysis of data for the study: NH, OU, Interpretation of data for the study: NH, SG, Drafting the manuscript: NH, Revising it critically for important intellectual content: NH, SG, SE, Final approval of the version to be published: NH, SG.

References

1. Agarwal A, Sharma RK, Nallella KP, Thomas AJ, Jr., Alvarez JG, Sikka SC. Reactive oxygen species as an independent marker of male factor infertility. *Fertil Steril*. 2006;86(4):878-85.
2. Schulte RT, Ohl DA, Sigman M, Smith GD. Sperm DNA damage in male infertility: etiologies, assays, and outcomes. *J Assist Reprod Genet*. 2010;27(1):3-12.
3. Moazamian A, Gharagozloo P, Aitken RJ, Drevet JR. OXIDATIVE STRESS AND REPRODUCTIVE FUNCTION: Sperm telomeres, oxidative stress, and infertility. *Reproduction*. 2022;164(6):F125-F33.
4. Aitken RJ, Drevet JR. The Importance of Oxidative Stress in Determining the Functionality of Mammalian Spermatozoa: A Two-Edged Sword. *Antioxidants* [Internet]. 2020; 9(2).
5. Hosen MB, Islam MR, Begum F, Kabir Y, Howlader MZ. Oxidative stress induced sperm DNA damage, a possible reason for male infertility. *Iran J Reprod Med*. 2015;13(9):525-32.
6. Boitrelle F, Shah R, Saleh R, Henkel R, Kandil H, Chung E, et al. The Sixth Edition of the WHO Manual for Human Semen Analysis: A Critical Review and SWOT Analysis. *Life (Basel)*. 2021;11(12).
7. Wiweko B, Utami P. Predictive value of sperm deoxyribonucleic acid (DNA) fragmentation index in male infertility. *Basic Clin Androl*. 2017;27:1.
8. Hologlu D, Gunes S, Asci R, Henkel R, Guvenc T. Association among sperm chromatin condensation, sperm DNA fragmentation and 8-OHdG in seminal plasma and semen parameters in infertile men with oligoasthenoteratozoospermia. *Andrologia*. 2022;54(1):e14268.
9. Hussain T, Kandeel M, Metwally E, Murtaza G, Kalhor DH, Yin Y, et al. Unraveling the harmful effect of oxidative stress on male fertility: A mechanistic insight. *Front Endocrinol (Lausanne)*. 2023;14:1070692.
10. Takeshima T, Usui K, Mori K, Asai T, Yasuda K, Kuroda S, et al. Oxidative stress and male infertility. *Reprod Med Biol*. 2021;20(1):41-52.
11. Henkel R, Sandhu IS, Agarwal A. The excessive use of antioxidant therapy: A possible cause of male infertility? *Andrologia*. 2019;51(1):e13162.
12. Humaidan P, Haahr T, Povlsen BB, Kofod L, Laursen RJ, Alsbjerg B, et al. The combined effect of lifestyle intervention and antioxidant therapy on sperm DNA fragmentation and seminal oxidative stress in IVF patients: a pilot study. *Int Braz J Urol*. 2022;48(1):131-56.
13. Padmavathi P, Raghu PS, Reddy VD, Bulle S, Marthadu SB, Maturu P, et al. Chronic cigarette smoking-induced oxidative/nitrosative stress in human erythrocytes and platelets. *Molecular & Cellular Toxicology*. 2018;14(1):27-34.
14. Saleh RA, Agarwal A, Sharma RK, Nelson DR, Thomas AJ, Jr. Effect of cigarette smoking on levels of seminal oxidative stress in infertile men: a prospective study. *Fertil Steril*. 2002;78(3):491-9.
15. Collodel G, Capitani S, Pammolli A, Giannerini V, Geminiani M, Moretti E. Semen quality of male idiopathic infertile smokers and nonsmokers: an ultrastructural study. *J Androl*. 2010;31(2):108-13.
16. Tang Q, Pan F, Wu X, Nichols CE, Wang X, Xia Y, et al. Semen quality and cigarette smoking in a cohort of healthy fertile men. *Environ Epidemiol*. 2019;3(4):e055.
17. Bundhun PK, Janoo G, Bhurtu A, Teeluck AR, Soogund MZS, Pursun M, et al. Tobacco smoking and semen quality in infertile males: a systematic review and meta-analysis. *BMC Public Health*. 2019;19(1):36.
18. Anna Jeng H, Sikdar S, Pan CH, Chang-Chien GP. Mixture Analysis of Associations between Occupational Exposure to Polycyclic Aromatic Hydrocarbons and Sperm Oxidative DNA Damage. *Ann Work Expo Health*. 2022;66(2):203-15.
19. Horta F, Catt S, Ramachandran P, Vollenhoven B, Temple-Smith P. Female ageing affects the DNA repair capacity of oocytes in IVF using a controlled model of sperm DNA damage in mice. *Hum Reprod*. 2020;35(3):529-44.
20. Esbert M, Pacheco A, Soares SR, Amoros D, Florensa M, Ballesteros A, et al. High sperm DNA fragmentation delays human embryo kinetics when oocytes from young and healthy donors are microinjected. *Andrology*. 2018;6(5):697-706.