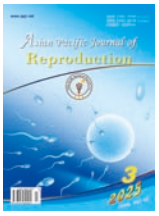

Original Article

Asian Pacific Journal of Reproduction

Journal homepage: https://journals.lww.com/apjr/

doi: 10.4103/apjr.apjr_145_24

The influence of individual and environmental factors on sperm DNA fragmentation index and its association with IVF outcomes

Harfsheno M[1], Shams E[2], Allahdadian M[3]✉, Ghasemi−Tehrani H[4], Ahmadi M[3]

[1]*Department of Biology, Faculty of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran*

[2]*Behbahan Faculty of Medical Sciences, Behbahan, Iran*

[3]*Department of Nursing and Midwifery, Fal.C., Islamic Azad University, Isfahan, Iran*

[4]*Fertility Department, School of Medicine, Isfahan University of Medical Science, Isfahan, Iran*

ABSTRACT

Objective: To investigate the influence of age, body mass index (BMI), varicocele, diabetes, tobacco use, and environmental occupational risks on sperm DNA fragmentation index (DFI) and its association with semen parameters and *in vitro* fertility (IVF) outcomes.

Methods: This cross-sectional study was conducted on 116 infertile men. Conventional semen parameters were analyzed according to the World Health Organization criteria. Sperm DNA fragmentation was evaluated using sperm chromatin dispersion method. After visiting the infertility center, the men's height and weight were measured, and blood tests were performed to check for diabetes, and medical records were reviewed for varicocele, tobacco use, and type of occupation. The sperm was then examined for DFI. Then, the association between sperm DFI and IVF failure rate was investigated.

Results: The study showed a significant association between DFI≥20% with BMI (*OR* 1.134, 95% *CI* 1.04-1.24, *P*=0.006), varicocele (*OR* 4.330, 95% *CI* 1.25-14.96, *P*=0.021), tobacco use (*OR* 3.066, 95% *CI* 1.06-8.90, *P*=0.039) and environmental and occupational risks (*OR* 2.694, 95% *CI* 1.08-6.75, *P*=0.034) as well as sperm motility (*P*<0.05). Although the amount of DNA damage increased in those aged ≥40 years, there was no significant association between the amount of DFI ≥20% and age, diabetes, sperm volume and concentration, morphology and progressive rate (*P*>0.05). The IVF failure rate was higher in people with a DFI ≥20%.

Conclusions: Factors such as BMI, varicocele, improper working conditions and environment cause damage to sperm DNA, and DFI ≥20% damage can have adverse effects on IVF outcomes.

KEYWORDS: DNA fragmentation index (DFI); Male infertility; Semen parameters; *In vitro* fertilization (IVF)

1. Introduction

Infertility is the concept of not becoming pregnant after a year of regular sexual contact without the use of contraception[1]. The studies found that 10%-15% of couples face the problem of infertility and the cause of 50% of infertility is men[2]. DNA fragmentation index (DFI) sperm in males assay can be used as a precision tool to evaluate fertility status[3]. Some factors and external substances affect the integrity of chromatin, and the accumulation of these factors can lead to irreversible damage to sperm DNA. Many factors are related, including reproductive

Key Points

Question: What are the factors of individual and environment influencing sperm parameters and IVF outcomes?

Findings: In the present study, factors such as BMI, varicocele, and poor working conditions and environment were associated with a higher sperm DNA fragmentation index (DFI).

Meaning: DFI≥20% can have adverse effects on IVF outcomes and reduced pregnancy success.

✉To whom correspondance may be addressed. E-mail: Maryamallahdadian@iau.ac.ir

How to cite this article: Harfsheno M, Shams E, Allahdadian M, Ghasemi-Tehrani H, Ahmadi M. The influence of individual and environmental factors on sperm DNA fragmentation index and its association with IVF outcomes. *Asian Pac J Reprod* 2025; 14(3): 122-128.

Article history: Received: 19 August 2024; Revision: 27 October 2024; Accepted: 21 December 2024; Available online: 15 May 2025

age, obesity and inactivity, testicular inflammation, environmental pollution, *etc*[4]. The DFI percentage is higher in older men (over 40 years) compared to younger men[5]. There have been several reports on the association between men's age and sperm DFI and assisted reproductive technology (ART) results, which have had conflicting results[6]. Today, with the change in lifestyle and machine life, inactivity has brought obesity and overweight, which has a negative effect on sperm DNA[7]. Body mass index (BMI) indicates body condition. A BMI value of 30 kg/m^2 is considered overweight and obese. Evidence reveals a link between obesity and reduced male fertility. It is also reported that the amount of DFI is higher in obese people[8,9]. The proper function of sperm in the process of fertilization and the formation of pronuclei is related to the integrity of sperm DNA[10]. In men, varicocele can cause hyperthermia and high temperature of the scrotum, which disrupts the function of the testicle and Leydig cell, and also causes shock to the sperm cell, and this condition causes damage to nuclear DNA and mitochondrial DNA[11]. Agents such as diabetes mellitus and cigarneys are also effective in infertility male[12]. A study conducted in the field of men's employment shows that the association between exposure to dangerous substances, pollutants and chemicals, high heat and prolonged sitting can increase the incidence of sperm DNA damage and thus decrease fertility[13]. Microscopic analysis of semen may be used as the first step in the diagnosis of infertility. Studies show that DNA damage >15% of sperm has a negative effect on semen parameters such as motility and morphology[14]. The integrity of sperm DNA is considered one of the important factors for the *in vitro* fertility (IVF) process, and in men whose sperm DNA damage is seen, the fertilization process fails[15]. Sperm efficiency is very important during fertilization and IVF[16]. The aim of this study was to investigate the effect of age, BMI, varicocele, diabetes and tobacco use on the amount of DNA damage and its association with semen parameters and IVF outcomes.

2. Methods

2.1. Study design

In this analytical cross-sectional study, which was performed from October 2021 to November 2023, semen samples were collected from 116 men suffering from infertility at private clinic in Esfahan, Iran. After visiting the infertility center, the men's height and weight were measured, and blood tests were performed to check for diabetes, and medical records were reviewed for varicocele, tobacco use, and type of occupation. In this study, men were divided into two groups: the control group DFI <20% and the test group DFI ≥20%. Based on reports from current articles around the world and the current study, it has been determined that in people with DFI more than 20%, the chance of successful pregnancy is reduced. All couples' height and weight were measured by a skilled nurse at the initial visit. Weight divided by height squared was used to compute BMI according to the classification standards of the global organization. Then, in the examinations and tests of men suffering from varicocele, diabetes, tobacco use, and the type of employment, including long-term sitting, contact with chemical agents or high heat, information was collected. All the wives of the studied men had reproductive system health[17].

The inclusion criteria for the study were: consent to participate in the research project, marriage for more than one year, and no successful pregnancy. The exclusion criteria were marriage for less than one year and having a child.

2.2. Analysis of semen parameters

Semen samples were collected after 2 to 7 days of abstinence from sexual intercourse in sterile containers through masturbation[18]. After liquefaction of the semen, sperm parameters were examined and analyzed according to the guidelines of the World Health Organization and DNA damage using the TUNEL test. Sperm parameters were analyzed by semen using a computer analysis (CASA system, Spain). Sperm counts were observed by a plate with a 10× objective lens. We used several key parameters, including volume, concentration, motility, morphology (Diff-Quick staining solution (Bred Life Science, Shenzhen, China) to facilitate morphological examination and to determine the proportion of sperm.

2.3. Analysis of sperm DFI

To investigate sperm DNA damage, the protocol of the manufacturer of the tunnel kit (Mannheim, Promega, Germany) was used. In summary, the semen samples were washed twice with phosphate buffered saline (PBS), and then fixed with 4% paraformaldehyde for 30 min. In order to penetrate, sperm samples were used for 5 min in Triton X-100 (Darmstadt, Germany Merck), and then propidium iodide dye with a concentration of 1 μg/mL was used to stain the nucleus for the purpose of cell counting. Sperm DNA was measured using a flow cytometer (BD Biosciences, San Jose, CA, USA).

2.4. Assessment of IVF outcomes

Oocytes were cultured for 2 to 4 h. Fertilization status was observed 16-18 h after inoculation. The state of zygote cell division 42 to 44 h and the formation of pronuclear after insemination was observed and the number of transplantable and high quality embryos was determined. Subsequently, we recorded blastocyst formation, implantation, early clinical pregnancy loss, and delivery or live birth in each cycle of IVF treatment. Patients were tested for serum β-hCG measurement 12-14 days after implantation. Implantation and clinical pregnancy were defined as the presence of a gestational sac confirmed by ultrasound.

2.5. Statistical analysis

Statistical analysis was performed at two descriptive and inferential levels. At the descriptive level, Freonian distribution tables and average, standard deviation, median and quartile deviation indices were used. At the inferential level, independent *t*-tests were used to compare quantitative variables, and Fisher's exact and *Chi*-square tests were used to compare qualitative variables between the studied groups. The selection of parametric and non-parametric tests was done according to the results of the Kolmogorov-Smirnov test in checking the normality of data distribution. Also, the logistic regression model was used in order to simultaneously investigate the effect of individual characteristics on the rate of DNA breakage of infertile men and to estimate its probability. The tests were performed using SPSS software version 27 and at a five percent error level.

All the assumptions of the logistic regression model were checked and confirmed and the final model was evaluated as suitable for predicting the association between independent and dependent variables. Among the individual characteristics, those that were univariately significant (P<0.05) or close to significant (P<0.1) as independent variables were included in logistic regression model.

The independence of the observations was checked according to the nature of the data and the data were considered as independent samples. Based on common criteria (at least 10 observations for each variable), the sample size was determined to be sufficient. Box-Tidwell test was used to ensure the assumption of linearity between independent variables and the logarithm of odds. The results showed that all independent variables were linear at a significance level of 0.05. Multiple collinearity was checked using the variance inflation factor (VIF). All variables had VIF less than 5, and therefore there was no significant multicollinearity.

2.6. Ethics statement

This cross-sectional study has been approved by the Ethics Committee for Humanities Research, Islamic Azad University Behbahan Branch, Behbahan, Iran (Code: IR IR.IAU.BEHBAHAN.REC.1400.004, dated: 15 September 2021). Written consent was obtained from the participants and a questionnaire containing personal information was completed for each person. The working conditions were explained to all the participants and all the participants were aware of the tests to evaluate the health of their reproductive organs and were free to continue the tests and could withdraw at any time during the project.

3. Results

3.1. Demographic characteristics

The 116 men in the age range of 24 to 55 years, whose wives were undergoing IVF treatment due to infertility, were examined. The individual characteristics of people based on the amount of DNA damage are presented in Table 1. The amount of DNA damage was less than 20% in 36 people (31%) and more than 20% in 80 people (69%). The average BMI of men with DFI <20% [(24.93±5.10) kg/m^2] was significantly lower than men with DFI≥20% [(28.17±4.87) kg/m^2] (P=0.001). The proportion of men with varicocele in people with DFI ≥20% (32.5%) was significantly higher than people with DFI <20% (11.1%) (P=0.015). Also, the proportion of men with high-risk jobs in people with DFI ≥20% (57.5%) was significantly higher than in people with DFI <20% (30.6%) (P=0.007). In other cases, no significant association between the amount of DNA damage and individual characteristics of men, including age (P=0.515), diabetes (P=0.675), tobacco use (P=0.054), and physical activity (P=0.745) was not observed. Of course, there is evidence of a significant association at the error level of ten percent, and the higher proportion of smokers among men with DFI ≥20% (37.5%) than among men with DFI <20% (19.7%) is also based on the test results of the *Chi*-square test.

In order to predict the presence of DNA damage in men based on individual characteristics, logistic regression model was used. Among the individual characteristics, those that were univariately significant (P<0.05) or close to significant (P<0.1) as independent variables (BMI, varicocele, tobacco use and high-risk occupation) and the amount of DNA damage (DFI ≥20%) were considered as dependent in this model.

Table 1. Association between DNA fragmentation index and demographic characteristics.

Variables	DFI <20% (n=36)	DFI ≥20% (n=80)	P-value
Age, years, mean±SD	35.72±6.13	36.59±6.81	0.515[a]
BMI, kg/m^2, mean±SD	24.93±5.10	28.17±4.87	0.001[a]
Varicocele, n(%)			
No	32(88.9)	54(67.5)	0.015[b]
Yes	4(11.1)	26(32.5)	
Diabetes, n(%)			
No	33(91.7)	76(95.0)	0.675[c]
Yes	3(8.3)	4(5.0)	
Tobacco use, n(%)			
No	29(80.6)	50(62.5)	0.054[b]
Yes	7(19.4)	30(37.5)	
Environmental and occupational risk, n(%)			
No	25(69.4)	34(42.5)	0.007[b]
Yes	11(30.6)	46(57.5)	
Physical activity, n(%)			
No	31(86.1)	67(83.8)	0.745[b]
Yes	5(13.9)	13(16.2)	

Note: a: Independent sample *t* test, b: *Chi* square test, c: Exact Fisher test. DFI: DNA fragmentation index; BMI: body mass index. Physical activity: 30 minutes a day for 5 days a week. Environmental and occupational risks: Physical hazards include excessive noise, poor lighting, vibrations, excessively high or low temperatures, and chemical hazards in the workplace, as well as exposure to harmful chemicals such as gases, liquids, and dust. Tobacco use: more than 3 times a week.

Table 2. Logistic regression model for DNA fragmentation index.

Variables	*OR*	95% *CI*	*P*-value
BMI*	1.134	1.04-1.24	0.006
Varicocele			
No (Referenc)			
Yes	4.330	1.25-14.96	0.021
Tobacco use			
No (Reference)			
Yes	3.066	1.06-8.90	0.039
Environmental and occupational risk			
No (Reference)			
Yes	2.694	1.08-6.75	0.034

OR: odds ratio; *CI*: confidence interval. *Body mass index (BMI) is considered as a continuous variable, and also categorized with a cut-off of 25 kg/m² based on WHO definitions. With an increase of one unit in men's BMI based on 25 kg/m², the chance of DNA damage in men will increase.

3.2. The effect of BMI, varicocele, tobacco use, and high–risk occupation on sperm DNA damage status

The results of the logistic analysis showed that the model based on four predictor variables leads to a significantly better prediction compared to the model with a fixed value [P<0.001, χ^2=25.284(4)]. Nagelcrack pseudo 2R showed that this model explains about 28% of the variance. This shows that BMI, varicocele, tobacco use, and high-risk occupation can properly differentiate the DNA damage status in people. The correct prediction rate for those with DFI <20% was greater than 91.3% and for people without DFI ≥20% was less than 38.9% and the overall prediction success rate was 0.75%, which indicates a relatively good prediction (Table 2).

According to the results of Table 2, four independent variables in the regression model including BMI (P=0.006), varicocele (P=0.021), tobacco use (P=0.039) and high-risk job (P=0.034) had a significant effect in predicting the condition of people in terms of DNA breakage (DFI ≥20%). The highest odds ratio value was for varicocele, so that the presence of varicocele increases the chance of DNA damage greater than 20% (DFI ≥20%) by 4.330 equals. The odds ratio for tobacco use was 3.066. Therefore, tobacco use increased the chance of DNA damage more than 20% (DFI ≥20%) by 3.066 times in men. The odds ratio for high-risk jobs was 2.694. Therefore, working in high-risk jobs in men increased the chance of DNA damage greater than 20% (DFI ≥20%) by 2.694 times. The probability value for BMI was 1.134. Therefore, by controlling the effect of other factors, with an increase of one unit in men's BMI, the chance of DNA damage in men will increase by 1.134 times.

3.3. Analysis results of sperm parameters

In comparing the parameters related to semen between two groups of men with DFI less than 20% and more than 20%, the results of the Mann-Whitney test showed a significant difference in the amount of sperm motility between the two groups at the five percent error level (P<0.001), and sperm motility was significantly higher in men with DFI <20%. No significant difference was observed between the two groups in the indices of volume (P=0.706), concentration (P=0.086), morphology (P=0.056) and advancing sperm (P=0.057). Of course, there was evidence of a significant difference in the ten percent error level in the three indices of concentration, morphology and progressing sperm (P<0.1) (Table 3).

Table 3. Sperm volume, concentration, motility, morphology and progressive rate stratified by the DNA fragmentation index (DFI).

Variables	DFI <20% (n=36)	DFI ≥20% (n=80)	*P*-value
Volume, mL	3.52±1.44	3.42±1.31	0.706
Concentration, 10^6/mL	69.91±23.31	63.73±21.01	0.086
Normal morphology, %	6.33±1.55	6.98±1.43	0.056
Total motility, %	46.39±15.12	34.68±12.67	<0.001
Progressive rate, %	36.33±11.01	33.28±5.50	0.057

Data are expressed as mean±SD.

Table 4. Sperm volume, concentration, motility, morphology and progressive rate base on the *in vitro* fertility (IVF) outcome.

Variables	IVF unsuccessful	IVF successful	*P*-value
Volume, mL	3.36±1.11	3.55±1.58	0.786
Concentration, 10^6/mL	65.79±21.64	65.49±22.26	0.973
Normal morphology, %	6.84±1.57	6.71±1.41	0.459
Total motility, %	36.92±13.63	39.86±15.33	0.312
Progressive rate, %	33.74±6.63	34.76±8.82	0.717

Data are expressed as mean±SD.

Table 5. Relationship between DNA fragmentation index (DFI) and *in vitro* fertility (IVF) treatment outcome.

Variables	*n*	IVF successful	IVF unsuccessful	*OR* (95 % *CI*)	*P*-value
DFI <20%	36	30(83.3)	6(16.7)	11 (4.06-29.78)	<0.001
DFI ≥20%	80	25(31.1)	55(68.8)		

Data are expressed as *n*(%).

There was also a significant difference in the amount of semen parameters, including volume (P=0.786), concentration (P=0.973), morphology (P=0.459), motility (P=0.312) and progressive movement (P=0.717) between men with successful and unsuccessful IVF treatment (Table 4).

3.4. The association between DNA damage and the result of IVF treatment

In the study, the result of IVF treatment was successful (pregnancy) for 55 people (47.4%) and failure (no pregnancy) for 61 people (52.6%). The association between DNA damage and the result of IVF treatment was significant (P<0.001). Thus, among men with DFI less than 20%, the result of IVF treatment was successful in 30 people (83.3%), and among men with DFI more than 20%, the result of treatment was successful in 25 people (31.3%). The odds ratio

was 11. Therefore, the presence of DNA damage of more than 20% in men increased, the chance of IVF treatment failure by 11 times (Table 5).

4. Discussion

Sperm DNA integrity is one of the most important factors related to successful reproduction and IVF. Therefore, doctors perform sperm DNA fragmentation test to analyze male infertility[19]. Nowadays, due to changes in lifestyle, fertility problems are on the rise, and it seems that the delay in having children is an important issue and cannot be underestimated[20]. Studies show that increasing the age of men over 40 years is directly related to the increase in sperm DFI[21,22]. In the present study, similar to the study of Sunil *et al*, the rate of DFI increased by more than 20% in men over 40 years old. Because most of the people studied were in the age range of over 35 years at the same time, there was no significant association between age and DFI[17]. Kaarouch *et al*'s study stated that there is no significant association between father's age >40 years and sperm DNA damage[23]. Lifestyle factors such as increased BMI and inactivity are directly related to increased sperm DNA damage[24]. In the present study, similar to the findings of Andrew *et al*, there was a positive and significant correlation between BMI >30 kg/m^2 and DNA damage[25]. The study of Elahi *et al*, similar to the present study, showed a significant and positive correlation between varicocele and DNA damage >25%[26]. Minas *et al*'s study reported that diabetes can damage sperm DNA and result in infertility, which was not consistent with the present study[27]. Jafari *et al* reported that there is no significant association between two groups of diabetic and non-diabetic infertile men in any of the sexual function factors[28]. Other studies showed no change in serum testosterone levels among diabetic patients, regardless of their sexual performance, which, similar to the present study, rejected the association between diabetes and infertility[29,30]. Studies have shown that occupational factors including heavy physical activity, prolonged sitting, exposure to hot environment, exposure to radiation, high temperature can increase sperm DNA speed[31]. Caliskan *et al*'s study, similar to the current study, showed a significant difference of <15% in men who were in inappropriate working conditions and environments[32]. Studies show an increase in sperm DFI in infertile men with abnormal semen parameters[33]. In the study of Okubo1 *et al*, there was no significant difference between sperm DNA damage and semen volume and concentration, which was consistent with the present study[34]. In Omrani *et al*'s study, there was a difference between DNA damage and motility and there was no difference between sperm morphology and DFI, which was consistent with the present study[35]. In Zhang *et al*'s study, there was no difference between progressive rate and DFI, which was consistent with the present study[36]. A study by Akhwaizadegan and colleagues similar to the present study showed that DNA damage can cause unsuccessful IVF and miscarriage[37]. In the study of Qi *et al*, there is a positive correlation between DFI ≥15% and unsuccessful IVF and lack of embryo growth quality[38]. In Mettlera *et al*'s study, associations between DFI ≥20% due to abnormal density of sperm nucleus after IVF procedure, repeated failures and non-formation of pronucleus eggs were reported[39]. The research conducted on the time of abstinence before receiving the semen sample of men shows that the shorter the time, the better the quality of the semen[40]. In the study of Barbagallo *et al*, which was consistent with the present study, it was stated that people who had fewer days of sexual abstinence had lower DFI levels than people who had more days of abstinence[41].

The limitations of the present study were the small sample size due to the investigation and follow-up of IVF results, and at the same time, the study was only of infertile men, so that their wives had reproductive system health.

In conclusion, the present study showed that factors such as BMI, varicocele, working conditions and unsuitable environment cause sperm DFI and more than 20% damage can have adverse effects on IVF results. Increasing age can also affect DFI >20%, but in the current study, the association between age, diabetes with DFI was not significant, and in the future, there is a need for research in a wider range of ages and the number of people on a large scale in order to study the effect. Different factors can affect sperm DNA and semen quality, both of which can affect artificial insemination.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Funding

The study received no extramural funding

Authors' contributions

Harfsheno M, Allahdadian M, and Shams E contributed to concept and design of the study, and final approval of the manuscript, and were responsible for overall supervision. Harfsheno M, Allahdadian M, Shams E, Ghasemi-Tehrani H, and Ahmadi M performed all the experiments, analyzed the data and interpreted them, and drafted the manuscript. All authors read, and approved the final version of the manuscript.

Publisher's Note

The Publisher of the *Journal* remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

[1] Pohl E, Gromoll J, Wistuba J, Laurentino S. Healthy ageing and spermatogenesis. *Reproduction* 2021; **161**(4): R89-R101.

[2] Repalle D, Saritha KV, Bhandari Sh. Sperm DNA fragmentation does not affect the clinical outcomes in the cumulative transfers of an ICSI cycle along with blastocyst transfers in couples with normozoospermic male patients. *Asian Pac J Reprod* 2022; **11**(3): 125-131.

[3] Nicopoullos J, Vicens-Morton A, Lewis SEM, Lee K, Larsen P, Ramsay J, et al. Novel use of COMET parameters of sperm DNA damage may increase its utility to diagnose male infertility and predict live births following both IVF and ICSI. *Hum Reprod* 2019; **34**(10): 1915-1923.

[4] Lu JC, Jing J, Chen L, Ge YF, Feng RX, LiangYJ, et al. Analysis of human sperm DNA fragmentation index (DFI) related factors: A report of 1010 subfertile men in China. *Reprod Biol Endocrinol* 2018; **16**(1): 23.

[5] Gonzalez D, Ory J, Blachman-Braun R, Nackeeran S, Best JC, Ramasam R. Advanced paternal age and sperm DNA fragmentation: A systematic review. *World J Mens Health* 2022; **40**(1): 104-115.

[6] Dong S, Chen C, Zhang J, Gao Y, Zeng X, Zhang X. Testicular aging, male fertility and beyond. *Front Endocrinol (Lausanne)* 2022; **13**: 1012119.

[7] Pooladi M, Sharifi M, Reza Dashti GH. Assessment of adiponectin and sperm function parameters in obese and non-obese: A comprehensive study. *Cell J* 2022; **24**(12): 715-722.

[8] Mykhailivna Kozopas N, Ihorivna Chornenka O, Zinoviyovych Vorobets M, Yevhenivna Lapovets L,Vasylivna Maksymyuk H. Body mass index and sperm quality: Is there a relationship? *J Hum Reprod Sci* 2020; **13**: 110-113.

[9] Asif M, Vijay AS, Maheshwari, Fyzullah S, Rani U, Swathi R, et al. Impact of chronological ageing on semen parameters in southern Indian men visiting infertility centre: A retrospective study. *Asian Pac J Reprod* 2023; **12**(1): 10-15.

[10]Panner Selvam MK, Ambar RF, Agarwal A, Henkel R. Etiologies of sperm DNA damage and its impact on male infertility. *Andrologia* 2021; **53**: 13706.

[11]Mongioì LM, Mammino L, Compagnone M, Condorelli RA, Basile A, Alamo A, et al. Effects of varicocele treatment on sperm conventional parameters: Surgical varicocelectomy versus sclerotherapy. *Cardiovasc Intervent Radiol* 2019; **42**: 396-404.

[12]Abbasihormozi SH, Babapour V, Kouhkan A, NiasariNaslji A, Afraz K, Zolfaghary Z, et al. Stress hormone and oxidative stress biomarkers link obesity and diabetes with reduced fertility potential. *Cell J* 2019; **21**(3): 307-313.

[13]Caliskan Z, Kucukgergin C, Aktan G, Bulut N, Ozdemirler G. Self-reported occupational exposure and its association with sperm DNA fragmentation in infertile men. *J Surg Med* 2023; **7**(10): 694-699.

[14]Tang L, Rao M, Yang W, Yao Y, Luo Q, Lu L, et al. Predictive value of the sperm DNA fragmentation index for low or failed IVF fertilization in men with mild-to-moderate asthenozoospermia. *J Gynecol Obstet Hum Reprod* 2021; **50**: 101868.

[15]González-Martínez M, Sánchez-Martín P, López-Fernández C, Johnston SD, Gosálvez J. The relationship between DNA fragmentation and the intensity of morphologically abnormal human spermatozoa. *Asian Pac J Reprod* 2024; **13**(1): 22-27.

[16]Zhang F, Li J, Liang Z, Wu J, Li L, Chen C, et al. Sperm DNA fragmentation and male fertility: A retrospective study of 5 114 men attending a reproductive center. *J Assist Reprod Genet* 2021; **38**: 1133-1141.

[17]Sunil CV, Jain A, Sunil KSS. Correlation between sperm DNA fragmentation index (DFI) with demographic characteristics, sexual history, social habits, chronic illness, BMI: A cross-sectionalstudy. *Indian J Obstet Gynecol Res* 2024; **11**(1): 33-38.

[18]World Health Organization. *WHO laboratory manual for the examination and processing of human semen*, 6th ed. Geneva: World Health Organization; 2021.

[19]Kaur Ghuman N, Kant Shukla K, Nandagopal S, Raikar S, Kumar Sh, Kathuria P, et al. Explaining the unexplained: Examining the predictive value of semen parameters, sperm DNA fragmentation and metal levels in unexplained infertility. *Hum Reprod Sci* 2023; **16**: 317-323.

[20]Molina-Garcra L, Hidalgo-Ruiz M, Arredondo-Lopez B, Colomino S, Delgado M, Martinez J. Maternal age and pregnancy, childbirth and the puerperium: Obstetric results. *J Clin Med* 2019; **8**: 672.

[21]Pino V, Sanz A, Valdés N, Crosby J, Mackenna A. The effects of aging on semen parameters and sperm DNA fragmentation. *JBRA Assist Reprod* 2020; **24**(1): 82-86.

[22]Evenson DP, Djira G, Kasperson K, Christianson J. Relationships between the age of 25 445 men attending infertility clinics and sperm chromatin structure assay (SCSA®) defined sperm DNA and chromatin integrity. *Fertil Steril* 2020, **114**: 311-320.

[23]Kaarouch I, Bouamoud N, Madkour A, Louanjli N, Saadani B, Assou S et al. Paternal age: Negative impact on sperm genome decays and IVF outcomes after 40 years. *Mol Reprod Dev* 2018; **85**(3): 271-280.

[24]Dutta S, Biswas A, Sengupta P. Obesity, endocrine disruption and male infertility. *Asian Pac J Reprod* 2019; **8**(5): 195-202.

[25]Andrew Peel A, Anmol Saini A, Deluao J, McPherson N. Sperm DNA damage: The possible link between obesity and male infertility, an update of the current literature. *Andrology* 2023; **11**: 1635-1652.

[26]Elahi M, Hojati V, Hashemitabar M, Afrough M, Mohammadpour Kargar H, Dastoorpoor M. Negative effect of varicocele on sperm mitochondrial dysfunction: A cross-sectional study. *Int J Reprod Bio Med* 2023; **21**: 323-332.

[27]Minas A, Camargo M, Alves M, Pimenta Bertolla R. Effects of diabetes-induced hyperglycemia on epigenetic modifications and DNA packaging and methylation during spermatogenesis; A narrative review. *Iran J Basic Med Sci* 2024; **27**: 3-11.

[28]Jafari Torkamani Z, Dolatian M, Omani-Samani R, Alizadeh A Navid B. Relationship between sexual function and type 2 diabetes in infertile men referred to Royan institute. *J Renal Inj Prev* 2021; **10**(4): 1-7.

[29]Boloña ER, Uraga MV, Haddad RM, Tracz MJ, Sideras K, Kennedy CC, et al. Testosterone use in men with sexual dysfunction: A systematic review and meta-analysis of randomized placebo-controlled trials. *Mayo Clin Proc* 2007; **82**(1): 20-28.

[30]Chiodini I, Di Lembo S, Morelli V, Epaminonda P, Coletti F, Masserini B, et al. Hypothalamic-pituitary-adrenal activity in type 2 diabetes mellitus: Role of autonomic imbalance. *Metabolism* 2006; **55**(8): 1135-1140.

[31]Abdoli1 S, Masoumi SZ, Kazemi F. Environmental and occupational factorsand higher risk of couple infertility: A systematic review study. *Middle East Fertil Soc J* 2022; **27**: 33.

[32]Caliskan Z, Kucukgergin C, Aktan G, Bulut N, Ozdemirler G. Self-reported occupational exposure and its association with sperm DNA fragmentation in infertile men. *J Surg Med* 2023; **7**(10): 694-699.

[33]Lu R, Chen X, Yu W, Jiang F, Zhou X, Xu Y, et al. Analysis of age-associated alternation of SCSA sperm DNA fragmentation index and semen characteristics of 1 790 subfertile males in China. *J Clin Lab Anal* 2020; **34**: e23548.

[34]Okubo1 T, Onda N, Hayashi T, Kobayashi T, Omi K, Segawa T. Performing a sperm DNA fragmentationtest in addition to semen examination based on the WHO criteria can be a more accurate diagnosis of IVF outcomes. *BMC Urol* 2023; **23**(78): 1-7.

[35]Omrani B, Al Eisa N, Javed M, Ghedan M, AlMatrafi H, Sufyan H. Associations of sperm DNA fragmentation with lifestyle factors and semen parameters of Saudi men and its impact on ICSI outcome. *Reprod Biol Endocrinol* 2018; **16**(1): 49.

[36]Zhang H, Zhu F, He X, Tang SH, Long T, Peng L, et al. The influence and mechanistic action of sperm DNA fragmentation index on the outcomes of assisted reproduction technology. *Open Life Sci* 2023; **18**: 1-11.

[37]Akhavizadegan H, Yamini N, Musavi AM, Moradi M, Khatami F. Sperm DNA fragmentation index in abortion or *in vitro* fertilization failure in presence of normal semen analysis. *Prague Med Rep* 2023; **124**(2): 166-171.

[38]Qi Q, Su Y, Peng J. Investigating the impact of the sperm DNA fragmentation index of male partners from infertile couples on *in vitro* fertilization-embryo transferoutcomes. *J Men's Health* 2024; **20**(5): 149-154.

[39]Mettlera A, Govindarajan M, Srinivas S, Mithraprabhu S, Evenson D, Mahendran T. Male age associede with sperme DND/choromatinintegrity. *Aging Male* 2020; **23**(5): 822-829.

[40]Sokol P, Drakopoulos P, Polyzos NP. The effect of ejaculatory abstinence interval on sperm parameters and clinical outcome of ART. A systematic review of the literature. *J Clin Med* 2021; **10**: 3213.

[41]Barbagallo F, Cannarella R, Crafa A, Manna C, la Vignera S, Condorelli RA, et al. The impact of a very short abstinence period on conventional sperm parameters and sperm DNA fragmentation: A systematic review and meta-analysis. *J Clin Med* 2022; **11**: 7303.

Edited by Lin LY, Lei Y