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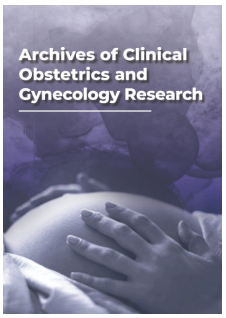
Original Article



DNA Fragmentation Level in Semen and Its Relation to Couple's Problems and Infertility

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Keywords

Sperm DNA fragmentation, male infertility, DFI, semen analysis, smoking, Libya

Abstract

Background: *Male infertility accounts for a substantial proportion of infertility cases globally, with Libya reporting a notably high prevalence (68.1%). Conventional semen analysis often fails to detect underlying molecular defects such as sperm DNA fragmentation (SDF), which can impair fertility even when standard parameters are normal.*

Objective: *This study aimed to assess the prevalence of high sperm DNA fragmentation index (DFI) and its correlation with conventional semen parameters, age, smoking, and history of recurrent pregnancy loss among infertile Libyan men.*

Methods: *A cross-sectional study was conducted at Lamis Infertility Clinic, Misurata, Libya, including 100 infertile men aged 20–50 years. Semen samples were analyzed according to WHO 2021 guidelines. Sperm DNA fragmentation was measured using the Sperm Chromatin Dispersion (SCD) test. Statistical analysis employed Chi-square and Spearman's rank correlation tests, with $p < 0.05$ considered significant.*

Results: *High DFI (>26%) was found in 25% of participants. Significant negative associations were observed between high DFI and impaired sperm motility ($p = 0.013$) and abnormal morphology ($p = 0.018$), though the morphology relationship was complex. Sperm concentration showed no significant association ($p = 0.113$). Smoking was significantly associated with high DFI ($p = 0.047$), with the highest prevalence among current smokers (36.6%). No significant associations were found with age ($p = 0.295$) or recurrent abortion history ($p = 0.242$).*

Conclusion: *The 25% prevalence of high DFI confirms its clinical significance in male infertility among Libyan men. Integrating SDF testing with conventional semen analysis is strongly recommended, particularly in unexplained infertility, impaired motility, abnormal morphology, and smokers. Smoking cessation should be prioritized as a modifiable intervention.*

Introduction

Infertility affects approximately 1 in 6 people worldwide, with male factors contributing to about 50% of cases [1]. In Libya, the situation is particularly pronounced, with studies reporting that 68.1% of infertility cases are attributable to male factors [2], a rate significantly higher than the global average of 20–30%. This disparity suggests the influence of region-specific environmental, behavioral, and genetic determinants.

Conventional semen analysis remains the cornerstone of male fertility evaluation, assessing parameters such as sperm concentration, motility, and morphology according to WHO guidelines [3]. However, these parameters alone are insufficient to exclude fertility issues, as approximately 30% of male infertility cases remain unexplained despite normal analysis results [4,5]. This diagnostic gap has highlighted the critical need for advanced biomarkers.

Sperm DNA fragmentation (SDF) has emerged as a key biomarker in this context [6]. SDF refers to structural breaks or damage within sperm DNA strands, resulting from oxidative stress, defective chromatin packaging, or abortive apoptosis [7,8]. Elevated SDF levels have been strongly associated with reduced fertilization rates, impaired embryo quality, increased miscarriage risk, and lower live birth rates, even in men with normal conventional semen parameters [4,9,10].

While international studies have established the clinical utility of SDF testing, data from North Africa, particularly Libya, remain scarce. Given the high prevalence of male factor infertility in Libya and the limited access to advanced diagnostic tools, this study aims to bridge this gap. The primary objective is to assess the prevalence of high DFI and its correlation with conventional semen parameters, age, smoking, and recurrent

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pregnancy loss among infertile Libyan men attending Lamis Infertility Clinic.

Methodology

Study Design and Setting

TThis observational, cross-sectional study was conducted at Lamis Infertility Clinic, Misurata, Libya, between December 2025 and February 2026.

Study Population

The study included 100 Libyan men aged 20–50 years who were partners of couples seeking infertility evaluation. Exclusion criteria were: known genetic/chromosomal abnormalities, current systemic infection, and history of chemotherapy or radiotherapy.

Sample Size and Sampling

Based on an expected prevalence of high SDF of 20–30% among infertile men [11,12], a minimum sample size of 100 participants was calculated to achieve 80% power at a 95% confidence level. Convenience sampling was employed, recruiting eligible participants consecutively during routine clinic visits.

Data Collection

Semen Collection and Conventional Analysis

Semen samples were collected by masturbation after 2–7 days of abstinence, allowed to liquefy at 37°C for 30–60 minutes, and analyzed according to WHO 2021 guidelines [3]. Parameters assessed included:

- Sperm concentration (normal: ≥40 million/mL)
- Progressive motility (normal: ≥30%)
- Normal morphology (normal: ≥4%)

Sperm DNA Fragmentation Assessment

SDF was quantified using the Sperm Chromatin Dispersion (SCD) test (Halosperm® kit) following the standardized protocol described by Fernández et al. [13]. Briefly:

1. Semen samples were embedded in 1% agarose on pre-coated slides.
2. Slides were treated with acid denaturation solution (7 minutes) and lysis buffer (10 minutes) to remove nuclear proteins
3. After dehydration in ethanol series (70%, 90%, 100%), slides were stained with SYBR® fluorescent dye.
4. Sperm nuclei were visualized under epifluorescence microscope at 400× magnification.
5. A minimum of 500 spermatozoa were counted per sample. Sperm with large halos were classified as non-fragmented, while those with small or absent halos were considered fragmented.

The DNA Fragmentation Index (DFI) was calculated as:

DFI (%) = (Number of fragmented sperm / Total sperm counted) × 100

A DFI > 26% was considered high, based on previously validated thresholds [14].

Statistical Analysis

Data were analyzed using SPSS version 26.0. Continuous variables were expressed as mean ± standard deviation. Categorical variables were presented as frequencies and percentages. Associations between DFI categories and categorical variables were assessed using Chi-square test. Correlations between DFI and sperm parameters were evaluated using Spearman's rank correlation coefficient. A p-value < 0.05 was considered statistically significant.

Ethical Considerations

The study protocol was approved by the Ethics Committee of Lamis Clinic. All participants provided written informed consent. Data were anonymized and stored securely to maintain confidentiality.

Results

Participant Characteristics

A total of 100 infertile men were enrolled, with a mean age of 38.82 years. Of these 41% were current smokers, 44% non-smokers, and 15% former smokers. A history of recurrent abortion was reported by 27% of participants.

Prevalence of High DFI

High DFI (>26%) was observed in 25% of participants (n=25), while 75% (n=75) had normal DFI (≤26%) (Table 1, Figure 1).

Table 1. *Prevalence of High Sperm DNA Fragmentation (DFI > 26%)*

DFI Category	Frequency	Precent
Normal (≤26%)	75	75%
High (>26%)	25	25%
Total	100	100%

Correlation Between DFI and Semen Parameters

Sperm Concentration: No statistically significant association was found between DFI and sperm concentration (p = 0.113). Among men with high DFI, 72% (18/25) had normal concentration, compared to 85.3% (64/75) in the normal DFI group.

Table 2: Correlation Between Sperm DNA Fragmentation Index (DFI%) and Sperm Concentration

Sperm concentration	DFI < 26%	DFI > 26%	Mean	Stander deviation	P-Value
≥ 40% normal	64	18	0.18	0.386	0.113
< 40 abnormal	11	7			
Total	75 (-75%)	25 (-25%)			

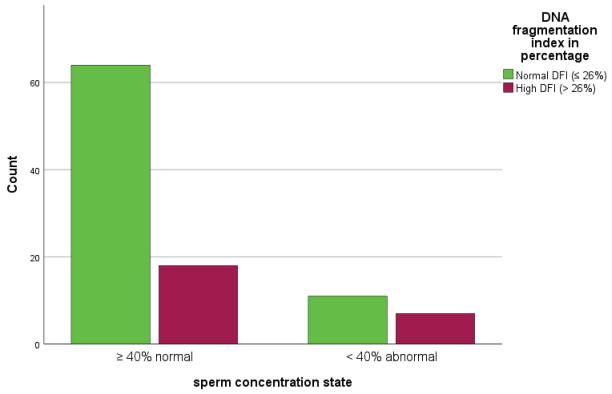

Figure 2: *Correlation Between Sperm DNA Fragmentation Index (DFI%) and Sperm Concentration State*

Sperm Motility

A statistically significant negative association was observed between high DFI and impaired sperm motility (p = 0.013). Among men with high DFI, only 40% (10/25) had normal motility, compared to 68% (51/75) in the normal DFI group.

Table 3: *Correlation Between DFI and Sperm Motility*

Sperm concentration	DFI < 26%	DFI > 26%	Mean	Stander deviation	P-Value
≥ 40% normal	64	18	0.18	0.386	0.113
< 40 abnormal	11	7			
Total	75	25			
	-75%	-25%			

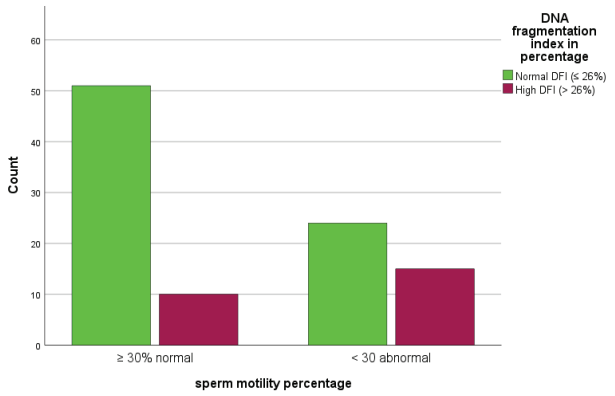

Figure 3: *Correlation Between Sperm DNA Fragmentation Index (DFI%) and Sperm Motility Percentage*

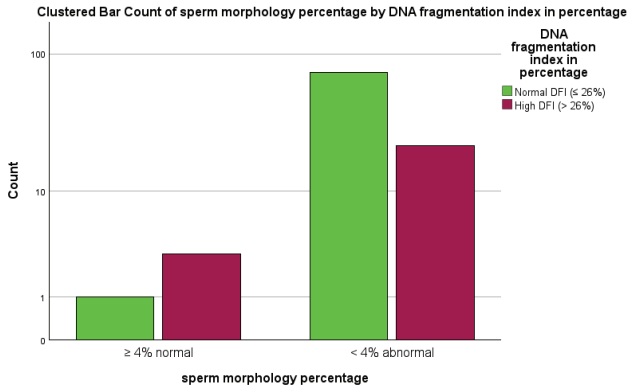

Figure 4: *Correlation Between Sperm DNA Fragmentation Index (DFI%) and Sperm Morphology Percentages*

Table 5: *Comparison of Sperm DNA Fragmentation Index (DFI%) Between Normal and Abnormal Semen Analysis*

Semen Analysis State	Frequency (%)	DFI ≤26%	DFI >26%	p-value
Abnormal	60 (60%)	42	18	
Total	100 (100%)	75	25	

Table 4: *Correlation Between Sperm DNA Fragmentation Index (DFI%) and Semen Morphology*

Sperm concentration	DFI < 26%	DFI > 26%	Mean	Stander deviation	P-Value
≥ 4% normal	1	3	0.960	0.197	0.018*
< 4% abnormal	74	22			
Total	75	25			
	(75%)	25%)			

Sperm Morphology

A statistically significant but complex association was found between DFI and morphology (P = 0.018). Notably, the high DFI group showed a higher prevalence of normal morphology (12%) compared to the normal DFI group (1.3%), suggesting that normal morphology does not guarantee DNA integrity.

Spearman's rank correlation revealed no significant correlation between DFI and specific abnormalities (head, tail, deviation), with all p-values > 0.7.

Comparison of DFI Between Normal and Abnormal Semen Analysis

No statistically significant difference in DFI was found between men with normal and abnormal conventional semen analysis (p = 0.157). In the normal semen analysis group (n=40), 17.5% (7/40) had high DFI, while in the abnormal group (n=60), 30% (18/60) had high DFI.

Relationship Between DFI and Age

No significant association was found between age groups and DFI status (p = 0.295). The prevalence of high DFI was 16.7% in men <30 years, 20.4% in men 30–40 years, and 33.3% in men >40 years.

Relationship Between DFI and Smoking

A statistically significant association was found between smoking and high DFI (p = 0.047). The highest prevalence of high DFI was observed in current smokers (36.6%, 15/41), followed by non-smokers (20.5%, 9/44), and former smokers.

Table 6: Relationship Between Age Groups and Sperm DNA Fragmentation Index (DFI%)

Variables/ Categories	Frequency (%)	Normal DFI ≤26%	Abnormal DFI >26%	Mean	Stander deviation	P-Value
Age Group						
<30 years	12 (12%)	10	2	38.82	8.025	0.295
30–40 years	49 (49%)	39	10			
>40 years	39 (39%)	36	13			
Total	100 (100%)	75	25			

Table 7: *Relationship Between DFI and Smoking Status*

Smoking	Frequency (%)	Normal DFI < 26%	Abnormal DFI > 26%	Mean	Stander deviation	P-Value
Yes	41 (41%)	26	15	0.71	0.71485	0.047*
No	44 (44%)	35	9			
former smoker	15 (15%)	14	1			
Total	100 (100%)	75	25			

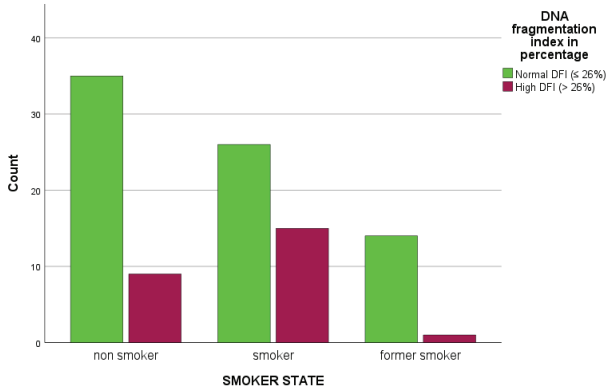

Figure 5: *Relationship Between DNA Fragmentation Index and Smoker State*

Relationship Between DFI and Recurrent Abortion 3.7

No significant association was found between DFI and history of recurrent abortion (p = 0.242). Among men with abortion history (n=27), 33.3% (9/27) had high DFI, compared to 21.9% (16/73) in those without abortion history.

Discussion

This study provides the first comprehensive assessment of sperm DNA fragmentation among infertile Libyan men, revealing a 25% prevalence of high DFI (>26%) and significant associations with impaired motility, abnormal morphology, and smoking

Prevalence of High DFI

The 25% prevalence of high DFI in our cohort aligns closely with international studies reporting DFI > 25–30% in 20–32% of infertile men [15,16,17]. This finding confirms that sperm DNA damage is not a rare phenomenon but a frequent pathological feature in male factor subfertility. Given that male infertility affects 68.1% of couples in Libya [2], this prevalence translates to a substantial clinical burden.

DFI and Sperm Motility

The significant negative association between high DFI and impaired motility (p = 0.013) is consistent with the oxidative stress model, where reactive oxygen species simultaneously damage flagellar structures and nuclear DNA [7,18]. This finding aligns with large-scale studies [19,20] and reinforces that poor motility serves as a visible indicator of hidden DNA damage. Men with asthenozoospermia face not only transport issues but also an elevated risk of transmitting damaged genetic material.

DFI and Sperm Morphology

The complex relationship between DFI and morphology (p = 0.018) requires careful interpretation. While a significant association was found, the paradoxical observation that men with high DFI had higher rates of normal morphology suggests that morphology and DNA integrity are influenced by distinct pathogenic mechanisms. This supports the findings of Liu et al. [15] and Le et al. [21], who reported no linear correlation. The lack of correlation with specific abnormalities (head, tail, deviation) in our Spearman analysis further underscores that normal morphology does not guarantee intact DNA, and vice versa. This reinforces the clinical necessity of DFI testing as a complement to morphology assessment.

DFI and Sperm Concentration

The absence of a significant association between DFI and concentration (p = 0.113) supports the concept of "isolated DFI" [22], where men with normal sperm count may still have compromised genetic integrity. This finding highlights that DFI provides an independent dimension of sperm quality, not captured by quantitative parameters alone [23].

DFI and Smoking

The significant association between smoking and high DFI (p = 0.047) aligns with the established literature [24,25,26]. Current smokers had nearly double the prevalence of high DFI compared to non-smokers (36.6% vs. 20.5%). Mechanistically, tobacco smoke induces oxidative stress and epigenetic alterations that compromise sperm DNA integrity [27,28]. This finding provides a clear, actionable target for intervention, as smoking cessation has been shown to partially reverse DFI [29].

DFI and Age

The lack of a significant association with age (p = 0.295) does not contradict the broader literature but likely reflects the specific characteristics of our cohort, including sample size and age distribution. International studies consistently report age-related DFI increases [30,31,32], and our null finding may be due to insufficient statistical power or the narrow age range of our sample.

DFI and Recurrent Abortion

The absence of a significant association with recurrent abortion (p = 0.242) contrasts with several meta-analyses [10,33]. However, the multifactorial nature of recurrent pregnancy loss and potential confounding by maternal factors may have obscured the paternal contribution in our study. We recommend prospective studies with larger samples and standardized definitions to further investigate this relationship.

Strengths and Limitations

Strengths

This is the first study to assess SDF in a Libyan cohort, providing region-specific data. The use of the validated SCD test

and WHO-standardized semen analysis strengthens reliability.

Limitations: The cross-sectional design and convenience sampling limit generalizability. The sample size, while adequate for primary objectives, may have been insufficient for subgroup analyses. Reliance on self-reported smoking and abortion history introduces recall bias.

Clinical Implications

Our findings strongly advocate for integrating DFI testing into routine male infertility evaluations, particularly in:

- Unexplained infertility
- Impaired motility or abnormal morphology
- Smokers
- Couples with recurrent ART failure

Smoking cessation should be prioritized as a first-line intervention.

Conclusion

This study demonstrates that 25% of infertile Libyan men have high sperm DNA fragmentation, confirming its clinical significance in male infertility. Significant associations with impaired motility, abnormal morphology, and smoking underscore the need for comprehensive assessment beyond conventional semen analysis. DFI testing should be integrated into diagnostic protocols, particularly in cases of unexplained infertility and when modifiable risk factors are present. Smoking cessation interventions are urgently needed to improve sperm genetic quality and reproductive outcomes.

Recommendations

1. **Clinical Practice:** Incorporate DFI testing in all male infertility evaluations, especially when conventional semen analysis is normal or when risk factors (smoking, advanced age) are present
2. **Public Health:** Implement smoking cessation programs targeting men of reproductive age.
3. **Research:** Conduct multicenter, prospective studies with larger samples to validate these findings and establish population-specific DFI thresholds.
4. **Policy:** Advocate for the inclusion of advanced sperm testing in national infertility management protocols

References

1. World Health Organization. 1 in 6 people globally affected by infertility: WHO 2023.
2. Eldib A, Tashani OA. The etiology of infertility in the western region of Libya. Libyan J Med Sci. 2021;5(2):70-4.
3. World Health Organization. WHO laboratory manual for the examination and processing of human semen. 6th ed. Geneva: WHO; 2021.
4. Liu K, Mao X, Pan F, Chen Y, An R. Correlation analysis of sperm DNA fragmentation index with semen parameters and the effect of sperm DFI on outcomes of ART. Sci Rep. 2023;13(1):2717.
5. McQueen DB, Zhang J, Robins JC. Sperm DNA fragmentation and recurrent pregnancy loss: a systematic review and meta-analysis. Fertil Steril. 2019;112(1):54-60.
6. Stavros S, Potiris A, Molopodi E, et al. Sperm DNA fragmentation: Unraveling its imperative impact on male infertility based on recent evidence. Int J Mol Sci. 2024;25(18):10167
7. Dutta S, Majzoub A, Agarwal A. Oxidative stress and sperm function: A systematic review on evaluation and management. Arab J Urol. 2019;17(2):87-97
8. Muratori M, Tamburrino L, Marchiani S, et al. Investigation on the origin of sperm DNA fragmentation: role of apoptosis, immaturity, and oxidative stress. Mol Med. 2015;21(1):109-22.
9. Dai Y, Liu J, Yuan E, Li Y, Shi Y, Zhang L. Relationship among traditional semen parameters, sperm DNA fragmentation, and unexplained recurrent miscarriage: A systematic review and meta-analysis. Front Endocrinol. 2022;12:802632.
10. Yao G, Dou X, Chen X, et al. Association between sperm DNA fragmentation index and recurrent pregnancy loss: results from 1485 participants undergoing fertility evaluation. Front Endocrinol. 2025;15:1493186.
11. Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. Reprod Biol Endocrinol. 2015;13(1):37.
12. Alahmar AT, Singh R, Palani A. Sperm DNA fragmentation in reproductive medicine. J Hum Reprod Sci. 2022;15(3):206-18.
13. Fernández JL, Muriel L, Rivero MT, Goyanes V, Vázquez V, Álvarez JG. The sperm chromatin dispersion test: a simple method for the determination of sperm DNA fragmentation. J Androl. 2003;24(1):59-66.
14. Gill K, Jakubik-Uljasz J, Rosiak-Gill A, Grabowska M, Matuszewski M, Piasecka M. Male aging as a causative factor of detrimental changes in human conventional semen parameters and sperm DNA integrity. Aging Male. 2020;23(5):1321-32.
15. Liu K, Mao X, Pan F, Yang X. Correlation of sperm DNA fragmentation index with seminal plasma biochemical index and semen parameters in infertile men. Research Square. 2020.
16. Yang T, Yu L, Xu J, et al. Correlation between standard sperm parameters and sperm DNA fragmentation from 11,339 samples. Syst Biol Reprod Med. 2024;70(1):91-100.
17. Pan M, Zhang Y, Qin N, et al. Predictive model of sperm DNA fragmentation in infertile men based on lifestyle factors. Front Endocrinol. 2025;16:1675168.
18. Wang Y, Fu X, Li H. Mechanisms of oxidative stress-induced sperm dysfunction. Front Endocrinol. 2025;16:1520835.
19. . Swornik M, Mirocki G, Przybylski M, et al. Evaluation of the sperm DNA Fragmentation Index in men from infertile couples. Ginekol Pol. 2025;96(9):765-75.
20. Chua SC, Yovich SJ, Hinchliffe PM, Yovich JL. How well do semen analysis parameters correlate with sperm DNA fragmentation? J Pers Med. 2023;13(3):518.
21. Le MT, Nguyen TAT, Nguyen HTT, et al. Does sperm DNA fragmentation correlate with semen parameters? Reprod Med Biol. 2019;18(4):390-6.
22. Ajayi AB, Ajayi V, Osuolale KA, et al. Understanding the impact of lifestyle and demographic factors on the DNA fragmentation index (DFI) among infertile men. BMC Urol. 2025;25(1):198.
23. Dutta S, Henkel R, Agarwal A. Comparative analysis of tests used to assess sperm chromatin integrity and DNA

fragmentation. Andrologia. 2020;53(2):e13718.

24. Cui X, Jing X, Wu X, Wang Z, Li Q. Potential effect of smoking on semen quality through DNA damage and the downregulation of Chk1 in sperm. Mol Med Rep. 2016;14(1):753-61.
25. Osadchuk L, Kleshchev M, Osadchuk A. Effects of cigarette smoking on semen quality, reproductive hormone levels, metabolic profile, zinc, and sperm DNA fragmentation in men. Front Endocrinol. 2023;14:1255304.
26. Gill K, Jakubik J, Rosiak-Gill A, et al. Utility and predictive value of human standard semen parameters and sperm DNA dispersion for fertility potential. Int J Environ Res Public Health. 2019;16(11):2004.
27. Cui J, Wang C, Zheng Y, et al. Mechanisms and reversibility of nicotine-induced spermatogenesis impairment and DNA methylation changes. Commun Biol. 2025;8(1):1053.
28. Firouzabadi AM, Henkel R, Niaki MT, Fesahat F. Adverse effects of nicotine on human sperm nuclear proteins. World J Mens Health. 2024;43(2):291.
29. Ragheb A, Abdelbary A, Massoud A, Elkhalek MA, Elbatanony A. Evaluating the effect of smoking and its cessation on semen parameters. Reprod Fertil. 2025;6(4).
30. Lu J, Jing J, Chen L, 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.
31. Yoshiakwa-Terada K, Takeuchi H, Tachibana R, Takayama E, Kondo E, Ikeda T. Age, sexual abstinence duration, sperm morphology, and motility are predictors of sperm DNA fragmentation. Reprod Med Biol. 2024;23(1): e12585.
32. Zhang F, Li J, Liang Z, et al. Sperm DNA fragmentation and male fertility: a retrospective study of 5114 men attending a reproductive center. J Assist Reprod Genet. 2021;38(5):1133-41.
33. Busnelli A, Garolla A, Di Credico E, et al. Sperm DNA fragmentation and idiopathic recurrent pregnancy loss: Results from a multicenter case-control study. Andrology. 2023;11(8):1673-81.