J Med Sci 2025;45 (4):142-151
DOI: 10.4103/jmedsci.jmedsci_46_25

ORIGINAL ARTICLE

Clinical Evaluation of Sperm DNA Fragmentation and its Influence on Male Infertility and Assisted Reproductive Technology Outcomes Using the TUNEL Assay

Saniya Imtiyaz Chamanmalik[1], R. B. Nerli[1], Shobhana Patted[2], Vijeta Shetty[2], Pankaja Umarane[1]

[1]*Department of Urology, J. N. Medical College, KLE Academy of Higher Education and Research (Deemed to be University), JNMC Campus,* [2]*Patted's Fertility and Research Centre, Belagavi, Karnataka, India*

Background: Male factors contribute to approximately 50% of infertility cases, however, conventional semen analysis does not detect molecular sperm abnormalities. Sperm DNA fragmentation (SDF) is crucial in fertilization, embryo development, and pregnancy outcomes. However, its impact on assisted reproductive technology (ART) success remains unclear. **Aim:** This study aims to investigate the clinical significance of SDF in ART outcomes and its potential as a biomarker for male infertility. **Methods:** This prospective study included 180 infertile males. Semen analysis was performed as per World Health Organization 2021 guidelines, sperm morphology was assessed using Diff-Quik staining and a TUNEL assay with flow cytometry to evaluate SDF. Based on semen parameters and SDF levels, participants underwent either *in vitro* fertilization (IVF, $n = 59$) or intrauterine insemination (IUI, $n = 121$). Pregnancy outcomes were assessed using beta-human chorionic gonadotropin levels and ultrasound. **Results:** Significant differences in semen parameters and SDF levels were observed between IVF and IUI groups ($P < 0.05$). IVF had higher pregnancy rates, with a negative correlation between SDF and conception success. Elevated SDF (>20% human chorionic gonadotropin) was associated with lower ART success. Lifestyle factors, including smoking (34.5%) and multiple substance use (35.6%), significantly increased SDF levels ($P = 0.0001$). **Conclusion:** SDF correlates with altered semen parameters and ART success, with lower SDF linked to improved IVF outcomes. Although a similar trend was observed in IUI, the association was not statistically significant. Lifestyle factors and paternal age significantly influenced SDF. SDF assessment can enhance the evaluation of male infertility and ART success.

Key words: Assisted reproductive technology, male infertility, reproductive outcomes, semen analysis, sperm DNA fragmentation

INTRODUCTION

Infertility is clinically defined as an inability to conceive after 12 consecutive months of unprotected sexual intercourse.[1] Infertility affects approximately 10%–15% of couples worldwide, and its prevalence has increased in recent years. Male factors account for nearly 50% of infertility cases, with 20% attributed solely to male infertility, and an additional 30% involving both male and female factors.[2,3] The evaluation of male infertility primarily relies on conventional semen analysis, which assesses parameters such as semen volume, sperm concentration, motility, and morphology.[4] However, approximately 15% of men with normal semen parameters are diagnosed as infertile, indicating that standard semen analysis may not fully capture the complexities of male reproductive potential.[5] Traditional semen analysis does not provide a comprehensive assessment of sperm fertilization capacity. With advancements in assisted reproductive technology (ART), it is increasingly recognized that conventional semen analysis alone is insufficient for a complete clinical evaluation of male fertility.[6] Therefore, identifying more precise clinical biomarkers is essential to elucidate the underlying causes of

Received: March 01, 2025; Revised: May 13, 2025; Accepted: May 27, 2025; Published: July 25, 2025
Corresponding Author: Dr. R. B. Nerli, Department of Urology, J. N. Medical College, KLE Academy of Higher Education and Research (Deemed to be University), JNMC Campus, Belagavi - 590 010, Karnataka, India. Tel: +91-831-2473777. E-mail: rajendranerli@yahoo.in

How to cite this article: Chamanmalik SI, Nerli RB, Patted S, Shetty V, Umarane P. Clinical evaluation of sperm DNA fragmentation and its influence on male infertility and assisted reproductive technology outcomes using the TUNEL assay. J Med Sci 2025;45:142-51.

male infertility and their impact on reproductive outcomes.[7,8] In particular, conventional semen analysis cannot effectively evaluate molecular and subcellular factors associated with male infertility. Among these, sperm DNA fragmentation (SDF) has emerged as a significant biomarker linked to male infertility and reproductive failure.[9,10] The sperm DNA fragmentation index (DFI) is a critical parameter for assessing sperm DNA integrity and identifying potential defects.[11] SDF has been shown to influence key reproductive processes, including fertilization, embryonic development, and the accurate transmission of paternal genetic material in both natural conception and ART procedures.[12] While SDF assessment is recognized as a valuable tool in evaluating male infertility, its role in predicting ART outcomes remains inconclusive. Studies exploring the impact of sperm DNA damage on ART success rates have produced conflicting results, highlighting the need for further investigation.[13,14] Recent research has explored the relationship between SDF and ART outcomes, yielding mixed results. A study by Jiang *et al*. investigated the impact of sperm DFI on early embryonic development during ART treatments. Their findings indicated that previously proposed DFI thresholds for diagnosing male infertility and predicting ART outcomes ranged between 15% and 30%, with no universally accepted standard. While DFI provides valuable insights into sperm quality, its predictive reliability for embryo quality and ART success remains limited, suggesting that DFI alone may not be a definitive indicator of ART outcomes.[15] Conversely, Li *et al*. examined the influence of DFI on ART pregnancy outcomes, categorizing patients based on their DFI levels. Their study revealed that elevated DFI was associated with reduced pregnancy rates in both *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) cycles, indicating a potential negative impact of SDF on ART success.[16] A comprehensive meta-analysis further supports a significant negative correlation between SDF and IVF outcomes, showing a notable reduction in implantation and pregnancy rates. While the effect of DFI on ICSI remains inconclusive, these findings highlight the importance of routine SDF testing in optimizing ART treatment strategies. However, further large-scale, well-controlled studies are necessary to refine clinical guidelines, particularly for ICSI.[17]

In contrast, Yao *et al*. concluded that sperm DNA damage does not directly impact ART pregnancy outcomes but may increase the risk of early miscarriage.[18] In addition, a recent study by Krog *et al*. suggests that assessing SDF should be strongly considered in couples with recurrent pregnancy loss before undergoing ART to mitigate the risk of early miscarriage.[19] The discrepancies in the literature may stem from differences in SDF detection methods across studies. Common techniques for assessing SDF include the sperm chromatin structure assay, sperm chromatin dispersion test, comet assay, and Terminal Deoxynucleotidyl Transferase dUTP Nick End Labelling (TUNEL).[20] The distinct mechanisms underlying these methods contribute to variations in findings. Even within the same detection technique, differences in threshold values and laboratory practices can lead to inconsistent results. Furthermore, many previous studies had relatively small sample sizes, increasing the likelihood of statistical errors.[21,22] In this study, we measured sperm DFI using the TUNEL assay, a widely used clinical approach. We analyzed the predictive value of DFI for pregnancy outcomes in 180 ART procedures (intrauterine insemination [IUI] and IVF) at our medical center, categorizing the DFI levels as <20 for normal and >20 for abnormal. Furthermore, we investigated the impact of sperm DFI on both positive and negative outcomes in IVF/ICSI.

MATERIALS AND METHODS

Study design

The study included 180 male participants from infertile couples undergoing infertility investigations. Subjects were recruited between November 2023 and November 2024. Comprehensive demographic information, including age, lifestyle habits, type of infertility, and prior medical records, was collected for all subjects. This study was conducted in accordance with the Declaration of Helsinki and approved by the University/Institutional Ethics Committee (ethical authorization number: KAHER/EC/22-23 dated July 27, 2022). Informed consent was obtained from each participant in this study. Inclusion criteria: Males aged 21 years or older attending a fertility clinic who provided informed consent. Only subjects with male infertility-related issues. Exclusion criteria: Males with infertility attributed to female factors, azoospermia, anatomical abnormalities, endocrine disorders, genetic conditions, genitourinary infections, malignancies, chronic illnesses, or a history of vasectomy or varicocele surgery were excluded.

Semen analysis

Semen samples were collected via masturbation into sterile containers with the sexual abstinence of 2–7 days and allowed to liquefy at room temperature for at least 30 min before analysis. Semen evaluation followed the World Health Organization (WHO) 6th edition criteria (2021), assessing standard parameters including pH, semen volume (mL), total sperm count (per ejaculate), concentration (per mL), total and progressive motility (%), vitality (%), and normal morphology (%). Sperm count and motility were measured using a Makler counting chamber with computer-assisted

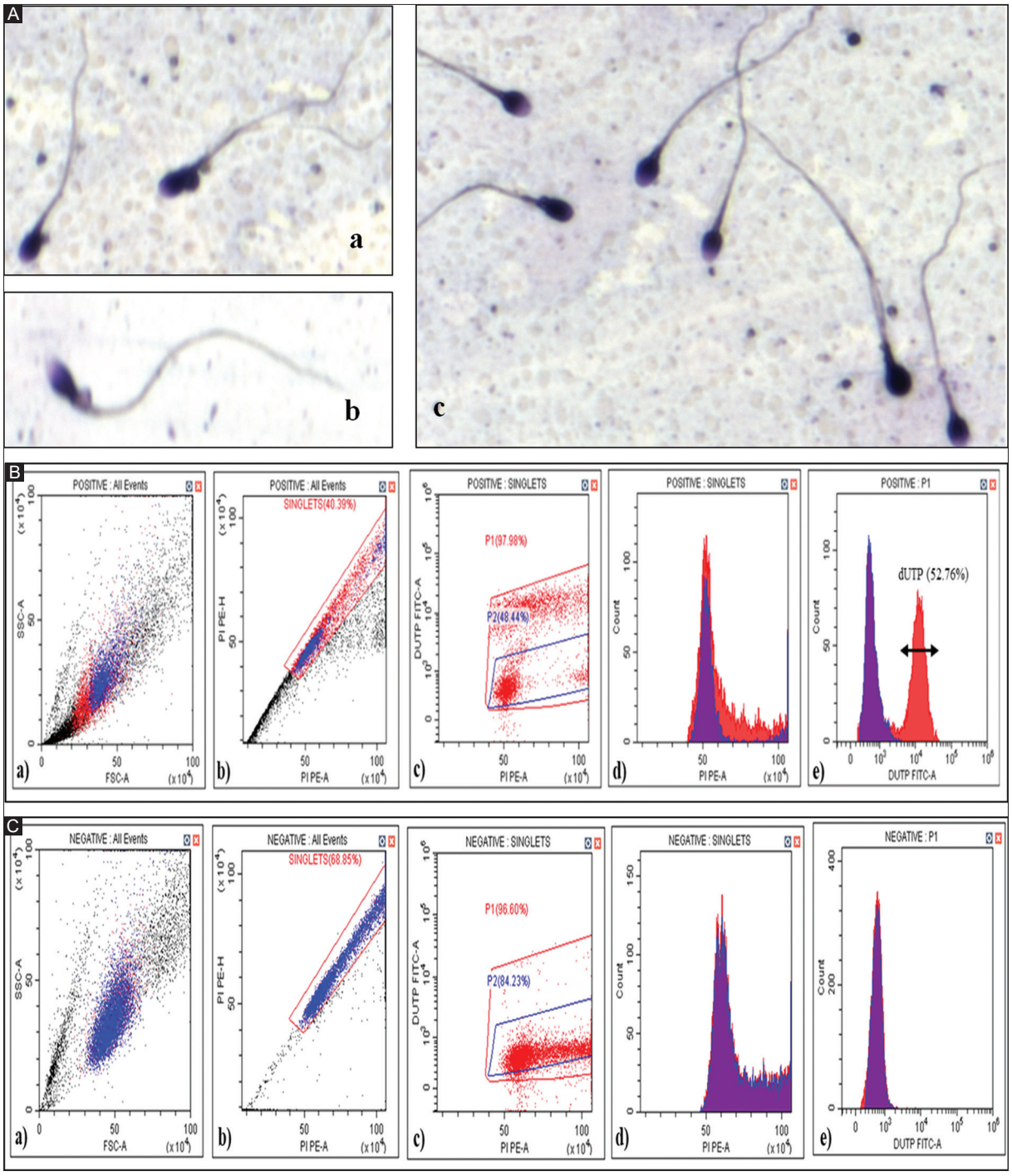

Figure 1: (A) Evaluation of sperm morphology using diff quick standing, an image representing altered semen morphology with head, mid-piece, and tail defects using a light microscope through a × 40 objective, (B) Flow cytometry gating strategy for positive sample, (a) Forward scatter - area versus side scatter - area (FSC-A vs. SSC-A): Scatter plot showing sperm distribution; red dots indicate fragmented sperm, (b) PI PE-A versus PI PE-H: Singlet gating (40.39%), lower than the negative control, indicating increased fragmentation, (c) PI versus dUTP-FITC-A: Higher SDF in P1 (97.98%) and P2 (48.44%) compared to the negative control, (d) PI histogram: Increased fluorescence suggests more PI-positive sperm, (e) TUNEL assay histogram: Elevated dUTP FITC-A fluorescence (52.76%) confirms higher sperm DNA fragmentation, (C) Flow cytometry gating strategy for negative sample, (a) FSC-A versus SSC-A: Sperm size (FSC-A) versus granularity (SSC-A); blue dots indicate sperm events, (b) PI PE-A versus PI PE-H: Singlet gating (68.85%) to exclude doublets and debris, (c) PI versus dUTP-FITC-A: SDF analysis showing P1 (96.60%) and P2 (84.23%) populations, (d) PI Histogram: PI fluorescence distribution of sperm. (e) TUNEL Assay Histogram: Minimal dUTP FITC-A fluorescence, indicating low DNA fragmentation, which indicates a negative sample. PI: Propidium Iodide, FITC: Fluorescein Isothiocyanate, DFI: DNA Fragmentation Index

semen analysis for accurate and reproducible results. Semen smears were air-dried, stained with Diff-Quik stain, and examined microscopically. Spermatozoa were classified as normal if no defects were present in the head, neck/midpiece, or tail [Figure 1A]. Based on semen analysis, participants were divided into normal and altered semen parameters.[23] Both groups were subsequently assessed for SDF, and based on both semen parameters and DFI, patients were selected for ART treatment.

Swim-up

The direct swim-up technique, without centrifugation, was employed for sperm preparation. Semen samples were carefully layered beneath PureSperm wash solution and incubated for 1 h at 37°C with 5% CO_2. Following incubation, the samples were fixed in 1% paraformaldehyde (1–2 × 10^6 cells/mL) in phosphate-buffered saline (PBS) (pH 7.4). Incubated on ice for 30–60 min, and centrifuged. After washing twice with PBS, the cell pellet was resuspended in 70% ice-cold ethanol and stored at −20°C. SDF was analyzed in all 180 test subjects using the benchtop flow cytometer.[24]

Sperm DNA fragmentation analysis

The terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay is a sensitive method initially developed for detecting DNA damage in somatic cells and later adapted for spermatozoa. It uses the TdT enzyme to catalyze the addition of fluoresceinated-dUTP at the 3'-OH ends of DNA fragments. In this study, SDF was analyzed using the TUNEL assay (BD Pharmingen™ APO-DIRECT™ Kit).

Part A: Control and test cells were centrifuged to remove ethanol, washed twice with Wash Buffer, and resuspended in 50 μL of DNA Labeling Solution. Cells were incubated for 60 min at 37°C (or overnight at RT for controls). After rinsing twice with rinse buffer, the pellet was resuspended in propidium iodide (PI)/RNase staining buffer (PI: Propidium Iodide) and incubated in the dark for 30 min before flow cytometry analysis. Part B: Flow cytometry, (Dxflex Beckman Coulter with Cytexpert software, Brea, California, USA) using a 488 nm Argon laser, employed PI (623 nm) for total DNA and FITC-dUTP (520 nm) for apoptotic cells. Dual-parameter displays gated nonclumped cells and resolved apoptotic cells, identifying their cell cycle stages. Apoptotic cells (P2 gate) showed increased FITC (Fluorescein Isothiocyanate) fluorescence, with most cells in the S-G2 phases as confirmed by PI staining. The DFI threshold value was obtained (by our choice through review) by 20% to divide patients into two groups: Group A (DFI <20%) and Group B (DFI ≥20%) [Figure 1B and C].[25]

Assisted reproductive technology treatment plan

The subjects were divided according to the ART procedure suggested by the clinician after thoroughly investigating the semen analysis and SDF reports. Out of 180 subjects, 59 (32.8) were taken for IVF, and 121 (67.2%) were considered for IUI.

Oocyte fertilization and embryo culture and transfer

For IVF fertilization, 59 subjects underwent pretreatment where fresh semen was processed using density gradient centrifugation (DGC) with sperm-grade 40% and 80% solutions. The sperm swim-up technique was then applied to achieve a final sperm density of 1 × 10^6/mL. The processed sperm were either cultured with oocytes in the standard IVF procedure or, following DGC, sperm with good morphology and viability were selected under a microscope and directly injected into the egg cytoplasm. Pronuclei of the oocytes were assessed 16–18 h postinjection to evaluate fertilization success. The normal fertilization (NF) rate was calculated as the number of double pronuclear embryos (2PN) divided by the number of metaphase II (MII) eggs (NF rate = a number of 2PN embryos/number of MII eggs). Fertilized embryos were cultured until day 3 (D3), and their development was monitored. Good-quality embryos on D3 or blastocysts on day 5 were selected for transfer, with progesterone support provided to the patient. The high-quality embryo rate was defined as the proportion of 2PN embryos cleaving to Grade I or II based on the Peter scoring system by D3. The good-quality embryo rate was determined as the proportion of Grade I or II embryos at the 8-cell stage relative to the total number of 2PN embryos.[26,27]

Intrauterine insemination

IUI is typically performed for couples with unexplained infertility, where standard investigations, including ovulation tests, tubal patency, and semen analysis. In this study, the IUI procedure was conducted on 121 subjects based on semen parameters, DFI, absence of female fertility issues, or patient preference. Ovulation was induced using clomiphene citrate, and once 1–2 follicles reached 17 mm in diameter, 10,000 IU of human chorionic gonadotropin (hCG) was administered. Thirty-six hours postinjection, fresh semen was collected and processed using DGC with sperm-grade 40% and 80% solutions. A 1 mL treated sperm suspension was then injected into the uterine cavity. After 16 days, serum β-hCG levels were measured to confirm pregnancy (>50 mIU/mL). Positive cases were followed by an ultrasound at 7 weeks to confirm clinical pregnancy.[28]

Evaluation of pregnancy outcome

Evaluation of pregnancy outcome Serum β-hCG levels were measured 14 days after embryo transfer, and biochemical pregnancy was confirmed by hCG levels >50 mIU/mL. Clinical pregnancy was confirmed by intrauterine pregnancy with a normal fetal heart rate seen on ultrasound 7 weeks after embryo transfer. Loss of the fetus within 12 weeks of pregnancy was classified as an early abortion.[29]

Statistical analysis

Statistical analysis was performed using SPSS 20 Software. Data from each group are expressed as the mean ± standard deviation. A comparative study between the two groups of interest was performed using the Student's *t*-test. Correlations between SDF and semen parameters were analyzed using Pearson's correlation coefficient. Statistical significance was defined as $P < 0.05$. Correlation analysis was performed using bivariate Pearson analysis, while categorical variables (smoking and alcohol consumption) were analyzed using a Spearman correlation.

RESULTS

In this study, the subjects were divided after a thorough investigation of semen parameters into normal and altered categories as given in Table 1.

Comparison of normal and altered semen parameters in 180 subjects

The two groups were compared to determine the relationship between normal and altered semen parameters. Table 2 shows the semen parameters, which include abstinence days, volume, concentration, motility, morphology, and other variables. There was a significant correlation between abstinence, sperm count, total sperm number, total motility, and morphology in the normal and altered groups, with *P* values of 0.046 and 0.001, respectively, which were $P < 0.05$*.

Table 1: Total diagnostic review of semen parameters

Total diagnostic review		
Semen parameters	Total number of subjects (*n*)	Valid (%)
Normal	109	60.6
Altered	71	39.4
Total	180	100.0

Subjects age-wise distribution of assisted reproductive technology procedure

Male age was also considered an important factor before selecting the state-of-the-art procedures. IUI and IVF have similar age distributions but slight variations in percentages. The majority of individuals undergoing both IUI and IVF are between 31 and 40 years old, i.e., 72 and 35, respectively. The percentage of older individuals (41–50 years) seeking IVF is slightly higher than that of those seeking IUI. The number of individuals seeking these treatments decreases significantly in the 51–60-year age range [Table 3].

Distribution of fertility type

Table 4 shows the distribution of primary and secondary infertility cases among patients undergoing IUI and IVF. Primary infertility was more common overall, with a higher proportion in both IUI and IVF groups.

Association of lifestyle habits with DNA fragmentation (180)

Table 5 shows that individuals with multiple habits have the highest percentage (35.6%) of SDF, followed by smoking (34.5%) and tobacco (16.1%). In contrast, individuals with no habits have the lowest percentage (9.2%) of SDF. The $P < 0.05$ signifies that the association between habits and SDF is statistically significant. Certain habits, especially multiple habits, smoking, and tobacco use, are connected with an increased risk of SDF. The statistical analysis confirms that this association is significant.

Table 2: Comparison of semen parameters in subjects with normal versus altered diagnostic reviews

Semen parameters tested	Diagnostics review			
	Normal (*n*=109), mean±SD	Altered (*n*=71), mean±SD	MD	*P*
Abstinence days	3.46±1.29	4.03±1.51	0.57	0.007*
Volume (mL)	2.29±0.85	2.23±1.05	−0.06	0.685
Sperm concentration (m/mL)	100.47±51.71	31.94±40.94	−68.53	0.001*
Total sperm number (m/mL)	220.53±143.92	66.63±88.35	−153.91	0.001*
Total motility (%)	78.04±13.81	48.79±19.21	−29.25	0.001*
Progressive motility (%)	89.44±333.09	95.69±544.29	6.25	0.92
Total morphology >4%	6.28±2.52	3.86±2.64	−2.42	0.001*

*P<0.05 Values are presented as numbers only or mean±SD. SD=Standard deviation; MD=Mean difference

Table 3: Age distribution among patients undergoing intrauterine insemination and *in vitro* fertilization procedures

Age distribution	Procedure	
	IUI, *n* (%)	IVF, *n* (%)
21–30	30 (24.8)	12 (20.3)
31–40	72 (59.5)	35 (59.3)
41–50	18 (14.9)	11 (18.6)
51–60	1 (0.8)	1 (1.7)

IUI=Intrauterine insemination; IVF=*In vitro* fertilization

Table 4: Distribution of fertility type among assisted reproductive technology procedures

Type of fertility	ART procedure		
	IUI, *n* (%)	IVF, *n* (%)	Total, *n* (%)
Primary	82 (67.8)	35 (59.3)	117 (65)
Secondary	39 (32.2)	24 (40.7)	63 (35)

IUI=Intrauterine insemination; IVF=*In vitro* fertilization; ART=Assisted reproductive technology

Table 5: Association between lifestyle habits and sperm DNA fragmentation

Habits	Sperm DNA fragmentation			χ^2	*P*
	>20	<20	Total		
None	8 (9.2)	52 (55.9)	60 (33.3)	65.84	0.0001*
Alcohol	4 (4.6)	9 (9.7)	13 (7.2)		
Smoking	30 (34.5)	25 (26.9)	55 (30.6)		
Tobacco	14 (16.1)	6 (6.5)	20 (11.1)		
Multiple	31 (35.6)	1 (1.1)	32 (17.8)		

*P<0.05 The categorical variables were represented by observational numbers and percentages

Association between diagnostic review and sperm DNA fragmentation

Table 6, we analyzed the seminal parameters and SDF of normal and altered subjects. Among 180 individuals, 41 (36.6%) had DNA fragmentation in normal semen analysis, while 46 (64.4%) had DNA fragmentation in altered semen. SDF was significantly correlated to semen parameters (P = 0.05*).

In vitro fertilization

Table 7, 59 subjects underwent IVF procedures in which semen parameters and SDF were assessed in males, and healthy female conditions were taken into consideration. Normal-appearing semen parameters (36) had a lesser fragmentation of 41.6%, and the altered semen parameters (23) had a higher fragmentation of 60.8%.

Table 6: Association between diagnostic review and sperm DNA fragmentation

Diagnostic review	DNA fragmentation			χ^2	*P*
	Yes	No	Total		
Normal	41 (36.6)	68 (62.4)	109 (60.6)	12.7	0.0001*
Altered	46 (64.8)	25 (35.2)	71 (39.4)		
Total	87	93	180		

*P<0.05 the categorical variables were represented by observational numbers and percentages

Table 7: Association between diagnostic review and sperm DNA fragmentation in *in vitro* fertilization patients

Diagnostic review	Art procedure (IVF *n*=59)		
	Total	Sperm DNA fragmentation	
		>20	<20
Normal	36 (61)	15 (41.6)	21 (58.3)
Altered	23 (39)	14 (60.8)	9 (39.1)
Total	59	29 (49.2)	30 (50.8)

The categorical variables were represented by observational numbers and percentages

Table 8: Correlation of DNA fragmentation and pregnancy outcome in assisted reproductive technology (*in vitro* fertilization)

Diagnostic review	Sperm DNA fragmentation (%)	Outcome			χ^2	*P*
		Negative	Positive	Total		
Normal	<20	9 (40.9)	12 (85.7)	21 (58.3)	7.066	0.008*
	>20	13 (59.1)	2 (14.3)	15 (41.7)		
Altered	<20	5 (31.3)	4 (57.1)	9 (39.1)	1.371	0.005*
	>20	11 (68.8)	3 (42.9)	14 (60.9)		

*P<0.05 The categorical variables were represented by observational numbers and percentages

Effects of sperm DNA fragmentation on pregnancy outcome by *in vitro* fertilization

The study compared normal and altered sperm parameters, including DNA fragmentation, and their impact on pregnancy outcomes [Table 8]. Among men with normal semen parameters, 15 (41.7%) had SDF, resulting in a 14.3% positive pregnancy outcome and 59.1% negative outcome. In contrast, 21 (58.3%) had normal semen parameters without DNA fragmentation, showing a significantly higher positive pregnancy outcome of 85.7% and a lower negative outcome of 40.9% (P = 0.008*). For men with altered semen parameters (23), 14 (60.9%) had SDF, leading to a 42.9% positive pregnancy outcome and 68.8% negative outcome. Meanwhile, 9 (39.1%) had altered semen parameters without DNA fragmentation, with a higher positive pregnancy outcome of 57.1% and a lower negative outcome

of 31.3% (P = 0.05*). These findings suggest a significant correlation between SDF and pregnancy success rates.

Intrauterine insemination

One hundred and twenty-one subjects underwent intrauterine procedures in which semen parameters and SDF were examined in males. Normal-appearing semen samples were higher in this procedure compared to altered ones. DNA fragmentation is observed higher in altered semen parameters, i.e., 66.6%, than in the normal semen parameters, i.e., 35.6% [Table 9].

Effects of sperm DNA fragmentation on pregnancy outcome in intrauterine insemination

Normal-appearing semen parameters had SDF in 26 (35.6) % of subjects, whereas altered semen parameters had 32 (66.7%) of SDF as shown in Table 10. Of 73 normal individuals, 15 had a positive pregnancy without DNA fragmentation, while had a positive pregnancy with fragmentation. Of 48 altered individuals, 5 had positive pregnancy outcomes without DNA fragmentation, while 4 had positive outcomes with DNA fragmentation. Negative pregnancy outcomes were comparatively higher in altered semen parameters with SDF than the normal ones.

DISCUSSION

Infertility has become a significant global health concern, influenced by societal pressures, psychological stress, and delayed parenthood. Advances in ART have improved treatment options, yet male infertility assessment remains largely dependent on conventional semen analysis based on WHO guidelines. Emerging evidence highlights the significance of SDF as a molecular biomarker complementing traditional semen parameters. Elevated SDF levels are associated with impaired sperm quality and poor ART outcomes; however, its definitive role in fertility remains under investigation.[30,31] This study utilized the WHO 6th edition guidelines for semen analysis and the TUNEL assay with flow cytometry to evaluate SDF. The association between semen parameters, SDF, and fertility outcomes was assessed, alongside the impact of lifestyle factors and age on sperm DNA integrity.

Our findings revealed significant variations in semen parameters between normal and altered diagnostic profiles, with the latter group exhibiting reduced sperm concentration, total count, motility, and morphology. Consistent with prior studies, sperm concentration was significantly lower in the altered group (31.94 M/mL) compared to the normal group (100.47 M/mL) (P = 0.001), reaffirming its strong correlation with reproductive potential.[32] Similarly, total motility was significantly reduced in the altered group (48.79% vs. 78.04%, P = 0.001), supporting prior research that links motility to fertilization success.[33] Sperm morphology also showed a notable decline in the altered group (−2.42% mean difference), emphasizing its role in male fertility assessments.[34]

Lifestyle factors such as smoking, alcohol consumption, and tobacco use were significantly associated with increased SDF (χ^2 = 65.84, P < 0.0001). Individuals without these risk factors had a higher prevalence of low SDF (<20%), whereas those engaging in multiple risk behaviors exhibited the highest proportion of high SDF (>20%). These findings align with previous studies indicating that smoking and excessive alcohol consumption induce oxidative stress, leading to sperm DNA damage and reduced fertilization potential.[35] Tobacco exposure was particularly associated with increased SDF, corroborating research linking tobacco use to DNA strand breaks and chromatin abnormalities.[36] Additionally, advancing paternal age was identified as a critical factor influencing ART outcomes, reinforcing the necessity of considering age in infertility evaluations.[37]

A significant association was observed between SDF and altered semen parameters, with 64.8% of individuals in the altered group exhibiting high SDF compared to 36.6% in the normal group (χ^2 = 12.7, P = 0.0001). Prior meta-analyses have reported that elevated SDF negatively impacts fertilization, embryo development, and pregnancy rates in ART, supporting our findings that increased SDF correlates with poor reproductive outcomes.[38]

Our analysis of ART outcomes further emphasized the impact of SDF on reproductive success. In IVF, individuals with

Table 9: Subjects with semen parameters and sperm DNA fragmentation intrauterine insemination

Art procedure (IUI n=121)			
Diagnostic review	n (%)	Sperm DNA fragmentation	
		<20	<20
Normal	73 (60.3)	26 (35.6)	47 (64.3)
Altered	48 (39.7)	32 (66.6)	16 (33.3)
Total	121	58 (47.9)	63 (52.1)

The categorical variables were represented by observational numbers and percentages. IUI=Intrauterine insemination

Table 10: Effects of sperm DNA fragmentation on pregnancy outcome in intrauterine insemination

Diagnostic Review	Sperm DNA fragmentation (%)	Outcome (IUI)			Chi-Square	P
		Negative N (%)	Positive N (%)	Total N (%)		
Normal	<20	32 (62.7)	15 (68.2)	47 (64.4)	2.588	0.108
	>20	19 (37.3)	7 (31.8)	26 (35.6)		
Altered	<20	11 (28.2)	5 (55.6)	16 (33.3)	2.46	0.117
	>20	28 (71.8)	4 (44.4)	32 (66.7)		

IUI = Intrauterine insemination

normal semen parameters and low SDF (<20%) demonstrated significantly higher pregnancy success rates (85.7%) compared to those with high SDF (>20%) (14.3%). Conversely, within the altered diagnostic group, high SDF was linked to lower pregnancy success (42.9%) and a higher proportion of negative outcomes (68.8%) [Table 8].

In IUI, participants with normal semen profiles and low SDF exhibited higher pregnancy success (68.2%) compared to those with high SDF (31.8%). However, in the altered diagnostic group, pregnancy success rates were lower for individuals with high SDF (44.4%) compared to those with low SDF (55.6%). Despite these trends, the Chi-square analysis (χ^2 = 2.588, P = 0.108) suggests that the association between SDF and IUI success did not reach statistical significance [Table 10], similar studies conducted stated that SDF might provide more insight into the male reproductive potential and in predicting IUI outcome. However, it fails to do so as an independent predictor.[39] Our study used an SDF threshold of 20% and demonstrated that elevated SDF negatively impacted IVF and IUI success rates, though statistical significance was reached only in IVF. A similar study used a lower SDF threshold of 15% and concluded that IVF-only was the most effective ART modality for women <40 years with SDF <15%. For those with SDF ≥15%, an IVF-ICSI split cycle showed higher blastocyst and high-quality blastocyst rates, but no significant differences were observed between IVF and ICSI.[40]

CONCLUSION

This study underscores the critical role of SDF as a molecular biomarker in male infertility assessment and its significant impact on ART outcomes. Our findings highlight a strong association between altered semen parameters and elevated SDF, emphasizing the importance of sperm DNA integrity in reproductive success. Individuals with normal semen profiles and low SDF exhibited significantly higher pregnancy success rates in IVF, whereas those with high SDF demonstrated poorer outcomes. While a similar trend was observed in IUI, the association between SDF and IUI success was not statistically significant.

Furthermore, lifestyle factors such as smoking, alcohol consumption, and tobacco use were strongly correlated with increased SDF, reinforcing their detrimental effects on sperm quality. Advancing paternal age was also identified as a key factor influencing fertility potential and ART outcomes. These findings align with existing research, supporting the integration of SDF analysis into routine male infertility diagnostics alongside conventional semen parameters.

Given the growing evidence linking SDF to fertilization, embryo development, and pregnancy success, incorporating SDF testing into ART protocols may enhance patient management and improve clinical outcomes. Future studies with larger cohorts and extended follow-up periods are necessary to further validate the prognostic significance of SDF and enhance its clinical utility in reproductive medicine.

Study limitations

- The sample size, particularly for the IUI subgroup, was limited and may affect the statistical power of the findings
- The DFI threshold of 20% was selected based on established literature and was validated through ROC curve analysis; however, the ROC data were not included in this manuscript and will be presented in detail in future research
- Detailed female partner data, such as age and ovarian reserve, were not included, which may influence ART outcomes.

Acknowledgments

We sincerely thank the patients for their participation and support in this study. We also thank the Department of Urology at KLE Hospital for their assistance, the patted fertility center staff, and the Minority Welfare Department, Belagavi.

Data availability statement

The data that support the findings of this study are available from the corresponding author, R. B. Nerli, upon reasonable request.

Financial support and sponsorship

This study was funded by the Government of Karnataka Minority Welfare Department, Belagavi.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Osadchuk LV, Osadchuk AV. Individual lifestyle and male fertility. Hum Physiol 2023;49:196-207.
2. Calogero AE, Cannarella R, Agarwal A, Hamoda TA, Rambhatla A, Saleh R, *et al*. The renaissance of male infertility management in the golden age of andrology. World J Mens Health 2023;41:237-54.
3. Huang B, Wang Z, Kong Y, Jin M, Ma L. Global, regional and national burden of male infertility in 204 countries and territories between 1990 and 2019: An analysis of global burden of disease study. BMC Public Health 2023;23:2195.
4. Tanga BM, Qamar AY, Raza S, Bang S, Fang X, Yoon K, *et al*. Semen evaluation: Methodological advancements

in sperm quality-specific fertility assessment – A review. Anim Biosci 2021;34:1253-70.
5. Llavanera M, Delgado-Bermúdez A, Ribas-Maynou J, Salas-Huetos A, Yeste M. A systematic review identifying fertility biomarkers in semen: A clinical approach through Omics to diagnose male infertility. Fertil Steril 2022;118:291-313.
6. Robertson MJ, Chambers C, Spanner EA, de Graaf SP, Rickard JP. The assessment of sperm DNA integrity: Implications for assisted reproductive technology fertility outcomes across livestock species. Biology (Basel) 2024;13:539.
7. Bieniek JM, Drabovich AP, Lo KC. Seminal biomarkers for the evaluation of male infertility. Asian J Androl 2016;18:426-33.
8. Andrabi SW, Ara A, Saharan A, Jaffar M, Gugnani N, Esteves SC. Sperm DNA Fragmentation: Causes, evaluation and management in male infertility. JBRA Assist Reprod 2024;28:306-19.
9. Agarwal A, Panner Selvam MK, Baskaran S, Cho CL. Sperm DNA damage and its impact on male reproductive health: A critical review for clinicians, reproductive professionals and researchers. Expert Rev Mol Diagn 2019;19:443-57.
10. Panner Selvam MK, Sengupta P, Agarwal A. Sperm DNA fragmentation and male infertility. Genet Male Infertil 2020:155-172.
11. Qiu Y, Yang H, Li C, Xu C. Progress in research on sperm DNA fragmentation. Med Sci Monit 2020;26:e918746.
12. Balder P, Jones C, Coward K, Yeste M. Sperm chromatin: Evaluation, epigenetic signatures and relevance for embryo development and assisted reproductive technology outcomes. Eur J Cell Biol 2024;103:151429.
13. 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;13:2717.
14. Cho CL, Agarwal A. Role of sperm DNA fragmentation in male factor infertility: A systematic review. Arab J Urol 2018;16:21-34. 3rd edition.
15. Jiang H, Xia X, Luo Y, Pan H, Qu S, Xu J. Sperm DNA fragmentation index: Limited effectiveness on predicting embryo quality in assisted reproduction technology treatments. Reprod Biol Endocrinol 2025;23:14.
16. Li F, Duan X, Li M, Ma X. Sperm DNA fragmentation index affect pregnancy outcomes and offspring safety in assisted reproductive technology. Sci Rep 2024;14:356.
17. Ribas-Maynou J, Yeste M, Becerra-Tomás N, Aston KI, James ER, Salas-Huetos A. Clinical implications of sperm DNA damage in IVF and ICSI: Updated systematic review and meta-analysis. Biol Rev Camb Philos Soc 2021;96:1284-300.
18. Yao G, Dou X, Chen X, Qi H, Chen J, Wu P, *et al*. Association between sperm DNA fragmentation index and recurrent pregnancy loss: Results from 1485 participants undergoing fertility evaluation. Front Endocrinol (Lausanne) 2025;15:1493186.
19. Krog MC, Nielsen JR, Slot A, Hviid KV, Kolte AM, Westergaard D, *et al*. Prospective reproductive outcomes according to sperm parameters, including DNA fragmentation, in recurrent pregnancy loss. Reprod Biomed Online 2024;15:1493186. Available from: https://doi.org/10.3389/fendo.2024.1493186.
20. Sengupta P, Dutta S, Samrot AV. Sperm DNA fragmentation testing in infertility. In: Genetic Testing in Reproductive Medicine. Singapore: Springer Nature Singapore; 2024. p. 47-66.
21. Ebrahimi M, Akbari Asbagh F, Tavakoli M, Eshraghi N, Poormand N, Ghaemi M. Correlation of sperm DNA fragmentation index with semen parameters, lifestyle and clinical pregnancy outcome after intracytoplasmic injection. J Family Reprod Health 2024;18:122-8.
22. Jiang H, Xia X, Luo Y, Pan H, Qu S, Xu J. Sperm DNA fragmentation index: Limited effectiveness on predicting embryo quality in assisted reproduction technology treatments. Reprod Biol Endocrinol 2025;23:14.
23. Björndahl L, Kirkman Brown J, other Editorial Board Members of the WHO Laboratory Manual for the Examination and Processing of Human Semen. The sixth edition of the WHO laboratory manual for the examination and processing of human semen: Ensuring quality and standardization in basic examination of human ejaculates. Fertil Steril 2022;117:246-51.
24. Amano K, Oigawa S, Ichizawa K, Tokuda Y, Unagami M, Sekiguchi M*, et al*. Swim-up method is superior to density gradient centrifugation for preserving sperm DNA integrity during sperm processing. Reprod Med Biol 2024;23:e12562.
25. 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:e12585.
26. Sobek A, Tkadlec E, Klaskova E, Prochazka M. Cytoplasmic transfer improves human egg fertilization and embryo quality: An evaluation of sibling oocytes in women with low oocyte quality. Reprod Sci 2021;28:1362-9.
27. Herbemont C, Labrosse J, Bennani-Smires B, Cedrin-Durnerin I, Peigne M, Sermondade N, *et al*. Impact of oxygen tension according to embryo stage of development: A prospective randomized study. Sci Rep 2021;11:22313.
28. Osmanlıoğlu Ş, Şükür YE, Tokgöz VY, Özmen B,

Sönmezer M, Berker B, *et al*. Intrauterine insemination with ovarian stimulation is a successful step prior to assisted reproductive technology for couples with unexplained infertility. J Obstet Gynaecol 2022;42:472-7.
29. Taniguchi M, Kanasaki H, Oride A, Okada H, Imamura K, Kyo S. Impact of current and previous sperm findings on outcomes of intrauterine insemination. Reprod Med Biol 2024;23:e12574.
30. Budiharto JM, Margiana R, William W, Supardi S, Narulita P. Relationship between spermatogenesis, DNA fragmentation index, and teratozoospermia index. Int J Health Sci (Qassim) 2022;16:1827-37.
31. Philipraj J, Rams K. Male infertility, current concepts and advances in evaluation and management, a review. J Urol Nephrol 2018;3:000145.
32. Gül M, Russo GI, Kandil H, Boitrelle F, Saleh R, Chung E, *et al*. Male infertility: New developments, current challenges, and future directions. World J Mens Health 2024;42:502-17.
33. Van de Hoek M, Rickard JP, de Graaf SP. Motility assessment of ram spermatozoa. biology (Basel) 2022;11:1715.
34. Agarwal A, Sharma R, Gupta S, Finelli R, Parekh N, Panner Selvam MK, *et al*. Sperm morphology assessment in the era of intracytoplasmic sperm injection: Reliable results require focus on standardization, quality control, and training. World J Mens Health 2022;40:347-60.
35. Szabó A, Váncsa S, Hegyi P, Váradi A, Forintos A, Filipov T, *et al*. Lifestyle-, environmental-, and additional health factors associated with an increased sperm DNA fragmentation: A systematic review and meta-analysis. Reprod Biol Endocrinol 2023;21:5.
36. 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: Results from a population-based study. Front Endocrinol (Lausanne) 2023;14:1255304.
37. Jimbo M, Kunisaki J, Ghaed M, Yu V, Flores HA, Hotaling JM. Fertility in the aging male: A systematic review. Fertil Steril 2022;118:1022-34.
38. 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 (Lausanne) 2021;12:802632.
39. Dong J, Lv Y, Zhu G, Zhao F, Wang J, Tian K, *et al*. Effect of sperm DNA fragmentation on the clinical outcomes of two assisted reproduction methods: IVF and ICSI. Int J Clin Exp Med 2017;10:11812-9.
40. Chua SC, Yovich SJ, Hinchliffe PM, Yovich JL. The sperm DNA fragmentation assay with SDF level less than 15% provides a useful prediction for clinical pregnancy and live birth for women aged Under 40 years. J Pers Med 2023;13:1079.