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Age, sexual abstinence duration, sperm morphology, and motility are predictors of sperm DNA fragmentation

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Abstract

Purpose: Sperm DNA fragmentation (SDF) has recently received attention as a cause of male infertility. However, SDF cannot be fully assessed using conventional semen parameter evaluations alone. Therefore, the authors aimed to elucidate the relationship between SDF and sperm parameters via computer-assisted sperm analysis (CASA) to improve treatment strategies in reproductive medicine.

Methods: This retrospective observational study analyzed the relationship between sperm parameters assessed by CASA and SDF values determined by the TUNEL assay in 359 patients who visited the Mie University Hospital for infertility treatment. The methodology involved semen analyses covering concentration, motility, and morphology, followed by SDF quantification using the flow cytometry.

Results: Statistical analysis revealed significant correlations between SDF and various factors, including age, sexual abstinence period, and specific CASA-measured parameters. Notably, lower sperm motility rates and abnormal head dimensions were associated with higher SDF values, indicating that these parameters were predictive of SDF.

Conclusions: This study highlights the importance of sperm motility and head morphology as indicators of SDF, suggesting their usefulness in assessing male fertility. These findings demonstrate the efficacy of detailed sperm analysis, potentially increasing the success rate of assisted reproductive technologies by improving sperm selection criteria.

KEYWORDS

abstinence, computer-assisted sperm analysis, sperm DNA fragmentation, sperm morphology, sperm motility

1 | INTRODUCTION

Infertility is a disease of the reproductive system, characterized by the inability to achieve clinical pregnancy after 12 months or more of regular, unprotected sexual intercourse.[1] Globally, an estimated 72.4 million couples experience challenges related to infertility.[2] Males are found to be solely responsible for 20%–30% of infertility cases and contribute to 50% of all cases.[3] Factors such as oxidative stress, hormonal and anatomical abnormalities, and genetic, lifestyle, and environmental elements contribute significantly to

male infertility.[4–8] Recently, sperm DNA fragmentation (SDF) has also emerged as a factor in male infertility. SDF refers to single- and double-strand breaks in sperm DNA.[9] SDF has been reported to negatively affect male fertility and reproductive outcomes, with men with higher SDF levels being less likely to conceive naturally.[10] High SDF levels are associated with a significantly increased risk of recurrent pregnancy loss (RPL).[11] Moreover, SDF levels can affect the outcomes of assisted reproductive technologies (ART), with higher SDF values being found to negatively impact pregnancy and delivery rates after intrauterine insemination (IUI).[12] Furthermore, SDF has been associated with lower pregnancy rates and increased miscarriage rates in in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI).[13,14]

Human sperm quality is typically defined by standard World Health Organization (WHO) semen analysis parameters (number, motility, and morphology). These factors are moderate predictors of reproductive outcomes. Several studies have investigated whether semen analysis parameters are associated with SDF. Gill et al.[15] reported that men with SDF greater than 18% had lower semen quality than men with SDF less than 18%; sperm concentration, sperm count, and forward motility rate were negatively correlated with SDF, and the sperm teratology index was positively correlated with SDF. However, it has been reported that even among men with normal semen analysis parameters, there are cases of advanced SDF.[16]

Moreover, in a study examining the prevalence of high SDF levels among infertile couples, a significant proportion of men with normal semen parameters had high SDF levels.[17] These findings suggest that SDF cannot be fully assessed using conventional semen parameter evaluations alone. A more detailed study on the relationship between sperm status and SDF is needed, and computer-assisted sperm analysis (CASA) may be a valuable tool for this purpose.

CASA is a computer-assisted technique for evaluating semen quality, offering more reproducible and accurate data than traditional manual microscopy. In addition, CASA allows for a detailed analysis of sperm motility characteristics and morphology.[18] Reports indicate that detailed sperm parameters obtained through CASA are associated with SDF, suggesting the potential utility of CASA in predicting male fertility.[19,20]

The primary objective of this study was to elucidate the correlation between SDF and sperm parameters measured using CASA, including motility patterns, velocity, and morphology. The authors aimed to identify key factors influencing SDF, thereby enhancing our understanding of male fertility issues and improving treatment strategies in ART.

2 | MATERIALS AND METHODS

2.1 | Study design

This retrospective study explored the correlation between sperm parameters measured by CASA and SDF assessed by the terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay with flow cytometry (FCM). Ethical approval was obtained from the Mie University Ethics Committee to ensure adherence to ethical standards (approval number: H2023-129). This study involved 359 male patients selected from a database of individuals who underwent SDF testing at the Mie University Hospital between March 2020 and December 2023. Patients were consented to participate through an opt-out process. Patients with a history of varicocele were not included in this study. Eligible patients whose sperm counts were too low to be analyzed by CASA were excluded from the study. Data on body mass index (BMI), sexual abstinence duration, alcohol consumption, and smoking status were collected using questionnaires.

2.2 | Semen analysis

Semen samples were allowed to liquefy for at least 30 min after collection. The semen volume was then measured and analyzed using a CASA system (LensHooke X1 PRO; Bonraybio, Taichung, Taiwan). This involved assessing the sperm concentration, motility (total and progressive), and morphology. The parameters measured by CASA included semen concentration, sperm count, motility rate, forward motility rate, motile sperm count, progressive sperm count, velocity average path (VAP), velocity straight line (VSL), velocity curvilinear (VCL), rapid motility sperm rate (VAP ≥ 25 μm/s), slow motility sperm rate (VAP < 25 μm/s), linearity (LIN), straightness (STR), wobble (WOB), amplitude of lateral head displacement (ALH), beat cross frequency (BCF), and normal morphology rate. The normal morphology rates were evaluated according to the WHO 5th edition criteria.[21] Additionally, the assessment included detailed measurements of head length, head width, head perimeter, head area, and tail length. All sperm within the analysis field of the LenseHooke X1 PRO were counted.

2.3 | Sperm DNA fragmentation measurement

Sperm cells (1×10^6) were fixed in 4% paraformaldehyde for 30 min and washed with phosphate-buffered saline. The cells were then permeabilized with 70% ethanol. To detect SDF, the authorsutilized the TUNEL assay, a method renowned for its efficacy in identifying DNA fragmentation. Staining was conducted following the protocol provided by the BD Pharmingen APO-DIRECT Kit (BD Biosciences, Franklin Lakes, NJ, USA). After permeabilization, the sperm were washed with wash buffer. Subsequently, a staining solution containing terminal deoxynucleotidyl transferase (TdT) and fluorescein-dUTP (dUTP-FITC) was added to the sperm and incubated at 37°C for 1 h. As a negative control, a similar solution without TdT was used to ensure the specificity of the staining process. This step allowed the labeling of DNA fragmentation sites with fluorescein isothiocyanate (FITC), a fluorescent marker. Following incubation, the sperm were washed with rinse buffer to remove the staining solution. Finally, propidium iodide (PI)/RNase was added to stain the sperm, facilitating the differentiation of fragmented DNA from non-fragmented DNA. Subsequent FCM analysis was performed using a Miltenyi

Biotec MACSQuant Analyzer 16 with the MACSQuantify software (Miltenyi Biotec, North Rhine-Westphalia, Germany). Initially, doublets were removed, and gating was conducted based on forward and side scatter. Following this, sperm positive for PI were gated. The threshold for FITC fluorescence intensity was determined using a negative control. Sperm with FITC fluorescence intensity above the threshold were considered FITC-positive. The number of FITC-positive sperm was counted, and the proportion of these sperm in the PI-positive population was determined as SDF. All measurements counted at least 5000 PI-positive sperm (Figure 1).

2.4 | Statistical analysis

The R software version 4.3.0 (The R Foundation for Statistical Computing, Vienna, Austria) was used for data analysis. Initially, a multiple regression analysis was performed, including all variables to evaluate their relationships with SDF. To refine the model and adjust for potential confounders, a stepwise selection method based on the Akaike Information Criterion (AIC) was subsequently applied. This process iteratively added or removed variables from the full model in a stepwise manner, optimizing the model by selecting the most significant predictors and achieving the lowest AIC. The model resulting from the stepwise selection was then utilized for a final multiple regression analysis aimed at investigating the factors associated with SDF. Similarly, for logistic regression analysis, all variables were initially included to assess risk factors for SDF >20%. The model was then refined using a stepwise selection method based on the AIC, ensuring that only the most significant predictors were included in the final logistic regression model. For comparisons between the two groups, the Mann–Whitney *U* test was used to examine differences in age, duration of sexual abstinence, BMI, and semen analysis parameters. Additionally, Fisher's test was employed to determine the impact of smoking and alcohol consumption on SDF. The odds ratio (OR) and its 95% confidence interval (CI) were used to indicate a higher risk of SDF >20%. Results were considered statistically significant when the *p*-value was <0.05.

3 | RESULTS

3.1 | Patient characteristics and each semen parameter

Our study included 359 men who visited the Mie University Hospital for infertility diagnosis and treatment. The semen parameters are listed in Table 1. The median age, body mass index (BMI), and sexual abstinence time of the subjects were 37 years, 23.5, and 4.5 days, respectively. The median SDF was 13.6% (Table 1).

3.2 | Multiple regression analysis of the relationship between sperm DNA fragmentation and each parameter

Table 2 presents the results of the multiple regression analysis, with SDF as the response variable and the other variables as explanatory

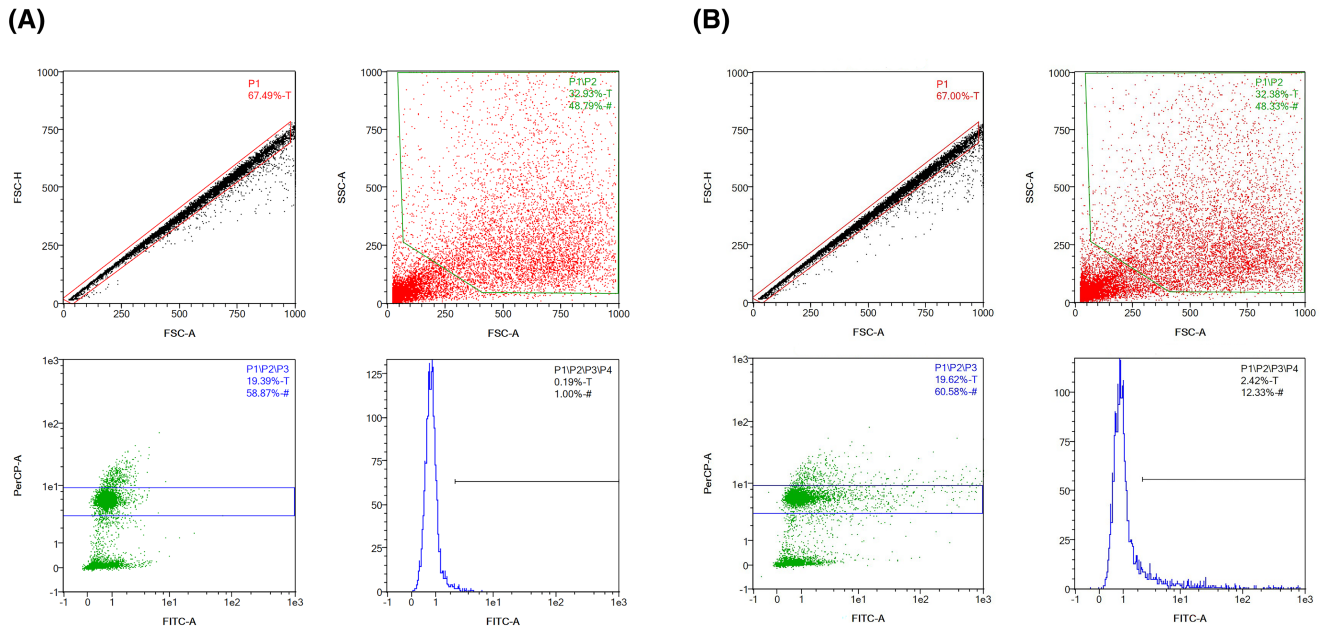

FIGURE 1 FCM analysis of TUNEL-stained sperm. (A) Negative control prepared using stain solution without TdT. For this negative control, the threshold was set at the fluorescence intensity at which the percentage of FITC-positive cells reached 1.0%. (B) FCM analysis of sperm stained with stain containing TdT. The percentage of sperm with FITC fluorescence intensity above the threshold among PI-positive cells was calculated as the SDF value. FCM, flow cytometry; FITC, fluorescein-dUTP; PI, propidium iodide; SDF, sperm DNA fragmentation; TdT, terminal deoxynucleotidyl transferase; TUNEL, terminal deoxynucleotidyl transferase dUTP nick-end labeling.

TABLE 1 Characteristics of the study population.

Parameter	Median (range)
No. of subjects	359
Age (year)	37 (20–55)
BMI	23.5 (9.5–39.9)
Abstinence period (day)	4.5 (0–30)
Smoking (*n*)	88
Alcohol consumption (*n*)	213
Semen volume (mL)	3 (0.1–8)
Concentration ($\times 10^6$/mL)	88.7 (4.1–354)
Sperm count ($\times 10^6$/ejaculate)	235.5 (0.6–1380)
Total motility (%)	73 (2–99)
Progressive motility (%)	55 (0–97)
Motility sperm count ($\times 10^6$/ejaculate)	151.3 (0.4–1003.6)
Progressive sperm count ($\times 10^6$/ejaculate)	116.5 (0–993.4)
VAP (μm/s)	5 (1–15)
VSL (μm/s)	42 (0–66)
VCL (μm/s)	26 (0–87)
Rapid motility sperm rate (%) [VAP ≥ 25 μm/s]	20.2 (2–42.3)
Slow motility sperm rate (%) [VAP < 25 μm/s]	15.4 (0.2–35)
LIN (%)	29.4 (4.4–62.6)
STR (%)	45 (1.7–99)
WOB (%)	66 (6–99)
ALH (μm)	63 (38–99)
BCF (Hz)	2.1 (0.4–18)
Morphology	
Normal morphology rate (%)	5.7 (3.3–8.7)
Head length (μm)	5.1 (4.2–6.1)
Head width (μm)	3.3 (2.8–3.8)
Head perimeter (μm)	12.9 (11.4–14.9)
Head area (μm^2)	13 (10.6–15.2)
Tail length (μm)	14.7 (2.5–36.8)
SDF (%)	13.6 (1.1–75)

Abbreviations: ALH, amplitude of lateral head displacement; BCF, beat cross frequency; BMI, body mass index; LIN, linearity; SDF, sperm DNA fragmentation; STR, straightness; VAP, velocity average path; VCL, velocity curvilinear; VSL, velocity straight line; WOB, wobble.

variables. The analysis revealed that age, duration of sexual abstinence, and VSL use were statistically significant. However, the variance inflation factor (VIF) exceeded 10 for several variables, indicating a potential multicollinearity problem. Furthermore, the relatively low degree of freedom adjusted *R*-squared of 0.083 suggests that this regression model may not adequately explain the variation in SDF. The authors performed a multiple regression analysis with variable deletion based on the AIC (Table 3) to address the multicollinearity issue and low degrees of freedom adjusted *R*-squared. After the deletion of the AIC-based variable, age, sexual abstinence time, rapid sperm motility rate, sperm motility rate, STR, head length, head perimeter, and head area were selected for the model. This variable selection improved the degrees of freedom adjusted *R*-squared from 0.083 to 0.124. The *p*-value of the *F*-test for the regression model was <0.05, indicating that the overall model was statistically significant. In particular, age, sexual abstinence time, rapid sperm motility rate, STR, head length, and head perimeter had $p < 0.05$, signifying statistical significance and suggesting potential associations with SDF. However, head length and perimeter did not significantly correlate with SDF. Moreover, the VIFs for head length and area remained high at 12.819 and 13.357, respectively, indicating that multicollinearity issues may persist for these variables.

3.3 | Comparison between two groups based on sperm DNA fragmentation level

Several studies have shown that the cutoff value of SDF measured using the TUNEL method is approximately 20%.[22,23] Based on this cutoff value, patients with SDF <20% were classified into the "low SDF group," and patients with SDF ≥20% were classified into the "high SDF group" for comparison (Table 4). The median SDF values of the high SDF group ($n = 253$) and low SDF group ($n = 106$) were 27.4% and 10.6%, respectively, which was a significant difference. The high SDF group was observed to have significantly higher age and sexual abstinence duration and significantly lower forward progressive motility, VSL, rapid sperm motility rate, slow sperm motility rate, and LIN compared to the low SDF group.

3.4 | Logistic analysis of risk factors for sperm DNA fragmentation

Although the differences described above suggest a potential relationship with SDF, no multivariate analysis was performed. This may have failed to account for the interactions among these variables or the effects of the other covariates. Therefore, a logistic analysis was performed. Variable deletion based on the AIC was performed, and five variables were selected in the final logistic regression model: age, sexual abstinence duration, rapid sperm motility rate, head length, and head perimeter. These variables were considered to potentially influence the probability of having an SDF of 20% or higher, and a logistic regression analysis was performed to assess their association. The analysis showed that sexual abstinence duration, rapid sperm motility rate, head length, and head perimeter were significantly associated with SDF. Specifically, a longer sexual abstinence duration and a lower rate of rapid sperm motility were correlated with a higher probability of having an SDF of 20% or higher. Furthermore, increasing head length decreased the probability of SDF being >20%, whereas increasing head perimeter increased that probability (Table 5).

4 | DISCUSSION

In this study, aging and longer sexual abstinence duration were associated with increased SDF. Our findings align with previous research,

TABLE 2 Evaluation of factors affecting sperm DNA fragmentation by multiple regression analysis.

	Estimate	Standard error	*p*-Value	VIF
Intercept	−96.009	53.508	0.074	
Age (year)	0.257	0.108	0.018	1.176
BMI	−0.022	0.166	0.893	1.119
Abstinence period (day)	0.480	0.140	0.001	1.347
Smoking	0.314	1.464	0.830	1.080
Alcohol consumption	−0.981	1.275	0.442	1.086
Semen volume (mL)	0.727	0.824	0.379	3.500
Concentration ($\times 10^6$/mL)	0.032	0.025	0.195	7.719
Sperm count ($\times 10^6$/ejaculate)	−0.006	0.014	0.675	27.238
Total motility (%)	0.117	0.137	0.395	33.650
Progressive motility (%)	−0.124	0.199	0.533	64.561
Motility sperm count ($\times 10^6$/ejaculate)	−0.024	0.030	0.424	102.552
Progressive sperm count ($\times 10^6$/ejaculate)	0.028	0.029	0.330	63.345
VAP (μm/s)	−0.168	0.468	0.720	1.579
VSL (μm/s)	−0.142	0.058	0.015	1.888
VCL (μm/s)	−0.100	0.148	0.501	13.533
Rapid motility sperm rate (%) [VAP ≥ 25 μm/s]	−0.188	1.130	0.868	126.438
Slow motility sperm rate (%) [VAP < 25 μm/s]	−0.123	0.718	0.864	40.486
LIN (%)	0.179	0.476	0.708	43.960
STR (%)	0.019	0.167	0.908	9.159
WOB (%)	0.186	0.171	0.277	7.164
ALH (μm)	0.052	0.275	0.849	11.470
BCF (Hz)	−0.260	0.703	0.712	1.565
Normal morphology rate (%)	0.674	1.426	0.637	1.485
Head length (μm)	−13.022	14.061	0.355	35.422
Head width (μm)	9.493	15.429	0.539	10.598
Head perimeter (μm)	16.408	8.717	0.061	50.050
Head area (μm^2)	−6.960	4.256	0.103	26.228
Tail length (μm)	0.007	0.177	0.968	1.827

Abbreviations: ALH, amplitude of lateral head displacement; BCF, beat cross frequency; BMI, body mass index; LIN, linearity; STR, straightness; VAP, velocity average path; VCL, velocity curvilinear; VIF, variance inflation factor; VSL, velocity straight line; WOB, wobble.

TABLE 3 Multiple regression analysis of influential factors on sperm DNA fragmentation with AIC-based variable reduction.

	Estimate	Standard error	*p*-Value	VIF
Intercept	−61.105	26.894	0.024	
Age (year)	0.258	0.101	0.011	1.074
Abstinence period (day)	0.456	0.122	<0.001	1.066
Rapid motility sperm rate (%)	−0.147	0.045	0.001	1.180
Slow motility sperm rate (%)	−0.059	0.040	0.148	1.050
STR (%)	0.157	0.065	0.016	1.082
Head length (μm)	−19.457	8.266	0.019	12.819
Head perimeter (μm)	17.208	7.508	0.023	38.870
Head area (μm^2)	−4.649	2.968	0.118	13.357

Abbreviations: STR, straightness; VIF, variance inflation factor.

reinforcing that age and duration of sexual abstinence are critical factors influencing SDF. The relationship between age and elevated SDF levels has been well-documented.[24,25] Oxidative stress, a major contributor to SDF levels, increases with aging.[26] This correlation was highlighted in a comprehensive study by Vaughan et al., who analyzed 16 945 semen tests and reported a significant increase in SDF and oxidative stress levels with advancing age.[27,28]

Furthermore, the impact of sexual abstinence duration on SDF has been a subject of interest.[29–31] Regarding the sexual abstinence duration, semen samples collected after a shorter sexual abstinence duration (24 h) exhibited decreased levels of intracellular reactive oxygen species (ROS) compared to those collected after a longer sexual abstinence duration (3–4 days).[30] This suggests that a shorter sexual abstinence duration might mitigate oxidative stress in the semen, potentially reducing SDF. These considerations lead us to believe that aging and sexual abstinence duration increase SDF levels via elevated oxidative stress.

The authors found that a decreased rapid sperm motility rate was associated with increased SDF. Additionally, logistic regression analysis suggested that a lower rapid sperm motility rate was associated with an increased risk of having an SDF of 20% or greater. Several studies have demonstrated a relationship between sperm motility and SDF. Cohen-Bacrie et al.,[32] in their study on the correlation between DNA damage and sperm parameters across 1633 participants, found a negative association between SDF and both total sperm count and rapid progression, corroborating our findings. Several studies have indicated that oxidative stress worsens sperm motility. Ferramosca et al.[33] reported that SDF associated with oxidative stress negatively affected mitochondrial respiration and reduced the forward motile sperm rate. In a study of 20 patients with idiopathic oligozoospermia, El-Taieb et al.[34] demonstrated that oxidative stress negatively affects the structure of the flagellar axoneme, leading to impaired sperm forward motility, These results

TABLE 4 Comparative analysis of clinical characteristics between the low and high sperm DNA fragmentation groups.

Parameter	Low SDF group (SDF < 20%)	High SDF group (SDF ≥ 20%)	*p*-Value
No. of subjects	253	106	
Age (year)	36 (20–54)	38.5 (25–55)	0.005
BMI	23.6 (9.5–39.9)	23.5 (17.2–36.5)	0.669
Abstinence period (day)	4 (0–30)	6 (0–30)	<0.001
Smoking (*n*)	63	25	0.8934
Alcohol consumption (*n*)	153	60	0.5561
Semen volume (mL)	3 (0.4–8)	2.8 (0.1–8)	0.285
Concentration ($\times 10^6$/mL)	87 (4.1–354)	91.7 (5.8–300)	0.219
Sperm count ($\times 10^6$/ejaculate)	235 (9.7–953)	240.1 (0.6–1380)	0.551
Total motility (%)	75 (2–99)	67 (2–99)	0.201
Progressive motility (%)	58 (0–95)	46.5 (0–97)	0.032
Motility sperm count ($\times 10^6$/ejaculate)	149 (1–924.4)	153.3 (0.4–1003.6)	0.956
Progressive sperm count ($\times 10^6$/ejaculate)	108 (0–829.1)	118.1 (0–993.4)	0.662
VAP (μm/s)	5 (1–15)	5 (1–11)	0.543
VSL (μm/s)	42 (0–61)	30 (0–66)	<0.001
VCL (μm/s)	26 (0–87)	22 (0–72)	0.061
Rapid motility sperm rate (%) [VAP ≥ 25 μm/s]	20.5 (2–42.3)	19.2 (3.5–37)	0.021
Slow motility sperm rate (%) [VAP < 25 μm/s]	15.6 (0.2–33.8)	14.3 (1.9–35)	0.045
LIN (%)	29.7 (4.4–62.6)	26.8 (7.5–52)	0.015
STR (%)	45 (1.7–99)	45 (2.2–80)	0.602
WOB (%)	66 (6–99)	65 (30–96)	0.440
ALH (μm)	63 (42–99)	62 (38–86)	0.818
BCF (Hz)	2.1 (0.4–18)	2 (0.7–3.4)	0.070
Normal morphology rate (%)	5.7 (3.3–7.2)	5.6 (4.4–8.7)	0.106
Head length (μm)	5.1 (4.2–5.9)	5.1 (4.7–6.1)	0.937
Head width (μm)	3.3 (3–3.7)	3.3 (2.8–3.8)	0.051
Head perimeter (μm)	12.9 (11.4–14.9)	12.9 (12–14.3)	0.206
Head area (μm^2)	12.9 (10.6–15.1)	13.1 (10.9–15.2)	0.142
Tail length (μm)	15.1 (2.5–30.3)	13.9 (5–36.8)	0.136
SDF (%)	10.6 (1.1–19.8)	27.4 (20.1–75)	<0.001

Abbreviations: ALH, amplitude of lateral head displacement; BCF, beat cross frequency; BMI, body mass index; LIN, linearity; SDF, sperm DNA fragmentation; STR, straightness; VAP, velocity average path; VCL, velocity curvilinear; VSL, velocity straight line; WOB, wobble.

indicate that oxidative stress increases SDF levels and impairs sperm motility, supporting our findings.

Our results showed that sperm with longer heads tended to have lower SDF, whereas sperm with a larger head perimeter had higher SDF. Logistic regression analysis also showed a significant reduction in the risk of higher SDF associated with longer sperm heads, while the risk of SDF was higher with larger head perimeters. Previous studies have highlighted the impact of sperm head morphology on IVF outcomes. Oehninger et al.[35] reported that severe sperm head abnormalities were associated with lower fertilization rates, pregnancy rates per cycle, and sustained pregnancy rates. Gao et al.[36] reported that the percentage of sperm with normal morphology and vacuole formation in the anterior part of the sperm, as observed by motile sperm organelle morphology examination (MSOME), was highly correlated with IVF rates.

Furthermore, Majzoub et al. demonstrated a potential association between abnormal sperm morphology, particularly head abnormalities, and increased SDF. They found a positive correlation between SDF and head defects and a negative correlation with normal morphology.[37] Jakubik-Uljasz et al.[38] showed that men with teratozoospermia often have higher SDF than men with normal sperm morphology.

To our knowledge, no study has examined the relationship between detailed morphological parameters and SDF in human sperm using CASA. However, a study using frozen canine sperm reported that round and large sperm were more prone to DNA

TABLE 5 An AIC-based variable reduction logistic regression analysis for predicting high sperm DNA fragmentation.

	Estimate	Standard error	*p*-Value	OR (95% CI)
Age (year)	0.035	0.022	0.109	1.04 (0.99, 1.08)
Abstinence period (day)	0.128	0.032	<0.001	1.14 (1.07, 1.22)
Rapid motility sperm rate (%)	−0.029	0.009	0.001	0.97 (0.95, 0.99)
Head length (μm)	−2.645	1.238	0.033	0.07 (0.01, 0.78)
Head perimeter (μm)	1.596	0.648	0.014	4.93 (1.41, 18.02)

Abbreviations: 95% CI, 95% confidence interval; OR, odds ratio.

damage,[39] consistent with our results, albeit from different species. The authors hypothesized that abnormal sperm DNA packaging might contribute to SDF, particularly in the presence of sperm head abnormalities. Protamine is the major nuclear protein in sperm, and human sperm nuclei contain two types: protamine 1 (P1) and protamine 2 (P2). These proteins condense sperm DNA into a compact form and protect genetic information.[40] Inadequate packaging of sperm DNA increases sperm vulnerability and may lead to SDF. Amor et al.[41] reported that the ratio of sperm P1 to P2 was associated with SDF. Manochantr et al.[42] reported that sperm deficiency in protamine, essential for sperm DNA packaging, correlated negatively with normal sperm morphology and positively with SDF. These findings suggest that a detailed analysis of sperm head morphology, particularly head length and circumference, is a potentially valuable tool for assessing SDF risk. However, its accuracy in assessing sperm morphology compared to sperm concentration and motility using CASA remains controversial.[43] The strength of this study lies in the correlation between sperm morphology parameters measured using CASA and SDF. This suggests that evaluating sperm morphology with CASA is associated with SDF and may offer important evidence supporting the validity of morphometry by CASA.

This study had several limitations due to its retrospective design, including the potential for bias in sample selection. Moreover, the outcomes of IVF were not examined, leaving it unclear how the sperm parameters measured by CASA correlate with IVF success rates. Future studies should address these factors to elucidate the relationships between IVF outcomes and sperm parameters. Additionally, unforeseeable confounding factors may introduce bias.

In conclusion, our study found that age, sexual abstinence duration, sperm motility rate, and sperm head morphology were associated with SDF. These findings allow for a more detailed assessment of male fertility. Moreover, they may provide crucial information to improve the criteria for sperm selection in ART.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

ETHICAL APPROVAL

Ethical approval was obtained from the Mie University Ethics Committee to ensure adherence to ethical standards (approval number: H2023-129).

HUMAN RIGHTS STATEMENT AND INFORMED CONSENT

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and its later amendments. Informed consent was obtained from all patients for being included in the study.

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