

Original Research Article

The role of sperm chromatin structure assay in improving the diagnosis of male factor infertility in Benin city

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ABSTRACT

Background: Male factor infertility accounts for about 30% of infertility cases worldwide. In Nigeria, evaluation relies almost exclusively on seminal fluid analysis (SFA), which does not assess chromatin integrity; up to 15% of men with normal SFA remain sub-fertile from unrecognized chromatin abnormalities. This study aimed to determine the prevalence of abnormal sperm chromatin structure, its relationship with semen parameters, and associated risk factors among male partners of infertile couples in Benin City, using the Sperm Chromatin Structure Assay (SCSA).

Methods: In this cross-sectional study, 120 male partners of infertile couples attending the Infertility Clinic, University of Benin Teaching Hospital from April to July 2026, underwent SFA and SCSA (flow cytometry with acridine orange staining) to determine the DNA Fragmentation Index (DFI). Data were analysed using descriptive statistics, correlation, and logistic regression; $p < 0.05$ was significant.

Results: Mean age was 45.3 ± 8.0 years and mean DFI was $22.4 \pm 7.1\%$. Abnormal DFI ($>25\%$) occurred in 39.2% (95% CI 30.9-48.1%) of participants. DFI correlated negatively with motility, progressive motility, morphology, and vitality (all $p < 0.05$), but not with volume or sperm count. Notably, 21.3% of men with normal SFA had abnormal DFI. Cigarette smoking was the only independent predictor of abnormal DFI (adjusted OR 3.57, $p = 0.002$); no seminal parameter achieved acceptable diagnostic accuracy ($AUC \geq 0.70$) as a DFI proxy.

Conclusions: Abnormal sperm chromatin structure is common among Nigerian men with infertility and is incompletely captured by semen analysis alone. SCSA should be incorporated into male infertility evaluation, particularly for semen with low vitality or progressive motility. Also, instructive to prioritize smoking cessation counselling as a modifiable risk factor.

Keywords: DNA fragmentation index, Male factor infertility, Nigeria, Seminal fluid analysis, Sperm chromatin structure assay

INTRODUCTION

Male factor infertility accounts for approximately 30% of infertility cases among couples worldwide.¹⁻³ This substantial contribution underscores the need to understand the diverse causes of male infertility in order to improve diagnosis and treatment outcomes. Among these causes, abnormalities in sperm chromatin structure represent a crucial, yet historically understudied, contributor. Chromatin in the sperm nucleus consists of tightly packed DNA and associated proteins that protect

the paternal genome and ensure its faithful transmission to the embryo. When this structure is compromised, sperm function is impaired in ways that conventional semen analysis cannot detect, with downstream consequences for fertilization and embryo development.

A growing body of evidence links abnormalities in sperm chromatin structure not only to reduced fertility but also to genetic problems in offspring and to higher rates of miscarriage after both spontaneous and assisted conception, including in vitro fertilisation (IVF).^{5,6} Despite

this, sperm chromatin integrity remains an overlooked component of routine infertility evaluation, particularly in resource-limited settings.

The Sperm Chromatin Structure Assay (SCSA) is a standardized, flow-cytometric method for evaluating sperm chromatin integrity.⁷ It uses the metachromatic dye acridine orange, which fluoresces green when bound to intact double-stranded DNA and red when bound to denatured, single-stranded (fragmented) DNA, to generate the DNA Fragmentation Index (DFI), an objective, reproducible measure of sperm nuclear quality that goes beyond the count, motility, and morphology assessed by conventional semen analysis.⁷ In many developed countries, SCSA is performed alongside Seminal Fluid Analysis (SFA) as part of a comprehensive fertility work-up, offering clinicians a fuller picture of sperm health and helping to predict treatment success.⁸ In Nigeria and much of sub-Saharan Africa, however, SCSA remains under-utilized because of the cost of equipment, the need for specialized laboratories, and a shortage of trained personnel, so SFA continues to serve as the sole diagnostic tool for male factor infertility in most centres.

This near-total reliance on SFA leaves an important diagnostic gap. Because SFA does not assess chromatin integrity or DNA fragmentation, it can reassure a couple that the male partner is fertile even when substantial nuclear defects are present. Previous work has shown that as many as 15% of men with normal SFA results remain sub-fertile because of underlying sperm chromatin defects.⁴ This gap can lead to men being wrongly classified as fertile, unexplained infertility, ineffective or poorly targeted treatment, and repeated cycles of assisted reproduction without an understanding of the true underlying cause, with attendant psychological, financial, and emotional costs to affected couples.

International data illustrate how common these defects are. In a study of 350 men from infertile couples, 28% had a DFI exceeding 20% and 12% had a DFI above 30%, and 15% of men with entirely normal semen parameters still showed high sperm DNA damage.⁹ Sperm DNA fragmentation also rises with advancing paternal age: men aged 40 years and above have significantly higher DFI than younger men even when their conventional semen parameters are normal, suggesting an age-related deterioration that standard analysis cannot detect.¹⁰ Nigerian data mirror this pattern. Among Nigerian men with unexplained infertility, 63% had a DFI above 20% compared with only 4% of fertile controls, and 15.2% had a DFI above 30%.¹¹ In South-West Nigeria, infertile men have been shown to have elevated seminal oxidative DNA damage and a mean DFI in the region of 15-16%, with DFI significantly and inversely correlated with sperm concentration.¹²

SCSA-derived parameters correlate, but imperfectly, with conventional semen indices. In a foundational study of 136 men, DFI and High DNA Stainability (HDS, a marker of

nuclear immaturity from incomplete protamination) showed moderate inverse correlations with sperm concentration, but these correlations were not observed in oligozoospermic men, suggesting SCSA adds diagnostic information beyond sperm count in low-count cases.¹³ Similar inverse relationships between DFI and motility, and between DFI and morphology, have been replicated in cohorts of thousands of men, with motility and concentration emerging as the strongest predictors of DFI on multivariate analysis.¹⁴ Elevated HDS is likewise associated with increased sperm abnormalities, higher DFI, and greater early pregnancy loss after IVF.¹⁵ Importantly, normal morphology does not preclude significant DNA damage, and a substantial proportion of Nigerian men with normal semen parameters and unexplained infertility have markedly elevated DFI, underscoring the limits of relying on conventional parameters alone.¹¹

Sperm chromatin integrity also has direct bearing on the outcomes of fertility treatment.^{16,17} High sperm DNA fragmentation is linked to delayed conception and low success with intrauterine insemination, with pregnancy rates per cycle falling sharply once DFI exceeds 25-30%.¹⁷ In IVF/ICSI cohorts, a DFI above 20% has been associated with reduced fertilization, fewer good-quality embryos, and fewer live births in conventional IVF, with miscarriage risk rising further once DFI exceeds 40%; ICSI outcomes appear comparatively less affected by elevated DFI.¹⁸ Protamine deficiency, DNA methylation abnormalities, and sperm DNA fragmentation similarly reduce fertilization rates in ICSI, although post-fertilization development is less affected.¹⁹ Meta-analyses confirm that elevated sperm DNA damage reduces implantation and pregnancy rates across assisted reproductive technologies (ART), with a more pronounced effect in IVF than ICSI.^{20,21} Mechanistically, high DNA fragmentation delays embryo cleavage and blastulation even with donor oocytes, and can trigger apoptotic pathways that culminate in embryo arrest or miscarriage, providing biological plausibility for these clinical associations.^{22,23} Emerging strategies such as morphologically selected sperm injection and testicular sperm extraction may reduce the DNA damage accumulated during epididymal transit and have shown improved live-birth outcomes in men with high sperm DNA fragmentation or recurrent ART failure.^{24,25} Professional bodies remain divided on how to act on this evidence: the European Society of Human Reproduction and Embryology supports selective sperm DNA fragmentation testing in ART, while the American Society for Reproductive Medicine advocates caution pending further standardisation.^{26,27}

Generally, this evidence indicates that a molecular assessment of sperm chromatin integrity captures clinically important information that conventional semen analysis misses, with direct implications for diagnosis, counselling, and treatment selection. In Nigeria, where SFA remains the only diagnostic tool routinely available

to most men presenting with infertility, the true burden of chromatin-related sub-fertility, its relationship with conventional semen parameters, the clinical and lifestyle factors associated with chromatin damage, and whether any routine semen parameter could serve as a practical proxy for chromatin damage in this resource-limited setting have not been well characterized. This study was therefore designed to evaluate the diagnostic utility of the sperm chromatin structure assay, using the DNA fragmentation index, among men attending a tertiary fertility clinic in Benin city. The specific objectives were to assess sperm chromatin structure using DFI among male partners of infertile couples; to determine the prevalence of abnormal DFI in this population; to investigate the relationship between DFI and standard seminal fluid parameters; and to identify clinical and lifestyle factors associated with abnormal DFI.

METHODS

Study design and setting

This was a cross-sectional analytical study conducted at the Human Reproduction Research Programme (HRRP)/IVF Unit, a tertiary fertility centre at the University of Benin Teaching Hospital (UBTH), and the Centre of Excellence in Reproductive Health and Innovation (CERHI) advanced molecular laboratory, University of Benin, Benin City, Edo State, Nigeria. The HRRP/IVF Unit is one of Nigeria's leading fertility centres, providing a dedicated laboratory for Seminal Fluid Analysis alongside a full range of assisted reproductive services, while CERHI is a research and training facility offering molecular and genetic testing, including SCSA, in support of reproductive health research.

Study population, duration and eligibility criteria

The study population comprised all consenting male partners of infertile couples attending the HRRP Infertility Clinic, UBTH from April to July 2026. Men who did not give informed consent, unable to supply a sufficient semen sample, or who were undergoing chemotherapy or being managed for testicular conditions by the urologist were excluded.

Sample size and sampling technique

The minimum sample size was calculated using the Cochran formula for descriptive cross-sectional studies:²⁸ $n = Z^2pq/d^2$, where $Z = 1.96$ (95% confidence level), $p = 0.075$ (prevalence of abnormal SCSA reported by Castilla et al.),²⁹ $q = 1 - p$, and $d = 0.05$ (precision). This yielded a minimum of 107 participants; after adjusting for an anticipated 10% non-response rate, the target sample size was approximately 120. Eligible men were recruited consecutively until this target was reached.

Sperm chromatin structure assay procedure

Semen samples were collected after 2-5 days of sexual abstinence and allowed to liquefy at room temperature for 30-60 minutes. Sperm concentration was adjusted to approximately 1-2 million sperm/mL with a buffer solution to standardize analysis across samples. Samples were then exposed to a low-pH solution for 30 seconds to partially denature DNA at sites of strand breakage or structural abnormality, before staining with acridine orange, which fluoresces green when bound to intact double-stranded DNA and red when bound to denatured, fragmented DNA. Stained samples were analysed by flow cytometry, and the ratio of red to total (red plus green) fluorescence was used to calculate the DNA Fragmentation Index (DFI), the percentage of spermatozoa with fragmented DNA. DFI was interpreted as follows: <15%, excellent DNA integrity; 15-25%, moderate DNA damage; and >25%, high DNA fragmentation (abnormal).

Data collection

A structured proforma was used to collect sociodemographic data (age, marital status, family setting) and clinical/lifestyle information (abstinence period, comorbidities, urogenital history, and exposures including alcohol, cigarette smoking, cannabis, caffeine, herbal tea, and tight underwear use), together with the results of standard Seminal Fluid Analysis (semen volume, sperm concentration, total motility, progressive motility, normal morphology, and vitality) and SCSA (DFI).

Statistical analysis

Data were entered and analysed in IBM SPSS version 25. Descriptive statistics (frequencies, percentages, means $\pm$ standard deviation, and medians with interquartile range [IQR]) summarized sociodemographic, clinical, and SCSA/SFA variables. The distribution of continuous variables was assessed using the Shapiro-Wilk test; as DFI and semen parameters were non-normally distributed (all $p < 0.05$), Spearman's rank correlation and the Mann-Whitney U test were used for continuous variables, and the chi-square test or Fisher's exact test (where expected cell counts were < 5) for categorical associations. Binary logistic regression, with abnormal DFI ($> 25\%$) as the outcome, was used to identify independent predictors of abnormal chromatin structure; variables with a univariate $p < 0.20$ were entered into the multivariable model. Because seminal fluid parameters were highly intercorrelated ($r = 0.21-0.90$), each was tested individually, adjusting for the significant/borderline clinical and lifestyle covariates identified in the multivariable model, rather than jointly. Receiver operating characteristic (ROC) curve analysis, with 95% confidence intervals calculated by the Hanley-McNeil method and optimal cut-offs selected by the Youden index, was used to evaluate the diagnostic performance of individual seminal fluid parameters for predicting abnormal DFI. A p -value < 0.05 was considered statistically significant throughout.

Ethical considerations

Ethical approval was obtained from the Health Research Ethics Committee (HREC) of the University of Benin Teaching Hospital prior to commencement of the study, and written informed consent was obtained from all participants.

RESULTS

A total of 120 male partners of infertile couples were analyzed. Descriptive statistics (frequencies, percentages, means $\pm$ SD, medians/IQR) were used to summarize variables. Data distribution was assessed with the Shapiro-Wilk test; DNA fragmentation index (DFI) and semen parameters were non-normally distributed (all $p < 0.05$), so Spearman's correlation and the Mann-Whitney U test were used where applicable, alongside Chi-square/Fisher's

exact test for categorical associations. Binary logistic regression was used to identify independent predictors of abnormal DFI. A p -value < 0.05 was considered statistically significant.

The mean age of respondents was 45.3 ± 8.0 years, with the majority (66.7%) aged 45 years and above. Most participants were married (91.7%) and in monogamous unions (93.3%), and 75.0% had abstained from ejaculation for 2-7 days prior to semen analysis (mean 2.8 ± 3.0 days). Lifestyle exposures were common: 63.3% consumed alcohol, 44.2% smoked cigarettes, 26.7% used cannabis, and 18.3% used caffeine (Table 1). Based on the assigned semen diagnosis, 39.2% of respondents had a normal semen analysis while 60.8% had an abnormal semen diagnosis (21.7% asthenozoospermia, 32.5% oligoasthenoteratozoospermia [OATS], 3.3% oligozoospermia, and 3.3% teratozoospermia).

Table 1: Demographic and clinical characteristics of respondents.

Variables	Frequency (N)	Percentage (%)
Age (years)		
<30	4	3.3
30-34	6	5.0
35-39	18	15.0
40-44	12	10.0
≥ 45	80	66.7
Mean $\pm$ SD	45.3 $\pm$ 8.0	
Days of sexual abstinence before SFA		
<2 days	28	23.3
2-7 days	90	75.0
>7 days	2	1.7
Mean $\pm$ SD	2.8 $\pm$ 3.0	
Hypertension		
Yes	26	21.7
No	94	78.3
Diabetes mellitus		
Yes	0	0.0
No	120	100.0
History of groin surgery		
Yes	2	1.7
No	118	98.3
History of testicular torsion		
Yes	2	1.7
No	118	98.3
History of mumps		
Yes	4	3.3
No	116	96.7
History of sexually transmitted illness		
Yes	20	16.7
No	100	83.3
Use of herbal teas		
Yes	20	16.7
No	100	83.3
Alcohol use		
Yes	76	63.3

Continued.

Variables	Frequency (N)	Percentage (%)
No	44	36.7
Cigarette smoking		
Yes	53	44.2
No	67	55.8
Caffeine consumption		
Yes	22	18.3
No	98	81.7
Tight underwear use		
Yes	20	16.7
No	100	83.3
Cannabis use		
Yes	32	26.7
No	88	73.3

The mean DFI was $22.4 \pm 7.1\%$ (median 23.0%, IQR 15.0-28.0%; range 12.0-35.0%) (Table 2). Using the standard classification, 25.0% of men had a low DFI (<15%),

35.8% a moderate DFI (15-25%), and 39.2% a high DFI (>25%) (Figure 1). Overall, 39.2% (95% CI 30.9-48.1%) of the study population had an abnormal DFI exceeding the 25% threshold (Table 3).

Table 2: Distribution of DNA Fragmentation Index (DFI) among male partners of infertile couples.

Variables	Mean $\pm$ SD	Median (IQR)	Minimum	Maximum
DNA fragmentation index (%)	22.4 $\pm$ 7.1	23.0 (15.0-28.0)	12.0	35.0

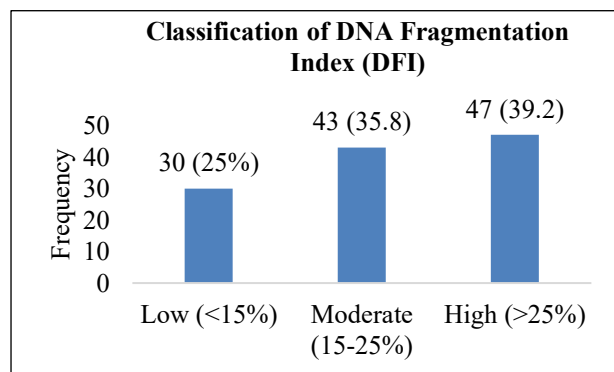


Figure 1: Classification of DNA Fragmentation Index (DFI) (n=120).

Table 3: Prevalence of abnormal sperm chromatin structure.

Variables	Frequency (N)	Percentage (%)	95% CI
Normal DFI ($\leq 25\%$)	73	60.8	51.9-69.1
Abnormal DFI ($> 25\%$)	47	39.2	30.9-48.1
Total	120	100.0	-

DFI correlated negatively and significantly with total motility ($r = -0.192$, $p = 0.036$), progressive motility ($r = -0.257$, $p = 0.005$), normal morphology ($r = -0.209$, $p = 0.022$), and vitality ($r = -0.260$, $p = 0.004$); semen volume and total

sperm count showed the same direction of association but did not reach significance ($r = -0.092$, $p = 0.315$ and $r = -0.131$, $p = 0.153$, respectively) (Table 4).

Table 4: Correlation between DNA Fragmentation Index (DFI) and seminal fluid analysis parameters.

Seminal fluid parameter	Correlation coefficient (r)	P value
Semen volume (mL)	-0.092	0.315
Total sperm count (million)	-0.131	0.153
Total motility (%)	-0.192	0.036*
Progressive motility (%)	-0.257	0.005*
Normal morphology (%)	-0.209	0.022*
Vitality (%)	-0.260	0.004*

Spearman's rank correlation (ρ) used as DFI and semen parameters were non-normally distributed (Shapiro-Wilk $p < 0.05$); $n = 120$ for all parameters (no missing values); *Statistically significant at $p < 0.05$

In Table 5, men with abnormal DFI had significantly lower progressive motility (12.9 vs 17.7%, $p = 0.020$) and vitality (32.7 vs 42.5%, $p = 0.011$) than men with normal DFI (Table 5); semen volume, total sperm count, total motility, and morphology showed the same direction of difference but did not reach significance. On categorical comparison of the clinician-assigned semen diagnosis, a normal semen analysis was significantly less frequent in the abnormal-DFI group (21.3% vs 50.7%, $p = 0.002$), asthenozoospermia was significantly more frequent in the abnormal-DFI group (34.0% vs 13.7%, $p = 0.016$), and

oligozoospermia, teratozoospermia, and OATS did not differ significantly between DFI groups (Table 5). Overall, an abnormal semen diagnosis was significantly more common in the abnormal-DFI group than the normal-DFI

group (78.7% vs 49.3%, $p=0.002$), indicating meaningful though incomplete overlap between DFI status and conventional semen analysis in this cohort.

Table 5: Comparison of seminal fluid parameters between normal and abnormal DNA fragmentation groups/association between abnormal DNA fragmentation and semen analysis diagnosis.

Variables	Normal DFI ($\leq 25\%$) Mean $\pm$ SD	Abnormal DFI ($>25\%$) Mean $\pm$ SD	Test statistic (U)	P value
Semen volume (mL)	2.95 $\pm$ 1.04	2.76 $\pm$ 1.02	1913.0	0.281
Total sperm count (million)	31.92 $\pm$ 18.96	28.94 $\pm$ 21.01	1958.5	0.192
Total motility (%)	39.44 $\pm$ 20.40	32.64 $\pm$ 21.62	2014.0	0.108
Progressive motility (%)	17.73 $\pm$ 11.55	12.87 $\pm$ 10.26	2145.5	0.020*
Normal morphology (%)	15.08 $\pm$ 9.90	12.43 $\pm$ 8.62	1981.0	0.152
Vitality (%)	42.47 $\pm$ 21.13	32.68 $\pm$ 21.26	2190.5	0.011*
Semen analysis diagnosis	Normal DFI, N (%)	Abnormal DFI, N (%)	χ^2 /Fisher's Exact	P value
Normal semen analysis	37 (50.7)	10 (21.3)	$\chi^2 = 9.181$	0.002*
Oligozoospermia	2 (2.7)	2 (4.3)	Fisher's exact	0.644
Asthenozoospermia	10 (13.7)	16 (34.0)	$\chi^2 = 5.825$	0.016*
Teratozoospermia	3 (4.1)	1 (2.1)	Fisher's exact	1.000
OATS (oligoasthenoteratozoospermia)	21 (28.8)	18 (38.3)	$\chi^2 = 0.789$	0.374

*Statistically significant at $p<0.05$.

On bivariate cross-analysis, no demographic or lifestyle factor was significantly associated with an abnormal semen diagnosis (Table 6); alcohol use showed a non-significant trend toward being more frequent among men with abnormal semen parameters (69.9% vs 53.2%, $p=0.098$). For DFI classification, cigarette smoking was

significantly more frequent among men with abnormal DFI (61.7% vs 32.9%, $p=0.004$), while hypertension showed a non-significant trend toward being less frequent among men with abnormal DFI (12.8% vs 27.4%, $p=0.095$). Alcohol use, caffeine use, and the remaining lifestyle/clinical factors were not significantly associated with DFI classification on bivariate analysis (Table 6).

Table 6: Association between demographic/clinical characteristics and abnormal semen parameters/ DFI classification.

Variables	Normal semen (n=47), N (%)	Abnormal semen (n=73), N (%)	Test statistic	P value
Age (years), Mean $\pm$ SD	45.7 $\pm$ 7.1	45.0 $\pm$ 8.6	U=1689.5	0.891
Abstinence days, Mean $\pm$ SD	3.3 $\pm$ 4.3	2.4 $\pm$ 1.5	U=1588.5	0.485
Hypertension	9 (19.1)	17 (23.3)	$\chi^2 = 0.10$	0.756
Groin surgery	2 (4.3)	0 (0.0)	Fisher's exact	0.151
Testicular torsion	1 (2.1)	1 (1.4)	Fisher's exact	1.000
Mumps	2 (4.3)	2 (2.7)	Fisher's exact	0.644
History of STI	10 (21.3)	10 (13.7)	$\chi^2 = 0.70$	0.403
Herbal teas use	7 (14.9)	13 (17.8)	$\chi^2 = 0.03$	0.867
Alcohol use	25 (53.2)	51 (69.9)	$\chi^2 = 2.74$	0.098
Cigarette smoking	16 (34.0)	37 (50.7)	$\chi^2 = 2.57$	0.109
Caffeine use	8 (17.0)	14 (19.2)	$\chi^2 = 0.00$	0.955
Tight underwear use	7 (14.9)	13 (17.8)	$\chi^2 = 0.03$	0.867
Cannabis use	11 (23.4)	21 (28.8)	$\chi^2 = 0.19$	0.662
Variable	Normal DFI (n=73)	Abnormal DFI (n=47)	Test Statistic	p-value
Age (years), Mean $\pm$ SD	44.9 $\pm$ 8.0	46.0 $\pm$ 7.9	U=1886.5	0.358
Abstinence days, Mean $\pm$ SD	3.0 $\pm$ 3.6	2.3 $\pm$ 1.6	U=1471.5	0.179
Hypertension	20 (27.4)	6 (12.8)	$\chi^2 = 2.80$	0.095
Groin surgery	2 (2.7)	0 (0.0)	Fisher's exact	0.519
Testicular torsion	0 (0.0)	2 (4.3)	Fisher's exact	0.151
Mumps	4 (5.5)	0 (0.0)	Fisher's exact	0.154

Continued.

Variables	Normal semen (n=47), N (%)	Abnormal semen (n=73), N (%)	Test statistic	P value
History of STI	14 (19.2)	6 (12.8)	$\chi^2 = 0.45$	0.503
Herbal teas use	15 (20.5)	5 (10.6)	$\chi^2 = 1.37$	0.242
Alcohol use	47 (64.4)	29 (61.7)	$\chi^2 = 0.01$	0.918
Cigarette smoking	24 (32.9)	29 (61.7)	$\chi^2 = 8.50$	0.004*
Caffeine use	17 (23.3)	5 (10.6)	$\chi^2 = 2.27$	0.132
Tight underwear use	14 (19.2)	6 (12.8)	$\chi^2 = 0.45$	0.503
Cannabis use	19 (26.0)	13 (27.7)	$\chi^2 = 0.00$	0.836

*Statistically significant at $p < 0.05$.

Cigarette smoking was the only independent predictor of abnormal DNA fragmentation index. Men who smoked cigarettes had over three-and-a-half times the odds of abnormal DFI compared with non-smokers (adjusted OR=3.57, 95% CI 1.60-7.96, $p=0.002$), after adjusting for hypertension, herbal teas use, and caffeine use.

Hypertension showed a protective trend that approached but did not reach significance (adjusted OR=0.37, 95% CI 0.13-1.10, $p=0.074$), while herbal teas use and caffeine use were not significant after adjustment (adjusted OR=0.50, $p=0.244$ and adjusted OR=0.55, $p=0.304$, respectively) (Table 7).

Table 7: Univariate and multivariable logistic regression of factors associated with abnormal DNA fragmentation index/univariate and adjusted association between individual seminal fluid parameters and abnormal DNA fragmentation index.

Variables	Crude OR (95% CI)	P value	Adjusted OR (95% CI)	P value
Age (years)	1.02 (0.97-1.07)	0.454	-	-
Abstinence days	0.90 (0.75-1.07)	0.230	-	-
Hypertension	0.39 (0.14-1.05)	0.063	0.37 (0.13-1.10)	0.074
History of STI	0.62 (0.22-1.74)	0.361	-	-
Herbal teas use	0.46 (0.16-1.37)	0.162	0.54 (0.16-1.77)	0.310
Alcohol use	0.89 (0.42-1.90)	0.766	-	-
Cigarette smoking	3.29 (1.53-7.06)	0.002*	3.57 (1.60-7.96)	0.002*
Caffeine use	0.39 (0.13-1.15)	0.088	0.55 (0.17-1.73)	0.304
Tight underwear use	0.62 (0.22-1.74)	0.361	-	-
Cannabis use	1.09 (0.48-2.48)	0.844	-	-
Seminal fluid parameter	Crude OR (95% CI)	p-value	Adjusted OR (95% CI)†	P value
Semen volume (mL)	0.83 (0.57-1.20)	0.315	0.79 (0.54-1.17)	0.242
Total sperm count (million)	0.99 (0.97-1.01)	0.419	0.99 (0.97-1.01)	0.479
Total motility (%)	0.98 (0.97-1.00)	0.085	0.99 (0.97-1.01)	0.243
Progressive motility (%)	0.96 (0.93-0.99)	0.023*	0.96 (0.93-1.00)	0.064
Normal morphology (%)	0.97 (0.93-1.01)	0.136	0.97 (0.93-1.02)	0.232
Vitality (%)	0.98 (0.96-1.00)	0.017*	0.98 (0.96-1.00)	0.035*

*Statistically significant at $p < 0.05$.

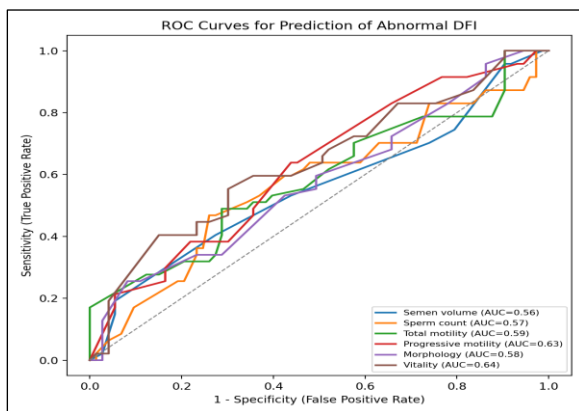
Seminal fluid parameters as potential proxy indicators of abnormal DFI

Because SCSA is costly and not routinely available in low- and middle-income settings, we examined whether any conventional seminal fluid parameter could serve as a practical proxy for abnormal DFI. Each semen parameter was first tested individually against abnormal DFI (univariate), then re-tested after adjusting for the significant/borderline clinical and lifestyle covariates from Table 7 (hypertension, cigarette smoking, and caffeine use). Semen parameters were added one at a time rather than together, as they were highly inter-correlated ($r=0.21-0.90$), which would make a combined model statistically unstable and its individual coefficients uninterpretable.

Sperm vitality was the only seminal fluid parameter that remained an independent predictor of abnormal DFI after adjustment: lower vitality was associated with higher odds of abnormal DFI both before and after adjusting for hypertension, cigarette smoking, and caffeine use (adjusted OR=0.98, 95% CI 0.96-1.00, $p=0.035$ per 1% increase in vitality), a finding consistent with its correlation in Table 4 and its group difference in Table 5. Progressive motility showed a similar trend that approached but did not retain independent significance after adjustment (adjusted OR=0.96, $p=0.064$). Semen volume, total sperm count, total motility, and normal morphology were not independently associated with abnormal DFI after adjustment.

Table 8: Diagnostic (ROC curve) analysis of seminal fluid parameters for predicting abnormal DNA fragmentation index.

Seminal fluid parameters	AUC (95% CI)	P value	Optimal cut-off	Sensitivity (%)	Specificity (%)
Semen volume (ml)	0.56 (0.45-0.66)	0.288	<1.8	19.1	94.5
Sperm count (million)	0.57 (0.46-0.68)	0.190	<18.0	46.8	74.0
Total motility (%)	0.59 (0.48-0.69)	0.106	<25.0	48.9	71.2
Progressive motility (%)	0.63 (0.52-0.73)	0.018*	<15.0	63.8	56.2
Morphology (%)	0.58 (0.47-0.68)	0.152	<3.0	25.5	91.8
Vitality (%)	0.64 (0.54-0.74)	0.009*	<15.0	40.4	84.9

**Figure 2: ROC curves of seminal fluid parameters for predicting abnormal DNA Fragmentation Index (>25%).**

None of the seminal fluid parameters reached the AUC ≥ 0.70 threshold conventionally regarded as acceptable for a screening test. Sperm vitality showed the best, though still only fair, discriminatory performance for abnormal DFI (AUC=0.64, 95% CI 0.54-0.74, $p=0.009$): a cut-off of <15% identified abnormal DFI with 40.4% sensitivity and 84.9% specificity. Progressive motility showed similarly fair discrimination (AUC=0.63, 95% CI 0.52-0.73, $p=0.018$; optimal cut-off <15%, 63.8% sensitivity, 56.2% specificity). Semen volume, sperm count, total motility, and morphology did not discriminate abnormal DFI status better than chance in this cohort (all $p>0.10$). Taken together with Tables 4, 5, and 7, sperm vitality and progressive motility show the most consistent, though still modest, signal that could help flag men for confirmatory SCSA testing; even so, an AUC below 0.70 for every parameter tested falls short of the conventional acceptability threshold, and none should be interpreted as a replacement for direct DFI testing.

DISCUSSION

This study evaluated the diagnostic utility of the Sperm Chromatin Structure Assay (SCSA), using the DNA Fragmentation Index (DFI), among 120 male partners of infertile couples attending a tertiary fertility unit in Benin City. Three principal findings emerged. First, abnormal sperm chromatin structure (DFI >25%) was present in a

substantial proportion (39.2%) of the study population. Second, DFI showed only modest correlation with conventional semen parameters, and a clinically important minority of men with an entirely normal semen analysis nevertheless had abnormal chromatin structure. Third, cigarette smoking was the only independent, modifiable predictor of abnormal DFI, while no single seminal fluid parameter, including the best performing marker (vitality), could reliably substitute for direct SCSA testing. These findings are further discussed below in relation to existing literature, as well as their clinical implications.

Prevalence of abnormal sperm chromatin structure

The observed DFI prevalence of 39.2% is considerably higher than several community-based estimates.^{9,29} It is, however, broadly consistent with reports from Nigerian tertiary fertility settings. Faduola and Kolade found that 63% of Nigerian men with unexplained infertility had a DFI above 20%, with a mean DFI of approximately 27.5% compared with about 14% in fertile donors, while Ibitoye et al reported a mean DFI of $15.78 \pm 8.52\%$ among infertile South-West Nigerian men and demonstrated a significant inverse relationship between oxidative sperm DNA damage and conventional semen quality.^{11,12} Our cohort's mean DFI of $22.4 \pm 7.1\%$ lies between these fertile and clearly sub-fertile benchmarks, which is plausible given that our sample was drawn from a mixed clinic population comprising both normal and abnormal semen analyses rather than a purely 'unexplained infertility' group. The relatively high prevalence found here most likely reflects a referral-population effect: this cohort was recruited from a dedicated tertiary IVF/infertility unit in which 60.8% already had an abnormal semen diagnosis and a majority were being prepared for IVF treatment, implying pre-selection toward men with more severe or longstanding fertility problems than would be seen in a first presentation or community sample. The relatively older mean age of participants (45.3 ± 8.0 years, with two-thirds aged 45 years or above) may also have contributed, since sperm DNA fragmentation has been shown to rise with advancing paternal age even when conventional semen parameters remain normal.¹⁰ Taken together, these findings confirm that abnormal sperm chromatin structure is not a rare phenomenon among Nigerian men presenting with infertility, and that the true burden in this population is

likely to be substantially higher than the figures historically used to justify screening thresholds.

Relationship between DFI and conventional semen parameters: confirming the diagnostic gap

DFI correlated negatively and significantly with total motility, progressive motility, normal morphology, and vitality, but not with semen volume or sperm count. These associations were directionally consistent with, though generally weaker in magnitude than, those reported by Sills et al ($r=-0.45$ for concentration and -0.40 for high DNA stainability) and by Shuai et al in a much larger Chinese cohort ($r\sim-0.53$ for motility, -0.52 for progressive motility).^{13,14} The weaker correlations observed here may partly reflect the composition of our cohort, in which a large proportion of men had oligoasthenoteratozoospermia (32.5%) or asthenozoospermia (21.7%); Sills et al similarly noted that correlations between DFI and conventional parameters can weaken or disappear once sperm concentration falls into the oligozoospermic range, suggesting that chromatin damage and conventional sperm-production defects, while related, are not simply interchangeable measures of the same underlying process.¹³ A noteworthy clinically important observation in this analysis is that 21.3% of men with a completely normal semen analysis nonetheless had an abnormal DFI and buttresses a justification for this study, that men with apparently normal sperm analysis may have infertility owing to occult DNA fragmentation. This finding closely reproduces, in a Nigerian tertiary-hospital population, the approximately 15% figure reported by Pourmasumi et al and provides direct local evidence that seminal fluid analysis alone will misclassify a clinically meaningful subset of sub-fertile men as normal.¹⁴ Conversely, most men with abnormal DFI (78.7%) also had an abnormal semen diagnosis, with asthenozoospermia disproportionately represented in the abnormal-DFI group (34.0% versus 13.7%, $p=0.016$). This pattern of substantial, but incomplete, overlap between chromatin status and motility defects is consistent with a shared underlying mechanism, whereby oxidative damage and defective protamination compromise both nuclear chromatin packaging and the mitochondrial/flagellar apparatus required for progressive motility.^{15,16} The practical implication is that while an abnormal semen analysis, especially progressive motility, should raise suspicion of chromatin damage, a normal one cannot be relied upon to exclude it.

Cigarette smoking as an independent, modifiable predictor of abnormal DFI

On multivariable logistic regression, cigarette smoking was the only independent predictor of abnormal DFI, with smokers having more than three-and-a-half times the odds of abnormal chromatin structure compared with non-smokers (adjusted OR 3.57, 95% CI 1.60-7.96, $p=0.002$), after adjustment for hypertension, herbal tea use, and caffeine consumption. This finding is consistent with other

research, Sepaniak et al first demonstrated significantly higher sperm DNA fragmentation among smokers than non-smokers (32% versus 25.9%) despite no difference in conventional semen parameters, concluding that DNA fragmentation should be regarded as an independent diagnostic parameter in its own right.³⁰ More recently, a large population-based study by Osadchuk et al reported that DFI increased by approximately 75% in heavy smokers relative to non-smokers, alongside reductions in semen volume, sperm count, and motility, while a systematic review and meta-analysis of 190 studies by Szabó et al identified smoking as the lifestyle factor most strongly associated with elevated sperm DNA fragmentation.^{32,31} Mechanistically, cigarette smoke is a potent source of reactive oxygen species that can overwhelm the limited antioxidant capacity of seminal plasma, producing oxidative base damage and strand breaks in sperm chromatin; this is consistent with the elevated levels of the oxidative-damage marker 8-hydroxy-2'-deoxyguanosine previously reported among infertile Nigerian men.¹² Given that smoking is the single modifiable exposure identified in this analysis, it represents an actionable target for pre-treatment counselling. By contrast, hypertension showed a non-significant trend toward a protective association with abnormal DFI (adjusted OR 0.37, 95% CI 0.13-1.10, $p=0.074$). This finding runs counter to the biologically plausible expectation that vascular and metabolic disease would increase oxidative stress and, therefore, DNA damage, and most likely reflects the small number of hypertensive men in this cohort ($n=26$) and the resulting imprecision of the estimate rather than a genuine protective effect; thus, it should not be over-interpreted and warrants confirmation in larger, adequately powered studies. No other demographic or lifestyle exposure examined, including alcohol, caffeine, cannabis, or tight underwear use, was independently associated with abnormal DFI, a pattern broadly in keeping with reports that, among common lifestyle factors, smoking has a more consistent and pronounced effect on sperm DNA integrity than other exposures.

Can conventional semen parameters substitute for direct SCSA testing?

Because SCSA is costly and not widely available in low- and middle-income settings, including Nigeria, a central practical question for this study was whether any routinely available seminal fluid parameter could serve as an adequate proxy for abnormal DFI.^{7,8} Sperm vitality was the only seminal parameter that remained independently associated with abnormal DFI after adjustment for hypertension, smoking, and caffeine use (adjusted OR 0.98 per 1% increase, $p=0.035$), and, together with progressive motility, showed the best discriminatory performance on ROC analysis (AUC 0.64 and 0.63, respectively). However, neither of these, nor any other seminal parameter tested, reached the AUC ≥ 0.70 threshold conventionally regarded as the minimum for an acceptable screening test. Using the optimal vitality cut-off of $<15\%$

would identify abnormal DFI with only 40.4% sensitivity, meaning that the majority of men with abnormal chromatin structure would be missed if vitality alone were used to decide who required confirmatory SCSA testing; the progressive motility cut-off of <15% performed only slightly better on sensitivity (63.8%) at the cost of considerably poorer specificity (56.2%). These findings reinforce, with local quantitative evidence, the conclusion drawn from earlier correlation-based studies that DFI provides diagnostic information that is only partially, and unreliably, captured by conventional semen parameters, in other words SCSA therefore cannot be replaced by inference from SFA results, even when the most strongly correlated parameters are used.^{13,14} In practical terms, although no combination of routine semen findings in this cohort could be used with confidence as substitute for DFI, however a combination of low/abnormal sperm vitality and progressive motility could be used to select/triage which men would require direct chromatin testing especially in resource limited setting like ours.

Clinical and public health implications

These findings have several implications for infertility practice in Nigeria. First, the demonstration that a fifth of men with a normal semen analysis nonetheless had abnormal chromatin structure lends direct local support our study objective, for supplementing SFA with SCSA, particularly in couples with unexplained infertility, recurrent implantation failure, or recurrent pregnancy loss, in whom sperm DNA damage has previously been linked to poorer fertilization rates, embryo quality, and live birth outcomes.¹⁶⁻²¹ Second, because cigarette smoking was the only independently modifiable risk factor identified, smoking cessation counselling should be considered an evidence-based component of pre-treatment fertility care, alongside consideration of confirmatory SCSA testing in men who smoke.

Furthermore, the finding that no seminal parameter can adequately proxy for DFI argues against ad hoc, resource driven strategies that rely on semen quality alone to decide who should undergo chromatin testing. Where SCSA cannot be offered to every patient, our data would support prioritizing testing in men with normal semen analysis but unexplained infertility, with low vitality or progressive motility, or a smoking history, while acknowledging that this triage approach would still miss some men with abnormal DFI, it may be beneficial in improving infertility management in our setting. These results also lend some support to the position of the European Society of Human Reproduction and Embryology, which recommends selective sperm DNA fragmentation testing in assisted reproduction, over the more conservative stance adopted by the American Society for Reproductive Medicine.^{26,27} It should be noted that this study did not capture fertilization, embryo quality, ongoing pregnancy, or live birth outcomes; the study could therefore address the diagnostic value of SCSA, but not its influence on fertility

treatment outcomes, which remains an important direction for future local research.

A major strength of this study is the use of a standardised flow-cytometry-based sperm chromatin structure assay (SCSA) alongside conventional semen analysis in all participants. The sample size exceeded the calculated minimum, while the statistical analyses allowed assessment of sperm DNA fragmentation and its relationship with conventional semen parameters.

However, the cross-sectional, single-centre design limits causal inference and generalisability. Self-reported lifestyle factors were subject to recall and social-desirability bias, while small subgroup sizes limited statistical power. Correlations among semen parameters also restricted their simultaneous inclusion in regression models. Finally, the absence of reproductive outcome data prevented assessment of the prognostic value of SCSA.

CONCLUSION

Abnormal sperm chromatin structure, defined by a DNA Fragmentation Index above 25%, was found in approximately two out of every five male partners of infertile couples attending a tertiary fertility clinic in Benin City, and was only partly explained by conventional seminal fluid parameters. A clinically important minority of men with an entirely normal semen analysis had abnormal chromatin structure, directly confirming the diagnostic gap that motivated this study. Cigarette smoking was the only independent, modifiable predictor of abnormal DFI, while no single seminal fluid parameter, including the best-performing marker (vitality), achieved acceptable diagnostic accuracy for identifying abnormal DFI. Our findings support the incorporation of SCSA into the evaluation of male infertility in Nigeria, particularly for men with normal semen analysis, unexplained infertility, low vitality or motility and/or a history of cigarette smoking. Health education on smoking cessation as an actionable intervention is highlighted. Larger, prospective, multi-centre studies that link DFI to fertilization, embryo, and pregnancy outcomes are recommended in order to establish locally relevant prognostic thresholds and cost-effective screening algorithms for Nigerian fertility practice.

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