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Contribution of Sperm DNA Fragmentation Testing to the Standard Male Infertility Work-Up

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Abstract

Introduction: Conventional sperm parameters do not systematically detect sperm DNA damage. To improve the management of infertility, integrating the sperm DNA fragmentation test into the standard work-up appears relevant and justified. **Primary objective:** To evaluate the contribution of sperm DNA fragmentation testing to the standard male infertility work-up. **Materials and methods:** This was a prospective cross-sectional analytical study conducted from September 2025 to February 2026 on a population of 37 infertile men. The analyses performed were semen analysis with sperm morphology assessment according to WHO 2021 and the sperm DNA fragmentation test using the Sperm Chromatin Dispersion (SCD) method. Statistical analyses were performed using R software. The significance threshold was set at $p < 0.05$. **Results:** The mean age was 40.40 ± 9.23 years. The most frequent abnormalities were teratozoospermia (94.59%) and necrozoospermia (37.84%). The prevalence of high DFI (>30%) was 48.65%. The analyses revealed significant correlations between continuous DFI-vitality ($r = -0.446$; $p = 0.006$), and continuous DFI-concentration ($r = -0.514$, $p = 0.001$). No significant correlation was found between continuous DFI and morphology ($r = 0.104$; $p = 0.541$). **Conclusion:** Sperm DNA fragmentation testing provides diagnostic information that complements standard semen analysis, supporting its consideration in the evaluation of male infertility.

Keywords

Sperm DNA Fragmentation, SCD Test, Semen Analysis, Male Infertility

1. Introduction

Infertility is defined as the inability of a couple to achieve a pregnancy after at least 12 months of regular, unprotected sexual intercourse [1]. It is estimated that approximately 80 million couples worldwide are infertile. Most of them live in developing countries [2] [3], including 20 to 35 million in Africa. Its medical, sociocultural, psychological, and financial impact makes it a genuine public health problem [4].

Male infertility is defined as the inability of a man to impregnate a fertile woman [5]. It is termed primary when there is no history of conception and secondary when a history of procreation is found [6]. Male infertility has multiple causes, and sperm DNA fragmentation is considered one of the major functional sperm abnormalities, particularly in cases of unexplained infertility and failures of assisted reproduction [7].

First-line male infertility assessment consists of analyzing the standard sperm parameters evaluated by semen analysis and sperm morphology assessment according to the 2021 criteria of the World Health Organization (WHO). However, this assessment remains insufficient to determine a man's fertility and does not allow analysis of the ability of spermatozoa to fertilize an oocyte, generate a good-quality embryo, and lead to a live birth [8] [9].

To improve the management of infertility, integrating the sperm DNA fragmentation test into the standard work-up appears relevant and justified. In recent years, the sperm DNA fragmentation test has emerged as a promising biomarker [8]. However, to date, few studies have assessed the prevalence of a high DNA fragmentation index (DFI) and its correlations with conventional sperm parameters in African settings in general, and in the Congolese setting in particular, which are characterized by limited resources. In light of this gap, a study in Brazzaville (Congo) was warranted. The present work was undertaken in this context.

The general objective was to evaluate the contribution of sperm DNA fragmentation testing to the standard male infertility work-up, by determining whether the DFI provides information that is complementary to conventional sperm parameters.

The specific objectives were to:

- determine the frequency of abnormalities in the different sperm parameters;
- determine the prevalence of a high sperm DNA fragmentation index;
- correlate conventional sperm parameters with the sperm DNA fragmentation index.

2. Materials and Methods

This was a cross-sectional analytical study with prospective data collection, conducted from September 2025 to February 2026 on a population of 37 patients followed for male infertility. It took place at the Fertility Laboratory of the MATETE Clinic Foundation in Brazzaville (Congo), which served as the site for sample collection, reading of the semen analysis and sperm morphology assessment, as well as staining and fixation of the slides for the sperm DNA fragmentation test.

This work was approved by the Health Sciences Research Ethics Committee (CERSSA) under number 048 CERSSA. After obtaining institutional approval from the Bio 24 laboratory in Dakar (Senegal), the fixed semen slides were forwarded to the site for their reading and standardized interpretation in the sperm DNA fragmentation test.

All married patients consulting at the clinic, eligible for the DNA fragmentation test for male infertility, and who had given written informed consent were included in our study. All patients whose semen analysis revealed azoospermia or a sperm concentration < 5 million spermatozoa/milliliter were excluded from the study, in accordance with the technical constraints of the method used.

Female infertility was not systematically explored or documented as part of this study.

The fragmentation test was provided at the laboratory's expense, and the result was transmitted directly to the prescribers and to the patients.

Data were collected using a questionnaire containing all the parameters to be studied, in order to meet the objectives of our study. The biological analyses performed were semen analysis with sperm morphology assessment and the sperm DNA fragmentation test. Semen was obtained by masturbation. The duration of sexual abstinence ranged from 2 to 5 days. Semen analysis and sperm morphology assessment were performed and interpreted according to the World Health Organization (WHO) 2021 recommendations [9]. Ejaculate volume was measured using a graduated wide-mouthed container. The reference volume ranges from 1.4 to 6.0 milliliters. A value below 1.4 milliliters defines hypospermia, whereas a volume above 6.0 milliliters defines hyperspermia. Seminal pH was assessed using a calibrated pH indicator paper (6.0 to 10.0) on which a drop of semen was placed within one hour of collection. The normal physiological value is ≥7.2. Progressive motility was assessed using the automated SCA analysis system (Casa-Sperm Analyser Evolution). Progressive motility is considered normal when it is ≥30%; a rate <30% defines asthenozoospermia. Sperm concentration was determined by counting spermatozoa using a Kova chamber, with a dilution adjusted to the concentration and a minimum threshold of 1/20. According to the laboratory reference standards, which are aligned with the WHO 2021 standards, the concentration is normal when it is ≥16 million/milliliter. A value below 16 million/milliliter defines oligozoospermia. Oligozoospermia is subdivided into moderate oligozoospermia, between 5 and 15 million/milliliter, and severe oligozoospermia, <5 million/milliliter. Vitality was assessed using the William test, with the *RAL Diagnos-*

tics Vita-Eosine kit; live spermatozoa are colorless or light pink, whereas dead spermatozoa are stained dark pink. The normality threshold is set at ≥54% live spermatozoa (if <54%: necrozoospermia). Morphology was assessed using the Sperm Stain staining kit, after reading the stained semen smear. Typical and atypical sperm forms were differentiated under a light microscope according to Kruger's strict criteria. The percentage of typical forms is considered normal when it is ≥4%. A value < 4% defines teratozoospermia.

The sperm DNA fragmentation test was performed on fresh semen using the Sperm Chromatin Dispersion (SCD) test technique. The ready-to-use Gold Cyto Sperm DNA kit was used for the SCD technique. The technique consists of melting the agarose contained in the Eppendorf tube in a water bath (90˚C - 100˚C) for 5 min, adding 30 µl of semen to the microtube containing the agarose and mixing well, then depositing 8 µl of the mixture on a slide and covering it with a glass coverslip, allowing it to gel for 5 min at 4˚C, immediately covering the smear with the denaturing solution for 7 min and then draining it, then covering the smear with the lysis solution for 25 min, successively dehydrating in 70% ethanol (2 min), 90% (2 min), and 100% (2 min), covering the smear with 50 µl of solution TA for 1 min and, on top of solution TA, adding 100 to 150 µl of solution TB (TB = 2 × TA or TB = 3 × TA) for 30 min, rinsing thoroughly with distilled water (gentle wash) and letting the slide air-dry, and then observing it under a bright-field or fluorescence microscope.

Spermatozoa with non-fragmented DNA are those with a large or medium halo; spermatozoa with fragmented DNA are those with a small halo or no halo, and degraded ones. The interpretation consists of calculating the sperm DNA fragmentation index (DFI) = (number of spermatozoa with fragmented DNA/total number of spermatozoa counted) × 100. A total of 300 spermatozoa are counted. A DFI < 15% is low, a DFI between 15 and 30% is moderate, and a DFI > 30% is high.

The categorization of patients according to the DNA fragmentation index (DFI) threshold was established in accordance with the clinical validation studies of the SCD test, which define an index above 30% as a marker of severe impairment of sperm DNA integrity associated with a significant decrease in fertility potential [10] [11].

2.1. Statistical Analyses

Age was expressed as mean ± standard deviation. Since the sperm parameters did not follow a normal distribution, the variables were expressed as median [interquartile range]. Bivariate correlations between the continuous sperm DNA fragmentation index (DFI) and the sperm parameters were calculated using the Spearman rank correlation coefficient (rho).

In a second step, the patients were divided into two groups according to the fragmentation threshold (DFI ≤ 30% = normal/DFI > 30% = high). Comparisons of medians between these two groups were performed using the non-parametric

Mann-Whitney test. The significance threshold was set at $p < 0.05$, with a 95% confidence interval (CI). Statistical analyses were performed using R software (R Core Team, 2026). Tables were prepared using Excel software.

2.2. Study Variables

The variables studied were: age, type of infertility, abnormalities of the sperm parameters, and the sperm DNA fragmentation index, as well as the correlation between the sperm parameters and the sperm DNA fragmentation index.

3. Results

This study included 37 men. The mean age was 40.40 ± 9.23 years; with extremes ranging from 29 to 61 years. The median was 39 [33.00 - 45.00] years. The mean duration of marriage was 9.8 ± 3.8 years.

3.1. Distribution of Patients by Age

Age is presented in Table 1 below. Indeed, the most represented age group was 30 - 39 years, with 17 patients, *i.e.*, a frequency of 45.95%.

Table 1. Distribution of patients by age group.

	Number (n)	Percentage (%)
<30 years	3	8.11
30 to 39 years	17	45.95
40 to 49 years	10	27.03
≥50 years	7	18.91
Total	**37**	**100.00**

3.2. Distribution of Patients by Age Group and Type of Infertility

As shown in Table 2 below, primary infertility was the most represented.

Table 2. Relationship between age group and type of infertility.

Age group	Infertility	
	Primary infertility	Secondary infertility
<30	3 (8.1%)	0 (0%)
30 to 39	17 (45.9%)	0 (0%)
40 to 49	10 (27.0%)	0 (0%)
≥50	2 (5.4%)	5 (13.5%)
Total	**32 (86.5%)**	**of inf3 (13.5%)**

3.3. Frequency of Sperm Abnormalities and High Sperm DNA Fragmentation Index (DFI > 30%)

These frequencies are presented in Table 3 below:

Table 3. Sperm parameter abnormalities and high sperm DNA fragmentation index (DFI > 30%).

Abnormalities	Number (n)	Percentage (%)
Hyperspermia	2/37	5.41
Moderate oligozoospermia	12/37	32.43
Asthenozoospermia	10/37	27.03
Teratozoospermia	35/37	94.59
Necrozoospermia	14/37	37.84
DFI > 30% (High)	18/37	48.65

Note: A patient may present with several abnormalities simultaneously.

3.4. Comparison of the Medians of the Different Sperm Parameters According to the Clinical Threshold of Sperm DNA Fragmentation (DFI ≤ 30%/DFI > 30%)

This comparison is presented in Table 4 below.

Table 4. Comparison of the medians of the different sperm parameters according to the clinical threshold of sperm DNA fragmentation (DFI ≤ 30%/DFI > 30%).

Variables	DFI ≤ 30% (n = 19)	DFI > 30% (n = 18)	*p*
Continuous DFI (%)	**21.00 [15.00 - 23.50]**	**44.00 [35.25 - 54.75]**	**<0.001**
Ejaculate volume (milliliter)	3.50 [2.95 - 4.60]	4.30 [3.42 - 5.00]	0.170
pH	8 [7.80 - 8.00]	8 [7.80 - 8.00]	0.515
Sperm concentration (million/milliliter)	**82.60 [32.95 - 182.70]**	**17.95 [11.40 - 30.60]**	**<0.001**
Progressive motility	49.00 [39.00 - 53.00]	36.50 [24.50 - 49.75]	0.091
Vitality (%)	**70.00 [62.00 - 72.50]**	**48.50 [38.25 - 63.50]**	**0.012**
Typical forms (%)	1.00 [0.00 - 2.00]	2.00 [0.25 - 2.00]	0.682

p = *p*-value ($p < 0.05$: significant).

3.5. Correlation between Sperm Parameters and the Continuous Sperm DNA Fragmentation Index (DFI)

As shown in Table 5 below, the analysis showed significant results between certain sperm parameters and the continuous DFI.

Table 5. Correlation between the sperm parameters and the continuous sperm DNA fragmentation index, n = 37.

Variables	*r* Spearman	IC 95%	*p*
Concentration - Progressive motility	0.22	[−0.110, 0.508]	0.189
Concentration - Vitality	**0.326**	**[0.002, 0.588]**	**0.049**

Continued

Concentration - Continuous DFI	**−0.514**	**[−0.720, −0.230]**	**0.001**
Progressive motility - Vitality	**0.786**	**[0.62, 0.880]**	**<0.001**
Progressive motility - Continuous DFI	−0.320	[−0.580, 0.00]	0.054
Vitality - Continuous DFI	**−0.446**	**[−0.670, −0.140]**	**0.006**
Typical forms - Continuous DFI	0.104	[−0.230, 0.410]	0.541
pH - Continuous DFI	−0.081	[−0.390, 0.250]	0.636
Volume - Continuous DFI	0.216	[−0.120, 0.500]	0.199

CI = Confidence Interval, *r* = *rho* Spearman correlation coefficient.

4. Discussion

This study was conducted on 37 married male subjects with a relatively stable occupational situation. This small sample size may be explained, on the one hand, by the absence of a public fertility center and by the still taboo nature of male infertility in our society, which made obtaining consent difficult for some patients. On the other hand, the Sperm Chromatin Dispersion (SCD) method de facto excludes azoospermic samples and those with a sperm concentration < 5 million spermatozoa/milliliter.

Regarding the sociodemographic parameters, our study revealed that 32 patients (86.49%) had primary infertility and 5 patients (13.51%) had secondary infertility. These results are markedly higher than those found by Odzébé *et al.* and Bolanga *et al.*, who reported 65.3% and 52% of cases of primary infertility, respectively [12] [13]. This difference could be explained by the larger populations in their studies compared with ours. The 5 cases of secondary infertility in our study population involved men aged 50 years and older, all of whom were polygamous. The presence of men aged 50 years and older could be explained by late information about advances in medicine in the field of reproductive biology and/or by the recent introduction of assisted reproductive technology (ART) in Brazzaville, but above all by the sociocultural context in which infertility is still frequently attributed to the woman, which may lead the man to seek a new union in order to have offspring, regardless of his age and at any cost.

The analysis of the age distribution showed that the age of our patients ranged between 29 and 61 years, with a mean of 40.40 ± 9.23 years. The most represented age group was 30 - 39 years, with 17 patients (45.95%), followed by the 40 - 49 age group, with 10 patients (27.03%). The predominance of relatively young individuals in the study population is explained by the fact that this age group corresponds, in men, to a period when the desire for children is heightened owing to a relatively stable social and professional situation. This result is consistent with those of Brzakowski in 2009 [14], Moungala *et al.* in 2021 [15], and Odzébé *et al.* in 2022 [12].

Regarding the biological analysis of semen, our results showed that 36 of the 37 patients had at least one major semen analysis abnormality. Teratozoospermia

was the sperm abnormality found in almost the entire study population, with a frequency of 94.59% (35/37 patients). It was followed by a high sperm DNA fragmentation index (DFI > 30%), which was found in nearly half of the study population, at a rate of 48.65% (18/37 patients). The high frequency of sperm abnormalities and of the continuous DFI led us to analyze the comparison of medians between the different sperm parameters and the DFI categories (≤30% and >30%) as well as the existence of a correlation between the continuous sperm DNA fragmentation index (DFI) and the sperm parameters. The prevalence of high DFI > 30% in our study population is comparable to that reported by Smith *et al.*, who found a prevalence of 45% - 50% in infertile populations [16]. The statistical significance threshold was set at $p < 0.05$. The correlation analysis showed that the continuous DFI was statistically significantly associated with sperm vitality and concentration. Vitality was significantly lower in the DFI > 30% group (48.50 [38.25 - 63.50] %; $p = 0.012$) with a negative correlation ($r = -0.446$; $p = 0.006$). These results suggest that samples with higher sperm vitality tend to have a lower DFI. Regarding concentration, the comparison of the two groups revealed that higher sperm concentrations were associated with the DFI ≤ 30% group and showed a correlation of $r = -0.514$ with $p = 0.001$ with continuous DFI. These results could indicate that sperm DNA fragmentation is higher in patients with moderate oligozoospermia and/or necrozoospermia. This shows that combined abnormalities of sperm concentration and vitality are strongly associated with a high sperm DNA fragmentation index, suggesting major impairment of sperm DNA integrity [17].

Regarding progressive motility, the statistical analyses showed a trend from 49.00 [39.00 - 53.00] % to 36.50 [24.50 - 49.75] %, $p = 0.091$ with a correlation of $r = -0.320$ and $p = 0.054$. There is a trend toward a negative association: when the DFI increases (DFI > 30%), progressive motility tends to decrease without reaching the threshold of significance. This could be explained by a lack of power (n = 37). In our cohort, it is not concentration that predicts motility, but rather vitality.

Regarding the continuous DFI and sperm concentration, the correlation we found is stronger than that reported in most studies, where it remains weak or sometimes non-significant. This divergence could be explained by the different study populations or by different DNA fragmentation techniques. These results corroborate those of Smith R *et al.* in 2007 [16] and Peng LJ *et al.* in 2026 [18]. Oligozoospermia associated with a high DFI suggests the existence of multiple mechanisms of sperm dysfunction (simultaneous reduction in number and genomic damage), worsening the reproductive prognosis compared with isolated oligozoospermia [19].

Conversely, no significant correlation was observed between the continuous DFI and the percentage of typical forms ($r = 0.104$; $p = 0.541$) nor with the different DFI categories (DFI ≤ 30%, DFI > 30%). Although the statistical analysis revealed a predominance of teratozoospermia of 94.59% in the study population, *i.e.*, in 35 patients, slightly less than half of the study population had excessive

sperm DNA fragmentation (DFI > 30%), *i.e.*, 48.65%. This result could suggest, on the one hand, that morphological abnormality is not the best reflection of sperm DNA integrity. In other words, teratozoospermia is not systematically associated with high sperm DNA fragmentation in our study population. And, on the other hand, that the morphological study of spermatozoa does not rely solely on conventional semen analysis with sperm morphology assessment. A morphologically normal spermatozoon can undergo severe DNA fragmentation, and vice versa. This result corroborates that of Bungum *et al.* in 2007, who likewise found no significant association between the continuous DFI and morphology [20].

Regarding the continuous DFI and its different categories, no significant correlation was found with pH and volume. In our study, ejaculate volume was not related to sperm DNA integrity [9].

Regarding the conventional sperm parameters, a strong positive correlation was observed between motility and vitality ($r = 0.786$, $p < 0.001$), indicating that the most motile spermatozoa were also the most viable. This confirms the internal consistency of our semen data. Motility and vitality are closely linked for the simple reason that a dead spermatozoon does not move. This is consistent with the WHO 2021 data, which state that only live spermatozoa are motile [9].

A weak-to-moderate positive association at the borderline of significance was observed between concentration and vitality (r = 0.326 and $p = 0.049$), suggesting that samples with a higher sperm concentration also tend to have better vitality.

5. Limitations of the Study and Methodological Considerations

- **Sample size**: our cohort of 37 patients is relatively modest.
- **Selection bias**: the population recruited at the Fertility Laboratory of the MATETE Clinic Foundation (a private fertility center in Brazzaville) may not be representative of the general population of infertile men in Brazzaville. Patients attending this laboratory generally have greater financial resources, potentially creating a bias toward "structured" forms of infertility.
- **Absence of a control group**: the study lacks a group of fertile men (control subjects). Without this group, the prevalence of high DFI cannot be compared between infertile and fertile men, which limits the assessment of the diagnostic specificity of the >30% threshold.
- **Cross-sectional design, causality:** the study is cross-sectional; causal relationships therefore cannot be established. The observed correlations reflect statistical associations, not cause-and-effect relationships.
- **Absence of a female infertility work-up:** not having explored the female side does not allow us to completely rule out the role of a mixed infertility factor or a bias related to maternal age in the overall interpretation of couples' fertility.

6. Conclusion

In light of the foregoing, this study made it possible to evaluate the sperm DNA

fragmentation index using the SCD method and its correlations with the standard semen analysis parameters. Thus, the population consulting for male infertility showed a high and diverse rate of sperm abnormalities, justifying the development of diagnostic markers that complement conventional parameters in order to identify the functional defects masked by standard morphological analysis.

The analysis of the correlations between the continuous DFI and the sperm parameters suggests that the fragmentation test provides information that complements standard semen analysis. Our data suggest that DFI assessment therefore appears to be a relevant complementary test for better stratifying the risk of infertility and adapting therapeutic management, particularly before assisted reproductive technology (ART).

These preliminary results justify larger prospective studies, with a control group and follow-up of reproductive outcomes (pregnancy, ART), before recommending systematic integration into routine practice.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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