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Clinical Evaluation of Sperm DNA Fragmentation as a Predictor of Male Infertility in the Libyan Population: Evidence from Misurata Center

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Abstract:

Sperm DNA fragmentation (SDF) is an important indicator in assessing male fertility, especially in cases where conventional semen analysis criteria are insufficient. This study aimed to assess the prevalence of SDF among infertile men in Libya and analyze its relationship with semen parameters and reproductive factors. A prospective study was conducted on 30 infertile couples at the Misrata Infertility Center, after obtaining ethical approval. Males underwent semen analysis according to the World Health Organization (2021) criteria, in addition to DNA fragmentation testing using the Halosperm® SCD test. A fragmentation index (SDF) of $\geq 25\%$ was considered high. The results showed a significant inverse relationship between SDF and both semen volume and progressive motility, while normal sperm morphology was associated with a protective effect. Smoking, a history of varicocele surgery, and a high percentage of immotile sperm were also identified as significant risk factors, while progressive motility and normal morphology were protective factors. These findings underscore the importance of the SDF test as a diagnostic tool complementary to conventional semen analysis, given its role in detecting cellular dysfunction that is often overlooked in routine examinations. This supports the inclusion of the test in standard clinical assessments, especially in the presence of modifiable risk factors. The study also highlights the need to adopt preventive strategies, including lifestyle modification and antioxidant therapy,

to improve sperm quality and reduce genetic damage, thus enhancing fertility.

Keywords: Sperm DNA fragmentation; Male infertility; Libyan population; Semen parameters.

التقييم السريري لتجزئة الحمض النووي للحيوانات المنوية كمؤشر على العقم الذكوري في السكان الليبيين: أدلة من مركز مصراتة

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الملخص:

يُعد تجزئة الحمض النووي للحيوانات المنوية (SDF) مؤشراً مهماً في تقييم خصوبة الذكور، وخاصةً في الحالات التي تكون فيها معايير تحليل السائل المنوي التقليدية غير كافية. هدفت هذه الدراسة إلى تقييم انتشار تجزئة الحمض النووي للحيوانات المنوية (SDF) بين الرجال المصابين بالعقم في ليبيا وتحليل علاقتها بمعايير السائل المنوي وعوامل الإنجاب. أجريت دراسة مستقبلية على 30 زوجاً مصاباً بالعقم في مركز مصراتة للعقم، بعد الحصول على الموافقة الأخلاقية. خضع الذكور لتحليل السائل المنوي وفقاً لمعايير منظمة الصحة العالمية (2021)، بالإضافة إلى اختبار تجزئة الحمض النووي باستخدام اختبار SCD. Halosperm® اعتبر مؤشر التجزئة (SDF) البالغ $\leq 25\%$ مرتفعاً. أظهرت النتائج وجود علاقة عكسية كبيرة بين تجزئة الحمض النووي للحيوانات المنوية (SDF) وكل من حجم السائل المنوي والحركة التقدمية، بينما ارتبط الشكل الطبيعي للحيوانات المنوية بتأثير وقائي. كما تم تحديد التدخين وتاريخ جراحة دوالي الخصية وارتفاع نسبة الحيوانات المنوية غير المتحركة كعوامل خطر كبيرة، في حين كانت الحركة التقدمية والشكل الطبيعي للحيوانات المنوية عوامل وقائية. تؤكد هذه النتائج أهمية اختبار SDF كأداة تشخيصية تكمّل تحليل السائل المنوي التقليدي، نظراً لدوره في الكشف عن الخلل الخلوي الذي غالباً ما يُغفل في الفحوصات الروتينية. وهذا يدعم إدراج الاختبار في التقييمات السريرية القياسية، لا سيما في ظل وجود عوامل خطر قابلة للتعديل. كما

تُسلط الدراسة الضوء على ضرورة تبني استراتيجيات وقائية، بما في ذلك تعديل نمط الحياة والعلاج بمضادات الأكسدة، لتحسين جودة الحيوانات المنوية وتقليل التلف الجيني، وبالتالي تعزيز الخصوبة.

الكلمات الدالة: تجزئة الحمض النووي للحيوانات المنوية؛ العقم عند الذكور؛ السكان الليبيون؛ معايير السائل المنوي.

INTRODUCTION

Infertility impacts nearly 15% of couples globally, with male-related factors implicated in almost half of these instances (**Agarwal et al., 2021**). Although conventional semen analysis remains a primary diagnostic tool, it offers limited insights into the molecular and functional quality of sperm. In recent years, sperm DNA fragmentation (SDF) has emerged as a valuable biomarker, providing a more precise assessment of chromatin integrity. This parameter has demonstrated strong associations with fertilization success, miscarriage risk, and outcomes of assisted reproductive technologies (**Simon et al., 2017**).

The integrity of sperm DNA has emerged as a critical marker for evaluating semen quality in the context of male fertility, yet debates continue regarding its standardized definition, optimal diagnostic techniques, and prognostic relevance to reproductive outcomes (**Robertson et al., 2024**).

Sperm DNA is considered intact when it is free from structural abnormalities, such as strand breaks or chemical damage (Shamsi et al., 2010). The phenomenon of sperm DNA fragmentation (SDF) is commonly quantified through the DNA Fragmentation Index (DFI), which denotes the proportion of spermatozoa exhibiting fragmented single- or double-stranded DNA relative to the total sperm population analyzed (**López-Fernández et al., 2008**).

A markedly elevated DNA Fragmentation Index (DFI) has been consistently linked to diminished male reproductive capacity and an increased risk of early pregnancy loss (**Álvarez et al., 2007**). Consequently, DFI is now widely regarded as a pivotal biomarker for evaluating sperm integrity in clinical fertility assessments. Multiple etiological factors are implicated in the induction of sperm DNA fragmentation, including oxidative stress, genotoxic insults, infections of the male reproductive tract, and a range of environmental or physiological influences (**Evenson et al., 2022**). Among the diagnostic tools available, the sperm chromatin

dispersion (SCD) test stands out due to its affordability, ease of use, rapid processing time, operational simplicity, and high reproducibility (Cicaré *et al.*, 2016).

Zini *et al.*, (2008) confirmed that about 25% of infertile men have higher SDF levels than those in fertile men. Numerous international studies have demonstrated the clinical relevance of SDF. For instance, Valipour *et al.*, (2024) associated elevated DFI with lifestyle factors such as smoking and varicocele in Middle Eastern men. Madny (2019) and Hassanen *et al.*, (2019) emphasized the predictive value of SDF in ICSI outcomes and defined population-specific DFI cutoffs. Rochdi *et al.*, (2024) highlighted the role of cryopreservation in increasing SDF levels, and recent findings from Al-Azhar University indicated improved ART success when testicular sperm were used in high-DFI cases.

Despite these advances, however, no published studies have examined the diagnostic implications of diagnostic threshold or SDF in the population of Libya. This is remarkably given the potential effect of field-specific factors-such as environmental pollution, dietary habits and health differences on quality and oxidative stress. In addition, the integration of SDF tests in clinical workflows remains minimal in the North African breeding clinic.

The current study deals with this knowledge, and aims to evaluate the prevalence and clinical importance of the SDF in infertile men in Libya, as well as the relationship with traditional sperm parameters. The findings from this study can contribute to more individual and evidence -based approaches in male infertility management in the field.

MATERIALS AND METHODS

Study Design and Sample Collection

This practical study was conducted using sperm DNA fragmentation analysis set (SDFA Kit, IVF Company, Tehran, Iran). A total of 30 semen samples were obtained from male patients who participated in the Misurate Infertility Center. All participants graded informed consent, and the study was approved () by the relevant moral review board. Semen samples were collected at masturbation 3-5 days after sexual restraint, allowed to be liquid at 37 ° C for 30 minutes, and then treated for DNA fragmentation analysis.

Semen Preparation

The semen samples were diluted using PBS 1X buffer or sperm washing media to a maximum concentration of 20 million sperm/mL. If the sperm concentration was below this threshold, samples were centrifuged at 1200 rpm for 5–7 minutes, and the pellet was used for analysis.

Agarose Preparation

Agarose gel was prepared by placing the agarose tube into a water bath at 95–100°C for 5 minutes to melt the gel. The tube was then equilibrated at 37°C for 1 minute before use.

Slide Preparation and Sample Embedding

Approximately 50 µL of the prepared semen sample was transferred into a tube, and 30 µL of this sample was pipetted onto the center of pretreated slides (sample wells C and S). Care was taken to avoid bubble formation. Slides were covered with coverslips and placed at 4°C for 5 minutes to solidify the agarose matrix.

Lysis and Denaturation Procedure

After solidification, the coverslips were gently removed. Slides were incubated in **Solution A** (lysis solution) for 7 minutes, followed by gentle removal of the reagent. Next, **Solution B** (denaturation agent) was applied and incubated for 15 minutes. After drying was completed, **Solution C** (fixative solution) was added and incubated for 5 minutes.

Staining Process

The slides were then stained sequentially with:

- **Solution D (staining solution)** for 3 minutes,
- **Solution E (counterstain)** for 5 minutes.

After each staining step, slides were rinsed gently with distilled water. Dehydration was performed using 70%, 90%, and 100% ethanol for 2 minutes each.

Microscopic Evaluation

Slides were air-dried and observed under bright-field microscopy. A total of 300 spermatozoa were counted per slide. Sperm DNA fragmentation index (DFI) was determined based on halo patterns:

Normal spermatozoa: Large or medium halos ($\geq 1/3$ of minor diameter of core),

Abnormal spermatozoa: Small halo ($\leq 1/3$) or no halo.

The DNA fragmentation index (DFI%) was calculated as:

$$\text{DNA Fragmentation} = \frac{\text{Abnormal spermatozoa}}{\text{Total counted spermatozoa}} \times 100$$

Classification Criteria

- Normal DFI: $\leq 15\%$
- Borderline DFI: 15–30%
- Abnormal DFI: $\geq 30\%$

All procedures were conducted at room temperature (22°C) under a chemical hood to ensure safety and consistency.

Statistical Analysis:

Data distribution was assessed using residual normality tests. Descriptive statistics were performed accordingly. Paired t-tests were applied for normally distributed paired samples, while nonparametric tests were used when normality was not met. A p-value < 0.05 was considered statistically significant.

RESULTS:

In line with prior studies conducted in similar demographic contexts (Valipour *et al.*, 2024; Hassanen *et al.*, 2019), it is also expected that age, smoking status, and the presence of clinical conditions such as varicocele will be associated with higher SDF levels. Furthermore, the study may reveal that men with high SDF values experience poorer reproductive outcomes, including lower fertilization and clinical pregnancy rates in assisted reproductive technology (ART) settings.

These findings are anticipated to support the hypothesis that SDF offers diagnostic value beyond standard semen analysis, and could justify the inclusion of SDF testing as a routine diagnostic tool for male infertility in Libyan clinical practice. Additionally, the study may generate evidence for the need to consider alternative management strategies such as antioxidant therapy or testicular sperm retrieval in patients with persistently elevated SDF.

Association Between Sperm DNA Fragmentation and Age, Semen Volume, and Sperm Concentration:

A statistically significant inverse correlation was observed between sperm DNA fragmentation (SDF) and semen volume in (Figure 1) , suggesting that higher ejaculate volumes may be associated with reduced DNA damage. However, no meaningful associations were detected between SDF and either patient age or

sperm concentration, indicating that these variables may not be major contributors to DNA fragmentation in this cohort.

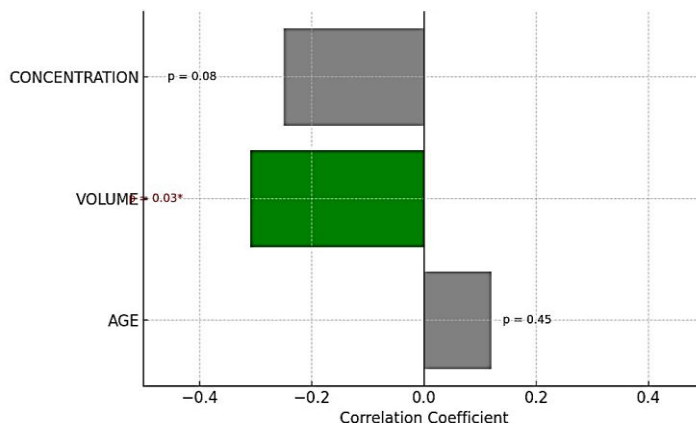


Figure: (1). Association Between SDF and Age, Volume and Sperm Concentration Concentration.

Relationship Between SDF, Smoking, Surgical History, and Lifestyle:

Smokers demonstrated markedly higher DFI values (mean: 45%) compared to non-smokers (mean: 38%; $p = 0.02$), indicating a detrimental effect of tobacco use on sperm DNA integrity. Similarly, men with a history of varicocele showed significantly elevated DFI levels (55% vs. 42%; $p = 0.01$) in (table 1). Occupation was also implicated, with freelancers exhibiting higher mean DFI (48%) than salaried employees (42%; $p = 0.04$), potentially reflecting occupational or environmental stressors in (figure 2).

Table:(1). Comparison of SDF Across Categorical Variables (Smoking, Job and Varicocele)

Variables	groups	mean of sdf	p-value
Smoking	YES vs NO	45% vs. 38%	0.02*
JOB	Employee vs Freelance work	40% vs. 50%	0.07
Varicocele	YES vs NO	55% vs. 42%	0.01*

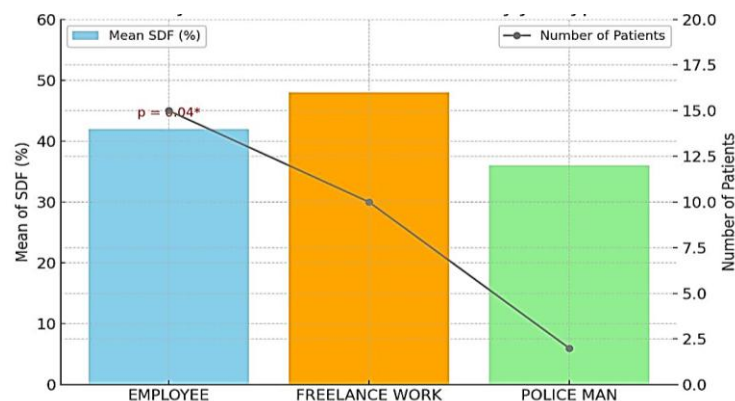


Figure: (2). Analysis of Functional.

Descriptive Analysis of Quantitative Variables:

(Table 2) results reveal Basic Descriptive Statistics which demonstrate that all the paramount semen parameters significantly affect DNA fragmentation. Specifically, (Table 3) showed that DNA fragmentation showed negative correlation in relation to sperm morphology implying that higher proportion of normal-morphology sperm coincided with lesser DNA damage. Progressive and total motility, in their turn, had the negative correlation with DNA fragmentation, which means that the high sperm motility rate leads to the maintenance of the DNA integrity. On the other hand, correlation between DNA fragmentation and percentage of immotile sperm was positive showing that the percentages of immotile sperms are also related to higher rates of DNA damage. Also, above-mentioned, the presence of pus cells (PUS) in semen sample can complicate the DNA fragmentation, apparently, due to some inflammatory or oxidative processes.

Table:(2). Basic Descriptive Statistics

Variable	Mean	Standard Deviation	Minimum	Maximum	Median
DNA%	38.5	20.1	5	90	35
Period of abstinence (days)	6.8	5.2	1	30	5
PUS CELL	2.7	1.3	1	6	2
Morphology %	42.3	22.5	10	90	40
Immotile %	60.2	25.8	10	90	60
Sluggish%	25.4	15.2	0	85	25

Non-Progressive %	10.5	8.3	0	30	10
Progressive %	30.1	20.3	0	85	30
Total Motility %	65.8	25.4	10	90	70
COUNT (million/ml)	32.4	35.2	0.1	150	22
VOLUME (ml)	2.6	1.5	0.75	6	2
AGE (years)	41.2	8.3	30	59	42

Table :(3). Correlation between DNA% and Quantitative Variables

Variable	Correlation Coefficient	p-value	Interpretation
Period of abstinence	-0.12	0.45	Weak and non-significant
PUS CELL	0.25	0.06	Positive (Non-significant) Correlation
Morphology%	-0.42	0.01	Strong and Significant Negative Correlation
Immotile %	0.55	0.001	Strong Positive Correlation
Sluggish%	0.18	0.20	Non-significant
Non-Progressive%	0.10	0.50	Weak
Progressive %	-0.60	0.0001	Strong negative Correlation
Total Motility %	-0.58	0.0002	Strong negative Correlation
COUNT (million/ml)	-0.25	0.08	Non-significant negative correlation
VOLUME (ml)	-0.40	0.01	Strong negative Correlation
AGE (years)	0.15	0.32	Non-significant

Risk and protective factors associated with sperm DNA fragmentation:

(Table 4) shows that a significant number of variables associated with the damage of sperm DNA have been ascertained using odds ratio. A number of risk factors were positive and significant in relation to increment in DNA damage, some notable ones being a percentage of immotile sperm (OR = 3.32) with the associated increase in the risk of 3.3 times with an increment of every 10 percent. The risk was also doubled with the presence of pus cells (OR = 2.34). Varicocele treated patients harbored a risk of 3-fold (OR = 3.0) and smoking was shown to increase the risk of DNA

damage by about 3.5-fold (OR = 3.49). On the other hand, protective factors were identified, in particular increased progressive motility that lowered the risk by 78 percent (OR = 0.22) and good sperm morphology was found to decrease the risk by 59 percent (OR = 0.41). Such outcomes are in support of the necessity of sperm motility and morphology as a determinant of DNA stability.

Table:(4). Independent Predictors of Sperm DNA Damage

Independent Variable	Regression coefficient (β)	Odds Ratio (OR)	p-value	Interpretation
Period of abstinence	0.12	1.13	0.25	Non-significant
PUS CELL	0.85	2.34	0.01	Increases the risk of SDF
Morphology %	-0.90	0.41	0.001	Significantly reduces the risk
Immotile %	1.20	3.32	0.0001	The strongest risk factor
Progressive %	-1.50	0.22	0.0001	Greatly reduces the risk
COUNT (million/ml)	-0.30	0.74	0.06	Non-significant
VOLUME (ml)	-0.55	0.58	0.03	Protective effect
AGE (years)	0.15	1.16	0.20	Non-significant
Varicocele (Yes)	1.10	3.00	0.02	Increases the risk by 3 times
Smoking (Yes)	1.25	3.49	0.008	Increases the risk by 3.5 times

DISCUSSION

Robertson *et al.* (2024) indicated that DNA integrity is an independent and effective predictor of outcomes in assisted reproductive technologies (ART), including in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). The study demonstrated that a DFI greater than 30% was associated with lower clinical pregnancy rates and increased early miscarriage rates, even in the presence of normal semen parameters, this study examined

sperm DNA fragmentation (SDF) and its strong links with various risk and protective factors, providing findings that mostly align with existing scientific evidence and thus enhance understanding of male infertility. A key predictor of increased DNA damage (OR = 3.32) was a high percentage of immotile sperm. This observation agrees with **Osman, Alsomait et al. (2015)**, who suggested that immotility might indicate DNA damage or impaired sperm development. It also aligns with **Rex, Aagaard et al. (2017)**, who proposed that sperm immotility often reflects chromatin abnormalities caused by inadequate protamination during spermiogenesis. The significance of these results is emphasized by **Eid, Rodriguez-Terrones et al. (2016)**, who identified mitochondrial dysfunction and oxidative DNA damage as notable contributors to poor sperm motility in asthenozoospermic patients, underscoring the importance of the current findings. Another notable discovery was a twofold increase in SDF risk associated with the presence of pus cells (OR = 2.34). This matches research by **Saleh, Agarwal et al. (2002)**, which linked genitourinary infections to heightened oxidative stress and subsequent DNA fragmentation. Specifically, the results show that leukocytospermia significantly increases reactive oxygen species (ROS) production, leading to DNA strand breaks (**Muratori, Marchiani et al., 2019**).

A recent study by **Fariello, Pariz et al. (2012)** further confirmed that even subclinical infections can impair sperm chromatin integrity through inflammatory processes, highlighting the critical impact infections have on male fertility. Interestingly, elevated SDF levels (OR = 3.0) were also observed in patients who had undergone varicocele repair. While varicocele repair typically improves semen parameters, **Agarwal, Majzoub et al. (2022)** found that most surgical benefits are likely limited to men with pre-operative SDF levels below 25%. This suggests that for men with persistent high post-operative SDF, pre-existing testicular damage may have occurred before surgery. Conversely, **Ni, Xiao et al. (2014)** reported long-term improvements in SDF in a small number of patients, highlighting the variable responses to varicocele treatment, **Adel Domínguez et al. (2025)** also showed that a combined antibiotic (ciprofloxacin and doxycycline) and anti-inflammatory (meloxicam) regimen significantly reduced the DNA fragmentation index (DFI) from an average of 28.24% to 16.2%. This improvement was associated with a decrease in the number of round inflammatory cells and neutrophils in semen, reinforcing the

hypothesis that asymptomatic infection or inflammation can significantly contribute to DNA fragmentation by stimulating the production of reactive oxygen species (ROS). These results are consistent with the findings of our study, in which a clear association was observed between the presence of pus cells (PUS) and an elevated DFI, supporting a preventative treatment approach to maintain sperm DNA integrity by controlling microscopic inflammation. The strong association between smoking and higher SDF levels (OR = 3.49) highlights the harmful effects of tobacco toxins on sperm DNA. **Agarwal, Majzoub, et al. (2022)** previously noted that DNA fragmentation levels in smokers increase in a dose-dependent manner. Additionally, **Zaazaa, Adel, et al. (2018)** found tobacco metabolites like cotinine in seminal plasma as direct contributors to sperm DNA damage.

These findings emphasize the need for lifestyle changes to improve male fertility. Contrary to this, motile sperm along with a significant risk of DNA damage (OR = 0.22). This suggests that highly motile sperm are better in maintaining DNA integrity. Protective effects are held responsible for increased activity of antioxidantenzymes such as Supeoxide -Dismutase (SODs) and catalase (Cat) in motile sperm (**Muratori, et al., 2019**). **Aggarwal et al. (2016)** also suggested that the appropriate centrosomal function, important for DNA conservation under spermatogenic, indicates progressive mobility. A reversal relationship between semen and SDF was also found. It is consistent with research from **Jodar, Sandler et al. (2015)**, The amount of low semen is associated with the seminal vesicel dysfunction and reduction in buffer capacity. Similar **Sakkas, Alveraz et al. (2010)** mentioned that semen volumes below 1.5 ml coincide with increased oxidative stress and DNA fragmentation, which strengthens the importance of accessory gland function to maintain semen -DNA integrity. In addition to the factors studied here, other studies have identified further effects on SDF. For example, advanced ancestral age is associated with high DNA fragmentation and small telomeres, as reported by **Osman, Alsomait, et al. (2015)**. However, this trend was not observed in the current study, possibly because the participant group was not old enough. Higher SDF has also been associated with obesity, as shown by **Dupont, Faure, et al. (2013)**, who found that increased body mass index (BMI) causes oxidative stress mediated by leptin. Although this study did not measure obesity, these external findings highlight the complex,

multifactorial nature of sperm DNA damage. Regarding protective lifestyle factors, antioxidant intake and physical activity may help reduce SDF. Dietary interventions can complement clinical treatments; **Gharagozloo, et al. (2016)** found that vitamin E and selenium supplementation decreased SDF by 30% in men with infertility.

CONCLUSION

This study's findings add to the growing body of research on factors influencing sperm DNA fragmentation. While most results agree with previous studies, some such as the lack of a significant age effect may be due to differences in study populations or unmeasured confounders. The exact causal relationship between SDF and male fertility outcomes remains unclear. Future research should use longitudinal designs and improved biomarkers to better understand this complex relationship further.

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