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# The role of sperm DNA integrity in seminal plasma of infertile men.

**Hamdya A. Ali[1], Ula Al-Kawaz[1], Wasan A. Abdulhamed[1]**

[1] **High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al Nahrain University, Baghdad, Iraq.**

**hhussny@gmail.com**

ABSTRACT

One-half of the genome is delivered by the sperm, and a healthy sperm is needed for fertilization, fetal development, and child wellbeing. Abnormal sperm DNA can impact any of these processes. The measurement of sperm DNA fragmentation has been added to some medical andrology laboratories as a crucial addition to the standard analysis of the semen. Sperm DNA fragmentation has also been linked to male infertility including idiopathic male infertility. Some degree of controlled DNA nicking is essential for adequate DNA compaction, but excessive sperm DNA fragmentation is usually associated with reduced male fertility potential, reduced fertilization, poor embryo quality, recurrent pregnancy loss and poor assisted reproductive techniques outcomes. Semen samples from 50 infertile males (as 19 asthenozoospermia, 7 oligozoospermia, 13 normospermia and 11 combined defects) were analyzed for sperm DNA fragmentation. This study followed the World Health Organization 2010 guidelines to assess the sperm parameters. Sample were collected by masturbation and put in a 37 °C incubator to be liquefied, then they were placed in a centrifuge (3000 rpm for 10 min) to extract the pellet, which was used for assessment of DFI% by anillin blue staining. Negative significant correlation (p = 0.023) between morphologically normal sperms and DFI % was found in normozoospermia group. The objective of this study is to examine the correlation of DFI among different seminal fluid categories (normozoospermia, oligozoospermia, asthenozoospermia and, combined).

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**KEYWORD**

Infertile men, DNA, Sperm DNA Fragmentation, DFI

## 1. Introduction

Infertility refers to couples unable to conceive after engaging in unprotected sexual activity for a minimum of twelve consecutive months (Magdum et al., 2022 [1]). The global spread of infertility is estimated to affect approximately 80 million individuals, with delayed conception impacting around fifteen percent of marriages trying to have successful pregnancy. Approximately half of these instances are caused by subfertility (Showell et al., 2014 [2]). Spermatozoa, which are cells that undergo a high degree of specialization and differentiation, are produced through the process of spermatogenesis within the seminiferous tubules of the testes. These cells originate from spermatogonial stem cells. Fertility disorders can arise because of the disruption of spermatogenesis or post-testicular events (Bisconti et al., 2021 [3]). One potential explanation for idiopathic male infertility could involve mechanisms such as DNA fragmentation or centriole dysfunction. Hence, the key lies in the development of reliable intracellular sperm analysis techniques (Pandruvada et al., 2021 [4]). DNA fragmentation was linked to difficulties in achieving natural conception, as well as in Artificial Reproductive Techniques such as Intrauterine Insemination and In Vitro Fertilization (Krzastek et al., 2020 [5]) . The DNA fragmentation index (DFI) serves as a measure to assess the extent of DNA damage, specifically in the form of single- or double-strand breaks, which can occur during the process of sperm generation and maturation before or after ejaculation (Deng et al., 2019 [6]).

The factors contributing to an increased DFI in testicular cells include smoking, alcohol consumption, exposure to high temperatures, exposure to chemicals and radiation, the presence of

varicocele, and undergoing chemotherapy treatment (Siddhartha et al., 2019 [7]) .

The presence of abnormal spermatozoa contributes to elevated levels of Reactive Oxygen Species, which negatively impacts the integrity of sperm DNA. Consequently, this condition is associated with infertility, compromised outcomes in Artificial Reproductive Techniques, and an increased risk of birth defects (Selvam et al., 2020 [8]).

Sperm DNA is acknowledged as an objective marker of sperm quality, potentially surpassing conventional sperm parameters in terms of diagnostic and predictive capabilities (Shamsi MB et al. 2011 [9]).

## *2. Patients and Methods*

### 1- The Subjects:

The research includes a total of 50 semen samples, which were obtained from individuals who were seeking treatment at the infertility clinics located at the High Institute for Infertility Diagnosis and Assisted Reproductive Technology affiliated with Al-Nahrain University. Individuals who suffered major systemic illness or had azoospermia or oligoasthenoteratospermia were not included. The research was conducted between 2022 and 2023. The age range of the participants in this study was between 21 and 64 years old, following the necessary approval from the ethical committee prior to the commencement of the research.

### 2- Methods (Semen Collection):

Samples of semen were collected after a period of sexual abstinence between 3 to 7 days, as recommended by (World Health Organization, 2010 [10]); the individual masturbated in a private room adjacent to the laboratory. The act of masturbation was performed directly into a sterilized wide-mouth container, which was provided by the laboratory. Subsequently, the collected samples

were placed in an incubator at a

temperature of 37°C, allowing for complete liquefaction. A skilled technician conducted a macroscopic and microscopic analysis of the semen samples and evaluated the seminal fluid parameters. The participants were categorized - based on criteria specified in (World Health Organization, 2010 [10]) - into four distinct groups: normozoospermic, asthenozoospermic, oligozoospermic, and males with combined defects.

Each sample was centrifuged to separate the supernatant from the pellet; one milliliter of the pellet was smeared on the slide with 4% formaldehyde for 30 minutes, and then a 5% aqueous anillin blue stain was added. Following the removal of any additional staining by tap water, the object was subsequently air-dried after being cleansed with running water. Sperms were examined under light microscopy with oil immersion, employing a high-resolution (100X) objective lens. Sperms displaying dark stain considered to have an abnormal DNA. To calculate the DFI%, a minimum of 200 spermatozoa were enumerated on each slide. An ejaculate containing less than 20% of sperm with stained nuclei was deemed to be within the normal range.

**3- Statistical analysis**

The data were analyzed using Statistical Package for Social Sciences (SPSS) version 23.0 and Microsoft Office 2010. The descriptive statistics, including mean and standard error, were measured to describe the data. The groups were compared by applying analysis of variance (ANOVA comparison between more than two groups).

The degree of association between continuous variables was calculated by Pearson's correlation coefficient (r), and the results were considered statistically significant when the p-value was equal to or less than 0.05.

## *3. Results*

**Classification of patients involved in the studied groups:**

All patients enrolled in the present study were divided into four groups **(Figure 1)**. Group 1 included 13 (26%) males with normozoospermia;

The second group involved 19 (38%) patients with asthenozoospermia; Group 3 included 7 (14.0%) patients with oligozoospermia, and finally, 11 (22.0%) patients with combined defects (Group 4). There were no significant correlations **(Table 2)** between DFI % with seminal fluids parameters of normozoospermia, asthenozoospermia, oligozoospermia, and combined groups, except a negative significant correlation between morphologically normal sperms and DFI% (r= -0.623 & p=0.023) in normozoospermia group.

***Table 1:*** Comparison of seminal fluids and DFI% between the study groups

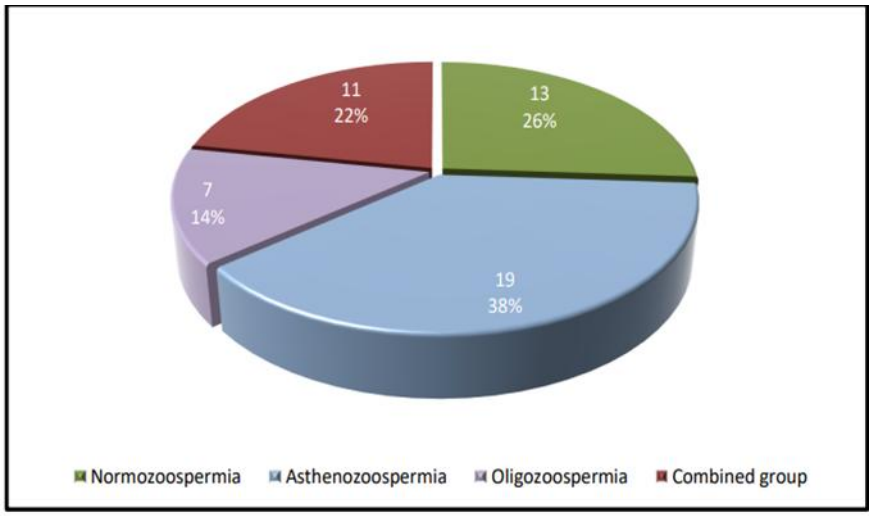
| Groups | DFI %<br>Mean ± SE |
|---|---|
| Normozoospermia group | 15.69 ± 2.74 |
| Asthenozoospermia group | 11.94 ± 1.61 |
| Oligozoospermia group | 15.00 ± 2.99 |
| Combined group | 19.82 ± 2.58 |
| p-value | 0.446 V NS |

SE: Standard error; NS: Not significant (p > 0.05); V: ANOVA test

***Table 2:*** Comparison between DFI% and semen parameters

| Parameters | | DFI % |
|---|---|---|
| Liquefaction time (min) | R | 0.104 |
| | p-value | 0.736 |
| Sperms count ($x10^6$/ml) | R | 0.289 |
| | p-value | 0.338 |
| Sperms concentration | R | -0.089 |
| | p-value | 0.772 |
| Progressively motile sperms % | R | 0.093 |
| | p-value | 0.762 |
| Non-Progressively motile sperms % | R | -0.285 |
| | p-value | 0.345 |
| Immotile sperms % | R | 0.097 |
| | p-value | 0.753 |
| Morphologically normal sperm % | R | **-0.623*** |
| | p-value | **0.023** |
| Round cells | R | -0.325 |
| | p-value | 0.303 |
| DFI% | R | 1 |
| | p-value | --- |

r=Pearson's correlation coefficient; DFI%: DNA fragmentation index




***(Figure 1)***: Patients' categorization into four distinct groups

## *4. Discussion*

No statistically significant differences were observed in seminal fluids and DFI% among the four groups under study (p=0.446), which aligns with Hussein (2023); however, the combined group exhibits a higher percentage of DFI% compared to other groups. Similar results were found by (Fadhil et al., 2020 [11]); their findings indicate that DNA fragmentation was significantly higher in patients with asthenozoospermia and oligo asthenozoospermia compared to those with normozoospermia. This may be explained by (Leach et al., 2015 [12]), in their study, they connected abnormal spermatid maturation, oxidative stress abortive apoptosis, and deficiencies in recombination during spermatogenesis to abnormal sperm chromatin/DNA structure.

In this study, a notable negative correlation between normal morphologically sperms and DFI % (r = -0.623 & p = 0.023) in the normozoospermia group was found, which goes with (Sakhil et al., 2021 [13]) and (Choucair et al., 2016 [14]). This can be explained by (Ferrigno et al., 2021 [15]), which

strongly suggested that spermatozoa exhibiting abnormal morphology are most likely to contain DNA damage. Additionally, the study found that sperm DNA fragmentation is associated with changes in sperm parameters, specifically count, motility, and morphology.

(Hussein E., 2023 [16]) Shows a negative correlation of DNA integrity levels with sperm parameters like concentrations, progressively motile sperm, sperm counts, total progressively motile sperm, and especially morphologically normal sperm; his results align with the current study. The same result demonstrated negative correlations in partners of couples attending IVF clinics with sperm DNA integrity and sperm quality, and he explains that decreased sperm DNA integrity due to sperm motility and morphology defects, which can be considered as premutagenic and can badly affect fetal outcome after IVF and has lifelong complication on the health of the baby (Atig et al., 2017 [17]). Additionally, (Brahem et al., 2011 [18]) found similar results, which indicated that spermatozoa in individuals with teratozoospermia have high DFI%. And also they found a significant correlation between DNA fragmentation and morphology. Also, this study aligns with (Andievia et al., 2022 [19]). Results showed that the correlation is positive on the morphological abnormalities of the sperm themselves, which aligns with this study.

## *6- Conclusion*

Sperm DFI% is a more accurate predictor of sperm quality and abnormalities than other analytic parameters. The standard semen analysis may fail to successfully detect abnormalities in spermatogenesis – high DFI% – in men. In conclusion, Infertile men, even those who have normal semen parameters, should consider the DFI% test as an essential test for their infertility evaluation.

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***Author Contribution***

Hamdya A. Ali performed the study, and Ula Al-Kawaz Wasan A. Abdulhamed supervised the work .

***Conflict of Interest***

The authors declare no conflict of interest .

***Ethical Clearance***

The study was approved by the Ethical Approval Committee.

***Financial Disclosure***

There is no financial disclosure.

***Ethical Clearance***

The study was approved by the Ethical Approval Committee

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