Contents list available at Journal of Science Letters

Journal of Science Letters

journal homepage: http://www.scienceletters.org/

Impact of the DNA Fragmentation Index on some Characteristic Parameters of Sperm Quality

Modou Mamoune Mbaye[1,2,3*], Ben Salah Kamal[1], Achraf Zakaria[2], Mustafa Zakaria[3], Bouchra ElKhalfi[1], Mohammed Zarqoui[4], Abdelaziz Soukri[1] and Noureddine Louanjli[6]

[1]*Laboratory of Physiopathology, Genetics Molecular and Biotechnology (PGMB), Faculty of Sciences Ain Chock, Research Center, Health and Biotechnology, University Hassan II of Casablanca, Morocco*
[2]*Laboratory of Medical Analyses, Reproductive Biology, Labomac, Casablanca, Morocco*
[3]*IRIFIV Fertility Center, IVF Laboratory, Casablanca, Morocco*
[4]*GYN OBT IRIFIV Center and IRIFIV-AISRG Scientific Research Group, Morocco*
[5]*Labomac Clinical Analysis Laboratory, IRIFIV Fertility Center, AFC Fertility Center, Casablanca, Morocco*

ARTICLE INFO

Article History
Received 18 August 2021
Revised 26 September 2021
Accepted 28 October 2021
Available Online 04 November 2021

Keywords:
DNA fragmentation index,
spermatozoon,
correlation,
sperm parameter

ABSTRACT

The DNA fragmentation index (DFI) is an essential factor in assessing the quality of the genetic material of human sperm. Its influence on the characteristic parameters of sperm quality remains a matter of debate. Ours work aim to find a correlation between the DNA fragmentation index and the characteristic parameters of sperm quality, namely: sperm count, motility, vitality, and morphology. The results showed a coefficient of determination between 0.11 and 0.17 suggesting a weakly negative correlation between DFI and motility, vitality, and even count. The correlation found between the sperm DNA fragmentation index and the rate of morphologically abnormal spermatozoa was much weaker, suggesting an absence of correlation.

1. Introduction

The sperm DNA fragmentation index (DFI) is a critical parameter in the integrity of sperm genetic material. It assesses the percentage of sperm with a high DNA fragmentation index to those with intact DNA. Sperm DNA fragmentation results from breaks in sperm DNA that can be caused by several factors, including oxidative stress, chromatin remodelling defects, and obstructive apoptosis.
For many years, the spermogram and spermocytogram have been the first-line tests for diagnosing male infertility. Even though they allow the evaluation of characteristic parameters of sperm quality and physicochemical properties of sperm. They remain ineffective in explaining the causes of male infertility. Indeed, we can find men with normal sperm parameters but with infertility disorders due to poor sperm quality of genetic material, which is often associated with reduced fertilization rates, abortions, and *in vitro fertilization* failures. In this sense, Benchaib, Boughali, *et al*., respectively showed a negative correlation between the DNA fragmentation index and the different parameters of the spermogram except for the morphologically abnormal sperm count (Benchaib *et al*., 2003; Boughali *et al.,* 2006).
Our work aims to find a correlation between the DNA fragmentation index and the characteristic parameters of sperm quality, namely: sperm count, motility, vitality, and morphology.

Patients and methods

Sample collection

This study was carried out at the Laboratoire d'analyses médicales et de biologie de la reproduction, "Labomac," Casablanca, Morocco. We established a study group of 50 standard and pathological samples. Informed consent was obtained from all included patients before using their semen in this study. Then, the samples were collected after 3 to 4 days of abstinence in sterile and labelled containers (S. E. Lewis-2007). For liquefaction, the samples were stored at 35°C until use (Mbaye et al., 2020). We monitored at a time interval of 10 min until liquefaction was complete.

*Corresponding Author: Modou Mamoune Mbaye
E-mail Address: mbayeass87@gmail.com
DOI: 10.10.46890/SL.2021.v02i06002

Microscopic analysis was performed in accordance with World Health Organization (WHO) standards and guidelines (S. E. Lewis-2007).

Motility

Sperm motility was assessed using the CASA system. We deposited a 10 μl aliquot of the mixture (semen/oil, control) on a 20 μm Makler counting chamber. Then we observed with CASA: Computer Assisted Semen Analysis Hamilton-Thorne version 10 HTM IVOS Analyzer (Hamilton-Thorne Biosciences, Beverly, MA, USA), immediately after depositing the mixture (Hirano *et al.,* 2001).

Vitality

Sperm vitality was assessed using a 2% eosin stain (Amelar 1973). We took a smear consisting of one drop of semen and one drop of 1% eosin. Subsequently, counting of live sperm (colorless) and dead sperm (pink head) via an optical microscope (objective × 40) using the same principle of the virtual line that was applied in the evaluation of motility, the percentage of live sperm and dead sperm on different fields was estimated using a counter (Amelar *et al*., 1973).

Counting

For the evaluation of the count, we added 380 µl of 1% formalin and 20 µl of semen (1:20 dilution) to a tube [8]. After carefully homogenizing the mixture with the micropipette, we placed a volume of this mixture inside one of the Kova cups (a single-use counting cell consisting of 10 cups, each cup consisting of 20 rows and 8 columns), using an optical microscope (objective × 40) (Ali *et al*., 2013). We counted the intact spermatozoa. The following relationship estimates the count:

$N = n \times 20 \times D \times 103 \times 10^{-6}$
N: the count in M/ml
n: the number of sperm counted in a column of 8 squares
D : dilution factor

Morphology

In a tube, we mixed 1 ml of Earl with a volume of sperm that is defined according to the sperm count; in the case of normal sperm, we added 0.5 ml of the sperm in the tube. In the case of oligozoospermic samples, 1 ml of semen was added to the tube containing Earl and centrifuged at 1000 rpm for 5 minutes. From the pellet, we made a smear which was left to dry and then fixed with 97% alcohol. The smear was left to dry once more and then stained with haematoxylin for 3 min. The slide was then rinsed and placed in a bottle containing ammonia (3 times). After rinsing, we placed the slide in another vial containing Schoor's dye for 1 min. Finally, the slide was rinsed and then observed under the light microscope (objective × 100) (Lannou *et al*., 2013).

The Sperm DNA Fragmentation Index

Sperm DNA integrity was assessed by the TUNEL test using a commercial kit (Roche Diagnostic, Lewes, UK). The principle of the TUNEL technique is to use an enzyme, terminal deoxynucleotransferase (TdT), capable of adding nucleotides to the 3'-OH ends of free DNA.
The semen sample was washed twice in phosphate-buffered saline (PBS, Sigma-Aldrich, Gillingham, UK) and adjusted to a concentration of 2 x 10^7 cells/ml in PBS. The cell suspension was then fixed in PBS containing 2% formaldehyde (Sigma-Aldrich) for 60 minutes at room temperature. After a double wash with PBS, the sample was centrifuged at 1200 rpm. This step was repeated twice. The prepared slides were immediately analyzed using a fluorescence microscope (Nikon Eclipse 80i) equipped with appropriate filters. Images were captured using a CCD camera and XytoGen software (Excilone, version 3.8.46, France) (figure 1) (Kaarouch *et al*., 2018).

Statistical analysis

The data obtained in our experiment were statistically analyzed. Our results on the correlation of the DNA fragmentation index and some characteristic parameters of sperm quality were performed by Student's t-test (t-test).

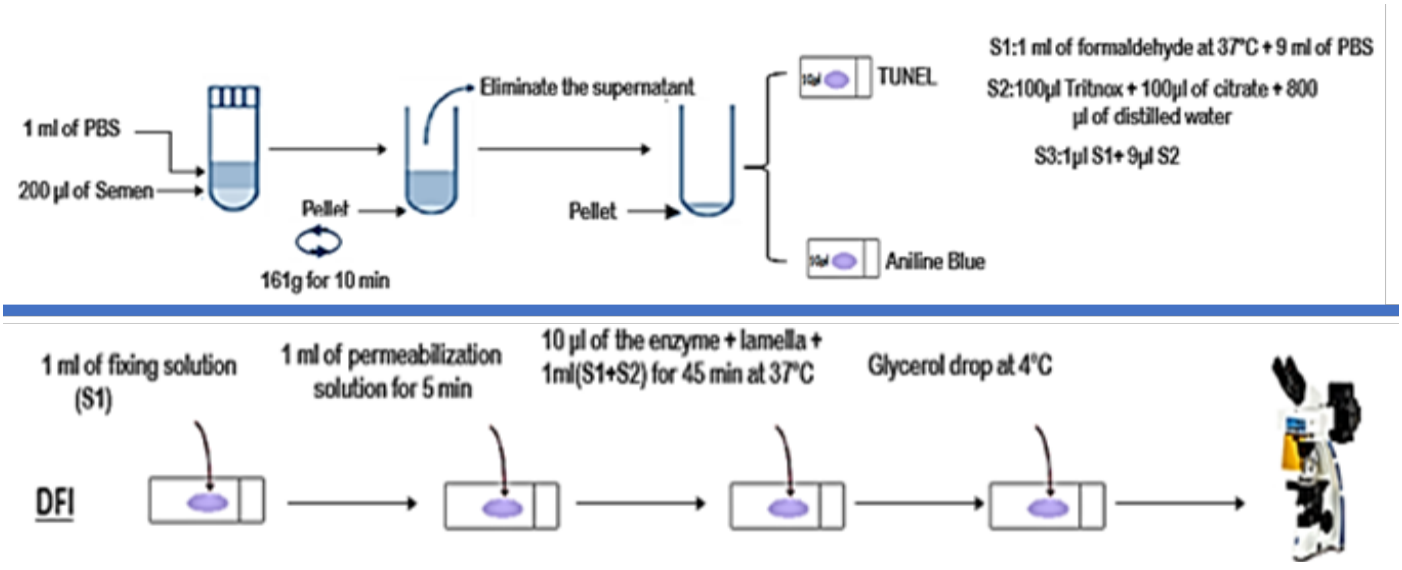

Figure 1: Processing method used for evaluating the DNA fragmentation index (DFI) [10].

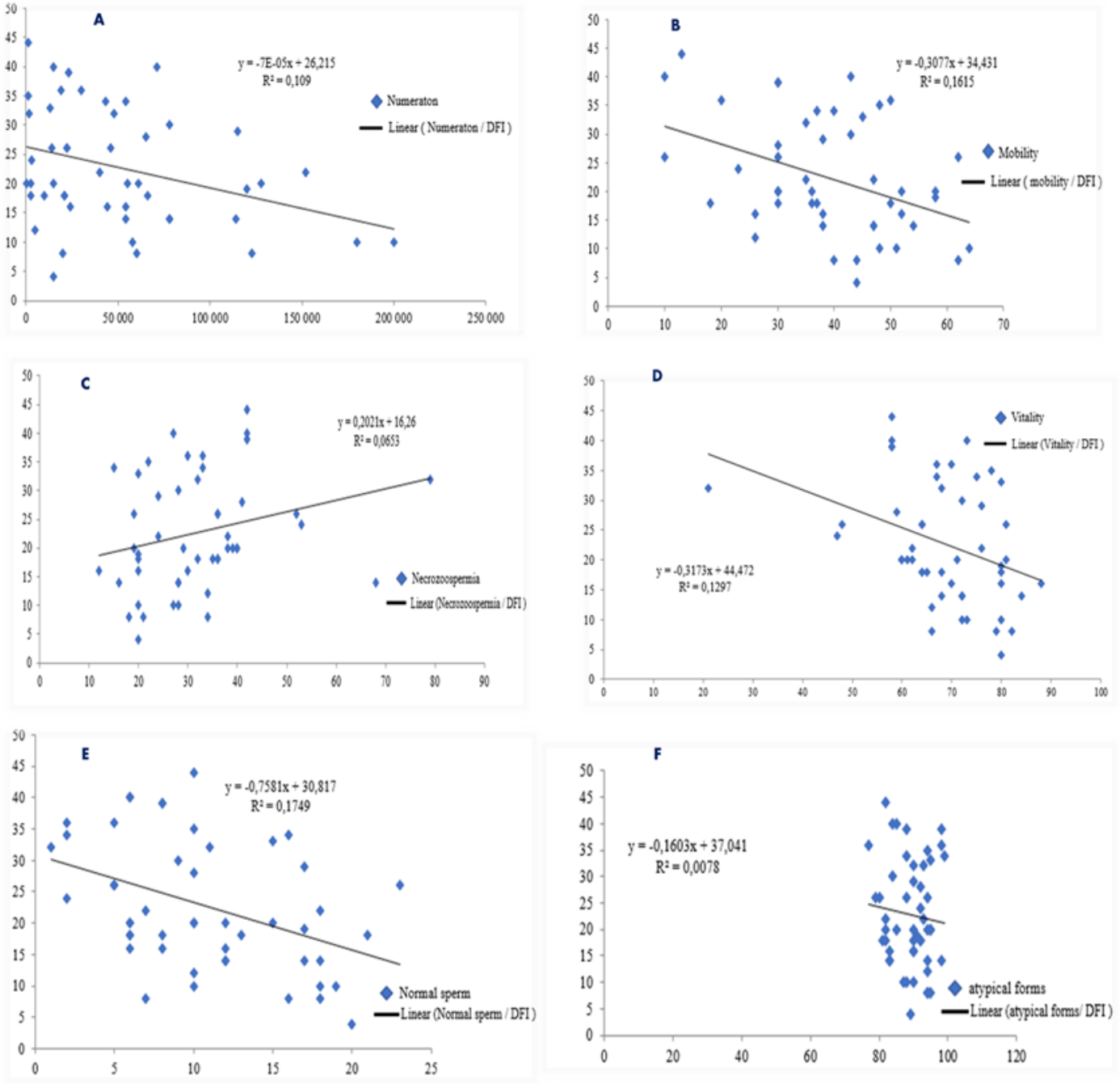

Figure 2: the distribution of the different parameters characteristic of sperm quality studied according to the DNA fragmentation index. A) count; B) motility; C) necrozoospermia; D) vitality; E) percentage of normal spermatozoa F) percentage of abnormal spermatozoa.

Student's t-test (t-test). All graphs and histograms shown in this article were produced using GraphPadPrism7.

Result

Correlation between DFI and some characteristic parameters of sperm quality

The linear coefficients of correlation of determination (R^2) found between the sperm DNA fragmentation index, and the different parameters typical of sperm quality studied are as follows:

-The coefficient of determination between the DNA fragmentation index and the count was R^2=0.11 (figure 2A).

-The coefficient of determination between the DNA fragmentation index and motility was R^2=0.16 (figure 2B).

-The coefficient of determination between the DNA fragmentation index and necrozoospermia was R^2=0.006 (figure 2C).

-The coefficient of determination between the DNA fragmentation index and vitality was R^2=0.13 (Figure 2D).

-The coefficient of determination between the DNA fragmentation index and the percentage of normal sperm was R^2=0.17 (figure 2E).

-The coefficient of determination between the DNA fragmentation index and the percentage of abnormal sperm

was R^2=0.16 (figure 2F).

The correlation coefficients R^2 found between the DNA fragmentation index and count, motility, vitality, and normal spermatozoa, respectively, are less than 0.5. This means that the linear relationships between DFI and the latter are fragile. In contrast, those for necrozoospermia and abnormal sperm percentage are much weaker, suggesting a lack of correlation.

Discussion

In this work, we are interested in evaluating the impact of fragmentations that occur in human sperm DNA on some parameter's characteristics of sperm quality, namely: sperm count, motility, vitality, and morphology, in order to prove the presence or absence of correlation between them and DFI.

The Sperm DNA Fragmentation Index is one of the main tests in genetic evaluation that explores the quality of the sperm genome by determining the percentage of sperm with fragmented DNA compared to those that have retained their genetic material intact (Evgeni *et al*., 2014).. DNA fragmentation is a physiological phenomenon due to several factors and mechanisms that cause alterations and breaks in sperm DNA, indeed sperm DNA fragmentation can influence different spermogram parameters, including sperm count, progressive motility, vitality, and morphology (Wright *et al.,* 2014). The linear correlation coefficient R^2 measures the strength and direction of the linear relationship between the two variables (Taylor *et al.,* 1990). The results of our work showed that some characteristic parameters of sperm quality studied showed a low negative correlation with DFI (coefficient of determination R^2 between 0.11 and 0.17). The count, motility, vitality and percentage of normal spermatozoa gave correlation coefficients with the fragmentation index of R^2=0.11 (figure 2A), R^2=0.16 (figure 2B), R^2=0.13 (figure 2D), R^2=0.17 (figure 2E) respectively. Thus, the linear relationships between DFI and these are fragile.

On the other hand, those for necrozoospermia and abnormal percentage are much weaker, suggesting an absence of correlation, as they are respectively R^2=0.006 (figure 2C) and R^2=0.07 (figure 2F).

Fragmentation of sperm DNA is a critical factor that mainly affects the percentage of live sperm and the rate of typical forms in the sperm, thus causing a slight decrease in the percentage of live sperm and morphologically normal spermatozoa. When spermatozoa have a high DFI, sperm DNA fragmentation does not affect the percentage of dead spermatozoa and the rate of abnormal forms (Gliozzi *et al.,* 2011).

Our results are in perfect correlation with those of the literature, namely: the study of Benchaib et al which showed a negative correlation between some parameters of sperm quality, notably necrozoospermia and the percentage of normal spermatozoa with DFI (Benchaib *et al*., 2003) and the study of Boughali et al, which found that the rate of abnormal spermatozoa was correlated with DFI (Boughali *et al*., 2006)

Conclusion

In our study, we found that the count and progressive motility are negatively correlated with the DNA fragmentation index; we also observed the absence of correlation between the two percentages of dead spermatozoa and morphologically normal spermatozoa with DFI, while there is a negative correlation between the two percentages of live spermatozoa and abnormal spermatozoa with the DFI

Contributions of the authors: This work is carried out in collaboration between the different authors who are equally involved in the review, drafting and correction. They also wrote, read and approved the manuscript.

Limitations of our study: In future studies, it is recommended to increase the number of samples.

Ethical statement: All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Hassan II University with a code of public health: 2008 – 321.

Funding: This research received no external funding

Conflicts of Interest: The authors declare no conflict of interest.

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