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Evaluation of the DNA Fragmentation of the Normozoosperms at the Pasteur Institute of Cote D'ivoire

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Sperm DNA fragmentation appears to be an important indicator of male fertility and an important parameter for evaluating the positive prognosis of a pregnancy. The objective of our study was to determine the prevalence of DNA fragmentation of normozoosperms received at the Institut Pasteur of Cote d'Ivoire (IPCI).
For this we determined the DNA Fragmentation Index (DFI) of spermatozoa as well as its impact in

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normozoosperms. The spermogram was carried out according to the criteria of the World Health Organization (WHO) and the integrity of the sperm DNA was verified by the acridine orange test.
Of the 18 normozoosperms samples selected, an average of 20.28 ± 11.22% for the DNA fragmentation index was obtained. About 27.78% of the samples presented a high level of DFI which reflects a poor pregnancy prognosis. Thus, 27.78% of normozoosperms men have abnormal DNA which does not allow these individuals to be able to conceive. This study showed that DNA fragmentation is an important factor in sperm quality.

Keywords: Spermatozoa; DNA; fragmentation; infertility.

1. INTRODUCTION

Infertility is a disease of the reproductive system characterized by the inability to achieve pregnancy after one year of regular unprotected sexual intercourse [1,2] estimated the median prevalence of infertility in couples to 9%, with rates of 3.5 to 16.7% in developed countries and 6.9 to 9.3% in developing countries. In Africa, infertility represents a major public health problem [3]. The male factor contributes to 50% of infertility problems. Male infertility has several etiologies. They can be endocrine, infectious, immunological, genetic, toxic, psychological, lifestyle-related or idiopathic [4]. The reference test for diagnosing male infertility is the spermogram. It is the biological test during which the different parameters of the man's sperm are analyzed [5]. In addition to the spermogram, the analysis of sperm quality DNA has an important impact in the analysis of male infertility and the results of Medical Assistance for Reproduction (MAR) [6]. The integrity of sperm DNA influences the fertility of the couple and makes it possible to predict the chances of pregnancy and its positive outcome. Therefore, the physiological and/or molecular integrity of sperm DNA is a new parameter of sperm quality and a potential predictor of fertility [7]. Several techniques have been developed to measure the extent of sperm DNA damage with the aim of identifying more objective parameters for the assessment of men infertility [8]. Also, in this study we will evaluate the prevalence of DNA fragmentation of normozoosperms received at the Institut Pasteur of Côte-d'Ivoire (IPCI).

2. MATERIALS AND METHODS

2.1 Materials

This study was carried out at the Biochemical and Physiology of Reproductive Cells Unit of the Department of Clinical and Fundamental Biochemistry of the Institute Pasteur of Côte d'Ivoire (IPCI). The biological material consisted of 18 normo-zoosperm samples which were collected from patients. The age of these patients were between 29 and 53 years. The sperm collection was carried out with the agreement of the patients for the use of their sample for research purposes, and the authorization was obtained from the National Commission for Ethics and Research of Côte d'Ivoire (CNER-THIS); order No. 036-13/CNESVS.

2.2 Methods

The semen samples were analyzed and then selected based on the protocol of the WHO Laboratory Manual for the examination and processing of human semen (WHO 2010).

To carry out the acridine orange test (AOT), semen smears were taken. After drying, these were fixed in a Carnoy solution which was made from a mixture of methanol/glacial acetic acid in the proportions 3:1 (v/v) for at least three hours. The smears were then rinsed with distilled water and then dried at room temperature. Then these were immersed in an acridine orange solution (2.5 mL of 1% AO stock solution, 10 mL of 0.1 mol/L citric acid and 400 µL of 0.3 mol $Na_2HPO_4.H_2O$ / L), for 5 min. Finally, the semen smears were then rinsed with distilled water. The slides were read on the same day from a fluorescence microscope using a 490 nm excitation filter and a 530 nm barrier filter.

2.3 Data Analysis

The Graph Pad Prism 10.0.2 software and Excel (2013) allowed the statistical analysis of the various data obtained. Results were reported as mean +/- standard deviation.

3. RESULTS

The production of the spermogram of the different samples allowed us to obtain the data in Table 1 which presents the average values of the parameters of the normo-zoospermic sperm selected for this study.

Table 1. Profile of spermogram parameters of normozoospermia analyzed

	Color	Viscosity	Volumes	pH	Number	%Mobility 1H	%Mobility 4H	% Normal forme
	Opalescent Gray	Normal						
Minimum			1,500	7,200	15,00	45,00	40,00	4,000
Maximum			5,800	7,500	160,0	70,00	65,00	11,00
Mean			2,889	7,400	64,22	59,44	54,44	6,722
Std. Deviation			1,243	0,1328	32,82	7,454	7,454	2,218

Table 2 presents the mean, maximum and minimum values for the percentage of sperm DNA fragmentation. Sperm with fragmented DNA fluoresce red while those with normal DNA fluoresce green. The interpretation of the DFI value allows it to be classified into four classes (Fig. 1):

0 < DFI ≤15% good pregnancy result
15% < DFI ≤ 25% good to average pregnancy potential
25% < DFI ≤ 40% fair to poor pregnancy potential
40% < DFI very poor pregnancy potential

4. DISCUSION

The acridine orange test (AOT) revealed the presence of fragmented DNA within the spermatozoa of normo-zoosperms examined at the IPCI. This study showed that 72.22% of patients presented a DFI between 0 and 25%, reflecting a good pregnancy result.

Indeed, fragmentation, or the accumulation of single and double-stranded DNA breaks in spermatozoa, is a characteristic of sperm [6]. It is linked to several factors which are either intrinsic such as deregulation of apoptosis processes, recombination defects during spermatogenesis, defects in histone methylation in the last stages of spermiation. There are also extrinsic causes which may be due to oxidative stress and also to the mechanisms of action of endocrine disruptors (EDCs, endocrine disruptor chemicals) [9].

The semen of this category of patients did not present any abnormalities to the spermogram and the acridine orange fragmentation test. Also, if these patients present infertility, that is to say an inability to procreate after one year of sexual intercourse, we can then deduce the presence of idiopathic infertility [10]. “Idiopathic” infertility to couple is the infertility of unknown origin, that is to say “unexplained” infertility [10]. Indeed, sperm analysis did not allow to determine a probable cause of abnormalities of the latter. The spermiological data does not make it possible to formally establish the diagnosis of male infertility. Also, in this case, many authors propose to further the exploration of fertility by analyzing new markers to explain idiopathic infertility [11,12]. Thus, the overall contribution of the sperm genome (including epigenetic regulation), transcriptome and proteome to the formation and development of the embryo must be studied in order to identify new molecular targets responsible for male infertility [11].

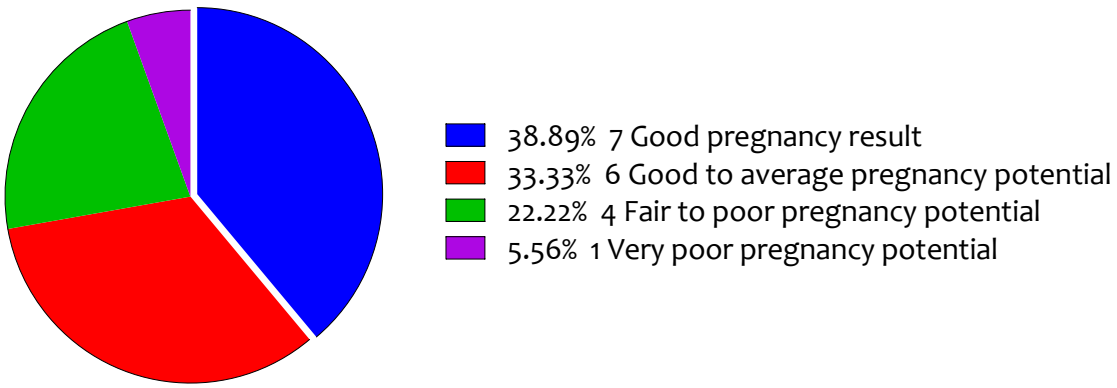

Fig. 1. DFI values and pregnancy potential of normo-zoosperms

Table 2. DFI values of normo-zoosperms examined at the IPCI

	Spermatozoa with green fluorescence	Spermatozoa with red fluorescence	% DFI
Mean	79,67	20,33	20,33
Standard error	11,20	11,20	11,20
Minimum	59	5	5
Maximum	95	41	41

A total of 27.78% of the sperm analyzed presented a DFI which reflects poor pregnancy potential. Numerous studies have shown that sperm DNA fragmentation influences natural reproduction. The integrity of sperm DNA plays an important role in the fertility of the couple and helps predict the chances of pregnancy and its positive outcome [7]. Indeed, various studies have analyzed the relationship between the degree of DNA damage and fertilization rate, embryo cleavage rate, implantation rate, pregnancy rate and live birth rate of offspring [8]. Therefore, it therefore appears important to implement the DNA fragmentation test in male fertility exploration examination.

5. CONCLUSION

DNA fragmentation is an important factor in sperm quality. Our study highlighted the presence of high DFI in normozoosperms. Also, the spermogram cannot alone define the quality of the sperm. The integrity of the paternal genome is therefore of paramount importance in the initiation and maintenance of a viable pregnancy.

CONSENT

As per international standards or university standards, patient(s) written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per international standard or university standards written ethical approval has been collected and preserved by the authors. Ethical approval number : (CNER- THIS); order No. 036-13/CNESVS

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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