



DFI and Chromatin Maturity Status Could be Considered as Independent Parameters for Male Fertility Assessment.

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Human sperm chromatin and integrity of DNA have multiple endogenous factors, such as sperm compression and nucleoproteins, with various mechanisms all through their own sperm production and transport. The integrity of the paternal genetic material in both regular and assisted pregnancy is essential for initiating and maintaining a viable pregnancy. The study included semen samples from 100 men taken from Al Nahrain University Medical Clinic and Babylon Teaching Hospital for Women and Children. The first sperm test was based on the World Health Organization (1999), followed by a DNA integrity test that uses a DNA fragmentation test, and the third intensification test Chromatin by aniline blue dye. It was correlated negatively with the sperm count in combination with DFI and chromatin sperm with semen characteristics ($r = -0.223$, $p = 0.026$). As well as DFI, sperm chromatin was being clinically significant associated with the rate of round cells ($r = 0.39$, < 0.001). DFI was positive and significantly associated with sperm chromatin in all infertile men ($r = 0.958$; $p < 0.001$). DNA isolation in all enrolled men has been adversely linked with sperm/ejaculation, has been associated with the round cell count positive way and is not understanding and resolving with other parameters. The width of abstinence and beneficial cell count in all register men were strongly linked with Sperm chromatin. There was a direct relationship between the fragmentation of DNA and chromatin. This could imply that one exam can be used instead of the other.

ABSTRACT

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KEYWORD

DNA, Sperm Chromatin, ART, Infertility.

1. Introduction

There, Sperm DNA is believed to lead about one-half of the genomic material to children, and normal sperm genetic material is needed for embryonic fertilization and brain development as well as post-natal child well-being (Qiu et al., 2020 [1]). In contrast to the relatively weak Chromatin structure throughout somatic cells, the unique association among DNA and nuclear sperm proteins means that sperm chromatin is heavily compacted (Imam et al., 2011 [2]). Thus, the whole different type of DNA synthesis has to be available in sperm kernels, where the formation of mature sperm is a unique procedure that involves the replacement of somatic cell-like histones with changeover proteins by a series and meiotic changes in the cytoplasmic design and the final addition of highly processed chromatin protamine's which take place during the later stage of life (Steger et al., 2000 [3]). The package is got at least six

times more compacted than mitotic chromosomes with a specific type of small protein molecules in a close status (Battulin et al., 2015 [4]). In fact, while the sperm DNA has been manufactured and transferred, it is prone to threats inherent and alien factors that could, in relation to other sperm parameters, impact both sperm moisture and DNA credibility. Medically, basic clinical diagnostic parameters conventional seminal fluid testing, have been implied as predictive markers in the male infertility prognosis. In the context of ART, whenever the major disadvantage of ART is that the natural selection barrier that subsists in all reproductive tracts is sidestepped by sperm and chromatin condensation and DNA (Jahmani et al. 2020 [5]). This study is developed to adapt to the link between chromatin condensation status and DNA integrity, based on the seriousness of sperm chromatin and on its interest in sperm chromatin status. And then, does one of

them have a correlation to sperm parameters?

2. Patients and Methods

This is prospective experimental trial study was carried out in the High Institute for Infertility diagnosis and Assisted Reproductive Technologies Al Nahrain University and Babylon Teaching Hospital for Women and Children from November 2020 until May 2021. This study was approved by the local ethical committee at the higher Institute of Infertility Diagnostics and Assisted Reproductive Techniques, Al Nahrain University, semen samples from 100 subject normozoospermic and patient's mild to moderate abnormalities according to WHO (1999). after 3-7 days of abstinence (inclusion criteria) Exclusion criteria were patients with azoospermia, genital infection and grade II or III Varicocele. a detailed questionnaire was designed based on the history and physical examination.

Collection and treatment of samples:

The masturbation approach was used to gather a sample in sealed tubes. Every person received only one sperm sample. If the sample is damaged or lost for any reason, the person is required to return to give another sample without sexual contact within 3-7 days. Following acquisition of samples, the samples were quickly placed into a 37 ° C incubator to fully liquefy the semen testing samples. The first is the WHO standard semen test (1999), and second the test of DNA integrity with DNA fragmentation reagents, which needs to take 50 microns of semen from liquid liquefaction, and the test series is Chromatin condensation by aniline blue dye. After such a liquefaction, 1 ml sperm is taken and multiple times washed in a buffer, then 10 microns are begun taking and a glass slide is spritzed. The relation between DNA integration and chromatin condensation was also analyzed with its relation to semen quality.

Analysis of Statistics

Data collected, summed up, analyzed and submitted using version case of normality distribution by Kolmogorov-Smirnov test, quantitative (numeric) variables were evaluated at first, and therefore the numeric variables, normally distributed, were expressed as average (centralized tendency index) and standard deviation. (Categorical) (an index of dispersion), While those not normally distributed input features have been expressed in median (center tendency index) and inter-quartile range (an index of dispersion).

3. Results

3.1. Correlation in all enrolled infertile men of DNA fragmentation and chromatin with seminal fluid characteristics

23 and Microsoft Office Excel 2010 of the Social Science Statistical Package, SPSS. In the

Table 1 illustrates the correlation of DNA and chromatin fragmentation with the seminal fluid characteristics. In the case of DNA fragmentation, the sperm count/ejaculation was significantly negative ($r=-0.223$, $p=0.026$) and was highly significantly related to round cell count ($r=0.390$, $p<0.001$). However, the correlation with other parameters was not significant. With respect to sperm chromatin, it was very positive.

3.2 Correlation of sperm chromatin fragmentation from DNA

In Figure 1 there is shown a correlation of fragmentation of DNA with sperm chromatin in all infertile males and a positive and significant correlation ($r = 0.958$; $p < 0.001$).

Table 1: DNA fragmentation correlation and sperm chromatin to the characteristic seminal fluid

Characteristic	DNA Fragmentation		Sperm Chromatin Immature	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Concentration million /ml	-0.184	0.067	-0.143	0.157
Count million/ejaculated	-0.223	0.026 *	-0.190	0.058
Progressive motility (A) (%)	-0.087	0.392	-0.046	0.646
Progressive motility (B) (%)	-0.092	0.364	-0.078	0.442
Progressive motility(A+B)	-0.098	0.331	-0.062	0.541
Non progressive motility (C) (%)	0.043	0.668	0.011	0.912
Immotile sperm (D) (%)	0.094	0.352	0.075	0.456
Normal morphology sperm (%)	0.058	0.567	0.026	0.800
Round cell count	0.390	< 0.001 **	0.427	< 0.001 **

r: correlation coefficient; *: significant at $p \leq 0.05$; **: highly significant at $p \leq 0.01$

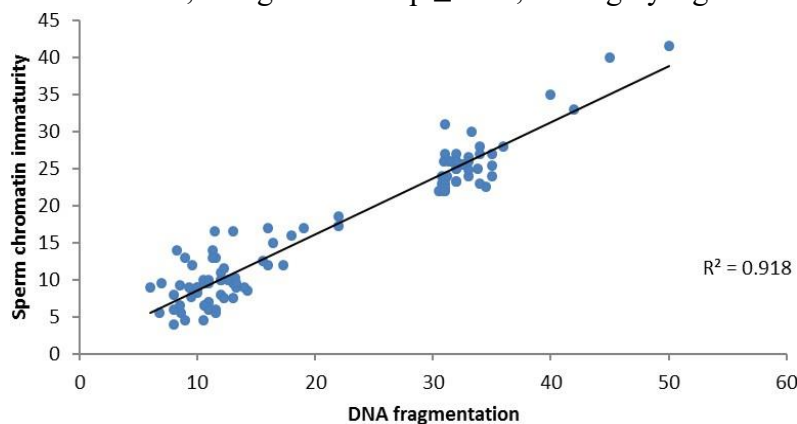


Figure 1: Display graph indicating the correlation of sperm chromatin DNA fragmentation in all infertile men

3.3 DNA fragmentation correlation with chromatin sperm, classified by seminal fluid properties into subgroups

A correlation between the

fragmentation of DNA and sperm chromatin in subgroups is presented in Table 2. These correlations have all been very important ($p < 0.01$). DNA fragmentation is also shown in Table 2.

Table 2: Sperm chromatin-classified correlation of DNA fragmentation into seminal fluid subgroups.

Group	<i>R</i>	<i>p</i>
Oligozoospermia	0.959	< 0.001**
Asthenozoospermia	0.943	< 0.001**
Teratozoospermia	0.983	0.003**
Oligoasthenozoospermia	0.969	< 0.001**
Asthenoteratozoospermia	0.939	< 0.001**
Normal	0.978	< 0.001**

r: correlation coefficient; **: highly significant at $p \leq 0.01$

4. Discussion

In the current study, the percentage of fragmentation of DNA and sperm chromatin condensation has shown a correlation with a number of sperm features when all infertile men are considered separately in a single group and in individual groups. In addition, the correlation between DNA fragmentation percentage and chromatin did sperm condensation

percentage was significantly positive. The highest positive correlation with the red cell in all enrolling men has been DNA fragmentation and sperm chromatin immature, which show that the higher the round cell count in the semen sample, the higher the risk of DNA fragmentation and Zeqiraj et al. concluded in 2018 that a negative correlation exists among male infertile

DNA fragmentation, movement and sperm morphology (Zeqiraj et al. 2018 [6]). On the contrary, the collective data from the Ferrigno et al study in 2021 revealed a strong significant correlation suggesting that the best candidates for damage caused by sperm with abnormal morphology are DNA ($p < 0.001$) (Ferrigno et al., 2021 [7]). In 2016, Choucaira et al. found the correlation of the sperm fragmentation with sperm alterations: count, motility and morphology. Where the connection among has been unfavorable and the count. In addition, it has been shown that 28% of patients with normalzoosperm show high fragmentation of sperm DNA. In the infertile group of patients, there is also a positive correlation between sperm DNA fragmentation and ART failure. Finally, fragmentation of sperm DNA with tobacco and environmental conditions is suggested (Choucaira et al. 2016 [8]). Other studies previously showed the relationship between integrity and morphology of sperm

DNA (Brahem et al., 2011 [9]) In this infertile group of men, the chromatin structure and packaging are far worse than normal. Therefore, we call for an independent and important factor in the diagnosis of infertility and for the selection of the proper treatment with chromatin and DFI status test.

6- Conclusion

In the present study it can be concluded that in all enrolled men, DNA fragmentation index was significantly negatively correlated to sperm count/ ejaculate, positively correlated to round cell count and not significantly correlated to other parameters. Also, their sperm chromatin immaturity was significantly positively correlated to round cell count and not significantly correlated to other parameters. There was no significant variation in DNA fragmentation and sperm chromatin among normoozoospermia oligozoospermia, asthenozoospermia, teratozoospermia,

oligoasthenozoospermia and asthenoteratozoospermia. This important finding suggests that both DNA fragmentation and sperm chromatin immaturity could be considered an independent parameter that affection male fertility potential. The DNA fragmentation and sperm chromatin immaturity were being positively correlated. This could make a suggestion that one test could be used instead of the other one.

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Author Contribution

Huda Abdalrazaq, performed the study, Ali I. Rahim, and Laith A. Abd AL- Hussein 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.

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References

- [1] Qiu Y, Yang H, Li C, Xu C. Progress in Research on Sperm DNA Fragmentation. Medical Science Monitor. International Scientific Information, Inc.; 2020 Feb 24;26. Doi.org/10.12659/msm.918746
- [2] Imam SN, Shamsi MB, Kumar K, Deka D, Dada R. Idiopathic recurrent pregnancy loss: role of paternal factors; a pilot study. J Reprod Infertil. 2011 Oct;12 (4):267-76.
- [3] Steger K, Pauls K, Klonisch T, Franke FE, Bergmann M. Expression of protamine-1 and -2 mRNA during human spermiogenesis. Molecular Human Reproduction. Oxford University Press (OUP); 2000 Mar 1;6 (3):219–25. Doi.org/10.1093/molehr/6.3.219
- [4] Battulin N, Fishman VS, Mazur AM, Pomaznoy M, Khabarova AA, Afonnikov DA, Prokhortchouk EB, Serov OL.

Comparison of the three-dimensional organization of sperm and fibroblast genomes using the Hi-C approach. *Genome Biol.* 2015 Apr 14;16 (1):77.

Doi: 10.1186/s13059-015-0642-0.

[5] Jahmani MY, Hammadeh ME, Al Smadi MA, Baller MK. Label-Free Evaluation of Chromatin Condensation in Human Normal Morphology Sperm Using Raman Spectroscopy. *Reprod Sci.* 2021 Sep;28 (9):2527-2539.

Doi: 10.1007/s43032-021-00494-6.

[6] Zeqiraj A, Beadini S, Beadini N, Aliu H, Gashi Z, Elezaj S, Bexheti S, Shabani A. Male Infertility and Sperm DNA Fragmentation. *Open Access Maced J Med Sci.* 2018 Aug 14;6 (8):1342-1345.

Doi: 10.3889/oamjms.2018.311.

[7] Ferrigno A, Ruvolo G, Capra G, Serra N, Bosco L. Correlation between the DNA

fragmentation index (DFI) and sperm morphology of infertile patients. *J Assist Reprod Genet.* 2021 Apr;38 (4):979-986.

Doi: 10.1007/s10815-021-02080-w.

[8] Choucair FB, Rachkidi EG, Raad GC, Saliba EM, Zeidan NS, Jounblat RA, et al. High level of DNA fragmentation in sperm of Lebanese infertile men using Sperm Chromatin Dispersion test. *Middle East Fertility Society Journal.* Springer Science and Business Media LLC; 2016 Dec;21 (4):269–76.

Doi.org/10.1016/j.mefs.2016.06.005

[9] Brahem S, Mehdi M, Elghezal H, Saad A. Detection of DNA fragmentation and meiotic segregation in human with isolated teratozoospermia. *J Assist Reprod Genet.* 2011 Jan;28 (1):41-8.

Doi: 10.1007/s10815-010-9482-8.