



Original Article

Relationship between sperm progressive motility and DNA integrity in fertile and infertile men

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ABSTRACT

Background: Progressive sperm motility was found to be predictive for in vitro and in vivo fertilization. There has been an increase in the literature of studies investigating whether DNA fragmentation could be associated with other semen parameters; however, few reports focused on the relationship between sperm DNA fragmentation and progressive sperm motility.

Purpose: We purposed here to determine the relationship between DNA fragmentation level and progressive sperm motility in different groups of infertile asthenozoospermic patients as well as in healthy men of proven fertility.

Study design: Retrospective study.

Setting: Andrology Laboratory of the Assisted Reproductive Technologies (ART) department of Elite fertility and gynecology center, Cairo, Egypt.

Patients and methods: Semen samples were collected and examined after liquefaction for 20 min at 37 °C from 182 patients. Patient were then classified as asthenozoospermic [(Mild asthenozoospermia; PR (progressive sperm motility) = 30–20%, n = 58), (Moderate asthenozoospermia; PR = 20–10%, n = 68) and (Severe asthenozoospermia; PR < 10%, n = 56)] and 32 fertile healthy men as a control.

Outcome results: Fertile healthy men showed lower sperm DNA fragmentation levels as compared with asthenozoospermic infertile men. There was a significant negative correlation of sperm DNA fragmentation using the modified sperm chromatin dispersion (SCD) test with motility ($r = -0.319$; $P < .001$) and progressive motility ($r = -0.474$; $P < .001$).

Conclusion: Overall, our data suggest that sperm DNA damage is strongly associated with both type and percentage of motility.

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1. Introduction

Overall, infertility affects 15% of couples. A male factor is solely responsible in about 20–25% of infertile couples and contributory in another 30–40% [1]. A routine semen analysis according to World Health Organization (WHO) guidelines [2] has been recognized as the most important tool of male infertility diagnosis; however, none of semen parameters reflect the DNA integrity of spermatozoa or their fertilization capacity. Thus, sperm DNA

fragmentation becomes a new essential parameter for estimating sperm quality [3].

Sperm motility is considered an important indicator of adequate metabolic activity of the sperm and strongly influences ICSI outcome [4]. Sperm motility has been categorized into three classes: progressive, non-progressive and immotile spermatozoa. Asthenozoospermia is one of the main causes of male seminal pathologies that affects about 19% of infertile men. It is characterized by decreasing in percentage of progressively motile (PR) spermatozoa below (32%). It affects approximately 19% of infertile men [5].

Sperm DNA fragmentation test provides approximate information about sperm DNA integrity that may help the clinicians to identify the cause of infertility [6], and therefore guide the couples

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to bespoke treatment for their particular needs. Various methods could be used to assess sperm chromatin abnormalities. These assays include the Sperm Chromatin Structure Assay (SCSA), the Sperm Chromatin Dispersion (SCD) test, the DNA Breakage Detection-Fluorescence in Situ Hybridization (DBD-FISH) assay, the In-situ nick translation (NT) assay, the Comet assay, and the TUNEL assay [7]. The Comet assay is simple and inexpensive; however, it is limited assay because it is time consuming and needs a highly skilled technician to interpret the results. The TUNEL and SCSA assays have been widely used in sperm DNA chromatin assessment, however, both of them are expensive. The clinical value of the DNA Breakage Detection-Fluorescence in Situ Hybridization (DBD-FISH) assay is limited because it is expensive, time consuming and less cumbersome. The NTL assay cannot be employed for routine clinical use because it lacks sensitivity and the results are not correlated with fertilization during in vivo studies [7,8]. Compared to other methods of assessing DNA fragmentation, the SCD test is a simple, fast and inexpensive technique. Data from several studies suggest that the sperm DNA fragmentation inversely correlate with the rates of fertilization and embryo development [9].

None of sperm parameters should be considered alone in male infertility assessment as all directly impact upon initiation a healthy ongoing pregnancy [10]. A significant negative correlation between DNA fragmentation and sperm concentration, motility and morphology has been previously reported [11].

The purpose of our study was to (1) determine the relationship between DNA fragmentation levels and progressive sperm motility in normozoospermic fertile men and asthenozoospermic infertile patients, and (2) verify whether the sperm progressive motility is correlated with DNA fragmentation in ejaculated semen using the modified sperm chromatin dispersion (SCD) test or not.

2. Materials and methods

This retrospective study was performed from September 2015 to June 2016. Semen samples were obtained from 214 men attending the andrology Laboratory of Assisted Reproductive Technologies (ART) department of Elite fertility and gynecology center, Cairo, Egypt.

The subjects, 214 enrolled in the study, were classified into two groups. Fertile group (Control; they were able to conceive and bear children through normal sexual activity two years ago) was 32 healthy men with normal semen characteristics (Normozoospermia) according to the criteria of WHO 2010 [5]. The second group were 182 infertile asthenozoospermic men that had at least one year of regular unprotected intercourse with no pregnancy achieved. They were subdivided into three sub-groups on the basis of their progressive motility (PR) [(Mild asthenozoospermia; PR = 30–20%, n = 58), (Moderate asthenozoospermia; PR = 20–10%, n = 68) and (Severe asthenozoospermia; PR < 10%, n = 56)]. Gynecologic evaluation and fertility workup of all female partners failed to reveal any abnormality.

2.1. Semen analysis

Semen samples of patients were collected in sterile containers by masturbation after 5 days of sexual abstinence and examined after liquefaction for 20 min at 37 °C. Volume, pH, concentration and motility were evaluated according to WHO guidelines [2].

2.2. Sperm morphology assessment

Air-dried seminal smears were fixed and stained with Diff-Quik stain (Baxter Healthcare, McGaw Park, IL), according to the

manufacturer's instructions. Normal sperm morphology was scored according to WHO criteria [2] and strict criteria by Kruger [12] using a x100 oil-immersion brightfield objective. At least 200 sperm were assessed.

2.3. Measurement of leukocytes

Leukocyte staining method (LeucoScreen; Ferti Pro M.V., Aalter, Belgium) was used to indicate the presence and number of leukocytes. A drop of 10 μ of semen mixed with 10 μ l of working solution (30 μ l of reagent 2–1 ml of reagent 1 of LeucoScreen). Yellow to brown stained cells were considered as peroxidase positive cells.

2.4. DNA fragmentation analysis

The SCD test processed using commercial kit (Halosperm[®], Laboratorios INDAS, Madrid, Spain). The spermatozoa were immersed in a melted agarose matrix at 37 °C. On a slide, a drop of 8 μ l of mixed sperm agarose initially treated with an acid solution (solution 1) for 7 min to denature the DNA with DNA breaks, and directly treated with lysing solution (solution 2) for 20 min to deproteinize the nucleoids. After removal of nuclear proteins, fixation was done using ethanol and then slide was stained using solution 3 for 6 min and solution 4 for 7 min. Non-fragmented sperm DNA appeared with a core and with a peripheral halo of dispersion of DNA loops. Fragmented sperm DNA appeared with very small or no halo of DNA dispersion.

2.5. Statistical analysis

The data were tabulated and analyzed using the computer program SPSS (Statistical package for social science) version 16. Data are presented as mean \pm standard deviation.

In the statistical comparison between the different groups, the significance of difference was tested using one of the following tests:

1. Student's *t*-test and Mann-Whitney test (*z* test):- Used to compare mean of two groups of quantitative data of parametric and non-parametric respectively.
2. ANOVA test (*F* value) and kruskal-wallis test (*x*²): Used to compare mean of more than two groups of quantitative data of parametric and non-parametric respectively.
3. Correlation coefficient: to find relationships between variables.

A *P* value < .05 was considered statistically significant (S), while > .05 was considered statistically insignificant. *P* value < .01 was considered highly significant (HS) in all analyses.

3. Results

Table 1 lists the comparison between the basic semen parameters, of the 214 men enrolled in the study. There were no significant differences between the two groups in terms of their age, semen volume, and BMI (Table 1). However, BMI was significantly higher of severe asthenozoospermic sub-group compared with control healthy men (BMI: 25.56 \pm 2.06 and 24.69 \pm 0.60 years, respectively, *P* = .015) (Table 2).

Differences between fertile and infertile sub-groups were non-significant for sperm count and morphology, as shown in Table 2. The mean leukocytes concentration comparable between the fertile and infertile sub-groups men was (0.156 \pm 0.37 versus 0.076 \pm 0.26, 0.097 \pm 0.27 and 0.35 \pm 0.57 \times 10⁶/ml, not significant).

Table 1
Data for semen parameters in two groups.

	Fertile (n = 32) (Mean ± SD)	Infertile (n = 70) (Mean ± SD)	t test	P value
Age	33.44 ± 7.84	32.47 ± 6.33	0.766	.444
BMI	24.69 ± 0.60	25.2 ± 1.72	1.67	.096
Volume (ml)	2.7 ± 1.2	3.08 ± 1.53	1.33	.184
Concentration ($\times 10^6$ /ml)	50.34 ± 17.57	51.36 ± 10.87	0.44	.66
Total motility (%)	63.44 ± 5.3	48.46 ± 15.25	5.49	<.001
Progressive motility (%)	35.0 ± 3.11	13.81 ± 8.79	13.47	<.001
Normal forms (%)	6.13 ± 2.06	5.49 ± 1.69	1.88	.061
Pus cells ($\times 10^6$)	0.156 ± 0.37	0.17 ± 0.40	Z = 0.387	.699
DNA Fragmentation (%)	13.43 ± 7.86	24.7 ± 11.75	5.22	<.001

SD: standard deviation; BMI: body mass index.

Values are a mean ± SD.

Note: $P < .05$ was considered to be significant when compared with fertile (control) group.

Table 2
Semen parameters assessed by WHO of different groups of infertile asthenozoospermic men as compared to control fertile normozoospermic men.

Variable	Fertile population		Infertile population		P value
	Normozoospermia Group (1) (n = 32) (Mean ± SD)	Mild asthenozoospermia Group (2) (n = 58) (Mean ± SD)	Moderate asthenozoospermia Group (3) (n = 68) (Mean ± SD)	Severe asthenozoospermia Group (4) (n = 56) (Mean ± SD)	
Age	33.44 ± 7.84	30.86 ± 5.04	33.79 ± 6.47	32.54 ± 7.04	.076
BMI (kg/m ²)	24.69 ± 0.60	25.56 ± 2.06	25.28 ± 1.67	24.74 ± 1.24	.015
Volume (ml)	2.7 ± 1.2	3.15 ± 1.62	3.29 ± 1.79	2.74 ± 0.93	.103
Concentration ($\times 10^6$ /ml)	50.34 ± 17.57	50.25 ± 12.53	53.83 ± 10.72	49.5 ± 8.59	.181
White blood cells ($\times 10^6$ /ml)	0.156 ± 0.37	0.076 ± 0.26	0.097 ± 0.27	0.35 ± 0.57	.95
Total motility (%)	63.44 ± 5.3	57.59 ± 7.21	48.97 ± 11.3	38.39 ± 19.1	<.001
Progressive motility (%)	35.0 ± 3.11	24.83 ± 3.37	12.5 ± 2.52	4.0 ± 2.53	<.001
Non progressive motility (%)	27.33 ± 6.12	33.04 ± 6.1	35.19 ± 11.1	29.29 ± 17.7	.01
Immotility (%)	37.0 ± 5.19	41.74 ± 6.77	52.41 ± 11.0	67.89 ± 21.5	<.001
Normal forms (%)	6.13 ± 2.06	5.21 ± 1.14	5.62 ± 1.99	5.64 ± 1.76	.122
DNA Fragmentation (%)	13.43 ± 7.86	19.75 ± 12.0	24.36 ± 8.96	30.25 ± 12.2	<.001

BMI: body mass index; WHO: World Health Organization; SD: standard deviation; *: significant.

Values are a mean ± SD.

Significant differences ($p < .05$) when compared by analysis with the fertile, normozoospermic group (control).

Table 3
Correlation analysis between sperm motility and sperm DNA.

	Correlation coefficient (r)	P-value
Motility	−0.319	<.001
Progressive motility	−0.474	<.001

As shown in Table 2, sperm motility and progressive motility were statistically different among three categories, mild, moderate and severe asthenozoospermic sub-groups compared to control group. There were also significant differences between the fertile men and patient groups with respect to DNA damage, all patients have higher DNA fragmentation levels (Table 1). When asthenozoospermic men were sub-divided into three categories, mild, moderate and severe asthenozoospermic sub-groups had higher DNA fragmentation levels 19.75 ± 12.0%, 24.36 ± 8.96 and 30.25 ± 12.2%, respectively compared to control group (Table 2).

Table 3 summarizes the results of a correlation analysis between sperm total motility and progressive motility with sperm DNA fragmentation in both fertile normozoospermic men and asthenozoospermic infertile men. A significant negative correlation was observed between progressive sperm motility and sperm DNA fragmentation ($r^2 = -0.474$; $P < .001$) in both fertile normozoospermic men and asthenozoospermic infertile men (Fig. 1).

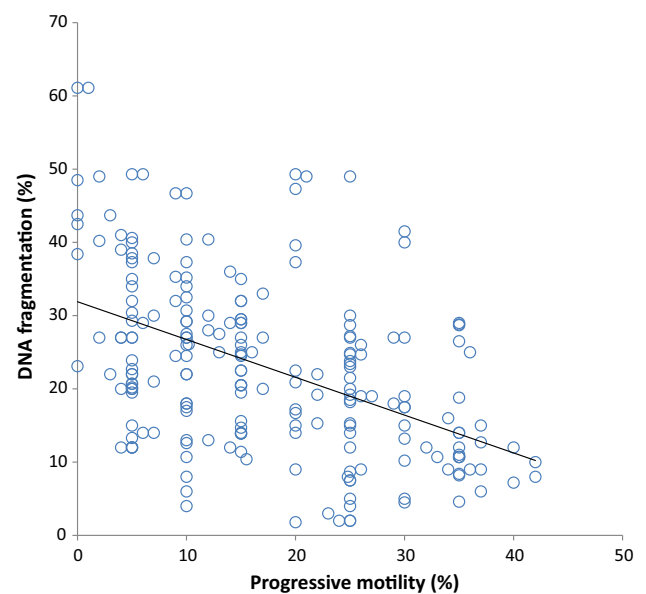


Fig. 1. Scatter graph illustrating associations between DNA fragmentation and sperm progressive motility ($r = -0.474$; $p = .001$).

4. Discussion

Results of the present study applied to a set of data obtained using SCD test to measure sperm DNA fragmentation. A total of 32 fertile normozoospermic men and 182 infertile asthenozoospermic men were divided into three sub-groups following the progressive motility parameter.

There has been a recent increase in the literature of studies investigating whether DNA fragmentation could be associated with male infertility. Our results showed significant differences in the sperm DNA fragmentation levels, as measured by SCD test, between the fertile normozoospermic and infertile asthenozoospermic populations. These finding is supported by data from many other studies [3,13,14]. For example, Irvine et al. concluded that high levels of sperm with damaged DNA are strongly correlated with infertile men [15]. The study by Boushaba and Belaaloui [16] reported significant association between the presence of nuclear DNA damage in the mature spermatozoa and male infertility. Also, data collected by Saleh et al. [14] found significant higher levels in DNA damage in normozoospermic infertile men compared with normozoospermic fertile men. However, Hughes and colleagues did not find any differences in sperm DNA damage between fertile and infertile, using a modified comet assay [17].

The infertile population enrolled this study are asthenozoospermia presented with good sperm count, morphology and relatively good motility but poor progressive motility according to WHO guidelines. Progressive motility is the most important used measure of semen quality [7]. The percentage of sperm that have forward progression is one of the most important prerequisites that affect both natural [18] and assisted pregnancy [19]. On the basis of the results of our study, decrease of spermatozoa progressive motility has been linked not only to male infertility but also to an increase in sperm DNA fragmentation. This is in agreement with a study by Irvine et al. has been reported a negative correlation of progressive motility and sperm DNA quality [15].

During sperm maturation, human spermatozoa first migrate through the epididymis from testis with little or no motility. The percentage of motile sperm increases gradually as they pass through the epididymis. Thus, impaired sperm motility may occur due to high levels oxidative stress (OS) caused by overproduced reactive oxygen species (ROS) [20] during sperm maturation process, or a consequence of unbalanced apoptosis [21]. Both OS and abortive apoptosis are also major causes of the generation of sperm chromatin damage in human spermatozoa [22]. Thus, it is not surprisingly in view of the above, an impairment of sperm progressive motility might be related with sperm DNA damage and, in turn, male infertility.

Although the effect of BMI was not the aim of our study, our data showed that it is significantly higher in severe asthenozoospermic infertile men when compared to a control fertile group with normal spermatozoa. This finding suggests that high BMI may cause impaired motility, increasing in DNA fragmentation and, in turn, associated with increasing in male infertility severity. This is in agreement with several studies showed that conventional sperm parameters and DNA fragmentation percentage to correlate inversely with BMI. A wide retrospective study by Nguyen et al. [23] reported that increasing in BMI is associated with increasing in risk of male infertility. The study by Hammoud et al. [24] reported a significant inverse correlation between the male obesity and progressively motile count. In another recent study by La Vignera et al. [25], overweight and obese men have significantly lower sperm progressive motility and higher DNA damage compared with normal weight men.

Our data conclusively showed that impaired progressive motility is associated with an increase of sperm DNA damage. Moreover,

the present research supports the idea that male infertility is associated with increased incidence of fragmented sperm DNA.

Conflict of interest

The authors declared that there is no conflict of interest.

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