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Sperm DNA fragmentation and male fertility: a retrospective study of 5114 men attending a reproductive center

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Abstract

Purpose The sperm DNA fragmentation index (DFI) was quantitatively measured and its relationship with age, semen quality, and infertility conditions was investigated.

Methods Semen routine test and sperm DFI were performed in 2760 infertile male and 2354 male whose spouse experienced at least one unexplained miscarriage to analyze the correlation between sperm DNA damage, semen routine parameters, and age.

Results Sperm DFI was significantly lower from patients whose wife experienced unexplained miscarriage compared to infertility males ($p = 0.000$). An inverse correlation between sperm DFI and sperm progressive motility was observed ($r_s = -0.465$, $p = 0.000$) and sperm DFI was positively correlated with age ($r_s = 0.255$, $p = 0.000$). However, the correlation between sperm DFI and sperm concentration, semen volume, total sperm count, and motile sperm count were not proved.

Conclusions Sperm DFI is an important indicator for evaluating the quality of semen. Sperm DNA integrity testing is preferentially recommended to those who have decreased sperm progressive motility, especially older men. An integrative analysis of sperm DFI, sperm progressive motility, age, and infertility conditions can provide a more comprehensive assessment of male fertility.

Keywords Sperm DFI · Semen analysis · Male infertility · Miscarriage · Age

Introduction

Conventional semen routine analysis and morphology examination are traditional strategies for evaluating male fertility. However, the restrictions of the traditional sperm analysis are gradually recognized by researchers. One the one hand, some fertile men attending for vasectomy often have poor sperm quality according to the WHO criterion [1]. On the other hand, men having values within the reference range still may be infertile [2]. What more important is that growing evidence suggests that general sperm parameters had shown little correlation or nothing with fertility outcome in populations of first pregnancy planners [3–5] and had not reliably predicted either male fertility or pregnancy outcome after infertility treatment [6]. So, semen microscopic analysis might remain the first stage of diagnosis but molecular testing is needed to provide a more robust tool in the evaluation of male fertility. Some novel methodologies, such as sperm DNA fragmentation index (DFI), an indicator for assessing sperm DNA damage, have been proposed in clinical practice [7, 8].

The integrity of the sperm DNA is undoubtedly essential for the accurate transmission of genetic information. Generally, a haploid genome is tightly packaged with protamines and compacted in sperm nucleus to avoid possible damage [9]. However, some intrinsic and extrinsic matters affect the integrity of chromatin, accumulatively leading to DNA damage. As we know, there are many factors that are known to be associated with sperm DNA damage, such as age, reproductive tract infections, systemic diseases, environmental pollution and lifestyle, *etc*. [10, 11], These factors can cause sperm DNA fragmentation, which not only acts as potential mediator for establishing an infertility conditions

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in men [12–14] but also increases the risk of female unexplained miscarriage [15, 16].

However, the association between sperm DFI and the traditional sperm analysis parameters and infertility conditions, i.e., primary or secondary infertility and unexplained miscarriage, remains under debate since its clinical usage. The reasons for the inconsistent literature might be as follows: firstly, the different mechanisms underlying the methods assaying sperm DNA fragmentation will result in discrepancies. Secondly, even the same method, the thresholds and reagents adapted and inconsistencies among laboratories also lead to different results and thus conclusions. Thirdly, another possible and important cause for the discrepancies might lie in the not big enough sample for analysis, which could totally change the conclusions. Herein, we performed a retrospective study of 5114 men facing fertility disorders in a reproductive center, aiming to provide a basis for clinical male infertility diagnosis and precise prediction of fertility of infertile couples.

Materials and methods

Participants

This retrospective study was performed at the Reproductive Medicine Center of the Women's Hospital of Zhejiang University, which is a clinical reproductive center of the academic hospital. Couples suffering from infertility were examined for fertility evaluation by a consultant at the center. In the present study, unexplained miscarriage refers to at least one pregnancy loss within 20 gestation weeks but without definite cause(s). After excluding the history of unexplained miscarriage of the female, male infertility refers to the inability of a fertile female to become pregnant (primary infertility) or pregnant again (secondary infertility) after ≥ 12 months of regular unprotected intercourse. We excluded male infertile patient whose spouse was identified with female infertile, such as anatomical abnormalities, endocrine diseases, genetic factors, infection and immune disorders, etc. At the same time, the patients whose sperm concentration is < 2 million/ml were also excluded for the cell number is too low to count and detect with sperm chromatin structure assay (SCSA). From December 2018 to December 2019, 2760 patients of male infertility and 2354 male patients whose spouse experienced at least one miscarriage were totally included. As approved by the Ethics Board of our facility, informed patient consent was not required for this study because of its retrospective design.

Semen routine analysis

Semen samples were obtained by masturbation into a wide-mouth sterile plastic graded container in the clinic after 2–7 days of sexual abstinence. After the semen was liquefied, analysis was performed by two independent technicians according to the World Health Organization (WHO) reference values [3]. Sperm concentration was determined with a Neubauer hemocytometer. A 1:20 dilution was made using 50 μL of semen and 950 μL of sperm diluent solution. Progressive motility (PR) refers to sperm that are swimming in a mostly straight line or in very large circles, which was also defined according to the WHO reference values. At least 200 spermatozoa were evaluated in a total of at least five fields to calculate the average percentage for progressive motility in the replicate wet preparations. During the study, internal quality control was taken to ensure that there was no significant difference between the technicians. The test was performed using a constant temperature console. Total progressive motile sperm count (TPMSC) was calculated by multiplying total sperm count by progressive motility divided by 100.

Sperm DFI detection

The sperm chromatin structure assay is a reliable and most commonly used assay for the determination of sperm DNA fragmentation. In the present study, a commercial kit special for sperm chromatin structure by staining with acridine orange (AO) (CellPro Biotech Co., Ltd. Ningbo, China) was used and the reference value for DFI was set to < 20%. The assay was performed according to the manufacturer's instruction and described as the following. Firstly, 40 μl semen samples were treated for 30 s with 400 μl of a solution containing 0.1% Triton X-100. After 30 s, 1.2 ml of staining buffer (6 μg/ml AO, 37 mmol/L citric acid, 126 mmol/L Na_2HPO_4, 1 mmol/L disodium EDTA, and 0.15 mol/L NaCl) was admixed to the test tube. Five minutes thereafter, the sample was placed into the FACSCalibur flow cytometer (BD, San Jose, CA) for the scanning and the analysis with software (DFIView 2010 Alpha11.15, CellPro Biotech, Ningbo, China). In the process, a minimum of 5000 cells of each sample were acquired and analyzed. The intra-batch variation was less than 5%.

Groups

With regard to the DFI criteria in our laboratory, it was divided into three groups: normal group (DFI < 20%), critical group (≥ 20% and < 30%), and abnormal group (DFI ≥ 30%). According to participants' age, it was classified into four groups: 20–29 years, 30–39 years, 40–49 years, and over 50 years. According to participants' clinical manifestation, it was classified into three groups: primary and secondary infertility group and unexplained miscarriage group. Meanwhile, four groups were defined as normospermia, oligozoospermia, asthenozoospermia, and oligoasthenozoospermia groups based on the WHO reference values [3]. In brief, lower

reference limits for sperm parameters are described as follows: total sperm number, 39 × 10^6/ejaculate; progressive motility, 32%; sperm concentration, 15 × 10^6/ml. Normospermia means both total number or concentration and PR are equal to or above the reference. Oligozoospermia is defined as the total number being below the reference. Asthenozoospermia is described as PR being below the reference, while oligoasthenozoospermia refers to the total number and PR value beimg both below the reference.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences software, version 20 (SPSS Inc., Chicago, IL, USA). Measurement data were presented as mean ± standard deviation/median and counting data were presented as count (percentage, %). Before comparison, the Kolmogorov-Smirnov test was first performed for normality. Then the Kruskal-Wallis test was used and the multiple tests were corrected by the Bonferroni method for non-normal distribution of the parameters. Spearman's rank correlation was utilized to test the relationship among sperm DFI, with age, semen volume, concentration, total sperm count, progressive motility, and TPMSC. $p < 0.05$ was considered statistically significant.

Results

Comparison of semen volume, concentration, total count, progressive motility, total motile count, and age among different DFI groups

In this study, 5114 patients were totally included, the median of age was 32.0 years; the median of sperm DFI was 15.9%; semen volume was 2.8 ml; the sperm concentration, total count, progressive motility, and total motile count were 52.5 × 10^6/ml, 135.0 × 10^6, 39.1%, and 54.1 × 10^6, respectively. According to the DFI values of spermatozoa, there are 3311 (64.7%) cases in the normal group, 1003 (19.6%) cases in critical group, and 800 (15.6%) cases in abnormal group. Comparing the results among the groups revealed significant differences in age, progressive motility, and TPMSC. Semen volume and total sperm count were significantly different only between DFI < 20% and DFI ≥ 30% groups (p = 0.024 and 0.001). As to sperm concentration, no significant difference was found between 20% ≤ DFI < 30% and DFI ≥ 30% groups. However, the parameter of the normal group (DFI < 20%) was the highest one and significantly different from those of other two groups (Table 1).

Table 1 Comparison of age and semen volume, concentration, total count, progressive motility, and TPMSC among different DFI groups

DFI	Number (%)	Sperm DFI (%)	Age (years)[#]	Semen volume (ml)[*]	Sperm concentration (× 10^6/ml) [◊]	Total sperm count (× 10^6) [△]	Progressive motility (%)[#]	TPMSC (× 10^6)[#]
< 20%	3311 (64.7)	12.3±4.1/12.3	32.8 ±5.4/32.0	2.8±1.2/2.8	62.6±40.5/54.0	164.8±118.8/137.6	42.3±9.9/42.2	71.2±53.8/58.9
20–30%	1003 (19.6)	24.2±2.8/23.8	34.9 ±6.4/33.0	2.9±1.3/2.8	60.2±43.8/51.0	165.5±131.8/133.1	35.6±10.6/35.8	63.0±56.3/49.1
≥ 30%	800 (15.6)	40.9±9.9/37.8	36.8 ±6.8/36.0	2.8±1.4/2.8	59.7±48.0/47.1	161.6±143.9/117.6	28.3±11.8/30.1	52.6±54.0/35.4
Total	5114 (100)	19.1±11.7/15.9	33.8 ±6.0/32.0	2.8±1.3/2.8	61.7±42.4/52.5	164.4±125.6/135.0	38.8±11.6/39.1	66.7±54.8/54.1
p value	-	-	0.000	0.023	0.000	0.001	0.000	0.000

Data presented as mean ± standard deviation/median or *n* (%) unless otherwise noted

TPMSC: total progressive motile sperm count

[#] Age, progressive motility, and TPMSC were significantly different between DFI groups (p = 0.000)

[*]Semen volume was significantly different only between DFI < 20% and DFI ≥ 30% groups (p = 0.024)

[△]Total sperm count was significantly different only between DFI < 20% and DFI ≥ 30% groups (p = 0.001)

[◊]Sperm concentration of DFI < 20% group was significantly higher than other two groups (p = 0.008 and 0.000, respectively) while no significant difference was indicated between 20% ≤ DFI < 30% and DFI ≥ 30% groups (p = 0.639)

Sperm DFI, volume, concentration, total count, progressive motility, and TPMSC difference among age groups

The subjects were allocated to four groups according to age, including 1319 cases aged 20–29, 2877 cases aged 30–39, 831 cases aged 40–49, and 87 cases aged ≥ 50. The results suggested that sperm DFI and concentration increased with age; however, a significant difference was found in sperm DFI (p = 0.000) but no significant difference was found in sperm concentration among age groups (p = 0.186), which was just like that of the DFI groups (Table 2). At same time, the pairwise comparisons of semen volume revealed a significant difference between every two groups ($p < 0.05$ or $p < 0.001$). As for the total sperm count, it decreased with age but the only significant difference was observed between 20 and 29 and 40–49 age group (p = 0.008). Also, TPMSC and progressive motility significantly decreased ($p < 0.05$ or $p < 0.001$), with the exception of two oldest groups, i.e., 40–49 and ≥ 50 age group (Table 2). Also, a logical and consistent result of age was revealed in the asthenozoospermia group (35.7 ± 6.8 years) and oligoasthenozoospermia group (34.9 ± 6.7 years, Table 4).

Sperm DFI among male infertility cases and patients whose spouse experienced unexplained miscarriage

In order to further analyze the effects of sperm DFI and the routine sperm variables on clinical manifestation, subjects were categorized into primary infertility group, secondary infertility group, and unexplained miscarriage group according to their medical history and diagnostic information. The results showed that the semen volume, sperm DFI of the unexplained miscarriage group, was significantly lower than those of the primary and secondary infertility groups (p = 0.000 or 0.001), while no significant difference was indicated between the primary infertility and secondary infertility groups. On the contrary, the semen volume, total sperm count, progressive motility, and TPMSC of the unexplained miscarriage group were significantly higher than those of the primary and secondary infertility groups (p = 0.000 or 0.002) while no significant difference was indicated between the latter two groups. Additionally, the only significant difference of sperm concentration was found between the primary infertility and unexplained miscarriage group (p = 0.005) (Table 3).

Age and sperm DFI among different sperm groups

The differences and distribution characteristics of age and sperm DFI among different sperm groups were performed too (Table 4). The results showed that a maximum average age appeared in the asthenozoospermia group (35.7 ± 6.8 years) and oligoasthenozoospermia group (34.9 ± 6.7 years) and no significant difference was observed between the two

Table 2 Sperm DFI, volume, concentration, total count, motility, and TPMSC among different age groups

Age	Number (%)	Age (years)	Sperm DFI (%)[#]	Semen volume (ml) [#]	Sperm concentration ($\times 10^6$/ml)	Total sperm count ($\times 10^6$)[*]	Progressive motility (%) [◊]	Total motile sperm count ($\times 10^6$)[◊]
20–29 years	1, 319 (25.8)	27.5±1.6/28.0	16.1±9.6/13.8	3.0±1.3/2.8	60.2±40.2/51.8	169.7±124.0/141.8	40.6±11.5/41.0	71.2±54.5/58.6
30–39 years	2, 877 (56.3)	33.5±2.7/33.0	18.7±11.4/15.7	2.8±1.3/2.8	61.3±42.5/51.8	164.8±125.2/135.0	38.9±11.4/39.2	67.0±55.1/54.2
40–49 years	831 (16.3)	42.9±2.5/42.0	24.1±13.0/21.1	2.6±1.2/2.6	64.6±45.1/55.7	158.2±131.7/121.4	36.2±11.4/36.7	60.6±54.4/46.5
≥ 50 years	87 (1.7)	53.1±3.4/52.0	30.2±15.7/26.8	2.2±1.5/1.9	69.7±46.7/59.4	134.9±95.9/ 122.0	32.2±14.7/32.4	46.8±38.7/39.2
p value	-	-	0.000	0.000	0.186	0.003	0.000	0.000

Data presented as mean ± standard deviation/median or n (%) unless otherwise noted

TPMSC: total progressive motile sperm count

[#] Pairwise comparisons of sperm DFI and semen volume categorized by age revealed a significant difference between every two groups ($p < 0.05$ or $p < 0.001$)

*Total sperm count of 40–49 age group was significantly lower than 20–29 age group (p = 0.008) and no other significant difference was observed

◊Progressive motility and TPMSC showed a significant difference between every two groups ($p < 0.05$ or $p < 0.001$) with the exception of the two oldest groups, i.e., 40 ~ 49 and ≥ 50 group

Table 3 Sperm DFI, semen volume, and routine sperm parameters in cases of primary and secondary male infertility and patients whose spouse experienced unexplained miscarriage

Clinical group	Number (%)	Age (years) [#]	Sperm DFI (%)[*]	Semen volume (ml) [△]	Sperm concentration ($\times 10^6$/ml)[◊]	Total sperm count ($\times 10^6$)[△]	Progressive motility (%)[△]	TPMSC ($\times 10^6$)[△]
Primary infertility	1442 (28.2)	33.6±5.2/33.0	20.3±12.4/17.2	2.8±1.2/2.8	59.3±43.0/48.7	155.3±125.9/124.9	36.9±12.8/37.4	61.7±54.8/48.4
Secondary infertility	1318 (25.8)	37.7±6.8/37.0	20.5±12.0/17.0	2.7±1.3/2.7	62.6±43.5/54.0	159.0±122.0/128.7	38.0±11.1/38.6	63.2±52.8/50.6
Unexplained miscarriage	2354 (46.0)	31.8±4.9/31.0	17.6±10.9/14.6	2.9±1.3/2.8	62.7±41.4/54.3	173.1±127.0/143.5	40.4±10.8/40.4	71.7±55.5/59.1
p value	-	0.000	0.000	0.000	0.005	0.000	0.000	0.000

Data presented as mean ± standard deviation/median or *n* (%) unless otherwise noted

TPMSC: total progressive motile sperm count

[#] Age was significantly different between the unexplained miscarriage, primary infertility, and secondary infertility groups ($p < 0.05$)

*Sperm DFI of the unexplained miscarriage group was significantly lower than that of the primary and secondary infertility groups ($p = 0.000$) while no significant difference was indicated between the infertility groups

△Semen volume, total sperm count, progressive motility, and TPMSC of the unexplained miscarriage group were significantly higher than that of the primary and secondary infertility groups ($p = 0.000$ or $p = 0.002$) while no significant difference was indicated between the infertility groups

◊Sperm concentration of the primary infertility group was significantly lower than that in unexplained miscarriage groups ($p = 0.005$)

Table 4 Comparison of age and sperm DFI among groups classified by semen variables

Sperm group	Number (%)	Age (years)[#]	Sperm DFI (%)[*]	Semen volume (ml)[△]	Sperm concentration ($\times 10^6$/m)[◊]	Total sperm count ($\times 10^6$) [◊]	Progressive motility (%)[*]	TPMSC ($\times 10^6$)
Normospermia	3828 (74.9)	33.4±5.7/32.0	16.4±9.1/14.4	3.0±1.3/2.8	69.3±41.0/61.6	188.4±121.9/158.1	43.3±7.9/42.1	81.0±53.7/68.4
Oligozoospermia	220 (4.3)	34.3±5.9/33.0	16.3±8.2/14.5	1.7±0.9/1.5	18.3±11.1/16.0	26.0±9.0/27.7	40.9±7.4/38.9	10.8±4.5/11.1
Asthenozoospermia	729 (14.3)	35.7±6.8/35.0	29.6±14.6/27.4	2.8±1.3/2.8	58.1±41.3/46.4	147.6±117.9/107.5	24.3±6.9/26.2	37.7±34.7/25.8
Oligoasthenozoospermia	337 (6.6)	34.9±6.7/33.0	28.8±14.9/26.3	2.0±1.0/1.8	11.9±9.3/9.0	19.6±10.1/19.2	17.7±8.5/18.0	3.7±2.8/2.9
p	-	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Data presented as mean ± standard deviation/median or *n* (%) unless otherwise noted

TPMSC: total progressive motile sperm count

[#] Age was significantly different between oligoasthenozoospermia and normospermia as well as asthenozoospermia and normospermia ($p = 0.000$)

*Sperm DFI was significantly different between every two groups ($p = 0.000$) with the exception of normospermia and oligozoospermia group ($p = 1.000$) and of asthenozoospermia and oligoasthenozoospermia ($p = 1.000$)

△Semen volume was significantly different between every two groups ($p < 0.001$ or $p < 0.005$)

◊Sperm concentration and total sperm count were significantly different between every two groups ($p = 0.000$) except the oligoasthenozoospermia and oligozoospermia group ($p = 0.102$)

*Sperm progressive motility was significantly different between every two groups ($p = 0.000$ or $p = 0.050$)

TPMSC was significantly different between every two groups ($p = 0.000$) with the exception of the oligoasthenozoospermia and oligozoospermia group ($p = 0.058$)

groups (p = 0.451). Significant difference was just found between the oligoasthenozoospermia and normospermia as well as the asthenozoospermia and normospermia group (p = 0.000). Sperm DFI also showed similar results, that is, the highest DFI value appeared in the asthenozoospermia group (29.6 ± 14.6%) and the oligoasthenozoospermia group (28.8 ± 14.9%). Actually, sperm DFI has a significant difference between every two groups (p = 0.000) with the exception of the normospermia and oligozoospermia group (p = 1.000) and of the asthenozoospermia and oligoasthenozoospermia group (p = 1.000).

Correlation analysis of sperm DFI with age and semen parameters

As described above, regardless of whether the participants were grouped by sperm DFI or age or clinical manifestation, the difference of sperm progressive motility, total motile count, and total sperm count could be observed among the groups. Since that sperm DFI and routine sperm parameters are both related with age, the associations of these parameters were worthy of further exploration. Spearman correlation analysis indicated that sperm DFI was positively correlated with age (r_s = 0.255, p = 0.000), while negatively correlated with sperm progressive motility (r_s = − 0.465, p = 0.000). However, the correlation between sperm DFI and sperm concentration (r_s = − 0.058, p = 0.000), semen volume (r_s = 0.046, p = 0.001), total sperm count (r_s = − 0.025, p = 0.079), and motile sperm count (r_s = − 0.153, p = 0.000) did not exist for the r_s values were so low (Table 5).

Discussions

Sperm DNA fragmentation index (DFI), a measure of the percentage of sperm with DNA damage present in an ejaculate, has been proposed as a novel sperm test due to the restrictions of traditional sperm analysis. Till now, the effective detection methods of sperm DFI include sperm chromatin structure analysis (SCSA), terminal transferase-mediated dUTP end labeling (TUNEL) assay, single-cell gel electrophoresis (SCGE) assay, sperm chromatin dispersion (SCD) test, etc. [17]. SCSA was first described by Evenson et al. [18] to evaluate sperm chromatin integrity and provide additional information about the fertilizing capacity of the sperm. Currently, SCSA is the preferred one for its high reproducibility [17] and has been proved to be an independent predictor of male fertility in vivo [19]. Owing to the high incidence of sperm DFI in the infertile men [20] and the couple with miscarriage [15], there is some information regarding the clinical value of assessing sperm DFI in male infertility besides an advanced sperm function test [6] to evaluate the fertility of the individual. However, the correlations of these parameters are inconsistent and still at issue for small sample size, variable patient populations, different methods for assessing DNA damage, and other factors, which have been mentioned in the "Introduction" section.

The routine sperm parameters were significantly different among groups classified by DFI value, especially between DFI < 20% and DFI ≥ 30% groups (Table 1). It is worthy of notice that progressive motility and TPMSC were significantly different between every two DFI groups (p = 0.000). Consistently, the significant increase of sperm DFI was observed in two groups with decreasing sperm motility, i.e., asthenozoospermia group (29.6 ± 14.6%) and the oligoasthenozoospermia group (28.8 ± 14.9%, Table 4). It was very interesting that, in the sense of statistics, there was completely no statistically significant difference with sperm DFI between the normospermia and oligozoospermia group (p = 1.000) and the asthenozoospermia and oligoasthenozoospermia group (p = 1.000) (Table 4).It could be interpreted as there was no correlation between sperm DFI and concentration, which is exactly consistent with the spearman correlation analysis (Table 5). Of course, its greater significance lies in the judgment of the integrity of the inherent sperm genetic material and effect on the outcome of pregnancy [6], which need more clinical evidence from obstetrical practice. A recent study from Winkle et al. [21] suggested that no significant correlation between male age and sperm DNA fragmentation was found between the patients and the controls as well as the different age populations. The conclusion was also supported by other previous documents [21–23]. However, our study revealed that a steady ascent in sperm DNA fragmentation with increasing age of the participants (r_s = 0.255, p = 0.000), i.e., sperm DNA damage, worsen gradually with the increase of age, which is consistent with another report [24] and some elder ones [23, 25–28]. Although the conclusions are not uniform, the majority of studies show a positive correlation between age and DNA damage [27]. It had been reported that sperm DFI in men with age ≤ 35 years was significantly lower than that in men with age between 36 and 39 years and above 40 years, and no significant difference in the latter two [26]. A homologous result was shown by our data that sperm DFI in men with age < 40 years was significantly lower than that in men with age

Table 5 Spearman correlation analysis of sperm DFI with age, sperm volume, concentration, count, progressive motility, and TPMSC

	Age	Sperm volume	Sperm concentration	Total sperm count	Progressive motility	TPMSC
r_s	0.255	0.046	− 0.058	− 0.025	− 0.465	− 0.153
p	0.000	0.001	0.000	0.079	0.000	0.000

TPMSC: total progressive motile sperm count

between 40 and 49 years and above 50 years. However, a significant difference was also observed in the latter two groups ($p < 0.050$). Actually, during the process of generation and maturation, the sperm will be affected by a variety of intra- and/or extratesticular adverse factors, such as temperature, radiation, chemical pollution, aging, etc. A clear relationship is known to exist between most of these situations and increased production of reactive oxygen species (ROS), which are detrimental to sperm DNA and cause its fragmentation after entering the cell nucleus [29, 30]. On the other hand, ROS seriously affect sperm motility too [31]. As a result, the sperm DNA fragments increase and the progressive motility gradually decreases, which was supported by our data that there is a significant correlation between sperm DNA fragmentation and sperm progressive motility ($r_s = -0.465$, $p = 0.000$, Table 5). Thus, chromatin integrity of spermatozoon is associated with increased age-related risk of infertility and a reduced probability of fathering a successful pregnancy. It coincided with the significant age difference between the infertility and unexplained miscarriage group in our study ($p = 0.000$, Table 3).

No significant correlation between male age and conventional semen parameters was recently reported by Winkle et al. [21]. However, our study manifested a significant correlation between sperm DNA fragmentation and sperm progressive motility ($r_s = -0.465$, $p = 0.000$). In addition, a tendency of sperm concentration improving with the increasing of age could be observed (Table 2) which was consistent to a series reports [21, 23, 28, 32, 33] but only one [28] showed a statistically significant. On the contrary, Levitas et al. [34]] detected a significant and inverse relationship between sperm quality and patients' age. The contradictory results might lay in many potential confounders, such as duration of abstinence, infertile or fertile male or volunteers, different classification of patients, different assays, etc.

As aforementioned, sperm concentration, count, and motility are traditional parameters essential to the semen quality evaluation but do not reveal sperm functional competence and reproductive potential. Sperm DFI is useful for determining sperm DNA integrity as a means to accurately assess the fertility of male and it is independent of semen parameters. Lu et al. [11] had found a negative correlation between sperm DNA fragmentation and sperm progressive motility ($r_s = -0.474$, $p < 0.001$) using the SCSA method. In our article, a very similar inverse correlation ($r_s = -0.465$, $p = 0.000$) was revealed too (Table 5). Identical conclusion had been drawn from a multitude of previous studies [35–37]. Consistently, there is a moderate or above correlation between sperm DFI and progressive motility in age and clinical subgroups with the exception of 20–29 years subgroup ($r_s = -0.387$, Table 5). In clinical ICSI procedure, the spermatozoa with fast progressive motility (means less DNA fragment) and normal appearance are often preferred, which may be the reason that ICSI improves the pregnancy outcome of infertile patients with high sperm DFI more than the IVF procedure [38].

Also as aforementioned, the greater significance of sperm FDI lies in the judgment of integrity of sperm genetic material and the effect on the outcome of pregnancy. However, research on unexplained miscarriage, including the recurrent one, has primarily focused on maternal causes, but there is a growing evidence demonstrating the impact of male factors. More than one meta-analysis has demonstrated a significant relationship between levels of DNA damage in sperm and pregnancy loss [15, 39]. In the current research, we study the characteristics of sperm DFI in both infertile males and patients whose partner had unexplained miscarriage. The results revealed that among primary and secondary infertility and patients whose spouse experienced unexplained miscarriage, the significance of semen volume difference was uncertain and it might only reflect the secretory function of accessary gland, while the function was closely related with age, which echoes the results in Table 2. As shown in Table 3, the total sperm count, progressive motility, and TPMSC of the unexplained miscarriage group was significantly higher than that of infertility group. It could be explained that the number of sperm, especially the number of progressive motility sperm, is the basic condition and guarantee in the fertilization process and DFI is the intrinsic molecular marker of sperm function [8]. Bungum et al. [40] proposed that sperm DFI detected by SCSA was an independent predictor of male fertility in vivo and Giwercman et al. [41] demonstrated that the patients with sperm DFI over 10% plus one abnormal standard sperm parameter had the increased odds ratio for infertility compared with fertile controls. Moreover, even men with normal sperm parameters, the odds ratio for infertility would increase when sperm DFI rise to 20%. As a result, chances of pregnancy achieved by intercourse or by intrauterine insemination decreased. Furthermore, when sperm DFI exceeded the level of 30%, the chances of conception would be close to zero. In this article, the primary and secondary infertility males had equivalent sperm DFI values less than 30% (20.30% and 20.49%, respectively). The other interesting finding was a lower DFI (17.6 %) in the unexplained miscarriage (Table 3). It might lie in that the distinction between the recurrent unexplained miscarriage and occasional one was not made in our study. However, most of published works focused on recurrent cases and the multiple definitions of recurrent unexplained miscarriage [42, 43]. However, they failed to father a pregnancy regardless of sperm parameters. In theory, sperm with DNA damage are capable of fertilizing an egg [38], while incapable of DNA repair, so they rely on the oocyte and/or fertilized eggs for repair post-fertilization. Yet, once the DNA fragments exceed their repair ability, the "late paternal effect" [16] during the activation of male gene expression would work and might contribute to poor blast development, implantation failure, and miscarriage [44]. On the other hand, it is widely acknowledged that oocyte quality is strongly attributed to female age, which is not documented in the current research. However, what worthy of notice is that the male age of miscarriage group is

significantly younger than the infertility group in our study (Table 3). It might lie in the duration of the attempt to conceive and the arrangement of the birth plan. On the other hand, aging has a significant impact on sperm parameters and fertility; both of which contribute to poor fecundability and increased time to pregnancy [45]. As a matter of fact, higher levels of DNA damage were confirmed in sperm of the older men as these populations have been shown to have more double-strand DNA breaks [46]. Therefore, the significant difference in sperm DFI between the male infertility group and miscarriage group is partly due to the difference in age.

In the present study, we did not conduct genetic tests other than sperm DNA damage, which is one shortcoming of this study. The other deficiency is the lack of restriction for female age and controls for normal fertility men. Future studies should carefully consider these variables.

Taken collectively, assaying sperm DFI and semen analysis in 2760 infertile male and 2354 male indicated that sperm DFI was positively correlated with age, but negatively correlated with sperm progressive motility. Based on the current data from a big sample, we may infer boldly that sperm DFI was an important indicator for evaluating the quality of semen. Till now, although there is insufficient evidence to recommend the routine use of sperm DNA integrity tests in the evaluation and treatment of the infertile couple (Level C) [2], sperm DNA integrity testing is preferentially recommended to those who have decreased sperm progressive motility, especially the elder man. An integrative analysis of sperm DFI, sperm progressive motility, age, and infertility conditions can provide a more comprehensive assessment of male fertility which will further be a benefit to the diagnosis, prognosis, and ultimately directional treatment of male infertility.

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Authors' contributions Yonghong Tian and Fan Jin conceived the idea and designed the study. Fengbin Zhang and Jinggen Wu collected the retrospective clinical data; Jingping Li and Zhongyan Liang analyzed data and conducted the statistical analysis. Fengbin Zhang and Chong Chen wrote the manuscript. Yonghong Tian and Lejun Li reviewed and assessed the manuscript.

Declarations

Ethical approval This study received ethical approval from the Institutional Review Boards of our facility (IRB-20200152-R).

Conflict of interest The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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