

ORIGINAL ARTICLE

Correlation of DNA fragments with routine semen parameters and lifestyle and their impact on assisted reproductive outcomes



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KEYWORDS

Semen analysis;
Embryonic
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fragmentation index

Abstract

Objective: This study investigated the correlation between sperm DNA integrity and routine semen evaluation parameters in male infertile patients, the influencing factors, and the impact of the DNA fragmentation index (DFI) on embryo quality and clinical outcomes in in vitro fertilization and embryo transfer (IVF-ET).

Methods: Sperm DFI and semen routine parameters of 6160 infertile men admitted between June 2017 and June 2018 were analyzed. Patients were divided into three groups according to their DFI: low-DFI (DFI < 15%), medium-DFI (15% < DFI ≤ 30%), and high-DFI (DFI > 30%). The correlations of DFI with patients' age, sperm concentration, sperm percentage of forward movement and sperm percentage of normal shape were analyzed. The clinical data of 5040 infertile couples who received IVF treatment between June 2016 and 2021 and had embryos transferred in a fresh cycle were reviewed. The fertilization rate, cleavage rate, blastocyst rate, and pregnancy rate in different DFI groups were compared.

Results: Semen evaluation parameters (concentration, spermatozoa with progressive motility, and the normal morphology rate), the high-quality embryo rate, blastocyst development rate, and pregnancy rate in the high-DFI group were significantly lower than those in the other two groups. The correlation analysis revealed that sperm DFI was negatively correlated with semen concentration, sperm motility, and normal sperm morphology and positively correlated with the man's age, BMI, and unhealthy habits (smoking and drinking). There was no significant difference in the number of mature eggs and normal fertilization rate among groups.

Conclusion: A strong correlation exists between sperm DFI and semen evaluation parameters. Smoking, drinking, and other unhealthy habits lead to an increase in DFI, reducing the high-quality embryo rate and blastocyst development rate and affecting pregnancy outcomes.

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Abbreviations: DFI, DNA fragmentation index; BMI, body mass index; ART, assisted reproductive technology; IVF-ET, in vitro fertilization and embryo transfer; SCSA, sperm chromatin analysis; FSH, follicle stimulating hormone; TZI, teratozoospermia index; SDI, sperm deformity index; PR, rapid forward motility sperm; NP, non forward motile sperm; IM, immobile sperm.

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PALABRAS CLAVE

Análisis seminal;
Desarrollo
embrionario;
Final del embarazo;
Índice de
fragmentación del
adn espermático

Correlación de la fragmentación de adn con los parámetros seminales convencionales y el estilo de vida y su impacto en los resultados de la reproducción asistida integridade do DNA espermático e resultados da gravidez

Resumo

Objetivo: Avaliar a correlação entre a integridade do DNA espermático de pacientes com infertilidade masculina e os parâmetros de avaliação seminal convencional, os fatores que a impulsionam, a influência do índice de fragmentação de DNA (DFI) na qualidade embrionária, o desfecho clínico na fertilização *in vitro* e a transferência de embriões (fiv-te).

Métodos: Foram analisados o DFI espermático e os parâmetros de rotina seminal de 6.160 homens com infertilidade admitidos entre junho de 2017 e junho de 2018. Os pacientes foram divididos de acordo com o DFI em baixo ($DFI < 15\%$), médio ($15\% < DFI \leq 30\%$) e alto ($DFI > 30\%$). Foi analisada a correlação do DFI com a idade dos pacientes, concentração espermática, porcentagem de espermatozoides motilizados para frente e porcentagem de espermatozoides com morfologia normal. Foram revisados dados clínicos de 5.040 casais inférteis que receberam tratamento de fertilização *in vitro* entre junho de 2016 e 2021 e transferiram embriões no novo ciclo. As taxas de fertilização, clivagem, blastocisto e concepção foram comparadas entre os diferentes grupos de DFI.

Resultados: Os índices de avaliação seminal (concentração, espermatozoides com movimento progressivo e morfologia normal), embriões de boa qualidade, desenvolvimento de blastocistos e prenhez foram significativamente menores no grupo com alto DFI. As análises de correlação mostraram que o DFI espermático está negativamente relacionado com a concentração seminal, motilidade espermática, morfologia espermática normal e positivamente relacionado com a idade dos homens, IMC e hábitos adversos (tabagismo e consumo de álcool).

Conclusão: Houve forte correlação entre o DFI espermático e os parâmetros de avaliação seminal. Hábitos indesejáveis, como tabagismo e consumo de álcool, levam ao aumento do DFI, diminuição da taxa de embriões de boa qualidade e desenvolvimento de blastocistos e comprometem o desfecho gestacional.

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Introduction

Infertility in approximately 50% of couples of childbearing age worldwide can be attributed to men, with the most common cause being abnormal semen quality.¹ Semen quality is the most basic index for evaluating male reproductive health. Smoking, drinking, increased body mass index (BMI), and age all lead to a decrease in semen volume, an increase in the abnormal sperm morphology rate, and a decrease in sperm motility, all of which may lead to worse pregnancy and live-birth outcomes in couples receiving therapy from assisted reproduction technologies (ART).² A study revealed that sperm DNA fragmentations increased significantly in men over 45 years old, almost twice as much as in younger men, and the integrity of sperm DNA was crucial for the success of in vitro fertilization (IVF) and normal embryonic development.³

In the process of spermatogenesis, maturation, and fertilization, the conversion of histone and protamine plays a role in maintaining DNA stability and inhibiting gene expression; these two structural regions are related to embryonic development after fertilization. Spermatozoa with defective DNA integrity can fertilize oocytes but may affect subsequent embryonic development.⁴ Both spermatozoa and high-quality oocytes have the ability to repair a certain

proportion of sperm DNA damage, but exceeding the range causes abnormal embryonic development. A high sperm DNA fragmentation index (DFI) increases sperm aneuploidy, which, after fertilization, may damage embryo quality and viability and the probability of a sustained pregnancy, finally leading to miscarriage.^{5,6}

In this study, we employed a sperm chromatin structure assay (SCSA) to detect sperm DFI. Through a case analysis of 5040 fresh cycles of IVF and embryo transfer (IVF-ET) at the Reproductive Medicine Center, Inner Mongolia Medical University, we explored the predictive value of DFI on IVF embryonic development and pregnancy outcomes and analyzed the correlation of sperm DFI with men's age, body mass index (BMI), lifestyle (smoking and drinking), and routine semen evaluation parameters to assess the role of DFI in evaluating male fertility and predicting pregnancy outcomes.

Materials and methods

Study participants

In this retrospective study, the semen test reports of 6160 male infertility patients who came to our center between June 2017 and December 2018 were reviewed. The clinical

data of 5040 infertile couples received IVF treatment and embryos transferred in fresh cycles in our center between January 2016 and June 2020 were also reviewed. A physical examination revealed no abnormalities in any of the patients. In terms of the female patients, their age was ≤ 35 years old, they had normal menstruation, follicle stimulating hormone levels were <10 IU/mL, simple infertility due to pelvic tubal factors, and the number of cleavage-stage embryos was ≥ 3 . All male patients recruited in this study were at an age of ≤ 45 years, healthy, and with obvious secondary sexual characteristics, no peripheral blood Chromosome abnormality or Y chromosome AZF microdeletions, no history of genital trauma, infection, and no family history of genetic disorder. The criteria were formulated according to the World Health Organization Laboratory Manual for the Examination and Processing of Human Semen, 5th Edition.⁶ None of the patients had chromosome abnormalities. Patients were informed of the details of the study. They provided informed consent and voluntarily participated in the study.

Research methods

Routine semen analysis

According to the World Health Organization Laboratory Manual for the Examination and Processing of Human Semen (5th edition),⁶ semen was collected by masturbation after 3–5 days of abstinence. Sperm concentration, total sperm count, and sperm motility were measured by a semen analysis system. The motility index was calculated according to the percentage of forward motile sperm (PR), non-forward motile sperm (NP) and immobile sperm (IM). The sperm survival rate was detected by EOSIN's staining and hypotonic swelling test. Normal sperm morphological rate and TZI were calculated.

Sperm DNA integrity test

Sperm samples were stained with acridine orange by the SCSA method using the nuclear integrity staining kit. The fluorescent signals were analyzed by the Navios Flow cytometry (Beckman Coulter, USA) and the streaming results were processed with the DFI Viewer software. The chromatin structure of damaged sperm is loose and the DNA is denatured by acid to form a single strand, while normal sperm DNA maintains a double strand. The acridine orange free base (AO) binds to double-stranded DNA and emits green fluorescence, while single-stranded DNA binds to yellow or red fluorescence. Sperm were stained with the acridine orange free base and analyzed by flow cytometry. A certain amount of semen was diluted to a final concentration of $(1-2) \times 10^6$ cells/mL. Then, 200 μ L of B solution was added for 30 s (cells were placed on ice), and 600 μ L of C solution was added. Each sample contained at least 5000 sperm cells for flow cytometry analysis, the results of which were analyzed by the flow cytometry software. The integrity of the DNA was expressed as a result of the DNA fragmentation index (DFI).

Controlled ovarian hyperstimulation program

All participants were treated with a standard protocol. When at least 1 follicle diameter was ≥ 18 mm or at least

3 follicle diameter was ≥ 16 mm, the dosage of Gn was adjusted according to hormone level and follicular development. After 36–38 h, the oocytes were collected by vaginal puncture under the guidance of ultrasound. The sperm of the man was extracted by masturbation and the sperm concentration was adjusted to $(0.7-1.0) \times 10^6$ /mL by density gradient centrifugation and upstream method. The pronuclear (PN) was observed at 16–18 h after fertilization, and the 2PN was a normal fertilized ovum. The embryo development was observed up to the 3rd–6th day and the blastocyst formation was observed. The blastocysts were evaluated by the Gardner blastocyst grading method.⁷ Serum B-human Gonadotropin (β -HCG) was measured 14 days after transplantation to confirm biochemical pregnancy, and intrauterine pregnancy sac was detected 28 days after transplantation to confirm clinical pregnancy.

Statistical analysis

The statistical analysis was conducted using SPSS version 26.0 software. The normality of the data was checked by the Lilliefors and Kolmogorov–Smirnov tests. Measurement data were expressed as mean $\pm$ standard deviation ($\bar{x} \pm SD$). Multiple means were compared by one-way ANOVA and multiple independent groups were compared by χ^2 test. The level of significance was set at $\alpha = 0.05$, and $P < 0.05$ was considered statistically significant. The Pearson and Spearman correlation coefficients were used to analyze the correlations, and $P < 0.05$ was considered statistically significant.

Results

Comparison of sperm DFI and routine semen evaluation parameters

A total of 5040 IVF cycles met the inclusion criteria. The semen concentration, spermatozoa with progressive motility, and normal morphology rate in the high-DFI group were considerably lower than those in the other two groups, and the differences were statistically significant ($P < 0.05$); no significant difference was identified in semen volume and the abnormal sperm index ($P > 0.05$, Table 1).

Correlation of sperm DFI with routine semen evaluation parameters and lifestyle

The analysis of the correlation of sperm DFI with routine semen evaluation parameters and lifestyle revealed that sperm DFI was positively correlated with age, BMI, abnormal sperm index, smoking, and drinking ($P < 0.05$), and the differences were statistically significant (Table 2). A statistical analysis of age, drinking, and smoking in each group was also performed, demonstrating that DFI values increased with age, and the difference was statistically significant ($P < 0.01$). The DFI values significantly increased with the frequency of smoking and drinking ($P < 0.05$, Table 3).

Table 1 Comparison of basic characteristics of sperm DFI and semen routine parameters in different groups.

Index	DFI $\leq$ 15% <i>n</i> = 1980	15% < DFI $\leq$ 30% <i>n</i> = 1760	DFI > 30% <i>n</i> = 1300	<i>F</i>	<i>P</i>
Semen volume (mL)	4.11 $\pm$ 1.82	4.03 $\pm$ 1.78	4.14 $\pm$ 1.96	0.038	>0.05
Sperm concentration ($\times 10^6$ /mL)	55.82 $\pm$ 19.86 ^b	42.07 $\pm$ 10.01 ^a	30.86 $\pm$ 18.36 ^{ab}	1.890	<0.05
PR (%)	40.74 $\pm$ 19.42	38.8 $\pm$ 18.94	33.25 $\pm$ 15.25 ^{ab}	2.719	<0.05
NP (%)	10.55 $\pm$ 6.13	11.66 $\pm$ 13.18	12.43 $\pm$ 8.77	0.029	>0.05
IM (%)	48.71 $\pm$ 19.12	49.54 $\pm$ 27.4	54.35 $\pm$ 20.96	0.585	>0.05
Normal morphology rate (%)	5.31 $\pm$ 3.61 ^b	3.42 $\pm$ 3.2 ^a	2.51 $\pm$ 2.0 ^{ab}	4.880	<0.01
Sperm deformity index (%)	1.27 $\pm$ 0.12	1.37 $\pm$ 1.41	1.28 $\pm$ 0.11	0.307	>0.05
Teratozoospermia index (%)	1.23 $\pm$ 0.12	1.35 $\pm$ 1.38	1.22 $\pm$ 0.12	0.316	>0.05

a: *P* < 0.05 versus the DFI $\leq$ 15% group.b: *P* < 0.05 versus the 15% < DFI $\leq$ 30% group.**Table 2** Correlation analysis between sperm DFI and semen parameters and lifestyle.

Parameter	<i>r</i>	<i>P</i>
Age	0.185	<0.05
BMI	0.121	<0.05
Abstinence days	0.031	>0.05
Duration of infertility	0.042	>0.05
Semen volume	0.019	>0.05
PH	0.024	>0.05
Sperm concentration	-0.562	<0.05
PR	-0.391	<0.01
NP	-0.017	>0.05
IM	0.229	<0.01
Normal morphology rate	-0.230	<0.01
TZI	0.105	<0.05
SDI	0.102	<0.05
Smoking(Y/N)	0.202	<0.01
Drinking(Y/N)	0.185	<0.05

Table 3 Relationship of sperm DFI to age, alcohol consumption and smoking.

Index	Sperm DFI	<i>F</i>	<i>P</i>
Age		6.32	<0.01
<30 (<i>n</i> = 1740)	13.36 $\pm$ 7.31		
30–40 (<i>n</i> = 2596)	14.68 $\pm$ 8.79		
>40 (<i>n</i> = 704)	20.35 $\pm$ 14.01		
Alcohol consumption (Times/week)		5.1	0.038
No drinking(<i>n</i> = 960)	15.01 $\pm$ 8.35		
1–2 (<i>n</i> = 1300)	15.98 $\pm$ 9.11		
>2 (<i>n</i> = 1800)	19.21 $\pm$ 11.58		
Smoke (cigarettes/day)		8.13	<0.01
No smoking (<i>n</i> = 523)	14.91 $\pm$ 9.18		
1–10 (<i>n</i> = 2134)	17.01 $\pm$ 9.56		
10–20 (<i>n</i> = 1577)	17.98 $\pm$ 11.5		
>20 (<i>n</i> = 806)	23.17 $\pm$ 13.1		

Impact of sperm DNA damage on embryo quality and pregnancy outcomes

No significant differences were identified between the different DFI groups in terms of basic data, such as age and the number of years of infertility (*P* > 0.05). Pregnancy assistance and pregnancy outcomes were compared, indicating that the high-quality embryo and blastocyst development rates in the high-DFI group (DFI > 30%) were significantly lower than those in the other two groups, and the differences were statistically significant (*P* < 0.05). No significant differences were revealed in the number of mature eggs and normal fertilization rate (*P* > 0.05, Table 4).

Discussion

Infertility is a global problem. Although its incidence varies from country to country, an upward trend, caused by environmental, lifestyle, and other factors, is evident. An increasing number of epidemiological studies have demonstrated that the quality of male semen is decreasing, with more than 50% of couples experiencing male reproductive

function defects.⁷ Up to 40% of unexplained infertility disorders may be related to a high DFI, and the duration of infertility is longer for those with a high DFI than for those with a low DFI.⁸ The sperm DFI is a parameter that reflects the sperm chromatin structure, and it can be used to evaluate male fertility, predicting the clinical outcomes of ART to some extent. In natural pregnancy, the decrease in male semen quality also plays a key role in recurrent miscarriage and fetal development stagnation. This study revealed that semen concentration, sperm motility, and normal morphology rates in the high-DFI group were significantly lower than those in the other two groups; with an increase in DFI, the sperm concentration and normal morphology rate decreased. These results are consistent with the conclusions provided by Luo et al.⁹ Some studies have concluded that patients with low sperm density had considerably higher semen DFI.¹⁰ However, Chi et al. revealed that¹¹ sperm DFI was closely related to sperm motility and morphology but not to other semen parameters. Studies have also revealed that the excessive accumulation of reactive oxygen species and the low expression of antioxidant enzymes in semen with a high DFI may cause damage to membrane lipids, sperm nuclei, mitochondrial DNA strands, the sperm

Table 4 Comparison of IVF pregnancy outcomes with different sperm DFI.

Index	DFI $\leq$ 15% <i>n</i> = 1980	15% < DFI $\leq$ 30% <i>n</i> = 1760	DFI > 30% <i>n</i> = 1300	χ^2	<i>P</i>
Duration of infertility (yr)	3.84 $\pm$ 2.33	3.82 $\pm$ 2.93	3.88 $\pm$ 2.83	0.286	0.413
Female age (yr)	31.5 $\pm$ 2.41	31.8 $\pm$ 4.01	31.33 $\pm$ 4.65	1.33	0.15
Female BMI (kg/m ²)	23.1 $\pm$ 3.15	23.9 $\pm$ 3.5	22.9 $\pm$ 3.8	0.88	0.32
Basic FSH (IU/L)	7.2 $\pm$ 3.58	6.9 $\pm$ 1.28	6.8 $\pm$ 2.33	0.87	0.33
Male age (yr)	32.5 $\pm$ 4.8	33.7 $\pm$ 6.0	34.1 $\pm$ 2.1	1.07	0.28
Male BMI (kg/m ²)	24.6 $\pm$ 5.8	24.3 $\pm$ 3.9	25.3 $\pm$ 3.1	1.29	0.24
Retrieved oocytes (<i>n</i>)	12.7 $\pm$ 5.58	12.59 $\pm$ 5.31	12.21 $\pm$ 4.68	1.159	0.25
Mature oocytes (<i>n</i>)	12 $\pm$ 4.66	11.6 $\pm$ 4.32	11.3 $\pm$ 4.81	8.65	0.096
Fertilization rate (%)	15,620/23,740 (65.8%)	12,833/20,020 (64.1%)	9109/14,300 (63.7%)	9.51	0.081
Cleavage rate of oocytes (%)	13,277/15,620 (85.0%)	10,664/12,833 (83.1%) ^a	7424/9109 (81.5%) ^{ab}	18.72	<0.05
High-quality embryo rate (%)	8986/14,085 (63.8%)	6036/10,498 (57.5%) ^a	2957/6958 (42.5%) ^{ab}	16.38	<0.05
Blastocyst development rate (%)	3777/5534 (68.25%)	2402/4050 (59.3%) ^a	1218/2583 (47.15%) ^b	19.13	<0.05
Biochemical pregnancy rate (%)	68.18 (1350/1980)	65.98 (1161/1760)	55.14 (717/1300) ^{ab}	15.12	<0.05
Clinical pregnancy rate (%)	66.87 (1324/1980)	65.17 (1147/1760)	54.11(703/1300) ^{ab}	14.14	<0.05
Miscarriage rate (%)	18.4% (244/1324)	5.0% (57/1147)	0.0% (5/703)	3.36	>0.05
Live birth rate (%)	50.0% (990/1980)	27.9% (491/1760)	16.7% (217/1300)	10.04	<0.05

a: *P* < 0.05 versus the DFI $\leq$ 15% group.

b: *P* < 0.05 versus the 15% < DFI $\leq$ 30% group.

ultrastructure, and genetic materials. Sperm chromatin not only protect the DNA of male parents but also provide epigenetic information supporting embryonic development, and a high DFI leads to chromatin cross-linking, affecting sperm maturation and nucleospermin formation,^{12,13} resulting in a decrease in semen quality.

Spermatogenesis is a complex process, which itself lacks effective anti-injury protection and repair mechanisms, and it is vulnerable to the joint influence of environmental and genetic factors during division and differentiation. The results of the present study revealed that sperm DFI was positively correlated with men's age, BMI, and unhealthy habits, which is consistent with the results of Albani and Deenadayal.^{14,15} A further group analysis of drinking and smoking revealed that DNA fragmentations increased significantly with the increase in men's age, and the sperm DFI of men significantly increased with the frequency of smoking and drinking. However, nicotine causes sperm cell apoptosis by activating the tumor necrosis factor signaling pathway and inhibiting telomerase activity, and sperm DNA instability reduces the number of spermatozoa and increases the number of sperm morphological defects.¹⁶ Sharma et al. revealed that smoking affected sperm quantity and motility, with the sperm quality of moderate and severe smokers decreasing considerably. This process was irreversible.¹⁷ The intake of ethanol is related to the central role of the hypothalamus in the reproductive system, which affects hormone secretion and leads to spermatogenesis disorders. An unhealthy lifestyle significantly changes the methylation pattern of sperm DNA, resulting in sperm DNA damage. Obesity can also cause changes in the testicular environment, increasing the level of inflammatory factors in the body and the oxidative stress response, and negatively affecting the niche of the spermatogenic epithelium, which is required for spermatogenesis, resulting in adverse effects on sperm structure and function.

Abnormalities at any stage of spermatogenesis affect the integrity of sperm genetic material, which then affects sperm quality and embryonic development.¹⁸ This study revealed that no significant difference existed between the different DFI groups in terms of the normal fertilization rate, and the high-quality embryo, blastocyst development, and pregnancy rates in the high-DFI group were considerably lower than those in the other two groups. A study noted that the fertilization of oocytes by spermatozoa is not restricted by the quantity of sperm DNA fragmentations but is related to the quality of oocytes. Because early embryonic development is controlled by maternal genes, a small amount of sperm DNA damage can be repaired. The male parent gene begins to be expressed at the 4–8 cell stage of embryonic development,¹⁹ and its effect on embryonic development is more obvious in the later stages of pregnancy. A large quantity of sperm DNA fragmentations may delay embryonic development and reduce the high-quality embryo rate, which has a significant impact on the miscarriage and live-birth rate.²⁰ Among the male factors leading to low embryo quality, routine semen evaluation parameters and the sperm DNA integrity index, as well as their impact on embryo quality, are attracting increasingly more attention from scholars. A large number of experimental studies and meta-analyses have revealed that sperm DNA fragmentations affect sperm–egg binding, embryo formation, and differentiation and developmental potential, and are even related to birth defects.^{21–23} However, some studies²⁴ demonstrated no significant correlation between sperm DFI and clinical pregnancy outcomes from assisted reproduction, concluding that the deformity rate was reduced after semen treatment, which weakens the impact of DFI on clinical pregnancy outcomes.

In this study, the correlation of male sperm DFI with routine semen evaluation parameters and lifestyle in Inner Mongolia was retrospectively analyzed, and the impact of

DFI on pregnancy outcomes was evaluated. The influencing factors for male infertility are complex because sperm DNA damage is the result of multiple factors. Although infertile men can reproduce by assisted conception, spermatozoa with damaged DNA may increase the risk of genetic diseases in children. Sperm DFI may be only one of the direct or indirect factors affecting natural pregnancy and ART outcomes. However, as our center did not have the initial sperm donor data, statistical analysis was not included. Further studies are needed to avoid selection bias and better guide clinical practice.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document.

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Conflict of interest

The authors do not have any possible conflicts of interest.

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