

## ORIGINAL RESEARCH

# Investigating the impact of the sperm DNA fragmentation index of male partners from infertile couples on *in vitro* fertilization-embryo transfer outcomes

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**Abstract**

This study was aimed to elucidate the relationship between sperm DNA fragmentation index (DFI) and semen parameters, and to investigate the impact of these parameters on *in vitro* fertilization-embryo transfer (IVF-ET) outcomes. The study was conducted on 159 couples undergoing IVF-ET treatment at the Department of Reproductive Health from January 2019 to October 2023. The case group was comprised of 79 patients with sperm DFI of  $\geq 15\%$ , and the control group had 80 patients with  $< 15\%$  fragmentation index. Comprehensive data on semen parameters and the reproductive outcomes were collected and analysed. Comparisons of the case and control groups depicted no significant differences in key parameters including semen volume, sperm concentration, total sperm count, number of retrieved oocytes, rates of mature (MII) oocytes, normal fertilization, cleavage, blastocyst formation, high-quality blastocysts, human chorionic gonadotropin (HCG) positivity, clinical pregnancy, implantation and miscarriage ( $p > 0.05$ ). However, marked differences were found in the rates of sperm progressive motility, total sperm motility, normal morphology, high-quality embryos, and transferable embryos ( $p < 0.05$ ). The correlation analysis between sperm DFI and semen parameters exhibited positive correlation between sperm DFI and total sperm count ( $p < 0.05$ ). The negative correlations were found between the sperm DFI and sperm progressive motility, total sperm motility, or normal morphology ( $p < 0.01$ ). The findings demonstrated that incorporating sperm DFI as a standard component of semen analysis was advisable, and the sperm DFI as reference tool assisted in predicting the early embryonic development in IVF-ET patients.

**Keywords**

Sperm DNA fragmentation index; Semen parameters; *In vitro* fertilization-embryo transfer

## 1. Introduction

Studies have revealed that  $\sim 15\%$  couples of reproductive age experience infertility or fertility complications [1]. Semen analysis is essential for assessing the male fertility and sperm quality in the clinical context [2]. However, the conventional semen parameters provide limited evaluations of sperm quality and male fertility, and fall short of the clinical demands. This emphasizes the robust and superior evaluation indices for boosting the clinical assessment of sperm quality [3].

The sperm DNA integrity is vital for transferring paternal genetic information to progeny, and it also affects fertilization and embryonic development [4]. Sperm efficacy in fertilization is linked to the sperm DNA integrity [5]. The pronuclei formation capacity after fertilization is affected by the compromised sperm DNA integrity. Furthermore, the poor sperm integrity contributes to detrimental outcomes including miscar-

riages, congenital anomalies, and hereditary diseases in offsprings [6]. Therefore, in the assisted reproductive medicine for infertile patients employing *in vitro* fertilization-embryo transfer (IVF-ET), the quality and developmental potential of embryos generated from DNA damaged sperm have been a concern. The impact of sperm DNA fragmentation on IVF-ET data metrics are thus in focus.

Male patients undergoing IVF-ET were investigated *via* the assisted reproductive medicine program at our institution. The semen samples were obtained, sperm DNA fragmentation indices (DFI) were determined, and association with semen parameters and sperm percentage having normal morphology were examined. Furthermore, the correlations between sperm DNA fragmentation indices and IVF-ET outcomes of their partners were analysed. The study herein was thus aimed to guide about its clinical interventions.

## 2. Materials and methods

### 2.1 Participants selection and study design

A retrospective analysis was conducted on the data from 159 couples undergone IVF-ET at our hospital from January 2019 to October 2023. The participants were divided into two groups based on the sperm DNA fragmentation indices of male partners. The case group had 79 males with sperm DFI  $\geq 15\%$  and average age of  $35.4 \pm 4.5$  years. The control group was comprised of 80 males with sperm DFI  $< 15\%$  and the average age of  $34.0 \pm 5.2$  years.

The inclusion criteria were developed for the participants selection to ensure validity and reliability of the study. Each selected partner had normal karyotype as per the chromosomal examination. Female partners had the basal follicle-stimulating hormone (FSH) levels  $< 10$  IU/L. The normal ovarian and uterine morphologies were confirmed by ultrasonography. The selected male partners had no abnormalities of secondary sexual characteristics or involving the penis, scrotum, spermatic cord, vas deferens, epididymis or testicles. Patients of severe oligoasthenospermia (sperm concentration  $< 5 \times 10^6$ /mL) were excluded to prevent potential factors affecting the results.

### 2.2 Semen sample collection

An abstinence of 2–7 days was instructed to the study participants prior to semen collection. The entire semen sample was collected in a sterile and clean collection cup *via* the masturbation and placed in a temperature box set at  $37^\circ\text{C}$  for the liquefaction. The complete liquefaction time was recorded and sample was removed. Sufficient amount was drawn by a pipette for the testing.

### 2.3 Sperm DNA fragmentation index testing

The sperm chromatin dispersion (SCD) method [7] was employed to determine the sperm DFI. Sperm DNA fragmentation staining kit was procured from Anhui Anke Biotechnology Co., Ltd (Anhui He Food and Drug Supervision and Measures (Quasi) No. 1400173, 2012, Hefei, China.). The presence of sperm DNA fragmentation was determined based on whether the sperm head had no halo or halo  $\leq 1/3$  of the smallest diameter of sperm head.

### 2.4 Routine semen quality analysis

Semen samples of the participants in both groups were collected. After semen liquefaction, the semen volume and pH were measured on constant temperature workbench. The semen parameters such as semen volume, sperm concentration, rate of progressive sperm motility, total sperm vitality, and total sperm count were analysed by Shanghai Bei'ang Computer-Aided Semen Analysis System. The procedures were conducted in accordance with the WHO criteria [8].

### 2.5 Sperm morphology testing

The normal sperm morphology rate was determined by Papanicolaou staining method *via* the sperm morphology rate detection staining kit from Zhuhai Beso Biotechnology Co., Ltd

(Yuezhu Food and Drug Administration Measures Production Preparation No. 20160002, Zhuhai, China). All procedures strictly followed the WHO criteria [8].

### 2.6 In vitro fertilization (IVF), embryo observation and transfer

The oocytes were fertilized ~39 hours after the injection of human chorionic gonadotropin (HCG) and checked for the expulsion of second polar body after 4 hours. The pronuclei were observed the next morning and scored. Fertilization was confirmed by the pronuclei presence. Embryos with 0, 1 or  $\geq 3$  pronuclei were considered abnormally fertilized, and those with 2 as normally fertilized. The embryos were observed on 2nd, 3rd, 5th and 6th days of culture (extended to D5 or D6 if necessary). The quality of cleavage-stage embryos and blastocysts was assessed using the guidelines for assisted reproductive technology. One to two high-quality embryos were selected for the transfer depending on patient's specific condition during fresh oocyte retrieval cycles or frozen embryo transfer cycles. Serum  $\beta$ -human chorionic gonadotropin ( $\beta$ -HCG) was measured after 14 days of transfer to confirm the biochemical pregnancy. The clinical pregnancy was confirmed *via* ultrasound by the presence of intrauterine pregnancy sac after 28 days of transfer [9].

### 2.7 IVF clinical outcomes: a retrospective analysis

A comprehensive analysis of the clinical outcomes of IVF procedures was made. A series of calculations evaluated the effectiveness of treatments.

The MII oocyte rate, calculated as (the number of MII oocytes/the total number of retrieved oocytes)  $\times 100\%$ , was used to elucidate the maturity level of the retrieved oocytes. The IVF normal fertilization rate was determined by the equation (the number of 2 pronuclear (PN) embryos/the number of retrieved oocytes)  $\times 100\%$ , providing a clear indication of fertilization success.

The cleavage rate of 2PN embryos, calculated as the ratio of the number of 2PN cleaved embryos to the number of 2PN fertilized embryos, was used to evaluate the early developmental stages of the embryos. The D3 high-quality embryo rate, calculated as (the number of high-quality embryos on day 3/the number of 2PN cleaved embryos)  $\times 100\%$ , indicated the embryo quality at a pivotal developmental juncture.

The transferable embryo rate was calculated as (the number of transferable embryos/the total number of cleaved embryos (2PN + 1PN + 0PN))  $\times 100\%$ , to obtain an overarching perspective on embryo availability. The high-quality blastocyst rate and blastocyst formation rate were calculated as (the number of high-quality blastocysts/the total number of embryos cultured to the blastocyst stage)  $\times 100\%$  and (the number of blastocysts formed/the total number of embryos cultured to the blastocyst stage)  $\times 100\%$ , respectively. These metrics provided essential insights into the later developmental phases of the embryos.

On the clinical spectrum, the HCG positivity rate was computed as (the number of biochemical pregnancies/the number of transfer cycles)  $\times 100\%$ , the clinical pregnancy rate was

calculated as (the number of clinical pregnancies/the number of transfer cycles)  $\times$  100%, and the embryo implantation rate was calculated as (the number of gestational sacs/the number of transferred embryos)  $\times$  100%. These metrics collectively provided a comprehensive view of the pregnancy outcomes.

Finally, the miscarriage rate was calculated as (the number of miscarriages/the number of clinical pregnancies)  $\times$  100%, providing crucial data on pregnancy maintenance. Through these rigorous calculations, we gained a thorough understanding of the diverse aspects of IVF outcomes, setting the stage for the development of improved protocols and an increase in pregnancy success rates.

## 2.8 Statistical analysis

The data were processed using SPSS version 20.0 (IBM, Armonk, NY, USA). Categorical data were presented as percentages (%) and subjected to chi-square ( $\chi^2$ ) test for analysis. Continuous data were expressed as mean  $\pm$  standard deviation and analysed by *t* test. Pearson correlation analysis identified the potential correlations. *p* value  $<$  0.05 was considered statistically significant.

## 3. Results

### 3.1 Comparison of demographic and spermatological parameters in case and control groups

The comparative analysis of case and control groups revealed no statistically significant differences in the ages of partners or in semen volume ( $F = 6.164, p = 0.104$ ), sperm concentration ( $F = 0.134, p = 0.221$ ), total sperm count ( $F = 19.586, p = 0.473$ ), or retrieved number of oocytes ( $F = 1.760, p = 0.386$ ) ( $p > 0.05$ ), as shown in Table 1. The differences were found in sperm progressive motility ( $F = 0.520, p < 0.001$ ), total sperm vitality ( $F = 2.182, p < 0.001$ ), and normal morphology rate ( $F = 0.888, p < 0.001$ ) between male patients in the case and control groups ( $p < 0.05$ ).

### 3.2 Comparison of embryo development and pregnancy outcomes between case and control groups

The transferable embryo rate ( $\chi^2 = 7.420, p = 0.006$ ) and high-quality embryo rate ( $\chi^2 = 5.316, p = 0.021$ ) in case group were lower than in the control ( $p < 0.05$ ), as shown in Table 2. However, the normal fertilization rate ( $\chi^2 = 0.976, p = 0.323$ ), cleavage rate ( $\chi^2 = 0.185, p = 0.667$ ), blastocyst formation rate ( $\chi^2 = 1.595, p = 0.207$ ), high-quality blastocyst rate ( $\chi^2 = 1.869, p = 0.172$ ), HCG positivity rate ( $\chi^2 = 0.528, p = 0.467$ ), clinical pregnancy rate ( $\chi^2 = 0.151, p = 0.697$ ), implantation rate ( $\chi^2 = 0.101, p = 0.751$ ), and miscarriage rate ( $\chi^2 = 0.438, p = 0.508$ ) had no significant differences in case and control groups ( $p > 0.05$ ).

### 3.3 Correlation analysis of DNA breakage rate with sperm parameters

The correlation analyses of sperm DFI and semen parameters depicted positive correlation between sperm DFI and total

sperm count ( $r = 0.166, p = 0.037$ ), as shown in Table 3. Negative correlations were found with sperm progressive motility ( $r = -0.655, p < 0.001$ ), total sperm motility ( $r = -0.609, p < 0.001$ ), and normal morphology ( $r = -0.432, p < 0.001$ ), however no correlation with semen volume ( $r = 0.139, p = 0.081$ ), or sperm concentration ( $r = -0.027, p = 0.734$ ).

## 4. Discussion

Sperm DNA integrity was a prerequisite for passing paternal genetic information to offspring and had role in fertilization and embryo development [10]. Studies demonstrated that exposure to radiations, high temperatures, anti-tumor drugs, infections and smoking could increase the rate of sperm DNA fragmentation in males [11]. The mechanisms of sperm DNA damage included abnormal chromatin assembly in spermatogenesis, oxidative stress in the body, and anomalies in sperm apoptosis [12].

Whether sperm DFI could represent semen parameters and provide clinical diagnosis of patient's semen quality, needed investigations [13–15]. Studies showed increased sperm DFI in infertile males with abnormal semen parameters [16].

This condition could also occur in male patients with unexplained infertility, and normal semen parameters [17]. Gu *et al.* [18] study on the relationship between sperm DFI and semen parameters exhibited that sperm DNA fragmentation was negatively correlated with sperm progressive motility and sperm concentration. Shuai *et al.* [2] and Osaka *et al.* [19] revealed that the sperm DFI was negatively correlated with semen parameters and rate of abnormal morphology. The study herein exhibited that sperm DFI in infertile patients was negatively correlated with sperm progressive motility, total sperm vitality, and normal morphology rate. Higher the sperm DFI, lower were the sperm progressive motility, total sperm vitality, and rate of normal sperm morphology. This finding was consistent with most studies analysing the correlations between sperm DFI and semen parameters. The sperm DFI test could thus be employed as a supplementary explanation for clinical diagnosis using routine semen parameters.

Sperm DNA was vital for the human genetic information and had role in the fertilization and subsequent development [20]. The impact of sperm DNA damage on embryo quality required investigations pertaining to the underlying mechanisms [21]. Martínez *et al.* [22] and Xue *et al.* [23] found no correlation between sperm DFI and fertilization rate in IVF-ET cycles which was consistent with this study. The findings herein depicted no significant difference in the rates of normal fertilization or normal cleavage between the two groups categorized by different sperm DFI values ( $p > 0.05$ ).

Du *et al.* [24] explored the relationship between IVF-ET and sperm DFI to reveal that lower sperm DFI group had higher fertilization rates and high-quality embryo rates than the higher sperm DFI group. The study herein demonstrated that both high-quality and transferable embryo rates were lower in the high fragmentation group (case group) than in low fragmentation group (control group) ( $p < 0.05$ ). This was aligned with the findings of Jiang *et al.* [25].

This study demonstrated that elevated levels of sperm DNA fragmentation contributed to reduction in embryo quality dur-

**TABLE 1. Comparison of patient characteristics and semen parameters between the two groups.**

Group	Control group (DFI <15%) (mean ± SD)	Case group (DFI ≥15%) (mean ± SD)	F	p
Sperm DFI (%)	8.6 ± 2.7	21.2 ± 5.3	15.111	<0.001**
Female age (yr)	32.8 ± 4.7	33.4 ± 5.3	0.602	0.429
Male age (yr)	34.0 ± 5.2	35.4 ± 4.5	1.707	0.066
Semen volume (mL)	2.9 ± 1.3	3.3 ± 1.6	6.164	0.104
Sperm concentration (10 <sup>6</sup> /mL)	125.9 ± 77.1	109.2 ± 92.7	0.134	0.221
Sperm progressive motility (%)	50.8 ± 13.3	30.1 ± 11.6	0.520	<0.001**
Total sperm vitality (%)	62.7 ± 14.0	41.5 ± 15.1	2.182	<0.001**
Total sperm count (10 <sup>6</sup> )	329.6 ± 198.8	360.7 ± 329.4	19.586	0.473
Normal morphology rate (%)	5.8 ± 1.8	4.1 ± 1.8	0.888	<0.001**
Oocytes retrieved (numbers)	11.1 ± 6.6	12.1 ± 7.9	1.760	0.386

\*\**p* < 0.01. DFI: sperm DNA fragmentation index; SD: standard deviation.

**TABLE 2. Comparison of embryo development and pregnancy outcomes between the two groups.**

Group	Control group (DFI <15%)	Case group (DFI ≥15%)	χ <sup>2</sup>	p
MII oocyte rate (%)	87.81 (778/886)	86.48 (825/954)	0.727	0.394
Normal fertilization rate (%)	63.66 (564/886)	61.43 (586/954)	0.976	0.323
Cleavage rate (%)	99.29 (560/564)	99.49 (583/586)	0.185	0.667
Transferable embryo rate (%)	84.50 (536/634)	78.70 (532/676)	7.420	0.006**
High-quality embryo rate (%)	70.71 (396/560)	64.30 (375/583)	5.316	0.021*
Blastocyst formation rate (%)	64.68 (249/385)	69.00 (256/371)	1.595	0.207
High-quality blastocyst rate (%)	27.01 (104/385)	31.54 (117/371)	1.869	0.172
Implantation rate (%)	44.03 (59/134)	42.11 (56/133)	0.101	0.751
HCG positivity rate (%)	68.75 (55/80)	63.29 (50/79)	0.528	0.467
Clinical pregnancy rate (%)	60.00 (48/80)	56.96 (45/79)	0.151	0.697
Miscarriage rate (%)	20.83 (10/48)	26.67 (12/45)	0.438	0.508

\**p* < 0.05, \*\**p* < 0.01. DFI: sperm DNA fragmentation index.

**TABLE 3. Correlations between the sperm DFI and semen parameters.**

	r	p
Semen volume (mL)	0.139	0.081
Sperm concentration (10 <sup>6</sup> /mL)	-0.027	0.734
Sperm progressive motility (%)	-0.655	<0.001**
Total sperm motility (%)	-0.609	<0.001**
Total sperm count (10 <sup>6</sup> )	0.166	0.037*
Normal morphology (%)	-0.432	<0.001**

\**p* < 0.05, \*\**p* < 0.01.

ing IVF-ET. There were no significant differences in the rates of blastocyst formation or high-quality blastocyst formation between high fragmentation and low fragmentation groups (*p* > 0.05). This could be because of the strategy employed in this clinical setting, where a decision was made to freeze high-quality cleavage-stage embryos on day 3 to ensure the availability of transferable embryos.

The impact of sperm DFI on pregnancy outcomes after IVF-ET was explored with various conclusions reached worldwide. Researchers had identified a correlation between sperm DNA integrity and the outcomes of pregnancies achieved *via* assisted reproductive technologies such as IVF-ET [26]. This connection was hypothesized to stem from diminished embryo quality because of sperm DNA damage which increased miscarriages and congenital anomalies as well as decreased the live birth rates [27]. The findings of retrospective analysis by Luo *et al.* [28] supported this perspective where sperm DFI was

lower in pregnancy group than in non-pregnancy after the IVF-ET. These findings were significant which led us to propose the sperm DFI as potential predictive marker for pregnancy outcomes in assisted reproduction.

In contrast, Xue *et al.* [23], Chen *et al.* [29], Yang *et al.* [30], Best *et al.* [31], and Sun *et al.* [32] identified no significant correlation between the sperm DFI and clinical outcomes of IVF and asserted that sperm DFI was not an independent predictor of clinical pregnancy outcomes. Our study was aligned with these findings as no significant differences were observed in the rates of biochemical and clinical pregnancy, embryo implantation or miscarriage in various DFI groups ( $p > 0.05$ ).

Few factors could explain these results. First, the relatively limited sample size of this study might have skewed the results to introduce potential bias. Second, certain infertile males with higher sperm DFI levels ( $\geq 15\%$ ) had undergone oral antioxidant treatment prior to fertilization. This might nullify any negative impact on the final pregnancy outcomes. Third, the focus on only the pregnancy outcomes of initial transfer cycle as opposed to all the embryo transfer cycles could have role, considering that the optimal embryo was chosen to transfer in this cycle.

This study was limited by its retrospective nature and relatively small sample size. Inclusion of additional samples in the next step would strengthen further research outcomes.

## 5. Conclusions

In summary, it is imperative to incorporate sperm DFI testing as a standard component of semen analysis because of the linkage between sperm DFI and factors such as sperm motility, total vitality, and normal morphology. This addition may improve the male fertility evaluations and guide to conducive treatment strategies. A high sperm DFI influences early embryo development in IVF-ET which leads to reduced rates of high-quality and available embryos. It may demonstrate the relevance of sperm DFI as reference tool for predicting early embryo development in IVF-ET patients. However, the sperm DFI is not correlated with the rates of biochemical pregnancy, clinical pregnancy, embryo implantation, or miscarriage which limits its predictive utility for the clinical outcomes in IVF-ET patients.

## AVAILABILITY OF DATA AND MATERIALS

The data presented in this study are available on reasonable request from the corresponding author.

## AUTHOR CONTRIBUTIONS

QLQ, YCS and JP—conceptualised and designed the research study. QLQ—was responsible for data collection, analysing and interpretation of the data as well as drawing the manuscript. YCS and JP—revised the paper and given final approval of the version to be published.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study protocol was reviewed and approved by the Medical Ethics Committee of Pingxiang Maternal and Child Health Hospital in Jiangxi Province (No. 2019-012-08). The requirement for informed consent from patients was waived because the study was a retrospective analysis of existing data.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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