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Original Research

Correlations Between Sperm DNA Fragmentation Index, Semen Parameters, and *In Vitro* Fertilization Embryo Culture Outcomes: A Single-Center Retrospective Study

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Abstract

Background: Sperm DNA fragmentation index (DFI) has emerged as a critical factor affecting male fertility, while the associations between DFI, conventional semen parameters and *in vitro* fertilization (IVF) embryo culture outcomes remain inconsistent in clinical studies. To investigate the impact of sperm DFI on embryo culture parameters in IVF treatment cycles. **Methods**: A comprehensive retrospective analysis was conducted on couples undergoing IVF treatment at the Reproductive Medicine Center of Wenzhou People's Hospital between January 2019 and April 2024. Correlation and regression analyses were performed to evaluate the associations between sperm DFI and male partner characteristics, including age, semen volume, sperm concentration, total motility, and progressive motility. Based on established clinical thresholds, sperm nuclear DNA integrity was categorized as optimal (DFI $\leq$15%), moderate (15% $<$ DFI $<$ 30%), or compromised (DFI $\geq$30%). To control for confounding variables, propensity score matching (PSM) was performed twice, using DFI cutoffs of 15% and 30, respectively. Following PSM, the first matched cohort comprised 36 couples in Group A (DFI $\leq$30%) and 36 couples in Group B (DFI $>$30%). The second matched cohort comprised 265 couples in the normal group (DFI $\leq$15%) and 135 couples in the abnormal group (DFI $>$15%). Differences in fertilization rate, cleavage rate, high-quality embryo rate, usable embryo rate, and blastocyst formation rate between the two groups were compared using the chi-square test. **Results**: Correlation and regression analyses revealed a significant positive correlation between sperm DFI and male age ($r = 0.244$, $p < 0.001$) and significant negative correlations with total motility and progressive motility ($r = –0.290$, $p < 0.001$; $r = –0.272$, $p < 0.001$). No significant correlation was found between DFI and semen volume or sperm concentration. The fertilization rate in Group B (60.48%) was significantly lower compared to Group A (66.93%) ($p < 0.05$). Additionally, the fertilization rate in the abnormal group (64.69%) was significantly lower compared to the normal group (68.35%) ($p < 0.05$). There were no statistically significant differences in cleavage rate (96.93% vs. 97.62%; 97.58% vs. 98.15%), high-quality embryo rate (38.60% vs. 44.64%; 35.46% vs. 35.63%), usable embryo rate (68.42% vs. 67.86%; 65.69% vs. 62.01%), and blastocyst formation rate (59.38% vs. 60.66%; 60.63% vs. 62.32%) (all $p > 0.05$) between Group B and Group A, as well as between the abnormal group and the normal group. **Conclusions**: Sperm DFI was positively correlated with male age and negatively correlated with sperm motility, but showed no association with other semen parameters. Elevated sperm DFI was associated with reduced fertilization rates in IVF cycles. In contrast, subsequent embryonic development parameters were unaffected by DFI levels, suggesting that sperm DNA damage primarily affects early fertilization events rather than later embryonic development stages.

Keywords: sperm DNA fragmentation index; *in vitro* fertilization; fertilization rate; embryo development; male infertility

1. Introduction

Infertility affects approximately one-sixth of couples of reproductive age, with male factor infertility contributing to approximately 50% of cases [1]. *In vitro* fertilization and embryo transfer (IVF-ET) represents a cornerstone treatment modality for infertility management. Since the birth of the world's first IVF baby in 1978, IVF technology has been widely used in reproductive medicine, enabling countless couples to achieve parenthood who would otherwise be unable to conceive naturally. However, sperm quality parameters significantly influence IVF treatment outcomes. While conventional semen analysis provides a fundamental assessment of male fertility potential, including sperm motility, which serves as a crucial predictor of IVF success rates [2], these parameters offer limited insight into the integrity of sperm nuclear DNA. In recent years, the sperm DNA fragmentation index (DFI) has attracted increasing attention. DFI reflects the extent of sperm DNA damage and is crucial for predicting sperm functional competence [3]. Sperm chromatin exhibits a highly specialized structural organization, wherein the vast majority of nucleosomal histones are replaced by protamines. This distinctive pattern of chromatin compaction is essential for mediating the biological and developmental functions of sperm, as it not only maintains the integrity of genetic material but also plays

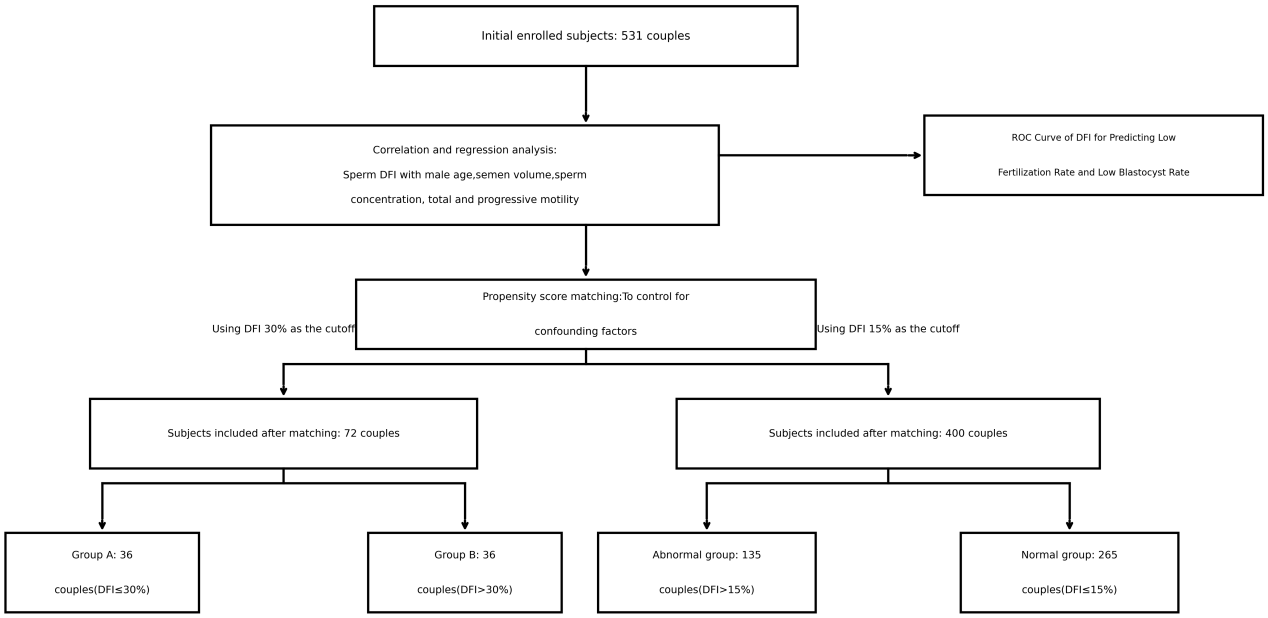

Fig. 1. Flowchart of couple sample screening and grouping. DFI, DNA fragmentation index; ROC, Receiver Operating Characteristic.

a pivotal role in ensuring accurate gene expression during early embryogenesis. Elevated sperm DFI has been associated with compromised paternal genetic contribution, potentially negatively affecting reproductive outcomes. Nevertheless, the precise mechanisms by which sperm DNA fragmentation influences early embryonic development remain incompletely understood.

The present study aims to elucidate the relationships between sperm DFI and both conventional semen parameters and IVF embryonic development outcomes, thereby contributing to the optimization of clinical decision-making in assisted reproductive technology (ART).

2. Materials and Methods

2.1 Patient Selection

Couples undergoing IVF treatment at Wenzhou People's Hospital's Reproductive Medicine Center between January 2019 and April 2024 were retrospectively included in the study. Inclusion criteria were as follows: (1) both partners fulfilled the clinical diagnostic criteria for IVF-assisted reproduction; (2) female partners had no anatomical abnormalities of the reproductive system; and (3) the fertilization method was conventional IVF. Exclusion criteria were: (1) chromosomal abnormalities in either partner. (2) male partners with genital tract malformations or acute genitourinary infections; and (3) use of intracytoplasmic sperm injection (ICSI) as the fertilization method.

2.2 Propensity Score Matching (PSM)

Initially, 531 couples were identified for potential inclusion. Correlation and regression analyses were conducted between sperm DFI and male age, semen volume, sperm concentration, total motility, and progressive motility. Subsequently, participants were stratified according to sperm DFI thresholds: 36 cases in Group B (DFI $>$30%) and 495 cases in Group A (DFI $\leq$30%), and 173 cases in the abnormal group (DFI $>$15%) and 358 cases in the normal group (DFI $\leq$15%). To account for the potential confounding effects of baseline characteristics, PSM was performed based on incorporating clinical variables including age, female body mass index (BMI), gonadotropin (Gn) dosage for ovarian stimulation, downregulation medication dose, semen volume, sperm concentration, sperm total motility. and sperm progressive motility. We performed PSM twice, using DFI cutoffs of 15% and 30%, respectively. Propensity scores were calculated for each participant using logistic regression analysis using Python (Version 3.11.4; Python Software Foundation, Wilmington, DE, USA). For the first PSM, a 1:1 nearest neighbor matching algorithm was implemented with no caliper value. Following matching, the final study cohort comprised 72 couples, with 36 couples in Group A and 36 in Group B. Since the number of cases in the group with DFI $>$30% was small during the first PSM, we performed a second PSM using a DFI cutoff of 15% and adjusted the parameters to make the matching more stringent. For the second PSM, a 1:2 nearest neighbor matching algorithm was implemented with a caliper value of 0.2. Following matching, the final study cohort comprised 400 couples, with 135 in the DFI abnormal group and 265 in the normal group. The patient selection and grouping process is summarized in Fig. 1. The normal group had a median DFI of 8.21 (interquartile range [IQR]: 5.70, 11.50), while the abnormal group had a median DFI of 23.00 (IQR: 17.25, 29.40). No statistically significant differences were observed in any baseline characteristics between the two groups (all $p > 0.05$), indicating a good balance.

2.3 Methods

2.3.1 IVF Procedures

2.3.1.1 Semen Collection. Male partners abstained from sexual activity for 3–7 prior to semen collection. Semen samples were obtained via masturbation in a private collection room within our reproductive center and collected into a disposable sterile wide-mouth container. Specimens were subsequently incubated in a 37 °C water bath until complete liquefaction.

2.3.1.2 Semen Analysis. Semen volume was quantified using precision electronic balance. Sperm concentration, total sperm count, total motility, and progressive motility were evaluated utilizing the CFT-9203 Computer-Assisted Semen Analysis (CASA) system (Jiangsu Geoffrey Software Technology Co., Ltd., Nanjing, Jiangsu, China). Sperm DFI was assessed employing a sperm nuclear integrity staining kit (fluorescent staining methodology; Tianjin Merit Medical Technology Co., Ltd.; Tianjin, China), in combination with a Mindray E6 flow cytometer (Shenzhen, Guangdong, China).

The analytical principle is based on acid-induced denaturation of sperm nuclear chromatin, in which damaged DNA becomes single-stranded and binds acridine orange dye, emitting red light or yellow fluorescence. Conversely, intact sperm nuclei maintain native double-stranded DNA structure and bind acridine orange to emit green fluorescence. A higher proportion of red fluorescence within processed specimens indicates increased sperm nuclear DNA damage.

2.3.1.3 Ovarian Stimulation. Controlled ovarian hyperstimulation was performed utilizing a long gonadotropin-releasing hormone (GnRH) agonist protocol during the follicular phase. When the majority of follicles achieved diameters of 18–21 mm, ovulation was triggered with an intramuscular injection of human chorionic gonadotropin (hCG) (OVIDREL®, Feltham, Greater London, UK) at doses of 4000–10,000 IU. Oocyte retrieval was performed under transvaginal ultrasound guidance 36 hours post-hCG administration.

2.3.1.4 Fertilization and Embryo Culture. Following semen liquefaction, sperm preparation was accomplished through Isolate® (Irvine Scientific, Santa Ana, CA, USA) density gradient centrifugation combined with swim-up processing [4]. Vitrolife's G-Series culture media (Vitrolife, Gothenburg, Västra Götalands län, Sweden) was utilized for insemination procedures and subsequent embryo culture. Oocytes were cultured for 2–4 hours prior to insemination. At 16–18 hours post-insemination, cumulus cells were removed to assess pronuclear formation, with the presence of two pronuclei indicating successful fertilization.

Embryo development was monitored daily throughout the culture period. All ART laboratory personnel were trained at reproductive medicine centers accredited by the National Health Commission of the People's Republic of China and obtained qualification certificates. Embryos were graded according to the Rijnders criteria [5]: Grade I embryos exhibited uniform blastomeres with $<5\%$ fragmentation; Grade II embryos demonstrated uniform or slightly uneven blastomeres with 5–20% fragmentation; Grade III embryos displayed uneven blastomeres with 21–50% fragmentation; and Grade IV embryos exhibited extremely uneven blastomeres with more than 50% fragmentation. Usable embryos were defined as those graded I, II, or III on Day 3 after oocyte retrieval. High-quality embryos were defined as those graded I and II on Day 3. After Day 3, embryos were cryopreserved or transferred, and the remaining embryos graded as I, II, and III were cultured to blastocysts. The embryos were cultured until Day 5 or Day 6 to observe whether blastocysts were formed. Blastocyst grading was performed according to the Gardner criteria [6]. Based on the size of the blastocyst cavity and the occurrence of hatching, blastocyst development was divided into six stages. Stage 1 was defined as a blastocyst cavity occupying less than half of the total embryo volume. Stage 2 was defined as a blastocyst cavity occupying half or more of the total embryo volume. Stage 3 was defined as a blastocyst cavity that completely filled the total embryo volume. Stage 4 was defined as a blastocyst cavity larger than the total embryo volume, with a thinning zona pellucida. Stage 5 was defined as a blastocyst in the process of hatching, with part of the trophectoderm protruding from the zona pellucida. Stage 6 was defined as a blastocyst that had completely hatched from the zona pellucida. Embryos cultured to Day 5 or Day 6 that formed Stage 2 or higher blastocysts were considered to have formed blastocysts.

2.3.2 Research Methods

2.3.2.1 Calculation Methods. Fertilization rate = Number of normally fertilized oocytes / Total number of oocytes $\times$ 100%.

Cleavage rate = Number of cleaved embryos / Number of normally fertilized oocytes $\times$ 100%.

Usable embryo rate = Number of usable embryos on Day 3 / Number of normally fertilized oocytes $\times$ 100%.

High-quality embryo rate = Number of high-quality embryos on Day 3 / Number of normally fertilized oocytes $\times$ 100%.

Blastocyst formation rate = Number of blastocysts formed / Number of embryos cultured to blastocysts $\times$ 100%.

2.3.2.2 Statistical Analysis. Statistical analyses were performed using SPSS version 22.0 (IBM Corp., Armonk, NY, USA). Correlation and regression analyses were conducted to evaluate between sperm DFI and male age, semen volu-

Table 1. Demographic and clinical characteristics after matching (Group A vs. Group B).

Variable	Group A (N = 36)	Group B (N = 36)	Standardized mean difference (SMD)		U/*t*	*p*-value
			Before PSM	After PSM		
Female						
Age (years)	35.64 ± 5.74	36.00 (31.75, 40.25)	0.431	0.050	664	0.861
BMI (kg/m^2)	22.45 ± 3.01	22.34 ± 1.97	0.031	0.045	0.190$^{\#}$	0.850
Total Gn dose (IU)	1675.00 (1500.00, 2081.25)	2009.03 ± 708.99	0.152	0.125	585	0.481
Downregulation medication dose (mg)	2.62 (0.69, 3.75)	3.75 (1.18, 3.75)	0.191	0.102	608	0.640
Initial Gn dose (IU)	600.00 (450.00, 750.00)	600.00 (450.00, 675.00)	0.062	0.129	667.5	0.827
Male						
Age (years)	39.58 ± 6.63	39.44 ± 7.87	0.902	0.019	0.081$^{\#}$	0.936
Semen volume (mL)	2.60 (1.95, 4.12)	3.00 (2.50, 3.60)	0.121	0.129	560	0.324
Sperm concentration (×10^6/mL)	71.77 (27.58, 126.15)	101.06 ± 49.28	0.312	0.076	539	0.222
Total motility (%)	47.69 ± 19.50	43.35 (35.00, 52.20)	0.934	0.117	719	0.427
Progressive motility (%)	33.80 ± 14.77	33.13 ± 14.34	0.803	0.046	0.197$^{\#}$	0.844
Total sperm count (×10^6)	201.25 (61.09, 390.15)	359.45 (198.25, 410.32)	0.439	0.003	484	0.066

$^{\#}$*t*-value. PSM, propensity score matching; BMI, body mass index; Gn, gonadotropin.

Table 2. Demographic and clinical variables after matching (Normal Group vs. Abnormal Group).

Variable	Normal Group (N = 265)	Abnormal Group (N = 135)	Standardized mean difference (SMD)		U/*t*	*p*-value
			Before PSM	After PSM		
Female						
Age (years)	32 (29, 37)	33 (28, 38)	0.056	0.048	17,505.5	0.727
BMI (kg/m^2)	22.00 (20.10, 24.60)	22.30 (20.70, 23.75)	0.130	0.034	17,878.0	0.993
Total Gn dose (IU)	2000.00 (1625.00, 2475.00)	2025.00 (1531.00, 2662.50)	0.070	0.007	17,931.0	0.969
Downregulation medication dose (mg)	3.75 (0.95, 3.75)	3.75 (0.95, 3.75)	0.032	0.019	17,656.0	0.821
Initial Gn dose (IU)	600 (450, 750)	600 (450, 750)	0.038	0.031	18,107.5	0.838
Male						
Age (years)	34 (31, 38)	34 (30, 40)	0.256	0.081	17,692.5	0.859
Semen volume (mL)	3.10 (2.20, 4.00)	2.90 (2.30, 3.60)	0.076	0.098	19,310.5	0.193
Sperm concentration (×10^6/mL)	65.20 (37.00, 109.70)	66.40 (41.55, 117.15)	0.052	0.038	16,821.0	0.330
Total motility (%)	60.20 (49.90, 70.20)	60.70 (47.60, 72.94)	0.402	0.045	18,388.5	0.647
Progressive motility (%)	44.03 ± 14.42	43.80 ± 14.62	0.406	0.015	0.147$^{\#}$	0.884
Total sperm count (×10^6)	184.60 (98.20, 347.20)	216.80 (127.05, 361.05)	0.194	0.027	16,688.0	0.273

$^{\#}$*t*-value.

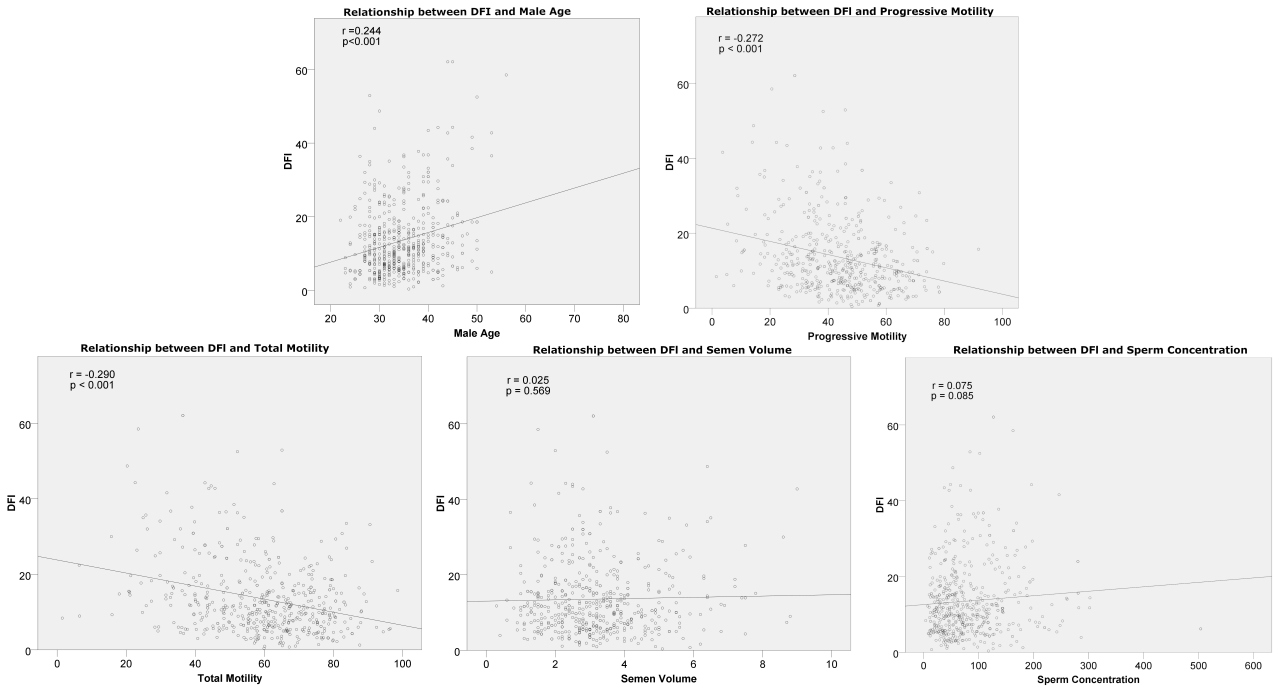

Fig. 2. Relationship between DFI and male age, sperm progressive motility, total motility, semen volume and sperm concentration. DFI, DNA fragmentation index.

me, sperm concentration, sperm total motility, and sperm progressive motility. Following PSM, normality testing was performed on all continuous variables.

Non-normally distributed continuous data were expressed as median (IQR, P25–P75), with intergroup comparisons performed using the Mann-Whitney U test. Normally distributed continuous variables were expressed as mean ± standard deviation (SD; $\bar{x} \pm s$), with intergroup comparisons using independent *t*-test. The chi-square test (χ^2 test) was used to compare the differences in fertilization rate, cleavage rate, usable embryo rate, high-quality embryo rate, and blastocyst formation rate between the two groups. A p-value of <0.05 was considered statistically significant.

2.4 Receiver Operating Characteristic (ROC) Curve Analysis of DFI to Predict Low Fertilization and Blastocyst Rates

In this study, a fertilization rate <60% and a blastocyst rate <30% were considered positive events, and corresponding dichotomous outcome variables were created. The ROC curve was used to analyze the predictive performance of DFI for these two outcomes. The optimal DFI cutoff point was determined using Youden's Index (Youden's Index = Sensitivity + Specificity – 1), and the area under the curve (AUC) was calculated to quantify its predictive ability. The AUC ranges from 0.5 to 1, where an AUC ≥0.7 indicates a good predictive ability, 0.5–0.7 indicates a moderate predictive ability, and <0.5 indicates no practical predictive value.

3. Results

3.1 Baseline Characteristics

Following PSM, no statistically significant differences were observed in any baseline characteristics between the two groups (all $p > 0.05$), including female age, BMI, total Gn dose, downregulation medication dose, initial Gn dose, and male age, semen volume, sperm concentration, sperm total motility, sperm progressive motility, and total sperm count, as detailed in Tables 1,2.

3.2 Correlation Analysis Between Sperm DFI and Semen Parameters

Correlation and regression analyses revealed significant positive correlations between sperm DFI and male age (r = 0.244, $p < 0.001$), as well as significant negative correlations with total motility (r = –0.290, $p < 0.001$) and progressive motility (r = –0.272, $p < 0.001$). No significant correlations were identified between DFI and semen volume or sperm concentration. The results are shown in Table 3 and Fig. 2.

3.3 Fertilization and Embryo Development Outcomes

The fertilization rate in Group B (60.48%) was significantly lower compared with Group A (66.93%) ($p < 0.05$). Similarly, the fertilization rate in the abnormal group (64.69%) was significantly lower than in the normal group (68.35%) ($p < 0.05$). However, no statistically significant differences were observed in cleavage rate (96.93% vs. 97.62%; 97.58% vs. 98.15%), high-quality embryo

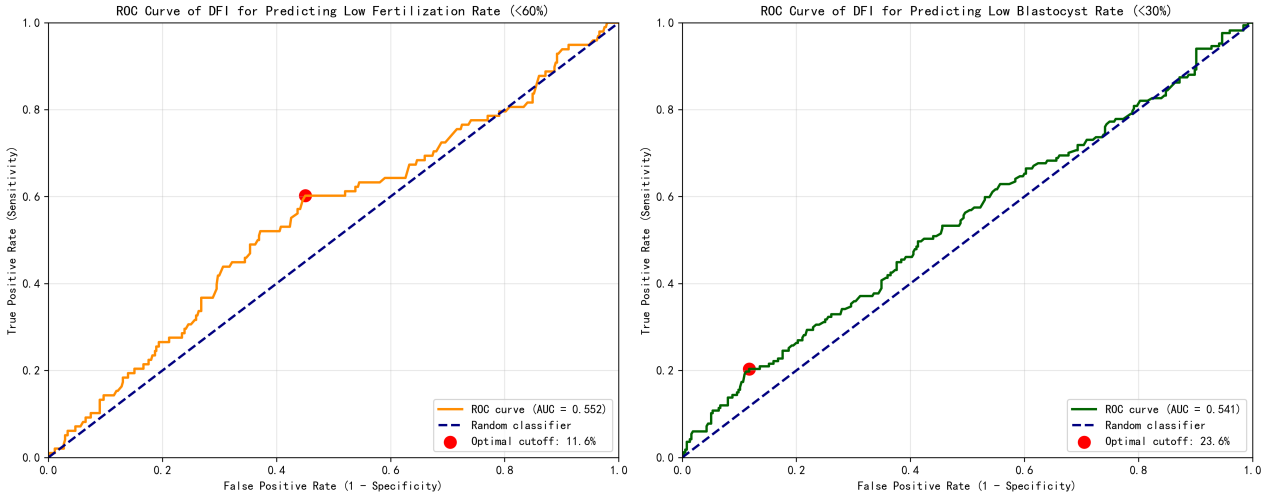

Fig. 3. ROC curve of DFI for predicting low fertilization rate and low blastocyst rate. ROC, Receiver Operating Characteristic; DFI, DNA fragmentation index.

Table 3. Correlation analysis between dperm DFI and male age, semen volume, sperm concentration, total motility, and progressive motility.

Variable	Pearson's Correlation Coefficient r	*p*-value
Male Age	0.244	<0.001
Semen Volume	0.025	0.569
Sperm Concentration	0.075	0.085
Total Motility	–0.290	<0.001
Progressive Motility	–0.272	<0.001

rate (38.60% vs. 44.64%; 35.46% vs. 35.63%), usable embryo rate (68.42% vs. 67.86%; 65.69% vs. 62.01%), or blastocyst formation rate (59.38% vs. 60.66%; 60.63% vs. 62.32%) (all $p > 0.05$) between Group B and Group A, as well as between the abnormal group and the normal group. Detailed results are presented in Tables 4,5.

3.4 ROC Analysis of DFI for Predicting Low Fertilization Rate (<60%)

As shown in the figure, the AUC of the ROC curve for DFI predicting a fertilization rate <60% was 0.552, with an optimal cutoff point of 11.6%. This AUC was slightly higher than 0.5, suggesting that DFI had only a very weak predictive ability for low fertilization rates. The degree of deviation of its ROC curve from the random classifier (blue dashed line) was small, indicating that its predictive performance was close to random.

3.5 ROC Analysis of DFI for Predicting Low Blastocyst Rate (<30%)

For the prediction of a blastocyst rate <30%, the AUC of the ROC curve of DFI was 0.541, with a optimal cutoff point of 23.6%. Similarly, the AUC was close to 0.5, indicating that the predictive ability of DFI for low blastocyst rate was also limited. Its ROC curve did not deviate significantly from the random classifier, further indicating that the predictive value of DFI as a single indicator was low, as detailed in Fig. 3.

4. Discussion

This comprehensive retrospective study provides valuable insights into the complex relationship between sperm DNA integrity and IVF outcomes. Our findings demonstrate that elevated sperm DFI significantly impairs fertilization rates, reinforcing the hypothesis that paternal sperm DNA damage primarily compromises early reproductive events rather than subsequent embryonic development.

We observed that sperm DFI correlates positively with male age and negatively with both total and progressive sperm motility, while no significant associations were found with other semen parameters. These findings align with existing literature [7–10]. A potential explanation is the age-related decline in antioxidant capacity against free radicals in the epididymis and reduced DNA repair capacity during spermatogenesis in the testes. The mechanisms underlying sperm DNA fragmentation are multifactorial [11], including defective chromatin packing, oxidative stress, and apoptosis. Among these, reactive oxygen species (ROS)-induced oxidative damage is widely regarded as the primary driver of DNA damage in spermatozoa [12,13].

Importantly, the present study demonstrated a statistically significant reduction in fertilization rate among the abnormal group (DFI >15%) compared with the normal group, while no significant differences were observed in subsequent embryonic development parameters, including cleavage rate, usable embryo rate, high-quality embryo rate, and blastocyst formation rate. These results suggest that high sperm DFI may specifically interfere with the

Table 4. Comparison of fertilization rate, cleavage rate, usable embryo rate, high-quality embryo rate, and blastocyst formation rate between Group A and Group B.

Outcome Variable	A Group (N = 36)	B Group (N = 36)	χ^2 Value	*p*-value
Fertilization Rate	66.93% (168/251)	60.48% (228/377)	130.522	<0.001
Cleavage Rate	97.62% (164/168)	96.93% (221/228)	0.173	0.766
Usable Embryo Rate	67.86% (114/168)	68.42% (156/228)	0.014	0.905
High-Quality Embryo Rate	44.64% (75/168)	38.60% (88/228)	1.460	0.227
Blastocyst Formation Rate	60.66% (74/122)	59.38% (95/160)	0.047	0.828

Table 5. Comparison of fertilization rate, cleavage rate, usable embryo rate, high-quality embryo rate, and blastocyst formation rate between Normal Group and Abnormal Group.

Outcome Variable	Normal Group (N = 265)	Abnormal Group (N = 135)	χ^2 Value	*p*-value
Fertilization Rate	68.35% (1782/2607)	64.69% (784/1212)	70.155	<0.001
Cleavage Rate	98.15% (1749/1782)	97.58% (765/784)	0.896	0.344
Usable Embryo Rate	62.01% (1105/1782)	65.69% (515/784)	3.168	0.075
High-Quality Embryo Rate	35.63% (635/1782)	35.46% (278/784)	0.007	0.932
Blastocyst Formation Rate	62.32% (870/1396)	60.63% (348/574)	0.494	0.482

initial fertilization process in IVF cycles, without significantly affecting early embryogenesis up to the blastocyst stage. This finding is consistent with Gu [14], who also reported that impaired sperm DNA integrity primarily influences fertilization rather than early embryo development. One proposed mechanism is that sperm DNA fragmentation may disrupt the precise sequence of molecular events required for syngamy, including paternal DNA decondensation and pronuclear formation, ultimately leading in fertilization failure.

Björndahl and Kvist [15] proposed the "zinc bridge model" to elucidate the dynamic stability of sperm chromatin. According to this model, zinc ions (Zn^{2+}) form reversible bridges between thiol groups (-SH) and imidazole residues in protamines, thereby maintaining chromatin compactness while allowing timely decondensation post-fertilization. This structure provides dual protection by safeguarding DNA against oxidative stress and ensuring rapid unpacking upon oocyte entry. Loss of zinc ions may cause premature formation of irreversible disulfide bonds (S-S), resulting in chromatin hyperstabilization. Such hyperstabilization can delay decondensation, hinder proper DNA exposure in the ooplasm, and consequently reduce fertilization rates, providing a plausible explanation for the inverse correlation between DFI and fertilization success.

In contrast, some studies have reported divergent findings. Li *et al*. [16] reported no significant association between DFI and fertilization rate but observed a positive correlation with miscarriage rate and a negative correlation with birth weight. Although the current study did not evaluate pregnancy outcomes such as miscarriage or neonatal weight, future investigations will include these clinical endpoints to provide a more comprehensive understanding of the long-term reproductive consequences of sperm DNA damage.

Moreover, the lack of significant correlations between DFI and embryological outcomes, including cleavage rate, usable embryo rate, high-quality embryo rate, and blastocyst formation rate, may result from the strong maternal regulatory mechanisms dominating early embryogenesis. Zeyad *et al*. [17] similarly reported no significant correlation between sperm DFI and clinical outcomes of assisted reproduction. This effect arises because sperm undergo a selection and optimization process during IVF, thereby reducing the impact of DFI on clinical pregnancy outcomes. Fang *et al*. [18] proposed that sperm with high DFI are naturally eliminated during fertilization. Additionally, several meta-analyses [19,20] have shown that sperm DFI does not significantly correlate with fertilization rate, blastocyst formation rate, clinical pregnancy rate, miscarriage rate, or live birth rate.

Prior to embryonic genome activation (around the 4–8 cell stage in humans), development relies heavily on maternally inherited mRNAs and proteins. Additionally, the oocyte contains robust DNA repair machinery capable of correcting certain sperm DNA defects, particularly single-strand breaks. To mitigate damage, the zygote may employ multiple regulatory mechanisms, including cell cycle arrest, delayed cleavage, modulation of DNA damage response and repair protein expression, as well as activation of the double-strand break repair pathway and base excision repair pathway, which address oxidative damage [21].

Nevertheless, the exact mechanisms underlying oocyte-mediated repair of sperm DNA damage remain incompletely elucidated [22]. Notably, conflicting results have been reported, such as those from Du and Tuo [23], who suggested that high DFI may adversely affect embryo quality and developmental kinetics, possibly because the extent of DNA damage exceeds the oocyte's repair capacity. Furthermore, other experimental studies and

meta-analyses [24,25] have demonstrated that sperm DNA fragmentation not only affects sperm-oocyte binding but also impacts embryo formation, embryonic differentiation, and developmental potential, and may even be associated with birth defects. These discrepancies with our findings highlight the need for further investigation. Our research group will continue to collect additional data for follow-up studies.

In this study, the AUCs of DFI for low fertilization rate and low blastocyst rate did not reach 0.7, suggesting that its predictive efficacy as a single indicator was limited. This limitation may reflect the multifactorial nature of fertilization and blastocyst rates: in addition to sperm DNA integrity, factors such as oocyte quality, endometrial receptivity, and laboratory standardization can also have significant effects.

Although the predictive performance was limited, the optimal cutoff points identified in this study still provide clinical reference value: when DFI $\geq$11.6%, clinicians should be alert to the risk of a fertilization rate $<$60%; when DFI $\geq$23.6%, attention should be given to the possibility of a blastocyst rate $<$30%. However, it should be emphasized that clinical decision-making must rely on a comprehensive assessment of multiple indicators, such as sperm concentration, motility, morphology, and oocyte parameters, rather than on DFI alone. Future studies may explore combining DFI with other sperm parameters or oocyte indicators to improve the accuracy of predicting fertilization rate and blastocyst rate.

From a clinical perspective, our findings support the integration of sperm DFI assessment into routine male fertility evaluation. Sperm DFI serves as a valuable predictor of fertilization success in ART and may guide clinical decisions regarding IVF protocol selection, including the use of ICSI or antioxidant therapies.

Limitations

A limitation of this study is that the DFI was measured within three months before oocyte retrieval, rather than using sperm samples collected on the day of oocyte retrieval. According to a report [26], the process from spermatogonia to mature spermatozoa in the testes takes approximately 74 days; when combined with subsequent maturation of spermatozoa in the epididymis, the total duration is about 90 days. Nevertheless, we consider that DFI measured within this three-month period reasonably represents sperm quality at the time of oocyte retrieval. This study has not yet included outcomes such as clinical pregnancy rate, implantation rate, miscarriage rate, or live birth rate; in future studies, our research group will continue to collect these data for further research.

5. Conclusions

In conclusion, sperm DFI is positively correlated with male age and negatively correlated with total and progressive sperm motility. Elevated sperm DFI negatively impacts fertilization rates in IVF treatment cycles, highlighting the importance of assessing sperm DNA integrity in the evaluation of male fertility. These findings support the integration of DFI assessment into routine clinical practice for couples undergoing IVF, potentially enabling more personalized treatment approaches and improved patient counseling regarding expected outcomes.

Availability of Data and Materials

The data sets generated and analyzed during the current study are not publicly available due to patient privacy concerns but are available from the corresponding author on reasonable request.

Author Contributions

XZ , HW and JW conceived and designed the research study. XZ and HP collected and processed the raw data. HP, ZX, SX, JZ, and CZ participated in specific experimental operations (e.g., sperm detection, oocyte collection, fertilization, embryo culture, and other related procedures) and provided critical technical advice for the study. XZ , HW and JW analyzed and interpreted the data. All authors contributed to drafting the manuscript, revising it critically for important intellectual content, and made editorial changes. All authors have read and approved the final version of the manuscript, and agree to be accountable for all aspects of the work, ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics Approval and Consent to Participate

The study was approved by the Ethics Committee of Wenzhou People's Hospital (Approval No. KY-202508-025). The requirement for informed consent was waived due to the retrospective nature of the study. The study was carried out in accordance with the guidelines of the Declaration of Helsinki.

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Conflict of Interest

The authors declare no conflict of interest.

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