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ORIGINAL ARTICLE

Male Infertility

Random sperm DNA fragmentation index is not associated with clinical outcomes in day-3 frozen embryo transfer

Qing-Xin Wang, Xia Wang, Min-Yan Yu, Hua Sun, Di Wang, Shu-Ping Zhong, Feng Guo

Damage to sperm DNA was proposed to play an important role in embryonic development. Previous studies focused on outcomes after fresh embryo transfer, whereas this study investigated the influence of sperm DNA fragmentation index (DFI) on laboratory and clinical outcomes after frozen embryo transfer (FET). This retrospective study examined 381 couples using cleavage-stage FET. Sperm used for intracytoplasmic sperm injection (ICSI) or *in vitro* fertilization (IVF) underwent density gradient centrifugation and swim up processing. Sperm DFI had a negative correlation with sperm motility ($r = -0.640$, $P < 0.01$), sperm concentration ($r = -0.289$, $P < 0.01$), and fertilization rate of IVF cycles ($r = -0.247$, $P < 0.01$). Sperm DFI examined before and after density gradient centrifugation/swim up processing was markedly decreased after processing (17.1% *vs* 2.4%, $P < 0.01$; 65 randomly picked couples). Sperm progressive motility was significantly reduced in high DFI group compared with low DFI group for both IVF and ICSI (IVF: 46.9% ± 12.4% *vs* 38.5% ± 12.6%, respectively; ICSI: 37.6% ± 14.1% *vs* 22.3% ± 17.8%, respectively; both $P < 0.01$). The fertilization rate was significantly lower in high (≥25%) DFI group compared with low (<25%) DFI group using IVF (73.3% ± 23.9% *vs* 53.2% ± 33.6%, respectively; $P < 0.01$) but was equivalent in high and low DFI groups using ICSI. Embryonic development and clinical outcomes after FET were equivalent for low and high DFI groups using ICSI or IVF. In this study, sperm DFI did not provide sufficient information regarding embryo development or clinical outcomes for infertile couples using FET.

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INTRODUCTION

About 8%–12% of couples worldwide experience infertility during their reproductive lives.[1] An increasing number of infertile couples seek medical support by assisted reproductive technology (ART).[2] Pregnancy success rates remain unpredictable because several possible factors are involved in the process, and nearly 50% of infertility reflects male factor infertility, either as a single factor or in combination with a female factor.[3]

Traditionally, analysis of male infertility involves the assessment of important factors that affect the ability of fertilization, in particular, sperm concentration and motility, and the percentage of sperm with normal morphology. In recent years, sperm DNA damage was proposed to play an important role in embryonic development. The integrity of sperm DNA has been considered as an auxiliary diagnostic tool to improve the prediction of fertility and potential biological markers of male infertility. The sperm DNA fragmentation index (DFI) has been used to reflect the levels of sperm damage. Sperm DNA fragmentation may have many potential causes, such as abortive apoptosis, oxidative stress or gonad-toxic therapy.[4–6] DNA-damaged sperm have the ability to fertilize oocytes, and oocytes also have the capacity to repair sperm DNA damage. However, embryonic development is related to the degree of DNA damage. Embryonic development will be affected when sperm DNA damage is beyond a certain level.[7,8]

Several studies have evaluated the influence of DFI on laboratory and clinical outcomes. Numerous studies have investigated the association between outcomes of ART and sperm DFI. Most studies indicated that sperm DFI had a negative impact on sperm quality and fertility[9–11] and was negatively correlated with fertilization, pregnancy, and live birth rates[12–16] and positively correlated with miscarriage rate.[13,17–19] Some research even suggested that sperm DNA fragmentation may be a useful predictor of outcomes after *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI).[20,21] Conversely, other research suggested that there was no correlation between sperm DFI and embryo development,[22] and DFI had no predictive value for pregnancy outcomes after IVF/ICSI.[23] In addition, most of these studies focused on fresh embryo transfers, and there have been few studies focused on the impact of DFI on outcomes after frozen embryo transfer (FET). Recently, an increasing number of clinics have been applying the freeze-all strategy in their practice of oocyte collection for ART. Frozen embryo transfers circumvent possible negative effects of high estrogen on the endometrium and reduce the risk of ovarian hyperstimulation syndrome in fresh cycles. The use of FET also increased the cumulative pregnancy rates of IVF/ICSI procedures[24] and take-home baby rates.[25]

The aim of this study was to investigate whether DFI was a useful indicator for clinical semen parameters in ART and to examine DFI

Center of Reproductive Medicine, The Affiliated Hospital of Nantong University, Nantong 226001, China.
Correspondence: Dr. F Guo (fguo@ntu.edu.cn)
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before and after sperm processing by density gradient centrifugation (DGC) and swim up collection. In addition, we investigated the effects of DFI on embryo development, blastocyst formation, and clinical outcomes after FET, to provide a more comprehensive guide for clinical practice.

PATIENTS AND METHODS

Patient selection

This was a retrospective study. A total of 381 couples using FET at the Center of Reproductive Medicine of the Affiliated Hospital of Nantong University (Nantong, China) were selected from May 1, 2016, to December 31, 2017. The study included infertile couples aged <38 years who had undergone ART for pure oviduct infertility or male factor infertility. Couples with adenomyosis, a chromosome abnormality, pelvic inflammation, uterine fibroid, and uterine malformation, all of which may adversely affect clinical outcomes of FET, were excluded. The study protocol was approved by the Ethics Committee of the Affiliated Hospital of Nantong University (2020-L031). Written informed consent was obtained from each patient.

IVF/ICSI procedures and laboratory outcomes

Controlled ovarian stimulation was performed using gonadotropin-releasing hormone agonist, gonadotropin-releasing hormone antagonist, and recombinant follicle-stimulating hormone/human menopausal gonadotropin. When three leading follicles reached a mean diameter of ≥17 mm, 250 μg of recombinant human chorionic gonadotropin (hCG; Ovidrel, Darmstadt, Germany) was injected. Oocytes were retrieved using transvaginal ultrasound guidance 36 h after hCG injection. Retrieved oocytes were incubated in G-IVF™ (Vitrolife, Gothenburg, Sweden) medium supplemented with 10% human serum albumin (Vitrolife). IVF or ICSI was performed 4–6 h after oocyte retrieval according to our center's protocol.

Oocytes were assessed to determine whether fertilization had occurred 16–20 h after insemination. Fertilization was considered to be normal if two pronuclei were identified. Embryo development was assessed at 48 h and 72 h after fertilization. At 72 h after oocyte retrieval, embryos were graded according to the number of cells, amount of fragmentation, and variation in cell size.[26] Grade I and II embryos were defined as high-quality embryos. Some day-3 high-quality embryos were cryopreserved, and the excess high-quality embryos and grade III and IV embryos were further cultured to blastocysts and high-quality blastocysts were cryopreserved. A freeze-all strategy was applied for patients (1) at increased risk of ovarian hyperstimulation syndrome, (2) that who on the day of trigging exhibited progesterone levels >1.5 ng ml^{-1} and/or an endometrial thickness <7 mm, and (3) who had other conditions which were not suitable for fresh embryo transfer.[27]

Sperm density gradient centrifugation and DNA fragmentation assay

On the day of oocyte retrieval, semen samples were collected by masturbation after 2–7 days of abstinence, and each sample was incubated at 37°C for a maximum of 60 min. After liquefaction, conventional semen analysis (sperm concentration and motility) was conducted according to the World Health Organization guidelines (WHO, 2010).[28] Semen samples for fertilization underwent DGC (Vitrolife) and swim up collection. The discontinuous density gradient centrifugation consisted of two (90% and 45%) 1-ml layers of SpermGrad (Vitrolife), with 2 ml of semen pipetted on the 45% layer. After centrifugation (ST16, Thermo Scientific, Waltham, MA, USA) at 300*g* for 20 min, the seminal plasma supernatant was discarded, and the sperm pellet was washed with 1 ml of embryo culture medium (Vitrolife) and centrifuged for 10 min, then the pellet was suspended in 1 ml culture medium for the sperm swim up collection.

Sperm DNA fragmentation was measured by the sperm chromatin dispersion (SCD) test (Shenzhen Huakang Co., Ltd., Shenzhen, China) for both the native and DGC plus swim up separated semen, according to the manufacturer's instructions. In brief, semen samples were diluted with phosphate-buffered saline to a concentration of 5×10^6–10×10^6 cells ml^{-1}, and the prepared spermatozoa were mixed with melted agarose, then pipetted onto precoated slides, and covered with a coverslip (22 mm × 22 mm). The slide was placed in the refrigerator at 4°C for 4 min to allow the agarose with sperm cells to solidify. The coverslip was gently removed, and the slide was incubated in an acid solution for 7 min and then in lysis buffer for 20 min. After washing the slide for 3 min in washing buffer, the slide was dehydrated in increasing concentrations of ethanol (70%, 90%, and 100%, each for 2 min) and then air-dried. After Wright-Giemsa staining (Sigma, San Francisco, CA, USA), at least 200 spermatozoa per sample were scored for holes by microscopy (E200, Nikon, Tokyo, Japan) using a 400× objective. Sperm with a small halo (similar to/smaller than a third of the minor diameter of the nucleus) or no halo was considered to have significant DNA fragmentation. DFI means percent of sperm with DNA fragmentation of total sperm counted. Sperm DFI analysis was performed for every patient before sperm DGC and swim up collection, and samples from 65 patients were randomly selected for DFI analysis after DGC and swim up.

Endometrium preparation and FET

For frozen embryo transfer, endometrium was prepared by the exogenous administration of estrogen–progesterone hormone. Estradiol valerate (DELPHARM Lille S.A.S., Paris, France) was administered orally at a starting dose of 2 mg daily from day 3 of the menstrual cycle. Endometrial thickness was monitored by ultrasonography every 3–5 days and estrogen dosage was adjusted until endometrial thickness reached 8 mm or more with a triple line pattern; 20 mg Dupbaston (Abbott Biologicals B.V., Raalte, the Netherlands) and 400 mg soft capsule progesterone (Cyndea Pharma, S.L., Olvega, Spain) via vaginal delivery were administered for endometrium transformation. Day-3 embryos were transferred on the 4th day of endometrium transformation. Blastocysts were transferred on the 6th day of endometrium transformation under the guidance of ultrasound. Couples with day-3 embryo transfers were selected for statistical analysis.

Confirmation of pregnancy

Serum hCG levels were measured 10 days after FET, with hCG >5 IU l^{-1} regarded as positive (pregnancy). Clinical pregnancy was defined as the presence of a gestational sac confirmed by ultrasound examination in the 4th week after embryo transfer. On-going pregnancy was defined as the completion of ≥20 weeks of gestation. First-trimester miscarriage rate was defined as the percentage of nonviable clinical pregnancy/clinical pregnancy, noted by ultrasound follow-up until gestational week 12 of pregnancy. On-going pregnancy rate was defined as the percentage of pregnancies with at least one fetal heart pulsation by ultrasound beyond 20 weeks of FET. The live birth rate was the percentage of FETs that led to a live birth.

Statistical analyses

All measurement data are represented as mean ± standard deviation (s.d.) and enumeration data as percentage (%). Statistical analysis was performed using IBM SPSS Statistics 20 (IBM Corp., New York,

NY, USA). The independent-samples *t*-test was used for comparison between groups. Correlations between parameters were examined using linear regression techniques with a Pearson's correlation coefficient. The Chi-square test was used to compare proportions between two groups. If the cell numbers were less than 5, Fisher's exact test was applied to compare frequencies between groups. Statistical significance was set at $P < 0.05$.

RESULTS

Optimal DFI cut-off value

The current study included 381 couples using FET during IVF/ICSI. Comparison of the receiver operating characteristic (ROC) curve for DFI and fertilization rate (≥60% means 1, <60% means 0) showed that there was statistical difference (area under the curve [AUC] = 0.559, 95% confidence interval [CI]: 0.501–0.617; $P = 0.049$; **Figure 1**). The best ratio of sensitivity and specificity was used as the cutoff value; in this study, it was 25% (sensitivity = 23.3%, specificity = 79.0%).

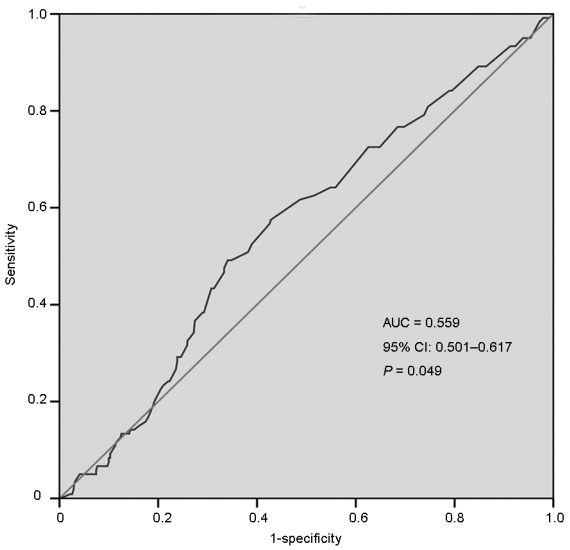

Figure 1: ROC curve for the sperm DNA fragmentation index. Fertilization rate was the criterion variable. ROC: receiver operating characteristic; AUC: area under the curve; CI: confidence interval.

Analysis of sperm DFI and baseline female characteristics in FET cycles

In the 381 couples who underwent FET (IVF: $n = 251$, ICSI: $n = 130$), the IVF and ICSI groups were further divided into two subgroups based on the DFI cutoff value (DFI <25% [low] and ≥25% [high]). For both IVF and ICSI, no statistical difference between the low and high DFI subgroups was found for female age, body mass index (BMI), hormone levels, total gonadotropin dose, days of stimulation, and number of oocytes collected (**Table 1**).

DFI impact on semen parameters

Pearson's correlation analysis showed that sperm DFI was negatively associated with sperm concentration and progressive motility (correlation coefficient [*r*]: −0.289 and −0.640, respectively; $P < 0.01$; **Table 2**). There were no correlations between sperm DFI and male age, BMI, or duration of infertility ($P > 0.05$; **Table 2**).

For semen parameters, progressive motility of the sperm was significantly higher in the low DFI group than that in the high DFI group (46.9% ± 12.4% *vs* 38.5% ± 12.6%, and 37.6% ± 14.1% *vs* 22.3% ± 17.8% for IVF and ICSI, respectively; both $P < 0.01$; **Table 3**), but there was no statistical difference in sperm concentration. All the sperm parameters are presented in **Table 3**.

Correlation between DFI and ART outcomes

For the clinical outcomes of FET cycles, there were no statistical differences in the implantation, clinical pregnancy, first-trimester miscarriage, on-going pregnancy, and the live birth rates between the two DFI groups (**Table 3**).

For the cycles of IVF, we investigated the relationship between sperm DFI and outcomes of embryonic development, including fertilization, cleavage, high-quality embryo, and blastocyst rates. The fertilization rate for IVF was significantly decreased in the high DFI compared with the low DFI group (73.3% ± 23.9% *vs* 53.2% ± 33.6%; $P < 0.01$; **Table 3**). For the ICSI cycles, no statistical differences were observed in the fertilization rate or other laboratory outcomes between the high and low DFI groups (**Table 3**). Sperm DFI values and IVF laboratory outcomes, such as fertilization, cleavage, high-quality embryo, blastocyst formation, and implantation rates are shown in **Table 4**. Sperm DFI was negatively associated with fertilization rate of IVF cycles (*r*: −0.247; $P < 0.01$; **Table 4**), but no significant correlation

Table 1: Baseline female characteristics in low (<25%) and high (≥25%) sperm DNA fragmentation index groups

Variable	*IVF*			*ICSI*		
	DFI <25%	*DFI ≥25%*	*P*	*DFI <25%*	*DFI ≥25%*	*P*
Patient (*n*)	227	24		77	53	
Female age (year), mean±s.d.	28.4±3.2	28.9±3.5	0.249	29.2±3.0	29.0±4.2	0.766
Infertility duration (year), mean±s.d.	3.1±2.4	3.7±2.6	0.141	4.0±2.7	3.6±2.9	0.409
Female BMI (kg m^{-2}), mean±s.d.	23.4±8.1	23.2±3.1	0.900	23.1±4.6	23.3±3.4	0.774
Basal FSH levels (IU l^{-1}), mean±s.d.	6.8±1.9	6.5±1.3	0.449	6.6±1.9	6.5±1.8	0.799
Basal LH levels (IU l^{-1}), mean±s.d.	5.1±2.5	4.4±2.1	0.157	4.8±2.4	5.2±3.6	0.530
Basal E2 levels (IU l^{-1}), mean±s.d.	49.3±16.9	46.2±13.8	0.313	47.2±16.4	48.3±14.4	0.691
Total gonadotropin dose (IU), mean±s.d.	1700.1±498.2	1520.5±630.9	0.126	1550.9±585.1	1627.9±625.9	0.470
Stimulation (day), mean±s.d.	7.6±1.2	7.6±1.2	0.957	7.9±1.2	7.7±1.5	0.361
E2 on hCG day (pg ml^{-1}), mean±s.d.	3514.7±2412.7	3650.4±2069.5	0.760	3026.0±1916.2	2930.1±1738.5	0.767
LH on hCG day (IU l^{-1}), mean±s.d.	2.0±1.4	1.6±1.0	0.231	2.0±1.3	1.6±1.0	0.081
Progesterone on hCG day (pg ml^{-1}), mean±s.d.	1.2±0.7	1.2±0.5	0.792	1.2±0.5	1.1±0.5	0.549
Oocytes retrieved (*n*), mean±s.d.	10.0±5.0	10.6±4.9	0.508	8.9±4.8	9.2±4.5	0.713

All couples used FET and data shown is divided into those using IVF and ICSI. DFI: DNA fragmentation index; BMI: body mass index; E2: estradiol; FSH: follicle-stimulating hormone; hCG: human chorionic gonadotropin; ICSI: intracytoplasmic sperm injection; IVF: *in vitro* fertilization; LH: luteinizing hormone; s.d.: standard deviation; FET: frozen embryo transfer

was found for other laboratory outcomes for the ICSI cycles ($P > 0.05$; **Table 4**).

Comparison of sperm DNA fragmentation index in semen samples before and after sperm collection

Sixty-five semen samples were randomly selected to compare the sperm DFI before and after sperm collection procedures. After DGC and swim up collection, the sperm DFI was markedly decreased (17.1% *vs* 2.4%; $P < 0.01$; **Figure 2**).

DISCUSSION

Frozen thawed embryo transfer plays an increasingly important role in ART. In this study, we focused on the effect of sperm DFI on clinical outcomes of FET. With the DGC plus swim up-processed sperm for fertilization, and the sperm chromatin dispersion test for sperm DFI detection, we did not find a negative impact of sperm DFI on embryo development and quality. The clinical pregnancy, ongoing pregnancy, and live birth rates after FET were equivalent in the high and low DFI groups.

Sperm DNA integrity evaluated by DFI has attracted much attention because of its diagnostic value for male infertility and its relationship with pregnancy outcomes.[23] At present, the correlation between DFI and clinical pregnancy outcomes remains controversial. Studies in animals showed that mammalian fertilization and embryonic development depended partly on the intrinsic integrity of sperm DNA. When DNA damage was above a certain threshold, the damage can interfere with normal fertilization and subsequent embryonic development, leading to pregnancy disorders.[8] In human research, it was found that sperm DNA fragmentation had a negative relationship with IVF/ICSI fertilization. High DFI was linked to the risk of cancelation of embryo transfer because of blocked embryo development. Infertile couples with a low DFI were more likely to achieve a pregnancy after IVF treatment.[29,30] Following IVF, the early abortion rate was significantly higher in the high DFI compared with medium and low DFI groups, even though there was no significant difference in the clinical pregnancy rate among the high, medium, and low sperm DFI groups.[31] These studies supported DFI as a prognostic indicator for the fertilization rate, pregnancy rate, and miscarriage rate of IVF/ICSI. Conversely, many studies did not find that sperm DFI had negative impact on IVF/ICSI clinical outcomes.[31–33] These studies found no significant differences in the rates of clinical pregnancy, early abortion, oocyte fertilization, or good-quality embryos among different DFI groups for IVF or ICSI cycles. These studies all focused on the clinical outcomes of fresh embryo transfer. In the present study, we analyzed sperm DFI and clinical outcomes of FET. For couples using FET, no significant difference was found in the clinical pregnancy, ongoing pregnancy, and live birth rates between the high DFI (≥25%) group and the low DFI (<25%) group.

Many potential issues may be associated with sperm DFI, leading to male factor infertility. Studies have investigated the correlation between sperm DFI and conventional semen parameters.[34–36] These studies found that infertile men tend to have more sperm DNA damage

Table 2: Correlation between the sperm DNA fragmentation index and male parameters

Variable	*DFI*	
	r	*P*
Male age (year)	0.010	0.841
Infertility duration (year)	0.007	0.893
Male BMI (kg m^{-2})	0.035	0.491
Sperm concentration (10^6 ml^{-1})	–0.289	0.000
Progressive motility (%)	–0.640	0.000

DFI: DNA fragmentation index; BMI: body mass index

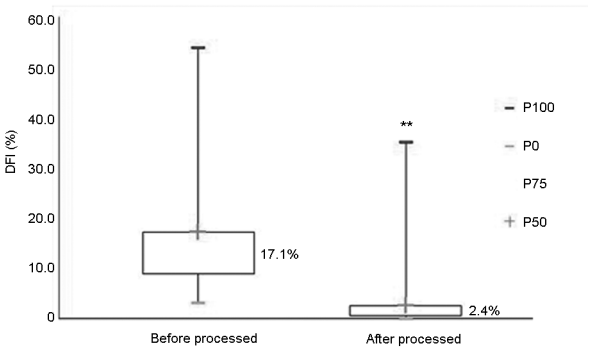

Figure 2: Comparison of the sperm DFI in samples (n = 65) before and after processing (density gradient centrifugation and swim up collection) showed a marked reduction in DFI after processing. $^{**}P < 0.01$. DFI: DNA fragmentation index.

Table 3: Male parameters and assisted reproductive techniques outcomes between DNA fragmentation index <25% and ≥25% groups, divided into intracytoplasmic sperm injection and *in vitro* fertilization cycles

Variable	*IVF*			*ICSI*		
	DFI <25%	*DFI ≥25%*	*P*	*DFI <25%*	*DFI ≥25%*	*P*
Male age (year), mean±s.d.	23.4±3.6	30.1±3.4	0.317	30.8±4.3	29.5±4.2	0.109
Male BMI (kg m^{-2}), mean±s.d.	24.4±3.7	23.4±3.3	0.212	24.0±3.7	24.6±3.4	0.365
Sperm concentration (10^6 ml^{-1}), mean±s.d.	70.4±36.3	64.1±48.4	0.441	50.1±40.6	37.3±33.3	0.059
Progressive motility (%), mean±s.d.	46.9±12.4	38.5±12.6	0.002	37.6±14.1	22.3±17.8	0.000
Fertilization rate (%), mean±s.d.	73.3±23.9	53.2±33.6	0.008	80.6±19.0	75.3±18.2	0.113
Cleavage rate (%), mean±s.d.	98.9±9.6	96.1±9.3	0.210	98.5±13.7	99.0±3.5	0.778
High-quality embryo rate (%), mean±s.d.	68.1±29.9	57.6±34.1	0.138	70.1±32.1	76.1±27.6	0.267
Blastocyst formation rate (%), mean±s.d.	70.8±30.9	77.8±30.7	0.977	78.5±30.0	86.6±29.9	0.307
Implantation rate (%), mean±s.d.	50.4±41.6	47.9±42.9	0.784	48.5±42.4	47.2±39.7	0.816
Pregnancy rate, % (*n*/total)	74.0 (168/227)	79.2 (19/24)	0.581	66.2 (51/77)	71.7 (38/53)	0.510
First-trimester miscarriage rate, % (*n*/total)	24.4 (41/168)	21.1 (4/19)	0.746	23.5 (12/51)	13.2 (5/38)	0.218
On-going pregnancy rate, % (*n*/total)	55.9 (127/227)	62.5 (15/24)	0.746	50.6 (39/77)	62.3 (33/53)	0.218
Live birth rate, % (*n*/total)	53.3 (121/227)	62.5 (15/24)	0.390	49.4 (38/77)	60.4 (32/53)	0.215

DFI: DNA fragmentation index; BMI: body mass index; ICSI: intracytoplasmic sperm injection; IVF: *in vitro* fertilization

Table 4: Correlation between sperm DNA fragmentation index and assisted reproductive techniques outcomes

Variable	*IVF*		*ICSI*	
	r	*P*	*r*	*P*
Fertilization rate (%)	–0.247	0.000	–0.157	0.074
Cleavage rate (%)	0.113	0.080	0.009	0.922
High-quality embryo rate (%)	–0.010	0.873	–0.055	0.537
Blastocyst formation rate (%)	0.068	0.449	0.114	0.393
Implantation rate (%)	0.053	0.405	–0.026	0.772

ICSI: intracytoplasmic sperm injection; IVF: *in vitro* fertilization

than fertile men, and sperm DFI was negatively associated with sperm concentration, vitality, and normal morphology. In addition, sperm DFI was positively correlated with age, abstinence time, and unhealthy lifestyles. In our study, sperm DFI was negatively associated with sperm concentration and progressive motility. Sperm progressive motility was significantly lower in the ≥25% DFI group. We found that sperm DFI was not significantly higher in the group with a lower sperm count, but this needs further investigation. In the current work, there was no correlation between sperm DFI and male age, duration of infertility, or male BMI, consistent with some research results.[37,38] Other researchers found that there was a positive correlation between the age of men and sperm DFI.[36,39,40] These previous studies included males ranging from 18 years old to 50 years old, whereas the present study only included reproductive age men receiving ART. These different ages may partly explain the different results.

Sperm wash is a standard process before ART fertilization, markedly reducing the number of dead sperm. Brahem *et al.*[41] observed a significant decrease in sperm DFI following DGC when compared with raw semen (approximately 17.0% *vs* 32.8%; $P < 0.01$). DGC and swim up provides an effective strategy to isolate motile sperm from semen.[37,42] Other researchers found that there was more DNA fragmentation in epididymal and ejaculated sperm than that in testicular sperm,[43] which indicates that DNA damage occurs later than spermatogenesis. DNA fragmentation may affect sperm motility more than concentration. In the present study, sperm DFI was markedly reduced after the DGC wash and swim up preparation. This finding suggests that the two-step preparation significantly removed spermatozoa with DNA breaks, as measured by the SCD test.

To evaluate sperm DNA integrity, commonly used methods include the sperm chromatin structure assay, terminal deoxynucleotidyl transferase dUTP nick end labeling, single-cell gel electrophoresis, and SCD assay. These methods act by incorporating either DNA probes/dyes or modified nucleotides at the site of damage, single or double DNA strand breaks, with or without the use of heat, acid, or lysis solution.[10] Although these DFI tests depend on different DNA fragmentation detection mechanisms, they are generally well correlated.[44] In this study, the SCD test was used to distinguish normal and fragmented sperm through acid denaturation. This test is simple and affordable, without the requirement of complex or expensive instrument,[45] and does not rely on the determination of either color or fluorescence intensity.[46] Spermatozoa with DNA fragmentation do not produce the characteristic halo of dispersed DNA loops that are observed in sperm without DNA fragmentation. The use of ICSI can provide a success pregnancy for a couple without the need to explore and develop assessment and treatment strategies targeting the potential cause of male infertility. Although the application of sperm DFI as a biomarker of male infertility remains controversial, the sperm DFI test still has clinical value.

Deciding whether to use day-3 embryo transfer or culture to blastocyst stage is a highly debated topic. At present, there is no standard protocol regarding the extended culture of day-3 embryos. *In vivo* fertilization occurs in fallopian tubes and human embryos enter the uterus during the blastocyst stage, which is better synchronized with the uterine environment. Several reports indicate that blastocyst transfer was associated with improved live birth rates in comparison with early cleavage stage embryo transfers,[47,48] while other studies debate whether embryo transfer at the blastocyst stage is superior to day-3 stage.[49,50] Glujovsky *et al.*[51] found that there was no difference in clinical pregnancy rate or miscarriage rate between early cleavage and blastocyst transfers. Further, extended embryo culture may lead to no embryos for transfer in some patients because of the risk of embryo developmental arrest and the risk of epigenetic changes due to the prolonged culture.[52] Currently, both day-3 embryo and blastocyst FET are widely used in the clinic. In this study, we analyzed day-3 embryo FET comparing high and low sperm DFI with clinical outcomes after FET.

Poor embryo quality may result in early miscarriage. High sperm nuclear DNA fragmentation has been correlated with lower fertilization rates,[53] poorer embryo development,[54] and lower blastocyst formation rates.[16,29,55,56] The fertilization rate in a DFI <14% group was significantly higher than the rate of a DFI ≥14% group.[57] However, other research found that the fertilization rates and number of high-quality embryos were not significantly different between defined DFI groups.[38] Some researches suggested that DFI did not correlate with blastocyst aneuploidy or morphological grading nor pregnancy rate and loss.[58,59] In the present study, there was a difference in the fertilization rate between the low and high DFI groups using IVF but not ICSI. No significant difference was found for embryonic development between the low and high DFI groups using either ICSI or IVF. Likewise, there was no difference in the clinical outcomes after FET between the two groups. These differences may be related to different sperm processing methods. In the present study, the sperm used for fertilization was processed through DGC and the swim up technique. Our analysis showed that sperm DFI was significantly decreased after this processing and became comparable with the control group. Another reason may be that the embryos being cryopreserved were of superior quality with better developmental potential. Ni *et al.*[60] reported findings similar to the current study, but they found that DFI was related to blastocyst formation after ICSI, so further investigation is needed.

There are limitations in this study. First, only day-3 embryo transfer was analyzed and the study lacked data from blastocyst transfer, which is widely used in clinical practice. Second, sperm DFI was measured with the SCD test for both native and processed semen from 65 randomly picked cases, but this was not done for every patient, possibly leading to selection bias.

Sperm DFI may be an efficient auxiliary diagnostic tool for predicting semen quality and fertilization ability, but the effect of DFI on embryo development and IVF/ICSI clinical outcomes remains controversial. The current study found that sperm DFI had no impact on embryo quality and clinical outcomes after FET even though the IVF fertilization rate was lower for the high DFI group. Sperm DNA fragmentation was significantly reduced by the DGC wash and swim up processing before sperm was used in IVF/ICSI fertilization. As a routine examination before IVF/ICSI, the sperm DFI test may be helpful for the embryologist to choose a suitable procedure to improve the fertilization rate. Sperm DFI had no significant effect on the clinical outcomes of FET.

AUTHOR CONTRIBUTIONS

FG has given substantial contributions to the conception and design of the present study, and critically revised the content. QXW performed data curation and drafted the manuscript. XW, MYY, HS, DW, and SPZ contributed to the acquisition of data. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declared no competing interests.

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