Taiwanese Journal of Obstetrics & Gynecology 57 (2018) 493–498

Contents lists available at ScienceDirect

Taiwanese Journal of Obstetrics & Gynecology

journal homepage: www.tjog-online.com

Original Article

Sperm DNA fragmentation index, as measured by sperm chromatin dispersion, might not predict assisted reproductive outcome

Tie-Cheng Sun [a, b], Yan Zhang [a], Hong-Tu Li [c], Xi-Ming Liu [d], Dong-Xu Yi [c], Li Tian [e], Yi-Xun Liu [a, *]

[a] State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing, 100101, China
[b] University of Chinese Academy of Sciences, Beijing, China
[c] Liao-Ning Family Planning Institute, Shen-Yang, 110031, China
[d] Changsha Reproductive Medicine Hospital Academician Workstation, Changsha, 410205, China
[e] Reproductive Medicine Centre Peking University People Hospital, Beijing, 100044, China

ARTICLE INFO

Article history:
Accepted 21 May 2018

Keywords:
Sperm DNA fragmentation (DFI)
Sperm motility
IVF/ICSI
Clinical pregnancy rate

ABSTRACT

Objective: Routine semen parameters have limited clinical diagnostic value for predicting male infertility. The aim of this study was to investigate the association between sperm DNA fragmentation index (DFI) and semen quality, and between DFI and clinical pregnancy rate of in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI).

Methods and materials: A total of 390 couples undergoing sperm fragmentation prior to receiving conventional IVF (n = 238) or ICSI (n = 152) were evaluated.

Results: We found that there were no significant differences in fertilization rate, good embryo rate, or pregnancy rate between high (≥30%) and low (<30%) DFI groups after IVF or ICSI. However, statistically different decreasing motility trends under higher DFI values in the IVF and ICSI groups were detected. Comparison of ROC curve of motility and DFI scores for achieved pregnancy revealed that the best DFI cut-off value was 20%. Also, no significant change was found when 20% DFI level was taken in IVF and ICSI outcomes.

Conclusion: DFI scores did not provide independent information regarding fertilization, embryo quality, or pregnancy for infertile patients who received IVF or ICSI, but were consistent with semen analysis for infertile couples, regardless of IVF or ICSI outcome.

Introduction

Routine semen parameters have limited clinical diagnostic and prognostic value for predicting male infertility. It has been estimated that more than 48.5 million couples are infertile worldwide, which has had a widespread global impact [1]. Male-related infertility is solely contribute to approximately 20% of all infertility cases; when combined with female factors, they contribute to 30–40% of cases [2]. To date, assessment of male infertility is still based on semen quality analysis according to World Health Organization (WHO) standards, including total sperm number, concentration, motility, and morphology [3]. In fact, many cases of male infertility are caused by sperm DNA defects, which routine semen quality analysis still fails to detect [4]. Therefore, routine semen have limited clinical diagnostic and prognostic value for predicting male infertility.

Recently, many studies have shown that sperm DNA fragmentation index (DFI) is used for prediction of male infertility, and it has better diagnostic and prognostic value than routine semen parameters [5–8]. It was reported that DNA integrity is essential to fertilize oocytes and is highly indicative of male infertility [9]. Recently, several studies have shown the damage rate of sperm DNA is higher in males with suspected infertility compared with fertile men [5,7,8,10–13]. Many factors can result in sperm DNA damage, including infection [14], drug use [15] and advanced age [16].

To date, various methods have been developed and introduced to measure sperm DNA fragmentation or damage, including terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling assay (TUNEL) [17], Comet assay

Abbreviations: DFI, Sperm DNA fragmentation; SCD, Sperm Chromatin Dispersion; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection.

* Corresponding author. State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, #1 Beichen West Road, Chaoyang District, Beijing 100101, China. Fax: +86 10 64807038.
E-mail address: liuyx@ioz.ac.cn (Y.-X. Liu).

https://doi.org/10.1016/j.tjog.2018.06.003

[18], DNA breakage detection-fluorescent in situ hybridization assay [19,20], sperm chromatin dispersion (SCD) [21], and sperm chromatin structure assay [6,22]. Recently, some investigators created a novel synthetic oligopeptide that showed significant value for detecting DNA damage in human spermatozoa [23].

More recently, contradictory findings have been published regarding the association between sperm DNA damage and outcome of assisted reproduction technology (ART). A systematic review showed that sperm DNA damage is associated with lower pregnancy rate in natural, intrauterine insemination [24], and in vitro fertilization (IVF) [25,26], and is associated with increased risk of pregnancy loss in couples who underwent IVF or intracytoplasmic sperm injection (ICSI) [25–28]. Alternatively, some studies suggested that DFI is not associated with ART outcome [29,30]. Therefore, evidence regarding association between sperm DNA damage and ART outcome is inconclusive.

In the present study, we investigate the associations among sperm DNA damage or fragmentation and semen parameters, fertilization rate, good embryo rate, and pregnancy rate after IVF or ICSI.

Materials and methods

Patients

This was a retrospective study at Peking University People's Hospital the data was collected during the month of September 2014 to June 2016. All experimental procedures and sample collection were approved by the Medical Ethics Committee of Peking University People's Hospital, and a written informed consent was obtained from each participant. A total of 390 infertile couples undergoing IVF or ICSI were included in this study. The following data were collected: sperm concentration, sperm motility, sperm morphology, DFI, fertilization rate, good embryo rate, and clinical pregnancy rate.

All female participants without poor ovary response had day 3 serum FSH levels <15 IU/L. Only freshly ejaculated sperm and sperm samples with a concentration of at least 1 million/mL were included for this study.

Semen analysis

Semen samples were collected from 390 men after 2–7 days of sexual abstinence and on the day of their partners' oocyte retrieval for IVF or ICSI. Semen analysis was performed according to WHO guidelines on a Makler R chamber (Sefi Laboratories, Tel Aviv, Israel) [3]. Sperm morphology was analyzed using strict criteria for all men [31]. Normal sperm samples were defined as those with concentrations $\geq 15 \times 10^6$/mL, progressive motility ≥32%, total motility ≥40%, and normal strict morphology ≥4%. Only normal sperm samples with concentrations $\geq 15 \times 10^6$/mL, motility ≥40%, and normal strict morphology ≥4% were used for IVF; and only sperm samples with at least one of the following criteria: concentration $<4 \times 10^6$/mL, and normal strict morphology <4% were used for ICSI.

ART procedures

All patients received ovarian stimulation using a standard luteal down-regulation regimen (long protocol) or flare-up short regimen (short protocol) [32–35]. Standard IVF or ICSI techniques were assessed as follows: the oocytes were assessed to determine whether fertilization had occurred at 16–18 h after insemination or microinjection. After 18 h, fertilization was determined to be normal if two pronuclei and two polar bodies were identified, and pronuclei size and position, as well as nucleoli size, distribution, and number were evaluated [36].

The day 3 embryo scoring system were observed according to their cell number, symmetry, blastomeres, type, and percentage of fragmentation [37]. Fresh embryo transfer was performed on day 3 after oocyte retrieval using the best quality embryos among a cohort of resultant embryos. The grading criteria were as follows: grade I: no fragmentation with equal-sized cells; grade II: <20% fragmentation with equal-sized cells; grade III: a lot of fragmentation with unequal-sized cells; grade IV: ≥20% fragmentation with unequal-sized cells; and grade V: ≥50% fragmentation. Embryos classified as grade I or II were denoted as good embryos. The day 5–6 blastocyst that were cryopreserved had at least grade 3BB [38]. Freezing and thawing were performed using a Kitazato Vitrification Freeze kit and Kitazato Thaw kit according to the manufacturer's protocols. No more than three surviving embryos were transferred into the uterine cavity. The luteal phase was routinely supported with progesterone 40–60 mg IM per day for 14 days and continued for another 4 weeks if pregnancy was established. Serum hCG levels were measured 14 days after embryo transfer. Clinical pregnancy was confirmed by ultrasound 4 weeks after embryo transfer.

SCD test

After liquefaction, an aliquot of 100 μL of the raw semen sample was used for SCD test [39]. Using the Halosperm® kit (INDAS Laboratories, Madrid, Spain), the SCD test was performed according to the manufacturer's protocol [40]. The procedure of measuring sperm DNA fragmentation by SCD test was performed as follows. A minimum of 500 spermatozoa per sample were scored under the × 100 microscope objective. The SCD test is based on the principle that sperm with non-fragmented DNA produce a big halo of dispersed DNA loops. Otherwise, sperm with fragmented DNA which size of halo smaller than 1/2 of minor diameter of the core [41]. It is widely accepted that a DFI value of 30% can be used as the cut-off to distinguish between potentially fertile and infertile men [5].

Semen preparation

IVF samples were prepared by swim up: raw semen were diluted 1: 1 (v: v) with Sperm Medium (SAGE, Cooper Surgical-Origio, Denmark). Then they were pelleted at 500 g for 5 min and the supernatants were discarded. Another process was to add 0.5–1 mL fresh medium and the incubation for 45 min of the tubes with 45° inclination. Finally, the upper 0.1–0.5 mL was taken for IVF procedures. ICSI samples were pelleted at 500 g for 5 min and the supernatants were discarded. Then, careful addition of 0.1 mL fresh medium was taken for ICSI procedures.

Statistical analysis

Statistical analysis was performed using SPSS (version 18.0, Inc., Chicago, USA). The Student's t-test for independent samples was used for comparison between groups. The correlations between parameters were examined using linear regression techniques with Pearson's correlation coefficient. ROC curves for variables were performed according to ROC analyses. The positive predictive value, negative predictive value, and their 95% CI were also calculated for significant variables. p values less than 0.05 were considered statistically significant.

Results

DFI and semen parameters

This study included 390 infertile couples undergoing IVF (n = 238) and ICSI (n = 152). The IVF and ICSI groups were further

subdivided into two subgroups based on the DFI cut-off value (≥30% and <30%). In the 238 couples undergoing IVF, 53 men were found to have an abnormal DFI value. No statistical significance was demonstrated between any DFI subgroups and serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), or testosterone (T). However, in the 152 couples undergoing ICSI, 71 men were reported to have an abnormal DFI value and not showed correlation with serum levels of FSH, LH, and T.

All characteristics of semen (volume, concentration, progressive rate, non-progressive rate, motility, morphology) and DFI are presented in Table 1. The sperm concentration was significantly higher in the IVF group compared with the ICSI group. Another difference was noted between sperm motility and DFI. In the IVF group, motility of ≥30% and <30% subgroups appeared dramatically discrepant (68.17. and 52.70, respectively). In addition, similar results were observed in couples undergoing ICSI (52.15 and 38.99, respectively). All other parameters (volume, normal morphology) were almost the same in the IVF group compared with the ICSI group (Table 1).

Correlation between motility and DFI level are shown (Fig. 1). Statistically significant negative correlations were found between DFI and motility in IVF couples ($r = -0.454$, $p < 0.001$) and ICSI couples ($r = -0.488$, $p < 0.001$).

DFI and fertilization rate, good embryo quality rate, and clinical pregnancy rate

In the IVF and ICSI groups, when DFI threshold had a cut-off value of ≥30%, there were no significant negative correlations between DFI and fertilization rate, good embryo quality rate, and pregnancy rate (Table 2). In the ICSI group, fertilization rate (69.31 ± 18.31 vs. 69.41 ± 18.9) and good embryo rate (52.49 ± 27.62 vs. 52.47 ± 25.44) were not significant. There was no difference in pregnancy rate (46.91% vs. 36.62%), although there was a decreasing trend in the ≥30% DFI subgroup.

Optimal DFI cut-off value

Comparison of ROC curve of motility and DFI for achieved pregnancy criterion showed there was no significant difference (Fig. 2). The value with the best ratio of sensitivity and specificity was defined as the cut-off value, and was 20% for DFI in both IVF and ICSI groups (Table 3). Also no significant change was found when 20% DFI level was taken in IVF and ICSI outcomes (Table 4 and Table 5).

Discussion

Sperm chromatin is well-organized and has high nuclear condensation, crystalline structure, haploid DNA, and heterogeneous proteins [42]. In spermatogenesis, any alterations that occur in the sperm chromatin could have detrimental effects on sperm functions [43]. It is widely accepted that sperm DNA fragmentation is correlated with semen quality [5,7,8,10,42,44,45]. In this study, we evaluated the association between DFI and human sperm parameters. A negative association between DFI and sperm motility was found, which is consistent with results of previous studies [7,13,29]. Our finding that there was a correlation between

Table 1
Semen parameters categorized according to the type of treatment: IVF or ICSI.

Group	IVF (n = 238)			ICSI (n = 152)		
DFI	<30%	≥30%	*P* value	<30%	≥30%	*P* value
Cycle (n)	185	53		81	71	
Male age (y)	37.72 ± 6.62	40.75 ± 7.18	0.004	36.46 ± 6.13	36.44 ± 7.47	0.981
FSH (IU ml^{-1})	5.16 ± 2.86	5.79 ± 3.1	0.167	7.0 ± 3.96	7.57 ± 4.34	0.402
LH(IU ml^{-1})	3.97 ± 1.97	3.91 ± 1.61	0.834	4.46 ± 2.12	4.33 ± 1.98	0.71
T (ng ml^{-1})	7.97 ± 6.0	3.67 ± 1.46	0.603	3.48 ± 1.49	9.06 ± 4.64	0.281
Volume (ml)	3.15 ± 1.32	3.16 ± 1.91	0.898	2.84 ± 1.24	3.09 ± 1.77	0.312
Concentration (M ml^{-1})	76.86 ± 10.45	67.43 ± 51.52	0.594	9.06 ± 13.93	8.75 ± 9.05	0.875
Progressive rate, PR (%)	**53.46 ± 15.65**	**40.58 ± 18.91**	**0.001**	**41.06 ± 19.49**	**32.72 ± 22.98**	**0.017**
Non-progressive rate, NP (%)	14.71 ± 7.41	12.13 ± 6.69	0.024	11.09 ± 6.66	6.28 ± 4.32	0.001
Motility (%)	**68.17 ± 17.98**	**52.70 ± 22.4**	**0.001**	**52.15 ± 23.23**	**38.99 ± 24.87**	**0.001**
Normal morphology (%)	4.67 ± 1.06	4.51 ± 1.25	0.331	3.86 ± 2.12	3.12 ± 1.58	0.017

Abbreviations: DFI, DNA fragmentation index; FSH, follicle-stimulating hormone; LH, luteinizing hormone; T, testosterone.
Significance was defined as $P < 0.05$.

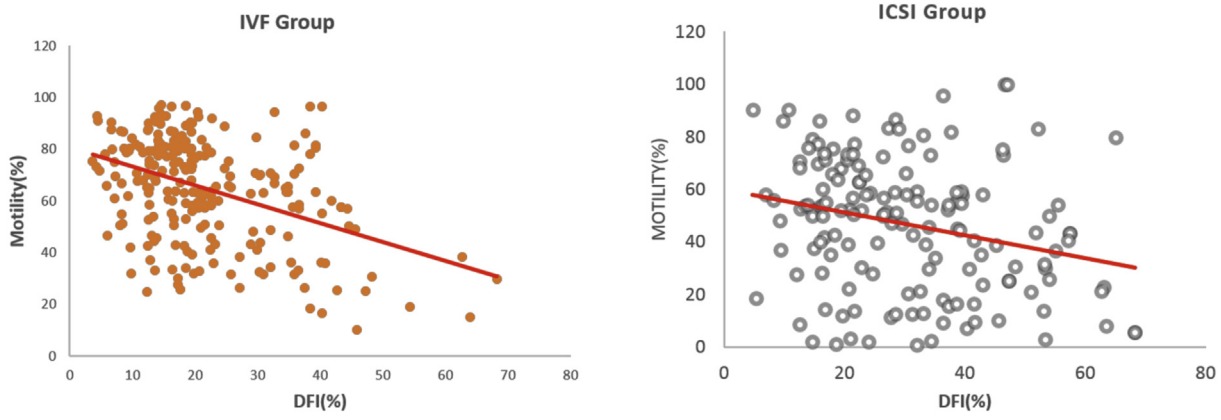

Fig. 1. Scatter graph illustrating the regression analysis of sperm motility and DFI.

Table 2
Comparison of sperm 30% DFI levels in outcomes of IVF or ICSI.

Group	IVF(n = 238)			ICSI(n = 152)		
DFI	<30%	≥30%	*P* value	<30%	≥30%	*P* value
Cycle (n)	185	53		81	71	
Female age (y)	35.73 ± 5.34	38.26 ± 4.94	0.002	34.37 ± 5.45	33.87 ± 5.81	0.587
Oocytes retrieved (n)	11.26 ± 5.94	10.23 ± 5.33	0.255	12.11 ± 6.78	14.56 ± 6.9	0.029
[a]Fertilization rate (%)	77.77 ± 18.99	80.51 ± 18.08	0.35	69.31 ± 18.31	69.41 ± 18.9	0.972
[b]Good embryo rate (%)	58.76 ± 27.26	61.3 ± 27.5	0.555	52.49 ± 27.62	52.47 ± 25.44	0.996
Clinical pregnancy rate (%)	43.24% (80/185)	56.6% (30/53)	0.074	46.91% (38/81)	36.62% (26/71)	0.202
Ongoing pregnancy rate (%)	87.5 (70/80)	93.33 (28/30)	0.325	81.58 (31/38)	80.77 (21/26)	0.625

Data values of reproduction outcome neither vary significantly in IVF nor ICSI group.
[a] Fertilization rate = (No fertilized oocytes)/(No inseminated oocytes) × 100.
[b] Good embryo rate = (No good embryos)/(No embryos) × 100.

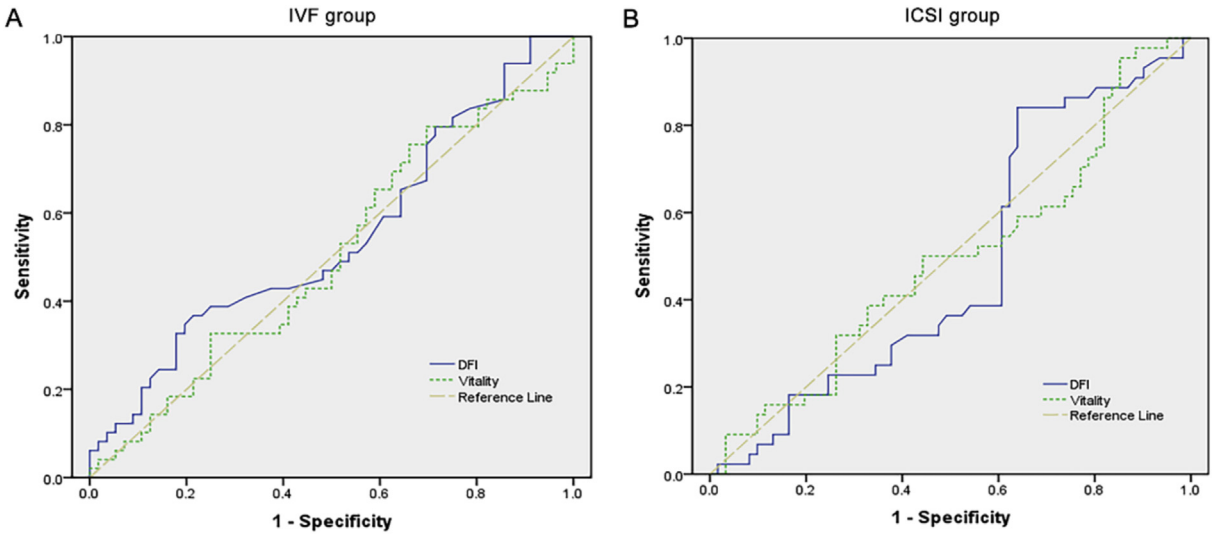

Fig. 2. Comparison of ROC curve for DFI and sperm motility in IVF or ICSI group. The criterion variable was achieved pregnancy. There were no significant differences [IVF group, motility (AUC = 0.502) vs. DFI (AUC = 0.543), $p = 0.448$; ICSI group, motility (AUC = 0.492) vs. DFI (AUC = 0.477), $p = 0.685$].

Table 3
Sensitivity and specificity, positive and negative likelihood and positive and negative predictive values with 95% confidence interval for sperm motility (%) and DFI. The cut-off value determined by ROC curve according to achieved pregnancy criterion.

Variable	Cut-off value	Sensitivity	Specificity	PLR	NLR	PPV	NPV
IVF group							
Motility	≥40	46	53	1.0	1.0	86	14
DFI	≥20	41	40	0.69	1.5	63	21
ICSI group							
Motility	≥40	33	48	0.6	1.4	41	39
DFI	≥20	55	63	1.5	0.7	36	79

DFI levels and sperm motility indicates that DFI can be used as a predictor of testicular spermatogenesis, and DFI should be a supplementary to WHO guideline. Additionally, some other parameters, such as semen volume and sperm morphology, were not associated with DFI, which is in contrast to findings of other studies; this may have been caused by using different methodologies [18,22], such as using of different editions of WHO guidelines, standardization of different sperm DNA fragmentation tests, sperm separation and use of different techniques.

Recently, some studies revealed that DFI levels were negatively correlated with fertilization rate, embryo quality rate, and pregnancy rate after IVF or ICSI [25,41,46,47]. However, our results confirmed that DFI was not related to fertilization rate, good embryo rate, or pregnancy rate. This discrepancy was probably due to some of the following reasons: first, when washing sperm, good sperm from semen are selected [47], and the good sperm observed under the microscope are selected and injected into the oocyte. Second, the good embryo rates of IVF or ICSI did not differ; this indicates that a rather high DFI value may not necessarily impact embryonic development, for which only mature and morphologically normal sperm are involved in fertilization or used in IVF or ICSI procedures. In addition, studies in mice [48] and humans [49,50] revealed that oocytes might repair DNA damage; therefore, the limited capacity of oocytes to repair sperm DNA damage might greatly affect ART outcome. Consequently, sperm DNA damage might not necessarily affect embryonic development and subsequent IVF/ICSI treatment. Third, the paternal genome is switched on after the 4–8 cell stage, which further affects embryo development [51,52]. An animal experiment showed that the oocyte can repair sperm dysfunction [53]. In the stage of in vitro development, the embryos with good quality does not means that its must be development to the blastocyst; otherwise the poor embryo might be reaching to blastocyst although the higher level of DFI fertilized the oocytes. Finally, some embryos causing its developing blocking may not be transferred.

When the association between DFI and pregnancy rate was analyzed, the ROC curve analysis results showed that sperm DNA damage assessment was a good predictive parameter of pregnancy success for infertile couples. The cut-off value was set at 20% sperm DNA fragmentation with greater sensitivity than the previously established sperm DNA fragmentation index of 30% [21]. However,

Table 4
Clinical data on semen parameters in IVF and ICSI cycles divided into according to DFI ≥20% versus DFI <20%.

Group	IVF (n = 238)			ICSI (n = 152)		
DFI	<20%	≥20%	*P* value	<20%	≥20%	*P* value
Cycle (n)	145	93		50	102	
Male age (y)	36.04 ± 5.07	38.03 ± 6.03	0.66	36.79 ± 4.77	36.14 ± 7.71	0.129
FSH (IU ml^{-1})	5.59 ± 2.66	4.33 ± 1.71	0.005	7.32 ± 3.47	8.25 ± 5.1	0.432
LH(IU ml^{-1})	4.07 ± 1.3	3.96 ± 1.11	0.78	4.76 ± 2.24	4.86 ± 2.23	0.833
T (ng ml^{-1})	3.72 ± 1.61	3.49 ± 1.29	0.445	3.82 ± 1.62	3.47 ± 1.3	0.26
Volume (ml)	3.3 ± 1.35	3.36 ± 1.94	0.857	3.0 ± 1.15	3.23 ± 1.66	0.492
Concentration (M ml^{-1})	76.86 ± 10.45	68.98 ± 58.39	0.849	13.39 ± 13.37	10.39 ± 9.23	0.195
Progressive rate, PR (%)	**54.63 ± 16.51**	**43.98 ± 17.03**	**0.004**	37.53 ± 21.52	35.34 ± 22.9	0.657
Non-progressive rate, NP (%)	14.82 ± 6.27	12.76 ± 6.11	0.128	8.16 ± 5.76	7.96 ± 5.85	0.872
Motility (%)	**69.45 ± 18.96**	**56.74 ± 19.33**	**0.003**	45.69 ± 24.88	43.29 ± 25.78	0.668
Normal morphology (%)	**4.58 ± 0.99**	**4.03 ± 0.9**	**0.01**	3.53 ± 2.01	3.3 ± 1.72	0.27

Abbreviations: DFI, DNA fragmentation index; FSH, follicle-stimulating hormone; LH, luteinizing hormone; T, testosterone.
Significance was defined as $P < 0.05$.

Table 5
Comparison of sperm 20% DFI levels in outcomes of IVF or ICSI.

Group	IVF(n = 238)			ICSI(n = 152)		
DFI	<20%	≥20%	*P* value	<20%	≥20%	*P* value
Cycle (n)	145	93		50	102	
Female age (y)	36.72 ± 5.53	37.47 ± 4.29	0.12	35.21 ± 5.0	35.46 ± 5.52	0.125
Oocytes retrieved (n)	10.86 ± 5.71	11.44 ± 6.2	0.855	12.35 ± 7.97	12.86 ± 7.0	0.622
[a]Fertilization rate (%)	79.31 ± 19.4	77.21 ± 16.46	0.164	68.76 ± 17.9	74.22 ± 19.52	0.458
[b]Good embryo rate (%)	62.28 ± 27.58	55.12 ± 25.75	0.927	49.06 ± 29.56	59.53 ± 26.82	0.278
Clinical pregnancy rate (%)	40 (58/145)	56 (52/93)	0.074	40 (20/50)	43 (44/102)	0.242
Ongoing pregnancy rate (%)	86.2 (50/58)	92.3 (48/52)	0.102	75 (15/20)	84.1 (37/44)	0.836

Data values of reproduction outcome neither vary significantly in IVF nor ICSI group.
[a] Fertilization rate = (No fertilized oocytes)/(No inseminated oocytes) × 100.
[b] Good embryo rate = (No good embryos)/(No embryos) × 100.

there also no significant change was found when 20% DFI level was taken in IVF and ICSI outcomes. Therefore, DFI cannot predict outcomes of fertilization rate, embryo quality, or pregnancy rate for infertile patients undergoing IVF or ICSI treatment.

Conclusions

Our observation indicates that DFI scores cannot provide independent information about fertilization rate, embryo quality, or pregnancy rate for infertile patients undergoing ART, but DFI could provide insight into male semen quality. Semen analysis remains a cornerstone of evaluation for male infertility because it provides basic information regarding male semen quality. We suggest that the DFI test should be included as a supplement to traditional semen analysis for couples with infertility, regardless of if they use IVF or ICSI treatment. Moreover, future studies should focus on the association between DFI and semen quality, especially in cut-off value of DNA fragmentation, because there is the clinical concern regarding whether DFI can be used for infertility diagnosis and prognosis.

Author contributions

Professor YX L and L T conceived and designed the experiments. We thank the staff of Reproductive Medical Center, Peking University People's Hospital, the Changsha Reproductive Medicine Hospital, and the Liao-Ning Family Institute for semen sample collection. We also thank Jia-Yi Duan, who performed the experiment, and Yang Cao, who analyzed the data.

Competing financial interests

All authors declare no competing financial interests.

Grant information

This work was supported by grants from Major Research Plan "973" Project (2012CB944702), Natural Science Foundation of China (31501953, 31471352, 31471400, 81270662 and 31171380) and Academician Workstation Support (Shenyang, Changsha and Shandong).

References

[1] Mascarenhas MN, Flaxman SR, Boerma T, Vanderpoel S, Stevens GA. National, regional, and global trends in infertility prevalence since 1990: a systematic analysis of 277 health surveys. PLoS Med 2012;9(12):e1001356.
[2] Nallella KP, Sharma RK, Aziz N, Agarwal A. Significance of sperm characteristics in the evaluation of male infertility. Fertil Steril 2006;85(3):629–34.
[3] Ford WC. Comments on the release of the 5th edition of the WHO laboratory manual for the examination and processing of human semen. Asian J Androl 2010;12(1):59–63.
[4] Agarwal A, Allamaneni SS. Sperm DNA damage assessment: a test whose time has come. Fertil Steril 2005;84(4):850–3.
[5] Sergerie M, Laforest G, Bujan L, Bissonnette F, Bleau G. Sperm DNA fragmentation: threshold value in male fertility. Hum Reprod 2005;20(12):3446–51.
[6] Bungum M, Bungum L, Giwercman A. Sperm chromatin structure assay (SCSA): a tool in diagnosis and treatment of infertility. Asian J Androl 2011;13(1):69–75.
[7] Evgeni E, Lymberopoulos G, Touloupidis S, Asimakopoulos B. Sperm nuclear DNA fragmentation and its association with semen quality in Greek men. Andrologia 2015;47(10):1166–74.
[8] Montjean D, Zini A, Ravel C, Belloc S, Dalleac A, Copin H, et al. Sperm global DNA methylation level: association with semen parameters and genome integrity. Andrology 2015;3(2):235–40.
[9] Winkle T, Rosenbusch B, Gagsteiger F, Paiss T, Zoller N. The correlation between male age, sperm quality and sperm DNA fragmentation in 320 men attending a fertility center. J Assist Reprod Genet 2009;26(1):41–6.
[10] Peluso G, Palmieri A, Cozza PP, Morrone G, Verze P, Longo N, et al. The study of spermatic DNA fragmentation and sperm motility in infertile subjects. Arch Ital Urol Androl 2013;85(1):8–13.
[11] Fei Q, Huang H, Jin J, Huang X. Diagnostic value of sperm DNA fragmentation for male infertility. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2014;31(1):60–4.

[12] Komiya A, Kato T, Kawauchi Y, Watanabe A, Fuse H. Clinical factors associated with sperm DNA fragmentation in male patients with infertility. ScientificWorldJournal 2014;2014:868303.
[13] Boushaba S, Belaaloui G. Sperm DNA fragmentation and standard semen parameters in algerian infertile male partners. World J Mens Health 2015;33(1):1–7.
[14] Sergerie M, Mieusset R, Croute F, Daudin M, Bujan L. High risk of temporary alteration of semen parameters after recent acute febrile illness. Fertil Steril 2007;88(4). 970 e971–977.
[15] Patel MN, Joshi HN, Patel CR. Interactions with herring sperm DNA and biological studies of sparfloxacin drug-based copper(II) compounds. Appl Organomet Chem 2012;26(11):641–9.
[16] Das M, Al-Hathal N, San-Gabriel M, Phillips S, Kadoch IJ, Bissonnette F, et al. High prevalence of isolated sperm DNA damage in infertile men with advanced paternal age. J Assist Reprod Genet 2013;30(6):843–8.
[17] Caglar GS, Koster F, Schopper B, Asimakopoulos B, Nehls B, Nikolettos N, et al. Semen DNA fragmentation index, evaluated with both TUNEL and Comet assay, and the ICSI outcome. In Vivo 2007;21(6):1075–80.
[18] Cui ZL, Zheng DZ, Liu YH, Chen LY, Lin DH, Feng-Hua L. Diagnostic accuracies of the TUNEL, SCD, and comet based sperm DNA fragmentation assays for male infertility: a meta-analysis study. Clin Lab 2015;61(5–6):525–35.
[19] Evenson DP, Darzynkiewicz Z, Melamed MR. Comparison of human and mouse sperm chromatin structure by flow cytometry. Chromosoma 1980;78(2):225–38.
[20] Enciso M, Alfarawati S, Wells D. Increased numbers of DNA-damaged spermatozoa in samples presenting an elevated rate of numerical chromosome abnormalities. Hum Reprod 2013;28(6):1707–15.
[21] Fernandez JL, Muriel L, Rivero MT, Goyanes V, Vazquez R, Alvarez JG. The sperm chromatin dispersion test: a simple method for the determination of sperm DNA fragmentation. J Androl 2003;24(1):59–66.
[22] Evenson DP. The Sperm chromatin structure assay (SCSA) and other sperm DNA fragmentation tests for evaluation of sperm nuclear DNA integrity as related to fertility. Anim Reprod Sci 2016;169:56–75.
[23] Enciso M, Pieczenik G, Cohen J, Wells D. Development of a novel synthetic oligopeptide for the detection of DNA damage in human spermatozoa. Hum Reprod 2012;27(8):2254–66.
[24] Zhang YS, Wei B, Chen B, Xu LH, Tang D, Peng CL, et al. Influence of the reference values for semen analysis proposed in the 5th edition of WHO laboratory manual on the indication spectrum for intrauterine insemination. Zhonghua Nan Ke Xue 2014;20(3):253–6.
[25] Osman A, Alsomait H, Seshadri S, El-Toukhy T, Khalaf Y. The effect of sperm DNA fragmentation on live birth rate after IVF or ICSI: a systematic review and meta-analysis. Reprod Biomed Online 2015;30(2):120–7.
[26] Zhang Z, Zhu L, Jiang H, Chen H, Chen Y, Dai Y. Sperm DNA fragmentation index and pregnancy outcome after IVF or ICSI: a meta-analysis. J Assist Reprod Genet 2015;32(1):17–26.
[27] Robinson L, Gallos ID, Conner SJ, Rajkhowa M, Miller D, Lewis S, et al. The effect of sperm DNA fragmentation on miscarriage rates: a systematic review and meta-analysis. Hum Reprod 2012;27(10):2908–17. https://doi.org/10.1093/humrep/des261. PubMed PMID: 22791753.
[28] Bounartzi T, Dafopoulos K, Anifandis G, Messini CI, Koutsonikou C, Kouris S, et al. Pregnancy prediction by free sperm DNA and sperm DNA fragmentation in semen specimens of IVF/ICSI-ET patients. Hum Fertil (Camb) 2016;19(1):56–62.
[29] Lin MH, Kuo-Kuang Lee R, Li SH, Lu CH, Sun FJ, Hwu YM. Sperm chromatin structure assay parameters are not related to fertilization rates, embryo quality, and pregnancy rates in in vitro fertilization and intracytoplasmic sperm injection, but might be related to spontaneous abortion rates. Fertil Steril 2008;90(2):352–9.
[30] Niu ZH, Shi HJ, Zhang HQ, Zhang AJ, Sun YJ, Feng Y. Sperm chromatin structure assay results after swim-up are related only to embryo quality but not to fertilization and pregnancy rates following IVF. Asian J Androl 2011;13(6):862–6.
[31] Cooper TG, Noonan E, von Eckardstein S, Auger J, Baker HW, Behre HM, et al. World Health Organization reference values for human semen characteristics. Hum Reprod Update 2010;16(3):231–45.
[32] Corfman RS, Milad MP, Bellavance TL, Ory SJ, Erickson LD, Ball GD. A novel ovarian stimulation protocol for use with the assisted reproductive technologies. Fertil Steril 1993;60(5):864–70.
[33] Palmer CB, Forstein DA, Higdon 3rd HL, Boone WR. Changes in long luteal protocol affects the number of days of stimulation: evolution of an assisted reproductive technology practice. J Reprod Med 2011;56(7–8):308–12.
[34] Polat M, Bozdag G, Yarali H. Best protocol for controlled ovarian hyperstimulation in assisted reproductive technologies: fact or opinion? Semin Reprod Med 2014;32(4):262–71.
[35] Vengetesh PM, Ramachandran A, Kumar P. Choosing GnRH antagonist protocol shows improved oocyte and embryo quality, coherent with the Peri-follicular Vascularity (PFV) in assisted reproductive techniques. J Clin Diagn Res 2015;9(11):QC24–8.
[36] Tesarik J, Greco E. The probability of abnormal preimplantation development can be predicted by a single static observation on pronuclear stage morphology. Hum Reprod 1999;14(5):1318–23.
[37] Alpha Scientists in Reproductive, M. and E. S. I. G. o. Embryology. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Hum Reprod 2011;26(6):1270–83.
[38] Ni W, Xiao S, Qiu X, Jin J, Pan C, Li Y, et al. Effect of sperm DNA fragmentation on clinical outcome of frozen-thawed embryo transfer and on blastocyst formation. PLoS One 2014;9(4):e94956.
[39] Muriel L, Garrido N, Fernández JL, Remohí J, Pellicer A, de los Santos MJ, et al. Value of the sperm deoxyribonucleic acid fragmentation level, as measured by the sperm chromatin dispersion test, in the outcome of in vitro fertilization and intracytoplasmic sperm injection. Fertil Steril 2006;85(2):371–83.
[40] Fernandez JL, Muriel L, Goyanes V, Segrelles E, Gosalvez J, Enciso M, et al. Halosperm is an easy, available, and cost-effective alternative for determining sperm DNA fragmentation. Fertil Steril 2005;84(4):860.
[41] Jin J, Pan C, Fei Q, Ni W, Yang X, Zhang L, et al. Effect of sperm DNA fragmentation on the clinical outcomes for in vitro fertilization and intracytoplasmic sperm injection in women with different ovarian reserves. Fertil Steril 2015;103(4):910–6.
[42] Smit M, Romijn JC, Wildhagen MF, Weber RF, Dohle GR. Sperm chromatin structure is associated with the quality of spermatogenesis in infertile patients. Fertil Steril 2010;94(5):1748–52.
[43] Didolkar AK, Patel PB, Roychowdhury D. Effect of aspirin on spermatogenesis in mature and immature rats. Int J Androl 1980;3(5):585–93.
[44] Angelopoulou R, Plastira K, Msaouel P. Spermatozoal sensitive biomarkers to defective protaminosis and fragmented DNA. Reprod Biol Endocrinol 2007;5:36.
[45] Samplaski MK, Dimitromanolakis A, Lo KC, Grober ED, Mullen B, Garbens A, et al. The relationship between sperm viability and DNA fragmentation rates. Reprod Biol Endocrinol 2015;13:42.
[46] Benchaib M, Lornage J, Mazoyer C, Lejeune H, Salle B, Francois Guerin J. Sperm deoxyribonucleic acid fragmentation as a prognostic indicator of assisted reproductive technology outcome. Fertil Steril 2007;87(1):93–100.
[47] Zhang XD, Chen MY, Gao Y, Han W, Liu DY, Huang GN. The effects of different sperm preparation methods and incubation time on the sperm DNA fragmentation. Hum Fertil (Camb) 2011;14(3):187–91.
[48] Tesarik J, Kopecny V, Plachot M, Mandelbaum J. Activation of nucleolar and extranucleolar RNA synthesis and changes in the ribosomal content of human embryos developing in vitro. J Reprod Fertil 1986;78(2):463–70.
[49] Lim AS, Tsakok MF. Age-related decline in fertility: a link to degenerative oocytes? Fertil Steril 1997;68(2):265–71.
[50] Ahmadi A, Ng SC. Developmental capacity of damaged spermatozoa. Hum Reprod 1999;14(9):2279–85.
[51] Tesarik J, Greco E, Mendoza C. Late, but not early, paternal effect on human embryo development is related to sperm DNA fragmentation. Hum Reprod 2004;19(3):611–5.
[52] Nasr-Esfahani MH, Salehi M, Razavi S, Anjomshoa M, Rozbahani S, Moulavi F, et al. Effect of sperm DNA damage and sperm protamine deficiency on fertilization and embryo development post-ICSI. Reprod Biomed Online 2005;11(2):198–205.
[53] Santos R, Palos-Ladeiro M, Besnard A, Porcher JM, Bony S, Sanchez W, et al. Relationship between DNA damage in sperm after ex vivo exposure and abnormal embryo development in the progeny of the three-spined stickleback. Reprod Toxicol 2013;36:6–11.