Received: 17 April 2021 | Revised: 29 May 2021 | Accepted: 12 June 2021

DOI: 10.1111/and.14180

REVIEW ARTICLE

First International Journal of Andrology
aNDROLOGIa
WILEY

The effect of sperm DNA fragmentation on intracytoplasmic sperm injection outcome

Kareim Khalafalla[1] | Ahmad Majzoub[1,2,3] | Haitham Elbardisi[1,3] | Ashni Bhathella[4] | Arth Chaudhari[5] | Ashok Agarwal[2] | Ralf Henkel[2,6,7] | Thoraya AlMarzooki[8] | Hasan Burjaq[9] | Mohamed Arafa[1,2,3,10]

[1]Urology Department, Hamad Medical Corporation, Doha, Qatar

[2]American Center for Reproductive Medicine, Cleveland, OH, USA

[3]Urology Department, Weill Cornell Medicine-Qatar, Doha, Qatar

[4]University of San Francisco, San Francisco, CA, USA

[5]Department of Animal Genetics and Breeding, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Sardarkrushinagar, India

[6]Department of Metabolism, Digestion and Reproduction, Imperial College London, London, UK

[7]Department of Medical Bioscience, University of the Western Cape, Bellville, South Africa

[8]Obstetrics and Gynecology Department, Hamad Medical Corporation, Doha, Qatar

[9]Assisted Reproduction Center, Hamad Medical Corporation, Doha, Qatar

[10]Andrology Department, Cairo University, Cairo, Egypt

Correspondence
Mohamed Arafa, Urology Department, Hamad Medical Corporation, P.O. Box: 3050, Doha, Qatar.
Email: mohamedmostafaarafa@gmail.com

Funding information
Qatar National Library

Abstract

Our study objective was to assess the effect of various sperm DNA fragmentation levels on clinical intracytoplasmic sperm injection outcome. This retrospective study included 392 patients who underwent ICSI and performed sperm DNA fragmentation testing before the procedure. Based on sperm DNA fragmentation cut-off values, the patients were differentiated into 3 groups as <20%, 20%–30% and >30%. According to the female status, patients were differentiated into favourable group (n = 259) with female age <35 years and anti-Mullerian hormone level ≥7.1 pmol/L; and unfavourable group (n = 133) with female age ≥35 years and anti-Mullerian hormone level ≤7.1 pmol/L. The patient's medical records were reviewed, and patient's demographic, laboratory data including semen analysis, sperm DNA fragmentation determined by means of sperm chromatin dispersion, hormonal profile and data regarding intracytoplasmic sperm injection cycle were collected. This cohort reported that the clinical reproductive outcomes of intracytoplasmic sperm injection showed no statistical significance with increase sperm DNA fragmentation levels. In sperm DNA fragmentation above 30%, favourable females had significantly higher clinical pregnancy rate and live birth rate than unfavourable females, while fertilisation rate and miscarriage rate showed no significance between the subgroups. High sperm DNA fragmentation is linked to poor semen parameters.

KEYWORDS

clinical reproductive outcomes, intracytoplasmic sperm injection, sperm chromatin dispersion test, sperm DNA fragmentation

1 | INTRODUCTION

Infertility is one of the predominant problems affecting around 15% of couples of reproductive ages worldwide with about 50% of causative factors from the male side (Irvine, 1998; Lotti and Maggi, 2018). In accordance with the World Health Organization (WHO) guidelines, the standard evaluation of fertility includes routine semen parameters such as ejaculate volume, pH, sperm count,

total and progressive motility, vitality and morphology (World Health Organization, 2010). Yet, these parameters do not always predict male fertility, as infertile men might have normal semen parameters in up to 15% of cases (World Health Organization, 2010).

Alternatively, sperm DNA fragmentation (SDF) testing provides a different parameter for the analysis of the male factors affecting fertility. In the recent years, SDF has been identified as a predictor of fertility with good reliability because sperm DNA integrity can impact fertilisation, embryogenesis, implantation and pregnancy outcomes (Majzoub, Agarwal, Cho et al., 2017; Majzoub, Agarwal, & Esteves, 2017). SDF may include breakage of single-strand DNA (ssDNA) or double-strand DNA (dsDNA), base deletion or modification, and inter- or intra-cross linkage (Sergerie et al., 2005). SDF occurs during late spermatogenesis due to defects in the repair system of DNA (Bui et al., 2018) which can be caused by different pathological mechanisms including apoptosis, elevated oxidative stress due to an increase in reactive oxygen species (ROS), and dysregulation of the chromatin protamine and histone components. These changes may occur as a result of various factors like drug use, tobacco smoking, environmental pollution, high testicular temperature, and advanced age (Sergerie et al., 2005). Furthermore, the impact of DNA damage on fertility outcome is not only believed to be influenced by sperm chromatin integrity, but also by the oocyte repair capacity. Oocytes play an important role in repairing SDF, depending on the oocyte's cytoplasmic and genomic quality (Fernández-Díez et al., 2016, García-Rodríguez et al., 2018, Setti et al., 2021). The exact mechanism by which the oocyte can repair the SDF remains unknown although human oocytes occupy DNA repair genes and can be linked to maternal mRNA repair (Osman et al., 2015).

Various tests for SDF have been identified with Sperm Chromatin Structure Assay (SCSA), Sperm Chromatin Dispersion (SCD), Terminal deoxynucleotidyl transferase dUTP nick-end labelling (TUNEL) and Single Cell Gel Electrophoresis (COMET) being most commonly applied (Majzoub, Agarwal, Cho et al., 2017; Majzoub, Agarwal, & Esteves, 2017).

Recent efforts have identified implications of SDF on fertility and hence clinical guidelines were published highlighting the importance of SDF testing in couples with unexplained/idiopathic infertility, recurrent natural pregnancy loss, clinical varicocele, lifestyle exposures, recurrent intrauterine insemination (IUI) and in vitro fertilisation (IVF) failure and recurrent miscarriages following ICSI (Agarwal et al., 2019). There is a contradiction in literature with respect to the effect of SDF on fertilisation and pregnancy rates with ICSI. Evidence extracted from three meta-analyses has indicated that higher SDF is not associated with a negative impact on ICSI outcomes (Zini, 2011; Li et al., 2006; Zhao et al., 2014). On the contrary, another meta-analysis by Simon et al. including 24 ICSI studies revealed that SDF can have a significant impact on the pregnancy rate with an OR of 1.31 (95% CI 1.08–1.59, $p = .006$) (Simon et al., 2017).

To bridge the above-mentioned gap regarding the role of SDF on ICSI, our aim in the current study was to investigate the effect of different levels of SDF on ICSI in our centre with regard to fertilisation, clinical pregnancy, live birth and abortion.

2 | METHODOLOGY

This is a retrospective cohort study which was carried out in the assisted conception unit at Hamad Medical Corporation, Doha, Qatar. The study duration was over a 5-year period from 1 August 2014 to 1 August 2019. Ethical Approval from the Institutional Review Board was obtained (IRB MRC-01-19-349) for the protocols and procedures of this study.

The charts of 1922 patients who underwent ICSI were screened for inclusion in the study. Inclusion criteria were patients that underwent ICSI using ejaculated spermatozoa and had a recorded sperm DNA fragmentation (SDF) test done within a week before ICSI. While couples with (a) severe male factor (severe oligozoospermia, azoospermia), (b) male genetic abnormalities, (c) female factor infertility (tubal factor, uterine abnormality, PCO), (d) history of mumps orchitis, (e) history of receiving chemotherapy or radiotherapy, and (f) history of testicular tumour were excluded.

The electronic medical records of male patients and their female partners were reviewed by two investigators for extraction of data including patients' age, related past medical history, any surgical procedure of relevance to fertility, consanguinity or history of infertility in the family. Clinical examination data involving general and local genital examination as well as laboratory data including semen analysis, SDF and hormonal profile (FSH, LH, Testosterone, PRL and E2) were extracted. Data retrieved for the female partners included basal FSH, anti-Mullerian hormone (AMH) and oestradiol. All information regarding the ICSI cycle was extracted from the medical records of the spouse including the protocol for ovarian stimulation and different ICSI outcomes.

2.1 | Procedures

2.1.1 | Semen analysis

Following 3–5 days of sexual abstinence, patients were asked to produce semen samples by masturbation. After liquefaction, semen analysis was carried out according to World Health Organization, 2010 standard protocol (WHO 5th edition guidelines).

2.1.2 | Sperm DNA fragmentation test

The Halosperm G2 test kit (Halotech, Madrid, Spain) was used to determine SDF. This test can be performed upon fresh and frozen semen samples. The SCD process is an indirect method of observing sperm DNA damage. During the test, controlled acid denaturation of the DNA and removal of nuclear proteins of the semen sample takes place. As a result, a large halo can be seen in sperm with intact DNA

by using fluorescence microscope, while spermatozoon with dispersed chromatin materials or fragmented DNA will not produce a halo. SDF cut-off value was taken as ≥30% to differentiate between infertile and fertile men using a standard protocol (Fernández et al., 2005).

2.1.3 | Reproductive hormones

Hormonal assays were done using the immunoassay chemiluminescence method, Architect i1000SR® (Abbott systems). The relevant blood samples were drawn in the early morning between 7 and 9 a.m. The testing included follicle-stimulating hormone (FSH) (range = 1–19 IU/L), luteinising hormone (LH) (range = 1–9 IU/L), prolactin (range = 73–407 mIU/L), total testosterone (range = 10.4–35 nmol/L) and oestradiol (range = 73–275 pmol/L).

2.1.4 | Study protocol for ICSI

Patients performed an ICSI trial using ejaculated spermatozoa. Ovarian stimulation in a controlled manner was started using recombinant FSH after desensitisation of pituitary with the dose guided by patient age, previous ovarian stimulation, oestradiol level and follicles scanned on ovarian transvaginal ultrasound scans (Yim et al., 2001). Continued monitored stimulation was done until at least two follicles matured and reached a mean diameter of 18 mm. Ovulation induction was then performed using 10,000 IU subcutaneous of hCG 36 hr before oocyte retrieval. Oocytes were aspirated guided by transvaginal ultrasound. Following ICSI, fertilised oocyte culturing was done, and the embryo quality was evaluated using the Veeck score. Embryo transfer was carried out on day 2 or 3 and patients were then daily given vaginal progesterone (Crinone 8%) for luteal phase support. (Veeck, 1988).

2.2 | Statistical analyses

The Shapiro-Wilk test for normality was performed to identify the distribution of the study variables. Frequencies were used to report categorical data, while median [95% CI] were used to present continuous values. Spearman's correlations were performed to assess the relationship between various study variables. The chi-square test was used to compare ICSI outcomes in various SDF levels. Kruskal-Wallis test was used to compare continuous variables between the three SDF levels. The Jonckheere-Terpstra test was used to test for an ordered difference in medians of the three SDF levels stating the direction of this order (trend). A *p*-value below .05 was considered statistically significant. All statistical analyses of collected data were performed using MedCalc® Statistical Software version 19.8 (MedCalc Software Ltd).

TABLE 1 Characteristics of study population

Clinical parameter	Value ($n = 392$) (median; mean ± *SD*)
Semen analysis data	
Volume (ml)	2.1; 2.2 ± 1.8
Concentration (10^6/ml)	32.0; 42.9 ± 36.8
Total motility (%)	42.0; 41.1 ± 18.4
Prog. motility (%)	14.5; 18.8 ± 18.7
Normal morphology (%)	6.0; 12.1 ± 12.6
DNA fragmentation (%)	20.5; 25.0 ± 14.6
Hormone data	
Oestradiol (pmol/L)	92.0; 104.3 ± 55.7
FSH (mIU/ml)	2.9; 3.6 ± 2.9
LH (mIU/ml)	3.3; 4.1 ± 2.9
Testosterone (nmol/L)	16.0; 17.8 ± 9.5

Abbreviations: FSH, follicle-stimulating hormone; LH, luteinising hormone; ORP, oxidation reduction potential; Prog. Motility, progressive motility.

3 | RESULTS

The records of 1,922 patients who underwent ICSI from 2014 to 2019 were screened in our study. A total of 392 patients had SDF data within 7 days before the ICSI procedure were included after applying the inclusion and exclusion criteria. There were 63.5% of the patients complaining of primary infertility ($n = 249$) and 36.5% of secondary infertility ($n = 143$). The mean male age was 37.32 ± 6.7 years while the mean female age was 33.8 ± 6.1 years.

Table 1 shows the clinical characteristics of the whole study population ($n = 392$) including semen parameters, hormonal analysis, and testicular size. As for the whole patients' samples, clinical pregnancy occurred in 44.1% (173/392) of the cases, live birth rate was 34.4% (121/352), and 9% (12/134) of patients had miscarriage.

Table 2 shows correlations between SDF and different clinical parameters. There was a highly significant negative correlation between SDF and sperm count and motility, while there was a highly significant positive correlation between SDF and abnormal morphology. No correlation could be found between SDF and male age and progressive motility.

The patients were grouped according to SDF value into three groups, SDF <20% ($n = 156$), SDF 20%–30% ($n = 149$), and SDF >30% ($n = 87$). Table 3 shows the comparison between the three SDF groups with regard to semen and hormone parameters. Semen volume, sperm total motility and normal morphology were significantly different between the 3 groups with higher values found in lower SDF groups. No statistical significance was observed for age, testis site, sperm concentration, sperm progressive motility and hormones between SDF groups. However, there was a trend of better sperm concentration and lower FSH levels in patients with low SDF.

Fertilisation ($p = .441$), clinical pregnancy ($p = .265$) and live birth rates ($p = .861$) were greater in patients with lower SDF than in those with higher SDF. However, the difference was insignificant. Similarly,

TABLE 2 Correlations between SDF and clinical parameters

	Male age	Sperm concentration (million/ml)	Total motility (%)	Progressive motility (%)	Abnormal morphology (%)	Fertilisation
SDF (correlation coefficient)	.121*	−.132*	−.294**	−.055	−.183**	.012
p value	.016	.009	.000	.278	.000	.819

Note: Spearman test (r^2). *Significant ($p < .05$), **Highly significant ($p < .001$)

TABLE 3 Comparison between three groups of SDF: semen and hormone parameters

	SDF <20 (*n* = 156) (mean ± *SD*)	SDF 20–30 (149) (mean ± *SD*)	SDF>30 (*n* = 87) (mean ± *SD*)	*p* value Kruskal-Wallis (Jonckheere-Terpstra)
Age	35.4 ± 6.7	37.3 ± 7.2	38.0 ± 9.1	.132 (0.016)
Semen				
Volume (ml)	2.3 ± 1.8	2.1 ± 1.8	2.2 ± 2.0	.049 (0.828)
Concentration (million/ml)	47.0 ± 42.2	44.2 ± 32.5	33.5 ± 31.5	.062 (0.008)*
Total motility (%)	46.2 ± 17.7	42.4 ± 16.9	30.0 ± 17.4	.002* (0.000)**
Prog. motility (%)	20.0 ± 20.1	20.1 ± 18.7	14.3 ± 15.6	.691 (0.254)
Normal morph. (%)	12.4 ± 11.6	13.5 ± 14.1	8.9 ± 11.1	.018* (0.000)**
Hormones				
Oestradiol (pmol/L)	97.0 ± 52.6	111.3 ± 59.3	105.5 ± 54.2	.371 (0.527)
FSH (IU/L)	3.2 ± 2.5	3.9 ± 3.3	3.9 ± 2.7	.269 (0.041)*
LH (IU/L)	3.8 ± 2.3	4.1 ± 2.8	4.5 ± 3.7	.179 (0.188)
Testosterone (mmol/L)	17.3 ± 9.6	18.6 ± 10.1	17.1 ± 7.9	.984 (0.931)

Abbreviations: FSH, follicle-stimulating hormone; LH, luteinising hormone; Normal morph., normal morphology; Prog. motility, progressive motility; SDF, sperm DNA fragmentation. *Significant ($p < .05$), **Highly significant ($p < .001$)

miscarriage ($p = .121$) was higher with high SDF but again the difference was insignificant (Table 4).

Patients were then subgrouped according to the female status into favourable group (female age <35 years and AMH value >/ = 7.1 pmol/L) which included 259 patients and unfavourable group (female age >/ = 35 years and AMH value <7.1 pmol/L) which included 133 patients. Table 5 shows the difference in the ICSI outcome within the 3 SDF groups with regard to favourability subgrouping. In groups with SDF <20% and 20−30%, fertilisation, clinical pregnancy and live birth rates were greater in the favourable subgroup compared to unfavourable subgroup; however, the difference was insignificant. In the SDF >30% group, clinical pregnancy and live birth rate were significantly higher in the favourable subgroup compared to unfavourable subgroup ($p = .005$ and $p = .024$ respectively) but fertilisation was insignificant. We compared ICSI outcome (fertilisation rate, clinical pregnancy, live birth rate and miscarriage) between different female status within each SDF group (young age/high AMH, young age/low AMH, old age/low AMH, old age/high AMH). We could confirm that favourable females (young age and high AMH) had significantly higher pregnancy rate and live birth rate than unfavourable females (Tables S1–S4).

4 | DISCUSSION

The current study examined the impact of SDF, measured with the SCD test, on the reproductive outcomes with ICSI; namely, clinical pregnancy, live birth, fertilisation, and miscarriage rates. The results showed that different SDF levels didn't affect the clinical outcome of ICSI with regard to fertilisation rate, clinical pregnancy, live birth rate and miscarriage rate in the whole sample. Patients with low SDF showed better outcome than higher SDF; however, the difference was insignificant.

High SDF has been linked with worse conventional semen parameters in a number of studies. Tie-Cheng et al. in 2018 used the SCD test in a cohort of 152 ICSI patients, that were subgrouped into <30% SDF and >30% SDF. They reported significantly lower total ($p = .001$) and progressive motility ($p < .001$) in the >30% SDF group in comparison to the <30% SDF group. Additionally, the authors showed a significant negative correlation between SDF and normal morphology. Similarly, Antonouli et al. revealed a significant negative correlation between total and progressive motility and SDF ($r = -.29$ total motility, $p = .012$: $r = -.27$ PM, $p = .019$) (Antonouli, 2019). Our present study showed significantly higher total motility and normal morphology in low SDF patients, but progressive motility was insignificant.

TABLE 4 Comparison between three groups of SDF: ICSI outcome

	SDF<20 (*n* = 156)	SDF 20–30 (*n* = 149)	SDF>30 (*n* = 87)	*p* value Kruskal-Wallis (Jonckheere-Terpstra)
Fertilisation (mean ± *SD*)	70.9 ± 23.1	74.3 ± 21.9	70.8 ± 20.7	.441 (0.814)
Clinical Pregnancy (% [*n*/total])	33.3% (52/156)	41.6% (62/149)	34.5% (30/87)	.265 (0.484)
Miscarriage (% [*n*/total])	6% (3/50)	8.6% (5/58)	15.4% (4/26)	.121 (0.550)
Live birth (% [*n*/total])	34.1% (47/138)	38.4% (53/138)	27.6% (21/76)	.380 (0.861)

Abbreviation: SDF, Sperm DNA Fragmentation

In the present study, we could not find a statistically significant correlation between male age and SDF. While male age is believed to be a risk factor for elevated SDF due to the existence of elevated levels of oxidative stress, defective sperm chromatin packaging, and disordered apoptosis that occur with ageing (Agarwal and Said, 2003), the reported evidence remains to be contradictory. Petersen et al. (2018) evaluated SDF across different male age subgroups, ≤35 years, 36–44 years, and ≥45 years in a cohort of 2,176 semen samples of infertile men, these authors reported lower levels of SDF (percentage of SDF; 15.9 ± 8.7, TUNEL) in the 35–44-year age group compared to ≥45 years age group (percentage of SDF; 16.2 ± 8.4; TUNEL). Moreover, the youngest age group, males ≤35 years, had the lowest level of SDF. Similarly, Antonouli et al. (2019) found a significant positive correlation between the SDF level and male age ($r = 0.23$, $p = .046$). On the other hand several other studies, echoing our results, failed to report any significant correlation between male age and SDF (Sun et al., 1997; Winkle et al., 2009; Colin et al., 2010; Brahem et al., 2011; Nijs et al., 2011).

Our data show that there is no significance between SDF groups as regards clinical pregnancy, fertilisation rate, miscarriage rate and live birth rate. This result is in agreement with other studies. Antonouli et al. used the SCD test to measure SDF within 150 patients and found no significant correlation between SDF and fertilisation rate in ICSI patients (Antonouli, 2019). Sun et al. also used SCD in ICSI patients and found no significant difference between <30% SDF and ≥30% groups as regards ICSI outcome ($p = .458$) (Sun et al., 2018). The impact of SDF on ICSI outcome is believed to be undermined by the sperm selection processes that usually choose the best quality sperm, i.e. with lower SDF, for the procedure. Indeed, Liffner et al. measured SDF levels in selected sperm using a colloid discontinuous gradient for patients with high SDF and reported levels that were even lower than those of normozoospermic donors (Liffner et al., 2019).

In the current study, SDF was found to have no significant effect on clinical pregnancy. This finding was in agreement with a study by Simon and coworkers who found that there was no significant correlation between SDF and clinical pregnancy following ICSI (Simon, 2013). Similarly, another systematic review by Agarwal and coworkers found that there was no correlation between SDF and clinical pregnancy rate (OR 0.94, 95% CI) (Cho and Agarwal, 2018). Moreover, in a study by Antonouli et al. (2019) in a cohort of 150 couples with donated oocytes, SCD was utilised at a cut-off value of 25% to test its correlation with ICSI outcome. The study found that there was no statistically significant difference in pregnancy outcome between low SDF (< 25%) and high SDF (> 25%) groups (pregnancies 68.2% and 65.1% respectively). Additionally, Sun and coworkers concluded that using a 30% cut-off value, that there was no significant effect of SDF on IVF and ICSI outcomes using SCD (Tie-Cheng, 2018). On the contrary, in a recent meta-analysis on 24 ICSI studies, the authors concluded that there was a negative significant correlation between SDF and clinical pregnancy rate (OR 1.31, 95% CI, $p < .007$) (Simon, 2013). The discrepancy whether SDF correlates to clinical pregnancy can be caused by the use of different SDF tests. Concerning SCD, used in both the present study and Tie-Cheng et al study, no significant correlation between SDF and ICSI clinical pregnancy rate. Regarding SCSA studies, a meta-analysis demonstrated a highly significant correlation between clinical pregnancy and SDF, where groups with SDF <27% are more likely to have a positive ART outcome than SDF >27% groups (OR = 1.437, 95% CI, $p = .000$) (Zhang et al., 2015). Besides, a meta-analysis and systematic review, pooled 8,068 treatment cycles of which there 2,359 SCD cycles (29.2%), and their random effects model combined 24 ICSI OR estimates (OR = 1.49; 95% CI: 1.11–2.01; $p = .0075$) showed a highly significant correlation between high SDF and low clinical pregnancy rate (Simon et al., 2017). However, since they used a variety of SDF tests, it was unclear if there was a specific correlation between SDF measured by SCD and clinical pregnancy following ICSI.

With regard to live birth rate (LBR), we could not find a significant effect of SDF in our results. A study conducted by Simon et al. (2013) involving 136 couples undergoing ICSI showed no relationship between sperm DNA damage and live birth rates. On the contrary, in 2015, Osman et al found a detrimental effect of high DNA fragmentation on LBR, in IVF and ICSI patients.

We subclassified the groups according to female factors (advanced age and AMH value) into favourable group (female age <35 years and AMH value >/ = 7.1 pmol/L) and unfavourable group (female age >/ = 35 years and AMH value <7.1 pmol/L, $n = 140$). We found no statistical difference in the fertilisation rate, clinical pregnancy rate, live birth rate and miscarriage rate between favourable and unfavourable subgroups in patients with SDF <20% and 20%–30%. However, in the high SDF group (>30%) clinical pregnancy rate and live birth rate were significantly higher in the favourable subgroup while fertilisation rate and miscarriage were insignificant. This could be explained by the fact that oocytes retrieved from

TABLE 5 Comparison of the incidence rates of reproductive outcome between three groups of SDF: ICSI outcome according to favourability measured by female factors

	SDF <20			SDF 20–30			SDF >30		
	favourable female ($n = 112$)	Un-favourable female ($n = 44$)	*p* value	favourable female ($n = 95$)	Un-favourable female ($n = 54$)	*p* value	favourable female ($n = 52$)	Un-favourable female ($n = 35$)	*p* value
Fertilisation	72.5 ± 21.1	66.6 ± 26.3	.297	74.9 ± 20.1	75.1 ± 23.1	.762	72.6 ± 17.7	73.5 ± 21.5	.677
Clinical pregnancy	36.9% (34/92)	22.5% (9/40)	.181	47.3% (35/74)	39.6% (19/48)	.540	48.8% (20/41)	10.7% (3/28)	.005
Miscarriage	3.1% (1/32)	0.0% (0/9)	.596	9.4% (3/32)	5.6% (1/18)	.713	18.8% (3/16)	25.0% (1/4)	1.000
Live birth	37.8% (31/82)	25.7% (9/35)	.312	42.6% (29/68)	37.0% (17/46)	.648	37.1% (13/35)	8.0% (2/25)	.024

Abbreviation: SDF, Sperm DNA fragmentation.

younger more favourable females had more capacity to repair SDF in the sperm during embryogenesis (Fernández-Díez et al., 2016, García-Rodríguez et al., 2018, Setti et al., 2021). This is contradictory to the results of the meta-analysis by Osman et al female subgroup analysis where she subclassified her patients accounting for female factors such as advanced age and ovarian reserve. They reported that SDF did not have an effect on LBR in ICSI patients (Osman et al., 2015).

The main limitation of our study was the retrospective nature of the study with the possibility of incomplete data. However, we extensively searched in all medical records of both spouses in addition to assisted conception unit records to overcome this limitation. The data were also retrieved from one ART centre in Qatar; therefore, our data lacked diversity within methodologies for IVF and SDF testing, as well as lacked geographical distribution. However, the geographical distribution should not be a problem in our patients' cohort because we are serving patients from more than 117 countries from different geographical and ethnic backgrounds. A prospective, multicentre study on the effect of SDF on ICSI outcome is highly recommended to consolidate the data on this issue.

5 | CONCLUSION

Sperm DNA fragmentation was found to be significantly correlated with conventional semen parameters highlighting its significance as a robust diagnostic test during male fertility evaluation. In this study, while patients with higher SDF values had worse reproductive outcomes with ICSI, the results did not reach statistical significance. In case of older female age with lower ovarian reserve, if the husband has high sperm DNA fragmentation this will significantly affect ICSI outcome with regard to clinical pregnancy and live birth rate. Therefore, in these cases, more intervention is needed through sperm selection methods either intracytoplasmic morphologically selected sperm injection (IMSI), physiological intracytoplasmic sperm injection (PICSI) or using testicular sperm for ICSI.

ACKNOWLEDGEMENT

Open Access funding provided by the Qatar National Library.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Kareim Khalafalla https://orcid.org/0000-0001-9476-4869
Ahmad Majzoub https://orcid.org/0000-0001-7423-6241
Haitham Elbardisi https://orcid.org/0000-0003-3902-7924
Arth Chaudhari https://orcid.org/0000-0002-9258-0860
Ashok Agarwal https://orcid.org/0000-0003-0585-1026
Ralf Henkel https://orcid.org/0000-0003-1128-2982
Mohamed Arafa https://orcid.org/0000-0003-0107-8857

REFERENCES

Agarwal, A., Panner Selvam, M. K., Baskaran, S., & Cho, C. L. (2019). Sperm DNA damage and its impact on male reproductive health: A critical review for clinicians, reproductive professionals and researchers. *Expert Review of Molecular Diagnostics*, *19*(6), 443–457. https://doi.org/10.1080/14737159.2019.1614916

Agarwal, A., & Said, T. M. (2003). Role of sperm chromatin abnormalities and DNA damage in male infertility. *Human Reproduction Update*, *9*(4), 331–345. https://doi.org/10.1093/humupd/dmg027

Antonouli, S., Papatheodorou, A., Panagiotidis, Y., Petousis, S., Prapas, N., Nottola, S. A., Palmerini, M. G., Macchiarelli, G., & Prapas, Y. (2019). The impact of sperm DNA fragmentation on ICSI outcome in cases of donated oocytes. *Archives of Gynecology and Obstetrics*, *300*(1), 207–215. https://doi.org/10.1007/s00404-019-05133-9

Brahem, S., Mehdi, M., Elghezal, H., & Saad, A. (2011). The effects of male aging on semen quality, sperm DNA fragmentation and chromosomal abnormalities in an infertile population. *Journal of Assisted Reproduction and Genetics*, *28*(5), 425–432. https://doi.org/10.1007/s10815-011-9537-5

Bui, A. D., Sharma, R., Henkel, R., & Agarwal, A. (2018). Reactive oxygen species impact on sperm DNA and its role in male infertility. *Andrologia*, *50*(8), e13012. https://doi.org/10.1111/and.13012

Cho, C. L., & Agarwal, A. (2018). Role of sperm DNA fragmentation in male factor infertility: A systematic review. *Arab Journal of Urology*, *16*(1), 21–34. https://doi.org/10.1016/j.aju.2017.11.002

Colin, A., Barroso, G., Gómez-López, N., Duran, E. H., & Oehninger, S. (2010). The effect of age on the expression of apoptosis biomarkers in human spermatozoa. *Fertility and Sterility*, *94*(7), 2609–2614. https://doi.org/10.1016/j.fertnstert.2010.04.043

Fernandez, J., Muriel, L., Goyanes, V., Segrelles, E., Gosalvez, J., Enciso, M., Lafromboise, M., & Dejonge, C. (2005). Simple determination of human sperm DNA fragmentation with an improved sperm chromatin dispersion test. *Fertility and Sterility*, *84*(4), 833–842. https://doi.org/10.1016/j.fertnstert.2004.11.089

Fernández-Díez, C., González-Rojo, S., Lombó, M., & Herráez, M. P. (2016). Impact of sperm DNA damage and oocyte-repairing capacity on trout development. *Reproduction*, *152*(1), 57–67. https://doi.org/10.1530/REP-16-0077

García-Rodríguez, A., Gosálvez, J., Agarwal, A., Roy, R., & Johnston, S. (2018). DNA damage and repair in human reproductive cells. *International Journal of Molecular Sciences*, *20*(1), 31. https://doi.org/10.3390/ijms20010031

Irvine, D. S. (1998). Epidemiology and aetiology of male infertility. *Human Reproduction*, *13*(Suppl 1), 33–44. https://doi.org/10.1093/humrep/13.suppl_1.33

Li, Z., Wang, L., Cai, J., & Huang, H. (2006). Correlation of sperm DNA damage with IVF and ICSI outcomes: A systematic review and meta-analysis. *Journal of Assisted Reproduction and Genetics*, *23*(9–10), 367–376. https://doi.org/10.1007/s10815-006-9066-9

Liffner, S., Pehrson, I., García-Calvo, L., Nedstrand, E., Zalavary, S., Hammar, M., Rodríguez-Martinez, H., & Álvarez-Rodríguez, M. (2019). Diagnostics of DNA fragmentation in human spermatozoa: Are sperm chromatin structure analysis and sperm chromatin dispersion tests (SCD-HaloSpermG2(®)) comparable? *Andrologia*, *51*(8), e13316. https://doi.org/10.1111/and.13316

Lotti, F., & Maggi, M. (2018). Sexual dysfunction and male infertility. *Nature Reviews Urology*, *15*(5), 287–307. https://doi.org/10.1038/nrurol.2018.20

Majzoub, A., Agarwal, A., Cho, C. L., & Esteves, S. C. (2017). Sperm DNA fragmentation testing: A cross sectional survey on current practices of fertility specialists. *Translational Andrology and Urology*, *6*(Suppl 4), S710–S719. https://doi.org/10.21037/tau.2017.06.21

Majzoub, A., Agarwal, A., & Esteves, S. C. (2017). Sperm DNA fragmentation: Overcoming standardization obstacles. *Translational Andrology and Urology*, *6*(Suppl 4), S422–S424. https://doi.org/10.21037/tau.2017.07.10

Nijs, M., De Jonge, C., Cox, A., Janssen, M., Bosmans, E., & Ombelet, W. (2011). Correlation between male age, WHO sperm parameters, DNA fragmentation, chromatin packaging and outcome in assisted reproduction technology. *Andrologia*, *43*(3), 174–179. https://doi.org/10.1111/j.1439-0272.2010.01040.x

Osman, A., Alsomait, H., Seshadri, S., El-Toukhy, T., & Khalaf, Y. (2015). The effect of sperm DNA fragmentation on live birth rate after IVF or ICSI: A systematic review and meta-analysis. *Reproductive BioMedicine Online*, *30*(2), 120–127. https://doi.org/10.1016/j.rbmo.2014.10.018

Petersen, C. G., Mauri, A. L., Vagnini, L. D., Renzi, A., Petersen, B., Mattila, M., Comar, V., Ricci, J., Dieamant, F., Oliveira, J. B. A., Baruffi, R. L. R., & Franco, J. G. Jr (2018). The effects of male age on sperm DNA damage: An evaluation of 2,178 semen samples. *JBRA Assisted Reproduction*, *22*(4), 323–330. https://doi.org/10.5935/1518-0557.20180047

Sergerie, M., Laforest, G., Bujan, L., Bissonnette, F., & Bleau, G. (2005). Sperm DNA fragmentation: Threshold value in male fertility. *Human Reproduction*, *20*(12), 3446–3451. https://doi.org/10.1093/humrep/dei231

Setti, A. S., Braga, D. P. A. F., Provenza, R. R., Iaconelli, A. Jr, & Borges, E. Jr. (2021). Oocyte ability to repair sperm DNA fragmentation: the impact of maternal age on intracytoplasmic sperm injection outcomes. *Fertility and Sterility*, *116*(1), 123–129. https://doi.org/10.1016/j.fertnstert.2020.10.045

Simon, L., Proutski, I., Stevenson, M., Jennings, D., McManus, J., Lutton, D., & Lewis, S. E. (2013). Sperm DNA damage has a negative association with live-birth rates after IVF. *Reproductive BioMedicine Online*, *26*(1), 68–78. https://doi.org/10.1016/j.rbmo.2012.09.019

Simon, L., Zini, A., Dyachenko, A., Ciampi, A., & Carrell, D. T. (2017). A systematic review and meta-analysis to determine the effect of sperm DNA damage on in vitro fertilization and intracytoplasmic sperm injection outcome. *Asian Journal of Andrology*, *19*(1), 80–90. https://doi.org/10.4103/1008-682x.182822

Sun, J. G., Jurisicova, A., & Casper, R. F. (1997). Detection of deoxyribonucleic acid fragmentation in human sperm: Correlation with fertilization in vitro. *Biology of Reproduction*, *56*(3), 602–607. https://doi.org/10.1095/biolreprod56.3.602

Sun, T. C., Zhang, Y., Li, H. T., Liu, X. M., Yi, D. X., Tian, L., & Liu, Y. X. (2018). Sperm DNA fragmentation index, as measured by sperm chromatin dispersion, might not predict assisted reproductive outcome. *Taiwanese Journal of Obstetrics and Gynecology*, *57*(4), 493–498. https://doi.org/10.1016/j.tjog.2018.06.003

Veeck, L. L. (1988). Oocyte assessment and biological performance. *Annals of the New York Academy of Sciences*, *541*, 259–274. https://doi.org/10.1111/j.1749-6632.1988.tb22263.x

Winkle, T., Rosenbusch, B., Gagsteiger, F., Paiss, T., & Zoller, N. (2009). The correlation between male age, sperm quality and sperm DNA fragmentation in 320 men attending a fertility center. *Journal of Assisted Reproduction and Genetics*, *26*(1), 41–46. https://doi.org/10.1007/s10815-008-9277-3

World Health Organization (2010). *WHO laboratory manual for the examination and processing of human semen* (5th ed.). World Health Organization.

Yim, S. F., Lok, I. H., Cheung, L. P., Briton-Jones, C. M., Chiu, T. T. Y., & Haines, C. J. (2001). Dose-finding study for the use of long-acting gonadotrophin-releasing hormone analogues prior to ovarian stimulation for IVF. *Human Reproduction*, *16*(3), 492–494. https://doi.org/10.1093/humrep/16.3.492

Zhang, Z., Zhu, L., Jiang, H., Chen, H., Chen, Y., & Dai, Y. (2015). Sperm DNA fragmentation index and pregnancy outcome after IVF or ICSI: A meta-analysis. *Journal of Assisted Reproduction and Genetics*, *32*(1), 17–26. https://doi.org/10.1007/s10815-014-0374-1

Zhao, J., Zhang, Q., Wang, Y., & Li, Y. (2014). Whether sperm deoxyribonucleic acid fragmentation has an effect on pregnancy and miscarriage after in vitro fertilization/intracytoplasmic sperm injection: A systematic review and meta-analysis. *Fertility and Sterility*, *102*(4), 998–1005.e1008. https://doi.org/10.1016/j.fertnstert.2014.06.033

Zini, A. (2011). Are sperm chromatin and DNA defects relevant in the clinic? *Systems Biology in Reproductive Medicine*, *57*(1–2), 78–85. https://doi.org/10.3109/19396368.2010.515704

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Khalafalla, K., Majzoub, A., Elbardisi, H., Bhathella, A., Chaudhari, A., Agarwal, A., Henkel, R., AlMarzooki, T., Burjaq, H., & Arafa, M. (2021). The effect of sperm DNA fragmentation on intracytoplasmic sperm injection outcome. *Andrologia*, 53, e14180. https://doi.org/10.1111/and.14180