



Relationship of Sperm DNA Fragmentation Index and Pregnancy Rates in ICSI Cycles for Iraqi Fertility Centres

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Abstract

Background: Sperm DNA fragmentation (SDF) is becoming a vital component in how we think about male infertility and the treatment using assisted reproductive technology (ART). The objective of the study was to evaluate the relationship between sperm DNA fragmentation index (DFI) and pregnancy rates in intracytoplasmic sperm injection (ICSI) cycles at Iraqi fertility centres.

Methods: A prospective cohort design study was performed across three private fertility centres in Baghdad, Iraq, between January 2022 to December 2023. In total, 485 ICSI cycles were included in the analyses; sperm DFI was measured using the sperm chromatin structure assay (SCSA). Patients were broken down into low ($\leq 15\%$), moderate (16-30%) and high ($> 30\%$) DFI.

Results: The clinical pregnancy rate was significantly higher in the low DFI (62.8%) group when compared to both the moderate (41.2%) and high (24.6%) DFI groups ($p < 0.001$). The live birth rate was reflected similarly in each group (54.3%, 33.7%, and 18.5% respectively). Multiple regression analysis indicated DFI as an independent predictor of pregnancy.

Conclusion: Sperm DNA fragmentation index was found to inversely correlate with pregnancy rates in ICSI cycles. Routine implementation of DFI testing in fertility practice in Iraq may improve results and offer better advice to patients.

Keywords: Sperm DNA fragmentation, ICSI, pregnancy rate, male infertility, Iraq

Introduction

Male factor infertility accounts for around 40-50% of all infertility worldwide and is one of the greatest challenges to reproductive health for couples worldwide (Agarwal et al., 2015). Traditional sperm parameters used in semen analysis assessing sperm concentration, motor activity and morphology have been used to assess male fertility for decades. However, traditional semen analysis parameters are not always able to predict pregnancy in an assisted reproductive technology (ART) cycle and particularly in intracytoplasmic sperm injection (ICSI) (Esteves et al., 2021). As such the search for more markers of sperm quality promoting successful outcomes in treatment has gained considerable momentum.

Sperm DNA fragmentation (SDF) is one of the newly developing biomarkers of male fertility and has received considerable attention in the literature for being one of the contributors to reproductive outcomes. DNA fragmentation is defined by breaks in the sperm DNA strand, either as single strand breaks (SSB) or double strand breaks (DSB), resulting in wasted and defective male gametes (Sergerie et al., 2005). The DNA fragmentation index (DFI) is a quantitative measurement of sperm DNA damage in a sperm population, contributing to an understanding of chromatin quality and potential outcomes in fertility.

Sperm DNA integrity is biologically significant beyond conception alone and impacts embryo development, implantation and maintenance of pregnancy. Sperm DNA damage can be due to many factors including oxidative stress, defective chromatin packaging, apoptosis, exposure to environmental factors, advanced paternal age, and lifestyle factors such as smoking and alcohol use (Aitken & Koppers, 2011). The spermatozoa harvested at ejaculation have undergone a complex journey of DNA repair mechanisms and DNA damage induction that impact the overall quality of their chromatin. There are several methods available to assess sperm DNA fragmentation, each with advantages and disadvantages. One of the most widely disseminated methods is the Sperm Chromatin Structure Assay (SCSA), which uses flow cytometry to assess the susceptibility of sperm DNA to acid-mediated denaturation (Evenson et al., 2002). Alternative methods include TUNEL assay, the Comet assay, and sperm chromatin dispersion (SCD) test. The SCSA method reports results in the form of a DNA Fragmentation Index (DFI), which indicates the proportion of sperm cells with DNA damage in a given sample.

Although the clinical relevance of sperm DNA fragmentation in natural conception has been established and well-characterized across multiple studies demonstrating inverse relationships between DFI, pregnancy rates, fertilization outcomes, and embryo quality (Simon et al., 2017), the concept remains debated in ICSI cycles. As a reminder, ICSI involves injecting a single sperm cell into the cytoplasm of the oocyte and theoretically bypasses many natural selection processes that would exclude damaged sperm from fertilization. Indeed, the selection of sperm cells for fertilization has been altered by ICSI, calling into question whether assessing sperm DNA fragmentation may provide prognostic value to ICSI cycles. Numerous recent meta-analyses have presented inconsistent evidence regarding the impact of sperm DNA fragmentation on clinical outcomes following ICSI fertilization. Several suggested that as DFI exceeds assessed thresholds or levels of normal DFI standards, fertilization rates of sperm cells are lower, less optimal embryo development is observed, and pregnancy rates are affected adversely, irrespective of being In statements contrary to the majority of other authors, some authors report to see a low to no relationship between DNA fragmentation and ICSI outcomes. They argue that the invasive nature of ICSI may enable an oocyte to repair sperm-related DNA damage. Differences in study populations included in their review of the literature, in how they assessed DFI, different threshold levels of DNA Fragmentation, and different outcome measures may explain this inconsistency.

The Middle East and Iraq have unique factors of design that may mediate the relationship between sperm DNA fragmentation and either maintain or attain sperm selection through ICSI. The region has a distinctive set of environmental stressors, ambient temperature, exposure to the dust, and factors associated with work both contributory to materiel products of petroleum products. These environmental stressors related to oxidative stress may also produce sperm DNA fragmentation. Differences in genetic polymorphism may also exist for ethnic population relative to DNA repair processes to find the clinical significance of measuring DNA fragmentation could be informative .

Fertility centers have seen consistent increases in ICSI use for a many different reasons over the last 10 years including increasingly higher rates of male factor infertility, or possibly cultural reasons (familial pressures) to produce a biological child. Unresolved clinical decisions, advice, changes to the social, or in workforce may also be an important or were likely second opinions facilitated by ART either through private or public sector access. The effectiveness rates of ICSI, as in many countries, varied in Iraq competitively to other fertility centers serving the same patient population.

Investigation of sperm DFI as a supplementary clinically informative metric for treatment outcomes in Iraq may allow for further examination of and practical significance of patient selection, treatment protocols, and counselling practices.

The financial implications of routinely implementing DFI testing as part of standard fertility assessment, even if at marginal cost, may be a consideration for some, especially the possible resource-limited health care systems. Although additional testing has upfront costs, the expected value of benefits related to leading to better patient selection, lower cycle cancellation rates, and better pregnancy outcomes could justify the costs from both clinical and economic perspectives. Since DFI has potential to direct treatment changes such as antioxidant supplementation, lifestyle changes, or sperm preparation methods that could improve the overall treatment outcome, DFI could serve as more than just a prognostic biomarker.

Current global recommendations about SDF testing vary around indication for testing (Schlegel et al., 2021). Some societies suggest routine sperm DNA fragmentation testing, while other recommendations suggest not ordering sperm DNA fragmentation testing except for specific clinical indications. The World Health Organization's (WHO) new manual provides recommendations to consider DNA fragmentation testing in cases of unexplained infertility, recurrent pregnancy loss in the same couple, or recurrent ART treatment failure. There are not yet standard operating guidelines or universal threshold values for determining the best assessment of SDF testing necessary to make viable clinical decisions regarding widespread clinical practice and implementation of protocols.

The Iraqi healthcare system faces challenges when implementing modern fertility testing options based on new technologies, decisively based on limited laboratory capabilities, knowledge-based expertise, and economics. However, several of the larger fertility centers in Baghdad and other governorates have begun using DNA fragmentation as part of additional assessment of male infertility as advancements in clinical practice, realizing the potential clinical value of a biomarker.

The aim of the present research was to shed light on an existing knowledge gap regarding sperm DNA fragmentation significance within ICSI cycles undertaken within Iraq population datasets. Analysis of data from several fertility centers in Iraq, the present research aimed to provide evidence-based recommendations for implementing protocols for DFI testing, and a threshold value for application in clinical practice local setting in Iraq. The outcomes of the research may be relevant to an international appreciation of population specific manifestations of the impact of variation in DNA fragmentation, whilst also supporting practical decision making for fertility specialists operating in Iraq.

The proposed studies aim to determine the relationship between sperm DNA fragmentation index and clinical pregnancy rates in ICSI cycles performed; identify the optimal threshold values of DFI for predicting cycle outcome; determine if DNA fragmentation testing can provide prognostic value independently; and evaluate if DFI testing could be cost effective in routine clinical practice in Iraqi fertility centres. These aims are aligned with both satisfying scientific inquiry and clinical necessity, with the potential to change practices in male fertility assessment in Iraq and other similar healthcare systems.

Methodology

This prospective cohort study was conducted across three private fertility centers in Iraq: Baghdad, spanning from January 2022 to December 2023. Written informed consent was obtained from all participants prior to enrollment.

Study Population and Inclusion Criteria

The study population comprised couples undergoing ICSI treatment for various indications of infertility. Inclusion criteria were established to ensure a homogeneous study population while maintaining adequate sample size for statistical analysis. Male partners were required to be between 18 and 60 years of age with complete semen analysis results and available sperm DNA fragmentation index measurements. Female partners were included if aged 18-45 years with at least one viable oocyte retrieved during the treatment

cycle. Couples must have provided complete demographic, clinical, and lifestyle information through standardized questionnaires administered by trained personnel.

Both primary and secondary infertility cases were included, with minimum infertility duration of 12 months. Couples undergoing their first through fourth ICSI attempt were eligible for participation, allowing analysis of treatment-naïve and previously treated populations. Various infertility etiologies were accepted, including male factor, female factor, combined factors, and unexplained infertility, reflecting real-world clinical practice diversity.

Exclusion Criteria

Comprehensive exclusion criteria were established to minimize confounding variables and ensure data quality. Couples were excluded if either partner had chromosomal abnormalities, active infections affecting reproductive organs, or malignancies requiring chemotherapy or radiation within the previous two years. Use of donor gametes, preimplantation genetic testing cycles, and frozen embryo transfers were excluded to focus specifically on fresh ICSI cycle outcomes.

Incomplete data sets, including missing DFI measurements, insufficient sperm for ICSI procedures, or lost to follow-up before primary outcome assessment, resulted in exclusion from final analysis. Couples participating in experimental protocols or receiving investigational treatments were excluded to maintain standard care consistency across the study population.

Semen Analysis and Processing

Semen samples were collected through masturbation after 2-5 days of sexual abstinence, following World Health Organization guidelines for semen analysis (World Health Organization, 2021). All samples were processed within one hour of collection using standardized protocols across participating centers. Conventional semen parameters were assessed using computer-assisted sperm analysis (CASA) systems (Hamilton-Thorne IVOS II, Beverly, MA, USA), with manual verification of concentration and morphology assessments.

Sperm concentration was determined using improved Neubauer hemocytometers, with duplicate counts performed for each sample. Progressive motility was classified according to WHO criteria, distinguishing rapid progressive (grade a), slow progressive (grade b), non-progressive (grade c), and immotile (grade d) spermatozoa. Morphological assessment followed strict Kruger criteria, with at least 200 spermatozoa evaluated per sample using Papanicolaou staining.

For ICSI procedures, sperm preparation employed density gradient centrifugation using commercially available media (IVF Online, Cooper Surgical, USA). Selected motile spermatozoa underwent additional washing steps with culture media before microinjection. Surplus processed sperm samples were utilized for DNA fragmentation index determination, ensuring consistency between tested and utilized gametes.

DNA Fragmentation Index Assessment

Sperm DNA fragmentation index was measured using the sperm chromatin structure assay (SCSA) performed with flow cytometry analysis (BD FACSCalibur, BD Biosciences, San Jose, CA, USA). This methodology was selected based on its excellent reproducibility, standardized protocols, and extensive validation in fertility research. All laboratory personnel underwent specialized training to ensure consistent technique application across centers.

Fresh sperm samples were processed immediately after semen analysis completion, with aliquots cryopreserved in liquid nitrogen for quality control purposes. The SCSA protocol involved acid treatment to denature damaged DNA strands, followed by acridine orange staining to differentiate between native and denatured DNA. Flow cytometry analysis quantified green (native DNA) and red (denatured DNA) fluorescence, with DFI calculated as the percentage of cells exhibiting predominantly red fluorescence.

Quality control measures included daily calibration using reference standards, duplicate testing of 10% of samples, and inter-laboratory comparison studies conducted quarterly. DFI measurements were performed by technicians blinded to clinical outcomes, maintaining analytical objectivity throughout the study period.

ICSI Procedures and Embryo Assessment

Controlled ovarian stimulation protocols were individualized based on patient characteristics, previous response patterns, and center-specific preferences. Gonadotropin-releasing hormone (GnRH) antagonist protocols were predominantly used, with recombinant follicle-stimulating hormone (FSH) as the primary stimulation agent. Trigger injection with human chorionic gonadotropin (hCG) or GnRH agonist was administered when at least three follicles reached 17mm diameter.

Oocyte retrieval was performed 34-36 hours post-trigger under transvaginal ultrasound guidance with conscious sedation. Retrieved oocytes underwent assessment for maturity, with only metaphase II oocytes selected for ICSI procedures. Microinjection was performed using standard techniques with inverted microscopes equipped with micromanipulation systems (Nikon Eclipse Ti, Nikon Instruments, Japan).

Embryo culture was conducted in sequential media systems (Global Media, Cooper Surgical, USA) under controlled environmental conditions (37°C, 6% CO₂, 5% O₂). Embryo assessment followed Istanbul consensus criteria, with daily monitoring of cleavage progression, cell number, fragmentation degree, and symmetry. Blastocyst development was evaluated using Gardner scoring system when applicable.

Embryo Transfer and Pregnancy Assessment

Embryo transfer timing was determined by individual center protocols and embryo development patterns, with day 3 and day 5 transfers both included in the analysis. Single embryo transfer was preferentially performed when high-quality embryos were available, with double transfers reserved for specific clinical indications or patient preferences. Transfer procedures utilized soft catheters under ultrasound guidance to optimize placement accuracy.

Luteal phase support was provided using progesterone supplementation (vaginal micronized progesterone 600mg daily or intramuscular progesterone 50mg daily) initiated on the day of embryo transfer and continued until pregnancy test results or discontinuation based on negative outcomes.

Primary and Secondary Outcomes

The primary outcome measure was clinical pregnancy rate, defined as visualization of gestational sac with fetal heartbeat activity via transvaginal ultrasound at 6-7 weeks of gestation. Secondary outcomes included positive pregnancy test (serum β -hCG >10 mIU/ml 12 days post-embryo transfer), implantation rate (number of gestational sacs divided by number of embryos transferred), ongoing pregnancy rate (viable pregnancy beyond 12 weeks gestation), live birth rate (delivery of viable infant after 24 weeks gestation), and miscarriage rate (pregnancy loss before 24 weeks gestation).

Statistical Analysis

Sample size calculation was performed using power analysis for correlation studies, targeting 80% power to detect moderate correlation coefficients ($r=0.3$) between DFI and pregnancy outcomes with alpha level of 0.05. Accounting for potential 15% dropout rate, minimum sample size was calculated as 450 ICSI cycles.

Statistical analysis was performed using SPSS version 28.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics included means with standard deviations for continuous variables and frequencies with percentages for categorical variables. Normal distribution was assessed using Shapiro-Wilk tests and histograms.

DFI was analyzed both as continuous and categorical variables, with predetermined threshold values of 15% and 30% creating three groups: low ($\leq 15\%$), moderate (16-30%), and high ($>30\%$) DNA fragmentation. These thresholds were selected based on previous literature recommendations and preliminary analysis of our dataset distribution.

Correlations between DFI and pregnancy outcomes were assessed using Pearson correlation coefficients for continuous variables and point-biserial correlations for dichotomous outcomes. Group comparisons employed one-way ANOVA for continuous variables and chi-square tests for categorical variables. Multiple logistic regression analysis identified independent predictors of pregnancy outcomes, controlling

for potential confounding variables including maternal age, BMI, infertility duration, previous ART attempts, and conventional semen parameters.

Results

Patient Characteristics and Demographics

A total of 485 ICSI cycles from 423 couples were included in the final analysis after applying inclusion and exclusion criteria. Patient demographics revealed mean maternal age of 31.4 ± 5.8 years (range 20-45 years) and mean paternal age of 36.2 ± 6.9 years (range 22-58 years). The majority of couples (62.8%) presented with primary infertility, while 37.2% had secondary infertility. Mean infertility duration was 4.7 ± 3.2 years, with 48.2% of couples undergoing their first ICSI attempt.

Table 1: Patient Demographics and Clinical Characteristics

Parameter	Overall (n=485)	Low DFI $\leq 15\%$ (n=178)	Moderate DFI 16-30% (n=204)	High DFI $>30\%$ (n=103)	p-value
Maternal age (years)	31.4 ± 5.8	30.8 ± 5.4	31.6 ± 5.9	32.1 ± 6.2	0.184
Paternal age (years)	36.2 ± 6.9	35.1 ± 6.2	36.8 ± 7.1	37.4 ± 7.5	0.023
BMI (kg/m ²)	26.8 ± 4.3	26.2 ± 4.1	27.1 ± 4.4	27.3 ± 4.6	0.089
Infertility duration (years)	4.7 ± 3.2	4.2 ± 2.8	4.9 ± 3.4	5.4 ± 3.6	0.007
Primary infertility (%)	304 (62.8)	118 (66.3)	124 (60.8)	62 (60.2)	0.442
First ICSI attempt (%)	234 (48.2)	96 (53.9)	98 (48.0)	40 (38.8)	0.045
Male factor infertility (%)	298 (61.4)	89 (50.0)	132 (64.7)	77 (74.8)	<0.001
Smoking status (%)	156 (32.2)	42 (23.6)	68 (33.3)	46 (44.7)	0.002

Semen Parameters and DNA Fragmentation Index

Conventional semen parameters demonstrated significant variations across DFI groups. Mean sperm concentration was 45.3 ± 28.7 million/ml, with progressive motility averaging $42.8 \pm 18.9\%$, and normal morphology $6.4 \pm 4.2\%$. The overall mean DNA fragmentation index was $21.3 \pm 12.4\%$, with 178 patients (36.7%) classified as low DFI, 204 (42.1%) as moderate DFI, and 103 (21.2%) as high DFI.

Table 2: Semen Parameters Across DNA Fragmentation Index Groups

Parameter	Low DFI $\leq 15\%$ (n=178)	Moderate DFI 16-30% (n=204)	High DFI $>30\%$ (n=103)	p-value
Concentration ($\times 10^6$ /ml)	52.8 ± 31.2	43.7 ± 26.8	35.9 ± 24.1	<0.001
Progressive motility (%)	48.9 ± 17.4	41.2 ± 18.7	35.6 ± 19.8	<0.001
Normal morphology (%)	8.1 ± 4.6	6.2 ± 3.8	4.5 ± 3.2	<0.001
Volume (ml)	3.2 ± 1.4	3.1 ± 1.3	2.9 ± 1.2	0.156
DFI (%)	10.8 ± 3.2	22.6 ± 4.1	38.4 ± 7.8	<0.001

IVF Laboratory Outcomes

Laboratory parameters showed significant differences across DFI groups. Mean number of oocytes retrieved was 11.8 ± 6.4 , with 8.9 ± 5.1 metaphase II oocytes available for ICSI. Fertilization rates were

significantly higher in the low DFI group compared to moderate and high DFI groups (78.4% vs 71.2% vs 64.8%, $p<0.001$). Similarly, good quality embryo rates on day 3 demonstrated inverse correlation with DFI levels.

Table 3: Laboratory Outcomes by DNA Fragmentation Index Groups

Parameter	Low DFI $\leq 15\%$ (n=178)	Moderate DFI 16-30% (n=204)	High DFI $>30\%$ (n=103)	p-value
Oocytes retrieved	12.3 $\pm$ 6.8	11.7 $\pm$ 6.2	11.1 $\pm$ 6.1	0.267
MII oocytes	9.4 $\pm$ 5.3	8.8 $\pm$ 4.9	8.2 $\pm$ 5.0	0.142
Fertilization rate (%)	78.4 $\pm$ 18.7	71.2 $\pm$ 21.4	64.8 $\pm$ 24.1	<0.001
Day 3 good quality embryos	4.2 $\pm$ 3.1	3.4 $\pm$ 2.8	2.7 $\pm$ 2.4	<0.001
Blastocyst formation rate (%)	45.8 $\pm$ 24.3	38.7 $\pm$ 25.1	31.2 $\pm$ 23.8	<0.001
Embryos transferred	1.8 $\pm$ 0.4	1.9 $\pm$ 0.3	1.9 $\pm$ 0.3	0.089

Clinical Pregnancy Outcomes

Clinical pregnancy rates demonstrated strong inverse correlation with DNA fragmentation index levels. The low DFI group achieved significantly higher clinical pregnancy rates (62.8%) compared to moderate DFI (41.2%) and high DFI (24.6%) groups ($p<0.001$). Live birth rates followed similar patterns, with 54.3%, 33.7%, and 18.5% respectively across the three groups.

Table 4: Clinical Outcomes by DNA Fragmentation Index Groups

Outcome	Low DFI $\leq 15\%$ (n=178)	Moderate DFI 16-30% (n=204)	High DFI $>30\%$ (n=103)	p-value
Positive pregnancy test	128 (71.9%)	104 (51.0%)	32 (31.1%)	<0.001
Clinical pregnancy rate	112 (62.8%)	84 (41.2%)	25 (24.3%)	<0.001
Implantation rate	112/318 (35.2%)	84/388 (21.6%)	25/196 (12.8%)	<0.001
Ongoing pregnancy rate	102 (57.3%)	75 (36.8%)	21 (20.4%)	<0.001
Live birth rate	97 (54.5%)	69 (33.8%)	19 (18.4%)	<0.001
Miscarriage rate	15/112 (13.4%)	15/84 (17.9%)	6/25 (24.0%)	0.387

Correlation Analysis

Pearson correlation analysis revealed significant negative correlations between DFI and various pregnancy outcomes. The strongest correlation was observed between DFI and clinical pregnancy rate ($r=-0.421$, $p<0.001$), followed by live birth rate ($r=-0.398$, $p<0.001$) and implantation rate ($r=-0.387$, $p<0.001$). These correlations remained significant after controlling for maternal age, previous ICSI attempts, and conventional semen parameters.

Multiple Regression Analysis

Logistic regression analysis identified DFI as an independent predictor of clinical pregnancy after controlling for confounding variables. For every 10% increase in DFI, the odds of clinical pregnancy decreased by 35% (OR: 0.65, 95% CI: 0.54-0.78, $p<0.001$). Other significant predictors included maternal age (OR: 0.92, 95% CI: 0.88-0.96, $p<0.001$) and number of good quality embryos (OR: 1.18, 95% CI: 1.08-1.29, $p<0.001$).

The analysis demonstrates clear inverse relationships between sperm DNA fragmentation index and pregnancy outcomes in ICSI cycles. Patients with low DFI levels achieved significantly superior clinical results across all measured parameters, supporting the clinical utility of routine DNA fragmentation testing

in Iraqi fertility centers. These findings provide evidence-based guidance for implementing DFI assessment as part of comprehensive male fertility evaluation protocols.

Discussion

The results of this extensive study provide substantial evidence for a significant inverse relationship between sperm DNA fragmentation index and pregnancy outcomes in ICSI cycles performed in Iraqi fertility centers. Our results indicate that patients record a clinical pregnancy rate of 62.8% with low DFI ($\leq 15\%$) which is significantly higher than the pregnancy outcome for when there is moderate (41.2%) and/or high DFI (24.6%). These percentage differences are clinically relevant differences that would inform treatment planning and counselling in fertility practice. The calculated correlation coefficients between DFI (overall level of DNA fragmentation) and relevant pregnancy outcomes ($r = -0.421$ for clinical pregnancy rate) indicate moderate to strong correlations even after accounting for historical prognostic factors such as age and previous attempts. This supports a clear difference to earlier assumptions that the ICSI procedure could fully account for sperm DNA damage effects by bypassing natural selection processes (Bungum et al., 2004; Virro et al., 2004).

The biological rationale supporting these associations is due to the fact that sperm paternal DNA integrity ensures sequential stages of embryonic development after the initial fertilization event (Morris et al., 2002). Although ICSI is successful in delivering sperm cells within the oocyte cytoplasm, regardless of the DNA quality, further development and progression beyond fertilization primarily relies upon paternal genetic material integrity (Borini et al., 2006). Sperm with fragmented DNA can enter the fertilization process, however their resulting embryos may not develop properly, leading to no implantation, early loss of pregnancy, or to abnormal specimens that develop due to compromised developmental potential (Tesarik et al., 2004). Our study indicating lower fertilization rates for high DFI groups (64.8% versus 78.4% low DFI) would indicate that DNA damage may have an effect at even early stages of embryonic development, for example possibly influencing sperm-oocyte activation mechanisms or defective paternal genome activation processes (Braude et al., 1988).

Further, the effect of DNA fragmentation on blastocyst rates we observed (31.2% high DFI versus 45.8% low DFI) is consistent with temporal effect of paternal DNA damage on embryonic development, in that there is emerging evidence suggesting that paternal genome activation occurs at or shortly after the 4-cell stage of human embryo development. This also coincides with the time when the effects of DNA fragmentation are most evidenced (Saleh et al., 2002; Kazerouni et al., 2009). The relationship between the progressive diminution of embryo quality being negatively correlated with increasing DFI also suggests a predictable, dose-dependent phenomenon that may guide the determination of thresholds for clinical decision making (Practice Committee of the American Society for Reproductive Medicine, 2008). Our demographic analysis reveal some interesting relationships between DFI levels and patient characteristics that would be important to factor into any clinical practice. Encouragingly, we noted the significant correlation between paternal age and DNA fragmentation index was consistent with the existing literature associating the negative effects of advanced paternal age with increased levels of oxidative stress and decreased chromatin packaging efficiency (Singh et al., 2003; Wyrobek et al., 2006). Similarly, the greater number of patients who were smokers with greater DFI levels (44.7% high DFI versus 23.6% low DFI) suggests that this practice is important to address during lifestyle counseling in a comprehensive fertility treatment (Saleh et al., 2002; Fraga et al., 1996). These and other recognizable lifestyle factors represent modifiable risk factors that could be subject to intervention and possibly improve treatment trajectories through dietary and antioxidant intake protocols (Showell et al., 2014; Zini et al., 2009). The inverse relationship between typical semen parameters and DFI levels seen in our study provides confirmation of the complementary not redundant value of such measures (Huang et al., 2005). Patients with high DNA fragmentation had significantly lower sperm concentration, motility and

morphology, indicating that DNA integrity measures can act as an additional layer of evaluation of sperm quality, not as a substitute for standard semen analysis (Benchabib et al., 2003; Larson et al., 2000). Thus, this points to the more generalized idea of a comprehensive sperm evaluation that incorporates measures of both functional and genetic integrity that will provide better prognostic assessment for couples having ICSI procedure) Agarwal et al., 2016).

While the relevance of our results has prognostic value, it also provides implications for treatment modifications and patient counseling guidelines. When clinicians recognize that a patient has high levels of DFI, they may apply some lifestyle advice, offer antioxidants, or use other sperm selection methods, such as magnetic activated cell sorting or microfluidic sperm sorting, to aim for improved outcomes (Greco et al., 2005; Parmegiani et al., 2010). Then, when clinicians relate high DFI levels to a need for improved and standardized psychological support, more timely expectations of treatment success rates and needed cycle numbers could be developed) Wdowiak et al., 2015.(

From a health economics standpoint, routinely incorporating DFI testing into practice calls for careful evaluations of cost-to-benefit ratios in a resource-poor health system like Iraq. There are obvious additional costs from DFI testing, but potential benefits could include improved cycle efficiency through better patient selection, less emotional distress from fewer failed cycles, and increased centres' overall success rate (Esteves et al., 2017; Collins et al., 2008; Lewis et al., 2013). Our results imply that couples exhibiting very high DFI (>30%) may benefit from counseling regarding alternative treatments or the possibility that they may require multiple treatment cycles, which may lower their treatment costs overall through more realistic treatment planning) Boe-Hansen et al., 2006.(

The thresholds that we used in our study (15% and 30%) were chosen from previous literature recommendations as well as institutional experience (Evenson & Wixon, 2006; Fernández et al., 2003). However, the most appropriate threshold for clinical decision making may require population-specific validation since genetic, environmental, and lifestyle factors may differ between population groups, including Middle Eastern populations (Evgeni et al., 2014). The continuum of DFI as observed in our study would suggest that fixed thresholds may be less useful than an individualized risk assessment using multiple prognostic factors) Spanò et al., 2000.(

With respect to local factors encountered in Iraqi fertility practice, there may be environmental factors that also lead to elevated rates of DNA fragmentation, including climate extremes, exposure to dust, occupational hazards, petroleum-industry activity, and dietary habits or practices (Al-Janabi et al., 2013; Durairajanayagam, 2018). These environmental factors may require population-specific reference ranges and treatment practices that address local conditions (Bakos et al., 2008). The markedly higher baseline DFI values in our study, compared with some European populations, could be due to varied environmental influences in the local population and the potential need for local validation studies) Giwercman et al., 2003.(

The prospective design and multi-center approach increase the generalizability of findings to representative practices in Iraqi fertility, while the meaningful sample size also provides adequate statistical power to identify clinically important differences (Irvine et al., 2000). Nonetheless, some caveats must be noted, namely the lack of longer follow-up data regarding offspring health outcomes, possible selection bias regarding couples who choose to participate in research studies, and the inability to blind personnel to DFI findings during treatment cycles (Chohan et al., 2006; Agarwal & Allamaneni, 2005). Future research directions could include longitudinal studies regarding the association between paternal DFI and offspring developmental outcomes, intervention studies assessing the effectiveness of antioxidant treatments in reducing levels of DNA fragmentation, and economic analyses to assess the effectiveness of the implementation of DFI in routine practice) Simon et al., 2010; Osman et al., 2015).

The standardization of DNA fragmentation testing in fertility centres is an important consideration for quality assurance and therefore a prerequisite for use in routine clinical practice (Evenson & Wixon, 2006).

Our study utilized standardized SCSA protocols with quality control, which provide the basis for setting regional standards for testing (Shamsi et al., 2011). It is possible that access to testing may be limited due to equipment and technical specialist limitations in smaller fertility centres; alternative testing approaches and centralized laboratory services could be considered to provide access without compromising quality (Feijó & Esteves, 2014).

Incorporating DFI testing into existing clinical fertility treatment protocols must include consideration of timing, interpretation, and clinical response (Practice Committee of the American Society for Reproductive Medicine, 2015). Based on the findings of our study, DFI testing would best be integrated into the initial evaluation of male fertility rather than only in the setting of unexplained infertility or repeated treatment failure (Zini et al., 2008). Integrating DFI testing early may allow for earlier identification of couples who may not have the same treatment options or who may benefit from additional supportive interventions (Cissen et al., 2016). The association we have reported between DNA fragmentation and pregnancy outcomes in our study is consistent with the biological plausibility of paternal DNA integrity acting as a key factor in reproductive potential even in ICSI cycles (Ribas-Maynou et al., 2013; Sivanarayana et al., 2014). This finding adds to the compelling rationale for routinely evaluating DNA fragmentation as one component of male fertility assessment, providing pilot data for establishing evidence-based clinical practice guidelines for Iraqi fertility centres (Esteves et al., 2012).

Conclusion

Our study demonstrates a significant and clinically relevant inverse association between sperm DFI and pregnancy outcomes (delivered biological child) in ICSI cycles occurring in Iraqi-based fertility centres. Patients with DFI values $\leq 15\%$ had higher clinical pregnancy rates (62.8%), live birth rates (54.5%) and embryological outcomes than moderate and high DNA fragmentation.

Identification of DFI as an independent clinical predictor of treatment success, even after controlling for established prognostic factors supports the clinical importance of routine testing for DNA fragmentation. The studies findings provide evidence-based rationales for starting DFI as a routine component of a holistic fertility assessment and management process by Iraqi fertility centres. The remnant threshold effects suggest that couples with DFI values above 30% may need treatment options changed, realistic expectations of success and possibly interventional strategies to maximize the potential for improving sperm DNA integrity.

The findings provide new and relevant population-specific data within the developing literature evidencing sperm DNA fragmentation and responds to real-time challenges for fertile specialists in Iraq. Routine assessment of DFI is anticipated to contribute to better treatment outcomes in patients, more accurate expectations during patient counselling not to mention better utilization of limited health care resources in Iraqi fertility centres.

Future work aims to develop interventional approaches to couples with elevated DNA fragmentation, document long-term offspring implications of paternal DNA damage and revisit the cost-effectiveness of routinely implementing DFI testing approaches in constrained healthcare contexts.

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