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Influence of Sperm Chromatin Immaturity on Intracytoplasmic Sperm Injection Outcomes

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ABSTRACT

Background: Assisted Reproductive Techniques (ART), particularly intracytoplasmic sperm injection (ICSI), bypass natural selection processes, allowing sperm with low deoxyribonucleic acid (DNA) integrity to fertilize eggs, which may adversely affect ICSI outcomes. Routine semen analysis provides limited insight into male reproductive potential, necessitating advanced assessments of sperm chromatin maturity.

Methods: Semen samples were collected from 92 patients after 1–21 days of sexual abstinence. Macroscopic and microscopic examinations were performed according to WHO standards (6th edition, 2021). A detailed questionnaire capturing history and physical examination was used. The relationship between sperm chromatin immaturity (SCI%) and ICSI outcomes, including fertilization rate and embryo quality, was evaluated.

Result: A weak and non- significant negative correlation was observed between SCI% and fertilization rate (CC = -0.051; p = 0.63) and between SCI% and grade 1 embryos (CC = -0.093; p = 0.38). Weak, non-significant positive correlations were found between SCI% and abnormal division (CC = 0.05; p = 0.64). However, a weak positive association was identified between SCI% and grade 2 embryos (CC = 0.242; p = 0.02) and grade 3 embryos (CC = 0.212; p = 0.04). SCI% showed no significant correlation with seminal fluid parameters.

Conclusion: This study concluded that sperm chromatin immaturity (SCI%) does not correlate with seminal fluid parameters but showed no significant correlation with fertilization rate or pregnancy rate and showed weak associations with some embryo grades.

INTRODUCTION

Sperm deoxyribonucleic acid (DNA) fragmentation is defined as any chemical alteration in the normal structure of sperm DNA. Among these alterations, single- and double-strand breaks are among the most common disruptions affecting the genetic material [1]. Sperm DNA fragmentation (SDF) can be induced by various processes, including defective DNA packaging during spermatogenesis, apoptosis, and oxidative stress. Sperm DNA damage negatively impacts the fertilization capacity of spermatozoa [2] and can also affect embryo development, implantation, and pregnancy outcomes in both natural and assisted reproduction [3].

The structure of sperm DNA consists of a protein core formed by an octamer of histones around which two loops of DNA (approximately 146 base pairs) are wrapped. These nucleosomes are further coiled into regular helices, known as solenoids [4]. This hierarchical packaging significantly increases the stability and reduces the volume of chromatin [5]. In sperm cells, DNA's pattern of organization involves a specific pathway marked by mitotic as well as meiotic transformations. Throughout the process, somatic histones are replaced step by step by transition proteins before finally being traded in for protamines. Consequently, the chromatin grows to be very dense—significantly denser than in normal mitotic chromosomes, with more than a six-fold level of compaction. The structural adjustments take place largely in the late stages of spermatogenesis and persist during spermiogenesis [6].

There is research indicating that sperm fragmentation in DNA largely accounts for male infertility, particularly if it happens to a great extent [7]. The common kinds of breaks in single- and double-stranded DNA in sperm make fragmentation analysis a diagnostic tool of great importance in assessing infertility [8]. The most common sources of sperm DNA fragmentation (SDF) include oxidative stress, apoptosis, and incorrect protamination. During passage through the epididymis as well as after ejaculation, proper protamination helps in keeping the integrity of DNA intact to avoid any harm to it [9]. Increased levels of SDF have been proven to relate to decreased fertility potential as well as semen parameters of poor quality [10]. SDF has also been found to relate to harmful effects in reproduction, wherein oxidative sperm lipid damage occurs as a result of an excess of reactive oxygen species (ROS) over antioxidants.

This study examined correlation between immature sperm chromatin — measured as sperm chromatin immaturity percentage (SCI%) — and critical intracytoplasmic sperm injection (ICSI) parameters, including fertilization success, embryo quality, and pregnancy. The semen samples from 92 subjects were collected after abstinence time of 1–21 days. Each was subjected to extensive macroscopic and microscopic examination as per WHO guidelines [3]. Clinical history and examination information was documented through a standardized questionnaire. Post-liquefaction, samples were divided: half was used for ICSI cycles, and half was examined for DNA fragmentation index (DFI) through aniline blue staining to assess immaturity of the chromatin. The research then probed correlations among SCI%, ICSI success parameters — fertilization success, embryo grades, and pregnancy—and analyzed if sperm DNA fragmentation affects the effectiveness of assisted reproduction.

METHODS

Before participating in this study, written consent forms from all participants were obtained after getting permission from the Institutional Review Board of Al-Nahrain University's High Institute for Diagnosis of Infertility and Assisted Reproductive Technologies. The informed consent process was built into the framework of the study's questionnaires. For the research, semen samples from 92 male subjects, consisting of 76 primary infertility and 16 secondary infertility cases, were obtained after a period of sexual continence of 1-21 days. Standard semen fluid analyses were carried out in accordance with traditional protocols [3].

Each collected semen specimen was divided into two aliquots: one was assigned for ICSI treatment, and the other was analyzed for chromatin maturity by acid aniline blue staining (SCI% test). The outcomes of all chromatin maturity tests were recorded. The embryo development was checked after 24 hours, with day-3 evaluation of embryos. Depending upon the clinical status of the recipient, viable embryos were either transferred immediately (1-4 per transfer) or cryopreserved for future use. The SCI% values were then compared with ICSI outcomes to assess the influence of high SCI% on fertilization rates, embryo quality, and pregnancy outcomes. The principal standards selected and deemed necessary for inclusion of males in the current study were unexplained infertility, asthenozoospermia, normospermia, oligozoospermia, oligoasthenoteratozoospermia (OAT), or teratozoospermia. The exclusion criteria included

cryopreserved samples, testicular biopsy or fine needle aspiration, azoospermia, and female partners with systemic diseases, uncontrolled endocrine disorders, congenital anomalies of the reproductive tract, large uterine fibroids, moderate to severe endometriosis, or hydrosalpinx.

RESULTS

Sociodemographic Factors of Enrolled Infertile Couples

The demographic and clinical characteristics of the couples who participated in this study are summarized in Table 1. The table presents data on age, body mass index (BMI), and infertility duration for both males and females, along with information on smoking status, sperm DNA integrity, and causes of infertility. A total of 92 infertile couples enrolled in the ICSI program. The age of male participants ranged from 24 to 64 years, with a mean age of 36.95 ± 0.82 years. Female participants were aged between 20 and 44 years, with a mean age of 32.07 ± 0.61 years. Regarding body mass index (BMI), males had a mean BMI of 28.13 ± 0.55 kg/m², while females had a mean BMI of 26.91 ± 0.49 kg/m². The duration of infertility varied from 1 to 25 years, with a mean of 7.35 ± 0.51 years. Among the couples, 83.7% presented with primary infertility. In terms of smoking habits among males, 52 participants (56.5%) were non-smokers, while 40 (43.5%) were smokers. Regarding sperm DNA integrity, 37 participants (40%) had normal sperm DNA integrity, whereas 55 participants (60%) exhibited abnormal sperm DNA integrity. The most common cause of infertility was male factor infertility, affecting 53 participants (57.6%). Mixed factors contributed to infertility in 25 participants (27.2%), while female factor infertility was observed in 11 participants (11.9%). Unexplained infertility was reported in 3 participants (3.3%).

Descriptive Information and Semen Parameters of Study Subjects

The semen parameters of the male participants in this study are summarized in Table 2, presenting various semen variables based on the WHO 2021 guidelines. The table provides an overview of the key parameters evaluated, including SCI and other semen analysis metrics. The participants had an average abstinence period of 4.61 ± 0.33 days. Semen samples liquefied within an average time of 30.00 ± 0.10 minutes. The pH of the semen was 7.48 ± 0.02, reflecting a slightly alkaline environment. The mean semen volume was 1.86 ± 0.08 mL.

The average sperm concentration was 22.37 ± 1.93 million per mL, with a total sperm count of 43.00 ± 4.36 million per ejaculate. Total motility was 36.86 ± 2.14%, and progressive motility was 22.84 ± 1.97%, both of which were below the WHO 2010 reference values. Non-progressive motility accounted for 14.02 ± 0.69%, while immotile spermatozoa represented 63.14 ± 2.14%. The percentage of total progressive motile spermatozoa was 13.93 ± 2.21%.

The percentage of sperm with normal morphology was 2.20 ± 0.14%, which is significantly below the threshold for normal values. The concentration of round cells was 2.52 ± 0.22 per mL. Additionally, the sperm chromatin immaturity percentage (SCI%) was 27.41 ± 1.77%, exceeding the normally accepted value of ≤ 20%, indicating impaired DNA integrity.

Oocyte quality and embryo features resulting from ICSI

The quality of oocytes and characteristics of embryos resulting from the ICSI procedure provide detailed insights into various parameters related to retrieved oocytes and embryo outcomes in this study (Table 3). The table summarizes key data on oocyte retrieval and embryo grading, including the quality of embryos obtained. These parameters include the average number of oocytes retrieved (12.14 ± 0.93), abnormalities, developmental stages, maturation and fertilization rates, zygote numbers, and embryo features such as the number of embryos transferred and their grades. The data present both the range and average values for each parameter, offering a comprehensive understanding of oocyte and embryo quality and development observed in the study.

Comparison of Male Patient Characteristics Based on Sperm Chromatin Immaturity

When the samples were categorized based on DNA integrity, the descriptive information and semen parameters showed no significant differences between the groups (Table 4), with the exception of SCI%. The table presents the mean ± standard error (SE), mean rank, and sum of

ranks for various semen parameters. A total of 92 semen samples were analyzed, with 37 samples classified as having normal SCI% and 55 samples classified as having abnormal SCI%. Statistical differences between groups were assessed using the Mann-Whitney test.

Comparing Male Patients' Characteristics Based on semen sample characteristics

When the samples were subdivided based on semen quality, as assessed through seminal fluid analysis, the findings (Table 5) revealed that individuals with normozoospermic samples exhibited significantly higher values for sperm concentration, total sperm count, total motility, progressive motility, and normal sperm morphology compared to those with single defects, double defects, and OAT. Notably, no significant differences were observed in sperm concentration, total sperm count, or total progressive motile spermatozoa between the normozoospermic samples and those with single defects. However, significant differences were identified between the normozoospermic group and the other groups.

The normozoospermic group showed the highest percentage of total progressive motile spermatozoa, with significant differences when compared to the other groups. Conversely, the OAT group exhibited the highest percentage of immotile spermatozoa, with significant differences observed between groups. Additionally, the normozoospermic group demonstrated the highest percentage of normal sperm morphology, with significant differences compared to the other groups. However, no significant differences were observed in non-progressive motility (%) or SCI% either within or between groups.

Comparing Male Patients' Characteristics Based on Infertility Factors

The comparison of sample characteristics based on different infertility factors reveals distinct trends. Individuals with male factor infertility exhibited significantly lower values for sperm concentration, total sperm count, total motility, progressive motility, total progressive motile spermatozoa, and normal sperm morphology compared to those with female factor infertility and mixed factors (Table 5). The table provides mean ± standard error (SE) values for various semen parameters, categorized by semen quality and infertility factors. A total of 92 semen samples were analyzed, including 14 normozoospermic samples, 21 samples with single defects, 25 samples with double defects, and 32 samples classified as OAT. Statistical analysis was conducted using the Kruskal-Wallis test, followed by the Mann-Whitney test, with significant differences indicated by $P \leq 0.05$.

In contrast, individuals with female factor infertility demonstrated higher values for these parameters than those with male factor infertility. Notably, no significant differences were observed in sperm concentration or total sperm count between individuals with female factor infertility and those with mixed factor infertility. Additionally, non-progressive motility and SCI% did not show significant differences among the infertility factor groups.

A two-factor ANOVA revealed no significant interaction between semen quality and sperm DNA integrity in relation to SCI% values. However, when the data were analyzed based on the classification of samples by sperm DNA integrity status, samples with normal sperm DNA integrity exhibited significantly lower SCI% values compared to those with abnormal sperm DNA integrity within their respective groups. A similar trend was observed when the samples were categorized based on infertility factors.

Correlations between the embryos' features and SCI%

Weak and non-significant negative correlations were observed between SCI% and fertilization rate (correlation coefficient [CC] = -0.051; p = 0.63), as well as embryo grade 1 (CC = -0.093; p = 0.38). Similarly, a weak and non-significant positive correlation was found between SCI% and abnormal division (CC = 0.050; p = 0.64). However, SCI% demonstrated a weak but significant positive association with embryo grades 2 and 3, with correlation coefficients of 0.242 (p = 0.02) and 0.212 (p = 0.042), respectively. Additionally, a weak and non-significant negative correlation was identified between SCI% and pregnancy rate (CC = -0.212; p = 0.076). All correlations were assessed using Pearson correlation analysis, with significant correlations determined at the 0.05 level (two-tailed). These findings highlight potential associations between SCI% and specific embryological outcomes while emphasizing the lack of strong correlations across most

parameters.

Tables

Parameters	Mean ± SE	Range
Male age (years)	36.95 ± 0.82	24 – 64
Male BMI (Kg/m²)	28.13 ± 0.55	16.0 – 44.8
Female age (years)	32.07 ± 0.61	20 – 44
Female BMI (Kg/m²)	26.91 ± 0.49	16.16 – 43.40
Duration of infertility (years)	7.35 ± 0.51	1 – 25
Parameters	**Frequency (n.)**	**Percent (%)**
Type of infertility	Primary = 77	83.7 %
	Secondary = 15	16.3 %
Smoking (male)	No = 52	56.5 %
	Yes = 40	43.5 %
Sperm DNA integrity	Normal = 37	40 %
	Abnormal = 55	60 %
Infertility factor	Male factor = 53	57.6 %
	Female factor = 11	11.9 %
	Mixed factors = 25	27.2 %
	Unexplained = 3	3.3 %

Table 1: Demographic and clinical characteristics of the couples in the present study. Values are expressed as mean ± standard error (SE) or percentages, where applicable

Semen variables	Mean ± SE	Range	Cut-off value (WHO 2021)
Days of Abstinence	4.61 ± 0.33	1 – 21	3 - 5
Liquefaction time (minutes)	30.00 ± 0.10	N. A	30 - 60 minutes
pH	7.48 ± 0.02	N. A	⩾ 7.2 - 8
Volume (mL)	1.86 ± 0.08	0.5 – 4	⩾ 1.5 mL
Sperm concentration (sperm $\times 10^6$/mL)	22.37 ± 1.93	1 – 90	⩾ 15 x 10^6/mL
Total sperm count (sperm $\times 10^6$ per ejaculate)	43.00 ± 4.36	1 – 270	⩾ 39 x 10^6 / ejaculate
Total motility % (PR+NP)	36.86 ± 2.14	0 – 80	40 %
Progressive motility % (A+B)	22.84 ± 1.97	0 – 70	⩾ 32 %
Non-progressive motility % (C)	14.02 ± 0.69	0 – 30	N. A
Immotile spermatozoa % (D)	63.14 ± 2.14	20 – 100	N. A
Total progressive motile spermatozoa %	13.93 ± 2.21	0 – 100	N. A
Normal Sperm Morphology %	2.20 ± 0.14	1 – 5	⩾14 % (WHO 1999) ⩾ 4% (WHO 2021)
Round cells	2.52 ± 0.22	1 – 16	⩽1 × 10^6 cells/mL
SCI%	27.41 ± 1.77	4 – 88	⩽ 20%

Table 2: Characteristics of the semen samples included in the study. Values are presented as mean ± standard error (SE) for continuous variables, N. A = not available

Parameters	Mean ± Std. Error	Range
Oocyte numbers	12.14 ± 0.93	1 – 47
Abnormal oocytes	1.30 ± 0.21	0 – 11
Germinal vesicles (GV)	1.26 ± 0.25	0 – 16
Metaphase I	1.04 ± 0.13	0 – 4
Metaphase II	8.57 ± 0.66	1 – 29
Maturation rate	71.70 ± 2.34	0 – 100
Fertilization rate	76.54 ± 2.61	0 – 100
Zygote numbers	6.23 ± 0.49	0 – 25
Embryo features		
Number of Embryos transferred	1.82 ± 0.14	0 – 4
Number of Embryos Day 3	4.95 ± 0.42	0 – 18
Abnormal division	0.32 ± 0.12	0 – 7
Embryos grade 1	3.08 ± 0.32	0 – 15
Embryos grade 2	1.05 ± 0.18	0 – 9
Embryos grade 3	0.49 ± 0.10	0 – 5

Table 3: Oocyte and embryo quality parameters of the study participants. Values are presented as mean ± standard error (SE)

Parameters	SCI groups	Mean	Std. Error	Mean Rank	Sum of Ranks
Sperm concentration (sperm x10^6/mL)	Normal	26.19	3.53	51.85	1918.50
	Abnormal	19.80	2.15	42.90	2359.50
Total sperm count (sperm x10^6 / ejaculate)	Normal	51.892	8.47	51.66	1911.50
	Abnormal	37.018	4.46	43.03	2366.50
Total motility% (PR+NP)	Normal	38.54	3.15	48.97	1812.00
	Abnormal	35.73	2.89	44.84	2466.00
Progressive motility % (A+B)	Normal	24.70	2.94	50.23	1858.50
	Abnormal	21.58	2.64	43.99	2419.50
Non-progressive motility% (C)	Normal	13.84	0.94	46.42	1717.50
	Abnormal	14.15	0.98	46.55	2560.50
Immotile spermatozoa% (D)	Normal	61.46	3.15	44.03	1629.00
	Abnormal	64.27	2.89	48.16	2649.00
Total progressive motile spermatozoa%	Normal	16.7355	4.05	51.96	1922.50
	Abnormal	10.7846	1.99	42.83	2355.50
Normal Sperm Morphology %	Normal	2.37	0.22	49.58	1834.50
	Abnormal	2.09	0.17	44.43	2443.50
SCI %	Normal	12.35*	0.81	19.00	703.00
	Abnormal	37.54	1.94	65.00	3575.00

Table 4: Comparison of semen sample characteristics based on SCI%. Superscript asterisks (*) indicate significant differences between groups (P ≤ 0.05)

Parameters	Semen quality				Infertility factors		
	Normozoospermic Samples	Samples with single defect	Samples with double defects	OAT samples	Male Factor	Female Factor	Mixed Factors
Sperm concentration (Sperm x10^6/mL)	44.14 ± 5.89^1	34.19 ± 3.42^1	19.44 ± 2.03^2	7.38 ± 0.68^3	13.79 ± 1.50^1	44.82 ± 7.52^2	29.44 ± 3.57^2
Total sperm count (Sperm x10^6 / ejaculate)	81.68 ± 16.92^1	68.29 ± 7.68^1	39.74 ± 4.91^2	12.03 ± 1.66^3	26.50 ± 3.71^1	77.96 ± 21.53^2	58.48 ± 7.27^2
Total motility % (PR+NP)	62.21 ± 2.91^1	50.10 ± 3.15^2	33.16 ± 3.06^3	19.97 ± 2.19^4	27.55 ± 2.31^1	62.36 ± 4.89^2	43.20 ± 3.49^3
Progressive motility % (A+B)	47.86 ± 5.30^1	35.48 ± 2.72^2	19.04 ± 2.80^3	6.56 ± 1.30^4	15.23 ± 1.82^1	48.18 ± 4.83^2	29.80± 3.24^3
Non-progressive motility % (C)	14.36 ± 1.67^{NS}	14.62 ± 1.17^{NS}	14.12 ± 1.22^{NS}	13.41 ± 1.43^{NS}	14.32 ± 1.02^{NS}	14.18 ± 2.04^{NS}	13.40 ± 1.01^{NS}
Immotile spermatozoa % (D)	37.79 ± 2.91^1	49.90 ± 3.15^2	66.84 ± 3.06^3	80.03 ± 2.19^4	72.45 ± 2.29^1	37.64 ± 4.89^2	56.80 ± 3.49^3
Total progressive motile spermatozoa %	38.33 ± 8.58^1	22.88 ± 2.79^1	6.38 ± 1.26^2	1.12 ± 0.31^3	5.12 ± 1.11^1	37.18 ± 11.15^2	20.05 ± 4.29^2
Normal Sperm Morphology %	4.19 ± 0.09^1	2.86 ± 0.17^2	1.96 ± 0.22^3	1.09 ± 0.07^4	1.59 ± 0.13^1	3.97 ± 0.32^2	2.50 ± 0.22^3
SCI %	24.29 ± 4.12^{NS}	24.63 ± 3.38^{NS}	28.60 ± 3.55^{NS}	29.68 ± 3.19^{NS}	28.21± 2.29^{NS}	26.82 ± 6.55^{NS}	26.43 ± 3.40^{NS}

Table 5: Comparison of semen sample characteristics based on semen quality and infertility factors. Different superscript numbers within a row indicate significant differences between groups, while NS denotes non-significant differences

DISCUSSION

The findings highlight the importance of timely infertility treatment, as many couples abandon it over time, emphasizing the need for time-sensitive interventions.

The mean BMI for males was 28.13 ± 0.55 kg/m², and for females, 26.91 ± 0.49 kg/m², slightly higher than previous reports [1]. Obesity may impair male fertility through mechanisms such as endocrinopathy and increased aromatization [2]. The mean infertility duration was 7.35 ± 0.51 years, with 83.7% of couples experiencing primary infertility, consistent with prior studies reporting rates of 77.3– 79.6% [1,11]. Male factor infertility was the most common cause (57.6%), followed by mixed factors (27.2%), female factor infertility (11.9%), and unexplained infertility (3.3%). Smoking, a known risk factor for male subfertility [12], was more prevalent in this study (43.5%) than reported by Alshahrani *et al.* (2014) (21.7%). A meta-analysis also linked smoking to reduced sperm counts [13].

Sperm chromatin immaturity was evaluated as an indicator of sperm DNA integrity. Among participants, 37 (40%) had normal DNA integrity, while 55 (60%) exhibited abnormalities. DNA fragmentation, reflecting spermatogenesis quality [14], was 27.41 ± 1.77% in this study, falling between the values reported by Zeqiraj *et al.* (2018) for infertile (34.53 ± 4.68%) and fertile men (14.91 ± 4.02%) [15]. Broader abnormalities included in this study may explain these differences. Prior research consistently demonstrates higher DNA fragmentation in infertile men [16– 18], highlighting its role as a diagnostic marker for male infertility. Collectively, these findings support incorporating the sperm DNA fragmentation index into male infertility assessments, as previously suggested [17]. DNA fragmentation functions as an independent factor influencing male fertility, emphasizing the critical role of maintaining DNA integrity for successful reproduction [19].

According to seminal fluid analysis, infertile men in this study were categorized into four groups: 14 normozoospermic samples, 21 with single defects, 25 with double defects, and 32 with OAT samples. The findings demonstrated interactions among levels of DNA fragmentation (DFI), levels of chromatin maturity (SCI), and morphological features of sperm. The findings of the current study demonstrated no statistically significant correlation among sperm DFI, fertilization success, or pregnancy outcome, being in accordance with Sun *et al.*'s (2018) findings depicting similar ICSI outcomes irrespective of levels of DFI (≥20% vs <20%) for fertilization, clinical pregnancy, and ongoing pregnancy rates [20]. This observation was further alleviated by a longitudinal cohort study that found no meaningful correlation among levels of DFI and ICSI outcomes in terms of fertility- related outcomes [21]. Paradoxically, however, our findings

suggest that DNA fragmentation might not impinge negatively upon ICSI success, yet conflicting evidence can be found in the literature. Zhu *et al.* (2020), for example, reported a correlation among high levels of DFI and recurrent pregnancy loss of unexplained aetiology [22], while Yang *et al.* (2019) described associations among elevated levels of DFI with pregnancy loss in early-stage intrauterine insemination, though in ICSI cases not in terms of any correlations [23]. These discrepancies can most likely be seen as inherent to the distinct aspects of ICSI protocols, viz., strict selection of morphologically normal sperm with limited DNA damage as well as preferential transfer of high-grade embryos [24]. Differences in outcome parameters (e.g., levels of β-HCG identification vs. maintenance rates of pregnancy) can also serve to explain these discrepancies. Simon *et al.* (2013), for instance, reported significantly lower rates of live births in standard IVF cycles among couples with high sperm DNA fragmentation, in contrast with unaltered ICSI outcomes [25]. This trend was substantiated by a systematic meta-analysis reporting compromised live birth rates in IVF but not in ICSI treatments [26]. Two possible reasons arise in mind to explain such discrepancies: (1) The fact that ICSI typically involves a generally younger female cohort who can have superior repair competence in their oocytes, and (2) The fact that IVF's prolonged gamete co-incubation can provoke oxidative stress, which ICSI avoids [27]. During ICSI, embryologists select spermatozoa with the most normal morphology for injection, which is associated with lower DNA fragmentation compared to abnormal spermatozoa [24]. Sperm DNA fragmentation levels can guide clinicians in optimizing assisted reproductive techniques (ART) [15]. Abnormal chromatin structures negatively impact ART outcomes, underscoring the importance of sperm chromatin integrity. This study highlights that protamine deficiency may impair embryo quality, suggesting improved methods for selecting sperm with intact chromatin. However, factors such as pre-ICSI processing and sperm selection based on morphology and motility can influence the evaluation of sperm chromatin status [28]. Assessing DNA integrity alongside morphology may enhance sperm selection for ICSI, as supported by previous studies [29,30]. High-magnification sperm selection (>6000×) has shown better clinical outcomes than conventional ICSI, with intracytoplasmic morphologically selected sperm injection (IMSI) reducing the risk of low fertilization rates [31,32].

In this study, weak and non-significant negative correlations were observed between SCI% and both fertilization rate and embryo grade 1, while a positive association was found with embryo grades 2 and 3. Sperm DNA fragmentation may activate DNA damage repair mechanisms in embryos to address paternal DNA damage [33]. These mechanisms, also active in somatic cells, appear to slow DNA replication and embryo development [34]. Sperm DNA damage has been linked to delays in pronuclei formation, first cleavage, and early embryo development, suggesting that zygotes repair chromatin damage at early stages [35–37]. Such mechanisms may explain delayed zygote formation and development observed with high sperm DNA fragmentation [38]. High levels of sperm DNA damage in this study were associated with increased embryo arrest. Previous studies link extensive DNA damage to embryo arrest after ICSI, likely due to impaired DNA replication and activation of S-phase or spindle-assembly checkpoints, which delay chromatid separation [39]. Additionally, apoptosis may be triggered at early blastocyst stages, arresting embryos with significant chromosomal damage [40]. These findings align with studies indicating that sperm DNA fragmentation significantly impacts ART outcomes, affecting embryo quality, pregnancy rates, miscarriages, live births, and fertilization success [40].

Sperm chromatin immaturity (SCI%) did not exhibit significant differences among infertile men categorized by seminal fluid analysis parameters or between infertility factor groups (male, female, and mixed factors). However, SCI% showed a positive correlation with grade 2 and grade 3 embryos, as well as with abnormal embryo division. These findings highlight the potential role of SCI% in influencing specific embryo development outcomes during ICSI. Future studies should explore advanced sperm selection techniques to mitigate the impact of DNA fragmentation on embryo quality. Limitations of this study include the absence of a long-term follow-up on clinical outcomes, such as live birth rates, and the potential influence of unexamined confounding factors.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest regarding the publication of this article.

AUTHOR CONTRIBUTIONS

Doaa Adnan Ajam, Amal Abdulwahid Mohammed, and Muayad Sraibet Abbood conceptualized, supervised, and reviewed the article. The authors performed the literature search and provided

valuable input during the manuscript writing process.

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