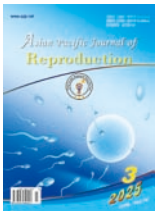

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DNA fragmentation and chromatin denaturation in various sperm categories: A prospective cohort study

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ABSTRACT

Objective: To evaluate how DNA fragmentation index (DFI) and chromatin denaturation index (CDI) relate to semen parameters across different types of male infertility, thereby improving the understanding and assessment of sperm quality.

Methods: A prospective and descriptive cohort study was conducted over two years at the Integrated Physiology Laboratory of the University of Carthage in collaboration with the Alyssa Fertility Group, Tunisia. A total of 163 participants were classified into five groups based on their semen parameters: normozoospermia, oligozoospermia, asthenozoospermia, teratozoospermia, and oligo-astheno-teratozoospermia. The normozoospermia group was selected from volunteers who had children. Semen samples were analyzed according to WHO guidelines. DFI was measured using Halosperm® and CDI was tested using aniline blue staining.

Results: Both DFI and CDI were significantly higher in all infertility groups, with the oligozoospermia group showing the highest DFI and CDI. Negative correlations were found between DFI/CDI and sperm motility, concentration, and morphology in the affected groups. The normozoospermia group served as a control with the lowest DFI and CDI values.

Conclusions: DFI and CDI are increasingly recognized as important biomarkers for evaluating sperm quality in cases of male infertility. Their elevated levels in patients with oligozoospermia, asthenozoospermia, teratozoospermia, and oligo-astheno-teratozoospermia underscore their potential role in not only diagnosing male infertility but also in assessing the overall reproductive outcomes for affected individuals, thus guiding more effective treatment strategies.

KEYSWORDS: Male infertility; DNA fragmentation index; Chromatin denaturation index; Sperm parameters; Semen analysis

1. Introduction

Infertility has been recognized as a real public health issue worldwide by the World Health Organization (WHO)[1]. In 2009, the WHO collaborating with other partners, provided a clinical definition of infertility as "a disease of the reproductive system

Key Points

Question: How do DNA fragmentation (DFI) and chromatin denaturation (CDI) relate to sperm quality and fertility outcomes in men with different sperm parameters (normospermia, oligospermia, asthenospermia, teratospermia, and oligo-astheno-teratospermia)?

Findings: In this cohort study, men with abnormal sperm parameters exhibited significantly higher levels of DNA fragmentation and chromatin denaturation compared to the normospermic group.

Meaning: These findings suggest that assessing sperm DNA integrity through DFI and CDI provides critical complementary information to conventional semen analysis, thereby enhancing the diagnostic accuracy of male fertility evaluations.

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defined by the failure of a clinical pregnancy after 12 months or more of regular, unprotected sexual relations"[2].

There are several possible causes of infertility affecting male and female or both. Male infertility contributes to up to 50% of cases in infertile couples[3]. Assessing male fertility is generally less complex than evaluating female fertility. A semen analysis is the initial examination conducted for couples seeking consultation regarding fertility issues. This analysis allows for the exploration of various sperm parameters[4]. However, the sperm profile for each man may not fully explain some issues of male infertility[5]. Additional tests, such as sperm DNA fragmentation and sperm chromatin maturity assessments, are useful in identifying potential causes of infertility[6].

Intact sperm DNA is essential for the transmission of genetic material to the next generation and is crucial for normal embryo development[7]. Although many studies have investigated the role of sperm DNA fragmentation in assisted reproductive technology (ART), there is no consensus on its clinical utility[8].

During spermiogenesis, the replacement of histones with protamine is essential for the compaction state of sperm chromatin, which helps protect the paternal genome as the sperm travels through the male and female reproductive tracts. Abnormal chromatin condensation can lead to DNA alterations such as denaturation and/or fragmentation. Sperm DNA damage is more common among infertile men and may contribute to poor reproductive outcomes[9].

Several studies on sperm DNA integrity have shown a significant correlation between DNA damage and pregnancy outcomes[10]. Fertile men with normal sperm parameters typically have high DNA integrity, whereas infertile men, particularly those with abnormal sperm parameters, often exhibit decreased DNA integrity. Moreover, many infertile men may have a compromised DNA integrity even if their sperm parameters are normal[11]. The link between DNA damage and reduced reproductive outcomes has led to the incorporation of sperm DNA integrity tests into the biological evaluation of male fertility.

The aim of our study is to determine the correlation of the DNA fragmentation index (DFI) and chromatin denaturation index (CDI) with semen parameters in patients with normozoospermia, oligozoospermia, asthenozoospermia, or teratozoospermia. These techniques are expected to be highly useful in the future for assessing sperm quality and help diagnose potential issues, particularly in the case of male infertility.

2. Methods

2.1. Study design

A prospective and descriptive cohort study was conducted from January 2021 to January 2023 at the Integrated Physiology Laboratory (LR17ES02) at the Faculty of Sciences of Bizerte (University of Carthage, Tunisia) in collaboration with the Assisted Reproductive Technology Center 'Alyssa Fertility Group' (A.F.G) at the Alyssa Clinic (Les Berges du Lac–Tunis).

2.2. Participants selection

A total of 163 male patients presented for infertility investigations or were eligible for an ART attempt. All had experienced a period of infertility exceeding one year. The recruitment strictly focused on male infertility; all cases of female infertility or female partners aged over 38 years were excluded.

After applying the inclusion and exclusion criteria, 163 patients were retained and classified into five groups according to the WHO 2021 semen parameter thresholds[4]: (1) Normozoospermia (n=39): Sperm concentration ≥15 million/mL, progressive motility ≥42%, normal morphology ≥4%; (2) Oligozoospermia (n=36): Sperm concentration <15 million/mL; (3) Asthenozoospermia (n=33): Progressive motility <42%; (4) Teratozoospermia (n=28): Normal morphology <4%; (5) Oligo-astheno-teratozoospermia (n=27): Combined alteration of all three parameters.

For the normozoospermia group, we selected male volunteers who had children in the year prior to the study. In our study, the normozoospermia group was considered as the control group. Exclusion criteria for men included a history of serious previous or concurrent illnesses, as well as having medications or antioxidant treatments in the last three months preceding the study. Additionally, patients suffering from azoospermia, urogenital infection, or a febrile condition were excluded. The flowchart of participants screening and group classification is presented in Figure 1.

The sample size for this study was determined using a statistical power analysis to ensure sufficient power to detect a meaningful effect size. Additionally, the inclusion criteria for participant selection, such as age, infertility status, and sperm quality parameters, were considered in determining the final sample size. A sample size calculation was performed to balance statistical reliability with practical constraints, such as time and resources. In some cases, a minimum number of participants per group was chosen based on standard recommendations for similar studies or the availability of subjects.

2.3. Sample collection and semen analysis

Spermograms were performed and analyzed according to WHO guidelines[4,12]. Sperm count was conducted using a semi-automated method with the Sperm-Class-Analyzer software (SCA5/6CASA system: Computer-Assisted Sperm-Analysis) and SCA-scope (Microptic®). All patients underwent a thorough examination of their sperm characteristics, with one or two sperm analyses conducted after sexual abstinence of two to three days.

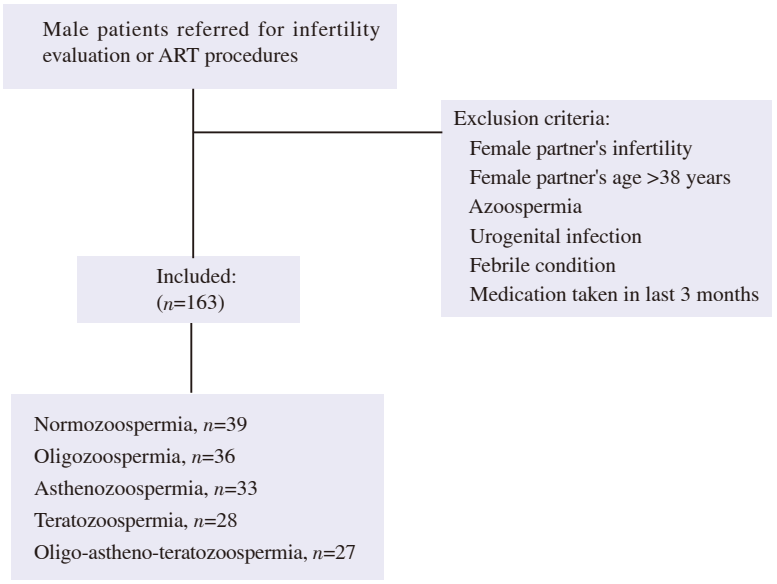

Figure 1. Flowchart of participants screening and group classification. Semen classification following WHO reference values. ART: assisted reproductive technology.

For sperm motility, following WHO recommendations[4], we used a classification system divided into four grades: Grade (a): Rapid progressive motility in a straight line (speed >25 μm/s), Grade (b): Slow progressive motility (speed of 5-25 μm/s), Grade (c): Non-progressive motility or slight movement, Grade (d): Immotile sperm. Sperm morphology was analyzed descriptively. For the head morphology, two main abnormalities were identified: microcephalic and macrocephalic head. For the intermediate piece morphology, defects such as asymmetry, thickening, bulging, irregularity, or the presence of residual cytoplasm were grouped. In terms of flagellum alterations, abnormalities included short, angular, coiled, double, or multiple flagella, as well as cases where spermatozoa were flagellum-free.

2.4. Evaluation of DFI

DNA fragmentation in all semen samples was analyzed using the Halosperm® HT-HS10 kit. Halosperm® is based the sperm chromatin dispersion (SCD) technique, developed by Halotech (Halotech DNA, Madrid, Spain) involving a controlled DNA denaturation to facilitate the removal of proteins within each spermatozoon. In sperm with intact DNA, this process results in the formation of halos, created by DNA loops around the sperm head. However, sperm with damaged DNA fails to form these halos, indicating DNA fragmentation.

The proportion of sperm without halos to the total number of sperm was calculated to determine the DFI using a selection of 200 or more spermatozoa.

DFI (%) = 100 × Number of spermatozoa without halos/Total number of spermatozoa counted.

2.5. Sperm chromatin denaturation assay

Aniline blue is an acidic dye used to evaluate the maturity of sperm chromatin by staining the sperm heads. This dye has a high affinity for histones rich in lysine present in the nuclei of immature spermatozoa, which stain blue, while the nuclei rich in protamine of mature spermatozoa remain uncolored[13]. The chromatin condensation of spermatozoa was assessed using aniline blue staining, following the method described by Terquem and Dadoune[13].

A drop of semen was smeared on a glass slide and air-dried. All smears were then fixed in a 3% buffered glutaraldehyde solution for 30 min. The slides were subsequently stained with a mixture of 5% of aniline blue in water and 4% acetic acid (pH 3.5) for 7 min.

Two types of staining intensity were observed in the sperm heads: unstained are considered normal (mature chromatin) and those blue stained were considered abnormal (immature chromatin). The percentage of stained sperm heads was calculated to determine the rate of sperm with denatured chromatin, known as the CDI.

2.6. Statistical analysis

Statistical analyses were performed using SPSS version 25.0 for Windows. The Kolmogorov–Smirnov test was employed to assess normality of the data. For normally distributed data, comparisons between groups were made using one-way analysis of variance (ANOVA) followed by Tukey's *post–hoc* multiple comparison test (*F* test), and data are expressed as mean±standard deviation (mean±SD). For non-normally distributed data, the Kruskal-Wallis test followed by Dunn's *post–hoc* test was used to compare differences between

groups, followed by Dunn's *post-hoc* test for pairwise comparisons, and data are expressed as median (interquartile ranges) (IQR). Spearman correlation test is used for the correlation between DFI and CDI. A *P*-value <0.05 was considered statistically significant.

2.7. Ethics statement

This study complies with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008[14]. This study was validated by the ethics committee of Alyssa Clinic (Ref: AFG/MBR/11/20) on November 13, 2020, and informed consent was obtained and signed by all study participants.

3. Results

3.1. Demographic and clinical profiles of patients

The clinical and demographic data of male patients from the different study groups are presented in Table 1. The normozoospermia group included 39 patients with a mean age of (36.4±3.2) years and a body mass index (BMI) of (23.2±3.2) kg/m². The oligozoospermia group included 36 patients with a mean age of (36.9±3.9) years and a BMI of (23.5±3.6) kg/m², with a duration of infertility of (6.1±2.3) years. The asthenozoospermia group included 33 patients with a mean age of (36.6±4.1) years and a BMI of (25.1±4.0) kg/m², with a duration of infertility of (7.2±2.5) years. The teratozoospermia group included 28 patients with a mean age of (37.1±2.9) years and a BMI of (26.2±3.3) kg/m², with a duration of infertility of (7.9±3.0) years. The oligo-astheno-teratozoospermia group included 27 patients with a mean age of (37.5±3.5) years and a BMI of (25.6±6.4) kg/m², with a duration of infertility of (7.3±2.5) years. In all groups except the normozoospermia group, the infertility was of primary type and attributed to a male factor.

3.2. Results of analysis of sperm parameters

No significant differences were observed in ejaculate volume (mL), semen pH, and liquefaction time (minutes) among the different groups. However, high semen viscosity was observed exclusively in the asthenozoospermia and oligo-astheno-teratozoospermia groups (Table 2).

Sperm concentration per milliliter showed a marked decline in all infertile groups compared to the normozoospermia group. The median (IQR) of sperm concentrations were as follows: 83.09 (67.38)×10⁶/mL for the normozoospermia group, 8.31 (7.57)×10⁶/mL for the oligozoospermia group, 16.53 (12.98)×10⁶/mL for the asthenozoospermia group, 16.21 (6.30)×10⁶/mL for the teratozoospermia group, and 2.16 (2.56)×10⁶/mL for the oligo-astheno-teratozoospermia group. These differences were statistically significant (P<0.001) compared to the normozoospermia group.

Similarly, the total sperm concentration per ejaculate decreased significantly across the infertile groups, with median (IQR) values of 224.23 (129.16)×10⁶/ejaculate in the normozoospermia group and 4.60 (7.94)×10⁶/ejaculate in the oligo-astheno-teratozoospermia group (P<0.001).

Sperm vitality assessed one-hour post-ejaculation, was significantly lower in all infertile groups compared to the normozoospermia group. Mean±SD values were (84.16±8.08)% in the normozoospermia group, dropping to (58.65±11.43)% in the oligo-astheno-teratozoospermia group (P<0.001).

Leukocyte counts did not differ significantly between groups, with no cases of leukospermia detected. Median (IQR) leukocyte concentrations ranged from 0.3 (0.6)×10⁶/mL to 0.5 (0.5)×10⁶/mL across the studied groups (Table 2).

Table 1. Clinical and demographic data of male patients.

Characteristics	Normozoospermia (n=39)	Oligozoospermia (n=36)	Asthenozoospermia (n=33)	Teratozoospermia (n=28)	Oligo-astheno-teratozoospermia (n=27)
Age, years	36.4±3.2	36.9±3.9	36.6±4.1	37.1±2.9	37.5±3.5
BMI, kg/m²	23.2±3.2	23.5±3.6	25.1±4.0	26.2±3.3	25.6±6.4
Duration of infertility, years	-	6.1±2.3	7.2±2.5	7.9±3.0	7.3±2.5

Data are expressed as mean±SD. BMI: body mass index.

Table 2. Various seminal parameters of patients.

Parameters	Normozoospermia (n=39)	Oligozoospermia (n=36)	Asthenozoospermia (n=33)	Teratozoospermia (n=28)	Oligo-astheno-teratozoospermia (n=27)
Ejaculate volume, mL	3.08±0.98	3.12±1.44	2.98±1.11	2.91±1.11	2.95±1.35
Semen pH	7.35±0.15	7.25±0.21	7.33±0.19	7.19±0.14	7.12±0.18
Abstinence, days	3.10±1.15	3.17±1.31	3.02±1.48	3.21±1.63	3.28±1.59
Liquefaction time, minutes	29.33±11.49	29.10±12.22	29.30±11.18	30.01±12.10	29.24±12.23
Sperm concentration[#], 10⁶/mL	83.09 (67.38)	8.31 (7.57)[*]	16.53 (12.98)[*]	16.21 (6.30)[*]	2.16 (2.56)[*]
Total sperm concentration[#], 10⁶/ejaculate	224.23 (129.16)	25.17 (28.28)[*]	50.07 (63.88)[*]	45.21 (36.2)[*]	4.60 (7.94)[*]
Vitality at 1 hour, % alive	84.16±8.08	64.94±8.77[*]	61.19±9.91[*]	63.59±10.62[*]	58.65±11.43[*]
Leukocytes[#], ×10⁶/mL	0.4 (0.6)	0.4 (0.5)	0.3 (0.6)	0.4 (0.7)	0.5 (0.5)

Data are expressed as mean±SD, analysis of variance (ANOVA) (F-test) followed by Tukey's *post hoc* test. [#]Median (IQR), the Kruskal-Wallis test followed by Dunn's *post-hoc* test. [*]P<0.001 compared to the normozoospermia group.

Table 3. Percentage of different grades of sperm motility.

Parameters	Normozoospermia (*n*=39)	Oligozoospermia (*n*=36)	Asthenozoospermia (*n*=33)	Teratozoospermia (*n*=28)	Oligo-astheno-teratozoospermia (*n*=27)
Total motility (Grade a + b + c)	78.08±7.34	48.03±19.22*	23.69±7.05*	31.15±4.93*	21.17±6.08*
Rapid progressive motility (Grade a)	38.31±9.85	28.86±38.53*	5.99±2.82*	6.19±3.07*	6.13±3.41*
Slow progressive motility (Grade b)	26.35±7.21	11.35±4.65*	13.04±6.42*	16.51±8.20*	6.39±4.74*
Progressive motility (Grade a + b)	58.31±8.62	40.43±12.91*	18.27±7.52*	22.95±10.40*	11.51±4.58*
Non-progressive motility (Grade c)	16.59±5.70	8.91±1.20*	12.93±5.65*	12.51±3.93*	12.26±5.25*
Immotile (Grade d)	29.50±8.70	50.89±44.37*	72.26±10.15*	63.69±10.41*	78.14±9.90*

Data are expressed as mean±SD, analysis of variance (ANOVA) (*F*-test) followed by Tukey's *post hoc* test. *P<0.05 compared to the normozoospermia group. Grade (a): Rapid progressive motility in a linear path (speed > 25 μm/s), Grade (b): Slow progressive motility (speed of 5-25 μm/s), Grade (c): Non-progressive or stationary motility, Grade (d): Immotile spermatozoa.

Table 4. Morphological values of spermatozoa.

Sperm morphology	Normozoospermia (*n*=39)	Oligozoospermia (*n*=36)	Asthenozoospermia (*n*=33)	Teratozoospermia (*n*=28)	Oligo-astheno-teratozoospermia (*n*=27)
Head size					
Normal	86.26±9.41	88.08±5.65	84.77±14.85	72.42±9.39*	71.80±7.15*
Microcephalic#	2.11 (2.52)	4.08 (11.43)	5.28 (7.50)*	5.00 (9.43)*	2.11 (12.50)
Macrocephalic#	6.26 (13.56)	5.00 (9.00)	4.00 (11.00)	11.00 (7.95)	12.00 (11.62)*
Head shape#					
Normal	15.00 (9.77)	11.00 (6.43)	8.94 (12.46)*	6.00 (4.57)*	6.00 (5.28)*
Rond	2.11 (2.47)	9.28 (9.24)*	7.29 (9.25)*	8.57 (6.50)*	10.00 (10.00)*
Thin/Elongated	1.02 (4.67)	2.00 (3.00)	3.61 (4.67)	10.00 (9.47)*	10.00 (15.00)*
Midpiece					
Normal	70.67±7.37	66.78±9.71*	68.42±6.28	62.93±5.64*	64.38±5.05*
Abnormal/Angulation#	12.00 (1.00)	11.21 (1.10)	11.00 (2.00)	14.29 (13.95)	12.16 (10.75)
Flagellum type					
Normal	88.71±7.96	77.71±14.75*	72.30±12.08*	71.95±12.77*	75.47±9.16*
Short#	0.00 (1.00)	0.00 (3.00)	1.00 (1.86)	2.06 (4.50)	0 .00 (4.50)
Coiled/Angular/ Multiple#	1.00 (5.00)	3.00 (5.02)	6.00 (10.00)*	5.00 (5.60)	4.52 (4.00)*
Absent#	0.00 (1.00)	0.00 (1.10)	0.00 (2.00)	0.00 (1.50)	0.50 (3.00)

Data are expressed as mean±SD, analysis of variance (ANOVA) (*F*-test) followed by Tukey's *post hoc* test. #Median (IQR), the Kruskal-Wallis test followed by Dunn's post-hoc test. *P<0.05 compared to the normozoospermia group.

3.3. Sperm motility and morphology assessment outcomes

Analysis of sperm motility demonstrated a significant reduction in both total motility (Grades a + b + c) and progressive motility (Grades a + b) in the oligozoospermia, asthenozoospermia, teratozoospermia, and oligo-astheno-teratozoospermia groups compared to the normozoospermia group (P<0.05). This decline was accompanied by a significant increase in the proportion of immotile spermatozoa (Grade d) in these groups (Table 3).

Regarding sperm morphology, the teratozoospermia and oligo-astheno-teratozoospermia groups showed a significant reduction in normal-sized sperm heads compared to the normozoospermia group (P<0.05). A significant increase in microcephalic sperm head in the asthenozoospermia and teratozoospermia groups (P<0.05) and also a significant increase in macrocephalic sperm head in the oligo-astheno-teratozoospermia group (P<0.05), when compared to the normozoospermia group. A similar trend was for abnormal flagellum shapes (Table 4).

3.4. Results of analysis of sperm DFI and CDI

The DFI was significantly higher in the oligozoospermia [(27.24±4.45)%], asthenozoospermia [(25.25±8.01)%], teratozoospermia [(22.11±7.57)%], and oligo-astheno-teratozoospermia [(22.27±6.70)%] groups compared to the normozoospermia group [(12.14±3.88)%] (P<0.001). Furthermore, significant differences were also observed between the oligozoospermia group and both the teratozoospermia and oligo-astheno-teratozoospermia groups (P<0.01) (Table 5).

The results of the CDI reflected the results of the DFI. The CDI in the normozoospermia control group was (13.01±4.31)% and it significantly increased in the other groups (P<0.001), reaching a maximum value of (26.16±6.66)% in the oligozoospermia group (Table 5).

The correlations between the DFI and various sperm parameters were evaluated. Regarding total motility, no significant correlation was found for the normozoospermia group (r=0.179, P=0.282), while a significant negative correlations were observed in the oligozoospermia (r=-0.659, P<0.01), asthenozoospermia (r=-0.758, P<0.05), teratozoospermia (r=-0.696, P<0.01), and oligo-astheno-teratozoospermia groups (r=-0.755, P<0.01). Concerning sperm concentration, significant negative correlations were found among all groups, with the strongest being in the teratozoospermia group (r=-0.808, P<0.01). Regarding sperm morphology, significant negative correlations were found in the normozoospermia, oligozoospermia, and asthenozoospermia groups. Conversely, a

Table 5. Sperm DNA fragmentation index and chromatin denaturation index.

Parameters	Normozoospermia (*n*=39)	Oligozoospermia (*n*=36)	Asthenozoospermia (*n*=33)	Teratozoospermia (*n*=28)	Oligo-astheno-teratozoospermia (*n*=27)
DNA fragmentation index (DFI), %	12.14±3.88	27.24±4.45*	25.25±8.01*	22.11±7.57*¥	22.27±6.70*¥
Chromatin denaturation index (CDI), %	13.01±4.31	26.16±6.66*	25.13±5.73*	24.53±5.85*	25.81±6.47*

Data are expressed as mean±SD, analysis of variance (ANOVA) (*F*-test) followed by Tukey's *post hoc* test. *P<0.001 compared to the normozoospermia group. ¥P<0.01: compared to the oligozoospermia group.

Table 6. Correlation between DNA fragmentation index (DFI) and chromatin denaturation index (CDI).

Parameters		Normozoospermia (*n*=39)	Oligozoospermia (*n*=36)	Asthenozoospermia (*n*=33)	Teratozoospermia (*n*=28)	Oligo-astheno-teratozoospermia (*n*=27)
Total motility of spermatozoa	DFI	0.179	-0.659*	-0.758#	-0.696*	-0.755*
	CDI	0.157	-0.735*	-0.760#	-0.718*	-0.780*
Progressive motility of spermatozoa	DFI	-0.148	-0.662*	-0.801*	-0.606*	-0.372#
	CDI	-0.054	-0.731*	-0.818#	-0.707*	-0.724*
Sperm concentration	DFI	-0.448#	-0.698*	-0.697*	-0.808*	-0.604*
	CDI	-0.632#	-0.740*	-0.864*	-0.400#	-0.202*
Sperm morphology	DFI	-0.247#	-0.285*	-0.565*	0.491*	0.043
	CDI	-0.190	-0.292#	-0.562#	0.350#	0.037

Data are the *r* values. Spearman correlation test is used. *P<0.01, #P<0.05.

significant positive correlation was observed in the teratozoospermia group, while no significant correlation was found in the oligo-astheno-teratozoospermic group (Table 6).

The correlations between the CDI and sperm parameters showed a significant negative correlation for total motility in the oligozoospermia (r=-0.735, P<0.01), asthenozoospermia (r=-0.760, P<0.05), teratozoospermia (r=-0.718, P<0.01) and oligo-astheno-teratozoospermia groups (r=-0.780, P<0.01). Likewise, we found a significant negative correlation between sperm concentration and CDI among all groups. This correlation is highly significant (P<0.01) in oligozoospermia, asthenozoospermia and oligo-astheno-teratozoospermia groups, and significant (P<0.05) in normozoospermia and teratozoospermia groups (Table 6).

A significant correlation between sperm morphology and the CDI was observed in the oligozoospermia (r=-0.292, P<0.05), asthenozoospermia (r=-0.562, P<0.05), and teratozoospermia groups (r=0.350, P<0.05). No significant correlation was found in normozoospermia (r=-0.190, P=0.09) and oligo-astheno-teratozoospermia groups (r=0.037, P=0.793) (Table 6).

4. Discussion

The study findings highlight several important factors associated with male infertility. The variation in infertility duration, particularly in the groups with pronounced sperm abnormalities such as teratozoospermia and oligo-astheno-teratozoospermia, aligns with existing research that links more severe reproductive issues to abnormal sperm morphology (teratozoospermia) and combined disorders such as oligo-astheno-teratozoospermia[4,15].

The age range [(36.4–37.5) years] and BMI values (23.2–26.2 kg/m^2) were relatively similar across all groups, with minor variations observed in the teratozoospermia and oligo-astheno-teratozoospermia groups. The teratozoospermia group had the longest infertility duration (7.9 years), suggesting a potential correlation between abnormal sperm morphology and infertility[16]. Elevated seminal viscosity, observed in the asthenozoospermia group, might impair sperm motility. This finding is supported by studies that associates hyperviscosity with difficulty in sperm migration through the female reproductive tract[17,18].

We did not observe any significant differences among the groups in the number of leukocytes. This result suggests that immune or inflammatory processes are not major contributors to infertility in our study. However, it is important to note that leukocytospermia is a sign of infertility caused by infections and its contribution to male infertility can reach up to 30%[19]. For the other sperm parameters, no differences were observed in ejaculate volume, pH, or liquefaction time between groups.

Significant differences were observed in sperm concentration (both per mL and per ejaculate) between the groups, which is critical since low sperm counts are a major indicator of infertility, particularly in the oligozoospermia and oligo-astheno-teratozoospermia groups[3,20].

In our study, the absence of significant differences in leukocyte count, volume, and pH, along with the sperm concentration data, highlights the importance of sperm morphology and motility as primary diagnostic parameters in evaluating male infertility[20,21]. Our results show that patients with teratozoospermia and oligo-astheno-teratozoospermia exhibit significantly more abnormalities in sperm head size and shape, including fewer normal-sized heads and a higher prevalence of microcephalic and macrocephalic heads.

The significant increase in both macrocephalic and microcephalic sperm heads in these groups is consistent with previous research

indicating that abnormalities in sperm head morphology are frequently linked to chromosomal irregularities, which can impact fertilization potential and the quality of embryos[22,23]. Furthermore, the abnormal flagellum morphology observed in our study could impair motility. Similar results are reported with a particular type of astheno-teratozoospermia known as the syndrome of multiple morphological abnormalities of the sperm flagella (MMAF) which is characterized by a combination of aberrant flagellar phenotypes (absent, short, bent, coiled, and aberrant flagella)[24].

According to several studies, flagellum abnormalities like coiled or angulated shapes, reduce the sperm's ability to progress through the female reproductive tract, decreasing the chances of successful fertilization[25]. Aberrant sperm morphology is considered a critical factor in assessing male fertility because it has been associated with reduced sperm function and increased DNA fragmentation[26]. Morphological defects should therefore be considered when evaluating male infertility, as they play a significant role in both natural conception and ART outcomes[27].

The measurement of DFI in spermatozoa from the different groups studied reveals that patients with oligozoospermia, asthenozoospermia, teratozoospermia and oligo-astheno-teratozoospermia had significantly higher DFIs compared to the control group. This is consistent with previous studies that show men with compromised sperm parameters tend to have higher levels of sperm DNA damage, which can negatively affect fertility outcomes[28].

Interestingly, a significant difference was observed in DFI between the oligozoospermia group and the teratozoospermia and oligo-astheno-teratozoospermia groups, suggesting a more severe impact on DNA integrity when morphology abnormalities are involved[29].

We also observed that the percentage of spermatozoa with CDI decreases significantly across the groups, indicating a potential compromise in chromatin integrity. However, no significant differences in CDI were noted among the different pathological groups. Our findings also reveal a negative correlation between sperm concentration and CDI across all groups studied, suggesting that lower sperm concentrations are associated with higher levels of chromatin decondensation.

Many studies have emphasized the importance of chromatin decondensation in sperm quality and their fertilization potential and sperm function[7,30]. Changes in sperm chromatin structure or epigenetic markers may be responsible for some cases of infertility[9]. This relationship underscores the importance of chromatin integrity in sperm functionality and overall fertility potential.

We found that DFI was negatively correlated with sperm concentration per mL, with this correlation being highly significant across the oligozoospermia, asthenozoospermia, teratozoospermia, and oligo-astheno-teratozoospermia groups. Studies have shown that high DFI is associated with impaired spermatogenesis, leading to disturbed sperm production and lower sperm concentrations, especially in men with oligozoospermia[11,31,32]. DNA damage appears to be a common issue regardless of the type of sperm dysfunction, as evidenced by the significant correlation observed, in our study, across various groups with sperm abnormalities.

No significant correlation was found between DFI and total sperm motility or progressive motility in the control group. However, a highly significant negative correlation was observed between the DFI and both total motility and progressive motility parameters in the oligozoospermia, teratozoospermia and oligo-astheno-teratozoospermia groups. A significant negative correlation was also noted in the asthenozoospermia group.

Additionally, we found a very significant negative correlation between CDI and sperm motility across all abnormal groups, reinforcing the link between chromatin integrity and functional parameters like motility.

In populations with oligozoospermia and asthenozoospermia, the increased CDI levels correspond to diminished sperm motility, suggesting that compromised chromatin structure may negatively impact the sperm's ability to move efficiently toward the oocyte.

The significant correlation observed in the abnormal sperm population could be explained by the possibility that sperm with higher DNA fragmentation may have damaged cellular structures, such as the tail, which is essential for motility. Similar to morphology, motility also shows a positive correlation with spermatozoa that have a high level of DFI[33].

Poor sperm morphology in teratozoospermia individuals may also affect the ability of sperm to maintain motility in the presence of adequate energy reserves. This is supported by the results of Evenson *et al* who suggest that sperm with higher DFI often have structural defects that impair their function[34]. Additionally, mitochondrial dysfunction and increased production of reactive oxygen species (ROS), may affect the sperm's ability to swim[35]. The oligo-astheno-teratozoospermia group shows the strongest correlation, probably due to the combined effects of low concentration, motility, and morphology, further exacerbating the effects of DNA damage on motility.

Correlation analysis also shows a significant positive correlation between DFI and sperm morphology in the normozoospermia group, and a highly significant correlation in the oligozoospermia, asthenozoospermia, and teratozoospermia groups. However, no significant correlation was found in the oligo-astheno-teratozoospermia group. This suggests that even in normally functioning sperm, DNA integrity is important for maintaining proper sperm structure.

Research by Saleh *et al* showed that even in a fertile population, suboptimal sperm morphology could be associated with increased DNA damage[36]. Zini *et al* highlighted the importance of assessing sperm DNA integrity even in men with normal semen parameters, as high DFI can lead to issues such as reduced embryo quality or early miscarriage[11].

In men with oligozoospermia or asthenozoospermia or teratozoospermia, higher levels of DFI correlate with poor sperm morphology and motility. These results suggest the increase of DFI may affect overall fertility potential among these groups of men[28].

Our findings show a significant positive correlation between sperm morphology and CDI in the oligozoospermia, asthenozoospermia, and teratozoospermia groups, but not in normozoospermia or oligo-astheno-teratozoospermia groups.

In men with oligozoospermia and asthenozoospermia, the positive correlation between sperm morphology and CDI suggests that as sperm morphology improves, chromatin decondensation quality also increases. The chromatin decondensation rate was found to be statistically higher in spermatozoa with enlarged heads compared to those with normal-sized heads[37]. Numerous elongated spermatozoa with a low chromatin condensation and a slight increase in aneuploidy rates were observed, suggesting potential mechanisms such as meiotic non-disjunction or anomalies during spermiogenesis[38].

Under normal circumstances, sperm undergoes a complex process of nuclear remodeling during spermiogenesis, characterized by the very tight packaging and condensation of chromatin. This process ensures that the genetic material is compacted efficiently, which is crucial for proper sperm function and successful fertilization[31].

One limitation of the study is that the sample size in each group might not have been large enough to fully represent the variability in sperm parameters within the different categories. Additionally, the lack of follow-up on clinical fertility outcomes such as pregnancy or live birth rates limits the interpretation of these findings regarding reproductive success.

In conclusion, our results highlight significant links between DNA fragmentation and chromatin denaturation with various sperm parameters, which may affect fertilization and embryonic development. This suggests that traditional semen analysis, which focuses on sperm count, motility, and morphology, may not fully reflect male fertility potential. Incorporating DFI and CDI measurements could improve the diagnosis and management of male infertility by offering additional information beyond conventional parameters. Elevated levels of DFI and CDI in conditions like oligozoospermia and asthenozoospermia indicate their importance not only for diagnosis but also for predicting reproductive outcomes. Thus, integrating these biomarkers into standard infertility evaluations is crucial, as they offer valuable information often missed by conventional analyses, leading to more tailored and effective treatment approaches.

Conflict of interest statement

Authors declare that they have no conflicts of interest.

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Authors' contributions

Maroua Ben Rhouma collected the data, conducted the experiments, reviewed the literature, and prepared the manuscript. Hatem Bahri carried out the ethical procedure as well as contributed to the data collection. Mustapha Ben Khalifa contributed to the interpretation of the results and the statistical analysis. Mohsen Sakly reviewed the manuscript and coordinated the work. Khemais Ben Rhouma contributed to the overall planning and supervision of the research. Moncef Benkhalifa and Olfa Tebourbi contributed to the conception and design of the study. All contributing authors approved the final manuscript.

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