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# Detection of sperm DNA damage in male infertility patients and evaluation of Levocarnitine efficacy using sperm chromatin diffusion (SCD) and AI-DFI methods: a cross-sectional study

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## Abstract

The objective of this study was to elucidate the relationship between sperm DNA damage and sperm parameters in male infertility patients and to assess the changes in sperm DNA fragmentation index before and after treatment with Levocarnitine in patients with asthenozoospermia and oligozoospermia. The results of 508 patients' semen samples tested between August 2021 and December 2022 in our Department of Urology and Reproductive Medicine were retrospectively analyzed. The 508 patients were divided into 3 groups: normal semen group ($n=181$), asthenozoospermia group ($n=170$), and oligozoospermia group ($n=157$). Their sperm DNA integrity was evaluated using the sperm chromatin diffusion (SCD) method and an artificial Intelligence-based DNA fragmentation index (AI-DFI). The patients were divided into two groups based on the assessment of sperm DNA integrity: a sperm DNA damage group and a sperm DNA integrity group. The two groups were then compared in terms of sperm concentration, motility, viability, and the proportion of normal sperm morphology. Pearson's correlation coefficient analysis was employed to examine the relationship between sperm DNA damage and semen parameters. The results showed that sperm concentration, progressive motility, viability, and normal morphology rate were significantly lower in the DNA damaged group, and correlation analysis showed that the results of sperm DNA damage detection was negatively correlated with these semen parameters. And the DNA fragmentation index (DFI) was highest in the asthenozoospermia group, followed by the oligospermia group and the normal group, with significant differences between the groups ($20.30\pm2.85$; $18.62\pm2.42$; $12.83\pm2.13$, $P=0.01$). Treatment of patients in the group with sperm DNA damage with Levocarnitine oral solution was found to significantly improve sperm concentration, progressive motility, viability, normal morphology rate, and DFI results after its use ($t=7.265$, 5.823, 7.750, 8.737, 8.355; $P=0.03$, 0.02, 0.02, 0.03, 0.01). This study concludes that men with asthenozoospermia and oligozoospermia have a high DFI, and Levocarnitine is effective in reducing DNA damage and improving sperm quality, suggesting that Levocarnitine has potential for clinical use.

**Keywords** Sperm DNA damage, Infertility, Semen quality, Levocarnitine, DFI

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## Introduction

Infertility is defined as not being able to conceive after 12 months of regular and unprotected intercourse. It results in about 10–15% of women of reproductive age being unable to conceive and is considered a major medical problem worldwide. Of these, approximately 50% of infertility cases is caused by male factors [1]. Currently, the evaluation and detection methods for male infertility rely on traditional semen analysis. However, about 15% of men with normal semen parameters are still diagnosed with infertility. Studies have shown that conventional semen analysis provides limited information about sperm function and does not fully reflect the fertilization potential of sperm [2]. In recent years, oxidative stress, induction of programmed cell death in spermatozoa (sperm apoptosis), and damage to genetic material (DNA damage) have been identified as important causes of the development and progression of male infertility [3, 4]. Sperm DNA damage can lead to miscarriage and infertility and can affect embryo quality, resulting in birth defects [5]. Sperm DNA Fragmentation Index (DFI) detects broken fragments of DNA and can reflect the extent of chromosomal damage in sperm, disruption of DNA integrity due to various causes during sperm production and maturation. In addition, DFI testing has been repeatedly described as a prognostic parameter for male fertility in a variety of assisted reproductive technologies and medical diagnostics [6, 7]. DFI testing has gradually been applied in clinical practice as an important adjunctive test for evaluating sperm quality [8].

Levocarnitine, also known as L-carnitine, as an important energy source for sperm motility, can promote fatty acid oxidation for energy in the mitochondria during sperm motility, reduce lipid droplet formation, and increase sperm membrane fluidity [9]. Levocarnitine in the epididymal fluid is closely related to sperm motility and fertilization ability [10]. Its concentration directly affects the maturation and metabolic process of sperm, and once there is a lack of Levocarnitine in the body, sperm quality is significantly reduced, leading to infertility [11].

In view of the above, we used the sperm chromatin diffusion method (SCD) and the latest artificial Intelligence-based DNA fragmentation index (AI-DFI) assay to more accurately detect sperm DNA damage in infertile patients and to analyze the relationship between DFI and sperm parameters. DFI was also assessed in two groups of patients (asthenozoospermia and oligozoospermia) before and after treatment with Levocarnitine.

## Materials and methods

### Patients' baseline characteristics

From August 2021 to December 2022, our hospitals Andrological Laboratory Examination and Reproductive Medicine Center observed and provided treatment in a cross-sectional study of 510 individuals diagnosed with male infertility. Their age ranged from 22 to 48 years, with a mean age of 35.60 years.

Two semen samples were excluded for their nonconformity to the inclusion criteria (one for medication history and one for missed biochemical data). Next, we evaluated the semen parameters and biochemical data of 508 males. The baseline characteristics are presented in Fig. 1. Based on the results of semen analysis, the patients were divided into the normal semen group ($n=181$), asthenozoospermia group ($n=170$), and oligozoospermia group ($n=157$). The percentage or proportion of DNA damage in the three groups of men was compared by DFI (Fig. 2) [12]. Based on the results of sperm DNA integrity testing in infertile patients, 508 cases of infertility patients were divided into the sperm DNA damage group (DFI > 25%) and the sperm DNA integrity group (DFI < 25%) [13]. Sperm concentration, sperm motility, sperm vitality, and sperm morphology were compared between the two groups.

After fulfilling the eligibility criteria, we evaluated the semen parameters and biochemical data of 508 men. According to the semen analysis results of patients, the patients were divided into the normal semen group ($n=181$), asthenozoospermia group ($n=170$), and

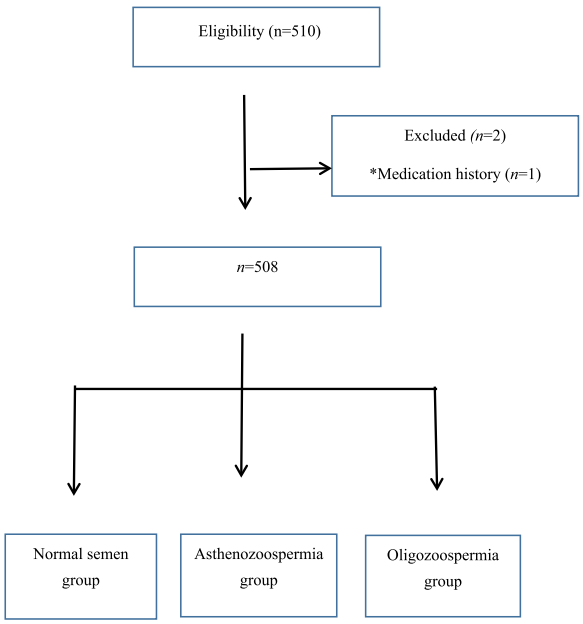


**Fig. 1** The flowchart of baseline characteristics of participants

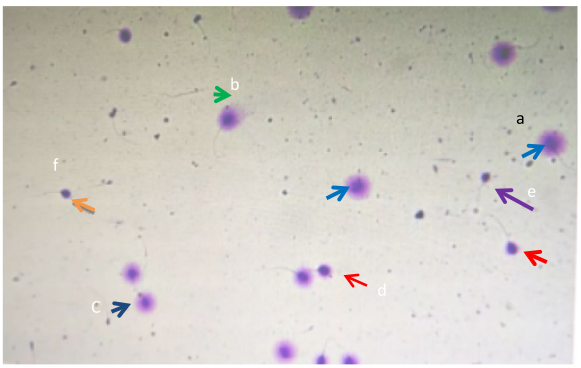


**Fig. 2** The SCD test results of a patient with severe oligozoospermia (a magnification of 100× [24]) (**a**) showing nuclei with large-sized halos, (**b**) showing nuclei with medium-sized halos, which are considered spermatozoa with non-fragmented DNA, while (**c**) showing nuclei with small-sized halos, (**d**) showing no halos, (**e**) showing no halos and degeneration, and (**f**) showing no halos and needles, which are considered as spermatozoa with fragmented DNA. SCD: sperm chromatin diffusion

oligozoospermia group ($n=157$), and the DFI of the percentage and biochemical data of males with DNA damage was compared among the three groups. DFI: DNA fragmentation index.

### Eligibility

Inclusion criteria were as follows: married male, reproductive age, couples with regular sexual life, no contraceptive measures after marriage, infertility for more than 1 year. Physical examination of all patients revealed no trauma or organic lesions of the genitourinary tract. Ultrasound examination showed no obvious abnormality of the testis, epididymis, spermatic vein, vas deferens, or prostate.

Exclusion criteria were as follows: total Sperm motility < 5%; sperm concentration < $1\times10^6$/mL; history of testicular hypoplasia; spousal infertility; leukocytospermia; presence of anti-sperm antibodies; history of endocrinopathy; environmental factors such as smoking and alcohol abuse; autoimmune disorders; history of taking any medication for infertility in the previous 2 months; antioxidant use in the 8 weeks prior to study entry; and non-cooperation with study.

### Treatment with Levocarnitine

The main ingredients of Levocarnitine oral solution are Levocarnitine, zinc and selenium-rich protein powder, and vitamin C. Patients with Sperm DNA damaged were treated with oral Levocarnitine solution (1.0 g each time, 3 times a day) for 3 consecutive months (Manufacturer: Northeast Pharmaceutical Group, Shenyang No.1 Pharmaceutical Co., LTD, National Drug Approval number: H19990372) [14, 15].

### Ethics

The present research received ethical approval from the Ethics Committee of the Obstetrics and Gynecology Hospital affiliated with Nanjing Medical University, adhering to the principles outlined in the Declaration of Helsinki. All participants provided written informed consent before participating in the study. This research was conducted in strict accordance with the relevant guidelines of the local institute.

### Semen collection

Semen specimens were acquired through masturbation after 2 to 7 days of abstaining from ejaculation, in accordance with the guidelines presented in the World Health Organization(WHO) Laboratory Manual for the Examination and Processing of Human Semen, 6th edition, and the WHO Manual for the Standardized Investigation, Diagnosis, and Management of Male Infertility (WHO, 2021) [16, 17]. Each sample was carefully directed into a sterile plastic container and placed in a thermostat at 37 °C for 20 to 30 min to allow complete liquefaction. A comprehensive analysis was then performed, including routine semen examination, assessment of sperm parameters, and DNA fragmentation analysis.

### Routine semen analysis

Based on the guidelines provided in the 6th edition of the WHO Laboratory Manual for the Examination and Processing of Human Semen and the WHO Manual for the Standardized Investigation, Diagnosis, and Management of Male Infertility [18], a semen quality detection system (CFT-9201, Jiangsu Ruiqi Life Science Instrument Co., LTD.) and appropriate reagents were employed to conduct routine semen analysis. The key parameters used in the analysis were as follows: a frame rate of 20 Hz for capturing images of low and medium sperm concentrations, while high sperm concentration images were captured at 7 Hz; the acquisition interval was 3 ms; a maximum velocity for sperm motility set at 200 μm $s^{-1}$; and detection of spermatozoa head area ranging from 7 to 60 $\mu m^2$. The evaluation of sperm motility was based on straight line velocity (VSL) and grayscale thresholds were established to isolate spermatozoa and exclude non-sperm granules. By applying the predetermined thresholds for sperm analysis, images of sperm were obtained and subjected to further analysis.

### Normal parameters

Semen volume ≥ 1.4 mL; pH ≥ 7.2; liquefaction time < 60 min; total number of sperm per

ejaculation $\geq 39\times10^6$; sperm concentration $\geq 16\times10^6$/mL; sperm vitality $\geq 54\%$; percentage of morphologically normal sperm $\geq 4\%$ [18]; and sperm with normal progressive motility $\geq 30\%$ [18].

#### Parameters of asthenozoospermia and oligozoospermia

According to the 6th edition of the WHO manual guidelines, asthenozoospermia was defined when progressive motility $< 30\%$ or total motility $< 42\%$ [19, 20] and oligozoospermia was defined as a total sperm count $< 39\times10^6$ or sperm concentration $< 16\times10^6$/mL [19, 20].

#### Sperm morphology assessment

For morphological evaluation, semen smears were stained with Diff-Quik (MICROPTIC S.L. Co., Barcelona, Spain) [21]. Approximately 10 μL of semen was smeared into a uniform thin layer on a clean glass slide and air-dried at room temperature for at least 10 min. The slides were then immersed in each kit solution in turn for 10–20 s, and then rapidly dipped in water to remove excess dye. The prepared slides were left to naturally dry and subsequently examined using a bright-field microscope (BH-2; Olympus, Tokyo, Japan) at 1000× magnification. A minimum of 200 spermatozoa (or the entire population if less than 200 were present on the slide) were enumerated using a double-blinded approach for each semen sample. The proportion of spermatozoa with normal morphology was then calculated [11]. Spermatozoa exhibiting abnormalities in the head, midpiece, or principal piece were classified as morphologically aberrant. The Sperm Deformity Index (SDI) was calculated by dividing the count of abnormal spermatozoa by the total number of spermatozoa observed [21].

#### Detection of sperm reactive oxygen species (ROS)

Luminol was used as the probe to detect the products. After being washed by PBS, the sperm concentration was adjusted to $2\times10^7$/mL. 10 μL luminol dissolved in dimethyl sulfoxide (DMSO) (5 mmol/L) was added to the sperm suspension (400 μL) and the fluorescence value was recorded immediately. Another 5 μL of luminol was added into 400 μL PBS solution as blank control. A suspension of 25 mL $H_2O_2$ mixed with 10 μL luminol was used as a positive control. The fluorescence signal was measured using a fluorescence spectrophotometer for 15 min. The result was presented in relative fluorescence unit [22].

#### SCD test (sperm chromatin dispersion test)

SpermFuncTM DNAf kit (BRED Life Science, Shenzhen, China) was used for SCD detection and DNA fragments were detected. The Eppendorf tube in kit was incubated at 80 ℃ for 20 min. After it was completely dissolved, the Eppendorf tube was transferred to a 37 ℃ constant temperature water bath for incubation.

(1) The liquefied semen specimen should be sufficiently mixed with the diluent (200–300 μL) and then 50 μL of the treated semen should be added to the Eppendorf tube. The mixture should be thoroughly mixed and then incubated at 37 ℃ for later use.
(2) Next, a volume of 30 μL of the semen–agarose mixture was meticulously dispensed onto the precoated slides, which were already equipped with a 22×22-mm coverslip as provided in the kit. The slides were then positioned on a refrigerated cold plate, set at a temperature of 4 °C, and allowed to rest for a duration of 5 min.
(3) The slide was carefully removed by "side extraction" and denatured with a 1–2 mL denatured solution at room temperature for 7 min, then the denatured solution was discarded. Subsequently, the slides were promptly submerged horizontally in solution A (0.09% hydrogen peroxide-acetic acid solution) and underwent an incubation period of 7 min. Following this, the slides were horizontally immersed in solution B (Tris–HCl buffer with 0.5% sodium dodecyl sulfate (SDS)) for a duration of 25 min.
(4) Finally, the slides were placed horizontally in the purified water for 3–5 min, during which the water was changed 1–2 times. The slides were subjected to a series of ethanol solutions with increasing concentrations (70%, 90%, and 100%) for 2 min each, then air-dried and placed in opaque closed boxes, ensuring protection from light, and stored at room temperature.
(5) For bright-field microscopy, Wright's staining solution (BRED Life Science, Shenzhen, China) and phosphate buffer solution (BRED Life Science, Shenzhen, China) were mixed at a ratio of 1:2, applied horizontally onto the slides to ensure coverage, and incubated for a duration of 15 min under continuous airflow. Subsequently, the slides were rinsed with running water for 10 s and left to air-dry. It is advisable to use strong staining solution to enhance the visibility of the periphery of dispersed DNA loop halos. A minimum of 500 spermatozoa were counted in each sample using a magnification of 100× [23].

In the halo test, a small-sized halo refers to the halo with a width under 1/3 the diameter of the sperm head. Spermatozoa with large and medium-sized halos were counted as non-fragmented DNA, whereas spermatozoa with small-sized halos, halos with degraded (irregularly

and weakly stained) heads, or halos with pinheads (minimal to no DNA content) as fragmented DNA. The reference value of DFI < 15% is for normal semen samples, while 15–30% is a borderline value and > 30% means a lot of DNA damage and poor fertility potential [24].

#### AI-DFI (artificial intelligence method) method

The advent of new AI techniques has led to a surge of interest in AI-based image analysis in the field of medical diagnostics [25] The capabilities of AI technology encompass high accuracy, self-learning, multi-dimensional signal specific identification, and parallel data analysis. Therefore, AI image recognition technology could potentially assist in detecting extreme samples with high impurities in DFI, like semen with low concentration and bacterial infections. These samples are challenging to evaluate accurately using the traditional flow SCSA method [26]. Such an approach would facilitate a greater alignment between DFI detection and the actual clinical requirements.

The sperm nuclear staining kit and fluorescence microscope were provided by Zhejiang Cellpro Biotech Co., LTD.

##### *Preparation and staining of cells*

Frozen samples (fresh samples need to liquefy at room temperature for 30 min, and then directly enter the next step after microscopic observation and counting) were taken out and thawed in a 37-degree water bath, and the ice samples should be taken out immediately when they just melt and perform cell count. A certain amount of semen was taken and diluted with reagent A (Semen Diluent) to 100 μL (final concentration: $1–2 \times 10^6$ sperm cells/mL). 10–20 μL diluted semen was taken and smeared directly, the slide was placed in the staining jar after drying, 200 μL reagent B solution (acid treatment solution) was added dropwise accurately for 30 s, and then 600 μL reagent C (i.e., AO dye, pre-mixed with reagents C1 and C2) was added. Staining should be done for 5 min protected from light, then the slide was picked up with forceps, it was rinsed well with distilled water and it was let dry in a dark way [27].

##### *Detection by high-power fluorescence microscope*

The number of red (DNA damaged spermatozoa) and green sperm (DNA undamaged spermatozoa) in 200 spermatozoa was counted, and the percentage of red fluorescent spermatozoa was calculated (Fig. 3). This method is particularly suitable for samples with oligospermia (extreme semen samples may contain a large number of self-fluorescent impurities, which may interfere with flow DFI results to some extent), a DFI < 25% is considered to be a normal result [28].

#### Statistical analysis

SSPS20.0 software (SPSS Inc., Chicago, IL, USA) was used for analysis. Paired t-test was performed for two groups of measurement data, and F-test (joint hypotheses test) was performed for more than two groups of measurement data, which was expressed as mean ± standard deviation. The chi-square test of the counting data is expressed in terms of frequency (percentage, %). Pearson correlation analysis was employed to investigate the relationship between sperm DNA damage and a number of other variables, including sperm concentration, sperm motility, sperm vitality, and the percentage of morphologically normal sperm in infertile patients. The final set of comparisons was made between the sperm concentration, sperm motility, sperm vitality, and rate of sperm with normal morphology before and after treatment. $P < 0.05$ was considered statistically significant.

## Results

#### Comparison of percentage or fraction of cases with DNA damage and DFI among different semen quality groups

Following semen analysis, of the 508 infertile patients, 170 cases were identified as asthenozoospermia, 157 cases were oligozoospermia, and the remaining 181 cases were normospermia. There were no significant difference ($P > 0.05$) between the three groups in terms of age, diet, physical strength, and work activity, and they were comparable. The DFI and percentage or fraction of cases with DNA damage exhibited statistically significant differences *($P < 0.05$)* between the

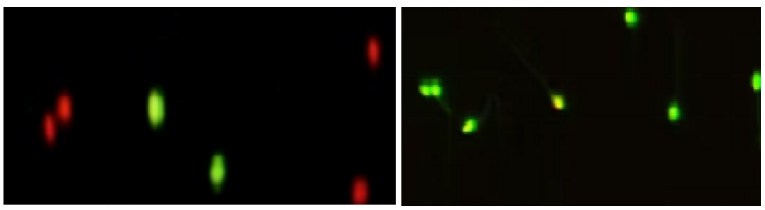


**Fig. 3** Artificial intelligence method-based fluorescence method about DFI. DFI: DNA fragmentation index

**Table 1** DFI comparison of sperm DNA damage frequency in different semen quality groups

| Group | n (508) | Age | Sperm DNA damage frequency, [n (%)] | DFI (x ± s) | Sperm concentration ($\times 10^6$/mL) | Progressive motility (%) | Sperm vitality (%) | Normal sperm morphology rate (%) |
|---|---|---|---|---|---|---|---|---|
| Asthenozoospermia | 170 | 35.13 ± 7.36 | 96 (56.46%) | 20.30 ± 2.85 | 20.63 ± 4.15 | 30.36 ± 6.83 | 38.41 ± 8.36 | 8.01 ± 2.67 |
| Oligozoospermia | 157 | 34.12 ± 6.71 | 79 (50.00%) | 18.62 ± 2.42 | 14.62 ± 3.05 | 36.17 ± 9.61 | 40.55 ± 9.13 | 7.15 ± 2.55 |
| Normospermia | 181 | 34.55 ± 6.82 | 72 (39.70%) | 12.83 ± 2.13 | 26.87 ± 5.34 | 43.05 ± 11.62 | 55.80 ± 11.05 | 12.36 ± 3.19 |
| *P* | | 0.82 | 0.02 | 0.01 | 0.03 | 0.02 | 0.03 | 0.03 |

*DFI* DNA fragmentation index

**Table 2** Semen parameters of infertile (astheno- and oligozoospermic) patients divided into groups based on their sperm DNA integrity status (according to DFI values)

| | n | Age | Sperm concentration ($\times 10^6$/mL) | Progressive motility (%) | Sperm vitality (%) | Normal sperm morphology rate (%) |
|---|---|---|---|---|---|---|
| Sperm DNA integrity group | 238 | 35. 20 ± 7.56 | 20.23 ± 6.85 | 42.80 ± 13.62 | 50.15 ± 7.93 | 12.50 ± 2.67 |
| Sperm DNA damage group | 270 | 33..8 ± 7.31 | 17.61 ± 5.22 | 35.35 ± 12.13 | 39.23 ± 7.06 | 7.82 ± 3.05 |
| *t* | | 6.032 | 9.322 | 8.973 | 11.336 | 10.275 |
| *p* | | 0.68 | 0.03 | 0.02 | 0.03 | 0.02 |

*DFI* DNA fragmentation index

asthenozoospermia, the oligozoospermia, and the normospermia group in descending order (Table 1).

### Semen parameters of infertile (astheno- and oligozoospermic) patients divided into groups based on their sperm DNA integrity status (according to DFI values)

The results of comparable sperm DNA integrity tests showed that a total of 270 patients had sperm DNA damage, and the remaining 238 patients had intact sperm DNA. No statistically significant difference was observed in the mean age between the two groups ($P > 0.05$). The concentration, progressive motility, vitality, and rate of normal morphology spermatozoa in the group with damaged sperm DNA were found to be significantly lower than in the group with intact sperm DNA ($P < 0.05$) (Table 2).

### Correlation analysis between index of sperm DNA damage and semen parameters in infertile patients

Following correlation analysis, a negative correlation was observed between the sperm DNA damage index in infertile patients and the following parameters: sperm concentration, progressive motility, spermatozoa vitality, and normal morphology sperm rate (Table 3).

**Table 3** Correlation analysis between sperm DNA damage and semen parameters in infertile patients

| Sperm parameter | Correlation (r) | *P* |
|---|---|---|
| Sperm concentration | − 0.652 | 0.002 |
| Progressive motility | − 0.587 | 0.003 |
| Sperm vitality | − 0.615 | 0.006 |
| Normal sperm morphology rate | − 0.620 | 0.005 |

### Comparison of semen parameters and DFI index before and after the treatment with Levocarnitine

After Levocarnitine treatment, sperm concentration, progressive motility, sperm vitality, and rate of normal morphology sperm were observed to be higher in patients with DNA damage than before treatment, with statistically significant differences ($P < 0.05$), and their DFI was lower than that before treatment, with statistically significant differences ($P < 0.05$) (Table 4).

## Discussion

In this study, semen analysis revealed 170 cases of asthenozoospermia, 157 cases of oligozoospermia, and 181 cases of normospermia out of 508 infertile patients. Significant differences in terms of DFI were observed between the asthenozoospermia, oligozoospermia, and normospermia groups (Table 1). The sperm concentration, progressive motility, vitality, and normal

**Table 4** Comparison of DFI of sperm analysis in patients with sperm DNA damage before and after treatment

| Group | n | Sperm concentration ($\times 10^6$/mL) | Progressive motility (%) | Sperm vitality (%) | Normal sperm morphology rate (%) | DFI (%) | Log (ROS + 1) |
|---|---|---|---|---|---|---|---|
| Before treatment | 270 | 17.61 ± 5.22 | 35.35 ± 12.13 | 39.01 ± 6.22 | 7.82 ± 3.05 | 19.63 ± 5.17 | 3.0 |
| After treatment | 270 | 23.17 ± 5.37 | 42.86 ± 13.85 | 55.63 ± 9.06 | 12.39 ± 5.66 | 13.18 ± 2.64 | 1.6 |
| *t* | | 7.265 | 5.823 | 7.750 | 8.737 | 8.355 | 6.521 |
| *P* | | 0.03 | 0.02 | 0.02 | 0.03 | 0.01 | 0.03 |

*DFI* DNA fragmentation index

morphology rate were observed to be lower in the sperm DNA damage group in comparison to the DNA intact group, with statistically significant differences (Table 2). A negative correlation was found between sperm DNA damage and sperm concentration, progressive motility, vitality, and normal morphology rate (Table 3). These findings are consistent with previous studies [24, 28–31]. The function of the sperm motility organ may be compromised due to DNA damage, affecting mitochondrial synthesis [32]. The resulting energy deficiency leads to reduced sperm motility or inactivity. The current study aligns with findings from other research [24, 30–32]. For instance, Al Smadi et al. identified three variants, 13708G > A, 4216 T > C, and 12506 T > A, to be negatively correlated with sperm motility and intracytoplasmic sperm injection (ICSI) outcomes [33, 34].

Numerous studies have explored the mechanisms behind sperm DNA damage, with a number of key factors emerging as prominent areas of interest. Excessive ROS removal and increased defense mechanisms can paradoxically cause DNA double-strand breaks in sperm. This may occur through direct oxidation of sperm DNA bases by ROS or the covalent attachment of lipid peroxidation byproducts to DNA, leading to chain breaks [33]. Abnormal chromatin assembly in sperm can also cause DNA double-strand breaks, with improper protamine incorporation during chromatin remodeling being a significant contributor [3, 35–38]. In damaged or immature sperm, a substantial amount of protamine sulfhydryl (SH) groups are unable to undergo oxidation into disulfide bonds, resulting in chromatin loosening and DNA instability. This ultimately leads to DNA denaturation into single-stranded fragments under acidic conditions, causing further DNA damage [39]. Measuring sperm DFI offers insight into the extent of defects in nuclear genetic material and the maturation status of sperm DNA.

Previous studies have indicated that oral Levocarnitine treatment could increase progressive sperm motility to 20.1 ± 8.8% and total motility to 38.3 ± 9.7% [40, 41]. Clinical studies have found a positive relationship between Levocarnitine concentration in semen and sperm count and motility [42]. Several randomized controlled trials have assessed the effects of different Levocarnitine doses on sperm parameters in men with idiopathic oligoasthenozoospermia. Trials involving 100 and 60 infertile men, respectively, reported improvements in sperm concentration, motility, and linearity after treatment [43]. In this study, patients with sperm DNA damage received Levocarnitine treatment, with dosage and duration consistent with other studies [44]. We observed that sperm concentration, progressive motility, vitality, and normal morphology rate were significantly improved in patients with DNA damage following treatment, consistent with findings by Abad (2013) [45] (Table 4). DFI decreased after treatment, indicating that Levocarnitine effectively improves semen quality and reduces DNA damage in infertile patients. Levocarnitine is essential for energy metabolism in the human body. Other research suggests that antioxidants like Levocarnitine play a protective role by scavenging free radicals, preventing the oxidation of purine and pyrimidine bases, and reducing the likelihood of DNA strand breaks or the formation of DNA–protein adducts [46, 47].

Moreover, antioxidants, including Levocarnitine, are believed to inhibit lipid peroxidation in sperm membranes, thus reducing oxidative stress and free radical damage to sperm cells [48]. This is consistent with our findings. Therefore, supplementing with Levocarnitine can effectively remove ROS, protect biofilm and sperm DNA integrity, alleviate epididymal dysfunction and sperm defects, and enhance sperm motility [49]. At the same time, the results of Liang Qi's study in 2012 showed that Levocarnitine can down-regulate TNF-a and ROS in the epididymal of patients with obstructive azoospermia, accordingly improve the environment of the epididymis, and then elevate the quality of sperm and the rate of adequate quality embryo of ICSI [50].

## Conclusions

This study utilized SCD combined with the latest AI-DFI method to accurately detect the relationship between DFI and sperm parameter changes in male infertility patients. Furthermore, the effects of Levocarnitine treatment on DFI were assessed in patients with

asthenozoospermia and oligozoospermia. The findings suggest that sperm DNA damage in infertile patients is associated with semen quality. In clinical practice, Levocarnitine therapy for sperm DNA damage effectively improves sperm quality and reduces DNA fragmentation. However, it should be noted that this study is subject to several limitations, including its reliance on single-center clinical data and a relatively small sample size. Further research is required in the form of larger, multicenter case studies.

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**Author contributions**
K.S.L, S.M.W wrote the main manuscript, Y.J.C, X.D.W, M.J.Z. prepared all the figures, R.F.A. provided assistance with data acquisition. All authors reviewed the manuscript.

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**Availability of data and materials**
No datasets were generated or analysed during the current study.

## Declarations

**Ethics approval and consent to participate**
The present research received ethical approval from the Ethics Committee of the Obstetrics and Gynecology Hospital affiliated with Nanjing Medical University, adhering to the principles outlined in the Declaration of Helsinki. All participants provided written informed consent before participating in this study. This research was conducted in strict accordance with the relevant guidelines of the local institute.

**Consent for publication**
Not required.

**Competing interests**
The authors declare no competing interests.

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