

Received: 2019.07.15

Accepted: 2020.01.15

Available online: 2020.02.24

Published: 2020.04.22

Progress in Research on Sperm DNA Fragmentation

Authors' Contribution:

Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDE Ying Qiu
BCD Hua Yang
BC Chunyuan Li
AEG Changlong Xu

The Reproductive Medical Center, Nanning Second People's Hospital, Nanning, Guangxi, P.R. China


Corresponding Author: Changlong Xu, e-mail: xuchanglong2011@hotmail.com

Source of support: This work was financially supported by the grants from the Guangxi Natural Science Fund Project (2017GXNSFAA198163, 2017GXNSFAA198149), the Nanning Scientific Research and Technology Development Project (20153124, 20163138, 20153011), the Youth Science Foundation of Guangxi Medical University (GXMUYSF201307, GXMUSF201543), the Scientific Research Project of Health Committee of Guangxi (Z2014456, Z2015197, Z20170110), and the Guangxi Key Laboratory of Biological Targeting Diagnosis and Therapy Research (KFJJ2014-02)

With the increasing incidence of male infertility, routine detection of semen is insufficient to accurately assess male fertility. Infertile men, who have lower odds of conceiving naturally, exhibit high levels of sperm DNA fragmentation (SDF). The mechanisms driving SDF include abnormal spermatogenesis, oxidative stress damage, and abnormal sperm apoptosis. As these factors can induce SDF and subsequent radical changes leading to male infertility, detection of the extent of SDF has become an efficient routine method for semen analysis. Although it is still debated, SDF detection has become a research hotspot in the field of reproductive medicine as a more accurate indicator for assessing sperm quality and male fertility. SDF may be involved in male infertility, reproductive assisted outcomes, and growth and development of offspring. The effective detection methods of SDF are sperm chromatin structure analysis (SCSA), terminal transferase-mediated dUTP end labeling (TUNEL) assay, single-cell gel electrophoresis (SCGE) assay, and sperm chromatin dispersion (SCD) test, and all of these methods are valuable for assisted reproductive techniques. Currently, the preferred method for detecting sperm DNA integrity is SCSA. However, the regulation network of SDF is very complex because the sperm DNA differs from the somatic cell DNA with its unique structure. A multitude of molecular factors, including coding genes, non-coding genes, or methylated DNA, participate in the complex physiological regulation activities associated with SDF. Studying SDF occurrence and the underlying mechanisms may effectively improve its clinical treatments. This review aimed to outline the research status of SDF mechanism and detection technology-related issues, as well as the effect of increased SDF rate, aiming to provide a basis for clinical male infertility diagnosis and treatment.

MeSH Keywords: **DNA Damage • Infertility • Reproductive Techniques, Assisted • Semen Analysis • Spermatogenesis**

Full-text PDF: <https://www.medscimonit.com/abstract/index/idArt/918746>

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Background

Sperm is the male gamete cell, which has the ability of reproduction. Sperm DNA integrity is the key to ensure reproductive function. At present, routine semen assessments are widely used to predict the fertilization ability of sperm. Research on sperm DNA fragmentation (SDF) is in the stage of preliminary exploration and the specific mechanism involved is unclear [1,2]. However, previous research has shown SDF has great predictive value in the field of assisted reproductive technology. Many factors affect SDF, including age [3,4], reproductive system diseases [5,6], cancer treatment [7], and smoking [8,9]. Some research has found that with aging, sperm concentration level is decreased, and the odds ratio of SDF in older men is more than double that of younger men [3,10]. A study of male infertility, which was performed for 2 years in a valley town with seasonal atmospheric smog pollution, demonstrated an increased risk for elevated SDF in young men who sprayed pesticides without protective gear [11]. Additionally, numerous animal experiments showed that treatment of different developmental germ cells with different reproductive toxicants led to various levels of SDF [11,12]. Understanding the mechanism of SDF is complicated. Despite the many methods used to detect SDF, there is no uniform standard. In this review, we discuss the mechanism involved in SDF, recent technical advances related issues, and the influences of increased SDF rate on assisted reproductive outcomes, aiming to provide a basis for clinical diagnosis for male sterility.

Sperm DNA Fragment Types and Forms

According to their sources, sperm DNA fragments include nuclear DNA fragments and mitochondrial DNA fragments. Several studies have disclosed that the high rate of SDF is associated with low rates of successful pregnancy and delivery [13]. Venkatesh et al. showed that there is a high incidence of mitochondrial DNA fragments inside of sperm cytoplasm in infertile males [14]. In terms of DNA fragmentation, sperm DNA fragments include DNA single-stranded fragments and DNA double-stranded fragments. To date, researchers have recognized forms of DNA strand damage including full-matrix patterns, base deletions (formation of abasic sites), base modifications (e.g., base oxidation, alkylation), and DNA cross-linking [15,16]. In general, DNA single-stranded fragments are more common than double-stranded fragments, and under certain conditions, if single-stranded DNA fragments are not repaired in time, some DNA single-stranded fragments will further destroy the formation of DNA double-stranded fragments, which in turn will cause DNA strands and DNA-proteins to entangle each other to form larger “DNA fragments”, which indicates that the production of DNA fragments is a dynamic and gradual process [17], and also shows that differences in the severity of SDF affect male fertility.

Mechanism of SDF

Abnormal spermatogenesis

Spermatogenesis refers to the process by which spermatogonial stem cells undergo a series of complex differentiation and development to produce spermatozoa. During spermatogenesis, the nuclear DNA inside of mature sperm is tightly bound to protamine, becomes highly concentrated, inhibits gene expression, and keeps genetic material stable. Protamine promotes DNA concentration and encapsulates genomic DNA into tiny sperm heads, which is the essential function of sperm. If the process of histone and protamine transformation occurs during spermatogenesis, sperm chromatin structure abnormalities can arise. Therefore, to reduce the adverse influences of the torsional stress between the sperm DNA double strands and to promote the adhesion of protamine to DNA strands, the DNA strand needs to be “melted” at its specific site and requires an enzyme repair link to return to normal structure. However, this process is very complicated, and is easily interfered with by various harmful factors from the internal and external environments, and even causes the sperm DNA single chain to be unable to be repaired normally (beyond its repair ability). This leads to production of sperm DNA double-stranded fragments and the destruction of chromatin structure, and this phenomenon has been confirmed in animal studies [17]. This shows that although sperm is capable of maintaining a strong penetration of the zona pellucida, the chromatin can finally develop a special highly concentrated, agglomerated structure that facilitates its function but simultaneously reduces its ability to repair internal and external environmental damage.

Gou et al. [18] showed that human PIWI gene deletion and mutation can lead to abnormal retention of histones, causing disordered histone and protamine transformation, and finally leading to male infertility. The PIWI subfamily has 3 main members – MIWI, MILI, and MIWI2 – which maintain the stability of genetic material in late spermatocytes. Animal experiments showed that knocking out the mouse *miwi*, *mili*, or *miwi2* gene results in significant defects in spermatogenesis in mice, which is characterized by male sterility [19,20].

Oxidative stress damage

In 1979, Jones first proposed that human sperm is especially sensitive to oxidative stress, speculating that this is involved in male infertility because excessive reactive oxygen species (ROS) exposure causes accumulation of sperm cells in the plasma membrane [21]. Peroxidation of saturated fatty acids causes destruction of double bonds in poly-fatty acids. These double bonds are essential for maintaining fluidity of the sperm membrane. Therefore, oxidative stress damages the membrane structure of sperm cells. In addition, oxidative

stress damages the membrane structure of sperm cells, and the metabolic function of sperm cells is inhibited or even lost, which can promote apoptosis of sperm cells, cause nuclear fragmentation of sperm cells, and increase nuclear DNA fragments.

Of note, the structural destruction of sperm cell membranes leads to direct exposure of sperm nuclear DNA to oxygen species in the seminal plasma (mainly white blood cells producing ROS in seminal plasma), which causes attack of sperm DNA by ROS and wide range of DNA single-strand and double-strand breaks and destruction, finally resulting in damage to sperm genetic structure and functional defects, leading to male infertility.

Subsequent experimental studies have shown that exposure of sperm to an artificially produced ROS environment can lead to various forms of damage to sperm DNA, including base modifications, frameshifts, deletions, and generation of abasic sites, cross-linking, DNA strand breaks, and rearrangement of chromosomes [22,23]. Under normal circumstances, the generation and elimination of ROS is in a stable state, and a small amount of active oxygen is necessary to regulate the normal functions of sperm, such as acrosome reaction, sperm capacitation, and normal egg binding [24]. However, when some of the internal and external environmental influences cause an imbalance between the sperm oxidation and antioxidant system, excessive reactive oxygen species cannot be removed in time, and high concentrations of reactive oxygen species interact with lipids, proteins, and DNA molecules, causing DNA fragmentation, eventually leading to abnormal sperm [25,26].

Previous studies reported that there are several reasons why sperm and spermatogenesis are susceptible to active oxygen attack. For instance, the sperm nuclear chromatin concentration period is highly sensitive, and the DNA repair mechanism is lacking in sperm cells. Moreover, the sperm membrane contains a high concentration of polyunsaturated fatty acids. The reactive oxygen is produced by the sperm itself, especially when sperms pass through the epididymis. Furthermore, sperm contains trace amounts of intracellular antioxidant enzymes, whereas most of the antioxidant enzymes are lost during spermatogenesis. In the process of fertilization, the phenotype and activity of sperm are also influenced by the anatomy of the female reproductive tract when acrosome reaction occurs. These changes in the environment of the female reproductive tract promote the survival of sperm and the success of fertilization [27–30].

Abnormal sperm apoptosis

Under physiological conditions, apoptosis is the genetically controlled cell death that occurs during normal development, and is distinguished by fragmentation of the genome and cleavage or degradation of several cellular proteins. The imbalance of apoptosis is the root cause of many diseases. Normal apoptosis

of sperm cells plays a decisive role in regulating the number of sperm, quickly removing sperm from chromosomal abnormalities in the body, and maintaining sperm quality. During spermatogenesis, 25% to 75% of sperm cells are destroyed by apoptosis. In 2010, Jana et al. reported that early apoptosis of spermatogenic cells is mainly regulated by spermatogenic cells and Fas/Fas ligands expressed on cell surface [31]. Abnormal expression of the Fas/Fas ligand system can occur when the testis is pathologically altered. This is consistent with the results of Lee et al. [32]. In addition, sperm “apoptosis escape” can cause spermatogenic cells with DNA fragmentation to escape certain apoptotic pathways and further differentiate into mature sperm carrying fragmented DNA [33]. Although these abnormal sperm and eggs can be normally fertilized, when the embryo develops to the 4-8 cell stage, the fragmented DNA will induce embryonic apoptosis, decrease the rate of blastocyst formation, and cause an abnormal increase in embryonic development and early abortion [34–36].

Method for Detecting SDF

A summary of detection methods of sperm DNA fragmentation with their advantages and deficiencies is provided in Table 1. The details are described below.

Sperm chromatin structure analysis

The SCSA method was proposed in 1980 to detect the presence of damaged sperm DNA and normal sperm using the chromatin specificity of acridine orange [37]. The principle is that the tight binding of normal double-stranded DNA has stability and acid resistance, and the chromatin structure of the damaged sperm DNA is relatively loose, and is easily denatured into a single chain by the action of an acidic substance. Flow cytometry detects the fluorescence of acridine orange combined with sperm DNA, which is finally processed by computer software to obtain SCSA parameters. Under good quality control, SCSA is a method with high reproducibility and a mutation rate of less than 2%, and the results obtained by different laboratories are highly comparable [38,39]. This method has become the criterion standard for detecting SDF.

Terminal transferase-mediated dUTP end labeling (TUNEL)

Fluorescein isothiocyanate (FITC) is used as a marker to specifically detect apoptosis-induced DNA fragmentation in sperm cells. The principle is first to increase the permeability of cell membrane from different tissue sections, so as to let the dUTP, which is labeled with rTDT and a biomarker, enter the cell membrane. Then, the dUTP binds to the nuclear cleavage DNA 3'-OH with the aid of rTDT. Also, the streptavidin labeled with HRP can bind to biotin of dUTP (there are at least 3 biotin

Table 1. Detection methods of sperm DNA fragmentation with their advantages and disadvantages.

Detection methods	Advantage	Deficiency	References
Sperm Chromatin Structure Analysis (SCSA)	<ul style="list-style-type: none"> • Large detection capacity • Lower mutation rate <2% • Highly comparable data • Sensitivity and specificity 	<ul style="list-style-type: none"> • Indirect assessment of DNA damage • Lower predictive power 	[37,38,39]
Terminal Transferase-mediated dUTP end Labeling (TUNEL)	<ul style="list-style-type: none"> • Uses flow cytometry • High accuracy • Low mutation rate <3.4% • Direct or indirect assessment 	<ul style="list-style-type: none"> • Needs aseptic operation • Microscope with low sensitivity to testing SDF 	[39,40,41]
Single-Cell Gel Electrophoresis (SCGE)	<ul style="list-style-type: none"> • Sensitivity and specificity • Rapid and cheap • Simple and repeatable 	<ul style="list-style-type: none"> • Related to detection thresholds, operating conditions 	[39,42,43]
Sperm Chromatin Diffusion Experiment (SCD)	<ul style="list-style-type: none"> • Easy to perform • Cheap materials • Higher accuracy 	<ul style="list-style-type: none"> • Avoids deep coloring • Indirectly assesses DNA damage 	[39,44,45]
DNA Flow Cytometry (FCM)	<ul style="list-style-type: none"> • Higher sensitivity and specificity • Objective and quick 	<ul style="list-style-type: none"> • Needs fresh specimens and unsecured tissue 	[39,46,47]

molecules that can be combined with streptavidin). Finally, with the oxidation and cyclization reactions, hydrogen peroxide, and horseradish peroxidase HRP of SP, there is production of a brown phenylhydrazine polymer, by which cell the apoptosis rate can be judged by the ratio of TUNEL-positive cells in different fields on each slice under fluorescence or optical microscopy. However, compared with flow cytometry, microscopy can underestimate the degree of SDF because of the low sensitivity of the microscope to detect DNA fragmentation [40]. TUNEL uses flow cytometry for higher accuracy and a mutation rate of less than 3.4% [39,41].

Single-cell gel electrophoresis (SCGE)

Single-cell gel electrophoresis (SCGE) was originally proposed by Ostling and Johanson in 1984, and has been gradually improved by researchers to become a fast, sensitive, and simple method for detecting SDF [42]. It relies on the principle that when the sperm DNA is damaged, DNA fragments of different sizes will appear. After treatment with detergent and lysate, DNA fragments, most proteins, and other cellular components in the sperm nuclei will seep out of the cell membrane and enter the lysate. Later, with the deepening of research on sperm DNA, successive research reports indicated that the neutral lysate and its temperature conditions in the former experimental conditions cannot completely separate the broken sperm DNA from its sperm nuclear protein, but only make it smaller. Part of the smaller fragment of the double-stranded DNA dissociates and moves out of the nucleus under the action of electric force to form a “comet-like” tail.

After continuous research and improvement, the conditions for electrophoresis were changed from neutral to weakly alkaline, which can better cleave macromolecules such as nucleic acids and proteins in sperm cells, and then fully under the action of electric field force. The sperm membrane is removed to form a more realistic comet image, reflecting the actual level of SDF. In 2012, Ribas-Maynou used an alkaline-neutral double-comet assay that not only significantly improved the accuracy of the test results, but also distinguished sperm single-strand DNA fragments together with double-stranded DNA fragments [43]. The SCGE analytical techniques might be the most effective visual method to assess the SDF *in vivo* or *in vitro* because it is a sensitive, rapid, cheap, simple, and repeatable operation [39].

Sperm chromatin diffusion experiment

The sperm chromatin dispersion (SCD) was proposed by Fernández in 2003 [44], and was subsequently improved to make the Halosperm kit. The optical microscope is used to observe the results and maintains the integrity of the sperm tail. It is a detection technology that is easy to operate, cheap, and highly accurate. The principle is that the sperm chromatid structure becomes loose after acid denaturation, so that the DNA ring adheres to the residual nuclear structure to form a characteristic halo. Sperm with impaired DNA integrity does not produce this characteristic halo. Therefore, the sperm DNA integrity can be judged by observing the presence or absence of the halo under a microscope. Zhang reported that SCD, TUNEL, and SCSA are equally effective for the detection of sperm DNA fragments, but SCD seems to be more sensitive than TUNEL [45].

The greatest advantage of SCSA is that it has a better ability to detect numerous cells by flow cytometry [39].

The application of flow cytometry in SDF

DNA flow cytometry (FCM) is also known as fluorescence activated cell sorting (FACS). The principle is that propidium iodide (PI) is used as a DNA-labeled fluorescent pigment, which is a membrane-impermeable DNA probe. However, only the spermatozoa damaged by the plasma membrane can be inserted into the base pair in the double-stranded nucleic acid, and the degree of chromatin condensation can be indirectly reflected by the FCM combined with the fluorescent dye, thereby using the maturity of the chromatin to judge the fertility. As the pathological degree of sperm increases, the degree of condensation of nuclear chromatin decreases, then the amount of binding of PI to DNA increases, and the mean value of fluorescence increases. In normal semen test results, DNA FCM can also detect abnormal changes in a small amount of semen, which indicates that DNA FCM is more sensitive and provides more information than the routine detection of semen and SDF, which may cause patient infertility [46]. FCM not only has the advantage of high sensitivity, but also objectively and quickly analyzes the pathological changes in spermatogenesis and maturation by analyzing the quality of sperm DNA [39,47].

Additional methods

In addition to the above detection methods, polymerase chain reaction (PCR) [48], fluorescence *in situ* hybridization [49], gene chip technology [50], and Raman spectroscopy [51] are also used in the detection of SDF. However, these detection techniques currently lack the standardization, standardized operational methods, and strict quality control required to ensure accurate SDF detection.

Gene Regulation and DNA Fragmentation.

Coding genes involved in DNA fragmentation

Spermatogenesis involves a process of spermatogonial cell division and proliferation, spermatocyte meiosis, sperm cell metamorphosis, and sperm maturation. In this highly complex and orderly process, at least 150 different genes are involved [52–54]. Animal model studies and population studies have found that some genetic variants are closely related to spermatogenesis disorders, including neuroendocrine-related genes (GnRH, FSH, LH, FSHR, LHR), gonadal development-related genes (AZF, WT1, PRDM1, SF), meiosis-related genes (MLH1, IRF1, PRDM9, SPO11), and other genes [55–62].

Follicle-stimulating hormone (FSH) is a glycoprotein heterodimer that regulates the development and functions of the male and female gonads. In the adult testis, FSH regulates spermatogenesis by acting on Sertoli cells, and there is evidence that follicle-stimulating hormone receptor (FSHR) polymorphisms are associated with male infertility [63]. FSHR KO mice present severe disturbances of testicular function, including small testis and aberrant gametogenesis [64–66]. Garolla et al. found that the intrauterine insemination with sperm from infertile male patients treated with FSH led to a 23.2% higher pregnancy rate due to a reduced SDF rate [67]. The normal course of spermatogenesis depends on proper pituitary secretion of FSH, luteinizing hormone (LH), and testicular secretion of testosterone. In a clinical trial of infertility treatment of a cohort of 186 men aged 25–35 years, with no pathological features observed in the female partner, patients were treated for infertility for more than 1 year and their blood sample were used to assess serum levels of testosterone, FSH, and LH; the results indicated that both extremely low and extremely high levels of FSH and LH caused a dramatic increase in SDF, and showed a negative correlation between SDF and testosterone level [68]. In another clinical study, all groups of patients without ovulatory dysfunction received an agonist or antagonist of GnRH in intracytoplasmic sperm injection (ICSI) cycles supplemented with recombinant LH (rLH), and showed a comparable level of apoptosis and SDF in granulosa cells [69]. The above studies suggest the relationships between some reproductive-related genes and SDF, which interact with each other to regulate biological reproductive activities. In addition, the regulatory role of DNA fragmentation is very extensive in mammals, although many aspects are unknown and need further study.

Non-coding genes involved in DNA fragmentation (miRNAs, lncRNAs, circRNAs)

Recently, non-coding RNAs, such as miRNA, piRNA, small interfering RNA (siRNA), circular RNA (circRNAs), and lncRNA, have been shown to play important roles in gene transcription and post-transcriptional regulation [70,71]. Numerous animal experiments and human experiments have found that miRNAs play an indispensable role in spermatogenesis [72]. Sperm lncRNAs have been investigated in rodents and humans, and were implicated in spermatogenesis and reproduction [73–75]. Similarly, numerous studies indicated that individual miRNAs are expressed during spermatogenesis and affect male fertility [70]. The regulatory relationships of miRNAs and SDF have been explored, but there have been few in-depth studies on this topic, and the much remains unclear. It was reported that IRF1, a member of the interferon regulatory factor (IRF) family, is a protein that is directly targeted by miR-383, which regulates interferon in cell apoptosis and cell cycle, and this regulatory axis is involved in testicular spermatogenesis and SDF [76]. Similarly, the role of miRNAs in infertile males has been explored in a

study including 5 groups of N (normozoospermic), MOAT (moderate oligoasthenoteratozoospermic), SOAT (severe oligoasthenoteratozoospermic), OA (obstructive azoospermia), and NOA (non-obstructive azoospermia), and the results indicated that the expression of miR-34c in the MOAT and NOA groups was significantly elevated. Additionally, the percentage subjects with oxidative stress and DNA fragmentation was significantly higher in the infertile groups (MOAT and SOAT) than in other groups in spermatozoa and testicular tissues [77], indicating there are direct and indirect regulatory connections between non-coding RNA and SDF. More extensive studies are needed to explore the regulation of SDF by non-coding genes and their involvement in male infertility.

SDF and DNA methylation

Methylation of DNA is considered to be one of the most important aspects of epigenetics and can affect gene expression and imprinting. In the fetal testis, Dnmt1 expression is associated with the proliferative state of PGCs, but its expression is terminated when *de novo* methylation occurs. Once the *de novo* methylation component was identified, Dnmt1 was re-expressed in testes in which there was strong proliferation of spermatogonial cells, demonstrating its important maintenance function in DNA methylation. Dnmt1-deficient fetuses are unable to maintain acquired DNA methylation and can experience spermatogonial cell death due to deletion of several site genomic imprints. However, Dnmt1 heterozygous mice have normal reproductive capacity, indicating that even a decrease in DNMT1 expression is sufficient to maintain a specific DNA methylation pattern in germ cells [78].

It is worth noting that Dnmt3L is only expressed in germ cells, and its expression pattern is expressed as a strong sex dimorphism. Dnmt3L inactivation can cause mitotic delay. Due to chromosomal association errors, spermatogenesis stops at the occlusion phase, and germ cells cannot continue to mature [79]. Interestingly, the phenotype of mice with specific excision of the Dnmt3A gene was essentially identical to that of Dnmt3L knockout mice [80]. Knockout of Dnmt3L or Dnmt3A in male germ cells, as well as DNA *de novo* methylation status, suggest that DNA methylation-modified transposon epigenetic silencing failures can lead to their reactivation and random remodeling in the genome, which can seriously affect the stability of the genome and gene expression. Deletion of the genomic imprint can contribute to genomic instability, leading to abnormalities in the chromosomal structure in which Dnmt3L and Dnmt3A are inactivated. Numerous studies have found that DNA methylation is strongly bound up with sperm DNA instability and infertility [81]. Filippini et al. found that the sperm DNAH19 gene is hypermethylated in normal people, but in patients with moderate to severe azoospermia, methylation levels are significantly reduced [82]. More importantly,

the abnormality of the maternal imprinted gene is more pronounced in patients than the paternal imprinted gene. In 2010, Wu et al. found that the proportion of methylenetetrahydrofolate reductase (MTHFR) gene promoter region in the hypermethylation state was higher than that in the control group when the methylation pattern of the gene promoter region was detected in 94 infertility patients using bisulfite sequencing technique. They speculated that hypermethylation of the MTHFR gene promoter caused silencing of the promoter, which in turn affected the spermatogenesis process [83]. Therefore, studying changes in DNA methylation will help better understand the pathogenesis of male infertility.

Potential diagnostic and therapeutic values of the above biomarkers (coding and non-coding genes in DNA fragmentation)

miRNAs are diverse and play an important regulatory role in spermatogenesis and spermatid function. Therefore, more and more scholars are paying attention to the diagnostic and therapeutic value of miRNAs in reproductive diseases such as male infertility. However, miRNA interaction sites are multiple and incompletely understood, so their clinical applications remain challenging. In addition, in terms of reproduction, the study of miRNAs remains part of the overall macroscopic analysis of miRNA, and more microscopic research is needed. With the maturity of high-throughput gene screening technology, lncRNAs are expected to become new biomarkers or therapeutic targets in the diagnosis and treatment of male infertility and even male reproductive diseases. In summary, further research is needed on the role of miRNAs and lncRNAs in male reproductive system diseases, the mechanism of action during sperm growth and development remains unclear, and the role of miRNAs in male infertility warrants further exploration. The current primary method for assessing male fertility is semen analysis, but traditional semen analysis does not accurately assess male fertility, which means there remains a need for an accurate, non-invasive assessment method to diagnose male infertility. Therefore, it is necessary to further study the role of miRNA and lncRNA in male infertility.

Potential Drugs for Mitigating or Treating DNA Fragmentation

There is very limited data on whether SDF is a separate causative factor affecting male fertility, and it is controversial whether SDF should be part of routine testing to assess sperm quality and predict male fertility. Most studies on SDF clearly show it is important in human reproduction. Research indicates that some treatment methods can improve SDF. At present, the main methods for treating SDF are anti-oxidation treatment, removal of the etiology, and traditional Chinese medicine.

Antioxidant therapy

Oxidative stress is an important mechanism for the production of SDF. Excessive ROS causes loss of the ability of the antioxidant system to scavenge ROS. Therefore, antioxidant supplementation can be effective. Both oral antioxidants and antioxidants can be added to the semen medium to improve sperm resistance. Commonly used exogenous antioxidants are natural antioxidants and synthetic antioxidants. Natural antioxidants are mainly derived from plants. The active ingredients are vitamins, alkaloids, polysaccharides, polyphenols and flavonoids, which can remove ROS with high efficiency and low toxicity [84]. Synthetic vitamins C and E and other macromolecular antioxidants are common synthetic antioxidants, but they are not easily absorbed and have toxic effects in long-term use. Trace element zinc has a positive effect on human sperm. Riffo found that zinc, as an antioxidant, protects sperm from oxidative stress damage and enhances sperm motility [85]. A study of 175 infertile Iranian men who consumed a diet based high in antioxidants assessed the relationship between dietary antioxidant intake and semen quality parameters. The results showed that men given β -carotene and vitamin C were less likely to have SDF, and the men given β -cryptoxanthin had higher sperm density, suggesting that antioxidants mitigate SDF and can improve sperm quality [86].

The antioxidant effects of L-carnitine (LC) and acetyl-L-carnitine from sperm cell of infertile patients have also been reported [87]. Compared with a single antioxidant, the combination of various antioxidants has a synergistic effect, but oxidative damage was still observed. It has obvious therapeutic effects and can reduce sperm damage caused by oxidative stress to varying degrees.

Removal of the causes of disease

There are many causes of abnormal increase of SDF. Many reports have confirmed that the cause of SDF in sperm cells is closely linked to environment and living habits, in addition to disease factors [88]. Genitourinary tract infection can lead to increased leukocytosis in semen, elevated levels of ROS in seminal plasma, and SDF through oxidative stress damage. Active treatment of primary infection of the urinary system can avoid abnormal production and increase of SDF. Abnormal rise in testicular temperature, varicocele, chemoradiotherapy, and drugs are common disease factors that may cause SDF. Cadmium and iron poisoning can cause DNA damage in testicular cells. The target of cytotoxic drugs is the testicular spermatogenic epithelium, which may be an important factor in spermatogenesis obstruction. Radiotherapy and chemotherapy can also damage the testicular spermatogenic epithelium, causing spermatogenic disorders and sperm damage. Therefore, for male patients with fertility problems, cryopreservation of

sperm before radiotherapy and chemotherapy is an important measure to ensure their fertility. Smoking, alcohol consumption, environmental toxins, prolonged use of hormonal drugs, unhealthy lifestyles, and certain working conditions can cause SDF. For example, heavy smoking and long-term alcohol consumption can lead to abnormal increase of ROS in semen and can cause sperm damage. Acrylonitrile (CAN) and its metabolites act as a multi-effect toxic substance, which can induce sperm DNA breaks and abnormal chromosome structure [89]. Therefore, to avoid risk factors, active treatment of primary disease is an effective measure to prevent and treat abnormal increases in SDF.

Traditional Chinese medicine treatment

Traditional Chinese medicine has long been used to treat male infertility. At present, traditional Chinese medicine combines traditional medicine with modern testing methods to achieve new developments in traditional Chinese medicine treatment. Wang, when using Shengjingsan to treat infertility patients, showed that Shengjingsan containing dodder, medlar, schisandra, raspberry, psyllium, antler, surimi, and agarwood could improve sperm quality, and reduce the extent of DNA fragmentation in sperm cell of infertile patients, as well as improve the conception rate of IVF-ET [90]. Traditional Chinese medicines such as Jujing Pill, Yishen Shengjing Recipe, Liuwei Dihuang Soft Capsule, Yangjing Zanyu Granule, Shizi Erxian Decoction, Epimedium Flavonoids, Rhodiola Extract, Cuscuta Water Extract, and Basil Day Water Extract, as well as flavonoids and equols, have been studied by scientists in China in recent years by combining the traditional Chinese medicine with research and testing techniques [91–101]. They all showed that the above-mentioned Chinese medicines or Chinese herbal extracts are effective in treating SDF.

Therapeutic challenges

SDF naturally exists in human semen, and the mechanism of its abnormal increase is not clear. Various factors contribute to SDF, including diseases, drugs, unhealthy living habits, environmental pollution, alcohol abuse, varicocele, and cancer treatments. SDF examination should be performed for patients with idiopathic infertility caused by non-azoospermia, unexplained recurrent miscarriage, and pre-fertility and pre-fertility sperm. The continuous development and updating of SDF detection technology will help to further deepen the understanding of SDF. However, inclusion of SDF in routine sperm detection needs further assessment.

Effects of SDF on Male Infertility

SDF is closely involved in male infertility. The degree of SDF in infertile men is significantly higher than that in fertile

men [102]. Especially in idiopathic infertility, about 20% of patients have high levels of SDF, which can affect fertilization, embryo implantation, and survival [103]. The current commonly used indicator for assessing sperm DNA damage is the DNA Fragmentation Index (DFI), but there is no uniform standard for the relationship between the threshold of SDF and male infertility. Bronet showed that conception rates and pregnancy rates significantly decreased when the value of DFI was above 27% [104]. If the DFI value is controlled at below 27% or even lower, the fertility of men will be greatly improved. In addition, some scholars believe that the prevalence of DNA double-strand breakage in healthy men is remarkably lower than in infertile patients, and most of them are single-strand DNA damage, probably because single-strand DNA damage can be repaired by itself without having much impact on fertility. However, double-stranded DNA-damaged sperm cannot repair itself, so double-stranded DNA damage may be the real cause of male infertility. Research on DNA single- and double-strand breakage in sperm nuclei is continuing, and most scholars believe that its reference value is better than DFI [17,105,106].

Relationship Between SDF and Pregnancy Outcomes in Assisted Reproductive Technology

Effects of SDF on pregnancy outcome in intrauterine insemination

Intrauterine insemination (IUI), as a traditional assisted reproductive technology, is an important method for the treatment of male infertility. The clinical pregnancy rate reaches 15% to 20% and male infertility is closely related to poor sperm quality. Sperm DNA integrity testing can more effectively assess sperm quality and predict male fertility.

Bungum showed that the sperm DFI value above 30% was the threshold for a notable margin in conception rate [107]. When the sperm DFI value is more than 30%, the natural pregnancy and IUI success rate was almost zero. The higher the sperm DNA integrity, the higher the semen quality, the higher the sperm count and the forward motor sperm rate, and the higher the clinical pregnancy rate, indicating that SDF can affect sperm quality through IUI success rate.

Effect of SDF on *in vitro* fertilization and intracytoplasmic injection

Epidemiological data on sperm DNA integrity shows that the degree of sperm DFI is negatively correlated with the success rate of *in vitro* fertilization (IVF). However, there is still controversy about use of intracytoplasmic sperm injection (ICSI) or traditional IVF in patients with high DFI. Anifandis showed

that there was no significant difference in pregnancy outcome between patients with high DFI and those with high DFI (ICSI) ($P>0.05$) [108]. However, it has been reported that patients with high levels of DFI who received ICSI had better fertilization rates, clinical pregnancy rates, and delivery rates than those who received IVF [109–111]. Chi also showed that there was no significant difference in pregnancy rates between patients with low DFI (DFI $\leq 30\%$) IVF or ICSI cycles ($P>0.05$), but in the high DFI group (In DFI $>30\%$), the pregnancy rate of ICSI cycle was higher than that of IVF cycle, and the difference was statistically significant ($P<0.05$) [108]. In addition, the difference in abortion rates between the high DFI group and low DFI group was not statistically significant ($P>0.05$).

Therefore, it is considered that ICSI improves the pregnancy outcome of infertile patients more than IVF in the high DFI group. This may be because the ICSI cycle is more selective for sperm with normal shape and fast movement. The results of Chi also showed that the difference in DFI between high DFI and low DFI in the ICSI cycle was statistically significant ($P<0.05$), but the difference in pregnancy rate was not statistically significant ($P<0.05$), which indicates that ICSI reduces the pregnancy rate caused by DNA damage [112].

Potential Effects of SDF on Offspring

The integrity of human sperm DNA plays an important role in the stable inheritance of paternal genes by offspring. With the development of assisted reproductive technology, especially the application of ICSI technology, sperm carrying DNA damage can be directly injected into the egg and fertilized by screening the egg zona pellucida and cervical mucus. Fertilized eggs and oocytes have some ability to repair damaged sperm DNA, but once they exceed their repair ability, DNA with genetic defects is transmitted to the next generation, causing hereditary diseases.

SDF is a mutagen. If the fertilized egg fails to be repaired before the first cleavage, it will cause mutation, and the same line mutation site will be fixed. This mutation may be related to male infertility, childhood tumors, and genetic imprinting defect-related diseases such as Angelman syndrome and Beckwith-Wiedemann syndrome [113]. The results of Fernández-Gonzalez confirmed that, compared with IVF, the number of tumors in the offspring of spermatozoon-damaged mice was significantly increased after ICSI [114]. However, Fernández-Gonzalez et al. found that the offspring produced by ICSI in mice with sperm DNA injury had high levels of genomic instability in somatic cells and germ cells, and they did not find differences in tumor susceptibility in mice within 1 year of survival [115].

Research also found that mice receiving high-dose radiation (10.0 Gy) had a mortality rate of 37% within 10 weeks after birth, which was significantly higher than that of non-irradiated mice. The rate was related to the cumulative damage of the paternal sperm DNA, but none of the mice died 10 weeks to 1 year after birth [116–118]. This may be because mice have a rapid developmental period at 10 weeks after birth, and the response to DNA fragmentation during early embryo development is phased, so it is speculated that the abnormal damage response pathway or genomic instability of the fetus ultimately leads to early postnatal death in the offspring [119]. Whether humans have this mechanism of action still needs further experimental confirmation. In addition, the mechanism by which SDF causes early death in offspring needs to be further clarified. However, some researchers have pointed out that even if DNA fragments can be detected in sperm nuclei, it is unclear what should be done with the obtained information, because it is not possible to “find” a particularly effective set of targeted measures to prevent the production of sperm DNA fragments.

Some researchers have suggested that there is no need to devote manpower and financial resources to detect the presence or absence of debris in sperm DNA, because even if we determine that sperm DNA is damaged, it can only be managed “hands-on”. However, Schlegel disagrees with that view, arguing that if a male patient has subclinical damage and a high

DNA fragmentation index, medical staff will find the cause of infertility [120]. For these patients, even if the clinicians cannot provide a particularly effective treatment, they give patients some appropriate symptomatic treatments, such as antioxidant drugs and essential trace element supplementation treatments and guide patients to avoid harmful environments and toxic exposure. These symptomatic treatments can help some patients overcome unexplained infertility to achieve a successful pregnancy outcome. Therefore, research on sperm DNA fragments is important. The relationship between SDF rate and male fertility needs to be further explored.

Conclusions

Male infertility, caused by various internal or external factors, is partly due to increased SDF rate. However, the relationships between SDF and physiological regulation activities in the body are quite complicated. Reviewing previous studies, we summarized the mechanism of SDF and also described some effective methods for detection of SDF, such as SCSA, TUNEL, SCGE, SCD, and FCM, which are valuable for the evaluation of male infertility. With lifestyle change, antioxidant supplementation, and gene therapy, male infertility can be effectively treated, and more patients with male infertility can be cured. We also provide valid references for further investigation of the mechanism of SDF.

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