Chapter 21
Sperm DNA Fragmentation: Treatment Options and Evidence-Based Medicine

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Key Points

- SDF is a valuable tool for male fertility evaluation as it can influence fertilization rate and embryo development.
- The most commonly used SDF testing methods include terminal deoxyribonucleotidyl transferase-mediated dUTP nick end labeling (TUNEL), single-cell gel electrophoresis (the comet assay), the sperm chromatin dispersion (SCD) test, and the sperm chromatin structure assay (SCSA).
- Indications for SDF testing include unexplained infertility, recurrent miscarriage, varicocele, recurrent assisted reproductive failure and men with lifestyle risk factors.
- Lifestyle modifications, frequent ejaculation, antioxidants, varicocelectomy, sperm selection, and use of testicular sperm for intracytoplasmic sperm injection are among the treatments that can be performed in patients with high SDF levels.

Introduction

According to the World Health Organization (WHO), infertility in men or women is known as the inability to conceive after 1 year of unprotected intercourse [1]. About 15% of couples of reproductive age are affected by infertility, with male factors

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contributing up to 50% of the subfertility [2]. Male infertility is defined as a male's inability to produce a pregnancy in a fertile female. "Male factor" infertility is diagnosed when there is an alteration in sperm count, motility, and/or morphology in at least one of two semen samples, collected 1 and 4 weeks apart [1]. Known causes for male infertility account for 30–50% of the cases, while the remaining 50–70% have unknown causes and are termed "Idiopathic" [3]. It remains a struggle to diagnose and to treat idiopathic infertile men who eventually would require assisted reproductive techniques (ART) to overcome their infertility [4].

While semen analysis remains the cornerstone test for male fertility evaluation, it is not always an optimal predictor of the true male fertility status due to the intra-individual variation in sperm quantity and quality. Furthermore, up to 40% of infertile men have semen parameters falling within normal reference ranges [2].

A DNA fragmentation test is defined as the percentage of spermatozoa with fragmented DNA in the ejaculate [5]. Sperm DNA fragmentation (SDF) is currently recognized as an important cause of male factor infertility [3]. DNA is a vital component of the cell or spermatozoa, so high levels of SDF may affect various markers of conception, including embryo quality and blastocyst development [6–8].

The objective of this chapter is to describe the most commonly utilized SDF testing methods, highlight the clinical indications for SDF testing and to explore possible treatment methods for high SDF.

SDF Testing Methods

Several techniques are being used in the clinical setting to quantify DNA damage. The most commonly used methods include terminal deoxyribonucleotidyl transferase-mediated dUTP nick end labeling (TUNEL), single-cell gel electrophoresis (the comet assay), the sperm chromatin dispersion (SCD) test, and the sperm chromatin structure assay (SCSA). The first two methods can directly detect DNA fragmentation, whereas the latter methods analyze the susceptibility of chromatin to denaturation, giving an idea about the status of the nucleus in terms of integrity and compaction of chromatin. Therefore, each method can interpret a different aspect of sperm DNA activity.

TUNEL Method

The TUNEL method is considered one of the most promising tools for SDF testing [8].

This method can measure both single- and double-strand breaks using an enzyme that incorporates a modified and labeled dUTP at the 3'-OH terminal end of damaged DNA strands. The modified dUTP can be labeled by many ways: either directly with fluorescein or indirectly by using labeled antibodies or streptavidin. After collection and addition of the labeled dUTP, the sperm is examined under a microscope, and the amount of damaged DNA can be quantified [8].

The TUNEL method is considered to be the method of choice for the detection of damaged DNA caused by automated cell death (apoptosis). However, it is not specific to this detection as it can also detect cell death caused by other ways, such as exposure to chemicals or other toxins [9].

Single-Cell Gel Electrophoresis (The Comet Assay)

Similar to the TUNEL method, the comet assay can also detect single- and double-strand breaks in DNA (Fig. 21.1). It is a sensitive and rapid technique. It was first developed by Ostling and Johansson in 1984 and was later revised by Singh et al. in 1988 [10]. In this method, sperm cells are placed in an agarose gel plate so that all the proteins in the cells are lysed. The DNA is placed in an alkaline/neutral medium, and electrophoresis takes place. This allows the damaged and broken DNA fragments to migrate away from the nucleus at a faster rate than do the undamaged DNA fragments. After staining with fluorescent dye, comet shapes are shown, where the undamaged DNA fragments are referred to as the "head" of the comet and the migrating damaged DNA fragments are referred to as the "tail." The interpretation of this assay is as follows: the higher the number of tails is, the higher is the number of damaged DNA strands [10].

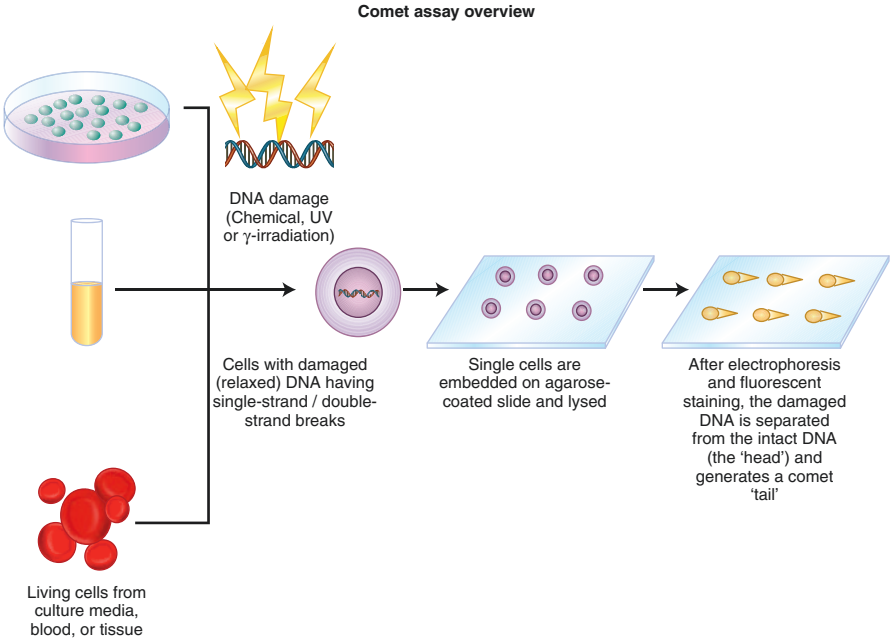

Fig. 21.1 Schematic illustration of the comet assay method

The Sperm Chromatin Dispersion Method (SCD)

The SCD method evaluates the ability of the sperm chromatin to disperse. It is a simple, fast, accurate, and highly reproducible method for the analysis of DNA fragmentation. Normally, upon the addition of hydrochloric acid, the sperm chromatin tends to denature and the acid will lead to the generation of single-stranded DNA. After this denaturation, a lysis solution is added, which will ensure the removal of all nuclear protein from the cell [11]. This procedure results in normal DNA spreading out of the center, producing halos that can be observed under the microscope [12]. However, fragmented DNA fails to show the same response to denaturation and lysis; hence, it does not form halos under the microscope. DNA fragmentation is therefore inversely proportional to the percentage of dispersion formed.

Unlike the TUNEL and the comet methods, the SCD method does not rely on the determination of color or intensity of fluorescence. Rather, it relies on the percentage of sperm cells with no dispersion (small or no formation of halos), which can be easily determined [13].

Sperm Chromatin Structure Assay (SCSA)

This method measures the susceptibility of DNA to denaturation, which occurs most commonly in fragmented DNA [7]. The SCSA is a flow cytometry test that measures two nuclear parameters simultaneously. After the addition of acid, fragmented DNA tends to denature to a higher extent than does normal DNA. Then an orange dye (acridine orange) is added to the solution, and flow cytometric analysis is performed. The sperm are passed under a beam of light with a specific wavelength, causing them to appear either orange (fragmented DNA) or green (normal DNA). A computer measures the percentage of green versus orange sperm cells, and a specific SCSA software plots the result, giving the two parameters: the DNA fragmentation index (DFI) and the percentage of sperm with high DNA stainability (HDS) [14].

A normal DFI is considered to be less than 15%. A sample with a percentage range of 16–29% is considered good or with fair fertility potential, whereas a sample with a percentage greater than 30% is considered to have a poor outcome for fertility [15].

As for the percentage HDS, it reflects the percentage of immature sperm, which is also predictive of pregnancy failure if elevated [15].

The advantages of using a flow cytometry test are high precision and accuracy, avoidance of human eye biases, and speed of measurement, in which about 250 cells per second can be assessed [14].

Indications of SDF Testing

SDF tests are increasingly being used in clinical practice for the evaluation of infertility in men. A recent survey of 65 professionals from 18 countries around the world reported that SDF testing is commonly ordered by 81.6% of responders who most commonly utilized the TUNEL and SCSA methods for SDF assessment [7]. This survey was part of a special issue on "Sperm DNA Fragmentation" in which we have identified specific clinical scenarios in which SDF testing would be most beneficial. These guidelines, endorsed by the society for Translational Medicine, have identified the following clinical indications for SDF testing:

Clinical Varicoceles

Varicocele is a vascular abnormality of the testicular venous drainage caused by the dilation and swelling of the pampiniform and/or cremasteric plexus. It is a very common condition prevalent in about 20% of the general male population [16]. While a good number of men with clinical varicocele are fertile, the condition is considered to be the most common correctable cause of infertility seen in about 40% of men with primary infertility and up to 80% of men with secondary infertility [16]. The impact of varicocele treatment on fertility status has been the subject of considerable debate. It is believed that proper patient selection is of utmost importance and that's where SDF testing may be beneficial. DNA damage from varicoceles can occur due to many factors mostly related to testicular hyperthermia and intratesticular blood stasis, resulting in hypoxia and oxidative damage [8].

Significantly worse SDF levels has been observed in infertile men with varicocele compared with counterparts without varicocele. This was associated with worse conventional semen parameters, early sperm apoptosis and abnormal mitochondrial membrane potential [17]. On the other hand, several studies have revealed a significant improvement in SDF levels after varicocelectomy that coincided with improved conventional semen parameters and most importantly with better pregnancy rates [18].

These findings lead Agarwal et al. to recommend SDF testing in patients with clinical varicocele to help in better selecting surgical candidates [8].

Unexplained Infertility, Recurrent Pregnancy Loss, or Intrauterine Insemination Failures

Male infertility may be present despite normal fertility evaluation and semen analysis. This occurs in 10–30% of couples seeking testing [8]. SDF testing in these men revealed a high DNA fragmentation index [8]. The same applies for couples with recurrent pregnancy loss and IUI failure [8].

SDF testing is indicated in men with unexplained infertility as studies have shown that even in men with normal conventional semen parameter results, high levels of sperm DNA fragmentation may be detected [8, 19, 20].

In a prospective study including 25 couples with unexplained infertility, the percentage of patients with SDF above 20% and 30% was 43% and 29%, respectively. All 25 couples were treated with ovarian stimulation and IUI. The proportion of couples who achieved pregnancy was significantly reduced when SDF rates were more than 20% [19].

Another study echoed similar results with successful pregnancy achieved at a higher rate (7–8.7 times) when the male partners had lower SDF levels [20, 21]. Saleh *et al*. observed that the SDF index, assessed by the SCSA assay, was higher in infertile men with normal SA (23%; interquartile range, 15–32%) than in fertile controls (15%; interquartile range, 11–20%) [22].

High level of SDF has been associated with recurrent spontaneous abortion, defined by two or more spontaneous miscarriages before 20 weeks of gestation. A study that evaluated 45 couples with RSA found that they have higher SDF rates (1.2 times) than controls (28.1 ± 4.9 vs. 21.7 ± 4.7, respectively; $P < 0.05$) [23].

Effect on IVF and ICSI

During conventional IVF, the prolonged exposure of the gametes to culture media would theoretically increase oxidative stress and the level of SDF, thereby imposing a risk on the IVF outcome. Conversely, during ICSI, the sperm is directly injected into the ovum, which utilizes its energy to repair any DNA damage right after fertilization [24]. This belief was, to a certain degree, proven by a number of systematic reviews reporting a significant negative impact for SDF levels on pregnancy rates with conventional IVF but not with ICSI [25, 26]. On the other hand, a significant relationship between SDF levels and miscarriage rate following both conventional IVF and ICSI has been reported [27]. A systematic review by Zini and Sigman showed that SDF was associated with a significant increase in the rate of miscarriage after IVF and ICSI with a combined OR of 2.48 (95% CI, 1.52–4.04; $P < 0.0001$) [28].

Risk Factors

SDF testing is indicated in men exposed to risk factors that can contribute to oxidative stress. Risk factors can be nonmodifiable, such as aging. Advancing age is associated with increased frequency of sperm DNA damage [6].

SDF testing can encourage infertile men to implant lifestyle modification to limit their exposure to modifiable risk factors, which include smoking, obesity, occupational exposure (lead and cadmium), organochlorine pollutants or pesticides (polychlorinated biphenyls and metabolites of dichlorodiphenyltrichloroethane), bisphenol A (compound widely used in plastic containers) [8].

A study evaluated the impact of cigarette smoking and alcohol consumption on semen parameters and sperm fragmentation measured by Halosperm. All parameters, including semen volume, percent of degenerated spermatozoa, and SDF, were significantly correlated with smoking status, and both smoking and alcohol consumption (separately or combined) were found to have deleterious effects on sperm parameters and SDF [29].

A study also correlated obesity with sperm DNA damage and found that the rate of sperm DNA damage measured by the TUNEL assay is increased in obese men with an odds ratio (CI of 95%) of 2.5 (1.2–5.1) [30].

Treatment

Conservative and Counseling Methods

Several conservative maneuvers can be performed aiming at reducing the SDF level. Ejaculatory abstinence time is believed to influence the SDF levels with shorter abstinence times through repetitive ejaculations, which have been found to lower SDF values [31, 32]. Agarwal et al. reported that a one- to two-day ejaculatory abstinence time resulted in significant reductions in SDF compared to longer abstinence. Although the ideal ejaculatory abstinence time is not yet determined, patients can be counselled to undergo repetitive ejaculations during the period of ovulation to minimize the effect of SDF on the likelihood of conception.

Patients can also be counselled to avoid risk factors that have been implicated in producing SDF. These include the following:

1. Physical factors such as radiation and heat, cigarette smoke, and airborne pollutants
2. Chemical agents such as anticancer drugs and sexually transmitted infections
3. Biological factors such as increasing male age, elevated body mass index, and diabetes

Infections should be controlled because several studies have shown that male genital infection and inflammation can increase SDF by 8–35% [33]. Inflammation can lead to the production of oxidative stress, which is known to cause DNA modification and damage.

Medical Treatment: Antioxidants

As mentioned before, oxidative stress is an important cause of SDF.

Antioxidants that are available in the semen are composed of enzymes such as glutathione peroxidase, superoxide dismutase, and catalase, as well as nonenzymatic compounds such as vitamins A, E, C, and B complex; pantothenic acid; coenzyme Q10 and carnitine; and micronutrients such as zinc, selenium, and copper. They provide protection against reactive oxygen species through either quenching or neutralizing their effects and maintaining a balanced redox potential.

Spermatozoa are particularly vulnerable to the harmful effects of reactive oxygen species (Fig. 21.2). They affect their activity, damage DNA structure, and accelerate apoptosis. Therefore, the use of antioxidants as a medical treatment for infertile men specifically those with SDF would be effective.

Antioxidants are compounds that could be consumed in the diet or can be taken as an oral supplement. They are the most common treatment prescribed for infertility, regardless of the cause [34, 35].

A study has shown that treatment of men with a DNA fragmentation index >30% with a 30- to 90-day course of antioxidant was associated with a statistically significant decrease in the DFI [36].

Many studies have shown the importance of antioxidants in infertile men and specifically in patients with SDF.

The combination of the following antioxidants has shown to improve sperm quality in terms of basic seminal parameters and DNA damage: L-Carnitine, vitamin C, CoQ10, vitamin E, zinc, vitamin B9, selenium vitamin B12 [37].

On the other hand, glutathione is a master antioxidant as it reduces oxidative damage by neutralizing the harmful free radicals. It has a synergistic effect with selenium. An observational study has shown that the use of glutathione for 2 months leads to a significant improvement in sperm concentration and a significant decrease in oxidative DNA damage [35].

Treatment of infertile men with docosahexaenoic acid (DHA) showed a significant decrease in SDF levels (p value <0.001), but there was an insignificant effect on semen parameters [38].

In addition to the previously mentioned antioxidants, L-carnitine has a pivotal role in cellular energy production; it necessary for mitochondrial oxidation of long-

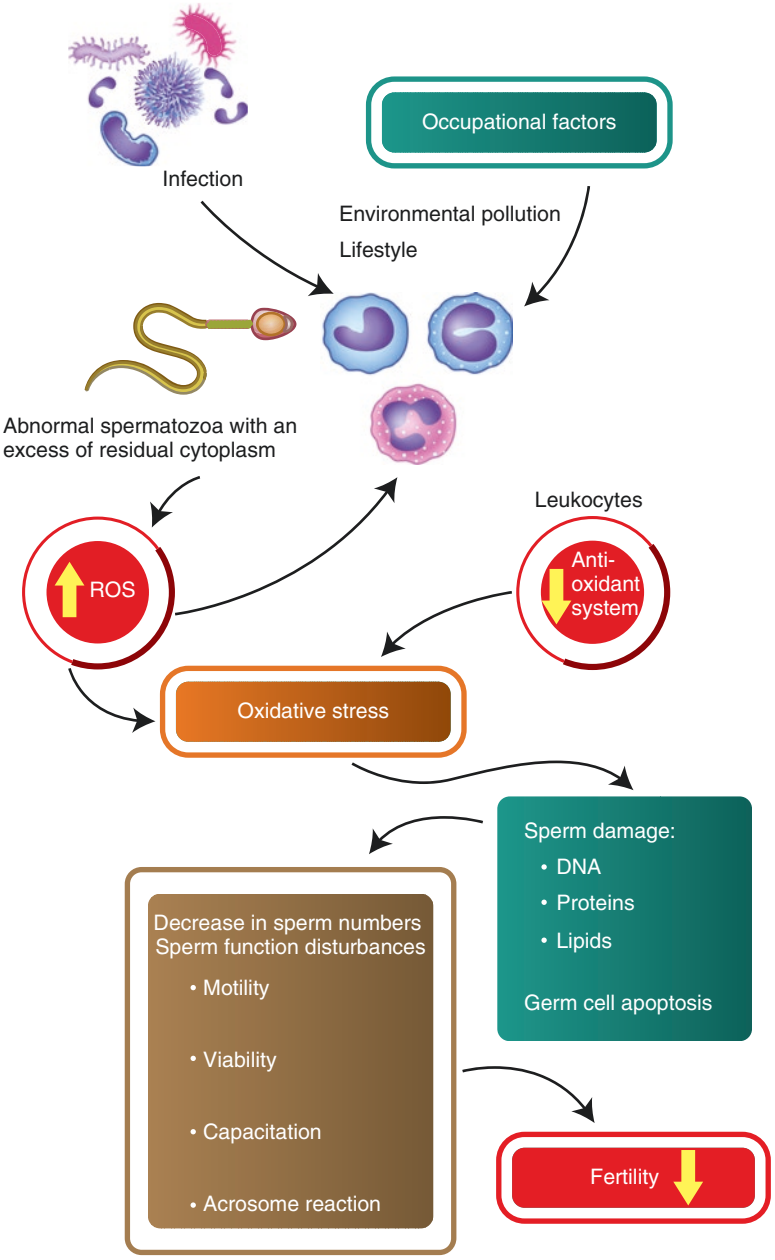

Fig. 21.2 The role of oxidative stress and antioxidants in male fertility

chain fatty acids. It also protects the cell membrane and DNA against damage induced by free oxygen radicals. The highest levels of L-carnitine in the human body are found in epididymal fluid, whose concentration is around 2000 times higher than in circulating blood [39]. A prospective observational study has shown that combining L-carnitine with vitamins C, E, B9, and B12; coenzyme Q10; zinc; and selenium results in decreased SDF levels, as well as increased sperm concentration in males with grade 1 varicocele [40].

Finally, lycopene, which is found in high levels in seminal fluid, provides protection against lipid and DNA oxidation and neutralizes ROS. A study published in 2015 was conducted on 21 normozoospermic males with idiopathic infertility and 23 males with semen abnormalities. After 3 months of therapy, the use of lycopene in infertile men has led to a significant improvement in the AA/DHA ratio in seminal plasma and has facilitated spontaneous pregnancy (16%), as well as IVF conception (42%) [41].

Surgical Treatment – Varicocele Ligation

Varicocele ligation, also known as varicocelectomy, is the most commonly performed surgery for the treatment of male infertility. This surgery can be performed at various anatomical levels, ranging from open to laparoscopic to microsurgical varicocelectomy [42]. The indications for varicocelectomy include the following: infertility with impaired semen parameters, hypogonadism, scrotal pain, testicular hypotrophy (mainly in children), or aesthetic issues with very large varicoceles [43].

Regarding varicoceles and infertility, the American Urological Association recommends that varicocele treatment should be given to the male partner of a couple attempting to conceive when all of the following are present: documented infertility, palpable varicocele, the female having normal fertility or potentially correctable infertility, and the male having one or more abnormal semen parameters or sperm function test results [44].

Varicoceles can be treated by either percutaneous occlusion/embolization (by the intravenous injection of specific materials to occlude the varicocele) or surgical ligation/clipping of the varicocele to prevent venous reflux [42].

Surgical ligation remains the most popular treatment of varicoceles, whereas percutaneous occlusion is reserved as a treatment option for persistent or recurrent varicoceles after surgical repair [42]. The effect of varicocelectomy on DNA damage was evaluated in an extended list of literature (Table 21.1).

A prospective study on 72 men with at least one-year history of infertility found that DNA fragmentation index (DFI) decreased significantly after varicocelectomy, from 34.5% to 28.2% ($P = 0.024$). All other sperm parameters (count, concentration, motility, and morphology) increased significantly [45].

Another meta-analysis on seven studies emphasized on the important role of varicocelectomy in restoring fertility, reducing DNA fragmentation, and concluded that it can improve sperm DNA integrity [46].

In a recent review on the role of varicocelectomy [47], Roque and Esteves concluded that the current evidence confirms the effectiveness of varicocelectomy as a means for both reducing oxidative stress, which results in sperm DNA damage, and potentially improving fertility [47].

Assisted Reproductive Treatment

Several treatments can be performed during the course of assisted reproduction in order to minimize or eliminate the detrimental effects of high SDF levels on the reproductive outcomes. These treatments include the following.

Table 21.1 Summary of studies evaluating the effect of varicocelectomy on sperm DNA fragmentation

Study	Design	Patients	Results
Zini, 2005	Retrospective cohort	37 patients with varicocele who had microsurgical subinguinal varicocelectomy performed	Mean SDF decreased after varicocelectomy (pre: 27.7%, post: 24.6%; $P = 0.04$).
Sakamoto, 2008	Retrospective cohort	30 infertile men with grade 2 or 3 varicocele (15 oligozoospermic and 15 normozoospermic) who had microsurgical subinguinal varicocelectomy performed	TUNEL-positive sperm decreased significantly 6 months after treatment (pre: 79.6%, post: 27.5%; $P < 0.001$).
Werthman, 2008	Retrospective cohort	11 patients with clinical varicocele and DFI >27% who had microsurgical subinguinal varicocelectomy performed	Ten of the 11 patients showed a significant decrease in SDF 3–6 months after varicocelectomy. Seven of the 11 patients showed a decrease in DFI to normal level, and the mean percent change in DFI was 24%.
Moskovtsev, 2009	Retrospective cohort	Patients with clinical varicocele treated with oral antioxidants alone (37 men) or subjected to both microsurgical subinguinal varicocelectomy and oral antioxidants (9 men)	SDF decreased in 78% of patients subjected to both varicocelectomy and oral antioxidants (pre: 44.7%, post: 28.4%; $P < 0.03$). No improvement in SDF was observed in patients on oral antioxidants alone (pre: 45.3%, post: 42.5%).

(continued)

Table 21.1 (continued)

Study	Design	Patients	Results
Smit, 2010	Prospective cohort	49 patients with clinical varicocele and oligozoospermia who had high inguinal ligation (36 men) or microsurgical varicocelectomy (8 men) performed	Improvement in SDF was observed after treatment (pre: 35.2%, post: 30.2%; $P = 0.019$). Thirty-seven percent of couples conceived naturally, and 24% achieved pregnancy with assisted reproduction after treatment. Mean postoperative DFI was significantly lower in couples who conceived naturally or with assisted reproduction than those who did not (spontaneous pregnancy: 30.1% vs 37.5%, assisted reproduction: 21.3% vs 36.9%).
Zini, 2011	Prospective cohort	25 patients with clinical varicocele and abnormal semen parameters who had microsurgical subinguinal varicocelectomy performed	Improvement in SDF was observed at 4 and 6 months after varicocelectomy (pre: 18%, 4 months: 10%, 6 months: 7%).
Lacerda, 2011	Prospective cohort	21 adolescents (ages 15–19) with grade 2 or 3 varicocele who had microsurgical subinguinal varicocelectomy performed	Sperm with intact nuclear DNA (comet class I) increased after varicocelectomy (49.6–64.5%, $P = 0.011$).
La Vignera, 2012	Not specified	30 patients with grade 3 left varicocele and oligoasthenoteratozoospermia who had microsurgical subinguinal varicocelectomy performed	There was significant reduction in SDF at 4 months after varicocelectomy (5.0–2.1%, $P < 0.05$), and postoperative results were similar to that of healthy controls (2.0%).
Li, 2012	Not specified	19 patients with clinical varicocele who had microsurgical subinguinal varicocelectomy performed	SDF was higher in men with varicocele than controls (28.4% vs 17.4%, $P = 0.007$). DFI decreased 3 months after operation (28.4–22.4%, P 0 0.018), and postoperative results were similar to that of controls.

Table 21.1 (continued)

Study	Design	Patients	Results
Baker, 2013	Retrospective cohort	24 patients with clinical varicocele who had microsurgical subinguinal varicocelectomy performed	SDF decreased after varicocelectomy (40.8–24.5%). A higher preoperative SDF was associated with a larger improvement postoperatively. Postoperative SDF in pregnant and nonpregnant couples showed no difference (22.2% vs 25.7%).
Kadioglu, 2014	Retrospective cohort	92 infertile patients with clinical left varicocele and abnormal semen analysis who had microsurgical subinguinal varicocelectomy performed	SDF decreased 6 months after varicocelectomy (42.6–20.5%, $P < 0.001$). A higher preoperative SDF was associated with a larger improvement postoperatively.
Ni, 2014	Prospective cohort	42 infertile men with clinical left varicocele and abnormal semen parameters who had microsurgical varicocelectomy performed	Higher DFI was observed in the preoperative group compared to controls (27.4% vs 11.5%, $P < 0.01$). DFI in patients who achieved pregnancy (20.6%) was lower than preoperative value (27.4%) and those of nonpregnant patients (24.7%). DFI in patients who achieved pregnancy after varicocelectomy was not significantly different from controls (20.6% vs 11.5%).
Pourmand, 2014	Randomized controlled trial	100 infertile patients with clinical left varicocele or subclinical varicocele who had varicocelectomy alone (group 1) or varicocelectomy, plus oral L-carnitine for 6 months (group 2)	Improvement in SDF was observed in both groups after varicocelectomy (group 1: 14.0–9.5%, group 2: 13.9–8.5%). The results were not different between groups.
Telli, 2015	Prospective cohort	72 infertile patients with clinical varicocele and oligozoospermia who had macroscopic inguinal varicocelectomy performed	SDF decreased after varicocelectomy (34.5–28.2%) with a mean follow-up of 6.2 months.

(continued)

Table 21.1 (continued)

Study	Design	Patients	Results
Tavalaee, 2015	Not specified	23 infertile patients with grade 2 or 3 left varicocele who had varicocelectomy performed	SDF improved 3 months after varicocelectomy (15.9–10.8%, $P < 0.001$).
Mohammed, 2015	Prospective cohort	75 infertile patients with clinical varicocele and altered semen parameters who had subinguinal varicocelectomy performed with loop magnification	Higher DFI was observed in preoperative patients than controls (32.4% vs 18.2%, $P = 0.003$). DFI deceased significantly after varicocelectomy (32.4–20.0%, $P = 0.05$). DFI in patients who achieved pregnancy at 1 year was significantly lower than that in patients who did not (16.4% vs 24.2%, $P = 0.04$).
Alhathal, 2016	Prospective cohort	29 infertile patients with clinical varicocele and abnormal semen parameters who had microsurgical subinguinal varicocelectomy performed	DFI was significantly higher in preoperative patients than controls (20.0% vs 7.4%, $P = 0.01$). DFI improved significantly after varicocelectomy (20.0–12.0%, $P = 0.001$).
Ni, 2016	Not specified	51 patients with clinical varicocele and abnormal semen analysis who had microsurgical retroperitoneal high ligation performed	SDF was higher in patients with clinical varicocele (range: 20.6–30.0%) compared to patients with subclinical varicocele (14.9%) and controls (12.0%). SDF reduced in patients with clinical varicocele and altered semen parameters, irrespective of clinical grade of varicocele. SDF was lower in patients who achieved pregnancy than in nonpregnant patients.
Abdelbaki, 2017	Prospective controlled cohort	60 infertile patients with clinical varicocele and abnormal semen parameters who had inguinal varicocelectomy performed with loop magnification	A higher DFI was observed in patients with varicocele than controls (29.9% vs 7.6%). DFI improved 3 months after varicocelectomy (29.9–18.8%, $P < 0.001$).

Sperm Selection Techniques

Sperm selection techniques are being recently employed in ART, most commonly in cycles of ICSI. These techniques are thought to improve the chance that structurally intact and mature sperm with high DNA integrity are selected for fertilization. These techniques include choosing the best spermatozoa according to surface charge, sperm apoptosis, sperm birefringence, sperm morphology under ultra-high magnification and ability to bind to hyaluronic acid [48]. Two techniques for excluding sperm with damaged DNA, namely, motile sperm organelle morphology examination (MSOME) and physiologic ICSI (PICSI) using hyaluronic acid-selected spermatozoa, received a significant amount of attention.

Studies investigating these sperm selection modalities have revealed conflicting results. Parmegiani et al. reported a SDF relative reduction by 67.9%, measured with SCD, while using PICSI [49]. While Rashki Ghaleno et al. reported that PICSI is an unreliable method for excluding sperm with high SDF prior to ICSI [50]. Similar findings were also reported in studies examining the effectiveness of MSOME [51, 52]. In a report evaluating 448 ICSI cycles from couples whose men were infertile due to high level of SDF, there were lower live-birth rates (24.2%) in the group with no intervention, compared to patients who underwent intracytoplasmic MSOME (28.7%), and PICSI (38.3%) [53]. The ability of other sperm selection techniques such as swim up technique and density gradient centrifugation to remove single and double strand DNA damage was tested. The results showed that such methods are equally efficient in eliminating spermatozoa containing double-strand DNA damage and sperm with highly damaged (degraded) DNA and that density gradient centrifugation is more efficient than swim up technique in selecting spermatozoa that are free from single-strand DNA damage [54].

Sperm Retrieval Techniques

The goal of sperm retrieval is to obtain sperm with best quality, adequate number for both immediate use and cryopreservation if possible, and to minimize the damage to the reproductive tract.

Sperm retrieval techniques are surgical methods originally developed to obtain spermatozoa from the epididymides and testicles of azoospermic men seeking ART.

However, their use in patients with high SDF stems from the understanding that in the majority of cases, such damage is accelerated during epididymal transit, indicating that the testicular sperm should contain lower levels of SDF than the ejaculated sperm. A few reports have confirmed this phenomenon by finding significantly higher levels of SDF in ejaculated sperm compared with testicular sperm [55, 56, 57].

Evidence shows that there is more DNA fragmentation in epididymal and ejaculated sperm than in testicular sperm [53]. In a systematic review and meta-anal-

ysis done in 2017 on five studies involving 143 patients, testicular and ejaculated sperm were compared for SDF. Clinical pregnancy rates were higher in the category of testicular sperm than in the category of ejaculated sperm, as were live-birth rates. On the other hand, miscarriage rates were lower with testicular sperm ICS [58].

We conducted a prospective study on 36 men with high-SDF levels who had a previous ICSI cycle from their ejaculates. A subsequent ICSI cycle was performed using spermatozoa retrieved through testicular sperm aspiration (TESA). Results of the prior ejaculate ICSI were compared with those of the TESA-ICSI. While there was no difference in the fertilization rate and embryo grading using ejaculate and testicular spermatozoa, clinical pregnancy was significantly higher in the TESA group compared to the ejaculated group (38.89% vs. 13.8%). Moreover, 17 live births were documented in the TESA group, and only three live births were documented in the ejaculate group ($p < 0.0001$).

The use of testicular sperm instead of ejaculated sperm assumes that the testicular sperm is of better quality. In comparing testicular to ejaculated sperm in the same patients, testicular sperm has been found to have lower SDF [59].

Conclusion

The role of SDF on male fertility has been a subject of great interest in this field of medicine. Several methods for SDF testing are available which is indicated in patients with clinical varicocele, unexplained infertility, recurrent miscarriage, assisted reproductive therapy failure and patients with lifestyle risk factors. Many interventions aiming to reduce SDF have been suggested including lifestyle changes, antioxidant use, varicocelectomy, sperm selection or use of testicular sperm prior to ICSI. Further studies are required to clarify the ideal treatment options for this group of patients.

Review Criteria
Extensive literature search was performed on search engines such as PubMed, Medline, Cochrane, Google Scholar, and ScienceDirect databases. Information from studies published for the past five decades until August 2018 was extracted. The literature search was limited only for the articles written in English language. “Sperm DNA damage and fragmentation” and “male infertility” were the main key terms used for conducting literature search. Book chapters and data published in scientific meetings relevant to sperm DNA damage were also included in this review.

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