Sperm DNA Fragmentation: A New Guideline for Clinicians

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Sperm DNA integrity is crucial for fertilization and development of healthy offspring. The spermatozoon undergoes extensive molecular remodeling of its nucleus during later phases of spermatogenesis, which imparts compaction and protects the genetic content. Testicular (defective maturation and abortive apoptosis) and post-testicular (oxidative stress) mechanisms are implicated in the etiology of sperm DNA fragmentation (SDF), which affects both natural and assisted reproduction. Several clinical and environmental factors are known to negatively impact sperm DNA integrity. An increasing number of reports emphasizes the direct relationship between sperm DNA damage and male infertility. Currently, several assays are available to assess sperm DNA damage, however, routine assessment of SDF in clinical practice is not recommended by professional organizations. This article provides an overview of SDF types, origin and comparative analysis of various SDF assays while primarily focusing on the clinical indications of SDF testing. Importantly, we report four clinical cases where SDF testing had played a significant role in improving fertility outcome. In light of these clinical case reports and recent scientific evidence, this review provides expert recommendations on SDF testing and examines the advantages and drawbacks of the clinical utility of SDF testing using Strength-Weaknesses-Opportunities-Threats (SWOT) analysis.
INTRODUCTION

Infertility is defined as the failure of a couple to achieve a clinical pregnancy after one year of regular, unprotected sexual intercourse [1]. Infertility affects more than 15% of couples globally with male factors alone or in combination with female factors, contributing to 50% of the cases [2]. Evaluation of infertile men still relies on conventional semen analysis, though it alone does not accurately predict male fertility potential and success of assisted reproductive technology (ART) [3]. In fact, about 15% of infertile patients have a normal semen analysis [4]. However, assessment of sperm concentration, motility and morphology may not fully reflect impaired sperm DNA integrity [5], which is detrimental for normal fertilization, embryo development and success of ART [6].

Sperm DNA fragmentation (SDF) can be caused by extrinsic factors (i.e., heat exposure, smoking, environmental pollutants, and chemotherapeutics) as well as intrinsic factors (i.e., defective germ cell maturation, abortive apoptosis, and oxidative stress [OS]) [7]. Compelling evidence demonstrates that OS is a major contributor to male infertility [8]. Reactive oxygen species (ROS) are vital for physiological processes such as apoptosis and capacitation, but an overproduction leads to various deleterious consequences including SDF [9]. Types of DNA damage include mismatch of bases, loss of base (abasic site), base modifications, DNA adducts and crosslink, pyrimidine dimers and single strand

![Fig. 1. Different types of DNA damage that can occur at DNA level: mismatched bases, abasic sites, base modifications (oxidation, alkylation, deamination), adducts and intrastrand crosslinks, pyrimidine dimers, and single and double strand fragmentation. ROS: reactive oxygen species, UV: ultraviolet.](image)

Keywords: Assisted reproductive techniques outcome; Clinical guidelines; Infertility, male; Oxidative stress; Sperm DNA fragmentation
breaks (SSB) and double strand breaks (DSB) (Fig. 1). Any of these alterations can induce SDF and compromise natural conception or ART outcomes.

Since 1999, there has been a significant increase in the number of studies reporting an association between SDF and male infertility. According to a recent scientometric analysis, the primary focus of SDF research in the past 20 years has emphasized lifestyle factors, varicocele, and asthenozoospermia [10]. Increased SDF levels have been implicated in male infertility while being associated with conditions such as varicocele, male accessory gland infection, advanced paternal age, cancer, chronic illness, exposure to environmental toxins and lifestyle factors [11].

Moreover, numerous studies have found that increased SDF adversely impacts conception rates [12-14]. Evidence shows that DNA damage in spermatozoa can affect the health and well-being of offspring [15]. Consequently, the negative impact of SDF on the male fertility potential may encourage more clinicians to utilize SDF testing in the clinical setting [16]. Interventions have also been explored to improve fertility outcomes and promote healthy offspring.

The present article aims to highlight the clinical utility of SDF testing by providing current evidence for its use in the management of the infertile male. This review begins by examining the underlying mechanisms and risk factors of SDF. It then describes the clinical tests associated with different types of DNA fragmentation, followed by the clinical indications for SDF testing. Finally, male infertility case scenarios with high SDF are presented along with expert recommendations on its management and an illustration of strengths, weaknesses, opportunities and threats (SWOT) of SDF testing.

Fig. 2. Overview of the origins of sperm DNA fragmentation (SDF). SDF result from underlying mechanisms such as defective maturation, abortive apoptosis, and oxidative stress. Moreover, clinical (age, infection, cancer, hormonal imbalances, obesity, diabetes) and environmental (heat exposure, environmental toxins, radiation, smoking, drug abuse, diet) risk factors lead to SDF. MAPK: mitogen-activated protein kinase, ERK: extracellular signal-regulated kinase, JNK: c-JUN N-terminal kinase, ROS: reactive oxygen species, ART: assisted reproductive techniques.
ORIGIN OF SPERM DNA FRAGMENTATION

1. Primary mechanisms underlying sperm DNA fragmentation

SDF is primarily induced by defective maturation and abortive apoptosis occurring within the testis, or by OS throughout the male reproductive tract [17]. During spermatogenesis, chromatin is compacted through histone exchange with transitional proteins and protamines [18]. This is facilitated by the endogenous nuclease topoisomerase II, creating DNA breaks to reduce torsional stress for histone disassembly and chromatin packaging [19-21]. If these breaks are not repaired, impairment of chromatin packaging may result in defective maturation and the appearance of sperm with increased SDF in the ejaculate [22-27]. SDF can also be induced by abortive apoptosis during spermatogenesis. Apoptosis ensures that no defective germ cells differentiate into spermatozoa, however failure of this process may result in the accumulation of spermatozoa expressing apoptotic markers in the ejaculated semen (Fig. 2) [28-30]. Extrinsic apoptosis is mediated through Fas-ligand binding to a death receptors, such as Fas, activating caspase-8 or 10 [31]. Indeed, the expression of Fas in the ejaculated sperm is an indicator of increased abortive apoptosis [32]. Excessive ROS can induce DNA damage [33] and also activate apoptotic pathways in spermatozoa [34]. Moreover, SDF can be indirectly induced by OS through by-products of lipid peroxidation, particularly malondialdehyde (MDA) and 4-hydroxynonenal (4HNE) which can introduce DNA adducts, such as 8-hydroxy-2′-deoxyguanosine (8-OHdG), 1,N6-ethenoadenosine, and 1,N6-ethenoguanosine, resulting in DNA damage [33,35-37]. On the other hand, direct oxidative damage to DNA bases results in formation of adducts such as 8-hydroxy-2-deoxyguanosine (8OHdG), particularly at sites with poor protamine shielding [24,25]. OS further activates the MAPK pathway, increasing p53 and caspase 3 expression and reducing bcl-2, thereby impairing maturation and promoting apoptosis [38]. OS activates intrinsic apoptotic pathways in spermatozoa, where externalization of phosphatidylserine is an early marker and SDF is a late marker of apoptosis [34]. This process is initiated through a mitochondrial-mediated pathway, where cytochrome c is released into the cytosol resulting in proteolytic activation of caspase 3, 6, and 7 [39,40].

2. Clinical and environmental risk factors of sperm DNA fragmentation

SDF increases with age, starting in reproductive years and doubling between the ages of 20 and 60 years [41-43]. This association has been attributed to higher exposure to OS, defective sperm chromatin packaging, and disordered apoptosis that occur with aging [44]. Clinical associations with increased SDF include varicocele, which induces testicular damage and SDF through increased intratesticular temperature and retrograde flow of renal and adrenal metabolites resulting in OS and apoptosis [45,46]. Genitourinary infections and subsequent leukocytospermia increases ROS production, increasing SDF [47-51]. Increase in SDF has also been reported in men with testicular cancer and other malignancies, which is suggested to be secondary to the associated endocrine alterations or OS in these pathologies [52-55].

Lifestyle and environmental factors induce SDF. Importantly, obese men have higher levels of OS and SDF compared to normal weight or overweight men [56-58]. Increased scrotal temperature, endocrine imbalance and chronic systemic inflammation are believed to be the mechanisms linking obesity with altered sperm function and reduced fertility potential. Indeed, studies have shown significant improvement in SDF and overall fertility with weight loss [59,60]. Men with diabetes demonstrate higher levels of SDF due to OS, in association with the generation of advanced glycation end products [61,62].

SDF and chromatin decondensation is observed with a subtle 2°C–3°C increase in physiologic scrotal temperature [63-66], partly mediated through OS induced apoptosis and elevated stress-inducible protein expression [67-69]. Increased scrotal temperature is induced by physical abnormalities such as cryptorchidism, retractile testes and varicocele, as well as in acute febrile illnesses and sedentary lifestyles [68,70-72].

Some studies demonstrate increased SDF with air pollution [73-75]; while others have found no difference [76-78]. Exposure to heavy metals such as lead, cadmium [79,80], fenvalerate (synthetic insecticide) [81] and organophosphorus pesticides [82] can cause DNA damage. The effect of occupational toxins depends on proximity and duration of exposure [83]. Bisphenol A and styrene found in synthetic rubber or polyesters, also alters sperm DNA integrity [84-87].

Cigarette smoking negatively impacts DNA integrity.
due to tobacco metabolites [92] such as nicotine [93], cadmium [79,94], lead [79,80,95] and benzopyrene [96]. Alcohol consumption can also increase SDF and cause apoptosis [97-99].

Electromagnetic waves, particularly from cell phones, increase mitochondrial ROS production and DNA aduct formation causing DNA damage [100-102]. Furthermore, radiation therapy for cancer can cause SDF [103].

These clinical and environmental risk factors increase the production of ROS by different mechanisms, leading to OS and ultimately result in SDF [104-108].

**SPERM DNA FRAGMENTATION: SINGLE-VERSUS DOUBLE-STRAND BREAKS**

DNA fragmentation is characterized by both SSBs and DSBs. In DNA with SSBs, the other strand can act as a template for replication. SSBs are caused by the action of abortive topoisomerase or DNA ligase activity adjacent to a lesion, which can covalently bind to phosphate and can thereby be fixed. The most commonly occurring lesions are base and sugar modifications and SSBs following oxidation, alkylation, deamination, and spontaneous hydrolysis [109]. When these lesions are not repaired, they can compromise the integrity of the genome [110]. Moreover, OS, lipid peroxidation and protein alteration may also lead to SSBs [111] (Fig. 3A).

In general, DSBs are considered harmful to the genomic DNA as they result in genetic rearrangements. DSBs are produced from endogenous sources as a consequence of SSBs during the DNA replication process [112], collapsed replication forks [113], or increased levels of free radicals [112] (Fig. 3B). Furthermore, exogenous causes such as ionizing radiation, genotoxic chemicals, radiomimetic drugs can also lead to DSBs [112,114,115].

Both SSBs and DSBs present in sperm DNA can affect the overall fertility and reproductive outcomes. DSBs negatively affect embryo kinetics and implantation rates, and have been associated with recurrent miscarriages in couples without a female factor [116,117]. In contrast, SSBs do not significantly impact embryo development or implantation rates [117]. Nonetheless, higher levels of SSBs are inversely related to the natural pregnancy outcome [118]. Thus, evaluation of SSB and DSB may provide important information during fertility evaluation of men [119]. Sperm DNA integrity can be determined using terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) assay and other direct tests, such as sperm chromatin structure assay (SCSA) and sperm chromatin dispersion (SCD). However, these assays cannot distinguish between SSBs and DSBs present in the DNA [117]. Depending on the methodology, i.e., either neutral (DSBs) or alkaline (SSBs and DSBs), this distinction can be made only by the Comet assay [120]. The two-tailed Comet assay can directly differentiate between the SSBs and DSBs [121]. On the other hand, the newest SDF test introduced for the immunodetection of gamma histone 2AX (γH2AX), can only assess DSBs [115]. The γH2AX is the phosphorylated form of γH2AX from the histone 2A family and is a highly specific and sensitive molecular marker of DSBs [122].

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**Fig. 3.** (A) Main insults that result in DNA single strand breaks are abortive topoisomerase, free radicals, and DNA ligase activity adjacent to lesion. (B) Main insults that result in DNA double strand breaks are free radicals, collapsed replication forks, replication in DNA strand with single-stranded breaks, ionizing radiation, genotoxic chemicals, and radiomimetic drugs.
| Assay | Reference | Pregnancy outcome | Cut off (%) | AUC | Sensitivity | Specificity | PPV | NPV |
|-------|-----------|-------------------|-------------|-----|-------------|-------------|------|------|
| TUNEL | Benchaib et al (2003) [322] | Evaluation of predictive power of different SDF values in IVF/ICSI | 4 | 76 | 25 | - | - |
|       |           |                   | 15 | 15 | 90 | - | - |
|       |           |                   | 18 | 14 | 95 | - | - |
|       |           |                   | 20 | 11 | 100 | - | - |
|       | Esbert et al (2011) [410] | SDF in morphologically normal sperm for ICSI | 17.6 | 0.70 | 61.5 | 82.6 | 66.7 | 79.2 |
|       |           | IVF performed with own oocytes | 11 | 0.60 | 76.7 | 54.3 | - | - |
|       |           | IVF performed with donor oocytes | 15 | 0.53 | 56.1 | 64.1 | - | - |
| Comet | Simon et al (2010) [311] | Native semen in IVF | 56 | - | 82.1 | 49.7 | 26.7 | 92.6 |
|       |           | Native semen in ICSI | 56 | - | 47.2 | 68.8 | 40.5 | 74.3 |
|       |           | DGC-selected sperm in IVF | 44 | - | 92.3 | 34.6 | 22.8 | 95.5 |
|       |           | DGC-selected sperm in ICSI | 44 | - | 54.6 | 63.4 | 44.4 | 72.2 |
|       | Ribas-Maynou et al (2012) [116] | Alkaline comet in natural conception | 45.6 | 0.97 | 93.3 | 90.7 | - | - |
|       | Simon et al (2017) [6] | Alkaline comet in ART | 52 | - | 68.7 | 63.5 | 36.7 | 86.8 |
| SCSA | Larson-Cook et al (2003) [323] | IVF/ICSI cycles | 27 | - | 16.4 | 100 | 100 | 35.4 |
|       | Jiang et al (2011) [126] | ICSI cycles | 10.3 | 0.75 | 50 | 94.9 | 85.7 | 75.5 |
|       | Zhang et al (2016) [127] | IVF cycles | 11.3 | 0.57 | 56.1 | 60.0 | 77.9 | 35.1 |
|       |           | ICSI cycles | 30.3 | 0.57 | 50.6 | 68.8 | 79.3 | 37.0 |
| SCD  | Meseguer et al (2011) [329] | Use of swim-up selected sperm and own oocytes in IVF/ICSI | 9.7 | - | 78.6 | 40.5 | - | - |
|       | Nuñez-Calonge et al (2012) [128] | ICSI cycles | 17 | 0.70 | 77.8 | 71.1 | - | - |
|       | Ribas-Maynou et al (2012) [116] | Natural conception | 22.5 | 0.90 | 76.8 | 92.9 | - | - |
|       | Gosálvez et al (2013) [129] | Swim-up selected sperm in ICSI | 17.5 | 0.74 | 81.0 | 73.0 | - | - |
|       |           | Neat semen in ICSI | 26 | 0.71 | 75.0 | 65.0 | - | - |
|       | Lópeze et al (2013) [130] | In IVF/ICSI cycles | 25.5 | 0.55 | 86.2 | 28.9 | 48.7 | 72.7 |
|       | Jin et al (2015) [131] | In IVF cycles of women with reduced ovarian reserve | 27.3 | 0.59 | 96.8 | 24.3 | 31.5 | 68.2 |
|       | Sun et al (2018) [408] | In IVF cycles | 20 | - | 41 | 40 | 63 | 21 |
|       |           | In ICSI cycles | 20 | - | 55 | 63 | 36 | 79 |

SDF: sperm DNA fragmentation, AUC: area under the curve, PPV: positive predictive value, NPV: negative predictive value, TUNEL: terminal deoxynucleotidyl transferase dUTP nick end labelling, SCSA: sperm chromatin structure assay, SCD: sperm chromatin dispersion, IVF: in vitro fertilization, ICSI: intracytoplasmic sperm injection, DGC: density gradient centrifugation, ART: assisted reproductive techniques.
WHAT TEST SHOULD I ORDER?

Several assays are used for SDF evaluation in clinical practice (Table 1). The TUNEL assay is based on labeling free 3′-OH nicks with dUTP [123]. While Comet assay identifies SDF based on electrophoretic separation of DNA where damaged DNA forms a comet-like profile. In the SCD test, a distinct “halo” (dispersed DNA loops) is observed after removal of DNA-linked proteins, while no or small halos indicate DNA damage [124]. The SCSA uses metachromatic acridine orange, which fluoresces green and red after binding to double- (native) or single-stranded (damaged) DNA, respectively [125].

As summarized in Table 1, several studies have attempted to identify clinical SDF cut-offs for the prediction of natural or ART-related pregnancy [126-131]. Although there remains no unanimous consensus on a specific cut-off value, a recent meta-analysis suggests that a cut-off of 20% can potentially differentiate between fertile and infertile men [108]. Different SDF values are reported for prediction of pregnancy in natural conception or ART (in vitro fertilization [IVF], intracytoplasmic sperm injection [ICSI], or both) settings by analyzing native semen, sperm processed by swim-up or density gradient centrifugation (DGC) as well as in cases where own or donor oocytes are used. Despite the heterogeneity of the published studies, the challenges related to the identification of unbiased cut-off values for the prediction of pregnancy and the use of SDF assays in clinical practice, it appears that the TUNEL assay is most commonly used [10], as it is accurate and reliable [132]. While it would be highly desirable if a globally accepted assay with strong predictive value would be performed by all clinics, in reality the choice of the SDF assay in individual clinics often depends on instrumentation availability, trained personnel and the cost of the assay to be performed in terms of reagents and run-time.

The diagnostic value of these tests for assisted reproduction can be increased by the evaluation of OS. A moderate correlation between ROS and SDF has been reported [133-135]. Decreased total antioxidant capacity, reflecting the amount of seminal antioxidants [136], has been associated with elevated SDF and male infertility [137] and an increased risk of spontaneous miscarriage [138]. By using oxidation reduction potential (ORP) as a measure of redox balance and SDF (by TUNEL) with cut-off values of 1.36 mV/10^6 sperm/mL and 32%, respectively, fertilization has been predicted with high sensitivity and specificity [139]. However, few studies report weak [140,141] or no correlation between ORP and SDF [134], suggesting that ORP and SDF testing might reflect the general impact of seminal OS on sperm functions and specifically on DNA, respectively. Therefore, ORP cannot be recommended as a stand-alone test in substitution of SDF evaluation, considering that other factors, such as abortive apoptosis or defects in DNA protamination, can render spermatozoa more susceptible to OS and DNA damage, even at relatively low ROS levels [142]. Moreover, defects in the protamination and in DNA condensation can leave unligated nicks [143] while a faulty DNA rearrangement may lead to severe DNA damage [144].

WHICH PATIENTS ARE SUITABLE FOR SPERM DNA FRAGMENTATION TESTING?

The considerable research conducted in recent years has improved our understanding of the clinical scenarios where SDF testing is most beneficial. We recently published clinical practice guidelines endorsed by the Society of Translational Medicine recommending SDF testing in patients with unexplained infertility, recurrent pregnancy loss (RPL), and clinical varicocele, prior to undergoing ART and in patients exposed to lifestyle risk factors and environmental toxicants [145]. An updated evidence supporting these recommendations are presented in Table 2.

For evidence-based reporting on SDF, the PubMed database was searched from the time of inception to December 2019. The search was limited to human studies published in English. The term ‘sperm DNA fragmentation’ was searched in combination with the following keywords using the Boolean expression “AND”: ‘intracytoplasmic sperm injection’, ‘fertilization in vitro’, ‘intrauterine insemination, ‘recurrent pregnancy loss’, ‘varicocele’, ‘idiopathic male infertility’, ‘unexplained male infertility’, ‘genital tract infections’, ‘male age’, ‘obesity’, ‘alcohol’, ‘smoking’, ‘air pollution’, ‘lifestyle’, ‘plastics’, ‘industrial’.

The inclusion criteria for the evidence-based reporting on SDF were (a) studies with male patients having primary or secondary infertility as target population and (b) studies reporting clinical outcome parameters
| Study reference          | Study design/participants                                                                 | SDF assay | Outcome measured       | Main results/findings                                                                                                                                                                                                 | Quality of evidence |
|--------------------------|-------------------------------------------------------------------------------------------|-----------|------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|
| Natural conception       | Malić Vončina et al (2016) [273]                                                          | TUNEL     | Conception rate        | Infertile men that did not conceive had significantly higher SDF than fertile couples. 31% infertile males conceived naturally and showed SDF values comparable to fertile males. Males with SDF < 25% and MMP > 62.5% had significantly higher odds ratio for natural conception.              | Level 4             |
| Natural conception       | Smit et al (2010) [381]                                                                  | SCSA      | Pregnancy rate         | SDF was not associated with change in pregnancy rate following vasectomy reversal.                                                                                                                                     | Level 4             |
| Natural conception       | Giwercman et al (2010) [274]                                                             | SCSA      | Fertility history      | Infertile men had significantly higher SDF than fertile men. SDF > 10% showed increased risk for infertility. Men with normal semen parameters and SDF > 10% had increased risk of infertility.                               | Level 4             |
| Natural conception       | Loft et al (2003) [275]                                                                  | 8-OHdG    | Pregnancy rate after 6 menstrual cycles | Increased oxidative damage negatively correlated with pregnancy rate.                                                                                                                                                   | Level 4             |
| Natural conception       | Spanò et al (2000) [149]                                                                 | SCSA      | Pregnancy rate after 24 months | SDF negatively correlated with pregnancy rate. SDF > 40% particularly detrimental.                                                                                                                                     | Level 4             |
| Assisted reproductive techniques (ART) | Muriel et al (2006) [397]                      | SCSA      | Pregnancy outcome     | SDF had no correlation with IUI pregnancy outcome.                                                                                                                                                                        | Level 2             |
| IUI                      | Oleszcuk et al (2016) [306]                                                              | SCSA      | Fertilization, embryo quality, pregnancy, miscarriage, and live birth rates | Negative association between increased SDF (> 20%) and standard IVF, good quality embryo and live birth rate. ICSI improved outcomes compared to IVF for SDF > 20%.                                                                 | Level 2             |
| IVF, ICSI                | Casanovas et al (2019) [117]                                                            | Comet     | Embryo kinetics and kinetics | dsSDF, not ssSDF, negatively affects embryo development in ICSI cycles.                                                                                                                                                 | Level 2             |
| ICSI                     | Wdowiak et al (2015) [327]                                                              | SCD       | Embryo morphokinetic parameters | Low SDF increased pregnancy outcome and rate of blastocyst development.                                                                                                                                                  | Level 2             |
| IVF                      | Chohan et al (2004) [400]                                                               | SCSA, SCD, TUNEL | Fertilization, embryo quality and pregnancy rates | SCSA correlated strongly with TUNEL and SCD. No significant effect of SDF for all outcomes.                                                                                                                                | Level 3             |
| Study reference          | Study design/participants                                      | SDF assay | Outcome measured                                      | Main results/findings                                                                 | Quality of evidence |
|-------------------------|----------------------------------------------------------------|-----------|-------------------------------------------------------|---------------------------------------------------------------------------------------|---------------------|
| Green et al (2020)      | Prospective cohort study of couples undergoing IVF             | SCSA      | Embryonic outcomes and clinical outcomes after euploid blastocyst transfer | Blastulation, euploidy, fertilization and miscarriage rates for SDF > 15% were not different from SDF < 15% fertilization | Level 3             |
| Al Omrani et al (2018)  | Longitudinal case-controlled cohort study of males of infertile couples undergoing ART | SCSA      | Fertilization rate and pregnancy outcome              | No significant difference in outcomes in low (<15%) and moderate (15%–30%) SDF groups while no pregnancy was achieved in case of high (>30%) SDF | Level 3             |
| Esbert et al (2018)     | Retrospective study of embryos from ICSI cycles                | TUNEL     | Embryonic cleavage, fertilization, implantation, pregnancy, and miscarriage rates | High SDF lead to delayed embryo cleavage time. No effect on fertilization, implantation, pregnancy, and miscarriage rates | Level 3             |
| Alvarez Sedó et al      | Prospective study of couples using donor eggs                  | TUNEL     | Fertilization, blastulation and pregnancy rates       | SDF > 15% negatively correlated with blastulation and pregnancy rates; with no effect on fertilization rate. Blastocytes had increased apoptotic rated in high SDF | Level 3             |
| Uppangala et al (2016)  | Cross-sectional study of couples undergoing ICSI               | Comet     | Metabolites from embryos                              | SDF was higher in the male infertility group. Embryo glutamine intensity was increased in lower SDF. No changes in amino acids, glucose, or other metabolites | Level 3             |
| Thomson et al (2011)    | Prospective cohort study of couples undergoing ART             | TUNEL and 8-OHdG | Clinical pregnancy                                      | Increased SDF and 8-OHdG negatively affected IUI but not ICSI                         | Level 3             |
| Speyer et al (2010)     | Prospective cohort study of couples undergoing ART             | SCSA      | Fertilization and implantation rates, rate of continuing pregnancies | Rate of continuing pregnancies reduced with high SDF (> 19%) in ICSI but not IVF. SDF had no effect on fertilization rate or number of embryos having more than 4 cells at day 3 after fertilization | Level 3             |
| Simon et al (2011)      | Prospective cohort study of couples undergoing ART             | Comet     | Fertilization rate, pregnancy rate and embryo quality | Increased SDF was associated with increased sperm protamination and reduced fertilization and pregnancy rates and embryo quality. SDF was lower in successful pregnancy for IVF but not ICSI | Level 3             |
| Study reference | Study design/participants | SDF assay | Outcome measured | Main results/findings | Quality of evidence |
|-----------------|---------------------------|-----------|------------------|-----------------------|---------------------|
| Xue et al (2016) [307] | Retrospective cohort of couples undergoing ART | SCSA | Fertilization, embryo cleavage rates and embryo quality grade | SDF was negatively correlated with fertilization rate in ICSI but not IVF cycles | Level 3 |
| Lin et al (2008) [314] | Prospective cohort study of couples undergoing ART | SCSA | Fertilization and pregnancy rates, good embryo quality | No significant differences between IVF and ICSI for fertilization and pregnancy rates or good embryo quality | Level 3 |
| Velez de la Calle et al (2008) [315] | Prospective cohort study of couples undergoing ART | SCSA | Fertilization and pregnancy rates, good embryo quality | Significant negative correlation for SDF and fertilization rate and embryo quality | Level 3 |
| Benchaib et al (2007) [317] | Prospective cohort study of couples undergoing ART | TUNEL | Fertilization and pregnancy rates, embryo development | SDF negatively correlated with fertilization rate in IVF and ICSI | Level 3 |
| Borini et al (2006) [318] | Prospective cohort study of couples undergoing ART | TUNEL | Clinical pregnancy and miscarriage rates, post-implantation development | SDF negatively affects embryo post-implantation in ICSI and may result in miscarriage | Level 3 |
| Zini et al (2005) [399] | Prospective cohort study of infertile couples undergoing ART | AO | Fertilization and pregnancy rates | No difference in low, moderate, and high SDF for all parameters | Level 3 |
| Huang et al (2005) [161] | Retrospective analysis of couples undergoing IVF with or without ICSI | TUNEL | Fertilization, good embryo and pregnancy rates | SDF>10% had a negative impact on fertilization rate only | Level 3 |
| Virro et al (2004) [402] | Prospective cohort study of infertile couples undergoing ART | SCSA | Fertilization, pregnancy and blastocyst rates | No impact of SDF on fertilization and pregnancy rates with IVF or ICSI | Level 3 |
| Benchaib et al (2003) [322] | Prospective cohort study of infertile couples undergoing ART | SCSA | Fertilization and pregnancy rates, embryo quality and development rate | SDF<10% resulted in increased fertilization rate | Level 3 |
| Hast et al (2000) [325] | Prospective cohort study of infertile couples undergoing ART | TUNEL | Fertilization rate | Negative correlation between SDF and fertilization rate in IVF but not for ICSI | Level 3 |
| Study reference            | Study design/participants                                                                 | SDF assay | Outcome measured                                                                 | Main results/findings                                                                 | Quality of evidence |
|---------------------------|------------------------------------------------------------------------------------------|-----------|-----------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|---------------------|
| IVF, ICSI                 | Retrospective study of embryos from couples undergoing IVF-ICSI and genetic pre-implantation cycles | SCSA      | Blastocyst aneuploidy and pregnancy rates                                          | No significant differences were found for high (>30%) moderate (15%–30%) and low (<15%) SDF | Level 3             |
| IVF, ICSI                 | Systematic review and meta-analysis (3,106 couples in 16 cohort studies included)         | Comet, SCSA, TUNEL, AO | Pregnancy and miscarriage rates                                                   | Meta-analysis showed that high-level SDF has a detrimental effect on IVF/ICSI outcome, with decreased pregnancy rate, and increased miscarriage rate | Level 3             |
| IVF, ICSI                 | Systematic review and meta-analysis (11 studies included in review)                      | SCSA, TUNEL | Influence of sperm DNA damage on risk of RPL after IVF and ICSI                    | Sperm DNA damage is predictive of pregnancy loss after IVF and ICSI                      | Level 3             |
| IUI, IVF, ICSI            | Prospective cohort study of couples undergoing ART                                         | SCSA      | Pregnancy and delivery                                                             | SDF>30% reduced IUI success                                                               | Level 3             |
| IUI, IVF, ICSI            | Prospective cohort study of infertile couples undergoing ART                               | SCSA      | Biochemical pregnancy, clinical pregnancy, and implantation rates                  | SDF>27% predicted no clinical pregnancy for IVF                                            | Level 3             |
| IUI, IVF, ICSI            | Prospective cohort of infertile couples undergoing ART                                     | SCSA      | Biochemical pregnancy, clinical pregnancy, and live birth rates                    | Reduced pregnancy and live birth rates for IUI with SDF>2.7%, but not IVF or ICSI        | Level 3             |
| IIU, IVF, ICSI            | Prospective cohort study of infertile couples undergoing IUI                               | TUNEL     | Clinical pregnancy                                                                 | Results for ICSI better than IVF for SDF>27%                                            | Level 4             |
| IVF                      | Prospective cohort study of infertile couples undergoing IVF                              | TUNEL     | D2 embryo quality, implantation, and ongoing pregnancy rates                       | High SDF showed lower clinical, ongoing pregnancy rates per embryo transfer, and lower implantation rates than low SDF High SDF spares fertilization and top embryo morphology rates but is associated with decreased IVF-ET outcome | Level 4             |
| IVF                      | Prospective cohort study of infertile couples undergoing IVF                              | TUNEL     | Pregnancy and blastocyst development rate                                         | Blastocyst development rate significantly higher in SDF<20% Clinical pregnancy was not significantly different | Level 4             |
| IVF                      | Prospective cohort study of infertile couples undergoing IVF                              | TUNEL, AO | Fertilization and pregnancy rates                                                  | High SDF (>36.5%) associated with reduced fertilization and pregnancy rates               | Level 4             |
| IVF                      | Prospective cohort study of infertile couples undergoing IVF                              | TUNEL     | Fertilization and embryo cleavage rates                                            | Negative correlation between SDF and fertilization rate and embryo cleavage rate         | Level 4             |
| IVF                      | Prospective cohort of infertile couples undergoing IVF                                    | SCSA      | Embryo cleavage                                                                   | Negative correlation between SDF and embryo cleavage                                    | Level 4             |
| Study reference | Study design/participants | SDF assay | Outcome measured | Main results/findings | Quality of evidence |
|-----------------|---------------------------|-----------|------------------|-----------------------|---------------------|
| **ICSI**        | Caglar et al (2007) [396] | Prospective cohort study of couples undergoing ICSI | TUNEL and Comet | No correlation between SDF and ICSI outcomes | Level 4 |
| **ICSI**        | Check et al (2005) [398]  | Prospective cohort study of infertile couples with previous ICSI failures | SCSA | No significant difference was found for low, moderate, and high SDF and outcomes for ICSI | Level 4 |
| **ICSI**        | Daris et al (2010) [394]  | Prospective cohort study of couples undergoing ICSI | TUNEL | No association between SDF and fertilization rate | Level 4 |
| **ICSI**        | Hammadeh et al (1996) [405] | Prospective cohort study of infertile couples undergoing ICSI | Aniline blue | No negative effect recorded for increased SDF | Level 4 |
| **ICSI**        | Avendaño et al (2010) [333] | Prospective cohort study of couples undergoing ICSI | TUNEL | Negative correlation for SDF and embryo quality; SDF<17.6% increased pregnancy probability by 3.5 times; no relationship between SDF and pregnancy outcome | Level 4 |
| **ICSI**        | Nasr-Esfahani et al (2005) [163] | Prospective cohort of infertile couples undergoing ICSI | Comet | Increased SDF did not affect fertilization rate, and embryos from high SDF sperm samples have less potential to reach blastocyst stage | Level 4 |
| **IVF, ICSI**   | Sun et al (2018) [408]    | Prospective cohort study of infertile couples undergoing ART | SCD | No difference between low (<30%) and high (>30%) SDF for all outcomes | Level 4 |
| **IVF, ICSI**   | Pregl Breznik et al (2013) [308] | Retrospective cohort study of infertile couples undergoing ART | SCD | Fertilization rate and embryo quality was negatively correlated to IVF but not to ICSI | Level 4 |
| **IVF, ICSI**   | Simon et al (2013) [164]  | Prospective cohort study of couples undergoing ART | Comet | Live birth rate was reduced in high SDF (>50%) compared to low SDF (<25%) for IVF. No relationship was found for SDF and live birth rate in ICSI | Level 4 |
| **IVF, ICSI**   | Simon et al (2010) [311]  | Prospective cohort study of couples undergoing ART | Comet | Increased SDF reduced all outcomes for IVF. No negative association was found for all parameters with ICSI | Level 4 |
| **IVF, ICSI**   | Tarozzi et al (2009) [313] | Prospective cohort study of couples undergoing ART | TUNEL | SDF and sperm protamination negatively correlated with fertilization and pregnancy rates in IVF. SDF positively correlated with pregnancy in ICSI, but not with fertilization | Level 4 |
| **IVF, ICSI**   | Tavalaee et al (2009) [395] | Prospective cohort study of couples undergoing ART | TUNEL | Negative correlation for SDF and fertilization rate in ICSI but not IVF. No effect of SDF on pregnancy rate | Level 4 |
| Study reference | Study design/participants | SDF assay | Outcome measured | Main results/findings | Quality of evidence |
|-----------------|---------------------------|-----------|------------------|-----------------------|---------------------|
| Gandini et al (2004) [403] | Prospective cohort study of infertile couples undergoing ART | SCSA | Fertilization, embryo quality and pregnancy rates | No differences between IVF and ICSI with high or low SDF | Level 4 |
| Henkel et al (2003) [321] | Prospective cohort study of infertile couples undergoing ART | TUNEL | Fertilization and pregnancy rates | SDF negatively correlated with pregnancy rate with no effect on fertilization rate in IVF and ICSI | Level 4 |
| Larson-Cook et al (2003) [323] | Retrospective cohort study of infertile couples undergoing ART | SCSA | Clinical pregnancy | SDF>27% resulted in no clinical pregnancies | Level 4 |
| Morris et al (2002) [404] | Prospective cohort study of infertile couples undergoing ART | Comet | Embryo quality | No association found between SDF and IVF outcomes or embryo quality | Level 4 |
| Larson et al (2000) [13] | Prospective cohort study of infertile couples undergoing ART | SCSA | Clinical pregnancy | SDF lower in men who achieved pregnancy with IVF and ICSI | Level 4 |
| Simon et al (2014) [328] | Cross-sectional study of infertile males undergoing ART | Comet | Fertilization rate, early (1–2 days) and late (3–4 days) paternal effect, and implantation stage | Low SDF (<30%) had higher percentage good quality embryos compared to high SDF (>71%) Implantation rate was higher in low SDF compared to intermediate (31%–70%) and high SDF ICSI had improved outcomes compared to IVF for increased SDF | Level 4 |
| Meseguer et al (2011) [329] | Prospective blinded cohort study of male partners undergoing IVF and ICSI | SCD | Pregnancy rate and oocyte quality | SDF negatively correlated with a pregnancy using the infertile couple oocytes No effect was observed for SDF and donated oocytes for pregnancy and embryo quality | Level 4 |
| Esbert et al (2011) [410] | Prospective cohort study of IVF and ICSI in own and donated oocytes in couple infertility | TUNEL | Fertilization rate and oocyte quality | No correlation for SDF and fertilization rate in IVF or ICSI SDF was similar in patients with < compared to >50% embryo utilisation SDF 36% threshold was not related to IVF or ICSI outcomes between own or donated oocytes | Level 4 |
| Janghorban-Laricheh et al (2016) [297] | Prospective, controlled study (35 men with grade 2–3 varicocele and primary infertility, 20 fertile men as controls) Clinical grades: 2–3 | Flow cytometry | SDF and phospholipase C | SDF higher in men with varicocele compared to fertile men Phospholipase C lower in men with varicocele vs. fertile controls | Level 3 |
| Study reference       | Study design/participants                                                                 | SDF assay         | Outcome measured | Main results/findings                                                                 | Quality of evidence |
|----------------------|------------------------------------------------------------------------------------------|-------------------|------------------|---------------------------------------------------------------------------------------|---------------------|
| Varicocele           | Prospective study (20 infertile men with varicocele and 20 fertile men)                  | SCD test, and DBD-FISH | SDF              | Infertile men with varicocele had higher SDF compared to fertile controls              | Level 3             |
| Varicocele           | Prospective study (44 men with varicocele, 15 fertile controls)                           | TUNEL             | SDF              | TUNEL higher in men with varicocele vs. fertile controls                              | Level 3             |
| Varicocele           | Case control (30 men with varicocele and 32 controls without varicocele)                 | Comet assay       | SDF, mitochondrial and acrosome activity | Varicocele group showed increased SDF, lower mitochondrial activity and lower acrosome activity vs. controls. No difference in lipid peroxidation levels between the groups | Level 3             |
| Varicocele           | Retrospective review (121 subfertile men with varicocele, 66 subfertile men without varicocele, 115 healthy fertile controls) | TUNEL             | SDF              | SDF was higher in varicocele group compared to subfertile men without varicocele, and fertile controls | Level 3             |
| Varicocele           | Observational study (157 men with >1 year of infertility and with varicocele)            | SCD               | SDF, semen parameters | Men with varicocele with abnormal sperm count, motility, morphology have higher DFI compared to men with normal SA | Level 4             |
| Varicocele           | Prospective controlled study (31 infertility patients [16 with varicocele], and 16 fertile controls) | SCSA              | SDF              | Infertile men with varicocele had significantly higher DFI% than fertile controls     | Level 4             |
| Varicocele           | Prospective study (179 infertile men with varicocele)                                    | SCD test          | SDF              | Infertile men with varicocele had higher DFI directly correlating with varicocele grade and inversely correlated with zinc concentration  | Level 4             |
| Varicocele           | Case control study (71 infertile men with varicocele and 30 healthy controls)            | TUNEL             | SDF              | Men with varicocele had higher TUNEL than healthy controls                             | Level 4             |
| Varicocele           | Case control study (55 men with testicular pain and varicocele and 25 healthy controls) | TUNEL & SCSA      | SDF              | Men with varicocele showed higher DFI and TUNEL positive cells                         | Level 4             |
| Study reference | Study design/participants | SDF assay | Outcome measured | Main results/findings | Quality of evidence |
|----------------|---------------------------|-----------|------------------|-----------------------|---------------------|
| Varicocele     | Enciso et al (2006) [304] | Case control study (18 infertile men with varicocele, 51 infertile men with normozoospermia, 103 infertile men with abnormal semen analysis, 22 fertile controls) Clinical grades: 1–3 | SCD test | SDF | Men with varicocele, infertile normozoospermic men, and infertile men with abnormal semen analysis showed higher SDF compared to fertile controls | Level 4 |
| Varicocele     | Dieamant et al (2017) [171] | Cross-sectional study, retrospective review (391 infertile men with varicocele, 2008 patients without varicocele) Clinical grades: 1–3 | TUNEL | SDF | Men with varicocele showed higher SDF than those without | Level 4 |
| Varicocele     | Tanaka et al (2020) [170] | Prospective case-control series (138 infertile men with varicocele and 102 infertile normozoospermic men without varicocele) Clinical grades: 1–3 | SCSA | SDF | Men with varicocele had higher DFI compared to those without varicocele | Level 4 |
| Varicocele     | Blumer et al (2008) [169] | Controlled prospective study (17 men with varicocele and 20 men without varicocele) Clinical grades: 2–3 | Comet assay | SDF | Men with varicocele had higher DNA fragmentation in Comet class II and Comet class IV compared to controls | Level 4 |
| Varicocele     | Bertolla et al (2006) [173] | Controlled prospective study (20 adolescent boys with and 20 adolescents without varicocele) Clinical grades: 2–3 | Comet assay | SDF | Higher class III and class IV SDF in adolescents with varicocele vs. no varicocele | Level 4 |
| Varicocele     | Zümürtbaş et al (2013) [305] | Retrospective case series (45 men with varicocele and 30 healthy men without varicocele) Clinical grades: 2–3 | AO | SDF | Varicocele patients showed higher red and green sperm colorations than the control group | Level 4 |
| Varicocele     | Vivas-Acevedo et al (2014) [174] | Case series (60 men with varicocele and 30 normal men as control) Clinical grades: 2–3 | SCD | SDF | Men with varicocele had higher SDF than controls | Level 4 |
| Recurrent pregnancy loss (RPL) | Carrell et al (2003) [283] | Retrospective controlled study (24 couples with RPL, 2 control groups: donors of known fertility and unscreened men from general population) | TUNEL | SDF and RPL | DNA fragmentation increased in RPL group (38±4.2) compared to donor (11.9±1.0) or general population (22±2.0) (p<0.001) In RPL group, there was no correlation between semen quality parameters and TUNEL data | Level 4 |
| Study reference     | Study design/participants                                                                 | SDF assay | Outcome measured                                                                 | Main results/findings                                                                                                                                                                                                 | Quality of evidence |
|--------------------|------------------------------------------------------------------------------------------|-----------|----------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|
| RPL                | Bellver et al (2010) [284]                                                                | SCD       | Y chromosome microdeletions and SDF                                              | Higher SDF in RPL and severe oligozoospermic patients compared to fertile donor group. Sperm DNA features do not seem to be related to unexplained RPL.                                                                        | Level 4             |
| RPL                | Gil-Villa et al (2010) [392]                                                              | SCSA      | Sperm factors associated with RPL                                               | RPL probably not due to alterations in sperm DNA package. Unclear how much DNA damage needed to negatively impact fertilization or embryo development. Importance of evaluating male factor with testing beyond semen analysis, such as lipid peroxidation and TAC. | Level 4             |
| RPL                | Absalan et al (2012) [285]                                                                | SCD       | DNA dispersion, semen parameters                                                 | RPL sperm showed significantly lower % big halo, higher % small halo and % without halo compared to control.                                                                                                                                                                   | Level 4             |
| RPL                | Imam et al (2011) [286]                                                                  | SCSA      | DFI, TAC, and ROS                                                              | Average mean DFI in RPL males was higher than controls. RPL male sperm also showed higher ROS and lower TAC.                                                                                                                                                                  | Level 4             |
| RPL                | Venkatesh et al (2011) [287]                                                             | SCSA      | Cytogenetic abnormalities, genetic abnormalities, OS, and SDF                  | Higher DFI in RPL couples regardless of abnormal or normal sperm compared to controls. ROS levels also higher in RPL men vs. controls.                                                                                                                                         | Level 4             |
| RPL                | Bronet et al (2012) [391]                                                                | TUNEL, FISH | SDF and aneuploidy rates in patients with RPL or implantation failure            | 76% of men had increased There was no correlation between SDF and aneuploidy rate in embryos or processed sperm samples.                                                                                                                                                         | Level 4             |
| RPL                | Kumar et al (2012) [182]                                                                 | SCSA      | DFI                                                                             | Mean DFI in cases 1.2 times higher than controls SDF threshold value of 26% to discriminate RPL cases from the control group Men with higher DFI are infertile, men with DFI<26% can conceive but experience RPL. | Level 4             |
| RPL                | Leach et al (2015) [288]                                                                 | SCSA      | SDF                                                                             | 70.5% of men had normal DFI (<15%), 23% had high levels (15%–30%) and 6.5% had very high levels (>30%) Couples with RPL had significantly higher DFI than those with other causes found on routine screening. | Level 4             |
| Study reference       | Study design/participants                                                                                     | SDF assay   | Outcome measured                      | Main results/findings                                                                                                                                  | Quality of evidence |
|-----------------------|-------------------------------------------------------------------------------------------------------------|-------------|---------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|
| RPL Ribas-Maynou et al (2012) [116] | Retrospective comparative study (20 donor males with RPL and 25 healthy donors with proven fertility and with no prior miscarriage) | Comet, SCD test | SDF                                   | Sperm from RPL men have lower SSB and higher DSB compared to fertile donors ssDNA damage may be able to predict fertilization potential, and dsDNA damage is related to risk of male-factor associated miscarriage | Level 4              |
| RPL Talebi et al (2012) [289]  | Retrospective comparative study (40 couples with RPL, 40 couples with proven fertility)                     | AO test, AB, TB, chromomycin A3, nuclear chromatin stability test | Sperm chromatin and DNA integrity | All sperm chromatin and DNA integrity tests showed significantly more abnormalities in males with RPL vs. control males Sperm from cases of RPL have lower chromatin condensation, hypostabilized chromatin, and lower DNA integrity compared to fertile men | Level 4              |
| RPL Zhang et al (2012) [290]  | Prospective case control study (111 couples with RPL and 30 fertile men as controls)                      | SCD         | Correlation between sperm factors with pregnancy outcome | Future pregnancy outcome may be predicted negatively by ASCI Sperm chromatin integrity has significant contribution to reproductive outcome ASCI significant predictor for future abortion and infertility | Level 4              |
| RPL Thilagavathi et al (2013) [291] | Retrospective comparative study (25 couples with RPL and 20 fertile couples)                                | SCSA        | Telomere length association with RPL   | Relative leukocyte mean telomere length in men and women in RPL group was significantly lower compared to controls Sperm DFI showed positive correlation with telomere length | Level 4              |
| RPL Khadem et al (2014) [180]  | Retrospective cohort study (30 couples with RPL and 30 fertile couples as controls)                         | SCD         | Reproductive outcome association with SDF | Abnormal SDF significantly higher in RPL group vs. control group Also, increased SDF negatively correlated with sperm with progressive motility (4=-0.613; p<0.001) | Level 4              |
| RPL Coughlan et al (2015) [390] | Retrospective cohort study (35 partners of women with RIF, 16 partners of women with RPL, and 7 fertile controls) | SCD and TUNEL | SDF, RIF, RPL                        | No obvious differences in SDF measured by either test SCD SDF statistically lower in prepared semen in all groups, however, this was not seen in TUNEL assay | Level 4              |
| RPL Ramasamy et al (2015) [292] | Retrospective comparative study (140 men with RPL and 5 normozoospermic controls providing 140 semen samples) | TUNEL, FISH | Prevalence of sperm autosomal and sex chromosome aneuploidy in men with RPL | RPL men had greater percentage of sperm aneuploidy within sex chromosomes, chromosomes 18 and 13/21 compared to controls There was no association between elevated SDF (>30%) and sperm aneuploidy | Level 4              |
| Study reference         | Study design/participants                                                                 | SDF assay          | Outcome measured                                      | Main results/findings                                                                                                                                                                                                 | Quality of evidence |
|-------------------------|-------------------------------------------------------------------------------------------|--------------------|-------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|
| RPL Bareh et al (2016)  | Prospective cohort study (26 males with RPL and 31 fertile males)                         | TUNEL, flow cytometry | Sperm DNA integrity                                   | Mean SDF significantly higher in men with RPL vs. controls                                                                                                                                                              | Level 4             |
| RPL Halim and Lubis (2016) | Prospective cohort study (40 males with RPL and 40 fertile males as controls)           | SCD                | SDF                                                   | Sperm DFI in case group higher than controls Significant association between sperm DFI≥30 and incidence of idiopathic early RPL                                                                                                                                 | Level 4             |
| RPL Zidi-Jrah et al (2016) | Retrospective comparative study (22 couples with RPL and 20 fertile men)                 | TUNEL, FISH        | RPL association with sperm aneuploidy, sperm DNA integrity, chromatin packaging, semen parameters | SDF and nuclear chromatin decondensation significantly higher in RPL group vs. controls Significantly higher sperm aneuploidy rate in RPL group                                                                                                                                 | Level 4             |
| RPL Carlini et al (2017) | Retrospective cohort study (114 infertile men in RPL couples, 114 fertile men with normal semen parameters) | TUNEL              | SDF                                                   | SDF levels higher in men with RPL vs. controls SDF positively correlated with age of patients with RPL and number of miscarriages                                                                                                                                 | Level 4             |
| RPL Eisenberg et al (2017) | Prospective observational study (344 couples with singleton pregnancy followed through 7 weeks gestation) | SCSA               | SDF                                                   | 28% of couples experience pregnancy loss after singleton pregnancy DFI≥30 positively associated with pregnancy loss Similar findings in those with 2nd loss Trend toward pregnancy loss with increased SDF                                                                                                                                 | Level 4             |
| RPL Esquerré-Lamare et al (2018) | Prospective case-control study (33 couples with unexplained RPL and 27 controls) | SCSA, TUNEL, FISH  | DFI and aneuploidy                                   | No difference in DFI or TUNEL results between cases and controls Total aneuploidy significantly higher in RPL group compared to controls                                                                                                                                 | Level 4             |
| RPL Kamkar et al (2018)  | Prospective case-control study (42 couples with RPL and 42 fertile men as controls)      | SCSA, TUNEL        | Sperm factors, SDF, ROS, TAC in male partners in couples with RPL | SDF significantly higher in case vs. control group in both SCSA and TUNEL Men with higher SDF had higher chance of miscarriage                                                                                                                                                     | Level 4             |
| Idiopathic male infertility (IMI)/unexplained male infertility (UMI) | Zandieh et al (2018) Cross-sectional study (evaluation of SDF in patients with UMI vs. fertile patients) | SCD                | SDF                                                   | Patients with UMI have significantly higher SDF than fertile patients                                                                                                                                               | Level 2             |
| IMI Aktan et al (2013)   | Cross-sectional study (evaluation of SDF in patients with IMI vs. fertile donors)        | TUNEL              | SDF                                                   | Patients with IMI have significantly higher SDF than donors                                                                                                                                                           | Level 2             |
| UMI Saleh et al (2003)   | Cross-sectional study (evaluation of SDF in patients with UMI vs. fertile donors)        | SCSA               | SDF                                                   | Patients with UMI have significantly higher SDF than donors                                                                                                                                                           | Level 2             |
| Study reference                  | Study design/participants                                                                 | SDF assay | Outcome measured | Main results/findings                                                                 | Quality of evidence |
|---------------------------------|-----------------------------------------------------------------------------------------|-----------|------------------|--------------------------------------------------------------------------------------|---------------------|
| IMI/UMI Pelliccione et al (2011) [276] | Cross-sectional study (evaluation of SDF in patients with IMI and UMI)                    | TUNEL     | SDF              | Patients with idiopathic OAT have significantly higher SDF than patients with UMI    | Level 3             |
| UMI Mayorga-Torres et al (2017) [388] | Cross-sectional study (evaluation of SDF in patients with UMI vs. fertile patients)       | SCSA      | SDF              | No significant difference in SDF levels of patients with UMI vs. fertile men         | Level 3             |
| IMI Komiya et al (2014) [190]    | Cross-sectional study (evaluation of SDF in patients with IMI vs. varicocele patients)    | SCD       | SDF              | No significant difference on SDF levels of patients with IMI vs. varicocele          | Level 3             |
| UMI Vandekerckhove et al (2016) [279] | Prospective cohort study (examining SDF and IUI outcomes in men with UMI)                  | SCD       | Pregnancy after IUI | Patients with SDF<20% has significantly higher pregnancy rate after IUI than those with SDF>20% | Level 3             |
| UMI Rybar et al (2009) [280]     | Cross sectional study (evaluating SDF on males with UMI vs. SDF in general population)    | SCSA      | SDF              | Patients with UMI have higher SDF when compared to general population               | Level 3             |
| UMI/IMI Rahimizadeh et al (2020) [281] | Cross-sectional study (evaluating SDF in UMI vs. idiopathic AT men vs. fertile men)        | SCSA      | SDF              | SDF in UMI or AT was significantly higher than in fertile men                        | Level 3             |
| UMI O’Neill et al (2018) [282]   | Prospective study (including males with UMI and poor IUI outcome undergoing SDF evaluation) | SCSA/TUNEL | Fertilization and clinical pregnancy rates | ICSI+high SDF group showed significantly higher fertilization and clinical pregnancy rates than IVF+normal SDF group | Level 4             |
| UMI Oleszczuk et al (2013) [185] | Retrospective study (evaluating SDF on males with UMI vs. SDF in general population reported in medical literature [10.5%]) | SCSA      | SDF              | Percentage of patients with high SDF (>30%) is significantly higher in the group with UMI when compared to general population | Level 4             |

**Risk factors**

| Radiofrequency electromagnetic field (RF-EMF) Avendaño et al (2012) [344] | Prospective study (assessing SDF in semen samples of 29 healthy donors) Each sample was divided in non-exposed (control) and exposed (experimental – 4-hour exposure to internet-connected laptop) | TUNEL     | SDF              | Exposed samples had higher SDF than unexposed                                      | Level 2             |
| Study reference | Study design/participants | SDF assay | Outcome measured | Main results/findings | Quality of evidence |
|-----------------|---------------------------|-----------|------------------|-----------------------|--------------------|
| RF-EMF          | Zalata et al (2015) [343] | Prospective study (assessing SDF in semen samples grouped according to semen parameters). Each sample was divided in non-exposed (control) and exposed (experimental – 1-hour exposure to EMF emitted from a cell phone). | Propidium iodide (PI) analyzed by flow cytometry | Samples exposed to RF-EMF showed increased SDF compared with non-exposed | Level 2 |
|                 |                           |           |                  |                       |                    |
| Heat exposure   | Zhang et al (2018) [198]  | Clinical trial (including healthy men exposed to testicular HS for three months). SDF was assessed before, during and after exposure | AB, AO, TUNEL | SDF was significantly increased during HS and until one month after interruption of HS | Level 2 |
|                 |                           |           |                  |                       |                    |
| Genital tract infection | Gallegos et al (2008) [47] | Cross-sectional study (evaluating SDF in 143 patients with genitourinary infection from *Chlamydia trachomatis* and *mycoplasma* vs. 50 healthy fertile patients) | SCD | Patients with infection showed higher SDF compared to healthy men | Level 2 |
|                 |                           |           |                  |                       |                    |
| Alcohol consumption | Komiya et al (2014) [190] | Cross-sectional study (evaluation of SDF in patients with and without alcohol use in a mixed population of IMI and varicocele patients) | SCD | Patients with chronic alcohol use have significantly higher SDF than counterparts | Level 3 |
|                 |                           |           |                  |                       |                    |
| Alcohol consumption and Smoking | Boeri et al (2019) [334] | Cross-sectional study (assessing SDF in the following groups: (1) non-smokers and abstainers; (2) at least one habit [smoking or alcohol]; (3) smokers and drinkers) | SCSA | SDF was higher in group 3 when compared to groups 1 and 2 | Level 3 |
|                 |                           |           |                  |                       |                    |
| Smoking         | Komiya et al (2014) [190] | Cross-sectional study (evaluation of SDF in smoking and non-smoking patients in a mixed population of IMI and varicocele patients) | SCD | No significant difference on SDF levels between the groups | Level 3 |
|                 |                           |           |                  |                       |                    |
| Smoking         | Antoniassi et al (2016) [195] | Cross-sectional study (comparing SDF between groups of smokers and non-smokers with normal semen parameters) | Comet | Smokers showed significantly higher SDF (Comet classes III and IV) than counterparts | Level 3 |
| Study reference       | Study design/participants                                                                 | SDF assay          | Outcome measured | Main results/findings                                                                 | Quality of evidence |
|----------------------|-------------------------------------------------------------------------------------------|--------------------|------------------|---------------------------------------------------------------------------------------|---------------------|
| Smoking Ranganathan et al (2019) [191] | Cross-sectional study (comparing SDF of men sub-grouped in (i) fertile smokers; (ii) fertile non-smokers; (iii) infertile smokers; and (iv) infertile non-smokers) | AB                 | SDF              | Infertile smoking subjects had significantly higher SDF than infertile non-smoking subjects | Level 3             |
| Smoking Elshal et al (2009) [331] | Cross-sectional study (comparing SDF of men sub-grouped in (i) fertile non-smokers; (ii) infertile non-smokers; and (iii) infertile smokers) | SCSA               | SDF              | Infertile smokers group showed significantly higher SDF than infertile non-smokers     | Level 3             |
| Smoking Taha et al (2012) [335] | Cross-sectional study (comparing SDF of men sub-grouped in (i) fertile non-smokers; (ii) fertile smokers) | SDF by flow cytometry, based on individual sperm stained with PI | SDF              | Smoker patients showed significantly higher SDF than non-smokers                       | Level 3             |
| Smoking Tawadrous et al (2011) [338] | Cross-sectional study (comparing SDF of men sub-grouped to (i) fertile smokers; (ii) fertile non-smokers; (iii) infertile smokers; and (iv) infertile non-smokers) | Enhanced apoptotic DNA ladder detection kit | SDF              | SDF correlated positively with the number of cigarettes smoked daily and smoking duration | Level 3             |
| Obesity Lu et al (2018) [192] | Prospective study (with a cohort of sub-fertile men)                                      | SCSA               | SDF              | No significant relationship between SDF and obesity                                   | Level 3             |
| Obesity Oliveira et al (2018) [199] | Cross-sectional study (comparing SDF between men sub-grouped by BMI)                      | TUNEL              | Correlation between SDF and BMI                                                       | No correlation was identified                                      | Level 3             |
| Obesity Fariello et al (2012) [56] | Cross-sectional study (with males seeking for infertility evaluation)                    | Alkaline Comet     | Correlation between SDF and BMI                                                       | BMI≥30 kg/m² was associated with higher SDF than BMI<30 kg/m²       | Level 3             |
| Obesity Kort et al (2006) [337] | Cross-sectional study (comparing SDF between men sub-grouped by BMI)                     | SCSA               | Correlation between SDF and BMI                                                       | A significant difference was found in DFI between the normal BMI group and both the overweight and obese groups | Level 3             |
| Obesity Chavarro et al (2010) [336] | Cross-sectional study (subfertile men seeking medical assistance for infertility were sub-grouped according to BMI) | Neutral Comet      | SDF              | Sperm with high DNA damage were significantly more numerous in obese men than in normal weight men | Level 3             |
| Obesity Bandel et al (2015) [411] | Cross-sectional study (comparing SDF between men sub-grouped by BMI)                     | SCSA               | SDF              | No significant correlation between SDF and BMI                                        | Level 3             |
| Study reference | Study design/participants | SDF assay | Outcome measured | Main results/findings | Quality of evidence |
|-----------------|--------------------------|-----------|------------------|-----------------------|---------------------|
| Diabetes mellitus (DM) | Condorelli et al (2018) [196] | Cross-sectional study (assessing SDF in infertile men with DM-1 and DM-2 and in a group of healthy fertile men [control]) | TUNEL | SDF | Patients with DM2 showed significantly higher SDF when compared to DM1 and control group | Level 3 |
| DM | Lu et al (2017) [340] | Cross-sectional study (assessing SDF in patients with DM and in a healthy group [control]) | SCSA | SDF | Patients with DM showed significantly higher SDF than control | Level 3 |
| Abstinence time | Lu et al (2018) [192] | Prospective study (with a cohort of subfertile men) | SCSA | Correlation coefficients between SDF and other parameters | Significant correlation between SDF and abstinence time | Level 3 |
| Age | Lu et al (2018) [192] | Prospective study (with a cohort of subfertile men) | SCSA | Correlation coefficients between SDF and other parameters | Significant correlation between SDF and age | Level 3 |
| Age | Rybar et al (2011) [342] | Cross-sectional study (evaluating SDF in infertile men divided into age groups: 20–30 years old; 31–40 years old and >40 years old) | SCSA | SDF | Patients aged >40 showed higher SDF than counterparts | Level 3 |
| Age | Alshahrani et al (2014) [42] | Cross-sectional study (evaluating SDF in infertile men divided into group 1: ≤30 years [n=69]; group 2: 31–40 years [n=298]; and group 3: >40 years [n=105]) | TUNEL | SDF | Men aged >40 showed significantly higher levels of sperm DNA damage, when compared to men in younger age groups | Level 3 |
| Age | Radwan et al (2016) [341] | Cross-sectional study (comparing SDF in patients aged >40 vs. <40 years) | SCSA | Association between age and SDF | Age >40 was significantly associated with SDF | Level 3 |
| Age | Belloc et al (2014) [332] | Cross-sectional study (on the correlation between SDF and age in normozoospermic men) | TUNEL | Correlation between age and SDF | Percentage of SDF was positively correlated with paternal age | Level 3 |
| Genital tract infection | Dehghan Marvast et al (2018) [200] | Cross-sectional study (assessing the correlation between C. trachomatis infection and SDF) | TUNEL | Correlation between SDF and C. trachomatis infection. | No significant differences in terms of DNA fragmentation between C. trachomatis-positive and C. trachomatis-negative men | Level 3 |
| Ionizing radiation | Zhou et al (2016) [193] | Cross-sectional study (assessing SDF in males exposed [n=46] and non-exposed [n=72] to ionizing radiation from hospital sources) | SCD | SDF | Exposed patients have significantly higher SDF than unexposed | Level 3 |
| RF-EMF | Radwan et al (2016) [341] | Cross-sectional study (evaluating relationship between cell-phone use and SDF) | SCSA | Association between HDS and cell phone use | Cell phone use for more than 10 years was positively related to HDS | Level 3 |
| Study reference          | Study design/participants                                                                 | SDF assay | Outcome measured                     | Main results/findings                                                                                   | Quality of evidence |
|-------------------------|------------------------------------------------------------------------------------------|-----------|--------------------------------------|--------------------------------------------------------------------------------------------------------|---------------------|
| Anxiety                 | Vellani et al (2013) [348] Cross-sectional study (evaluating SDF and anxiety)            | TUNEL     | SDF                                  | State anxiety and trait anxiety were related to increased SDF                                           | Level 3             |
| Nutritional factors     | Vujkovic et al (2009) [349] Cross-sectional study (evaluating SDF and diet patterns in subfertile patients) | SCSA      | SDF                                  | “Health Conscious” diet pattern (rich in fruits and vegetables) is inversely associated with SDF         | Level 3             |
| Nutritional factors     | Jurewicz et al (2018) [197] Cross-sectional study (evaluating SDF and diet patterns [western, mixed, prudent] in infertile patients) | SCSA      | SDF                                  | Prudent dietary pattern was identified to decrease the SDF index                                        | Level 3             |
| Occupational stress     | Radwan et al (2016) [341] Cross-sectional study (evaluating relationship between SDF and occupational stress [assessed by Subjective Work Characteristics Questionnaire]) | SCSA      | Association between SDF and occupational stress | A positive significant association was observed between occupational stress and SDF | Level 3             |
| Air pollution           | Rubes et al (2005) [74] Cross-sectional study (evaluating SDF in patients with high and low exposure to air pollution [the cut off was United States air quality standards]) | SCSA      | SDF                                  | A significant association between air pollution and SDF was reported                                    | Level 3             |
| Heat exposure           | Zhang et al (2015) [65] Prospective study (evaluating SDF before and after 3-month heat exposure in healthy males) | AB, TUNEL | SDF, abnormal chromatin condensation | SDF and abnormal chromatin condensation were significantly higher during exposure and returned baseline levels 3 months after the exposure | Level 3             |
| Styrene exposure        | Migliore et al (2002) [84] Cross-sectional study (evaluating SDF in styrene-exposed workers) | COMET     | SDF                                  | Styrene-exposed patients have significantly higher SDF than unexposed                                   | Level 3             |
| Exposure to perfluorinated compounds (PFC) | Governini et al (2015) [345] Cross-sectional study (evaluating SDF in PFC-contaminated and non-contaminated subjects) | TUNEL     | SDF                                  | SDF was significantly increased in PFC-contaminated subjects compared to PFC-non-contaminated subjects | Level 3             |
| Exposure to phthalate   | Hauser et al (2007) [346] Cross-sectional study (evaluating the correlation between SDF and exposure to phthalate in a cohort of infertile men) | Neutral Comet | Correlation between SDF and exposure to phthalate | SDF was positively associated with MEP and with MEHP (metabolites of phthalates) | Level 3             |
| OP exposure             | Miranda-Contreras et al (2013) [347] Cross-sectional study (including patients exposed and unexposed to OP) | SCSA      | SDF                                  | SDF was significantly increased in exposed men when compared to unexposed                              | Level 3             |
| Study reference | Study design/participants | SDF assay | Outcome measured | Main results/findings | Quality of evidence |
|-----------------|---------------------------|-----------|-----------------|----------------------|-------------------|
| Sun et al (1997) [88] | Case-control study (assessing smoking history and SDF on infertile men undergoing semen analysis) | TUNEL | Correlation between smoking and SDF | Smokers showed significantly higher SDF than counterparts | Level 4 |
| Bojar et al (2013) [412] | Cross-sectional study (comparing ranges of SDF [<15%; 15%–19%; 20%–25%; >25%] between groups of smokers and non-smokers) | SCSA | SDF | No significant correlation between SDF levels and smoking | Level 4 |
| Kumar et al (2014) [201] | Retrospective study (assessing SDF in males exposed and non-exposed to ionizing radiation) | SCSA | SDF | Exposed patients have significantly higher SDF than unexposed | Level 4 |
| Kumar et al (2014) [339] | Cross-sectional study (evaluating SDF in patients with and without environmental [toxic substances such as pesticides and solvents] and/or lifestyle [smoking/alcohol intake] factors) | AO | SDF | The group with environmental and/or lifestyle exposure history showed higher SDF | Level 4 |
| Sánchez-Peña et al (2004) [82] | Cross-sectional study (including patients exposed to OP) | SCSA | SDF | Men exposed to OP showed significantly higher SDF than general population (compared to a cohort of the same research group with unexposed men) | Level 4 |

For quality of evidence, used Oxford Centre for Evidence-Based Medicine 2011 Levels of Evidence (https://www.cebm.net/wp-content/uploads/2014/06/CEBM-Levels-of-Evidence-2.1.pdf). For more details: https://www.cebm.net/2011/06/explanation-2011-cebm-levels-evidence/.
including changes in semen parameters or SDF levels, fertilization, pregnancy, birth, and miscarriage rates.

The search yielded a total of 1,584 publications. The title and abstracts were cross-checked by three independent researchers and 251 articles were considered in this review. Relevant information was extracted from the studies that fulfilled the selection criteria and presented in Table 2.

1. Natural conception

As stated previously, sperm DNA integrity plays a crucial part in the fertilization process and in early embryo development, thereby directly influencing the likelihood of natural conception. Reports have linked SDF to low cleavage rates [146,147] and to the arrest of embryonic development after the second cleavage state [148]. Using the SCSA on 215 Danish first pregnancy planners, Spanò et al [149] reported an inverse relationship between the level of SDF and probability of natural pregnancy in a menstrual cycle. Moreover, evidence linking SDF to natural pregnancy rates can be drawn from the meta-analysis by Zini [146] which included three studies and 616 couples and revealed that high SDF, determined by the SCSA test, was associated with failure to achieve natural pregnancy with an odds ratio (OR) of 7.01 (95% confidence interval [CI]=3.68–13.36).

2. Assisted reproductive technology outcomes

Numerous reports investigating the predictive role of SDF on ART outcomes have reported contradictory results [41,88,146,150-164]. This controversy can be attributed to a number of factors, such as the study selection methods used by these reviews, heterogeneity of the conducted studies, differences in the SDF testing methods and female age and fertility status to name a few. With regards to intrauterine insemination (IUI), Chen et al [150] analyzed the results of 10 articles and demonstrated that high SDF levels were associated with significantly lower pregnancy (relative risk [RR]=0.34, 95% CI=0.22–0.52; p<0.001) and delivery rates (RR=0.14, 95% CI=0.04–0.56; p<0.001). This result was also echoed by two other meta-analyses reporting that patients with low SDF had an OR for clinical pregnancy ranging between 7.01 and 16 [41,146]. However, a recent meta-analysis by Sugihara et al [151] analyzed the results of 3 studies and reported that while low SDF was associated with better pregnancy rates with a RR of 3.30 (95% CI=1.16–9.39), the test performance was low as it had a low positive predictive value (17%).

As for SDF impact on IVF/ICSI, various meta-analyses have been published assessing the rates of pregnancy, miscarriage and live birth. Four meta-analyses [146,152,154,157] reported that high SDF was associated with lower pregnancy rates with conventional IVF with an OR ranging between 0.68 and 1.7. With regards to ICSI, only Simon et al [157] reported significantly lower pregnancy rates with high SDF, while the remaining three meta-analyses failed to find a significant association [146,152,154].

Live birth rate was examined by one meta-analysis and was found to be significantly lower in men with high SDF following both IVF and ICSI with a combined OR of 1.17 (95% CI=1.07–1.28; p=0.0005) [158]. Three meta-analyses examined the miscarriage rate following ART in relation to SDF [146,153,154]. Overall, high SDF was associated with greater risk for miscarriage following both IVF and ICSI with a combined OR ranging between 2.28 and 2.48.

Contrary to the abovementioned studies, two meta-analyses of slightly different design reported rather discouraging results. Cissen et al [155] analyzed 30 studies to assess the value of SDF in predicting the chance of ongoing pregnancy with IVF or ICSI. Overall, SDF testing had fair to good sensitivity with poor specificity. The authors constructed a hierarchical summary receiver operating characteristic curve, which reported fair predictive performance for TUNEL and Comet assays, while the predictive power for SCSA and SCD was poor. The authors concluded that the current SDF testing methods had a limited ability in predicting the chance of pregnancy in the context of ART. Furthermore, Collins et al [156] analyzed 13 studies having an extensive study heterogeneity and reported random effect model of the diagnostic OR rather than sensitivities and specificities. While the authors detected that sperm DNA integrity was significantly associated with pregnancy following IVF and ICSI with a diagnostic OR of 1.44, 95% CI=1.03–2.03; p=0.04 the likelihood ratios (LR(+)=1.23, LR(−)=0.81) were in a range suggesting that testing did not alter the outcome and was hence not clinically relevant. Subgroup analyses showed that the test accuracy was not materially affected by the testing method (TUNEL or SCSA) or the ART modality (IVF or ICSI).

Taking the abovementioned results all together,
there is reasonable evidence to state that SDF is relevant in the context of ART. A high SDF value is associated with decreased pregnancy rate with IUI and IVF and with increased miscarriage rate following both IVF and ICSI.

3. Varicocele

Varicocele is the most common correctable cause of male infertility, prevalent in about 40% of men with primary infertility and up to 80% of men with secondary infertility [165]. Improper patient selection for varicocele ligation was an important reason for the controversy regarding the effect of treatment on pregnancy outcome. This has been resolved by indicating surgery only for patients with clinically evident disease and abnormal semen parameters [166]. However, even when proper patient selection is practiced, pregnancy is observed in only, 40% to 50% of patients following surgery [167]. Hence efforts have been made to refine the indications of surgery in varicocele patients and interest in SDF has emerged after finding a significant positive association with varicocele [168].

Studies have shown that men with varicocele have significantly higher levels of SDF than controls regardless of their fertility status, suggesting that varicocele is independently associated with impaired DNA integrity [168-175]. A recent cross-sectional study was carried out on 2,399 men attending a fertility clinic, 16.3% (391/2,399) of whom were diagnosed with varicocele [171]. Men with varicocele had a significantly increased percentage of seminal SDF (p=0.03), abnormal chromatin packaging (p=0.001), and abnormal mitochondrial membrane potential (p=0.03) in comparison to men without varicocele. It is important to note that varicocele treatment is associated with a reduction in the SDF level. A meta-analysis conducted by Wang et al [176] involving 12 studies (7 studies assessed SDF in patients with varicocele, while 6 studies determined the outcome of surgery) revealed that varicocele was associated with significantly higher levels of SDF compared with controls with a mean difference (MD) of 9.84% (95% CI=9.19–10.49; p<0.00001). Varicocele treatment resulted in significant reduction of SDF levels when compared to control group (MD of -3.37%; 95% CI -4.09 to -2.65; p<0.00001).

A recent review by Roque and Esteves [177] investigated 21 studies, including >1,200 subjects in whom the effect of varicocele ligation on SDF was assessed. The authors observed that all studies reported a significant decrease in SDF following varicocele ligation during a follow-up period ranging from 3 to 12 months. Few studies reported the pregnancy outcome following treatment and generally identified lower SDF values in couples who conceived compared with those who did not. Smit et al [178] utilized the SCSA in 49 patients before and after varicocelectomy and reported significant reduction in SDF values following surgery (MD=5%; p=0.019). Out of the 49 subjects, 18 (37%) conceived spontaneously and 11 (22%) conceived with ART. The SDF levels were significantly lower in patients who conceived spontaneously or with ART (26.6%±13.7%) in comparison to patients who did not conceive at all (37.3%±13.9%) (p=0.013). Another study by Ni et al [179] assessed the ratio between protamine 1 and 2 mRNAs (P1/P2) as well as SDF levels using PCR and SCSA, respectively, in 42 infertile men with varicocele and 10 fertile donors with normal semen parameters. The study group underwent varicocelectomy and pregnancy was achieved by 23.81% of patients 6 months after surgery. Compared with couples who failed to conceive following varicocelectomy, pregnant couples had significantly lower mean P1/P2 mRNA ratio and SDF levels.

4. Recurrent pregnancy loss

RPL, defined as spontaneous loss of 2 or more pregnancies. Prior to 20 weeks of gestation, has been linked to elevated levels of SDF in several investigations. Studies performed using different SDF testing methods such as SCD [180], TUNEL assay [181] and SCSA [182] reported significantly higher SDF levels among patients with RPL in comparison to normal controls [183]. Aiming to understand the male contribution to RPL, Tan et al [184] recently conducted a meta-analysis on 12 prospective and 2 retrospective studies including 530 men with RPL and 639 fertile controls. The study revealed a significant association between RPL and SDF with an average MD of 11.98, 95% CI 6.64–17.32; p<0.001 indicating that men with RPL had significantly higher SDF values than the control group. Similar result was reported during subgroup analysis according to the SDF testing method.

5. Idiopathic and unexplained male infertility

Unexplained male infertility (UMI) is a term given to couples who otherwise have a completely normal fertility evaluation. Studies have revealed that men
with normal semen parameters may still have elevated levels of SDF. Oleszczuk et al [185] have shown that about 1 out of 5 men with unexplained infertility had a SDF level above 30%. Another prospective study conducted on 25 men with unexplained infertility revealed that a SDF level above 30%, measured by SCD, was detected in 29% of the subjects [185]. Similarly, idiopathic male infertility, a term given to describe men with one or more abnormality in semen parameters without an identifiable etiology, has been associated with high SDF. Studies have confirmed a significant inverse correlation between the SDF level and sperm count, motility and normal morphology [140,186,187]. A few comparative studies also have revealed that men with idiopathic male infertility tend to have significantly higher SDF than normal fertile controls [188] (Table 2).

6. High risk patients
Various studies have been conducting linking several lifestyle factors/environmental exposures to elevated levels of SDF [78,94,189-198]. These factors include (i) Physical agents such as radiation and heat; (ii) Chemical agents such as cigarette smoke, airborne pollutants, and chemotherapeutic drugs; and (iii) Biological factors including sexually transmitted infections, increasing male age, elevated body mass index (BMI) and medical conditions such as insulin dependent diabetes [78,94,189-197,199-201]. Elevated OS levels is believed to be the main mechanism resulting in SDF with these exposures. Moreover, occupational exposures have been considerably linked to SDF and altered fertility potential. Examples of such exposures include lead and cadmium [83], organochlorine pollutants found in pesticides [82], and bisphenol A, a compound widely utilized in plastic containers used in food and drink industries [86].

MANAGEMENT OF HIGH SPERM DNA FRAGMENTATION

1. Oral antioxidant therapy
Antioxidants play an important role in general health by scavenging excess free radicals and thus, preventing oxidative damage to macromolecules. However, the benefits of exogenous antioxidant therapy are less clear [202]. Clinicians commonly utilize antioxidant therapy to maintain redox balance by scavenging ROS [203]. Several clinical trials have demonstrated the positive effects of antioxidants on SDF in infertile men (Table 3) [204-208]. However, with no validated guidelines on antioxidant supplementation, they are frequently used empirically. Antioxidants can be easily purchased over the counter and are commonly considered safe. However, excess antioxidant supplementation may have a paradoxical effect on OS and SDF, a condition referred to as reductive stress [209]. As a result, the indiscriminate use of oral antioxidants in men without elevated OS should be avoided [210].

2. Control of infections/inflammation/leukocytospermia
Among infertile men, the incidence of infection ranges from 2% to 18% [211]. Sexually transmitted infections or prostatitis are associated with elevated OS and leukocytospermia, which may result in elevated SDF and impaired fertility [212,213]. Antibiotic therapy has been reported to be effective in treating infection-induced elevated SDF levels (Table 3) [47]. Moreover, empirical antibiotic therapy for leukocytospermia may improve natural pregnancy rates [214].

3. Varicocelectomy
Varicocele has been consistently associated with increased SDF values. It has been established that varicocele repair can improve OS markers and reduce SDF indices [11]. Current data supports the value of varicocele repair in reducing SDF and improving fertility (Table 3). In a systematic review of 21 studies evaluating the effect of varicocelectomy on SDF, all studies reported a significant decrease in SDF by an average of approximately 8% [177]. Moreover, varicocele repair has demonstrated improvements in pregnancy success in both natural conception and assisted reproduction by way of improved SDF indices [178]. Given these observations, the association between palpable varicocele and SDF should be considered, and varicocelectomy discussed with patients as a potential solution to improving fertility.

4. Lifestyle modifications
Exposure to environmental and lifestyle factors have far-reaching implications on male fertility. Current data has consistently associated smoking with higher SDF values when compared to non-smokers [91,99,191,215], however no study has yet evaluated the impact of smoking cessation on SDF. There have also
| Study reference                     | Study design/participants                                                                 | SDF assay | Outcome measured         | Main results/findings                                                                                   | Quality of evidence |
|-----------------------------------|------------------------------------------------------------------------------------------|-----------|--------------------------|--------------------------------------------------------------------------------------------------------|---------------------|
| **Reduction of abstinence time**   |                                                                                         |           |                          |                                                                                                        |                     |
| Gosálvez et al (2011) [350]       | Prospective study 21 infertile men and 12 donors                                          | SCD       | SDF                      | Lower baseline levels of SDF reported after shorter periods of abstinence between ejaculations than those recommended | Level 2              |
|                                   | SDF evaluated on neat sperm after 24 hours of abstinence with recurrent ejaculation (one every 24 hours) |           |                          |                                                                                                        |                     |
|                                   | SDF assessment before and after DGC (3 hours abstinence)                                  |           |                          |                                                                                                        |                     |
| Agarwal et al (2016) [222]        | Prospective study Normozoospermic samples analyzed after 1, 2, 5, 7, 9, and 11 days of abstinence | TUNEL     | SDF                      | The least amount of DNA fragmentation observed after 1 and 2 days of abstinence                          | Level 3              |
| Mayorga-Torres et al (2015) [223]| Prospective study Samples were collected daily over a period of 2 weeks                  | SCSA      | SDF, MMP, ROS             | Two weeks of ejaculation did not influence any functional parameters                                      | Level 3              |
| Uppangala et al (2016) [352]      | Prospective study 76 samples collected by 19 healthy volunteers after 1, 3, 5, and 7 days of abstinence | Aniline blue, SCD, Immuno detection of 5-methyl cytosine | SDF, sperm maturity and methylation                                                                   | Level 3              |
|                                   |                                                                                         |           |                          | The duration of abstinence positively correlated with semen volume and concentration                    |                     |
|                                   |                                                                                         |           |                          | After 1-day abstinence, sperm showed higher sperm chromatin immaturity than after 3 and 5 days while SDF was lower after than in sperm collected after 5 and 7 days |                     |
|                                   |                                                                                         |           |                          |                                                                                                        |                     |
| Shi et al (2018) [351]            | Prospective study 328 subjects assessed lifestyle and demographic factors associated with human semen quality and sperm function | SCSA      | SDF and lifestyle associated factors | DFI was significantly associated with abstinence time                                                  | Level 3              |
| Sánchez-Martín et al (2013) [227]| Retrospective cohort 40 men practicing recurrent ejaculation before ICSI, 150 men whose samples were collected following 4 days of abstinence | SCD       | SDF                      | Higher ICSI pregnancy rate in recurrent ejaculation group DGC selection resulted in lowering of SDF in recurrent ejaculation group | Level 3              |
| **Lifestyle modification**        |                                                                                         |           |                          |                                                                                                        |                     |
| Surgical/ non-surgical weight loss | Samavat et al (2018) [219] Prospective study 31 morbidly obese men (23 underwent laparoscopic roux-en-Y gastric bypass, 8 non-operated) evaluated after 6 months from surgery or recruitment | TUNEL     | SDF                      | SDF did not change from baseline to follow-up in both groups                                           | Level 3              |
| Surgical weight loss              | Carette et al (2019) [220] Prospective study 46 men (20 gastric bypass and 26 sleeve gastrectomy) | TUNEL     | SDF                      | The SDF was decreased at 12 months follow-up after surgery                                            | Level 3              |
Table 3. Continued 1

| Study reference                  | Study design/participants                                      | SDF assay | Outcome measured | Main results/findings                                                                 | Quality of evidence |
|---------------------------------|----------------------------------------------------------------|-----------|------------------|----------------------------------------------------------------------------------------|---------------------|
| **Non-surgical weight loss**    | Mir et al (2018) [60]                                               | SCD       | SDF              | Reduced SDF after weight loss                                                          | Level 3             |
| **exercise program**            |                                                                  |           |                  |                                                                                        |                     |
| **Diet modification**           | Faure et al (2014) [221]                                            | TUNEL     | SDF              | Subsequent to following dietary advice, all men showed a reduction of SDF             | Level 4             |
| **Treatment of infections/inflammations** | Gallegos et al (2008) [47]                                       | SCD       | SDF              | Patients with infection had higher SDF than control group prior to treatment while antibiotics treatment resulted in decreased SDF | Level 2             |
| **Oral antioxidant therapy**    | Omu et al (2008) [354]                                              | SCSA      | SDF, semen parameters | SDF and sperm motility improved after treatment                                          | Level 1             |
| **Grec et al (2005) [208]**     | Randomized placebo-controlled study                               | TUNEL     | SDF, semen parameters | Significant decrease in SDF levels                                                    | Level 1             |
| **Martinez-Soto et al (2016) [356]** | Randomized, double blind, placebo-controlled, parallel-group study | TUNEL     | SDF              | Reduced SDF after supplementation while no changes were reported for the placebo group | Level 1             |
| **Stenqvist et al (2018) [202]** | Randomized placebo-controlled, double-blind study                 | SCSA      | SDF              | Increased sperm concentration after three months of treatment                          | Level 1             |

*SCD* = Spectroscopic Correlation Detector; *SDF* = Sperm DNA fragmentation; *SCSA* = Sperm Chromatin Structure Assay; *TUNEL* = Terminal Deoxynucleotidyl Transferase-Linked Nick End-Labeling.
| Study reference | Study design/participants | SDF assay | Outcome measured | Main results/findings | Quality of evidence |
|-----------------|---------------------------|-----------|------------------|-----------------------|---------------------|
| Kumar et al (2012) [182] | Prospective study | Comet | SDF | Significant decrease in alkaline-labile sites and mean tail length of the comet in comparison with the control group | Level 2 |
| Tunc et al (2009) [207] | Prospective study | TUNEL, CMA3 assay | SDF | Significant decrease in SDF and protamine packaging | Level 3 |
| Ménézo et al (2007) [205] | Prospective study | SCSA | SDF | Significant decrease in SDF | Level 3 |
| Kodama et al (1997) [353] | Prospective study | 8OHdG | Oxidative damage, semen parameters | Significant improvement in sperm concentration and decrease in 8OHdG | Level 3 |
| Greco et al (2005) [355] | Prospective study | TUNEL | SDF, semen parameters | After therapy, significant decreased SDF levels and improvement in ICSI clinical pregnancy and implantation rates compared with the pre-treatment ICSI outcomes | Level 3 |
| Fraga et al (1991) [357] | Prospective study | 8OHdG | Oxidative damage | Vitamin C depletion/repletion was inversely associated with seminal vitamin C levels and 8OHdG measures | Level 3 |
| Abad et al (2013) [358] | Prospective study | SCD following various periods of sperm storage (0, 2, 6, 8, and 24 hours) at 37°C | SDF | Significant decrease in SDF levels at all incubation times and in highly degraded sperm | Level 3 |
| Gual-Frau et al (2015) [360] | Prospective study | SCD | SDF | Significant decrease in SDF levels and in highly degraded sperm cells | Level 3 |

*SDF: Semen Diamine Oxidase; Comet: Comet assay; TUNEL: Terminal deoxynucleotidyl transferase dUTP nick-end labeling; CMA3: Chromosome Metaanalysis and Mutational Analysis for Trisomies 13 and 18; SCSA: Scanning Chromatographic Spot Analysis; 8OHdG: 8-Oxoguanine; SCD: Sperm Chromatin Decondensation; ICSI: Intracytoplasmic Sperm Injection.*
| Study reference          | Study design/participants                                                                 | SDF assay          | Outcome measured | Main results/findings                                                                                                                                                                                                 | Quality of evidence |
|--------------------------|-------------------------------------------------------------------------------------------|--------------------|------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|
| Piomboni et al (2008) [361] | Prospective study 36 men with leukocytospermia and 15 controls supplemented for 3 months with beta-glucan (20 mg), fermented papaya (50 mg), lactoferrin (97 mg), vitamin C (30 mg), and vitamin E (5 mg) | AO staining       | SDF              | No significant decrease in SDF Significant increase in sperm morphology and total progressive motility Significant reduction in leukocyte number                                                                                                                                                      | Level 3             |
| Negri et al (2017) [362]   | Retrospective study 15 men had no treatment, 55 were treated with a SOD-based antioxidant supplementation plus hydroxytyrosol and carnosol, 48 took different antioxidant combinations for 2 months | SCD                | SDF              | The SOD-based supplementation was associated with improved SDF                                                                                                                                                                                                                               | Level 3             |
| Varicocelectomy           |                                                                                           |                    |                  |                                                                                                                                                                                                                                                                                                                                                      |                     |
| Sun et al (2018) [382]     | Randomized controlled trial 358 men (179 unilateral repair and 179 bilateral repair)        | SCSA               | SDF              | DFI was significantly reduced in both varicocelectomy groups at 1-year follow-up                                                                                                                                                                                                               | Level 1             |
| Ni et al (2016) [363]      | Prospective study 15 infertile patients with subclinical varicocele, 22 normozoospermic clinical varicocele patients, 51 astheno/oligozoospermic clinical varicocele patients, and 25 healthy controls Clinical grades: 1–3 Intervention: retroperitoneal ligation | SCSA               | SDF              | Improved sperm DFI status post repair of clinical varicocele in all 3 grades at 3 and 6 months                                                                                                                                                                                             | Level 2             |
| Mohammed et al (2015) [264] | Prospective study 75 men with >1 year infertility and varicocele, 40 fertile controls Clinical grades: 1–3 Intervention: subinguinal varicocelectomy | AO Assay and Flow Cytometry | SDF and chromatin condensation | Improved DFI after intervention AO is a more reliable method vs. flow cytometry in evaluation of sperm DNA integrity after varicocelectomy                                                                                                                                                   | Level 2             |
| Afsin et al (2018) [365]   | Prospective case controlled 40 infertile men (15–30 years) with clinical varicocele Clinical grades: 2–3 Intervention: NA | TUNEL              | SDF              | Significantly improved SDF after varicocele repair at 3, 6, and 12 months after surgery                                                                                                                                                                                                          | Level 2             |
| Alhathal et al (2016) [368] | Prospective study 29 infertile varicocele men, 6 donors Clinical grades: clinical varicocele Intervention: microsurgical varicocelectomy | Aniline blue staining, IAF fluorescence, SCSA | Semen parameters and SDF | Significant improvement in sperm concentration and motility at 6 months Significant reduction in DFI, % HDS, % positive aniline blue staining, % positive S IAF                                                                                                                                 | Level 2             |
| Study reference                      | Study design/participants                                                                 | SDF assay | Outcome measured                                                                                   | Main results/findings                                                                                   | Quality of evidence |
|-------------------------------------|--------------------------------------------------------------------------------------------|-----------|---------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------|---------------------|
| Showell et al (2014) [203]          | Prospective study 42 subfertile patients with varicocele and 10 healthy controls with proven fertility Clinical grades: 1–3 Intervention: microsurgical subinguinal varicocelectomy | SCSA      | DFI                                              | DFI was higher pre-intervention in men with varicocele vs. controls DFI improved significantly after varicocele repair | Level 2             |
| Abdelbaki et al (2017) [379]        | Prospective study 60 infertile men with varicocele, 20 normozoospermic fertile men Clinical grades: 1–3 Intervention: inguinal varicocelectomy | SCSA      | DFI                                              | DFI levels were higher in men with varicocele vs. control DFI and ROS levels decreased after varicocelectomy | Level 2             |
| Lacerda et al (2011) [364]          | Prospective study 27 adolescents (15–19 years) Clinical grades: 2–3 Intervention: bilateral subinguinal micro varicocelectomy | Comet     | DNA integrity, mitochondrial activity, and lipid peroxidation | Improved sperm DNA integrity and mitochondrial activity Comet class 1 cells (undiamaged DNA) increased after repair | Level 3             |
| Zini and Dohle (2011) [168]         | Systematic literature review 16 clinical articles included Clinical grades: NA Intervention: multiple approaches | 8-OHdG, TUNEL, Comet, SCSA, aniline blue | SDF                                              | Most studies demonstrated higher SDF in varicocele patients, improved after varicocele repair | Level 3             |
| Zini et al (2005) [367]             | Retrospective study 37 men with varicocele Clinical grades: clinical, subclinical, and no varicocele Intervention: microsurgical varicocelectomy | AO, flow cytometry | SDF                                              | Improved DNA damage after varicocele repair                                                                 | Level 3             |
| Smit et al (2013) [178]             | Prospective study 49 infertile men Clinical grades: 1–3 Intervention: high inguinal or microsurgical sub inguinal | SCSA      | SDF                                              | Significant improvement in % DFI post intervention 37% conceived spontaneously 24% achieved with ART Mean postoperation DFI significantly higher in couples who did not conceive | Level 3             |
| Zini and Sigman (2009) [268]        | Prospective trial 25 infertile men with varicoceles Clinical grades: NA Intervention: microsurgical varicocelectomy | SCSA      | SDF                                              | Sperm DFI improved 4- and 6-month post-intervention                                                                 | Level 3             |
| Ghazi and Abdelfattah (2011) [369]  | Prospective study 81 infertile men with clinical varicoce Clinical grades: NA Intervention: microsurgical inguinal varicocelectomy | TUNEL     | SDF                                              | SDF improved 6 months post intervention                                                                 | Level 3             |
| Study reference | Study design/participants | SDF assay | Outcome measured | Main results/findings | Quality of evidence |
|-----------------|---------------------------|-----------|------------------|------------------------|---------------------|
| Roque and Esteves (2018) [177] | Literature review Clinical grades: NA Intervention: multiple approaches | Multiple | SDF | Men with varicocele have higher SDF SDF decreases after varicocele repair | Level 3 |
| Zaazaa et al (2018) [370] | Prospective cohort study 120 infertile men Clinical grades: 2–3 Intervention: microsurgical subinguinal varicocelectomy | SCD | SDF | Improved SDF after varicocelectomy with or without mast cell stabilizers | Level 3 |
| La Vignera et al (2012) [371] | Prospective study 30 men with varicocele and 30 fertile controls Clinical grade: 3 Intervention: microsurgical subinguinal varicocelectomy | TUNEL | SDF | Control group showed lower SDF In varicocele patients, SDF was lower after varicocelectomy | Level 3 |
| Kadioglu et al (2014) [372] | Retrospective study 92 men with varicocele Clinical grades: 1–3 Intervention: microsurgical varicocelectomy | SCSA | SDF | Significant decrease in DFI post varicocelectomy | Level 3 |
| Lara-Cerrillo et al (2020) [373] | Retrospective study 20 men with varicocele and 12 controls Clinical grades: 1–3 Intervention: microsurgical subinguinal varicocelectomy | Comet assay | Single and double strand DNA breaks | Significant decrease in the percentage of single and double DNA strand breaks after varicocelectomy | Level 3 |
| García-Peiró et al (2014) [374] | Retrospective study 15 untreated varicocele patients (clinical grade 1), 16 with subclinical varicocele, 19 patients with surgically treated clinical varicocele, 10 with surgically treated subclinical varicocele and 21 fertile controls Clinical grades: clinical grade 1 and subclinical found on ultrasonography Intervention: NA | TUNEL, SCD, SCSA | SDF | Infertile men with clinical and subclinical varicocele showed similar elevated SDF levels Only men with clinical varicocele showed improvement after varicocele repair | Level 3 |
| Cho et al (2016) [375] | Literature review Clinical grades: clinical and subclinical varicocele Intervention: multiple approaches | SCD, SCSA, TUNEL | Semen parameters SDF and pregnancy rate | Improved semen parameters, decreased SDF, increased pregnancy rates after clinical varicocele repair No benefit on fertility potential for repairing subclinical varicocele | Level 3 |
| Study reference            | Study design/participants                                                                 | SDF assay | Outcome measured | Main results/findings                                                                 | Quality of evidence |
|---------------------------|-------------------------------------------------------------------------------------------|-----------|-----------------|---------------------------------------------------------------------------------------|---------------------|
| Baker et al (2013) [263]  | Retrospective study 83 infertile men with varicocele Clinical grades: 1–3 Intervention: microsurgical subinguinal varicocelectomy | TUNEL     | SDF             | SDF decreased post intervention 51% of couples were able to conceive naturally or with ART | Level 3              |
| Tahamtan et al (2019) [376] | Retrospective study 18 infertile men with clinical varicocele and 20 fertile controls Clinical grades: 2–3 Intervention: NA | TUNEL     | SDF             | Men with varicocele had higher SDF than fertile controls                                | Level 3              |
| Wang et al (2012) [176]  | Literature review and meta-analysis 12 studies included in review Clinical grades: 1–3 Intervention: all surgical approaches considered | SCSA, TUNEL, Comet | SDF             | Men with varicocele showed higher sperm DNA damage over controls Varicocelectomy can improve sperm DNA integrity | Level 3              |
| Li et al (2012) [377]     | Retrospective, case control series 19 infertile men with varicocele and 19 normozoospermic men Clinical grades: 1–3 Intervention: microsurgical subinguinal varicocelectomy | SCSA      | SDF             | DFI higher in varicocele group pre-intervention compared to controls and decreased at 3 months post-intervention, which was similar to levels for normal control group | Level 3              |
| Sakamoto et al (2008) [378] | Retrospective study 28 azoospermic, 30 oligospermic (15 with varicocele), 30 normozoospermic (15 with varicocele) Clinical grades: 1–3 Intervention: Microsurgical subinguinal varicocelectomy | TUNEL     | SDF             | TUNEL positivity significantly decreased after varicocele repair                         | Level 3              |
| Telli et al (2015) [380]  | Prospective study 72 men with at least 1-year history of infertility, a palpable varicocele and oligospermia | AO test   | SDF             | The mean DFI decreased after varicocelectomy                                           | Level 3              |
| Testicular sperm          | Arafa et al (2018) [269] Prospective cohort study comparing testicular vs. ejaculated spermatozoa | SCD       | Fertilization rate, embryo grading and live births                                      | Use of testicular ICSI significantly improves clinical pregnancy and live birth rates | Level 2              |
| Testicular sperm          | Greco et al (2005) [252] Prospective cohort study comparing testicular sperm and ejaculated sperm | TUNEL     | Pregnancy rate, implantation rate, ongoing clinical pregnancy                           | Higher pregnancy rate was achieved using testicular sperm in ICSI                     | Level 2              |
| Study reference | Study design/participants | SDF assay | Outcome measured | Main results/findings | Quality of evidence |
|-----------------|--------------------------|-----------|------------------|-----------------------|---------------------|
| Testicular sperm Esteves et al (2015) [387] | Prospective study comparing testicular sperm and ejaculated sperm | SCSA | Clinical pregnancy, miscarriage and live-birth rates | Lower SDF in testicular than ejaculated spermatozoa; ICSI outcomes improved with testicular sperm | Level 2 |
| IMSI Maettner et al (2014) [384] | Prospective study To establish the relationship between the IMSI selected spermatozoa and their DNA integrity in 45 patients | SCD | SDF | By analyzing normozoospermic, oligoa-sthenozoospermic and oligoa-sthenotheratozoospermic samples, the IMSI technique alone is not enough for the selection of spermatozoa with intact nuclei | Level 2 |
| PICSI Parmegiani et al (2010) [247] | Randomized study involving 206 couples that compared conventional PVP-ICSI to ICSI, in which the spermatozoa are selected for their capacity to bind to HA | SCD | SDF | The best-quality embryo rate (grade 1) in the HA-ICSI group was significantly higher than in the PVP-ICSI group | Level 2 |
| Microfluidics, DGC-swim up Quinn et al (2018) [246] | Blinded, controlled study evaluating semen parameters and SDF in samples from infertile men (n=70) processed by microfluidics or DGC-swim up | SCD | SDF | Microfluidics was associated with the best sperm recovery in terms of SDF | Level 2 |
| Testicular sperm Moskovtsev et al (2010) [251] | Prospective study 12 men with high SDF Evaluation of DNA damage of ejaculated and testicular spermatozoa | TUNEL | SDF | Lower SDF in testicular samples than ejaculated spermatozoa | Level 3 |
| Testicular sperm Pabuccu et al (2017) [386] | Retrospective study comparing testicular vs. ejaculated spermatozoa of normozoospermic males with high SDF (>30%) and previous ART failures | SCSA | Clinical and on-going pregnancy rates and miscarriage | Clinical and on-going pregnancy rates were significantly improved while miscarriage rate was reduced when testicular spermatozoa were used in ICSI | Level 3 |
| PICSI; IMSI; Testicular sperm Bradley et al (2016) [253] | Retrospective cohort analysis of ICSI cycles | SCIT | Pregnancy, blastocyst transfer and live birth rates | High SDF (>29%) without intervention had lower fertilization rate or poor outcomes for blastocyst transfer; High SDF with intervention (ICSI) had improved blastocyst transfer rate; TESA samples showed the highest live birth rate | Level 3 |
| PICSI Mongkolchaipak and Vutyavanich (2013) [248] | Prospective study Samples from 50 patients with severe male factor cases, processed through DGC, and subjected to sperm selection by using the conventional method (control), high magnification at 36,650 or HA binding | TUNEL | SDF | Spermatozoa selected under high magnification had a lower SDF rate than those selected by the HA binding method; Spermatozoa selected by both methods had much lower aneuploidy and SDF rate than the controls | Level 3 |
| Study reference | Study design/participants | SDF assay | Outcome measured | Main results/findings | Quality of evidence |
|-----------------|---------------------------|-----------|------------------|-----------------------|--------------------|
| **IMSI**        | Hammoud et al (2013) [383] Prospective study To evaluate the potential value of IMSI for 8 patients with high SDF | TUNEL | SDF | Motile normal spermatozoa with a vacuole-free head selected at 6,300× magnification showed lower SDF than all other types of spermatozoa | Level 3 |
| **DGC, Swim-up** | Jayaraman et al (2012) [232] Comparison of DGC and swim-up, either alone or in combination to select sperm with high DNA integrity | TUNEL | SDF | By using different techniques for sperm selection, no difference was observed in the SDF percentage | Level 3 |
| **DGC, Swim-up** | Volpes et al (2016) [233] Comparison of direct swim-up, pellet swim-up, DGC, and DGC followed by swim-up to select sperm with high DNA integrity | SCD | SDF | Pellet swim-up and DGC followed by swim-up selected the highest number of sperm with intact DNA | Level 3 |
| **Swim-up, hyaluronan (HA)-binding methods** | Vozdova et al (2012) [234] 12 patients who carried balanced chromosomal translocations 10 controls Comparison of swim-up and HA-binding methods for the evaluation of the frequency of spermatozoa with abnormal karyotypes and altered chromatin quality | SCSA | SDF | Higher SDF in the group of translocation carriers compared to controls | Level 3 |
| **Swim-up, DGC** | Enciso et al (2006) [304] Comparison of swim-up and DGC to select spermatozoa with low SDF | Comet, SCD | SDF | Both techniques are equally efficient in eliminating DNA-damaged spermatozoa DGC is more efficient in selecting spermatozoa with low percentage of single-strand DNA damage | Level 3 |
| **DGC, MACS** | Zhang et al (2018) [243] Comparison of DGC and DGC - MACS to select viable spermatozoa with lower SDF | TUNEL | SDF | The lowest SDF rate was observed in DGC-MACS selected sperm | Level 3 |
| **DGC, swim-up** | Ogu et al (2018) [235] Comparison of DGC and swim-up to select less damaged sperm from unexplained and mild male factor subfertile patients undergoing IUI | SCD | SDF | Swim-up selected sperm showed a reduction in the SDF compared to basal rates | Level 3 |
| **DGC** | Zini et al (2000) [236] Comparison of DGC and swim-up technique to select sperm with better sperm DNA integrity | SCSA | SDF | Swim-up selected sperm showed lower percentage of denaturated sperm DNA integrity | Level 3 |
| **Microfluidics** | Nosrati et al (2014) [249] Samples from 8 healthy donors were separated by using a microfluidic device | SCSA | SDF | DNA integrity significantly improved after microfluidics selection | Level 4 |

For quality of evidence, use Oxford Centre for Evidence-Based Medicine 2011 Levels of Evidence (https://www.cebm.net/wp-content/uploads/2014/06/CEBM-Levels-of-Evidence-Introduction-2.1.pdf and https://www.cebm.net/wp-content/uploads/2014/06/CEBM-Levels-of-Evidence-2.1.pdf). For more details: https://www.cebm.net/2011/06/explanation-2011-ocebm-levels-evidence/.

SDF: sperm DNA fragmentation, ICSI: intracytoplasmic sperm injection, IMSI: intracytoplasmic morphologically selected sperm injection, PICSI: physiological intracytoplasmic sperm injection, DGC: density gradient centrifugation, HA: hyaluronic acid, SCD: sperm chromatin dispersion, TUNEL: terminal deoxynucleotidyl transferase dUTP nick end labeling, SCSA: sperm chromatin structure assay, MMP: mitochondrial membrane potential, ROS: reactive oxygen species, DFI: DNA fragmentation index, DHA: docosahexaenoic acid, FSH: follicle stimulating hormone, LH: luteinizing hormone, CMA3: chromomycin A3, OHdG: 8-hydroxy-2’-deoxyguanosine, AO: acridine orange, SOD: superoxide dismutase, NA: not available, IAF: iodoacetamide fluorescein, HDS: high sperm DNA stainability, ART: assisted reproductive techniques, SCIT: sperm chromatin integrity test, TESA: testicular sperm aspiration, PVP: polyvinylpyrrolidone, MACS: magnetic-activated cell sorting, IUI: intrauterine insemination.
been numerous environmental factors such as airborne pollutants, ionizing radiation, and pesticides linked with increased SDF values [74,82,193,216,217]. Several studies have demonstrated higher SDF in obese men, yet a recent meta-analysis found no robust association between BMI and SDF [218]. No concrete evidence in lifestyle modification impact on SDF exists [219], however, weight loss and dietary changes have been proposed to benefit SDF indices in patients [220,221] (Table 3).

5. Short ejaculatory abstinence
The negative impact of prolonged ejaculatory abstinence (EA) on SDF has been reported without significant detrimental effect on conventional semen parameters [222,223]. Therefore, short-term recurrent ejaculation may be a simple noninvasive maneuver to improve SDF. Although the beneficial effect of short EA on natural conception is unclear, application of the technique to assisted reproduction may have its value [224-226]. In addition to higher pregnancy rates in ICSI, recurrent ejaculation has been associated with a significantly lower SDF [227] (Table 3).

6. Sperm processing and preparation
Laboratory conditions (i.e., prolonged incubation, centrifugation, cryopreservation and use of different media) can significantly impact SDF by increasing OS-mediated DNA damage [228-231]. Conventional (swim-up, DGC) and advanced techniques can select sperm with low levels of SDF [232-236] (Table 3). Magnetic-activated cell sorting (MACS), based on the detection of phosphatidylserine [237], shows a better selection alone [238,239] or in combination with DGC [240-243]. Intra-cytoplasmic morphologically selected sperm injection (IMSI) uses high magnification to select the most morphologically normal sperm, as the presence of vacuoles in the nuclear region has been associated with high SDF [244,245]. Other approaches include the physiological intracytoplasmic sperm injection (PICSI), based on sperm binding to hyaluronic acid, and microfluidic devices, allowing sperm migration along microchannels [246-249].

7. Use of testicular sperm for intracytoplasmic sperm injection
Testicular sperm has been explored as a treatment option for high SDF based on the finding of lower SDF in testicular sperm than ejaculated sperm [250,251], and better ICSI outcome [12,252,253]. However, surgical sperm retrieval [254] carries risk of anesthetic and surgical complications. Furthermore, possible higher aneuploidy rate in testicular sperm is a concern [255] despite a recent report of opposing view [256]. Therefore, the use of testicular sperm in clinical management of non-azoospermic patients with high SDF is still debated.

CLINICAL CASE REPORTS

1. Case 1
A 37-year-old male, presented to the male infertility unit complaining of primary infertility of 3 years duration. He is a navy lieutenant and is physically fit. He does not have a history of recent febrile illness, genitourinary infections or trauma. He does not have a significant past medical or surgical history. He smokes half a pack of cigarettes a day for the past 15 years. There is no family history of infertility. His wife is 26 years old with regular menses and normal fertility evaluation. There is no consanguinity between the couple. On physical examination, he was of normal BMI (26 kg/m²). Genital examination revealed normal phallus, normal testis size, palpable vasa bilaterally and no palpable varicocele. An outside semen analysis demonstrated a volume of 3 mL, sperm concentration of 11 million/mL, total motility of 40% (progressive motility 20%) and normal morphology of 10%.

Repeat semen analysis with SDF testing performed at our center demonstrated a volume of 4.5 mL, sperm concentration 9 million/mL, total motility 30% (progressive motility 10%), normal morphology of 3% (WHO, 2010) and SDF of 45% (using the SCD test [Halosperm], normal<30%). His serum hormone levels were as follows: testosterone 17.5 nmol/L (normal=10.4–30.86 nmol/L), follicle stimulating hormone (FSH) 2.5 IU/L (normal=1.5–12.4 IU/L), luteinizing hormone (LH) 2 IU/L (normal=1.7–8.6 IU/L), estradiol 122 pmol/L (normal=94.8–223 pmol/L), and prolactin 245 mIU/L (normal=85–323 mIU/L). Scrotal ultrasound demonstrated normal testicular volume echogenicity, and vascularity and absence of epididymal cysts and varicoceles.

The patient was diagnosed with idiopathic oligoasthenoteratozoospermia and high SDF. He had a lifestyle risk factor and was counselled about the importance of smoking cessation on his overall health and fertility potential.
He was prescribed the following antioxidants: vitamin C 500 mg twice daily, L-carnitine+zinc 1,000 mg twice daily, and folic acid 0.5 mg once daily for 3 months.

On the 3-month follow-up visit, a repeat semen testing showed a volume of 3 mL, sperm concentration of 17 million/mL, total motility 45% (progressive motility 25%), normal morphology 5%, and SDF 26%. There was still no pregnancy and hence, the couple were advised to undergo IUI. The patient was kept on vitamin C and L-carnitine+zinc regimen.

The procedure was performed 5 weeks following the last patient visit. The prewash total motile sperm count was 22.5 million and the post-wash total motile sperm count was 13 million. The patient was seen in the clinic 6 weeks following his IUI and reported that his wife was pregnant.

2. Case 2

A 41-year-old male, presented for fertility evaluation after failure of conception for 3 years. His past medical and surgical history were unremarkable. He did not smoke or consume alcohol. On physical examination, the patient had normal built (height 176 cm, weight 82 kg, BMI 26.5 kg/m²). On genital examination, both testes were normally descended with normal size and consistency, both epididymides were normal, vasa deferentia could be felt bilaterally, and no varicocele could be appreciated either side. The spouse was 32 years old with regular menses, no gynecological problems and normal ovarian reserve (anti-mullerian hormone: 18.9 pmol/L, normal=0.071–52.4 pmol/L).

Semen analysis demonstrated a volume of 2.5 mL, sperm concentration of 34 million/mL, total motility of 8%, 0% progressive motility and 5% normal morphology. SDF testing was performed using the Halosperm Kit and was found to be high (90%). Hormonal profile assessment showed normal levels of testosterone, FSH, LH, prolactin and estradiol. The patient was given antioxidants in the form of L-carnitine 1,000 mg+zinc twice daily, vitamin C 1,000 mg once daily, co-enzyme Q10 and selenium for 3 months and on repetition of SDF, it was still high (85%). The couple were counseled and decided to go for a trial of ICSI using ejaculated sperm. The female was started with standard long protocol. On the day of ICSI, 16 cumulus-oophorus complexes were collected, 13 of which were in metaphase II (MII) and were used in ICSI. After 24 hours only one oocyte was fertilized and at day 3, it showed no division therefore no embryo transfer was done.

After 4 months, SDF was still elevated (85%) and the couple were scheduled for a second ICSI trial using testicular sperm which was retrieved by testicular sperm aspiration on day of ICSI. In total, 22 oocytes were collected, 15 of which were MII. At 24 hours, 9 oocytes were fertilized. Two embryos were transferred on day 5. Pregnancy test was positive after 2 weeks and the spouse delivered a healthy girl.

3. Case 3

A 44-year-old male, presented for fertility evaluation after failure of conception for 6 years. He had an unremarkable past medical and surgical history. He was a non-smoker and non-drinker. On physical examination, the patient has normal built (height 177 cm, weight 77 kg, BMI 24.6 kg/m²). On genital examination, both testes were normally descended with normal size and consistency, both epididymides were normal, vasa deferentia could be felt bilaterally, and varicocele could be appreciated on both sides clinically (left grade III and right grade I). The spouse was 30 years old with regular menses and no gynecological problems. The couple performed one IUI trial 3 years ago but it was unsuccessful.

Semen analysis showed oligoasthenoteratozoospermia with a sperm concentration of 6 million/mL, total motility of 34%, 4% progressive motility, and 2% normal morphology. SDF testing with the Halosperm Kit was high (45%). Hormonal profile assessment showed normal levels of testosterone, FSH, LH, prolactin and estradiol. Scrotal ultrasound confirmed bilateral varicocele with vein diameters of 4.8 mm and 3.2 mm on left and right sides, respectively. The couple were counseled on treatment options and consented to proceed with surgical varicocelectomy. Bilateral microsurgical subinguinal varicocelectomy was performed without any complications.

After 3 months, the patient repeated the semen analysis which demonstrated improvement but with continued oligoasthenoteratozoospermia with a sperm concentration of 9 million/mL, total motility of 65%, 8% progressive motility, and 3% normal morphology. SDF was normalized (25%). The couple were counseled for assisted conception, but they opted to try for natural conception for another 3 months. At 6 months following surgery, they achieved a spontaneous pregnancy.
and subsequently delivered a healthy girl.

4. Case 4
A 34-year-old male, presented for fertility evaluation. His wife was 33 years old with regular menses and no gynecological problems. They were married for 8 years and had a 6.5-year-old boy who was conceived spontaneously. The husband had an unremarkable past medical history. He underwent left orchidectomy following failed orchiopexy for an intra-abdominal left undescended testis at the age of 6 years. He was a non-smoker and did not consume alcohol. On physical examination, the patient had normal built (height 182 cm, weight 98 kg, BMI 29.6 kg/m²). On genital examination, the left scrotal sac was empty and underdeveloped. The right testis, epididymis, and vas deferens were normal, and there was no palpable varicocele.

Semen analysis showed a sperm concentration of 14 million/mL, total motility of 45%, 15% progressive motility and 4% normal morphology. SDF was tested twice using Halosperm Kit and was high (47% and 49%). ORP was assessed using the MiOXSYS system and was high (29 mV/10⁶ sperm/mL, normal=1.34 mV/10⁶ sperm/mL). Hormonal profile assessment showed normal levels of testosterone, FSH, LH, prolactin and estradiol. The patient was given antioxidants (containing mainly selenium, L-carnitine, L-arginine, Coenzyme Q10, Lycopene, N acetylle l-cysteine, vitamin C, and E) for 3 months. On repetition of semen analysis, it showed 31 million/mL, total motility of 50%, 25% progressive motility and 8% normal morphology, while SDF (25%) and ORP (1.2 mV/10⁶ sperm/mL) normalized. One month later, his wife achieved a spontaneous pregnancy and she delivered a healthy boy.

Fig. 4. Clinical algorithm to elucidate the applications of sperm DNA fragmentation (SDF) testing in clinical practice. ICSI: intracytoplasmic sperm injection.
EXPERT RECOMMENDATIONS ON SPERM DNA FRAGMENTATION TESTING

The extensive literature search conducted in this review reveals that SDF significantly impacts male fertility and its testing in specific clinical circumstances may augment the treatment strategy resulting in better outcomes. Accordingly, a clinical algorithm is set forth by this expert panel to elucidate the application of SDF testing in clinical practice (Fig. 4). Patients presenting with infertility should be evaluated with a complete medical and reproductive history, undergo physical examination by a reproductive specialist or urologist and provide at least two semen specimens for conventional analysis [257-259].

Men with idiopathic and UMI, RPL, and modifiable lifestyle risk factors should undergo SDF testing (grade C recommendation). This recommendation is based on the evidence linking high SDF levels in the abovementioned conditions. It is also aimed at providing pertinent treatment strategies directed at lowering SDF levels. Oral antioxidant therapy may be considered in these regards (grade C recommendation). While its benefit in alleviating SDF and improving live birth rates in infertile men has been reported by a Cochrane meta-analysis [260], further research is needed to refine the ideal candidates and treatment regimen.

Diet modification and weight reduction may help in reducing SDF (grade C recommendation). However, further research is needed to confirm the role of lifestyle modifications in improving sperm DNA integrity and possibly translate into better reproductive results. Nonetheless, the information provided by SDF testing might help to monitor patient compliance and treatment prognosis.

Another indication for SDF testing is in patients who are diagnosed with clinical varicocele (grade C recommendation). The findings of higher SDF in both fertile and infertile men with varicocele than controls [168] and significant decrease in SDF levels after varicocele repair [261] provide the rationale of SDF testing in refining the selection of varicocelectomy candidates. In addition, reduction in SDF seems to translate into better reproductive outcomes [262-264]. Although the association between SDF and high-grade varicocele is much stronger, patients with low-grade varicocele had achieved improvement in natural pregnancy rate that were similar to those with high-grade varicocele after surgery [265].

SDF testing should also be offered to infertile couples prior to initiating or after failure of IUI/IVF (grade C recommendation). The relationship between SDF and ART outcomes has been extensively investigated. Controversies persist in view of heterogeneous nature of the studies [16,266]. In general, high SDF is one of the etiologies in patients with recurrent IUI or IVF failure [267]. In contrast to the association between SDF and IUI/IVF outcomes, there is compelling evidence suggesting that SDF has a negligible effect on ICSI outcome measures [154,158,268]. These results signify the potential role of ICSI in the treatment of men with high SDF. Patients with persistently high SDF result should be directed towards ICSI, such recommendation will avoid unnecessary delay in definitive treatment which is particularly important in couples with limited reproductive window (grade C recommendation).

Finally, SDF testing is indicated in couples with recurrent miscarriage following ICSI (grade C recommendation). While high levels of SDF appear not to have a significant impact on ICSI pregnancy rates [146,152,154,157], a greater risk of miscarriage following ICSI has been reported by several meta-analyses [146,153,154]. A number of interventions have been explored in the context of ICSI to reduce SDF levels and consequently achieve a better outcome. Various sperm selection methods (swim-up, DGC, MACS, IMSI, PICSI) are able to identify sperm with intact DNA integrity for injection [237,240-244,246]. The significantly lower SDF levels in testicular compared to ejaculated sperm supports the use sperm harvested from testis as a plausible maneuver to bypass sperm DNA damage which occurs during the epididymal transit [250]. A meta-analysis of five studies favored the use of testicular sperm by demonstrating better clinical pregnancy and live birth rates [12]. The utilization of testicular sperm is further supported by recent reports and better reproductive outcomes that have been reported in both oligozoospermic and normozoospermic men with prior ICSI failure [269,270]. Nonetheless, the invasive nature of sperm retrieval procedures and the higher rates of sperm aneuploidy with testicular sperm can be considered as potential disadvantages for this treatment approach which certainly warrants further investigation [271,272].
STRENGTHS-WEAKNESSES-OPPORTUNITIES-THREATS (SWOT) ANALYSIS ON THE CLINICAL UTILITY OF SPERM DNA FRAGMENTATION TESTING IN SPECIFIC MALE INFERTILITY SCENARIOS

SWOT analysis, a system that was originally developed for financial studies, has been recently applied to health sciences. It explores the strengths and weaknesses of a given method in an attempt to identify the threats and opportunities accessible to overcome certain gaps hindering its broad application. Studies included in this review (Table 2) were analyzed using the SWOT method to understand the perceived advantages and drawbacks for the clinical utility of SDF in specific clinical scenarios (Fig. 5).

1. **Strengths**

SDF testing can serve as an ancillary test to conventional semen analysis in specific clinical scenarios. Evidence indicates that higher levels of SDF are observed in patients who are unable to conceive naturally [149,273-275], who present with UMI/idiopathic infertility [185,188,277-278], have RPL [116,138,180-183,283-285], are diagnosed with varicocele [169,174,296-305], have a negative ART outcome [13,41,88,117,306-330] and who are found to have lifestyle/environmental risk factors [42,47,65,74,82,84,88,190-193,195-197,243,331-349].

The widespread use of SDF testing has been hampered by the belief that no effective treatment exists to alleviate high SDF in clinical practice. On the contrary, studies have shown that a number of interventions can be utilized in this regard. Examples of such interventions include recurrent ejaculation to shorten the abstinence time [222,227,350-352], oral antioxidant therapy [205,207,208,353-362], performing varicocelectomy for patients with clinical varicocele [168,176,177,179,263,264,360,363-382], treating genitourinary infections when diagnosed [47], and utilizing advanced sperm selection techniques for ICSI such as PICSI/IMSI [383-385] or using testicular sperm instead of ejaculated sperm [251,252,269,386,387].

2. **Weaknesses**

Perhaps the main limitation of SDF testing is the lack of a definitive cut-off value above which a sample is considered anomalous. It is worth mentioning that various SDF thresholds may be determined based on the predicted outcome measure (fertility/infertility, ART success/failure, etc.). Indeed, several cut-off values were reported having a fair to good overall accuracy in predicting various outcome measures (Table 1). Despite the differences in the reported cut-offs, a recent meta-
analysis by Santi et al. compared the SDF results of four different assays (TUNEL, SCD, SCSA, and Comet) between 2,883 infertile men and 1,294 fertile men. The authors identified a SDF cut-off of 20% which had a good predictive power in differentiating between fertile and infertile men with a sensitivity of 79% and a specificity of 86% (area under the curve=0.844) [108].

Another weakness for the utility of SDF testing is the existing moderate to low evidence in support of its use in the above-mentioned clinical scenarios. The heterogenous nature of the conducted studies and the scarcity of randomized clinical trials are possibly the main reasons behind the obtained level of evidence. Furthermore, few contradictory studies have been reported in almost every clinical scenario. A number of studies failed to find a significant association between high levels of SDF and UMI idiopathic infertility [190,388], RPL [389-392], and likelihood of conception whether natural [381], or following ART [314,393-410]. While 50%–60% of patients with varicocele have elevated SDF levels, it is not uncommon to find a normal SDF result in infertile men with varicocele who might have a conventional semen parameter abnormality. As for lifestyle/environmental risk factors, no solid evidence exists to support the benefit of lifestyle modification on the SDF level [199,411,412].

3. Threats

The lack of sufficient high-quality evidence supporting the utility of SDF testing resulted in international societies (American Society for Reproductive Medicine, American Urologic Association, European Association of Urology [EAU]) not to recommend its routine use for the evaluation of male infertility. However, since many confounding factors can impact the likelihood of conception, it is not uncommon in the field of reproduction to provide recommendations for diagnostic tests based on lower quality of evidence. Nonetheless, the increasing number of publications exploring the utility of SDF testing in recent years should provide enough fuel for an update to reproductive society guidelines. This is recently witnessed in the latest update of the EAU guidelines on male infertility which recommends SDF testing for the assessment of couples with RPL

| Assay          | Principle                                      | Type of damage detected | Pros                                                                 | Cons                                                                 | Estimated price (US dollars) |
|----------------|-----------------------------------------------|-------------------------|----------------------------------------------------------------------|----------------------------------------------------------------------|-----------------------------|
| TUNEL          | Labeling of free break ending 3-OH DNA        | SSB/DSB                 | High sensitivity and reliability                                       | Protocols and thresholds are still not standardized                | 150                         |
|                |                                               |                         | Minimal inter-observer variability                                     | Expensive equipment and trained personnel required                 |                              |
|                |                                               |                         | Evaluation by both fluorescent microscopy and flow cytometry          |                                                                      |                              |
|                |                                               |                         | Analysis of both fresh and frozen samples                             |                                                                      |                              |
| Comet          | Single cell electrophoretic separation        | SSB and/or DSB          | High sensitivity                                                       | Poor repeatability                                                  | ~400–600                    |
|                |                                               |                         | Correlation with semen parameters                                       | High inter-observer variability                                    |                              |
|                |                                               |                         | Possibility to discriminate between SSB and DSB                        | Variable protocols and thresholds                                  |                              |
|                |                                               |                         |                                                                      | Time-consuming                                                       |                              |
|                |                                               |                         |                                                                      | Evaluation of a low number of cells                                |                              |
|                |                                               |                         |                                                                      | Appropriate imaging software required                              |                              |
| SCSA           | Evaluation of DNA integrity by using the meta-chromatic acidine orange | SSB/DSB                 | Simultaneous examination of a large number of cells                    | Commercial kits not available                                       | 300                         |
| SCD or Halosperm test | Evaluation of the dispersed chromatin (“halo”) after lysing treatment | SSB/DSB                 | Highly repeatability                                                   | Expensive equipment and trained personnel required                   |                              |
|                |                                               |                         | Analysis of frozen or fresh samples                                   |                                                                      |                              |
|                |                                               |                         | Commercial kits available                                              | Inter-observer variability                                          | 175                         |
|                |                                               |                         | Repeatable and consistent results in 45 minutes                        |                                                                      |                              |
|                |                                               |                         | No expensive equipment required                                        |                                                                      |                              |

SDF: sperm DNA fragmentation, TUNEL: terminal deoxynucleotidyl transferase dUTP nick end labelling, SCSA: sperm chromatin structure assay, SCD: sperm chromatin dispersion, SSB: single strand breaks, DSB: double strand break.
following natural or ART conceptions and in men with unexplained infertility [259].

Case scenarios are commonly used in medical literature to describe a certain clinical condition. However, they may not accurately represent all the possible presentations that might be seen in the clinic. Despite this, we have utilized this method to personalize the message giving it a clinical perspective.

Finally, the cost of SDF testing ranges between $150–300 (Table 4) which is another important factor limiting its routine use in clinical practice. However, cost is also a major drawback to several fertility related therapeutic interventions that are usually not covered by medical insurance [413]. While SDF testing may be considered an additional cost for patients undergoing fertility treatments, such as ART or varicocelectomy, understanding the circumstances where this assay is most beneficial should help in improving the outcome of treatment and may possibly impact the overall treatment cost.

4. Opportunities

Further studies of adequate power and controlled design are necessary to enhance our understanding of the clinical utility of SDF. This review inspected the available literature with regards to various applications for SDF testing in clinical practice. However, a number of gaps remain and are considered potential areas of research. These areas are particularly involved with demonstrating the impact of interventions on SDF reduction and more importantly on fecundity.

CONCLUSION

SDF is detrimental for normal fertilization, embryo development and success of ART and therefore, SDF testing is increasingly being utilized in the evaluation of male infertility. SDF can be induced endogenously by defective maturation and abortive apoptosis occurring within the testis, or by OS throughout the male reproductive tract. It can also result from exogenous sources including clinical disease states (varicocele, cancer, diabetes), lifestyle risk factors (smoking, alcoholism, obesity), and environmental exposures (air pollution, pesticides, industrial chemicals). Various SDF testing methods are available; while a single specific cut-off value has not been unanimously identified, a threshold of 20% is believed to be hold a good discriminative accuracy between fertile and infertile men. The thorough literature review presented in this manuscript identifies specific clinical scenarios where SDF testing is most beneficial. These include patients with unexplained and idiopathic infertility, RPL, varicocele, opting for ART and in those with lifestyle/environmental risk factors. A number of therapeutic interventions can be undertaken in patients with high SDF result to improve their likelihood of conception. Recurrent ejaculation, antioxidant therapy, lifestyle modification, varicocelectomy, and the use of advanced sperm selection techniques or testicular sperm for ICSI are examples of treatment methods that can be utilized in such patients.

Key points

1) Sperm DNA integrity is crucial for fertilization and development of healthy offspring.
2) SDF results from defective maturation, abortive apoptosis and OS and can be induced by a number of disease states and lifestyle/environmental exposures.
3) There are several assays available to assess sperm DNA damage and most commonly utilized tests include TUNEL, SCD, SCSA and single cell gel electrophoresis assay.
4) Evidence indicates that SDF testing is most beneficial in patients with unexplained and idiopathic infertility, RPL, varicocele, opting for ART and in those with lifestyle/environmental risk factors.
5) High SDF fragmentation can be treated by recurrent ejaculation, antioxidant therapy, lifestyle modification, varicocelectomy, and the use of advanced sperm selection techniques or testicular sperm for ICSI.

ACKNOWLEDGEMENTS

Authors are thankful to the artists from the Cleveland Clinic’s Center for Medical Art & Photography for their help with the illustrations. The study was supported by the American Center for Reproductive Medicine (Andrology Research Fund #500000105879).

Conflict of Interest

The authors have nothing to disclose.
Author Contribution

Conceptualization: AA. Writing – original draft: all the authors. Writing – review & editing: all the authors.

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