Review Article
Male reproductive health and infertility

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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.

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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

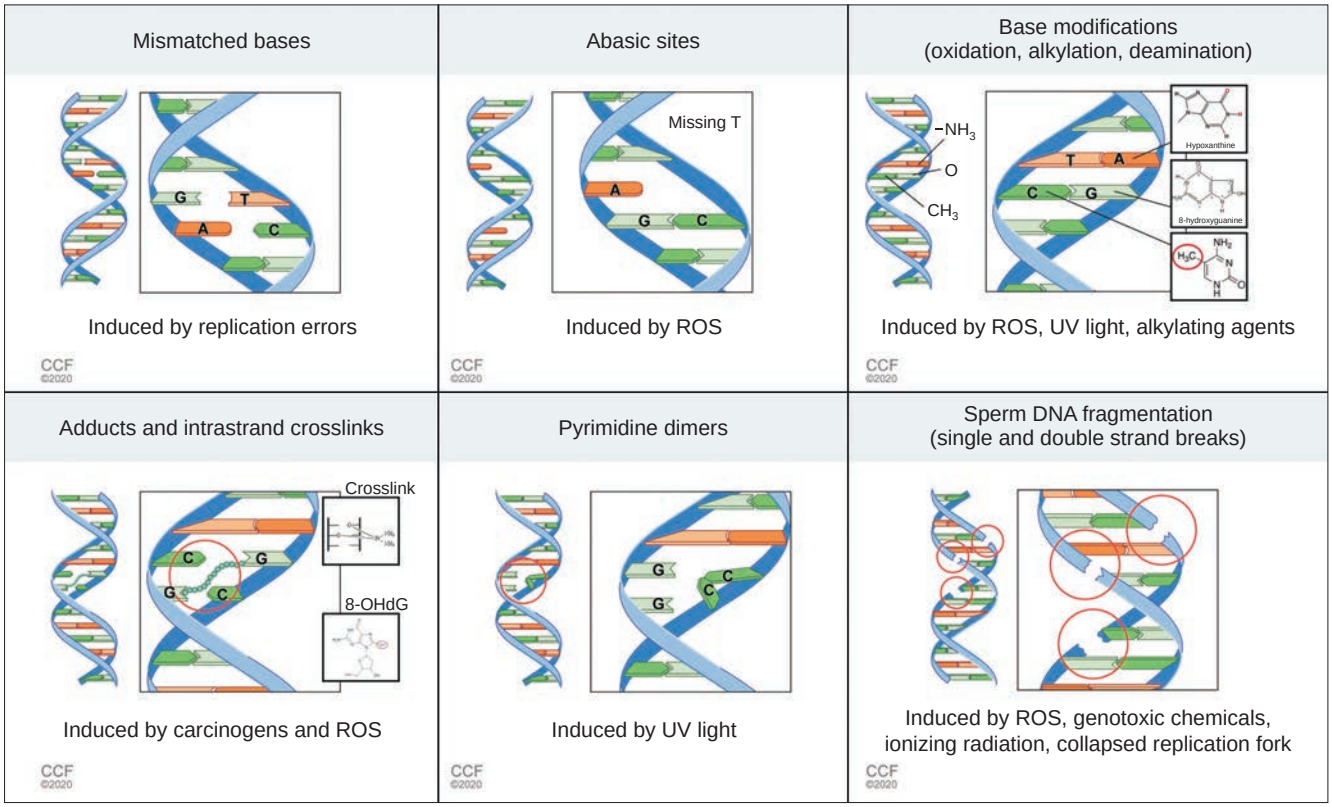

Fig. 1. Different types of DNA damage that can occur at DNA level: mismatched bases, abasic sites, base modifications (oxidation, alkylation, deamination), adducts and intrastrand crosslinks, pyrimidine dimers, and single and double strand fragmentation. ROS: reactive oxygen species, UV: ultraviolet.

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.

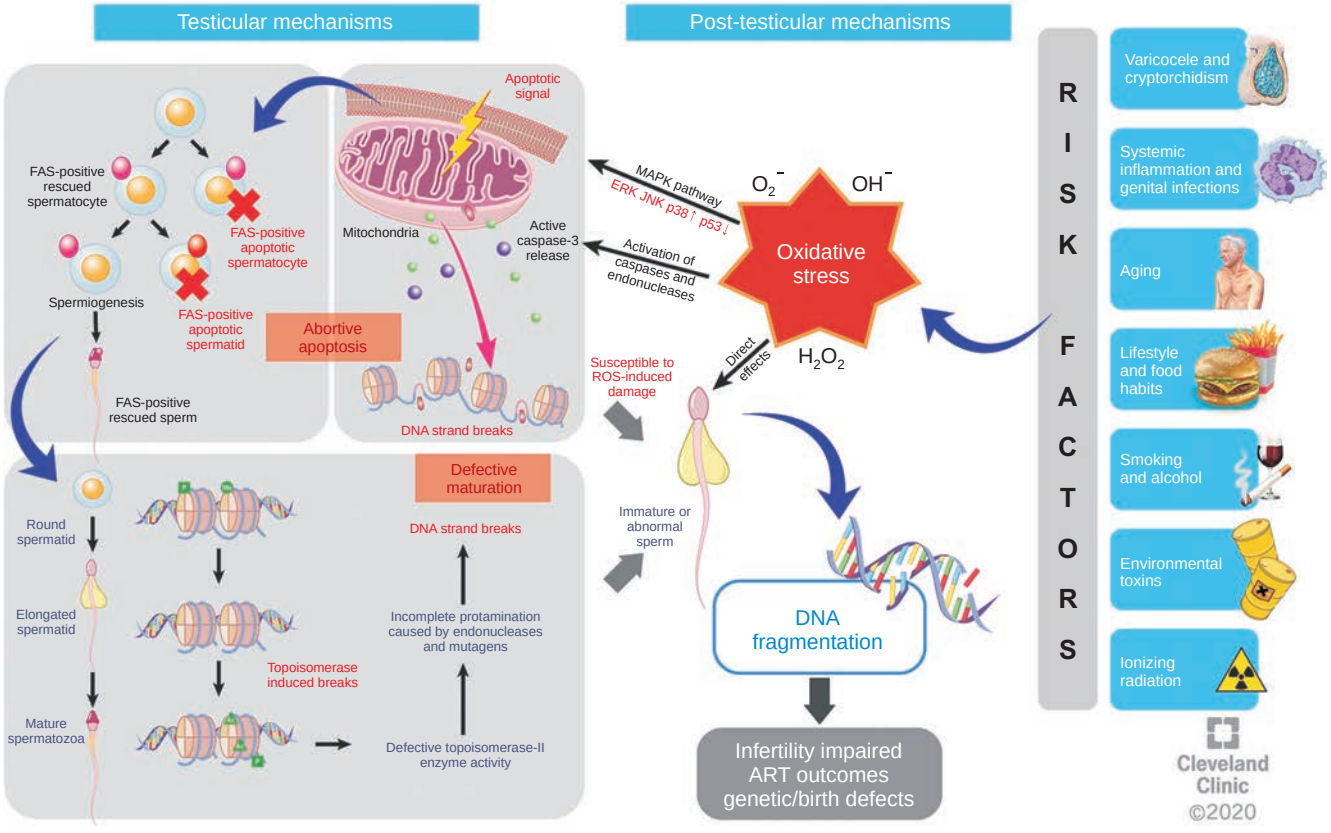

Fig. 2. Overview of the origins of sperm DNA fragmentation (SDF). SDF result from underlying mechanisms such as defective maturation, abortive apoptosis, and oxidative stress. Moreover, clinical (age, infection, cancer, hormonal imbalances, obesity, diabetes) and environmental (heat exposure, environmental 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-20-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

[88-91] 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 adduct 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].

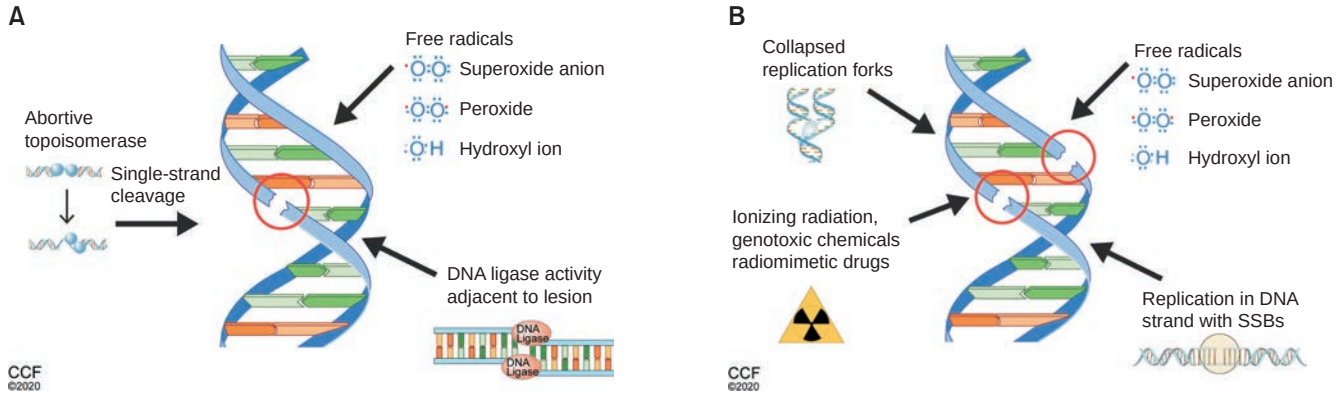

Fig. 3. (A) Main insults that result in DNA single strand breaks are abortive topoisomerase, free radicals, and DNA ligase activity adjacent to lesion. (B) Main insults that result in DNA double strand breaks are free radicals, collapsed replication forks, replication in DNA strand with single-stranded breaks, ionizing radiation, genotoxic chemicals, and radiomimetic drugs.

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Table 1. Published SDF cut-off values for the prediction of pregnancy outcomes using different laboratory assays

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	-	-
	Avendaño et al (2010) [333]	SDF in morphologically normal sperm for ICSI	17.6	0.70	61.5	82.6	66.7	79.2
	Esbert et al (2011) [410]	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	-	-
		Use of swim-up selected sperm and donor oocytes in IVF/ICSI	26.7	-	35.1	85.0	-	-
		Use of native sperm and own oocytes in IVF/ICSI	27.1	-	64.4	67.6	-	-
		Use of native sperm and donor oocytes in IVF/ICSI	28.5	-	48.8	67.6	-	-
	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	-	-
	López 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	98.6	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 labelling 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 standalone 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

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Table 2. Correlation between clinical outcomes and SDF testing: evidence-based report

	Study reference	Study design/participants	SDF assay	Outcome measured	Main results/findings	Quality of evidence
Natural conception						
Natural conception	Malić Vončina et al (2016) [273]	Prospective case-controlled study of fertile and infertile males	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]	Prospective case control study assessing the impact of SDF in males following vasectomy reversal	SCSA	Pregnancy rate	SDF was not associated with change in pregnancy rate following vasectomy reversal	Level 4
Natural conception	Giwercman et al (2010) [274]	Cross-section case-controlled study comparing infertile men with no known female factors to fertile men	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]	Prospective cohort study in couples planning pregnancy for the 1st time	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]	Prospective cohort study in couples planning pregnancy for the 1st time	SCSA	Pregnancy rate after 24 months	SDF negatively correlated with pregnancy rate SDF>40% particularly detrimental	Level 4
Assisted reproductive techniques (ART)						
IUI	Muriel et al (2006) [397]	Prospective cohort study of couples undergoing IUI	SCSA	Pregnancy outcome	SDF had no correlation with IUI pregnancy outcome	Level 2
IVF, ICSI	Oleszczuk et al (2016) [306]	Retrospective cohort study in couples undergoing ART	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
ICSI	Casanovas et al (2019) [117]	Prospective blinded cohort study analysing SSB and DSB in the same samples	Comet	Embryo kinetics and kinetics	dsSDF, not ssSDF, negatively affects embryo development in ICSI cycles	Level 2
ICSI	Wdowiak et al (2015) [327]	Prospective cohort study of couples undergoing ICSI	SCD	Embryo morphokinetic parameters	Low SDF increased pregnancy outcome and rate of blastocyst development	Level 2
IVF	Chohan et al (2004) [400]	Prospective cohort study of infertile couples undergoing IVF	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

Table 2. Continued 1

	Study reference	Study design/participants	SDF assay	Outcome measured	Main results/findings	Quality of evidence
IVF	Green et al (2020) [159]	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
ICSI	Al Omrani et al (2018) [393]	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
ICSI	Esbert et al (2018) [406]	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
ICSI	Alvarez Sedó et al (2017) [326]	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
ICSI	Uppangala et al (2016) [160]	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
IUI, ICSI	Thomson et al (2011) [310]	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
IVF, ICSI	Speyer et al (2010) [312]	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
IVF, ICSI	Simon et al (2011) [309]	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

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Table 2. Continued 2

	Study reference	Study design/participants	SDF assay	Outcome measured	Main results/findings	Quality of evidence
IVF, ICSI	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 No association between SDF and embryo cleavage rate or quality in IVF or ICSI cycles SDF>22.3% prognostic indicator for reduced fertilization rate following ICSI	Level 3
IVF, ICSI	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 SDF>15% increased miscarriage rate in IVF	Level 3
IVF, ICSI	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 SDF threshold determined at <18% for positive fertilization	Level 3
IVF, ICSI	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 Miscarriage rate was higher for high SDF in ICSI but not IVF	Level 3
IVF, ICSI	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
IVF, ICSI	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
IVF, ICSI	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
IVF, ICSI	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 SDF>30% increased risk for low blastocyst and ongoing pregnancy rates	Level 3
IVF, ICSI	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 SDF correlated negatively with embryo development rate but not quality	Level 3
IVF, ICSI	Høst 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

Table 2. Continued 3

	Study reference	Study design/participants	SDF assay	Outcome measured	Main results/findings	Quality of evidence
IVF, ICSI	Gat et al (2017) [409]	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	Zhao et al (2014) [154]	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	Zini et al (2008) [153]	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	Bungum et al (2007) [319]	Prospective cohort study of couples undergoing ART	SCSA	Pregnancy and delivery	SDF>30% reduced IUI success SDF>30% increased ICSI success compared to IVF	Level 3
IUI, IVF, ICSI	Boe-Hansen et al (2006) [162]	Prospective cohort study of couples undergoing ART	SCSA	Biochemical pregnancy, clinical pregnancy, and implantation rates	No difference between IVF and ICSI for low SDF (<27%) SDF>27% predicted no clinical pregnancy for IVF	Level 3
IUI, IVF, ICSI	Bungum et al (2004) [41]	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>27%, but not IVF or ICSI Results for ICSI better than IVF for SDF>27%	Level 3
IUI	Duran et al (2002) [324]	Prospective cohort study of infertile couples undergoing IUI	TUNEL	Clinical pregnancy	No samples with SDF>12% achieved clinical pregnancy	Level 4
IVF	Frydman et al (2008) [316]	Prospective cohort study of 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	Seli et al (2004) [401]	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	Henkel et al (2004) [320]	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	Sun et al (1997) [88]	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	Filatov et al (1999) [330]	Prospective cohort of infertile couples undergoing IVF	SCSA	Embryo cleavage	Negative correlation between SDF and embryo cleavage	Level 4

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Table 2. Continued 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	ICSI outcome	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	Clinical pregnancy and miscarriage rates	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	Fertilization rate	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	Fertilization, cleavage and pregnancy rates	No negative effect recorded for increased SDF	Level 4
ICSI	Avendaño et al (2010) [333]	Prospective cohort study of couples undergoing ICSI	TUNEL	Embryo quality and pregnancy outcome	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	Fertilization rate, embryo quality and embryo cleavage score	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]	Retrospective cohort study of infertile couples undergoing ART	SCD	Fertilization, good embryo and pregnancy rates	No difference between low (<30%) and high (>30%) SDF for all outcomes	Level 4
IVF, ICSI	Pregl Breznik et al (2013) [308]	Prospective cohort study of couples undergoing ART	SCD	Fertilization rate and embryo quality	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	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	Fertilization rate, embryo cumulative scores, total number of embryos, embryo transfer, clinical pregnancy, and miscarriage	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	Fertilization and pregnancy rates	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	Fertilization and pregnancy rates	Negative correlation for SDF and fertilization rate in ICSI but not IVF No effect of SDF on pregnancy rate	Level 4

Table 2. Continued 5

	Study reference	Study design/participants	SDF assay	Outcome measured	Main results/findings	Quality of evidence
IVF, ICSI	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
IVF, ICSI	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
IVF, ICSI	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
IVF, ICSI	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 In ICSI cycles, SDF positively correlated with impaired embryo cleavage	Level 4
IVF, ICSI	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 No pregnancy resulted for SDF>27% for IVF and ICSI	Level 4
IVF and ICSI	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
IVF and ICSI	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
IVF and ICSI	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
Varicocele						
Varicocele	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

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Table 2. Continued 6

	Study reference	Study design/participants	SDF assay	Outcome measured	Main results/findings	Quality of evidence
Varicocele	Cortés-Gutiérrez et al (2016) [298]	Prospective study (20 infertile men with varicocele and 20 fertile men) Clinical grades: 1–3	SCD test, and DBD-FISH	SDF	Infertile men with varicocele had higher SDF compared to fertile controls	Level 3
Varicocele	Bahreinian et al (2015) [175]	Prospective study (44 men with varicocele, 15 fertile controls) Clinical grades: 2–3	TUNEL	SDF	TUNEL higher in men with varicocele *vs*. fertile controls	Level 3
Varicocele	Blumer et al (2012) [299]	Case control (30 men with varicocele and 32 controls without varicocele) Clinical grades: 2–3	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	Moazzam et al (2015) [172]	Retrospective review (121 subfertile men with varicocele, 66 subfertile men without varicocele, 115 healthy fertile controls) Clinical grades: 1–3	TUNEL	SDF	SDF was higher in varicocele group compared to subfertile men without varicocele, and fertile controls	Level 3
Varicocele	Park et al (2018) [296]	Observational study (157 men with >1 year of infertility and with varicocele) Clinical grades: NA	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	Saleh et al (2003) [300]	Prospective controlled study (31 infertility patients [16 with varicocele], and 16 fertile controls) Clinical grades: 1–3	SCSA	SDF	Infertile men with varicocele had significantly higher DFI% than fertile controls	Level 4
Varicocele	Nguyen et al (2019) [301]	Prospective study (179 infertile men with varicocele) Clinical grades: 1–3	SCD test	SDF	Infertile men with varicocele had higher DFI directly correlating with varicocele grade and inversely correlated with zinc concentration	Level 4
Varicocele	Tang et al (2012) [302]	Case control study (71 infertile men with varicocele and 30 healthy controls) Clinical grades: 1–3	TUNEL	SDF	Men with varicocele had higher TUNEL than healthy controls	Level 4
Varicocele	Smith et al (2006) [303]	Case control study (55 men with testicular pain and varicocele and 25 healthy controls) Clinical grades: 2–3	TUNEL & SCSA	SDF	Men with varicocele showed higher DFI and TUNEL positive cells	Level 4

Table 2. Continued 7

	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	Zümrütbaş et al (2013) [305]	Retrospective case series (45 men with varicocele and 30 healthy men without varicocele) Clinical grades: clinical	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)						
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

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Table 2. Continued 8

	Study reference	Study design/participants	SDF assay	Outcome measured	Main results/findings	Quality of evidence
RPL	Bellver et al (2010) [284]	Prospective cohort study (30 men with RPL, 30 men with severe oligozoospermia, 30 fertile donors)	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]	Descriptive study (23 couples with history of RPL and 11 fertile men)	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]	Prospective cohort study (30 couples with RPL and 30 fertile couples)	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]	Retrospective case control study (20 infertile men with history of RPL and 20 fertile controls)	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]	Retrospective comparative study (48 couples with RPL [16 males with abnormal sperm, 32 males with normal sperm], and 20 fertile controls)	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]	Prospective study (154 embryos from 38 couples with RPL undergoing IVF/ICSI)	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]	Retrospective comparative study (45 men with idiopathic RPL and 20 controls)	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]	Retrospective study (108 couples with RPL)	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

Table 2. Continued 9

	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 autosome 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

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Table 2. Continued 10

	Study reference	Study design/participants	SDF assay	Outcome measured	Main results/findings	Quality of evidence
RPL	Bareh et al (2016) [181]	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) [183]	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) [293]	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) [294]	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) [295]	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) [389]	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) [138]	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)						
UMI	Zandieh et al (2018) [277]	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) [278]	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) [188]	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

Table 2. Continued 11

	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) Normal SDF: IVF Abnormal SDF: ICSI	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

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Table 2. Continued 12

	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	SDF	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	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	SDF	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	SDF	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	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	SDF	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	SDF	Smokers showed significantly higher SDF (Comet classes III and IV) than counterparts	Level 3

Table 2. Continued 13

	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/m^2 was associated with higher SDF than BMI<30 kg/m^2	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

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Table 2. Continued 14

	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

Table 2. Continued 15

	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 patents 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

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Table 2. Continued 16

	Study reference	Study design/participants	SDF assay	Outcome measured	Main results/findings	Quality of evidence
Smoking	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
Smoking	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
Ionizing radiation	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
Environmental and lifestyle factors	Kumar et al (2014) [339]	Cross-sectional study (evaluating SDF in patents 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
OP exposure	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-ocebm-levels-evidence/.

SDF: sperm DNA fragmentation, IUI: intrauterine insemination, IVF: *in vitro* fertilization, ICSI: intracytoplasmic sperm injection, OP: orpganophosphorus pesticide, TUNEL: terminal deoxynucleotidyl transferase dUTP nick end labelling, MMP: matrix metalloproteinases, SCSA: sperm chromatin structure assay, 8-OHdg: 8-hydroxy-2'-deoxyguanosine, SSB: single strand breaks, DSB: double strand break, dsSDF: double strand SDF, ssSDF: single strand SDF, ICSI: intracytoplasmic sperm injection, SCD: sperm chromatin dispersion, AO: acridine orange, IVF-ET: *in vitro* fertilization and embryo transfer, DBD-FISH: DNA breakage detection-fish, NA: not available, DFI: DNA fragmentation index, SA: sperm analysis, TAC: total antioxidant capacity, ROS: reactive oxygen species, RIF: recurrent implantation failure, ASCI: abnormal sperm chromatin integrity, FISH: fluorescence in situ hybridization, TB: toluidine blue, ssDNA: single strand DNA, dsDNA: double strand DNA, OAT: oligoasthenoteratozoospermia, AT: asthenoteratozoospermic, HS: heat stress, AB: aniline blue, BMI: body mass index, HDS: high sperm DNA stainability, MEP: monoethyl phthalate, MEHP: mono-2-ethylhexyl phthalate.

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] invloving 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 conducted 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

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Table 3. Therapeutic interventions for SDF: evidence based report

	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 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)	SCD	SDF	Lower baseline levels of SDF reported after shorter periods of abstinence between ejaculations than those recommended	Level 2
	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 after1 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	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	Level 3
	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 exercise program	Mir et al (2018) [60]	Prospective study 105 men with infertility and obesity	SCD	SDF	Reduced SDF after weight loss	Level 3
Diet modification	Faure et al (2014) [221]	Prospective case-series 6 idiopathic infertile men with SDF≥25% and significant abdominal fat	TUNEL	SDF	Subsequent to following dietary advice, all men showed a reduction of SDF	Level 4
Treatment of infections/inflammations						
Antibiotic	Gallegos et al (2008) [47]	Prospective study 95 men with *Chlamydia trachomatis* or Mycoplasma (under macrolide, tetracycline, or quinolone therapy), 50 fertile men	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]	Randomized placebo-controlled study 11 patients: Zn (400 mg) 12 patients: Zn (400 mg)+vitamin E (20 mg) 14 patients: Zn (400 mg)+vitamin E (20 mg)+ vitamin C (10 mg) 8 patients: placebo group	SCSA	SDF, semen parameters	SDF and sperm motility improved after treatment	Level 1
	Greco et al (2005) [208]	Randomized placebo-controlled study 64 men with unexplained infertility and high SDF levels supplemented for 2 months with vitamin C (1,000 mg)+vitamin E (1,000 mg)	TUNEL	SDF, semen parameters	Significant decrease in SDF levels	Level 1
	Martínez-Soto et al (2016) [356]	Randomized, double blind, placebo-controlled, parallel-group study 74 subjects randomly assigned to either the placebo group (n=32) or to the DHA supplemented group (n=42) (three 500-mg capsules of oil per day over 10 weeks)	TUNEL	SDF	Reduced SDF after supplementation while no changes were reported for the placebo group After supplementation, DHA group presented a lower DNA fragmentation rate than the placebo group	Level 1
	Stenqvist et al (2018) [202]	Randomized placebo-controlled, double-blind study 77 men from infertile couples, with normal testosterone, LH and FSH levels and DFI≥25% Of those, 40 were the placebo group while 37 received combined antioxidant treatment (vitamins, antioxidants and oligoelements) for 6 months	SCSA	SDF	Increased sperm concentration after three months of treatment No variation in the DFI during the 6 months of antioxidant therapy	Level 1

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Table 3. Continued 2

Study reference	Study design/participants	SDF assay	Outcome measured	Main results/findings	Quality of evidence
Kumar et al (2012) [182]	Prospective study 120 men exposed to lead, and 120 healthy human subjects supplemented for 3 months with vitamin C (1,000 mg) 5 consecutive days in a week	Comet	SDF	Significant decrease in alkaline-labile sites and mean tail length of the comet in comparison with the control group Significant improvement in all semen parameters	Level 2
Tunc et al (2009) [207]	Prospective study 50 patients supplemented for 3 months with vitamin C (100 mg), E (400 IU), Se (26 μg), Zn (25 mg), folic acid (0.5 mg), and garlic (1 mg)	TUNEL, CMA3 assay	SDF	Significant decrease in SDF and protamine packaging No significant improvement in semen parameters	Level 3
Ménézo et al (2007) [205]	Prospective study 58 supplemented for 3 months with b-carotene (18 mg), vitamin C (400 mg), vitamin E (400 mg), Zn (500 μmol), Se (1 μmol)	SCSA	SDF	Significant decrease in SDF	Level 3
Kodama et al (1997) [353]	Prospective study 14 infertile men supplemented for 2 months with vitamin C (200 mg), vitamin E (200 mg), glutathione (400 mg)	8OHdG	Oxidative damage, semen parameters	Significant improvement in sperm concentration and decrease in 8OHdG	Level 3
Greco et al (2005) [355]	Prospective study 29 patients with high SDF and prior failed ICSI supplemented for 2 months with vitamin C (1,000 mg)+vitamin E (1,000 mg)	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 10 volunteers supplemented for 15 weeks with vitamin C (250 mg)	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 20 infertile patients diagnosed with asthenoteratozoospermia supplemented for 3 months with L-carnitine (1,500 mg); vitamin C (60 mg); coenzyme Q10 (20 mg); vitamin E (10 mg); Zn (10 mg); vitamin B9 (200 μg); selenium (50 μg); vitamin B12 (1 μg)	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 Significant improvement in sperm concentration, motility and morphology	Level 3
Gual-Frau et al (2015) [360]	Prospective study 20 infertile men with grade 1 varicocele supplemented for 3 months with L-carnitine (1,500 mg), vitamin C (60 mg), coenzyme Q10 (20 mg), vitamin E (10 mg), Zn (10 mg), vitamin B9 (200 μg), selenium (50 μg), vitamin B12 (1 μg)	SCD	SDF	Significant decrease in SDF levels and in highly degraded sperm cells Significant increase in total sperm count	Level 3

Table 3. Continued 3

	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 5 IAF	Level 2

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Table 3. Continued 4

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	SDF	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	SDF	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 (undamaged 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 varicocele Clinical grades: NA Intervention: microsurgical inguinal varicocelectomy	TUNEL	SDF	SDF improved 6 months post intervention	Level 3

Table 3. Continued 5

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

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Table 3. Continued 6

	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
Sperm selection/testicular sperm for ICSI						
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

Table 3. Continued 7

	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, oligoasthenozoospermic and oligoasthenotheratozoospermic 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

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Table 3. Continued 8

	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	Oguz 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	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, DHA: docosahexaenoic acid, 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]. Intracytoplasmic 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/m^2). 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.

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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/m^2). On genital examination, both testes were normally descended with normal size and consistency, both epididymes 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/m^2). On genital examination, both testes were normally descended with normal size and consistency, both epididymis 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/m^2). 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 (2.9 mV/10^6 sperm/mL, normal=1.34 mV/10^6 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 acetyle 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/10^6 sperm/mL) normalized. One month later, his wife achieved a spontaneous pregnancy and she delivered a healthy boy.

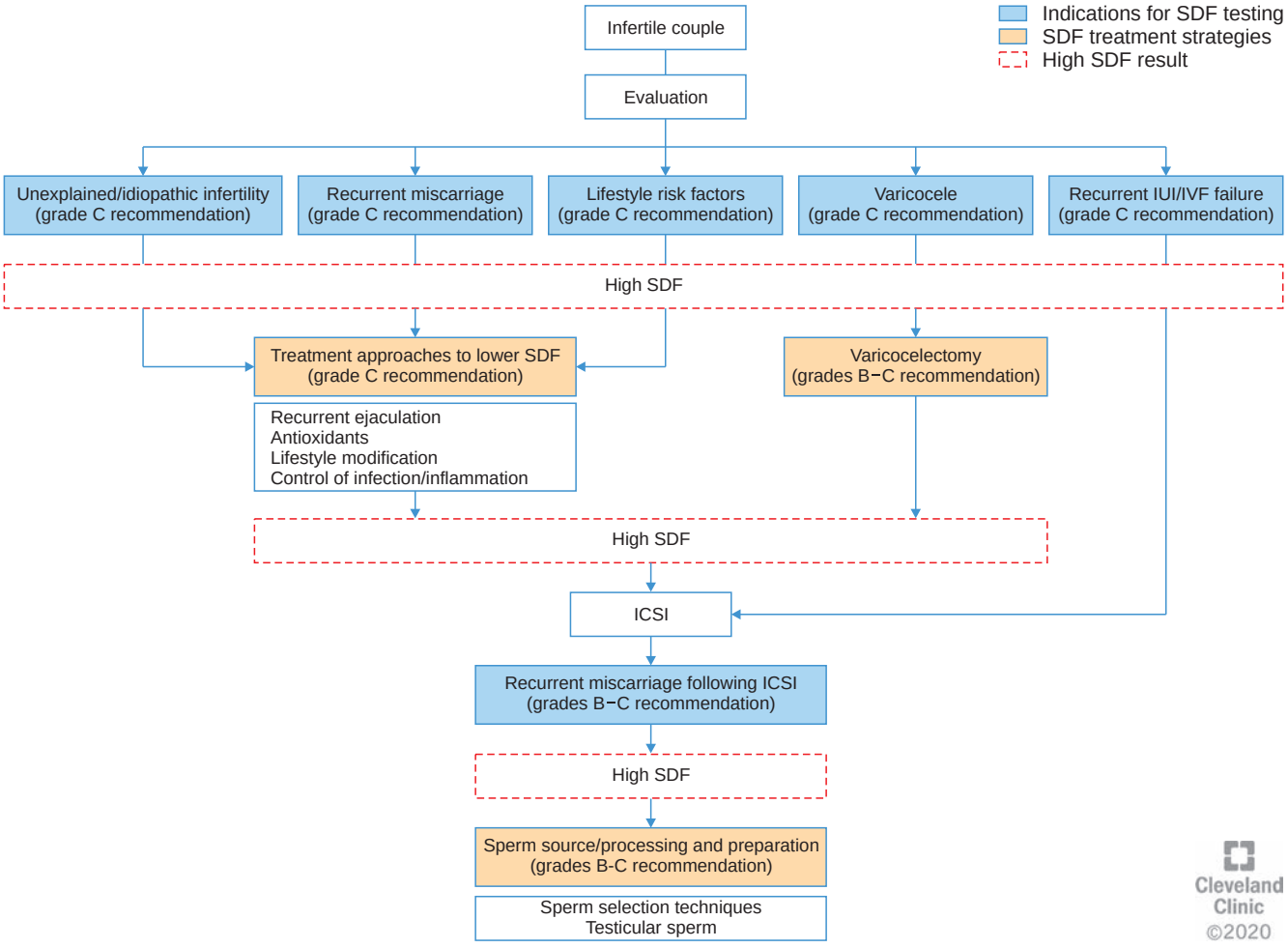

Fig. 4. Clinical algorithm to elucidate the applications of sperm DNA fragmentation (SDF) testing in clinical practice. ICSI: intracytoplasmic sperm injection.

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 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].

Fig. 5. Strengths-Weaknesses-Opportunities-Threats (SWOT) analysis on the clinical utility of sperm DNA fragmentation (SDF) testing in specific male infertility scenario. ART: assisted reproductive techniques.

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 [185,188,276-282], have RPL [116,138,180-183,283-295], 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 [42,47,56,65,74,82,84,88,190-193,195-197,243,331-349].

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

Table 4. Comparison of different SDF tests

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 Minimal inter-observer variability Evaluation by both fluorescent microscopy and flow cytometry Analysis of both fresh and frozen samples	Protocols and thresholds are still not standardized Expensive equipment and trained personnel required	150
Comet	Single cell electrophoretic separation	SSB and/or DSB	High sensitivity Correlation with semen parameters Possibility to discriminate between SSB and DSB	Poor repeatability High inter-observer variability Variable protocols and thresholds Time-consuming Evaluation of a low number of cells Appropriate imaging software required	~400–600
SCSA	Evaluation of DNA integrity by using the meta-chromatic acridine orange	SSB/DSB	Simultaneous examination of a large number of cells Highly repeatability Analysis of frozen or fresh samples	Commercial kits not available Expensive equipment and trained personnel required	300
SCD or Halosperm test	Evaluation of the dispersed chromatin ("halo") after lysing treatment	SSB/DSB	Commercial kits available Repeatable and consistent results in 45 minutes No expensive equipment required Easy to perform	Inter-observer variability	175

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.

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Conflict of Interest

The authors have nothing to disclose.

Author contribution

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