

Sperm DNA Fragmentation in Reproductive Medicine: A Review

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ABSTRACT

Approximately 15% of the world's couples suffer from infertility during their reproductive period of which the male factor is responsible for 50% of cases. Male factor infertility is multifactorial in origin, and sperm DNA fragmentation (SDF) has also been linked to male infertility including idiopathic male infertility. Some degree of controlled DNA nicking is essential for adequate DNA compaction, but excessive SDF is usually associated with reduced male fertility potential, reduced fertilisation, poor embryo quality, recurrent pregnancy loss and poor assisted reproductive techniques (ARTs) outcomes. Although semen analysis remains the gold standard for diagnosis of male factor infertility worldwide, its limitations motivated the search and the development of complementary tests of sperm function and integrity. SDF assay is an emerging diagnostic tool in infertile men, and several indications for SDF testing in infertile couples have also been proposed. The use of SDF in routine male infertility assessment is, however, still controversial. Furthermore, both direct and indirect SDF tests are now available. Hence, the present review was conducted to summarise the recent evidence of SDF, underlying mechanisms, clinical indications, diagnostic tests, as well as the role of SDF in male factor infertility, pregnancy and ART outcomes.

KEYWORDS: *Assisted reproductive techniques, male infertility, pregnancy, sperm DNA fragmentation*

INTRODUCTION

Infertility is defined as the failure of couples to achieve pregnancy within 12 consecutive months of unprotected intercourse, affecting approximately 15% of couples of reproductive ages.^[1] Infertility has medical, psychological, social, and financial consequences. Factors underlying male infertility include genetic and anatomical abnormalities, varicocele, endocrine disorders, systemic diseases, infections, immunological and environmental toxins, lifestyle, radiotherapy, chemotherapy and medications.^[2] In approximately 30% of infertility cases, the underlying cause of semen abnormalities is unknown and referred to as idiopathic male infertility (IMI). Oxidative stress (OS) and sperm DNA fragmentation (SDF) have been suggested as potential mechanisms for IMI.^[3,4] SDF involves sperm DNA single- or double-stranded (ss or ds) breaks and has

been linked to reduced male fertility potential, reduced fertilisation, decreased pregnancy rates, suboptimal embryo quality, increased risk of spontaneous abortions and poor assisted reproductive technique (ART) outcomes.^[5] Recent studies have also reported an increased incidence of childhood malignancies and genetic and neuropsychological diseases in the offspring of men with high SDF.^[6,7] Lower semen parameters have been observed in men with high SDF, and a recent study has also observed a correlation between SDF and sperm morphology among infertile men.^[8,9]

Although semen analysis remains the gold standard for the evaluation of male factor infertility worldwide, it has many limitations including data obtained from fertile rather than infertile men, unequal population distribution, inability to assess sperm function and the lack of cut-off

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values to differentiate fertile from infertile patients.^[1,10] Therefore, additional markers of male fertility such as genetic markers, OS, SDF and sperm function tests have also been explored to overcome the limitations of conventional semen analysis.

Although the routine use of SDF testing in assessing infertile men is still controversial, the American Urological Association and European Association of Urology guidelines have acknowledged the value of test.^[11] The evidence supporting the utilisation of SDF testing in the clinical setting of infertility is increasing, and the guidelines for SDF testing have been proposed.^[12] However, data on the impact of SDF on male fertility potential, pregnancy and ART outcomes, indications of SDF tests, optimal techniques for SDF tests and treatments that effectively reduce SDF are limited.^[13,14] Therefore, in this review, we have provided updated evidence regarding SDF, underlying mechanisms, diagnostic tests and the impact of SDF on male fertility potential, pregnancy and ART outcomes.

METHODS

This narrative review included a systematic search of electronic scientific databases PubMed, Medline, Google Scholar, and Cochrane review to include published articles from 2010 to 2022. The search involved keywords and combinations of search terms ‘male infertility’, ‘sperm DNA fragmentation’, ‘sperm DNA damage’, ‘sperm DNA fragmentation tests’, ‘semen parameters’, ‘pregnancy’, ‘fertilization’, ‘assisted reproductive techniques’, ‘IUI’, ‘IVF’ and ‘ICSI’, and ‘genetic diseases’. Articles were perused and their reference lists were checked for relevant publications. We included articles published in English only.

CAUSES OF SPERM DNA FRAGMENTATION

Sperm DNA undergoes compaction during the process of spermatogenesis. For effective condensation, the sperm DNA encircles histone proteins which are gradually substituted by highly basic protamine.^[1] Torsional stress is exerted by dsDNA during the process of condensation resulting in nicks and breaks in the DNA, followed by a restoration of appropriate reordering of chromatin.^[15]

A range of cellular events is implicated in the impairment of fertility and SDF [Figure 1]. The reduction of protamination from failure to repair the nicks could result in sperm DNA damage.^[1] Abnormal chromatin packing and remodelling during spermatogenesis,^[16] as well as apoptosis during sperm maturation within the epididymis^[17] also contribute to SDF. SDF is also caused by OS, varicocele, infections, inflammation of the

male genital tract, drugs, chemotherapy, radiotherapy, cancer, obesity, advanced age, as well as environmental pollutants and toxins.^[18-22] Furthermore, SDF is a potential mechanism that may explain the inability to conceive in couples with idiopathic infertility.^[23] We and others have reported lower seminal antioxidants markers and higher SDF in infertile men compared to fertile controls and these abnormalities were ameliorated with oral antioxidants.^[1,3,5,6,24-26]

TESTS FOR SPERM DNA FRAGMENTATION

The available tests for the assessment of SDF are generally categorised into two main types: direct and indirect tests. Direct tests are used to measure the degree of sperm DNA damage through the use of probes and dyes. The indirect tests are used for evaluating the DNA vulnerability to denaturation, which is more characteristic of fragmented DNA.^[27] The most frequently used tests for the evaluation of SDF include terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL), the sperm chromatin structure assay (SCSA), and the sperm chromatin dispersion (SCD) assay.^[1] SDF tests are summarised in Table 1.

Direct sperm DNA fragmentation tests

Acridine orange assay

The principle underlying acridine orange (AO) test is similar to that of SCSA which is based on the evaluation of the degree of DNA denaturation by quantification of the metachromatic shift of AO from green to red.^[28] Visual interpretation is utilised in carrying out AO tests by using fluorescent microscopy without the use of flow cytometry. The test does not require extensive training.^[28] This makes AO test more affordable and simpler than the SCSA test. However, the test lacks reproducibility and is associated with significant interlaboratory differences.^[29]

Terminal deoxynucleotidyl transferase dUTP nick end labelling

TUNEL assay is utilised to identify ‘nicks’, or free ends of DNA by using fluorescent nucleotides.^[30] The TUNNEL assay was invented by Mitchell *et al.* by relaxing the whole chromatin structure with dithiothreitol (DIT) before fixation to permit contact with all ‘nicks’.^[31] A recently modified TUNEL protocol utilising bench top flow has been used recently.^[32] The assay measures the integration of dUTP into dsDNA or ssDNA breaks via an enzymatic reaction. It further creates an indication that is multiplied by the number of DNA breaks. Evaluation of the sample is carried out through the use of flow cytometry or a standard fluorescence microscope. The test is limited by the lack of strict standardisation which makes the comparison between laboratories more difficult.^[19]

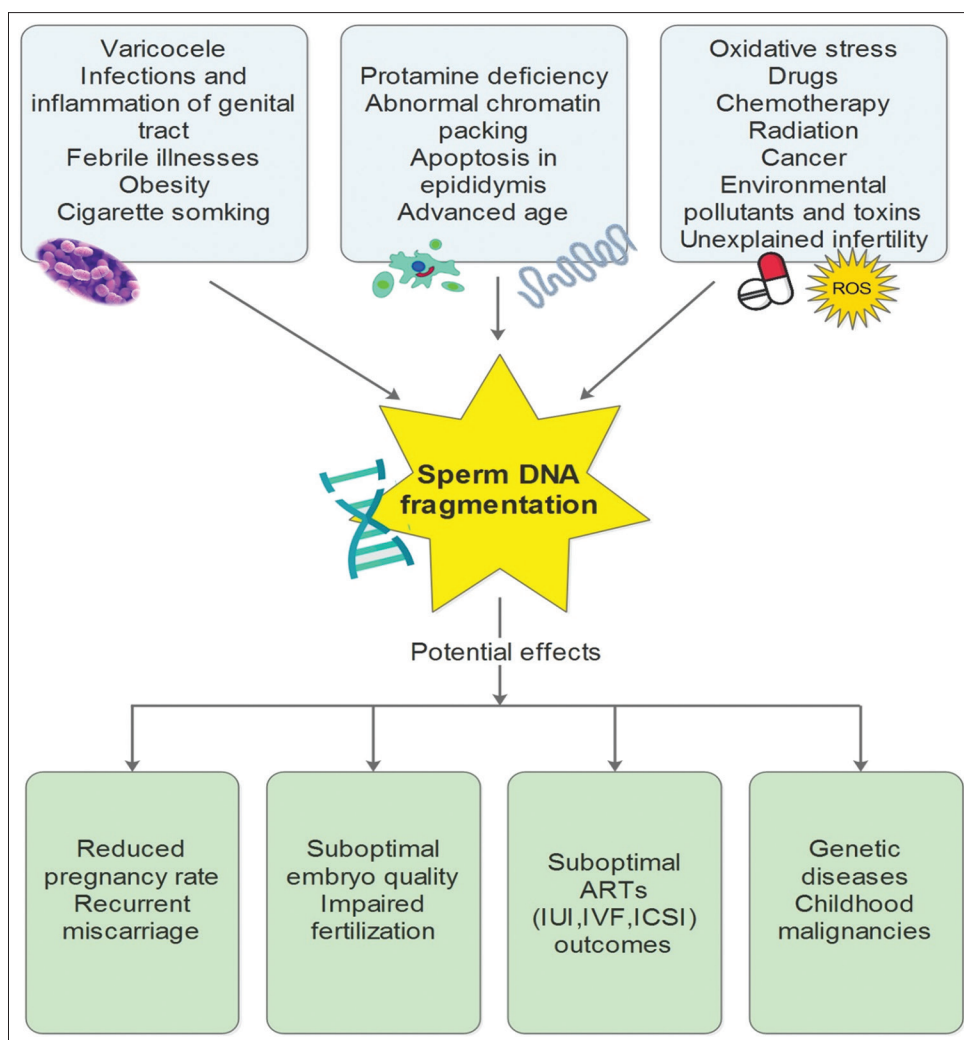


Figure 1: Causes of SDF and consequences of SDF on fertility potential and well-being of the newborn. IUI: Intrauterine insemination; IVF: *In vitro* fertilisation; ICSI: Intracytoplasmic sperm injection; SDF: Sperm DNA fragmentation

In situ nick translation assay

In situ nick translation test is used to detect DNA strand breaks in the tissue section at the cellular level. Therefore, it can be used for sperm cells as well. NT test can also be applied to detect DNA damage in a single cell, and hence, it is useful to assess DNA damage, stress and apoptosis. The NT and TUNEL assays are similar. Both of them quantify the integration of dUTP into DNA breaks. The difference is that while TUNEL targets the identification of both ssDNA and dsDNA breaks, the NT assay targets the identification of ss breaks in a reaction catalysed by DNA polymerase I. The test is simple, but it is less sensitive than the other tests.^[33]

Single-cell gel electrophoresis assay (Comet)

Single-cell gel electrophoresis or Comet assay quantifies the aggregate of DNA damage per spermatozoon, as a single cell can be followed on the gel.^[34] Because the test can detect sperm DNA damage at a single cell level, it can be used for the assessment of cases with severe

oligozoospermia.^[35] The staining power of the comet test depends on the quantity of migrated DNA, which is an indication of different degrees of SDF.^[36] Comet assay can not only detect ss and ds breaks but also identify altered bases. The method is inappropriate for quick diagnosis and needs highly experienced staff for the analysis of results. However, the method is informative because of its ability to analyse different kinds of DNA damage in a single cell by utilising electrophoresis.^[16]

Indirect sperm DNA fragmentation tests

Toluidine blue staining

Toluidine blue (TB) staining is used for evaluating the integrity of sperm chromatin DNA. TB is a thiazine metachromatic dye with great attraction for sperm DNA phosphate residues. This microscopic assay stains the damaged chromatin nuclear structure of the spermatozoa. Optical microscopy is utilised for viewing the extent of damage after staining. It should be noted that intermediate coloration increases the interobserver variability.^[1]

Table 1: Sperm deoxyribonucleic acid fragmentation testing techniques, principle, advantages, and disadvantages

Test	Principle	Method	Interpretation	Advantages	Disadvantages
Direct assays AO test	AO is nucleic acid selective dye and causes a metachromatic shift in fluorescence upon binding to DNA breaks	Air-dried semen sample smears are first fixed in Carnoy's fixative for 2 h followed by AO staining for 5 min. Spermatozoa stained with AO are then excited at 488-nm wavelength	Intact DNA gives green fluorescence and damaged DNA red	Rapid, easy, and cost-effective test	Interlaboratory variations
AB staining	AB test detects the degree of sperm chromatin maturation and chromatin defects	Optical microscopic visualization of AB stained chromatin	Histone-rich nuclei of immature sperm stain blue, while mature protamine-rich nuclei remain unstained	Rapid, easy, and cost-effective test	Interlaboratory variations and results are dependent on staining efficiency
TUNEL	TUNEL assay identifies DNA breaks by the addition of template-independent DNA polymerase to the 3'hydroxyl (OH) breaks-ends of ssDNA and dsDNA	Fluorescein isothiocyanate conjugates with dUTPs and the fluorescent signal thus received is measured by flow cytometer or fluorescence microscopy	Sperm having DNA breaks show fluorescence and the results are presented as a percentage of fluorescent sperms	Direct test Can be used to detect dsDNA and ssDNA breaks Can be performed on fresh/frozen samples	Inconsistent with high variability in the reference values Time-consuming
SCGE/comet assay	SCGE assessment of fragmented DNA	Sperm are embedded in agarose and lysed using detergent and high salt to form nucleoids with supercoiled DNA loops followed by electrophoresis	Fragmented DNA appears as a tail, while intact DNA remains in the sperm head	Direct assay High sensitivity and specificity Can detect different types of DNA damage in single sperm	Inter-observer variation Time-consuming Requires fresh semen samples Expensive
Indirect assays TB staining	TB is an acidophilic metachromatic dye with a high affinity for sperm DNA phosphate residues	Optical microscopic visualization of stained damaged DNA chromatin	Sperm heads with high chromatin DNA integrity are stained blue and damaged ones are stained violet-blue/purple	Rapid, easy, and cost-effective test	Inter-observer variations and results are dependent on staining efficiency
CMA3 staining	CMA3 is an anthraquinone antibiotic glycoside that binds reversibly to DNA and competes with protamine for the same site	Air-dried seminal smear fixed with glacial acetic acid-methanol (1:3) solution for 20 min at 4 °C followed by staining with CMA3	Spermatozoa with low protamination stains light yellow, and those with high DNA damage stains bright yellow	Strong correlation with other SDF assays	Interlaboratory and inter-observer variations
SCSA	SCSA is a flow cytometric test that identifies sperm DNA breaks indirectly by acid-induced DNA denaturation	Acid-induced denaturation of sperm DNA, followed by AO staining and measurement by flow cytometry	Intact DNA fluoresces green and denatured DNA as orange-red	Rapid, simple, precise, and repeatable test Can be performed on fresh/frozen semen samples	Indirect assay Requires expensive set-up and skilled technicians
SCD/halo test	Detects fragmented DNA dispersion after acid denaturation	Sperm embedded in agarose microgel are acid denatured to remove nuclear proteins followed by staining with nuclear stain DAPI	Sperm with fragmented DNA do not produce the halo of dispersed DNA loops as produced by sperm with non-fragmented DNA, following acid denaturation	A simple test with easy availability of commercial kits Can be performed on neat and washed seminal samples	Indirect assay Time-consuming Inter-observer variations

SCD=Sperm chromatin dispersion, TUNEL=Terminal deoxynucleotidyl transferase dUTP nick end labeling, SCSA=Sperm chromatin structure assay, SCGE/COMET: Single-cell gel electrophoresis, AO=Arcidine orange, AB=Aniline blue, TB=Toluidine blue, DNA=Deoxyribonucleic acid, SDF=Sperm DNA fragmentation, ssDNA=Single-stranded DNA, dsDNA=Double-stranded DNA, CMA3=Chromomycin A3, DAPI=4',6-diamidino-2-phenylindole, dUTPs= 2'-Deoxyuridine 5'-Triphosphate, OH=Hydroxyl

Chromomycin A3 staining

Chromomycin A3 (CMA3) and protamine compete for the same binding sites on the DNA. In CMA3 staining, a highly positive test is an indicator of a low DNA protamination state which is related to poorly packaged sperm chromatin.^[37] CMA3 is a guanine-cytosine-specific fluorochrome, and its result has been shown to correlate well with that of aniline blue staining for sperm chromatin assessment.^[1]

Sperm chromatin structure assay

Sperm chromatin structure assay (SCSA) evaluates the susceptibility of sperm DNA to denaturation. The test uses metachromatic characteristics of AO for this purpose.^[38] The principle underlying SCSA is based on increased susceptibility of abnormal chromatin structure in the sperm DNA to acid or heat denaturation.^[33] SCSA is a flow cytometry-based assay that assesses a large number of cells quickly and strongly.^[39]

The degree of DNA denaturation is evaluated by the quantification of the metachromatic shift of AO from green to red after treatment with acid. This is done by utilising flow cytometry.^[40] SCSA has the advantage that it has a standardised protocol for lowering interlaboratory differences. DNA fragmentation index (DFI) is used in SCSA as the measure of SDF.^[1]

Sperm chromatin dispersion test

The principle underlying the sperm chromatin dispersion (SCD) test is that sperm with fragmented DNA fails to produce the characteristic halo of dispersed DNA loops after acid denaturation and removal of nuclear proteins, normally seen in sperms with nonfragmented DNA. The test is also known as Halosperm[®] test.^[33] The test is limited by the interobserver variation resulting from its feature of subjective evaluation under the microscope. One advantage of the SCD test is that there is no need for complex instruments.

Indications for sperm DNA fragmentation testing

Measurement of SDF in infertile men is a promising diagnostic and prognostic tool and many indications for SDF tests have been suggested.^[41,42] The main indications for SDF testing are summarised in Table 2.

Clinical varicocele

Various clinical reports have suggested a significant relationship between SDF and varicocele, in both infertile and fertile men.^[10] SDF levels in men with varicocele were significantly high, and it was observed that, after varicocelectomy, the levels were significantly reduced, thereby leading to enhanced fertility potential.^[43] Varicocele results in venous stasis and OS that are implicated in the development of SDF and testicular dysfunction.^[44]

Table 2: Recommended indications for sperm DNA fragmentation testing

Clinical varicocele
Unexplained infertility
Recurrent pregnancy loss
Borderline semen analysis
ART (IUI/IVF/ICSI) failure
Prognosis of ART treatment and natural pregnancy
Lifestyle and environmental factors

ART=Assisted reproductive techniques, IUI=Intrauterine insemination, IVF=*In vitro* fertilization, ICSI=Intracytoplasmic sperm injection

The existing body of literature shows little evidence of the clinical usage of SDF testing in low-grade varicocele, but there is stronger evidence for men with high-grade varicoceles.^[43] Recent studies have revealed that DNA fragmentation testing could help clinicians to select varicocelectomy candidates.^[44-46] SDF tests are applicable in men with grade II/III varicocele with conventional semen factors and endorsed in men with grade I varicocele with borderline/abnormal semen parameters.^[19]

Assisted reproductive techniques (intrauterine insemination, in vitro fertilisation/intracytoplasmic sperm injection) failure

It has been reported that in IVF and ICSI, high SDF is related to a higher incidence of pregnancy loss.^[4] Systematic reviews by Zini and Sigman^[47] and Osman^[48] have demonstrated that there is a significant correlation between high SDF and poor ART outcomes. However, these two systematic reviews were not without limitations due to heterogeneous study design and several potential confounding factors that could affect the results of the reviews which were not controlled. Nonetheless, there is sufficient evidence to suggest an association between high SDF and pregnancy loss.^[47]

A significantly higher level of SDF was observed in ejaculated sperm compared to testicular sperm.^[49] Moreover, recent studies have shown that the testicular sperm was associated with a higher success rate of IVF and ICSI.^[50] Hence, testicular sperm instead of ejaculated sperm in ICSI might have several benefits in men with recurring IVF failure, high SDF and oligozoospermia. SDF can therefore provide useful prognostic information, especially on the outcome ART cycles in patients with recurring ART failure [Table 3]. Different therapeutic approaches, including oral antioxidants and sperm selection methods, have been tried to reduce SDF and its impact on ART results.

Borderline semen parameters

A key contributing factor to male infertility is OS.^[67] Antioxidants in the semen counteract excessive reactive

Table 3: Summary of studies on sperm deoxyribonucleic acid fragmentation in male infertility

Author and year	SDF test	Participants/intervention	Outcome measures	Results
Zandieh <i>et al.</i> , 2018 ^[51]	SCD	Fertile men (<i>n</i> =30), unexplained infertility group (<i>n</i> =28)	Semen parameters, hydrogen peroxide, superoxide anion levels, and SDF	In the infertile group, sperm motility and normal morphology were significantly lower than in the control group. Levels of hydrogen peroxide and superoxide anion were higher in the infertile group.
Carlini <i>et al.</i> , 2017 ^[52]	TUNEL	Fertile men with proven fertility (<i>n</i> =114), recurrent pregnancy loss (<i>n</i> =112)	Seminal parameters and SDF	SDF was higher (15%) in the infertile group. Sperm DNA integrity was impaired in the recurrent pregnancy loss group and the values were higher (8%) when compared to the fertile group.
Atig <i>et al.</i> , 2017 ^[53]	TUNEL	Fertile men (<i>n</i> =50), infertile men (<i>n</i> =100; 40 OAT, 31 teratozoospermia, 29 asthenozoospermia)	SDF, malondialdehyde levels, and semen parameters	SDF and malondialdehyde levels were increased in the infertile group and SDF correlated with semen parameters.
Wiweko and Utami, 2017 ^[54]	SCD	Fertile group (<i>n</i> =36), the infertile group with abnormal semen analysis except for azoospermia (<i>n</i> =78)	Sperm DFI	Sperm DFI was significantly higher in the infertile group when compared to the control fertile group.
Martínez-Soto <i>et al.</i> , 2016 ^[55]	TUNEL	Treatment group (<i>n</i> =32), docosahexaenoic acid (1500 mg for 10 weeks), placebo group (<i>n</i> =25)	SDF and semen parameters	Significantly lower SDF levels in treatment group (-17.2%±2.8%) versus placebo group (+11.2%±1.9%).
Malić Vončina <i>et al.</i> , 2016 ^[56]	TUNEL	Fertile group (<i>n</i> =51), unexplained couple infertility (<i>n</i> =85)	SDF, MMP levels, semen parameters and natural conception rate	Insignificant effect on sperm parameters. 31% of infertile men conceived naturally.
Bareh <i>et al.</i> , 2016 ^[57]	TUNEL	Fertile (normozoospermic) group (<i>n</i> =31), unexplained recurrent pregnancy loss (<i>n</i> =26)	SDF	Infertile group SDF values <25% and with MMP values >62.5% had significantly increased odds for conception (odds ratio 5.22). SDF was significantly higher (27%) in men with RPL compared to fertile controls.
Ni <i>et al.</i> , 2016 ^[58]	SCSA	Healthy donors (<i>n</i> =25), abnormal semen analysis, and varicocele (<i>n</i> =15)	DFI and malondialdehyde	Sperm DFI was significantly lower in healthy donors with proven fertility. A strong correlation between sperm DFI and malondialdehyde levels.
Muratori <i>et al.</i> , 2015 ^[59]	TUNEL	Fertile group with proven fertility (<i>n</i> =86), couples with unexplained infertility (<i>n</i> =348)	SDF with receiver operating characteristic curves	After matching for both age and semen parameters, only brighter and total SDF predicts male fertility. At high values of total SDF, brighter SDF predicts natural conception better than total SDF.
Gual-Frau <i>et al.</i> , 2015 ^[60]	SCD	20 infertile men with Grade 1 varicocele treated with combined antioxidants for 3 months	SDF and sperm concentration	Significantly lower SDF levels (-22.1%), 31.3% fewer highly degraded sperm cells, significantly higher sperm concentration.
Abad <i>et al.</i> , 2013 ^[61]	SCD measurement done following various periods of sperm storage 37°C	20 infertile patients with asthenoteratozoospermia treated with combined antioxidants for 3 months	SDF and sperm parameters	Significant decrease in SDF with time. Significant improvement in sperm concentration, motility, and morphology.

Contd...

Table 3: Contd...

Author and year	SDF test	Participants/intervention	Outcome measures	Results
Mangiarini <i>et al.</i> , 2013 ^[62]	TUNEL	Normozoospermia (<i>n</i> =18), teratozoospermia (<i>n</i> =14)	SDF and sperm morphology	In normozoospermic individuals, TUNEL positive normal morphology sperm was 4% Spermatozoa with vacuolated head or a small non-oval head had a significantly higher incidence of DNA fragmentation in both groups (12 and 13%, 19 and 13% respectively) Significantly lower alkaline-labile sites and mean tail length of the COMET when compared to the control group Significant improvement in all semen parameters
Vani <i>et al.</i> , 2012 ^[63]	COMET	120 healthy human subjects, 120 men exposed to lead received treatment with Vitamin C (1000 mg) 5 consecutive days per week for 3 months	SDF and semen parameters	
Brahem <i>et al.</i> , 2011 ^[64]	TUNEL	Fertile group with proven fertility (<i>n</i> =30), men with teratozoospermia (<i>n</i> =70)	SDF and chromosomal aneuploidy	Teratozoospermic individuals show increased levels of SDF and chromosomal aneuploidy
Venkatesh <i>et al.</i> , 2011 ^[65]	SCSA	Fertile men (<i>n</i> =50), couples with unexplained couple infertility (<i>n</i> =100)	DFI, sperm count, motility, and morphology	DFI in infertile men was significantly higher (9%) in infertile men when compared to controls
Alahmar <i>et al.</i> ^[9]	SCD	Fertile controls (<i>n</i> =40), infertile men with idiopathic oligoasthenospermia (<i>n</i> =65) treated with CoQ10	Semen parameters, seminal SDF, seminal antioxidant markers and seminal CoQ10 level	Semen parameters were negatively correlated with DFI Increased sperm concentration, motility, seminal antioxidant markers, and CoQ10 level and reduced SDF and ROS
Alahmar <i>et al.</i> ^[66]	SCD	Fertile controls (<i>n</i> =50), infertile men with idiopathic oligoasthenoteratospermia (<i>n</i> =50) treated with CoQ10	Semen parameters, seminal SDF, seminal antioxidant markers, seminal CoQ10 level and sex hormones	Increased sperm concentration, progressive and total motility, seminal antioxidant and CoQ10 levels, and reduced SDF and ROS
Alahmar and Singh ^[26]	SCD	Fertile controls (<i>n</i> =58), infertile men with idiopathic oligoasthenospermia (<i>n</i> =130) treated with CoQ10 or centrum multivitamin	Semen parameters, seminal SDF, seminal antioxidant markers, seminal CoQ10 level and sex hormones	Both CoQ10 and centrum were effective in improving semen parameters, antioxidant capacity, and SDF, but the improvement was greater with centrum than with CoQ10
Alahmar and Naemi ^[25]	SCD	Fertile controls (<i>n</i> =84), infertile men with idiopathic oligoasthenospermia (<i>n</i> =178) treated with CoQ10 and followed up for 24 months	Semen parameters, seminal SDF, seminal antioxidant markers, seminal CoQ10 level and pregnancy rate, time to pregnancy, and their predictors	Increased sperm concentration, motility, seminal antioxidant markers, and CoQ10 level and reduced SDF and ROS CoQ10 level, sperm concentration, motility, male age, ROS and GPx were independent predictors of pregnancy and time to pregnancy

SCD=Sperm chromatin dispersion, TUNEL=Terminal deoxynucleotidyl transferase dUTP nick end labeling, DNA=Deoxyribonucleic acid, SDF=Sperm DNA fragmentation, DFI=DNA fragmentation index, OAT=Oligoasthenoteratospermia, SCSA=Sperm chromatin structure assay, COMET=Single-cell gel electrophoresis, ROS=Reactive oxygen species, COQ10=Coenzyme Q10, MMP=Mitochondrial membrane potential, dUTP=2'-Deoxyuridine 5'-Triphosphate, RPL=Recurrent Pregnancy Loss, GPx=Glutathione Peroxidase

oxygen species (ROS). The imbalance between the antioxidant and ROS levels could trigger a state of OS which could damage sperm.^[68] According to Majzoub *et al.*,^[69] antioxidant treatment with selenium and zinc resulted in a statistically significant fall in SDF levels by 19%, with a significant decrease in SDF and improvement in sperm concentration.

Age and smoking are other important factors linked with increased sperm DNA defects, reduced overall fertility, reduced fertilisation and reduced semen parameters.^[70] It has been shown that DNA fragmentation is considerably lower in infertile non-smokers than in infertile smokers.^[71] Abnormalities in semen parameters have been also linked to obesity.^[72] Certain organochlorine pollutants such as metabolites of dichlorodiphenyltrichloroethane and polychlorinated biphenyls are also known to cause SDF.^[61] Infertility and DNA damage are also associated with environmental and occupational exposure to metals such as cadmium and lead.^[73]

Unexplained infertility/recurrent pregnancy loss

The prevalence of unexplained infertility is believed to exist in 20% of fertile couples.^[19] SDF is considered an independent predictor of male fertility and helps in assessing unexplained infertility and impairment in sperm DNA integrity in men with normal conventional semen parameters.^[74] Studies have shown that SDF could be used as a prognostic factor for natural pregnancy and IUI success rate.^[19,75] Some reports have also revealed significantly higher SDF levels in couples with recurring pregnancy loss (RPL) than in controls [Table 3].^[76-78]

Effect of sperm DNA fragmentation on male infertility

Conventional semen analysis remains the standard initial investigation for male infertility evaluation.^[1] Conventional semen analysis has limitations and is unable to predict male fertility, and there is a need to identify new diagnostic and prognostic markers for male infertility.^[2] In the last years, the SDF test has been generally recognised as a valuable tool for the evaluation of male infertility.^[12]

SDF can be induced by OS, poor sperm compaction and abortive apoptosis.^[4] OS impairs spermatogenesis, resulting in the generation of sperm with poorly chromatin condensation; these defective cells initiate an apoptotic pathway associated with an increase in ROS production by the mitochondria.^[5] OS is also implicated in the activation of endogenous caspases and endonucleases that increase the damage of sperm DNA.^[6] Mature sperms have limited mechanisms to repair DNA damage which makes the sperm vulnerable to oxidative stress-mediated DNA damage.^[7]

The impact of SDF on male fertility potential has been supported by numerous studies.^[8] Current data advocate different levels of SDF among patients with male infertility.^[9] Further, SDF is negatively correlated with male fertility, and infertile men with poor reproductive outcomes exhibit high levels of SDF.^[10] Progress in research on SDF has enhanced our understanding of the mechanisms involved in different infertility pathologies. SDF has been proposed to be the underlying cause of poor semen quality in men with IMI^[12] and can be considered a promising diagnostic, prognostic and therapeutic tool in the management of male infertility.^[13]

Effect of sperm DNA fragmentation on natural pregnancy

Sperm DNA damage negatively correlates with the chances of natural conception. The likelihood of natural pregnancy is decreased when the SDF index assessed by SCSA is 20%–30%^[16] [Figure 1]. Some studies also suggest that SDF levels of more than 30% are associated with reduced pregnancy rates.^[16,39] Similarly, SDF has been correlated with recurring miscarriages.^[76] A study on 30 recurrent spontaneous abortion couples and 30 controls found higher SDF in the patients' group.^[77]

A relatively large number of couples fail to achieve pregnancy despite the absence of a male or female factor of infertility.^[13] Gestation and subsequent embryo development depend on the integrity of the gamete's DNA.^[15] Both sperm and oocyte contribute equally to the formation of the embryonic DNA; therefore, normal sperm chromatin structure is essential for safe and healthy transmission of parental genetic materials. The defect in sperm DNA structure compromises fertilisation and subsequent development of the embryo.^[16]

Evidence from numerous studies supports the association between high SDF and failure to achieve natural pregnancy.^[17] Different studies have shown that sperms with high SDF are associated with a longer time to achieve pregnancy and lower rates of natural conception.^[20] Bungum *et al.* showed that a significant proportion of couples with unexplained infertile have a significantly high level of SDF. In addition, men with SDF level of more than 30% have a low probability of achieving natural pregnancy.^[13] Miscarriage is a common pregnancy complication, occurring in 15% of all clinically recognised pregnancies, and is also related to SDF level.^[16] Couples whose natural pregnancy resulted in miscarriage have lower sperm DNA integrity.^[79]

Sperm DNA fragmentation and assisted reproductive techniques outcomes

There are controversies regarding the incorporation of DNA damage tests in the routine assessment of

male factor infertility. While some reports showed that sperm DNA damage had a crucial effect on pregnancy and should be integrated as a part of routine clinical examination,^[80] others suggested that SDF tests should not be integrated into the routine clinical examination.^[39]

In a recent study carried out by Simon *et al.*, out of the 92 studies that were evaluated for the association between SDF and ART, it was observed that only 35 studies indicated a considerably inverse association between SDF and the fertilisation rate.^[81] The remaining 57 studies showed no substantial association. The association between sperm DNA damage and low fertilisation rate for every method was higher in IVF (59%) than in ICSI (24%).^[33]

Intrauterine insemination

SDF levels higher than 30% may predict reduced pregnancy rates after IUI.^[5] A study showed that the pregnancy was lower in men with SDF level of 12% or above.^[33] However, Kimura and Nagao^[82] reported no association between sperm DNA damage and clinical pregnancy rates after IUI. Measurement of SDF could help in the prediction of IUI results. When SDF is high, other therapeutic approaches such as ICSI could be adopted to treat infertile couples.

In vitro fertilisation

Several studies that have explored the impact of SDF on IVF and IVF/ICSI outcomes have provided variable results. While some studies suggested that SDF does not affect fertilisation or embryo quality,^[83] others have implicated paternal factors and SDF in poor embryo development and early pregnancy loss.^[80] In a review carried out by Zini *et al.*,^[84] a significant association was observed between abnormal sperm DNA damage tests and lower pregnancy rates. Another study conducted by Zhang *et al.*^[85] showed that higher clinical pregnancy rates were correlated with a DFI lower than 27%. A major challenge associated with these studies is the interpretation of results because of the heterogeneous designs and different protocols used. In a study to evaluate the clinical outcomes of SDF in 550 Chinese couples of which 415 underwent IVF and 135 ICSI, it was concluded that high levels of SDF were not related to alterations in pregnancy or live birth rates in both ICSI and IVF groups.^[86]

Intracytoplasmic sperm injection

It has been reported that SDF has a considerable effect on pregnancy rates in different meta-analyses on IVF and ICSI.^[33] A meta-analysis conducted by Zini and Sigman^[47] revealed that the variation in the pregnancy rate between a group with high SDF and a group with low SDF was 11%. Another study revealed no

significant correlation between the percentage of SDF and clinical pregnancy rates.^[87] The percentage of SDF was also associated with the clinical pregnancy rate after ICSI.^[88]

The probable implication of SDF in ART outcomes has been presented in numerous meta-analysis studies, but the strength of association and the association with ICSI are still debated. A meta-analysis by Sugihara *et al.* showed a limited capacity of SDF in predicting IUI outcome (risk ratio [RR]: 3.15, 95% confidence interval [CI]: 1.46–6.79),^[1] while Chen *et al.* indicated that high SDF was significantly associated with lower pregnancy rate (RR: 0.34, 95% CI: 0.22–0.52) and birth rate of IUI cycle (RR 0.14, 95% CI: 0.04–0.56).^[29]

Another two different studies on of total 25,639 and 8068 IVF/ICSI cycles, respectively, concluded that SDF has negative effect on clinical pregnancy (RR = 0.83; 95% CI: 0.73–0.94) (and odds ratio [OR] = 1.31; 95% CI: 1.08–1.59), respectively, following IVF and/or ICSI treatment.^[29,30]

Effects of sperm DNA fragmentation on birth defects

There are natural mechanisms that prevent the transmission of the defective genome to the next generation. However, ART treatment for infertility may facilitate the transmission of the defective genome, which could affect the well-being of children born using assisted reproduction. However, increased aneuploidy and high prevalence of genomic abnormalities in ICSI candidates were related to increased SDF levels.^[68] Animal studies in mouse models have indicated adverse effects of increased SDF on offspring including abnormal growth and behaviour, premature ageing and increased prevalence of tumours.^[37] Recent studies have linked increased SDF to a higher incidence of childhood malignancies, inherited diseases and neuropsychiatric disorders in the offspring.^[66,90] Nonetheless, the evidence of the association between SDF and genetic diseases is inconsistent.

Treatment and Prevention of sperm DNA fragmentation

OS is a key player in the development of SDF.^[31,32] OS triggers a harmful chain reaction that leads to sperm membrane lipid peroxidation and nuclear DNA damage.^[33] Treatment and prevention of OS-induced damage could be achieved by, oral antioxidant therapy, varicocele repair, infection control and lifestyle modifications.^[36] Although there are no standardised guidelines for the treatment of OS-induced sperm injury, antioxidant therapy is a promising strategy for the treatment of infertile men with high levels of

oxidative damage.^[37] The rationale behind antioxidant therapy is that SDF is recognised as a significant factor in infertility, pregnancy loss and ART failure, and antioxidants are believed to counteract these negative effects.^[12]

There is growing evidence of antioxidant supplementation to minimise SDF in infertile men. However, beneficial effects on pregnancy and live birth outcomes are inconsistent.^[41] Different antioxidants showed beneficial effects against SDF.^[89] Early *in vitro* studies showed a protective effect for antioxidants flavonoids and catalase against OS-induced SDF.^[42] A combination of zinc, D-aspartate and coenzyme Q10 protected human sperm against SDF using *in vitro* culture.^[39] Ménézo *et al.* observed that an oral antioxidant treatment consisting of Vitamin C, Vitamin E, β -carotene, zinc and selenium could decrease SDF.^[91]

The integrity of the sperm genome is a critical factor in ART success, and pregnancy and implantation rates may improve after antioxidant therapy in couples undergoing ICSI treatment.^[19] In another study, the administration of Menevit[®] combined antioxidants significantly improves pregnancy rates in couples subjected to IVF/ICSI treatment.^[46] Therefore, antioxidant treatment may improve fertilisation potential and reproductive outcomes.^[41] However, large well-designed randomised placebo-controlled trials are essential to formulate a definitive conclusion.

CONCLUSION

SDF is a major factor involved in IMI and poor reproductive outcomes including reduced semen quality, reduced fertilisation, embryo quality, pregnancy rates, recurrent pregnancy loss and poor ART outcomes. With the advent of various novel, validated, sensitive SDF assays, SDF could provide diagnostic, therapeutic and prognostic information that allows better management of infertile couples, and can predict the outcomes of natural pregnancy and ART. Although the routine use of SDF tests is still not widely recommended, SDF testing in selected patients could still provide complementary data that cannot be provided by the traditional semen analysis. Hence, there is fair evidence indicating that SDF testing is a useful diagnostic and prognostic tool in male fertility evaluation in selected patients. Future studies should be focussed on optimising SDF tests, target patients groups, impact on fertility and ART outcomes as well as therapies that could reduce SDF levels in infertile men.

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Conflicts of interest

There are no conflicts of interest.

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