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Sperm DNA fragmentation in male infertility: From bench to bedside

Ala'a Farkouh [a], Ramadan Saleh [a,b,c], Rupin Shah [a,d] and Ashok Agarwal [a,e]

[a]Global Andrology Forum, Ohio, USA; [b]Department of Dermatology, Venereology and Andrology, Faculty of Medicine, Sohag University, Sohag, Egypt; [c]Ajyal IVF Center, Ajyal Hospital, Sohag, Egypt; [d]Division of Andrology, Department of Urology, Lilavati Hospital and Research Centre, Mumbai, India; [e]Cleveland Clinic Foundation, Cleveland, Ohio, USA

ABSTRACT

Objectives: Sperm DNA fragmentation (SDF) is a molecular marker of sperm chromatin health. Elevated SDF is associated with male infertility, recurrent pregnancy loss, and failure of assisted reproductive technologies (ART). In 2021, the sixth edition of the World Health Organization (WHO) Manual for the Laboratory Examination and Processing of Human Semen has listed SDF as an extended test of semen that can be ordered under certain circumstances. However, the manual neither explained the indications for testing nor provided clear guidance on diagnostic thresholds.

Methods: This article summarizes the current body of knowledge regarding clinical applications of SDF, including the appropriate population to test, methods of testing, and management strategies.

Results: Several etiologic factors and pathophysiologic mechanisms for SDF have been described including poor lifestyle habits, noxious exposures, and varicocele. Four SDF assays are included in the WHO manual and may be utilized based on resources and expertise. Strategies to lower SDF levels in infertile men include addressing underlying causes, supplementation with antioxidants, shorter abstinence periods, and use of testicular sperm for intracytoplasmic sperm injection.

Conclusion: SDF testing can be implemented in the evaluation of infertile men and couples experiencing ART failure and appropriate management strategies can be offered to improve reproductive outcomes. There is vast potential for future research regarding the clinical utility of SDF in the evaluation and treatment of infertile couples.

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Introduction

Although conventional semen analysis is the cornerstone of male fertility evaluation, it is limited in its ability to capture the functional and molecular aspects of spermatozoa, such as fertilization potential and DNA or chromosomal integrity [1,2]. Over the past few decades, sperm DNA fragmentation (SDF) and its diagnostic utility for male infertility have captured the interest of many reproductive scientists and clinicians worldwide [3,4].

As such, the sixth edition of the World Health Organization (WHO) Manual for the Laboratory Examination and Processing of Human Semen incorporated assessments beyond the routine semen analysis and included SDF as an extended semen examination, citing it as a promising biomarker [5,6]. One limitation of the WHO manual is that it does not provide clinical circumstances for testing [7]. This is further compounded by limited recommendations on SDF testing from international society guidelines.

Consequently, clinicians are often challenged with defining the appropriate patients for SDF testing and the various management options they can provide to their infertile patients with elevated SDF. To bridge this gap, the Global Andrology Forum (GAF) [8] group conducted a global survey targeted towards reproductive clinicians to explore worldwide practice patterns on indications and techniques of SDF testing as well as management strategies of infertile men with elevated SDF [9–11].

When to order an SDF test?

Several etiologies and risk factors have been associated with elevated SDF, including advanced paternal age, smoking, obesity, diabetes mellitus, genital tract infections, exposure to chemicals, pollutants, and radiation, as well as clinical varicocele. Evidence has shown that elevated SDF is detrimental for male reproductive potential, being associated with infertility, recurrent pregnancy loss (RPL), and failure of assisted reproductive technologies (ART) [12–15]. Infertile men that benefit from SDF testing include those with no known etiology for their infertility, whether with normal conventional semen parameters (i.e. unexplained infertility) or abnormal parameters (i.e. idiopathic male infertility), those with known risk factors or clinical varicocele, and couples experiencing RPL or ART failure [9]. For subclinical varicocele, SDF testing is not recommended for this purpose, due to lack

CONTACT Ashok Agarwal agarwaa32099@outlook.com Global Andrology Forum, Moreland Hills, Ohio 44022, USA

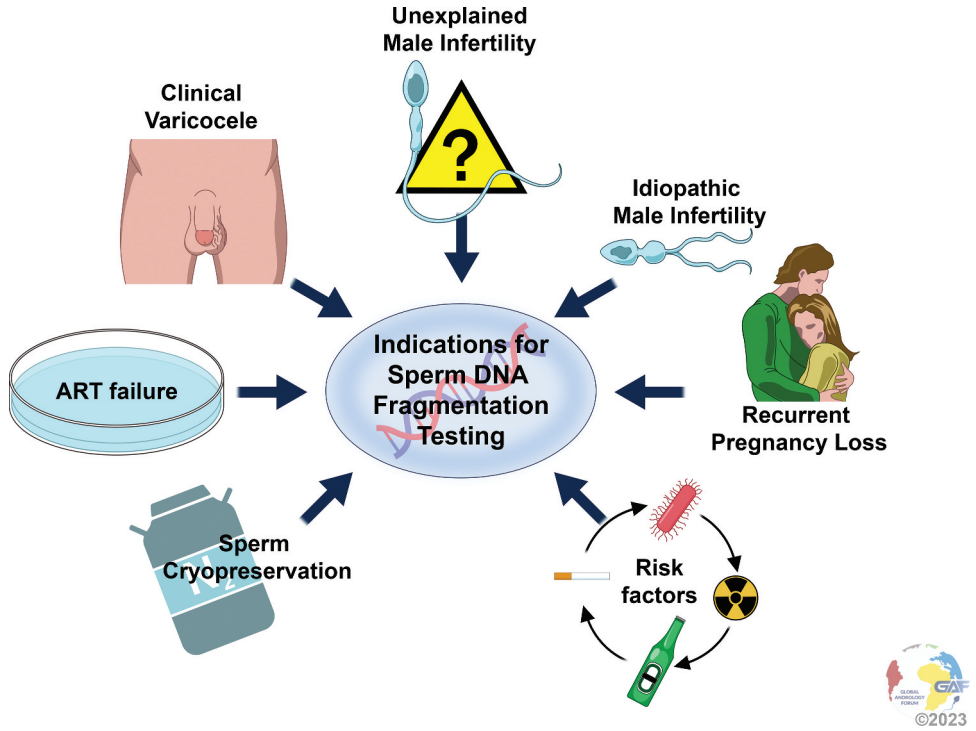

Figure 1. Indications for sperm DNA fragmentation testing. Reproduced from Agarwal A, et al. Controversy and consensus on indications for sperm DNA fragmentation testing in male infertility: a Global survey, Current guidelines, and expert recommendations. World J Mens Health. 2023 Jul;41(3):575–602 with permission, Global Andrology Forum ©2023. All rights reserved. ART: assisted reproductive technologies.

of evidence on a significant association or benefit from correction [10,16]. Finally, although there is evidence of the damaging effect of cryopreservation on sperm DNA integrity, no clear recommendation is available, and clinicians should use a case-based approach when deciding on testing [9]. Potential indications for SDF testing are summarized in Figure 1.

How to test for SDF?

Currently, four assays for measuring SDF levels are included in the WHO manual and can be used in clinical practice, based on availability, expertise, and resources [5,11]. These include the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL) assay, the sperm chromatin structure assay (SCSA), the sperm chromatin dispersion (SCD) assay, and the Comet assay. Table 1 summarizes the techniques, advantages, and disadvantages of these four assays. Since prolonged ejaculatory abstinence may result in elevated SDF levels [17], SDF testing is recommended with less than 5 days of abstinence duration [11]. Regarding interpretation of SDF results, the consensus is that each testing laboratory establishes and reports its own reference values [11]. This recommendation acknowledges a major limitation in SDF testing, where no standardized cut-off exists.

How to treat elevated SDF?

Generally, the initial step in management involves lowering SDF using various measures. Interventions targeting known etiologies or risk factors have been proven to be successful in reducing SDF and improving

Table 1. Summary of the four currently recommended assays for measuring sperm DNA fragmentation. TUNEL: terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling; SCSA: sperm chromatin structure assay; SDF: sperm DNA fragmentation.

Assay	Technique	Advantages	Disadvantages
TUNEL	Fluorescent-marked deoxynucleotides incorporated in sites of breaks and detected by microscopy or flow cytometry	• Sensitive • Reliable • Minimal interrater variability	• Expensive • Requires specialized equipment and training
SCSA	Depends of different fluorescence of acridine orange dye if bound to intact or denatured DNA (at sites of breaks)	• Standardized protocol • Reproducible • Can examine a large number of cells	• Expensive • Requires specialized equipment and training
SCD	Acid denaturation followed by dispersion of fragmented DNA	• Simple equipment • Commercial kits available	• High interobserver variability
Comet	Electrophoresis detecting migration of DNA fragments	• Sensitive • Reproducible • Can be performed with very low sperm counts	• Interobserver variability • Requires experienced observer • Variable protocols

reproductive outcomes. These include repair of clinical varicocele [18], antibiotics for genital tract infections [19], and weight loss for obese men [20]. Although no strong evidence exists on the effects of smoking and alcohol cessation on SDF, almost all reproductive experts advocate for and recommend lifestyle modification and avoidance of all potential risk factors [10]. Since reduced ejaculatory abstinence is associated with lower SDF levels and improved reproductive outcomes, it is recommended as a general conservative measure in these patients [10].

Another general approach is the use of antioxidants for 3–6 months [10]. Given the association between oxidative stress and the pathogenesis of SDF, investigators have studied various antioxidant formulations and have reported their effects in lowering SDF levels [21]. On the contrary, the evidence behind empiric hormonal therapy for SDF is not as robust and a recommendation is made for the selective use of hormones only by well-trained fertility experts in specific indications [10]. In summary, management of elevated SDF may include treatment of underlying conditions, avoidance of risk factors, reduced ejaculatory abstinence, and empiric antioxidants [10].

For couples experiencing ART failure with elevated SDF in the male partner, management should consider the clinical context, including the type of ART. For intrauterine insemination and *in vitro* fertilization (IVF) failure, the aforementioned conservative options are recommended [10]. A reduced ejaculatory abstinence on the day of ART may also be utilized as a means to obtain sperm with low SDF. Alternatively, after IVF failure, intracytoplasmic sperm injection (ICSI) may be considered, as evidence shows no effect of SDF levels on clinical pregnancy rates after ICSI [13,14]. For ICSI, sperm selection techniques, such as magnetic activated cell sorting, intracytoplasmic morphologically selected sperm injection, and microfluidic sperm sorting, may be considered as an attempt to improve ART outcome by selecting spermatozoa with lower SDF [10,22]. Testicular sperm extraction followed by ICSI may also be employed to reduce SDF levels after ICSI failure with ejaculated sperm; however clinicians should be aware that evidence behind this recommendation is weak, with no validated SDF testing method on testicular sperm [10,15]. The different strategies for managing infertile men with elevated SDF are summarized in Figure 2.

Clinical case scenario

A couple is referred for andrological evaluation after three episodes of RPL following spontaneous conception. The woman is 27 years old with completely normal gynecological assessment. The man is a 28-year-old engineer with unremarkable history and physical examination, other than smoking. Semen analysis, laboratory evaluation, and ultrasound revealed no remarkable abnormality, while a TUNEL assay revealed elevated SDF. This couple's RPL may be attributed to

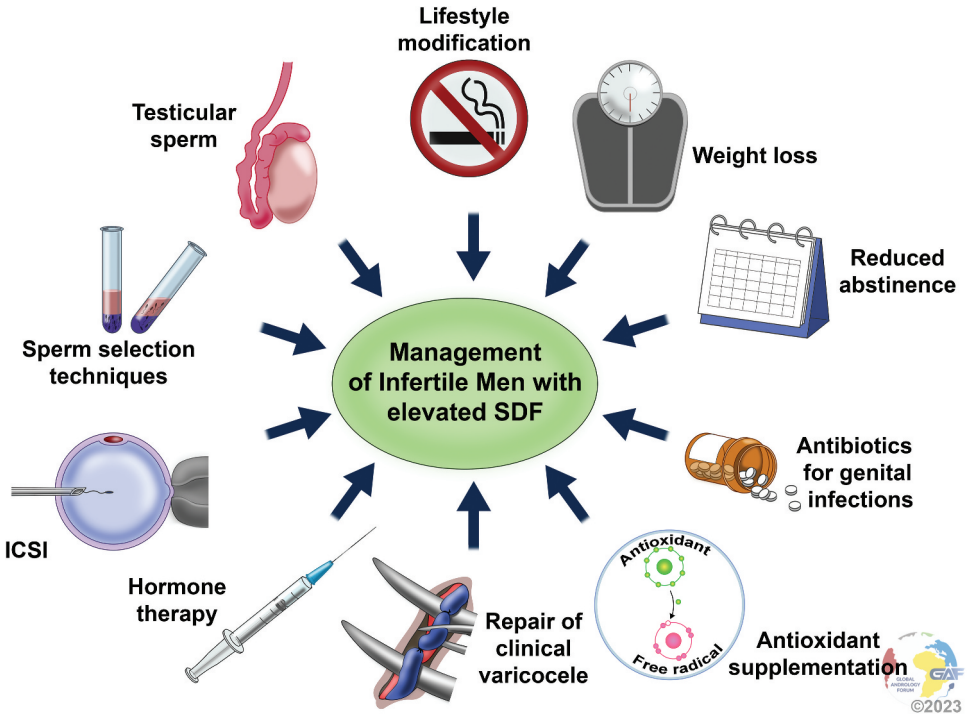

Figure 2. Treatment strategies of elevated SDF. Reproduced from Farkouh A, et al. Controversy and consensus on the management of elevated sperm DNA fragmentation in male infertility: A Global survey, Current guidelines, and expert recommendations. World J Mens Health. 2023 Apr 20 with permission, Global Andrology Forum ©2023. All rights reserved. ICSI: intracytoplasmic sperm injection.

the elevated SDF. Smoking cessation and a trial of empiric antioxidants for 3–6 months may help alleviate SDF levels and allow the couple to conceive naturally if SDF is found to be low on repeat assessment. If elevated SDF persists, the couple may be counselled to proceed with ICSI using sperm selection techniques. Additionally, the couple should be counselled to pursue recommended evaluations for RPL, such as karyotype, cytogenetic analysis of products of conception, and preimplantation genetic testing for aneuploidy [23].

Future prospects

With the evolution of research methodology and technology, future studies may further highlight the benefit of SDF testing when evaluating infertile couples and may help answer the question of whether SDF should be part of the routine infertility assessment. This is particularly important, as there is no unanimous recommendation or consensus by professional societies. For example, the American Urological Association/American Society for Reproductive Medicine guidelines recommend against routine evaluation of SDF in infertility evaluation [24]. The predictive potential of SDF for ART outcomes should also be explored, which may enable the use of this diagnostic marker as an aid in clinical decision-making when discussing an optimal management strategy for infertility. Future studies should also focus on establishing a standardized method of SDF testing and interpretation with uniform cut-off points that can be used by all clinicians worldwide. This may be accomplished with the incorporation of artificial intelligence, which may help interpret and report DNA damage in a more systematic and objective way [25]. Finally, very few recommended treatment strategies for elevated SDF are rooted in high quality randomized controlled trials. Areas for potential exploration include determining an optimal type, dose, and duration of antioxidant therapy for SDF, investigating the efficacy and outcomes of the various sperm selection techniques on SDF reduction, and defining the proper indications and role of testicular sperm extraction for non-azoospermic infertile men with elevated SDF.

Key points

- SDF is associated with aging, infections, clinical varicocele, and poor lifestyle habits, leading to male infertility.
- TUNEL, Comet, SCSA, and SCD are the four assays included in the recent WHO manual. However, the optimal test and cut-off have not been defined and it is recommended that cut-off values be individually determined.
- SDF management consists of elimination of underlying causes, lifestyle changes, and antioxidants. If SDF remains elevated, then measures at the time of ART include short abstinence, various sperm selection techniques, or use of testicular sperm.
- There is vast potential for future research regarding the clinical utility of SDF in the evaluation and treatment of infertile couples.

Experts' comment

With the advent of ICSI, many of the older sperm function tests have become redundant. However, tests that assess the quality of sperm DNA still have important clinical utility. There is controversy over which test to use, interpretation of results and which treatment to offer. The guidelines in this mini-review provide the clinician with practical suggestions based on the best current knowledge.

Abbreviation

ART: assisted reproductive technologies; GAF: Global Andrology Forum; ICSI: intracytoplasmic sperm injection; IVF: *in vitro* fertilization; RPL: recurrent pregnancy loss; SCD: sperm chromatin dispersion; SCSA: sperm chromatin structure assay; SDF: sperm DNA fragmentation; TUNEL: terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling; WHO: World Health Organization

Disclosure statement

No potential conflict of interest was reported by the author(s).

ORCID

Ala'a Farkouh http://orcid.org/0000-0002-0362-9109
Ramadan Saleh http://orcid.org/0000-0003-0503-3533
Rupin Shah http://orcid.org/0000-0002-7868-5949
Ashok Agarwal http://orcid.org/0000-0003-0585-1026

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