



Italian Journal of Gynæcology & Obstetrics

June 2021 - Vol. 33 - N. 2 - Quarterly - ISSN 2385 - 0868

The Sperm DNA Fragmentation Study Group (SFRAG) Guideline and its relevance for practicing gynecologists

S. C. Esteves¹⁻³, A. Conforti^{2,4}, C. Alviggi^{2,4}

¹ANDROFERT, Andrology and Human Reproduction Clinic, Referral Center for Male Reproduction, Campinas, SP, Brazil

²Department of Surgery, Division of Urology, University of Campinas (UNICAMP), Campinas, SP, Brazil

³Faculty of Health, Aarhus University, Aarhus, Denmark

⁴Department of Neuroscience, Reproductive Science and Odontostomatology, Federico II University of Naples, Naples, Italy

ABSTRACT

This editorial highlights the key recommendations of the novel evidence-based *Sperm DNA Fragmentation Guideline* (SFRAG guideline) for gynecologists providing infertility care. Sperm DNA fragmentation is a biomarker of sperm's chromatin quality. Elevated sperm DNA fragmentation rates contribute to couple's infertility and negatively impact medically assisted reproduction outcomes. There are five main clinical scenarios in which gynecologists should consider sperm DNA fragmentation testing to guide their decision-making process. They include unexplained infertility, recurrent pregnancy loss, before (or after failed) medically assisted reproduction, and the presence of male infertility risk factors. The SFRAG guideline emphasizes the importance of corrective measures to decrease sperm DNA fragmentation rates and selection of the best medically assisted reproduction modality for the affected couples. The intended goal is to provide the foundation for standardizing care in this area while maintaining clinicians' autonomy.

SOMMARIO

Questo editoriale mette in evidenza le raccomandazioni chiave delle nuove *linee guida per la frammentazione del DNA dello sperma* basata sulle evidenze (linea guida SFRAG) per i ginecologi che forniscono cure per l'infertilità. La frammentazione del DNA dello sperma è un biomcatore della qualità della cromatina dello sperma. I tassi elevati di frammentazione del DNA dello sperma contribuiscono alla sterilità della coppia e hanno un impatto negativo sui risultati della riproduzione assistita. Esistono cinque scenari clinici principali in cui i ginecologi dovrebbero prendere in considerazione i test di frammentazione del DNA dello sperma per guidare il loro processo decisionale. Includono infertilità inspiegabile, interruzione della gravidanza ricorrente, prima (o dopo il fallimento) della riproduzione medicalmente assistita e la presenza di fattori di rischio di infertilità maschile. La linea guida SFRAG sottolinea l'importanza di misure correttive per diminuire i tassi di frammentazione del DNA dello sperma e la selezione della migliore modalità di riproduzione medicalmente assistita per le coppie affette. L'obiettivo prefissato è quello di fornire le basi per la standardizzazione dell'assistenza in quest'area mantenendo al contempo l'autonomia dei medici.

Corresponding Author: Sandro C. Esteves

E-mail: s.esteves@androfert.com.br

Copyright 2021

DOI: 10.36129/jog.33.02.01

Key words

Infertility; sperm DNA fragmentation; medically assisted reproduction; unexplained infertility; miscarriage.

INTRODUCTION

Sperm DNA integrity is essential for the birth of healthy progeny (1). Sperm DNA fragmentation (SDF), a marker of damaged chromatin, has an independent and critical role in male infertility diagnosis and reproductive success (2). The reasons relate to the often higher SDF levels in ejaculated sperm of infertile men (*vs* fertile counterparts) and the adverse impact of SDF on the sperm's ability to fertilize the egg and promote healthy embryo development (2, 3). Consequently, elevated SDF has been associated with longer time-to-pregnancy, increased risk of pregnancy loss, and decreased success in medically-assisted reproduction (MAR) (*e.g.*, intrauterine insemination [IUI] and in vitro fertilization/intracytoplasmic sperm injection [IVF/ICSI]) (4, 5). The adverse effect of SDF on reproductive success is modulated by the oocyte's DNA repair capacity, which is intrinsically related to female age (6). Sperm DNA damage exceeding the oocyte's repair capacity – or the oocyte's failure to repair DNA damage – negatively influences the embryo's development potential and offspring's health (7).

Routine semen analysis – the laboratory backbone of infertility investigation – has low diagnostic discriminatory power (unless at extremely lower levels) as there is considerable overlap between semen characteristics (*e.g.*, sperm count, motility, and morphology) of fertile and infertile men (8). The need for more robust male infertility diagnosis methods has been the driving force of the ongoing efforts to develop and implement SDF testing in clinical practice.

While it is not a replacement for the current tools for infertility diagnosis, SDF testing may add independent information about sperm quality at the molecular level, and its integration into practice may provide better counseling, diagnosis, and treatment planning. Despite that, SDF testing is not routinely recommended during the infertility evaluation by infertility societies. Insufficient clinical data, tests' technical limitations and lack of effective treatment options to overcome SDF related-infertility have been the common grounds for the reluctance to endorse the clinical application of SDF tests (9). However, evidence on these areas has increased steadily, justifying the development of clinical practice guidelines to refine efficiency in diagnosing and treating clinical conditions associated with SDF.

This editorial aims to highlight a recently published evidence-based guideline for the investigation and treatment of SDF – the SFRAG guideline (10). This consensus guideline provides a comprehensive evidence summary about the role of SDF on infertility and offers best practice advice on testing and care of infertile couples affected by SDF. The primary goals of the SFRAG guideline are to provide clinicians – gynecologists, reproductive endocrinologists, urologists, and andrologists – with clear advice on best practices in SDF. The SFRAG recommendations were developed based on the best available evidence, ranging from moderate to low quality. Like other guidelines (11), the SFRAG guideline may be used to help standardize care while securing physician autonomy, making it an invaluable resource for a broad range of professionals providing infertility care, including gynecologists.

In the first part, the SFRAG guideline outlines the SDF pathophysiology and explains the existing laboratory tests available to measure SDF. Several conditions, including varicocele, chronic illnesses, male accessory gland infections, advanced paternal age, inadequate lifestyle (*e.g.*, smoking, obesity), occupational and environmental factors, use of medication with potential gonadotoxic effect, and exposure to ionizing and non-ionizing radiation have been associated with high SDF levels. These conditions can promote abortive apoptosis or increase the generation of reactive oxygen species (ROS). Excessive ROS promote oxidative stress, representing a significant causative factor of SDF in live sperm. The SFRAG guideline highlights the four reliable tests to measure SDF, namely:

1. terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL assay);
2. sperm chromatin structure assay (SCSA);
3. sperm chromatin dispersion test (SCD);
4. the Comet assay.

Although the results provided by these tests do not necessarily line up, there is a good correlation between SDF rates reported by TUNEL, SCSA, SCD, and alkaline Comet. Under this section, the guideline provides 13 recommendations on how testing should be carried out and results analyzed. For example, it underlines the importance of using a standardized protocol with strict quality control for achieving reliable test results. Besides, it explains that a neat semen sample should be used for SDF testing, collected after ejaculatory abstinence of 2-5 days. Thresholds of about 20% by TUNEL, SCSA,

SCD, and alkaline Comet, assessed on neat semen should be used to discriminate fertile from infertile men. Additionally, thresholds of 20-30% evaluated by SCSA, alkaline Comet, and SCD are clinically useful for classifying infertile couples into a statistical probability of longer time to achieve natural pregnancy, decreased pregnancy by MAR, and increased miscarriage. Lastly, the SFRAG guideline emphasizes use of a fixed ejaculatory abstinence length for SDF testing, particularly when monitoring the effects of medical and surgical interventions aimed at decreasing SDF levels.

The second part describes seven clinical situations that may benefit from SDF testing, including i. Varicocele, ii. Unexplained/idiopathic infertility, iii. Recurrent pregnancy loss (RPL), iv. Intrauterine insemination (IUI), v. In vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI), vi. Infertility risk factors and vii. Sperm cryopreservation. The guideline provides specific recommendations for each condition – 28 in total – and best practices for treatment. The recommendations with higher clinical implications for practicing gynecologists are summarized below.

Unexplained infertility

Among couples with unexplained infertility, elevated SDF rates are found in up to 20% of individuals. These couples should be informed that abnormal SDF levels may adversely impact their chances of achieving a live birth. It may be therefore prudent to offer SDF testing in couples with unexplained or idiopathic infertility, as an abnormal test result may indicate that damaged sperm chromatin might be the underlying infertility factor. An abnormal test result should prompt a complete male evaluation to help identify and possibly treat conditions associated with poor sperm DNA quality. A decrease in SDF may allow these couples to achieve natural conception or eventually optimize reproductive outcomes of MAR. ICSI can be considered if no correctable male factor is identified, or if abnormal SDF levels persist after treatment, an advice that is particularly useful for couples with a limited reproductive time window.

Recurrent pregnancy loss

A plausible female factor-independent relationship exist between RPL and SDF. Indeed, miscar-

riage rates are increased in couples whose male partners have elevated SDF. It has been hypothesized that DNA fragmentation not repaired by the oocyte may contribute to poor blastocyst development, implantation failure, and miscarriage – the proposed mechanism involves oxidative stress. SDF testing in couples with RPL may help identify the cases in which SDF contributes to the condition, thus helping in patient counseling and guiding clinical management. For instance, a couple with RPL found to have elevated SDF should have the male partner evaluated to rule out male factors possibly associated with oxidative stress and SDF. If no causative factor is identified, ICSI may be a reasonable alternative to overcome the problem.

Medically assisted reproduction

Infertile couples eligible for MAR treatment should be informed that abnormal SDF levels may adversely impact their chances of achieving a live birth. IUI and IVF/ICSI pregnancy rates decrease in these couples, whereas the risk of miscarriage increases in those who achieve pregnancy. On this basis, SDF testing may have value not only in couples experiencing unexpected MAR failures, but also those about to embark on this type of treatment. Likewise, the male partner should be evaluated to rule out and/or fix any underlying male factors possibly causing SDF. The guideline goes on by adding that among couples with ICSI failure and persistently elevated SDF (*i.e.*, despite correctable measures taken), sperm retrieved from the testis may be considered for sperm injection in subsequent treatment cycles due to the lower SDF rates in testicular *vs* epididymal/ejaculated sperm and higher ICSI success rates with use of testicular sperm than ejaculated sperm in men with abnormal SDF levels.

Risk factors

SDF testing is recommended in men with infertility risk factors (*e.g.*, tobacco smoking, obesity, metabolic syndrome, exposure to environmental or occupational toxicants, use of licit or illicit drugs with gonadotoxic effects, and advanced paternal age). An abnormal SDF test may be used for counseling, reinforcing the importance of lifestyle changes and avoiding exposure to toxins, and monitoring the effect of lifestyle changes. It should also prompt a urological/andrological evaluation to help identi-

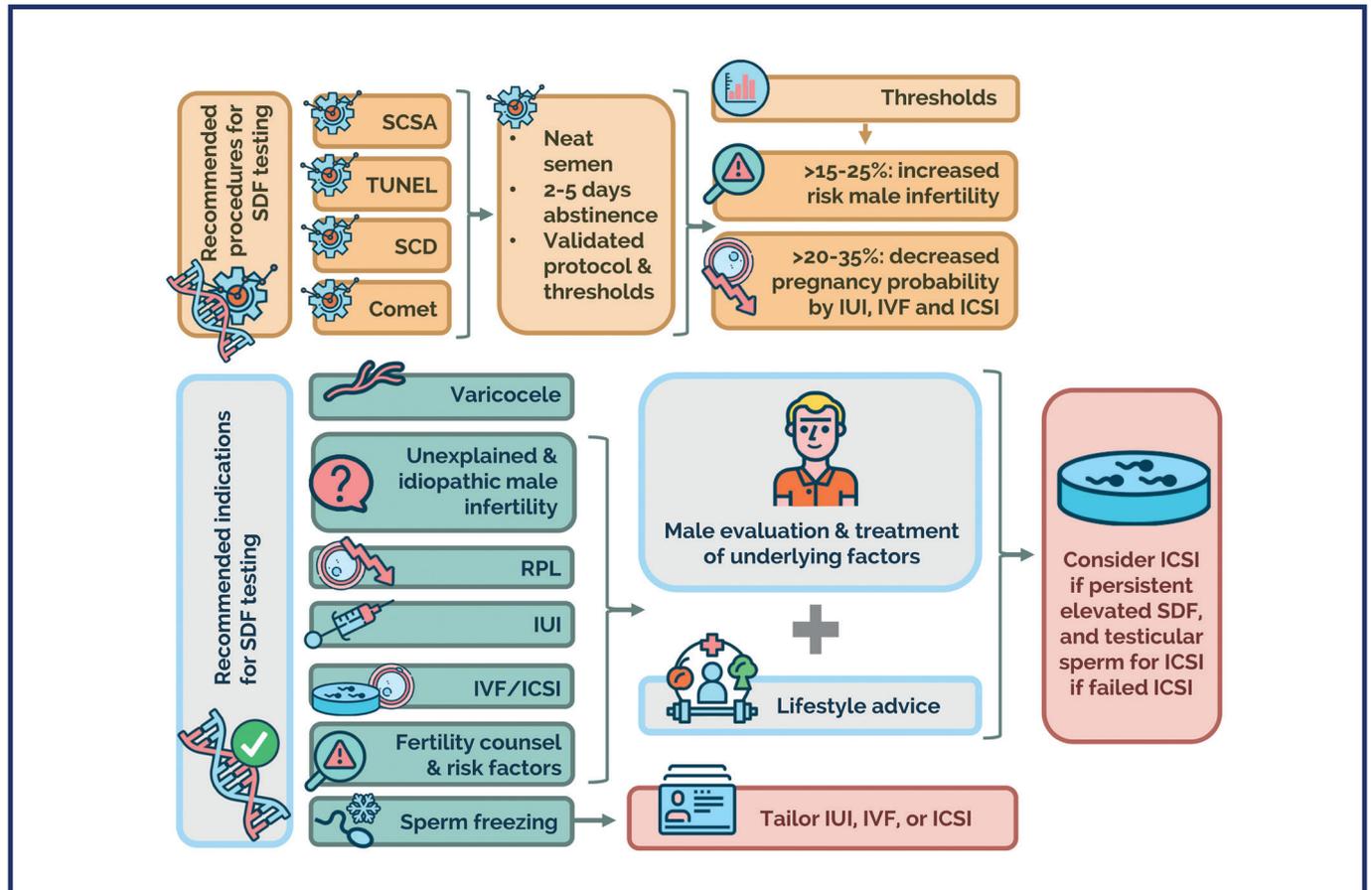


Figure 1. A pictorial summary of the recommendations for testing and management in infertile couples with high sperm DNA fragmentation (9).

IUI: intrauterine insemination; IVF: in vitro fertilization; ICSI: intracytoplasmic sperm injection; RPL: recurrent pregnancy loss.

*This is an open-access article distributed under the Creative Commons Attribution License. The license permits unrestricted use, distribution, reproduction in any medium, provided the original work is properly cited.

fy other hidden and potentially correctable conditions linked to SDF.

The SGRAG guideline has united reproductive urologists and gynecologists with clinical experience in diagnosing and treating male factor infertility. Moreover, the guideline working group included scientists pivotal in developing the main SDF assays, who deciphered each test and made it easy to interpret results and understand their limitations. For each recommendation, a strength rating based on both expert judgment and evidence levels is provided. The guideline emphasizes the central role of reproductive urologists/andrologists in the evaluation of the male partner and highlights the importance of corrective measures to improve the male reproductive health and SDF (figure 1). It also stresses the importance of selecting the adequate MAR modality for the affected couples. Lastly, the SFRAG guideline discusses the main gaps in knowledge and provides a series of recommendations for future research. We strongly believe gynecologists providing care to infertility patients should be fully

aware of the adverse impact of SDF on fertility and reproductive outcomes. The SFRAG guideline is an important document providing the foundation for standardizing care in this subject area while maintaining clinicians' autonomy.

CONFLICT OF INTERESTS

SCE is a member of the Sperm DNA fragmentation Group and leading author of the SFRAG guidelines, distributed as an open-access article under the Creative Commons Attribution License. The license permits unrestricted use, distribution, reproduction in any medium, remixing, transformation, and building upon the material for any purpose provided the original work is properly cited. The full version can be found at <https://onlinelibrary.wiley.com/doi/10.1111/and.13874>. The authors declare that they have no conflict of interests.

REFERENCES

1. Krawetz SA. Paternal contribution: New insights and future challenges. *Nat Rev Genet* 2005;6:633-42.
2. Esteves SC, Santi D, Simoni M. An update on clinical and surgical interventions to reduce sperm DNA fragmentation in infertile men. *Androl* 2020; 8:53-81.
3. Champroux A, Torres-Carreira J, Gharagozloo P, Drevet JR, Kocer A. Mammalian sperm nuclear organization: resiliencies and vulnerabilities. *Basic Clin Androl* 2016;26:17.
4. Esteves SC, Santi D, Simoni M. An update on clinical and surgical interventions to reduce sperm DNA fragmentation in infertile men. *Androl* 2020;8:53-81.
5. Iorio GG, Conforti A, Vallone R, *et al.* Reproductive function of long-term treated patients with hepatic onset of Wilson's disease: a prospective study. *Reprod Biomed Online* 2021;42(4):835-41.
6. Jin J, Pan C, Fei Q, *et al.* Effect of sperm DNA fragmentation on the clinical outcomes for in vitro fertilization and intracytoplasmic sperm injection in women with different ovarian reserves. *Fertil Steril* 2015;103:910-6.
7. Aitken RJ. DNA damage in human spermatozoa; important contributor to mutagenesis in the offspring. *Transl Androl Urol* 2017;6:S761-S764.
8. Esteves SC. Clinical relevance of routine semen analysis and controversies surrounding the 2010 World Health Organization criteria for semen examination. *Int Braz J Urol* 2014;40:443-53.
9. Esteves SC, Agarwal A, Cho CL, Majzoub A. A Strengths-Weaknesses-Opportunities-Threats (SWOT) analysis on the clinical utility of sperm DNA fragmentation testing in specific male infertility scenarios. *Transl Androl Urol* 2017;6(Suppl 4):S734-S760.
10. Esteves SC, Zini A, Coward RM, *et al.* Sperm DNA fragmentation testing: Summary evidence and clinical practice recommendations. *Andrologia* 2021;53(2):e13874.
11. Picarelli S, Conforti C, Buonfantino C, *et al.* IVF during Coronavirus pandemic: who comes first? The POSEIDON viewpoint. *J Gynaecol Obstet* 2020;32:223-8.