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COMPREHENSIVE REVIEW AND CRITICAL EVALUATION OF CLINICAL PRACTICE GUIDELINES ON SPERM DNA FRAGMENTATION

Niranjan Babu Mudduluru[*1], Sirisha Stephen[2], Hamad Basha Shaik[3]

[1]Department of Pharmacognosy, Seven Hills College of Pharmacy, Tirupati, A.P., India
[2,3]Department of Pharmacy Practice, Seven Hills College of Pharmacy, Tirupati, A.P., India

Corresponding Author
Dr. M. Niranjan Babu
Professor, Department of Pharmacognosy Seven Hills College of Pharmacy, Tirupati, A.P., India – 517561, Contact: 7702484513, Email: principal.cq@jntua.ac.in

ABSTRACT
Sperm DNA fragmentation (SDF) is associated with male infertility and reproductive challenges. SDF testing is recommended for individuals with adaptable lifestyles and risk factors for infertility, those experiencing recurrent pregnancy loss, grade 1 varicocele, infertile couples with recurrent pregnancy loss (RPL) or undergoing intrauterine insemination (IUI), cases of unexplained infertility, repeated failure in assisted reproductive technology (ART) procedures, and patients with either abnormal or normal semen parameters. To assist clinicians in utilizing SDF for male fertility evaluation, guidelines are necessary. Two recent guidelines by Agarwal et al. and Esteves et al. have been evaluated and compared. While the guidelines share similar recommendations, they also highlight differences. The best practice recommendations from these guidelines have been combined to provide a comprehensive understanding of SDF in male fertility.

Keywords: ART, infertile couples, lifestyle, SDF, unexplained infertility

INTRODUCTION
Sperm DNA fragmentation (SDF) refers to single-stranded or double-stranded breaks in the sperm genome, which can adversely affect male fertility and reproductive outcomes. SDF can result from three primary mechanisms: abortive apoptosis, defective chromatin maturation, and oxidative stress. DNA damage can occur within the testes, during passage through the reproductive ducts, after ejaculation during sperm processing, or during cryopreservation[1].

Infertility is a global concern, and DNA fragmentation is a significant factor contributing to the condition. Infertile men typically have higher levels of DNA fragmentation compared to fertile men, underscoring the importance of examining sperm count and motility. New diagnostic techniques, such as DNA fragmentation testing, are essential for effectively addressing infertility, as overlooking this factor can lead to ineffective medical approaches and mismanagement [2].

High levels of SDF are associated with an increased risk of recurrent pregnancy loss (RPL), lower pregnancy rates, and higher miscarriage rates in in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) [3]. Various conditions, disorders, and exposures, such as varicocele, male genital tract infections, advanced age, smoking, obesity, radiation, and environmental toxins, have been linked to increased SDF. Shorter ejaculatory abstinence times have been reported to reduce SDF levels. Clinical trials have also shown that antioxidants can improve SDF levels. Additionally, the DNA fragmentation index (DFI) has been reported to decrease by more than 5% after varicocelectomy [4].

Tests to measure SDF include the terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) assay, sperm chromatin dispersion (SCD), sperm chromatin structure assay (SCSA), and comet assay. These tests are utilized to study SDF within the context of assisted reproductive technologies (ART). However, most studies on the use of testicular sperm in non-azoospermic men with high SDF for ICSI consist of small cohorts or case series, lacking adequate control groups or live birth reporting [5].

There is a growing body of studies and reports highlighting the detrimental impact of SDF on male fertility and reproductive outcomes. These studies also review various factors that can increase or decrease SDF and influence reproduction. Given the breadth of research on SDF, there is significant potential for its application in clinical practice [6]. Clinical practice guidelines are essential to direct physicians and reproductive specialists on the appropriate use of SDF testing, including which assays to use, indications for testing, and strategies to reduce SDF. Recently, leading scientists in andrology have formulated and published two new guidelines on SDF, with recommendations based on high-quality reports and meta-analyses. This article aims to compare and contrast these guidelines, summarizing and unifying them to provide a comprehensive guide for clinicians on the use of SDF testing in their practice [7].

MERITS

Sperm DNA fragmentation testing is a valuable tool for assessing the likelihood of natural conception in male patients. It evaluates the integrity of the DNA package, determines the degree of DNA damage, and measures single and double-stranded breaks. This method helps identify patients who may benefit from varicocelectomy, predict outcomes of subsequent ART cycles, and select sperm with appropriate DNA for initiating ART procedures. Compared to standard semen analysis, SDF tests provide more specific and significant insights [8]. The American Urological Association, American Society of Reproductive Medicine, and European Association of Urology recognize that sperm DNA fragmentation contributes to male infertility, making SDF testing essential for identifying male infertility [9].

GUIDELINE AND RECOMMENDATION

Agarwal et al. provided a summary of their recommendations and a clinical algorithm for using SDF testing in the evaluation of infertile couples. They suggested six indications for SDF testing and seven management strategies, each graded according to the Oxford Centre for Evidence-Based Medicine (OCEBM) levels. Esteves et al. offered two tables with 41 recommendations, 13 related to technical aspects and 28 to indications. Each recommendation was graded using OCEBM levels and given a strength rating based on expert judgment, categorized as either strong (applicable to most individuals) or conditional (appropriate depending on the situation) [10]. Both guidelines relied on meta-analyses and high-quality articles to recommend SDF testing and treatment strategies. Agarwal et al. summarized studies correlating clinical conditions with SDF, suggesting testing indications and management strategies [11]. Esteves et al. provided statements summarizing evidence and supporting studies, presented in two tables for technical aspects and clinical indications. Each study was rated based on OCEBM levels of evidence [12].

DISCUSSION

SDF testing is crucial for assessing male reproductive potential and influencing reproductive outcomes. It can be used for investigative or predictive purposes and lead to targeted management strategies. However, many ART centers neglect fertility evaluation in men with

normozoospermia or available spermatozoa for ICSI, resulting in multiple failed ART cycles. Prompt assessment of the male partner, including SDF levels, can help identify underlying pathological factors and guide treatment paths, reducing unnecessary interventions or repeated failed ART cycles [13]

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The Society for Translational Medicine (STM) advocated for SDF testing in 2017, discussing indications, recommendations, tests for SDF, and management strategies. However, other international societies have not provided clear guidelines on its implementation, particularly regarding specific tests or conditions for testing. The European Society of Human Reproduction and Embryology (ESHRE) discussed SDF testing as a means to explain reproductive polymorphism (RPL), the European Academy of Andrology (EAA) suggested adding SDF testing to basic semen analysis in men with oligoasthenoteratozoospermia considered for ART, and the European Association of Urology (EAU) recommended SDF testing only for men with unexplained infertility or after RPL. The American Urological Association (AUA) and American Society for Reproductive Medicine (ASRM) published guidelines on male infertility, recommending against SDF testing in the initial evaluation of fertility but advocating its use and importance in couples experiencing RPL [14].

The two new guidelines offer a unique perspective on SDF testing, discussing how, when, and why to test, as well as treatment options. They expand the indications and role of SDF testing beyond existing international society guidelines, providing clinicians and specialists with valuable insights into the use of SDF and treatment approaches.

a. Testing for Sperm DNA Fragmentation

The guidelines recommend the TUNEL assay, Comet assay, SCSA, and SCD assay as the four validated tests for Sperm DNA Fragmentation (SDF) in assisted reproductive technology (ART). According to a meta-analysis a 20% cutoff for SDF distinguishes fertile from infertile men. Studies with published cutoff values for SDF tests across various settings and reproductive outcomes. They also discussed the role of measuring oxidation-reduction potential as a marker of oxidative stress to enhance the diagnostic value of SDF tests for ART, though they did not recommend it as a replacement for these tests. Esteves et al. offered comprehensive evidence and technical recommendations on SDF tests, including factors influencing SDF levels during testing, such as ejaculatory abstinence duration, timing of sample processing after ejaculation or thawing, cryomedia and freezing techniques, and sperm processing methods. They recommended testing after 2-5 days of abstinence, maintaining consistent abstinence to monitor intervention effects, and conducting SDF testing within 30-60 minutes after liquefaction of raw semen or immediately after thawing if frozen. They concluded that thresholds of 20%-30% are associated with adverse pregnancy outcomes, acknowledging that this prediction is not absolute.

b. Indications for Sperm DNA Fragmentation Testing

DNA fragmentation analysis is crucial for males experiencing unexplained fertility issues, recurrent pregnancy loss, or failed assisted reproductive techniques. Male factor infertility affects 40% of registered cases worldwide. A sperm DNA fragmentation index of 26% or higher is considered abnormal and correlates with poor outcomes for natural conception and assisted techniques. Guidelines recommend SDF testing for various situations, including evaluating pregnancy outcomes, assessing patient conditions, and identifying factors contributing to infertility. They review the adverse impact of SDF on natural pregnancy and ART outcomes, providing specific recommendations for testing in cases of IUI or IVF failure and recurrent miscarriage post-ICSI. SDF testing before initiating ART following ART

failure. The guidelines discuss and recommend SDF testing for clinical varicocele, idiopathic male infertility, unexplained male infertility, and recurrent pregnancy loss (RPL), also reviewing the adverse impact of lifestyle and exposure risk factors. Esteves et al. include sperm cryopreservation as an indication for SDF testing due to the detrimental effects of freezing on sperm caused by increased oxidative stress.

c. Treatment and Management of Sperm DNA Fragmentation

Sperm DNA integrity is influenced by various environmental and dietary factors, including physical elements like radiation and heat, tobacco smoke, airborne pollutants, chemical elements like anticancer medications, sexually transmitted infections, and biological elements like advancing male age, increased body mass index, and diabetes. Lifestyle adjustments, such as wearing loose-fitting clothing, avoiding hot environments, and adhering to appropriate ejaculatory abstinence periods, are recommended for men with poor sperm quality. Reactive oxygen species (ROS) from male genital tract infections can damage sperm DNA. Patients can receive medication for 2-12 weeks to reduce ROS production by their white blood cells, potentially increasing sperm fertility. Oral antioxidant vitamin therapy is the standard of care for male infertility patients to reduce ROS production and enhance fertility. Sperm DNA fragmentation decreases after antioxidant treatment, indicating ROS's role in its deterioration.

Varicocele repair, which is integral to varicocele's pathogenesis, has been shown to improve infertility caused by oxidative stress and strengthen sperm nuclear DNA. A meta-analysis of six studies involving 177 patients demonstrated that varicocelectomy enhances sperm DNA integrity.

Selecting sperm with minimal DNA damage for ART is ideal, as it helps mitigate adverse ICSI reproductive outcomes attributed to sperm DNA damage. Testicular sperm typically exhibits lower DNA damage and better DNA integrity than ejaculated sperm. A recent prospective comparative study with 172 patients with elevated SDF levels found that SDF levels in testicular sperm were five times lower than those in ejaculated sperm. Even after oral antioxidant therapy, using testicular sperm for ICSI was associated with better reproductive outcomes, yielding a birth rate of 46.7% compared to 26.4% in the ICSI group using ejaculated sperm.

Management strategies for men with elevated SDF, including treating underlying factors, providing lifestyle advice, proceeding with ICSI if SDF levels remain elevated, and considering testicular sperm if ICSI fails. They emphasized the importance of comprehensive evaluation by a specialist upon detecting abnormal SDF levels. A section on management strategies, citing evidence supporting the benefits of antioxidants, varicocelectomy, and antibiotics for treating genital tract infections. They also recommended frequent ejaculation as a treatment strategy for men with persistently elevated SDF levels.

ICSI for men with persistent elevated SDF levels, while also suggesting sperm selection techniques as a less invasive method to improve SDF levels in cases where ICSI fails. They highlighted the lack of validation for testicular sperm testing, insufficient evidence on the use of testicular-derived sperm in ICSI, and the absence of consensus regarding its application in ICSI. They emphasized the necessity for randomized controlled trials to substantiate surgical interventions for men with elevated SDF. The combined recommendations from the guidelines, serving as a practical guide for optimal clinical practice.

CONCLUSION

Sperm DNA integrity is influenced by environmental and dietary factors. Lifestyle adjustments, management of reactive oxygen species (ROS), oral antioxidant therapy, varicocele repair, and the selection of sperm for ART can all contribute to improving fertility outcomes. Testicular sperm generally exhibits lower DNA damage and better DNA integrity compared to ejaculated sperm, which translates to improved reproductive outcomes even after antioxidant therapy. The guidelines provide comprehensive insights and recommendations on SDF testing, with Esteves et al. focusing on technical aspects and offering numerous recommendations, while Agarwal et al. concentrate on treatment strategies and present a management algorithm. The guidelines are comprehensive and accessible, providing valuable insights into SDF and complementing each other effectively.

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