International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 844-848 www.ijprajournal.com ISSN: 2456-4494

A Comprehensive Approach and Critical Evaluation of Clinical Practice Guidelines for Sperm Dna Fragmentation

Dr. Lokesh. K, Dr. Borus Purushothaman, Dr. Harini. V, Veerammal, Dr. Suman Sharma
Dr. Borus Andro Lan And Reasearch Center, Chennai

Date of Submission: 05-05-2024 Date of Acceptance: 15-05-2024

ABSTRACT: Sperm DNA fragmentation (SDF) is linked to male infertility and reproductive issues. SDF testing is recommended for individuals with adaptable lifestyles with risk factors for infertility, and those with recurrent pregnancy loss, grade 1 varicocele, infertile couples with RPL or IUI, unexplained infertility, repeated failure in ART procedures, patients with abnormal or normal semen parameters. Guidelines are needed to aid clinicians in using SDF for male fertility evaluation. Two recent guidelines, Agarwal et al and Esteves et al, have been evaluated and compared. Guidelines have similar recommendations but also highlight differences. The best practice recommendations from guidelines have been combined for a comprehensive understanding of SDF in male fertility.

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

I. INTRODUCTION

Sperm DNA fragmentation (SDF) refers to single-stranded or double-stranded breaks in the genome of spermatozoa, which can negatively impact male fertility and reproductive outcomes. Three primary mechanisms can lead to SDF: abortive apoptosis, defective chromatin maturation, and oxidative stress [2]. Damage to sperm DNA can occur within the testes, during passage along the reproductive ducts, after ejaculation during sperm processing, or during cryopreservation [1]. Infertility is a global concern, with DNA fragmentation being a key factor contributing to the disease. Infertile men have higher levels of DNA fragmentation than fertile men, making it crucial to examine sperm count and motility [3]. Newer diagnostic techniques, such as DNA fragmentation testing, are essential in addressing infertility, as ignoring this can lead to ineffective medical approaches and mismanagement of infertility.

High SDF levels are associated with a significantly increased risk of recurrent pregnancy loss (RPL) [3,4], lower pregnancy rates, and increased miscarriage rates for in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) [6,7,15]. Several conditions, disorders, and exposures in men have been associated with SDF [8], including varicocele, male genital tract infection, advanced age, smoking, obesity, radiation, and environmental toxin exposures [11]. Shorter ejaculatory abstinence time has also been reported to lessen SDF levels [10,12]. Clinical trials that have studied the influence of antioxidants on sperm DNA have also reported improvement in the amount of SDF [13,14]. DNA fragmentation index (DFI) has been reported to decrease by more than 5% after varicocelectomy [9].Tests that measure SDF include terminal deoxyNucleotidyl transferase dUTP nick end labelling (TUNEL) assay, sperm chromatin dispersion (SCD), sperm chromatin structure assay (SCSA), and comet assay. These tests have been used to study SDF within the context of assisted reproductive technologies (ART) [5,17]. Majority of the articles published on the use of testicular sperm in non – azoospermic men with high SDF for ICSI consist of small cohorts r case seies, without adequate control groups or reporting live birth [16].

There is an increasing number of studies and reports on the deleterious impact of SDF on male fertility and reproductive outcomes. These studies also review the various factors that increase or decrease SDF and can influence reproduction. Given the various aspects relating to SDF that have been studied, there is vast potential for implementation into clinical practice. It is important to have clinical practice guidelines that help direct physicians and reproductive specialists towards the use of SDF testing, including which assays to use, indications for testing, and strategies to reduce SDF. Leading scientists in the field of andrology have recently formulated and published two new guidelines on SDF with recommendations based on high-quality reports and metaanalyses.

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 844-848 www.ijprajournal.com ISSN: 2456-4494

This article aims to compare and contrast guidelines and summarize and unify them to provide a complete guide for clinicians regarding the use of SDF testing in their practice.

MERITS

Sperm DNA fragmentation testing is a valuable tool for determining the likelihood of conceiving naturally in male patients. It evaluates the integrity of the DNA package, determines the degree of DNA damage, and measures single/double stranded breaks. This method helps identify patients who would benefit from varicocelectomy, predict subsequent ART cycles, and select sperm with inappropriate DNA for initiating ART procedures. Compared to standard semen analysis, SDF tests are exclusive and have greater significance. The American Urological Association, American Society of Reproductive Medicine, and European Association of Urology have accepted that sperm DNA fragmentation contributes to male infertility, making SDF testing essential for identifying male infertility.

GUIDELINE AND RECOMMENDATION.

Agarwal et al [18]. provided a summary of their recommendations and a clinical algorithm for using SDF testing in infertile couple evaluation. They suggested six indications for SDF testing and seven management strategies. They graded each recommendation using the Oxford Centre for Evidence-Based Medicine (OCEBM) grades. Esteves et al. provided two tables with 41 recommendations, 13 relating to technical aspects and 28 relating to indications. They graded each recommendation using OCEBM grades and gave a strength rating based on expert judgement. Each recommendation was either strong (applicable to most individuals) or conditional (different choices might be appropriate for the situation). guidelines used metaanalyses and high-quality articles to recommend SDF testing and treatment strategies. Agarwal et al [18] summarized studies correlated with clinical conditions and SDF, recommending indications for testing and treatment strategies. Esteves et al [19] provided statements summarizing the evidence and supporting studies, presenting it in two tables for technical aspects and clinical indications, respectively. guidelines rated each study based on OCEBM levels of evidence.

II. DISCUSSION

Male factor (SDF) testing is crucial for assessing male reproductive potential and influencing reproductive outcomes. It can be implemented for investigative or predictive purposes and can lead to targeted management strategies. However, many ART centers neglect fertility evaluation of men with normozoospermia or those with available spermatozoa for ICSI, leading to multiple failed ART cycles. Prompt assessment of the male partner, including SDF levels, can help identify underlying pathological factors and direct treatment paths, reducing the cost and burden of unnecessary interventions or repeated failed ART.

The society for Translational Medicine (STM) advocated for SDF testing in 2017, discussing indications, recommendations, and tests for SDF and management strategies [20]. However, other international societies have not provided clear guidelines regarding its implementation, particularly regarding tests used or conditions for testing. The European Society of Human Reproduction and Embryology (ESHRE) discussed SDF testing as a means to explain Reproductive Polymorphism (RPL) [21], the European Academy of Andrology (EAA) suggested adding SDF testing to initial basic semen analysis in men with oligoasthenoteratozoospermia considered for ART [22],the European association of urology (EAU) recommend SDF test only for men with unexplained infertility or after RPL [23] and the American Urological Association (AUA) and American Society for Reproductive Medicine (ASRM) published a guideline on male infertility, recommending against SDF testing in initial evaluation of fertility but advocating its use and importance in couples experiencing RPL [24].

The two new guidelines offer a uni”ue perspective on SDF testing, discussing how to test for it, when to test, and how to treat. They expand the indications and role of SDF testing beyond 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 TUNEL assay, Comet assay, SCSA, and SCD assay as the four valid tests for Serum Deposition Factor (SDF) in ART. They cite a metaanalysis by Santi et al.,[25]. which states that a 20% cut-off value for SDF can distinguish fertile from infertile men. Agarwal et al [18]. also provided a table summarizing studies with published cut-off values for SDF tests in various settings and for different reproductive outcomes. They also discussed the role of

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 844-848 www.ijprajournal.com ISSN: 2456-4494

measuring oxidation reduction potential as a marker of oxidative stress in increasing the diagnostic value of SDF tests for ART, but did not recommend it in lieu of these tests. Esteves et al. provided more extensive evidence and technical recommendations on the use of SDF tests. They discussed factors affecting SDF levels during testing, such as abstinence length, time between ejaculation/thawing and testing, cryomedia and freezing technique, and sperm processing techniques. They recommended testing after 2-5 days of abstinence, fixed abstinence to monitor intervention effects, and SDF testing within 30-60 minutes after liquefaction of neat semen and immediately after thawing if frozen. They concluded that thresholds of 20%-30% are associated with adverse pregnancy outcomes, but acknowledged 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 abnormal and correlates with poor outcomes for natural conception and assisted techniques.

Guidelines recommend sperm density factor (SDF) testing for various situations, including pregnancy outcomes, patient conditions, and factors contributing to infertility. They review the adverse impact of SDF on natural pregnancy and assisted reproductive technology (ART) outcomes, providing specific recommendations for testing in IUI or IVF failure and recurrent miscarriage after ICSI. Esteves et al [19] also recommend testing for SDF before initiating ART after ART failure. guidelines discuss and recommend SDF testing for clinical varicocele, idiopathic male infertility, unexplained male infertility, and recurrent sperm loss (RPL), and review the adverse impact of lifestyle and exposure risk factors. Esteves et al [19] also include sperm cryopreservation as an indication for SDF testing, as freezing can adversely impact sperm due to 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 pants, staying away from hot environments, and refraining from ejaculation for the appropriate amount of time, are recommended for men with poor sperm quality. Reactive oxygen species (ROS) can damage sperm DNA due to infections of the male genital organ. For 2-12 weeks, patients can receive medicine to reduce the quantity of reactive oxygen species their white blood cells create, 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 decreased after antioxidant treatment, indicating that ROS were responsible for some of the deterioration.

Varicocele repair, which is the main component of the pathogenesis of varicocele, has been shown to reduce infertility caused by oxidative stress and strengthen the nuclear DNA of the sperm. A meta-analysis of six studies with 177 patients found that varicocelectomy enhances sperm DNA integrity.

Isolating sperm with little DNA damage for ART is a desirable approach, as it helps decrease adverse ICSI reproductive outcomes caused by sperm DNA damage. Testicular sperm tends to have less DNA damage and better DNA integrity than ejaculated sperm. A recent prospective comparative study involving 172 patients with elevated SDF found that SDF levels in testicular sperm were five times lower than in ejaculated sperm. Even after oral antioxidant therapy, using testicular sperm for ICSI was associated with better reproductive outcomes, with a birth rate of 46.7% compared to 26.4% for the ICSI group using ejaculated sperm.

Esteves et al [19] discussed management strategies for men with elevated SDF, including treatment of underlying factors, lifestyle advice, ICSI if SDF levels remain elevated, and testicular sperm if failed ICSI. They stressed the importance of a comprehensive evaluation by a specialist if abnormal SDF levels are detected. Agarwal et al [18] provided a section for management strategies, citing evidence of the benefits of anti-oxidant use, varicocelectomy, and antibiotics in treating genital tract infections. They also recommended recurrent ejaculation as a treatment strategy for men with persistent elevatedSDF.

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 844-848 www.ijprajournal.com ISSN: 2456-4494

Agarwal et al [18] advocated for ICSI for men with persistent elevated SDF, but also recommended sperm selection techniques for ICSI failure as a less invasive method for improving SDF levels. They cited the lack of validation for testicular sperm testing, poor evidence on the use of testicular derived sperm in ICSI, and a lack of consensus on its use in ICSI. They stressed the need for randomized controlled trials to justify a surgical approach for men with elevated SDF. The combined recommendations from guidelines are summarized in Table 3, which can be used as a guide for best practice.

III. CONCLUSION

Sperm DNA integrity is influenced by environmental and dietary factors. Lifestyle adjustments, reactive oxygen species (ROS), oral antioxidant therapy, varicocele repair, and sperm isolation for ART can improve fertility. Testicular sperm has less DNA damage and better DNA integrity than ejaculated sperm, leading to better reproductive outcomes, even after antioxidant therapy. guidelines offer extensive knowledge and recommendations on SDF testing, with Esteves et al [19] evaluating technical aspects and providing numerous recommendations. Agarwal et al [18] focus on treatment strategies and offer an algorithm for management approaches. guidelines are comprehensive and accessible to low readers, providing ample insight into the topic of SDF and complementing each other.

REFERENCES

[1]. González-Marín C, Gosálvez J, Roy R. Types, causes, detection and repair of DNA fragmentation in animal and human sperm cells. Int J Mol Sci 2012;13:14026-52.

[2]. Muratori M, Marchiani S, Tamburrino L, Baldi E. Sperm DNA fragmentation: mechanisms of origin. Adv Exp Med Biol 2019;1166:75-85.

[3]. Evenson DP, Jost LK, Marshall D, Zinaman MJ, Clegg E, Purvis K, et al. Utility of the sperm chromatin structure assay as a diagnostic and prognostic tool in the human fertility clinic. Hum Reprod 1999;14:1039-49.

[4]. McQueen DB, Zhang J, Robins JC. Sperm DNA fragmentation and recurrent pregnancy loss: a systematic review and meta-analysis. Fertil Steril 2019;112:5460.e3.

[5]. Chen Q, Zhao JY, Xue X, Zhu GX. The association between sperm DNA fragmentation and reproductive outcomes following intrauterine insemination, a meta analysis. ReprodToxicol 2019;86:50-5

[6]. Zhao J, Zhang Q, Wang Y, Li Y. Whether sperm deoxyribonucleic acid fragmentation has an effect on pregnancy and miscarriage after in vitro fertilization/intracytoplasmic sperm injection: a systematic review and metaanalysis. Fertil Steril 2014;102:998-1005

[7]. Zini A. Are sperm chromatin and DNA defects relevant in the clinic? SystBiolReprod Med 2011;57:78-85.

[8]. Tanaka T, Kobori Y, Terai K, Inoue Y, Osaka A, Yoshikawa N, et al. Seminal oxidation-reduction potential and sperm DNA fragmentation index increase among infertile men with varicocele. Hum Fertil (Camb) 2020.

[9]. Qiu D, Shi Q, Pan L. Efficacy of varicocelectomy for sperm DNA integrity improvement: a meta-analysis. Andrologia 2021.

[10]. Gallegos G, Ramos B, Santiso R, Goyanes V, Gosálvez J, Fernández JL. Sperm DNA fragmentation in infertile men with genitourinary infection by Chlamydia trachomatis and Mycoplasma. Fertil Steril 2008;90:328-34.

[11]. Panner Selvam MK, Ambar RF, Agarwal A, Henkel R. Etiologies of sperm DNA damage and its impact on male infertility. Andrologia 2021.

[12]. Agarwal A, Gupta S, Du Plessis S, Sharma R, Esteves SC, Cirenza C, et al. Abstinence time and its impact on basic and advanced semen parameters. Urology 2016;94:102-10.

[13]. Greco E, Iacobelli M, Rienzi L, Ubaldi F, Ferrero S, Tesarik J. Reduction of the incidence of sperm DNA fragmentation by oral antioxidant treatment. J Androl 2005;26:349-53.

[14]. Jannatifar R, Parivar K, Roodbari NH, Nasr-Esfahani MH. Effects of N-acetylcysteine supplementation on sperm quality, chromatin integrity and level of oxidative stress in infertile men. ReprodBiol Endocrinol 2019;17:24.

International Journal of Pharmaceutical Research and Applications
Volume 9, Issue 3 May-June 2024, pp: 844-848 www.ijprajournal.com ISSN: 2456-4494

[15]. Esteves SC, Roque M, Bradley CK, Garrido N. Reproductive outcomes of testicular versus ejaculated sperm for intracytoplasmic sperm injection among men with high levels of DNA fragmentation in semen: systematic review and meta-analysis. Fertil Steril 2017;108:456-67

[16]. Ambar RF, Agarwal A, Majzoub A, Vij S, Tadros NN, Cho CL, et al. The use of testicular sperm for intracytoplasmic sperm injection in patients with high sperm DNA damage: a systematic review. World J Mens Health 2020.

[17]. Dutta S, Henkel R, Agarwal A. Comparative analysis of tests used to assess sperm chromatin integrity and DNA fragmentation. Andrologia 2021.

[18]. Agarwal A, Majzoub A, Baskaran S, Panner Selvam MK, Cho CL, Henkel R, et al. Sperm DNA fragmentation: a new guideline for clinicians. World J Mens Health 2020;38:412-71

[19]. Esteves SC, Zini A, Coward RM, Evenson DP, Gosálvez J, Lewis SEM, et al. Sperm DNA fragmentation testing: summary evidence and clinical practice recommendations. An drologia 2021

[20]. Agarwal A, Cho CL, Majzoub A, Esteves SC. The Society for Translational Medicine: clinical practice guidelines for sperm DNA fragmentation testing in male infertility. TranslAndrolUrol 2017.

[21]. ESHRE Guideline Group on RPL, Bender Atik R, Christiansen OB, Elson J, Kolte AM, Lewis S, et al. ESHRE guideline: recurrent pregnancy loss. Hum Reprod Open 2018.

[22]. Colpi GM, Francavilla S, Haidl G, Link K, Behre HM, Goulis DG, et al. European Academy of Andrology guideline management of oligo-astheno-teratozoospermia. Andrology 2018.

[23]. Tharakan T, Bettocchi C, Carvalho J, Corona G, Jones TH, Kadioglu A, et al.; EAU Working Panel on Male Sexual Reproductive Health. European Association of Urology guidelines panel on male sexual and reproductive health: a clinical consultation guide on the indications for performing sperm DNA fragmentation testing in men with infertility and testicular sperm extraction in nonazoospermic men. EurUrol Focus 2021.

[24]. Schlegel PN, Sigman M, Collura B, De Jonge CJ, Eisenberg ML, Lamb DJ, et al. Diagnosis and treatment of infertility in men: AUA/ASRM guideline,American Urological Association; 2020.

[25]. Santi D, Spaggiari G, Simoni M. Sperm DNA fragmentation index as a promising predictive tool for male infertility diagnosis and treatment management - metaanalyses. Reprod Biomed Online 2018;37:315-26.