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Sperm DNA Fragmentation: A Critical Assessment of Clinical Practice Guidelines

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Sperm DNA fragmentation (SDF) is implicated in male infertility and adverse reproductive outcomes. With the publication of many studies regarding the etiologies and contributors to SDF, as well as the effects of SDF, guidelines are necessary to aid clinicians in the application of SDF for male fertility evaluation. Two recent clinical practice guidelines were published by Agarwal et al and Esteves et al. In this article, we have evaluated and compared both guidelines. We have found fairly similar recommendations between the two guidelines and have also highlighted the differences between them. Finally, we have summarized and combined the best practice recommendations from both guidelines.

Keywords: DNA fragmentation; Male infertility; Oxidative stress; Practice guidelines as topic; Reproductive techniques, assisted

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INTRODUCTION

Sperm DNA fragmentation (SDF) refers to single-stranded or double-stranded breaks in the genome of spermatozoa. Because the mature male gamete lacks the ability to repair DNA damage [1], these breaks tend to persist and can negatively influence male reproductive potential and outcomes.

Three primary mechanisms can lead to SDF: (1) abortive apoptosis, where spermatozoa destined for apoptosis fail to complete the process and are released with fragmented DNA due to the action of endonucleases; (2) defective chromatin maturation, where during the normal process of sperm chromatin compaction, DNA nicks are not repaired, leading to persistent breaks as well as less compact DNA that is more susceptible to damage by exogenous factors; and (3) oxidative stress, where reactive oxygen species can directly induce DNA breaks in the testes or as spermatozoa move along the male reproductive tract [2]. Damage to the sperm DNA can occur within the testes, during passage along the reproductive ducts, after ejaculation during sperm processing, or during cryopreservation.

SDF can have an adverse impact on male fertility and reproductive outcome. In fact, it was reported that men with higher SDF levels were less likely to conceive naturally [3]. Elevated SDF is also associated with a significantly increased risk of recurrent pregnancy loss (RPL) [4]. Furthermore, SDF levels can affect the outcomes of assisted reproductive technologies (ART). High SDF values have been found to negatively impact pregnancy and delivery rates after intrauterine insemination (IUI) [5]. SDF has also been associated with lower pregnancy rates and increased miscarriage rates for *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) [6,7].

Several conditions, disorders, and exposures in men have been associated with SDF. SDF is found to be significantly higher among infertile men with varicocele [8] and DNA fragmentation index (DFI) has been reported to decrease by more than 5% after varicocelectomy [9]. Male genital tract infection is also associated with elevated SDF and treatment with antibiotics can lead to reduction of SDF levels [10]. Advanced age, smoking, obesity, radiation and environmental toxin exposures have also been linked to increased sperm DNA damage [11]. On the other hand, shorter ejaculatory abstinence time has been reported to lessen SDF levels [12]. Clinical trials that have studied the influence of antioxidants on sperm DNA and have also reported improvement in the amount of SDF [13,14]. Finally, testicular sperm appears to have less SDF compared to ejaculated sperm, and therefore, some studies advocate the use of testicular sperm with better outcomes including clinical pregnancy rates and reduced miscarriage rates [15]. Theoretically, testicular sperm is less exposed to epididymal and external oxidative stress and can therefore be used as a last resort after failure of less invasive methods. However, the majority of articles published on the use of testicular sperm in non-azoospermic men with high SDF for ICSI consist of small cohorts or case series, without adequate control groups, or reporting of live birth rates [16].

Tests that measure SDF include: (1) terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) assay, which adds a labelled nucleotide to sites of DNA nicks and later assess extent of fluorescence; (2) sperm chromatin dispersion (SCD), which relies on formation of a halo by intact DNA around the nucleus after denaturation and a smaller or absent halo with fragmented DNA; (3) sperm chromatin structure assay (SCSA), which uses Acridine orange (AO) and measures the DFI, defined as the ratio between red fluorescence (AO bound to single stranded DNA at sites of breaks) and green fluorescence (AO bound to double stranded intact DNA); and (4) comet assay, which is a single cell electrophoresis in which fragmented DNA forms a tail while intact DNA remains in the comet head [17]. All of these assays have been used to study SDF within the context of ART.

There has been 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 hence 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 therefore 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 meta-analyses. Agarwal et al [18] published

their guideline in "The World Journal of Men's Health" in August of 2020, while Esteves et al [19] published theirs in "Andrologia" in October of 2020.

In this article, we aim to compare and contrast both guidelines that have been recently published, and to summarize and unify them in order to provide a complete guide for clinicians regarding the use of SDF testing in their practice.

METHODOLOGY

We critically reviewed the guidelines by Agarwal et al [18] and Esteves et al [19] and evaluated the following aspects:

1) The recommendations made by each guideline.
2) The grading given to each recommendation.
3) The evidence upon which the recommendations were made.

We then compared the recommendations of both guidelines, extracting the similarities and highlighting the differences between them.

RESULTS

1. Recommendations made by each guideline

Agarwal et al [18] provided a text summary of their recommendations and produced a clinical algorithm regarding the application of SDF testing in the evaluation of an infertile couple. They recommend six indications for SDF testing and offered seven management strategies. They then graded each recommendation using the Oxford Centre for Evidence-Based Medicine (OCEBM) grades of recommendation. The recommendations by Agarwal et al [18], together with the grading given to each, are summarized in Table 1.

Esteves et al [19], on the other hand, provided 2 tables (Tables 4 & 5 in their guideline) that list their recommendations. They gave a total of 41 recommendations, 13 relating to the technical aspects of SDF testing and 28 relating to the indications of SDF testing. They graded each recommendation using the OCEBM grades and also gave another strength rating based on expert judgement; with each recommendation being either strong (the recommendation should be applied to most individuals in the situation) or conditional (different choices might be appropriate for the situation).

Table 1. Recommendations by Agarwal et al (2020) [18]

The following men should undergo SDF testing
1. Men with unexplained or idiopathic male infertility (Grade C)
2. Couples experiencing recurrent pregnancy loss (Grade C)
3. Men with modifiable lifestyle risk factors (Grade C)
4. Men with clinical varicocele (Grade C)
5. Infertile couples prior to initiating or after failure of IUI or IVF (Grade C)
6. Couples with recurrent miscarriage following ICSI (Grades B-C)
The following treatment approaches can lower SDF (Grade C)
1. Oral antioxidant therapy
2. Lifestyle modification, including diet modification and weight loss
3. Recurrent ejaculation
4. Control of infection and inflammation
Men with varicocele and high SDF should undergo varicocelectomy (Grades B-C)
Patients with persistently high SDF should be directed towards ICSI (Grade C)
Sperm processing and preparation or testicular sperm can be used for ICSI with recurrent miscarriage and high SDF (Grades B-C)

SDF: sperm DNA fragmentation, IUI: intrauterine insemination, IVF: *in vitro* fertilization, ICSI: intracytoplasmic sperm injection.

2. Evidence used to make recommendations

Both guidelines used meta-analyses and high quality articles to base their recommendations upon. They only differed in how they presented this evidence. Agarwal et al [18] summarized studies that correlated clinical conditions and SDF (Table 2 in their guideline) and used this to recommend indications for SDF testing. They also summarized studies that correlated interventions and SDF (Table 3 in their guideline) and used this to recommend treatment strategies for SDF. In their tables, they gave each study a rating based on OCEBM levels of evidence. Esteves et al [19] provided statements that summarized the evidence and provided the studies that back each statement. They presented the evidence in two tables (Tables 2 & 3 in their guideline) pertaining to technical aspects and clinical indications respectively.

3. Comparison of the guidelines

Table 2 offers a comparison between recommendations in both guidelines.

DISCUSSION

SDF is an important factor that can influence male reproductive potential and affect reproductive outcomes. The implementation of SDF testing in clinical practice can be done for investigative or predictive

Table 2. Comparison of recommendations by both guidelines

	Agarwal et al (2020) [18]	Esteves et al (2020) [19]
Technical aspects regarding SDF testing		
Assays used to measure SDF are: TUNEL, SCSA, SCD, and Comet	The four assays were discussed as those used for SDF testing, along with pros, cons, and estimated cost No explicit recommendations	The four assays were described and any of the four is recommended to test for SDF (Grade B)
Conditions for testing	No recommendation	Recommendations regarding abstinence length and dealing with frozen specimens were made (various grades)
Thresholds for discriminating fertile and infertile men	Cut-off of 20% described in text No explicit recommendations	Cut-off of 20% (SCSA, TUNEL, SCD) and 26% (Comet) (Grade B)
Thresholds for predicting reproductive outcomes	Various cut-off values in different conditions summarized in a table No explicit recommendations	Thresholds exceeding 20-30% indicate a higher likelihood of adverse reproductive outcomes (Grade B)
Indications for SDF testing		
UMI & IMI	Recommended (Grade C)	Recommended (Grades B-C)
RPL	Recommended (Grade C)	Recommended (Grades B-C)
IUI	Recommended (Grade C) (before or after failure)	Recommended (Grades B-C) (before or after failure)
IVF	Recommended (Grade C) (before or after failure)	Recommended (Grades B-C) (before or after failure)
ICSI	Recommended (Grades B-C) (only after failure due to recurrent miscarriage)	Recommended (Grades B-C) (before and after failure)
Clinical varicocele	Recommended (Grade C)	Recommended (Grade C)
Risk factors	Recommended (Grade C)	Recommended (Grade C)
Sperm cryopreservation	No recommendation	Recommended (Grade D)
Management of SDF		
Antioxidant use	Recommended (Grade C)	No recommendation
Recurrent ejaculation	Recommended (Grade C)	No recommendation
Control of inflammation	Recommended (Grade C)	No recommendation
Lifestyle modification	Recommended (Grade C)	Recommended within the context of testing (Grade C)
Varicocelectomy	Recommended (Grades B-C)	Discussed within the context of testing No explicit recommendation
ICSI if persistently high SDF	Recommended (Grade C)	Recommended (Grades B-D) - grades varied depending on context of elevated SDF (UMI/IMI/RPL or IUI or IVF or risk factors)
Alternative Method of Sperm Selection after failed ICSI	Recommended (Grades B-C)	No recommendation
Testicular Sperm after failed ICSI	Recommended (Grades B-C)	Recommended (Grade B)

Grades for recommendations are based on Oxford Centre for Evidence-Based Medicine (OCEBM) grades of recommendation.
SDF: sperm DNA fragmentation, TUNEL: terminal deoxynucleotidyl transferase dUTP nick end labelling, SCSA: sperm chromatin structure assay, SCD; sperm chromatin dispersion, UMI: unexplained male infertility, IMI: idiopathic male infertility, RPL: recurrent pregnancy loss, IUI: intrauterine insemination, IVF: *in vitro* fertilization, ICSI: intracytoplasmic sperm injection.

purposes and can also allow targeted management strategies. Unfortunately, it is common practice in many ART centers to neglect fertility evaluation of men with normozoospermia or those who have spermatozoa available for ICSI. This can lead to several failed ART cycles, before referral to a urologist or andrologist is done for male factor evaluation. Prompt assessment of the male partner, including the level of SDF may allow early identification of underlying pathological factors and can direct towards targeted treatment paths, reducing the cost and burden of unnecessary interventions or repeated failed ART.

Table 3. Summary of the guidelines on the clinical use of SDF testing

Testing for SDF
• **Any of the following assays can be used to provide valid and reliable information on SDF levels: TUNEL, Comet, SCSA, and SCD**
• **A cut-off level of 20% can be used to distinguish fertile from infertile men**
• Cut-off values for prediction of various pregnancy outcomes differ according to various factors, but adverse outcomes are generally associated with levels above 20%–30%
• Testing for SDF should be done after 2–5 days of ejaculatory abstinence
• SDF tests should be performed within 30–60 minutes after liquefaction or immediately after thawing
Indications for SDF testing
• **Unexplained or idiopathic male infertility**
• **Recurrent pregnancy loss**
• **Clinical varicocele**
• **Lifestyle risk factors**
• **Before or after failure of ART–IUI, IVF**, ICSI
• Recurrent pregnancy loss after ICSI
• Sperm freezing
Management of SDF
• **Lifestyle advice and modification**
• Use of antioxidants
• Recurrent ejaculation
• **Treatment of underlying conditions** – varicocelectomy, antibiotics
• **Use of ICSI if SDF persistently elevated**
• Another method for sperm selection after failed ICSI
• **Testicular sperm for failed ICSI**

Items in bold are common to both guidelines.
SDF: sperm DNA fragmentation, TUNEL: terminal deoxynucleotidyl transferase dUTP nick end labelling, SCSA: sperm chromatin structure assay, SCD: sperm chromatin dispersion, ART: assisted reproductive technologies, IUI: intrauterine insemination, IVF: *in vitro* fertilization, ICSI: intracytoplasmic sperm injection.

The first society to advocate SDF testing were The Society for Translational Medicine (STM) in 2017, who have discussed the indications for SDF testing and provided recommendations, and also examined the tests for SDF and management strategies [20]. Although the discussions and recommendations put forward by this guideline are fairly similar to the new guidelines, it served as the base upon which recommendations for SDF testing were further elaborated and expanded, such that the two new recent guidelines were able to make more solid recommendations based on robust evidence that has emerged since the publication of STM guideline.

Other international societies have mentioned and recommended SDF testing, but do not provide clear guidelines regarding its implementation, particularly with respect to tests used or conditions for testing. The European Society of Human Reproduction and Embryology (ESHRE) discussed the role of SDF testing as a means to explain RPL [21]. The European Academy of Andrology (EAA) suggest adding SDF testing to initial basic semen analysis in men with oligoasthenoteratozoospermia who are considered for ART [22]. The European Association of Urology (EAU) recommend SDF testing only for men with unexplained infertility or after RPL [23]. Recently, the American Urological Association (AUA) and American Society for Reproductive Medicine (ASRM) published a guideline on male infertility and they recommend against SDF testing in initial evaluation of fertility, but advocate its use and importance in couples experiencing RPL [24].

Therefore, the two new guidelines offer a unique point of view with respect to SDF testing as they discuss how to test for it, when to test, and how to treat. They expand the indications and role of SDF testing beyond those in the aforementioned international society guidelines and offer clinicians and specialists considerable insight into the use of SDF and approach to particular patients.

1. Testing for sperm DNA fragmentation

Both guidelines recommend TUNEL assay, Comet assay, SCSA, and SCD assay as the four valid tests for SDF. They both also cite a meta-analysis 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. Moreover, they discussed the role of 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 [19], on the other hand, provided more extensive evidence and technical recommendations on the use of SDF tests. They discussed that results obtained by the four recommended tests are reported differently and may not necessarily yield similar results but have been well-correlated to each other with good correlation within and among laboratories. They also discussed factors that affect the levels of SDF measured during testing and these include: (1) abstinence length; (2) time between ejaculation/thawing and testing; (3) type of cryomedia and freezing technique

influencing post-thaw SDF levels; and (4) sperm processing techniques. They provided recommendations to control for these factors: (1) testing after 2–5 days of abstinence; (2) fixed abstinence to monitor effect of any intervention; and (3) SDF testing should be done within 30–60 minutes after liquefaction of neat semen and immediately after thawing if frozen.

They further discussed the predictive potential of SDF in ART and concluded that thresholds of 20%–30% are associated with adverse pregnancy outcomes whether natural or assisted, but also acknowledged that this prediction is not absolute. They went on to discuss factors that influence the predictive power of these tests, including: (1) type, site, and extent of DNA damage; (2) number of cells affected; (3) oocyte's ability to repair SDF; (4) female age in predicting pregnancy with IVF/ICSI; and (5) semen processing.

Both guidelines provided tables comparing the four SDF tests. Esteves et al provided a more extensive explanation on the principle of each assay, cut-off values, and the specimen requirements for each, while Agarwal et al provided pros, cons, and estimated cost of each.

2. Indications for sperm DNA fragmentation testing

In general, both guidelines listed similar conditions in which SDF testing may be warranted. Agarwal et al summarized extensive data from the literature in a table linking SDF to various situations, whether pregnancy outcomes for both natural and assisted reproduction or patient conditions and factors that might contribute to infertility. They then reviewed the adverse impact of SDF on natural pregnancy and ART outcomes and provided specific recommendations for SDF testing in IUI or IVF failure and recurrent miscarriage after ICSI. Esteves et al also extensively reviewed the impact of SDF on ART and recommended testing for SDF be done before initiating ART (IUI, IVF, or ICSI), after ART failure.

Both guidelines discussed and recommended testing for SDF in clinical varicocele as a means to guide surgery recommendation. Esteves et al also recommended against testing in subclinical varicocele. Both guidelines recommended SDF testing for idiopathic male infertility (IMI), unexplained male infertility (UMI), and RPL. They also both reviewed the adverse impact of lifestyle and exposure risk factors.

Esteves et al also included sperm cryopreservation as an indication for SDF testing, providing evidence that freezing can adversely impact sperm due to increased oxidative stress. They endorse SDF testing in this scenario as a means to optimize the freezing method used and to steer towards a particular ART method.

3. Management of sperm DNA fragmentation

Esteves et al discussed evidence regarding management within the context of indications for testing and proposed management strategies for the condition being discussed. These include: treatment of underlying factors, lifestyle advice, ICSI if SDF levels remain elevated, and testicular sperm if failed ICSI. They also stressed on the importance of a comprehensive evaluation by a specialist, should abnormal SDF levels be detected.

Agarwal et al, however, dedicated a section for management strategies and provided another table summarizing evidence regarding each strategy. They cited level 1 evidence regarding the benefit of anti-oxidant use. They provided extensive evidence regarding the benefit of varicocelectomy and discussed the role of antibiotics in treating genital tract infections.

While Esteves et al evaluated length of abstinence and its impact of SDF levels as a factor to consider when testing, Agarwal et al have recommended it as a treatment strategy, endorsing recurrent ejaculation as a means to reduce SDF.

Like Esteves et al, Agarwal et al also advocate that men with persistent elevated SDF should be directed towards ICSI and that testicular sperm can be used in ICSI failure. However, Agarwal et al also provided evidence and recommended use of sperm selection techniques for ICSI failure as a less invasive method for improving SDF levels. Their reasoning is based on the fact that: (1) SDF testing has not been validated for testicular sperm, making interpretation of testicular SDF levels difficult; (2) even though a few studies report that the use of testicular sperm has a positive impact on ART outcomes, the quality of evidence is poor; and finally (3) there is a lack of consensus on the use of testicular derived sperm in ICSI. Furthermore, different practices based on clinical judgement are employed for using testicular sperm as there are no clear-cut indications for its use in non-azoospermic men, therefore it is important to stress the need for randomized controlled trials to justify a surgical approach for men with elevated SDF.

Finally, we have combined and summarized the recommendations from both guidelines in Table 3 which can be used as a guide for best practice.

CONCLUSIONS

In conclusion, both guidelines provide extensive evidence and insight regarding SDF testing and recommend testing for similar situations. Esteves et al widely evaluate the technical aspects of SDF testing and provide many recommendations in that regard. While Agarwal et al focus more on treatment strategies and provide an algorithm regarding possible management approaches. Both guidelines are comprehensive and allow readers to gain ample insight into the topic of SDF and are in fact complementary to each other.

Conflict of Interest

The authors have nothing to disclose.

Author Contribution

Conceptualization: AA. Writing – original draft: all the authors. Writing – review & editing: all the authors.

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