A Strengths-Weaknesses-Opportunities-Threats (SWOT) analysis on the clinical utility of sperm DNA fragmentation testing in specific male infertility scenarios

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Background: Sperm DNA fragmentation (SDF) is recognized as a leading cause of male infertility because it can impair the paternal genome through distinct pathophysiological mechanisms. Current evidence supports SDF as a major factor in the pathophysiology of several conditions, including varicocele, unexplained infertility, assisted reproductive technology failure, and environmental lifestyle factors, although the mechanisms involved have not been fully described yet. Measurement of the levels of DNA fragmentation in semen provides valuable information on the integrity of paternal chromatin and may guide therapeutic strategies. A recently published clinical practice guideline (CPG) highlighted how to use the information provided by SDF testing in daily practice, which triggered a series of commentaries by leading infertility experts. These commentaries contained an abundance of information and conflicting views about the clinical utility of SDF testing, which underline the complex nature of SDF.

Methods: A search of papers published in response to the CPG entitled "Clinical utility of sperm DNA fragmentation testing: practice recommendations based on clinical scenarios" was performed within the *Translational Andrology and Urology* (*TAU*) website (http://tau.amegroups.com/). The start and end dates for the search were May 2017 and August 2017, respectively. Each commentary meeting our inclusion criteria was rated as "supportive without reservation", "supportive with reservation", "not supportive" or "neutral". We recorded whether articles discussed either SDF characteristics as a laboratory test method or clinical scenarios, or both. Subsequently, we extracted the particulars from each commentary and utilized the 'Strengths-Weaknesses-Opportunities-Threats' (SWOT) analysis to understand the perceived advantages and drawbacks of SDF as a specialized sperm function method in clinical practice.

Results: Fifty-eight fertility experts from six continents and twenty-two countries contributed commentaries. Overall, participants (87.9%; n=51) were supportive of the recommendations provided by the CPG on the utility of SDF testing based on clinical scenarios. The majority of participants made explicit remarks about both the clinical scenarios and SDF assays' characteristics. Among 'not supportive' and 'supportive with reservation' participants, 75% (n=30/40) and 77.5% (n=31/40) expressed concerns related to technical limitations of SDF testing methods and clinical utility of the test in one or more clinical scenarios discussed in the CPG, respectively. The SWOT analysis revealed that the CPG provides a reasonable evidence-based proposal for integration of SDF testing in the routine daily practice. It also uncovered gaps of knowledge and threats limiting the widespread application of SDF in everyday practice, thus allowing the identification of opportunities to further refine SDF testing and its clinical utility.

Conclusions: The understanding of the role of SDF in male infertility requires an in-depth analysis of

the multifactorial pathophysiological processes and the theories involved. The SWOT analysis allowed an objective evaluation of CPG on the clinical utility of SDF testing based on clinical scenarios and its accompanying commentaries written by global experts in all possible angles. Implementation of SDF testing in the clinic may not only increase the outcome of ART but more importantly improve the health of both fathers to be and resulting offspring.

Introduction

Infertility affects approximately 15% of couples globally, with male infertility directly or indirectly responsible for about 60% of the cases (1,2). Conditions compromising male fertility potential include varicocele, congenital and genetic abnormalities, endocrine disorders, genitourinary infection, systemic diseases, and exposure to gonadotoxins, which can be identified through a comprehensive male infertility workup (3). A medical history, physical examination, and semen analysis are the minimum standards for the evaluation of men seeking fertility (3). Among these, routine semen analysis is the central laboratory test in male infertility evaluation (4). However, assessment of semen volume, sperm count, sperm motility, and sperm morphology rarely provides robust discriminatory information of the male fertility potential, unless at extremely low levels (5,6). As a result, half of the male infertility cases are deemed as unexplained or idiopathic due to currently existing limitations in diagnostic modalities (7,8).

Sperm DNA plays a critical role in embryo development and therefore influences the chances of establishing a pregnancy and delivering a healthy baby, both natural and assisted (9,10). The conditions that compromise male fertility may cause deterioration in semen parameters and damage sperm DNA (2,11). However, DNA damage is also seen in sperm of men with unexplained and idiopathic infertility (7,12,13). Although no mechanism has been suggested to explain sperm DNA damage conclusively, oxidative stress (OS) has been implicated as an important mediator of infertility in such cases (2,10,12-15). Nonetheless, different intrinsic susceptibility must exist among infertile men with various etiologies, which culminates in the variable effect of OS on sperm DNA (11).

Development of assays to evaluate sperm DNA made

it possible to measure the proportion of sperm with damaged chromatin in a given ejaculate. Yet, although often equalized, one has to distinguish between damages directly to the DNA and disturbed chromatin condensation due to improper exchange of the histones by protamines, which in turn renders the DNA vulnerable to damage as the necessary protection provided by protamines is missing. Probes or dyes are used to identify the existence of DNA breaks in specimens examined by fluorescence and optical microscopy or flow cytometry (10,16,17). The term 'sperm DNA Fragmentation (SDF)' has been used to name these tests. At present, sperm chromatin structure assay (SCSA), terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), sperm chromatin dispersion test (SCD), and single gel electrophoresis (Comet) are the most commonly used methods to measure SDF (18).

With increasing evidence on the role of sperm DNA damage in infertility (19) and the possible consequences of damaged sperm chromatin to the health of offspring (9), measurement of SDF in semen became popular as a means of providing additional information to that of routine semen analysis (18). However, a poor understanding of assays' characteristics and a general belief that SDF is an untreatable condition has hampered the implementation of SDF testing in clinical practice. Among many questions faced by clinicians is the type of test to be used for SDF measurement, the patient populations that might benefit from this test, and the type of remedies to offer the affected patients.

To shed light on these critical issues, a clinical practice guideline (CPG)—the first of its kind—was recently issued concerning the clinical utility of SDF testing in specific clinical scenarios (20). The prime objective of the guideline was to underline the actual indications of SDF testing and to explain the management options available to patients

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with increased SDF. The authors examined original and review articles concerning the significance of SDF testing and arranged their CPG into two sections. In the first part, they presented the current tests for SDF evaluation, pointing out their core principles as well as the main advantages and shortcomings whereas in the second part they carried out an evidence-based analysis of test utility in clinical scenarios commonly found by urologists and reproductive specialists. Specifically, varicocele, unexplained infertility, recurrent (natural) pregnancy loss, recurrent intrauterine insemination (IUI) failure, *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) failures, and lifestyle risk factors were analyzed. In each clinical scenario, after a detailed discussion of the rationale involved, a clinical recommendation was made by the expert panel (20).

With the publication of the above-mentioned CPG, a group of urologists, andrologists and scientists with expertise in male infertility, as well as gynecologists, reproductive endocrinologists and embryologists were invited to contribute commentaries concerning its utility. These commentaries contained an abundance of information and conflicting views about the clinical utility of SDF testing, which underline the complex nature of SDF. In this review, we examine these commentaries to identify and analyze the strengths and weaknesses of SDF testing as perceived by fertility experts. For this purpose, we applied a Strengths-Weaknesses-Opportunities-Threats (SWOT) analysis, a method that was originally developed for business (21), but has also been adapted to health sciences (22,23). The SWOT analysis focuses on the strengths and weaknesses as a means of identifying threats and opportunities available to circumvent existing gaps that may limit the broad application of a method or system.

Methods

A search of papers published in response to the guideline article by Agarwal *et al.* (20), entitled "Clinical utility of sperm DNA fragmentation testing: practice recommendations based on clinical scenarios", was performed within the *Translational Andrology and Urology* (*TAU*) website (http://tau.amegroups.com/). The start and end dates for the search were May 2017 and August 2017, respectively. The overall strategy for study identification was based on hand search of 'ahead of print' articles related to the guideline mentioned above. Articles' type included 'editorial', 'commentary', and 'letter to the editor'. Additionally, we requested *TAU*'s editorial office and its senior editor (Dr. Lucine M. Gao) to provide us with full

texts of articles to be published by *TAU* journal that met our inclusion criterion. Fifty-eight relevant articles—from now on termed "commentaries"—were identified and the full (24-43) texts (44-62) were examined (63-81). Each commentary was rated as "supportive without reservation", "supportive with reservation", "not supportive" or "neutral" concerning the contents and recommendations provided by the CPG mentioned above. We also noted whether or not the authors specifically discussed the SDF test characteristics and clinical scenarios, and recorded if the concerns raised by them related to SDF as a laboratory test method, clinical scenarios, or both.

Subsequently, we extracted the particulars from each commentary about SDF testing as a lab method and utility of SDF in the clinical scenarios, as applicable. Clinical varicocele with borderline to normal conventional semen parameters, unexplained infertility/recurrent pregnancy loss/IUI failure, IVF/ICSI failure, and borderline abnormal (or normal) conventional semen analysis with lifestyle risk factors were the scenarios contemplated (20). The information was entered into an excel spreadsheet and was categorized further into one of the four areas, namely, "Strengths (positives)", "Weakness (negatives)", "Threats (external factors with possible impact)", and "Opportunities". Tables outlining the SWOT issues were developed. Then, a critical analysis of each item listed was carried out using both data from existing literature and relevant supporting information provided by a series of 'reply to author' published in *TAU* journal. The latter (82-98) were (99-116) responses (117-133) provided by the guideline's authors (20) to the commentaries (24-43), editorials (44-62), and letters (63-81).

Results

Descriptive analysis

Fifty-eight fertility experts from six continents and twenty-two countries contributed commentaries. Overall, participants (87.9%; n=51) were supportive of the recommendations provided by the CPG on the utility of SDF testing based on clinical scenarios. Among these, 27.6% (n=16) supported the CPG without reservation and 60.3% (n=35) with reservation, whereas 8.6% (n=5) participants were not supportive or neutral (3.5%; n=2) (*Figure 1*). The vast majority of participants made specific comments about either the clinical scenarios or SDF assays' characteristics, or both (*Table 1*). Among 'not supportive' and 'supportive with reservation' participants, 75%
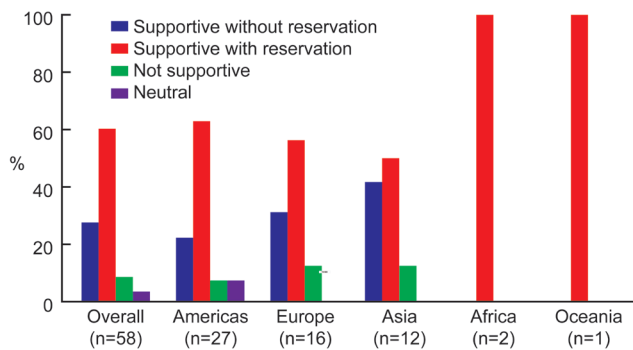
Countries (number of participants): United States (n=19), Brazil (n=4), Canada (n=3), and Argentina (n=1) [Americas]; Italy (n=4), France (n=3), Spain (n=3), Turkey (n=2), United Kingdom (n=1), Denmark (n=1), Belgium (n=1), Poland (n=1) [Europe]; India (n=6), China (n=1), Iran (n=1), Malaysia (n=1), Qatar (n=1), Pakistan (n=1), Israel (n=1) [Asia]; South Africa (n=1), Egypt (n= 1) [Africa], Australia (n=1) [Oceania]

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Figure 1 Expert opinion about the clinical utility of the guideline on sperm DNA fragmentation testing based on clinical scenarios (20).

(n=30/40) and 77.5% (n=31/40) expressed concerns related to technical limitations of SDF testing methods and clinical utility of the test in one or more clinical scenarios discussed in the CPG, respectively (*Table 1*).

SWOT analysis

The (24-43) expert (44-62) commentaries (63-81) covered a broad range of issues concerning SDF, which reflect the mixed profile and expertise of participants, as discussed elsewhere (18). As seen in *Table 1*, authors of commentaries focused either on SDF as a laboratory test method or the utility of such testing in specific clinical scenarios, with most authors covering both areas. Given such heterogeneity, we developed the SWOT analysis in two parts. In the first part, we discuss participants' concerns regarding SDF as a lab method (*Figure 2*) and in the second part, we reason their

Table 1 Distribution of expert opinions according to the focused area: (I) SDF as a laboratory test method; (II) clinical scenarios; or (III) both, stratified by endorsement category (supportive without reservation, supportive with reservation, not supportive, neutral)

Variables	SDF testing as a lab method, N (%)	Clinical scenarios, N (%)	Both, N (%)	Authors (reference)
Total (n=58)	10 (17.2)	15 (25.9)	33 (56.9)	–
Supportive without reservation (n=16)	5 (31.2)	5 (31.2)	6 (37.6)	Cho (24), Kosgi (26), Gosálvez (30), Menezo *et al.* (36), Vandekerckhove (46), Rey (47), Mirzazadeh & Sadri-Ardekani (48), Drevet (52), Mathur (55), Carrel & Hotaling (61), Christensen & Humaidan (63), Samplaski (69), Hallak (72), Tandon (75), Evenson (78), Harlev (81)
Supportive with reservation (n=35)	6 (17.1)	6 (17.1)	23 (65.8)	Henkel (25), Arafa & ElBardisi (27), Tadros & Sabanegh Jr (28), Turek (32), Garrido *et al.* (33), Oehninger (34), El-Sakka (35), Yovich & Keane (37), Hsiao (38), Khandwala & Eisenberg (39), Muratori & Baldi (40), Khalili & Agha-Rahimi (41), Tesarik & Galán-Lázaro (42), Basar & Kahraman (43), Franco Jr (44), Borini *et al.* (45), Pool (49), Glina (50), Amiri-Yekta *et al.* (51), Durairajanayagam (53), Lynne & Brackett (54), Jarvi (57), Kadioglu (59), Marmar (60), Tatem & Brannigan (62), Cunha-Filho (64), Herati & Lamb (66), Ahmad (70), Kovac (71), Dada (73), Potdar (74), Varghese *et al.* (76), O'Flaherty (77), Malhotra (79), Ferlin (80)
Not supportive (n=5)	0 (0.0)	1 (20.0)	4 (80.0)	Pandiyan *et al.* (29), Benagiano *et al.* (31), Fraczek & Kurpisz (56), Johnson & Sandlow (58), Mehta (65)
Neutral (n=2)	2 (100.0)	0 (0.0)	0 (0.0)	Veeramachaneni (67), Ward (68)

SDF, sperm DNA fragmentation.

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Figure 2 A Strengths-Weaknesses-Opportunities-Threats (SWOT) analysis of sperm DNA fragmentation (SDF) testing as a specialized sperm function laboratory method.

concerns about the clinical utility of such testing in specific clinical scenarios (*Figure 3*).

Discussion

Part 1: SWOT analysis of SDF as a laboratory test method

Strengths

SDF tests assess the quality of DNA package
During spermiogenesis, histones are replaced by transition proteins and subsequently with protamines (104,134). Cysteine residues of protamines further undergo intra- and intermolecular disulfide cross-linking resulting in a highly condensed chromatin arranged in a toroid (116). This complex packaging protects the sperm chromatin during its transport from the male to female reproductive tracts and ensures delivery of intact paternal genetic material to the oocyte. A certain amount of histones is, however, retained in human sperm chromatin making it vulnerable to injury (10).

Sperm DNA damage is a complex process involving multiple non-mutually-exclusive causative mechanisms that generate a variety of insults to DNA. Varicocele, infection, and inflammation of the genital tract, cancer, genetic mutations, chromosomal abnormalities, aging, environmental exposure and lifestyle factors are the main conditions associated with sperm DNA damage (2). Furthermore,

testicular apoptotic processes during spermatogenesis, aberrations during chromatin remodeling, and OS are among the primary mechanisms postulated to cause nuclear and mitochondrial sperm DNA damage (10,13,49).

SDF testing was originally developed to detect DNA damage in ejaculated sperm (78). Although the term 'fragmentation' is widely used to refer to any test that assesses sperm chromatin, not all insults break the DNA into "fragments" (63). In addition to single- or double-stranded DNA breaks, chromatin damage includes DNA nicks, nuclear protein defects, and alteration of chromatin configuration (10). Mostly, the tests measure the proportion of sperm with: (I) real DNA breaks (e.g., TUNEL); (II) combination of actual DNA breaks and potentially denaturable DNA due to the preexistence of single-stranded DNA breaks (SS-DB) or double-stranded DNA breaks (DS-DB) (e.g., SCSA, SCD, and Comet); or (III) poor chromatin packaging (20,104,116). As noted above, not all types of damage are detected by the same test although SDF test results are interrelated to a greater or lesser extent via properties of the DNA (23).

Since the levels of SDF in the neat semen have been associated with infertility, both natural and assisted, it is therefore suggested that SDF testing reflects the overall quality of the DNA package in a given semen specimen, not just the damaged sperm detected in the test result (135).

Strengths (Positives)

- The Clinical Practice Guideline (CPG) is timely to guide urologists and fertility specialists in requesting sperm DNA fragmentation (SDF) tests in proper clinical scenarios
- The four case scenarios cover the spectrum of difficult clinical decisions that most fertility specialists encounter in clinical practice. The evidence-based recommendations are extremely valuable for the assessment of infertile males and couples undergoing ART
- There is a common belief that SDF is untreatable. The guideline clarifies this issue and offers evidence-based guidance for interventions

Weaknesses (Negatives)

- Clinical scenario 1 (Varicocele with normal/borderline semen parameters): there is no evidence that SDF alone can predict improvements in semen parameters or affect pregnancy outcomes after varicocele repair
- Clinical Scenario 2: Unexplained infertility/recurrent pregnancy loss/intrauterine insemination (IUI) failure: little data is available to support the usefulness of SDF testing
- Clinical Scenario 3: In vitro fertilization and/or intracytoplasmic sperm injection (IVF/ICSI) failure: Controversy exists regarding the predictive value of SDF testing and the association between SDF and ICSI outcomes
- Clinical scenario 4 (Borderline abnormal or normal conventional semen analysis with life-style risk factors for SDF): there is insufficient evidence that lifestyle modification will result in resolution of SDF or improve fertility outcomes
- Overall, the quality of current evidence regarding SDF use leads to level C recommendation

Threats (External Factors With Possible Impact)

- Specialty societies and National Institutes do not recommend the use of SDF testing in the routine workup of the infertile male patient
- These guidelines are not inclusive of all clinical conditions that may benefit from using SDF testing

Opportunities

- More studies should be conducted to clarify the clinical utility of SDF test to determine the course of treatment according to specific clinical scenarios and beyond

Figure 3 A Strengths-Weaknesses-Opportunities-Threats (SWOT) analysis of the clinical utility of sperm DNA fragmentation testing in specific clinical scenarios.

SDF tests provide results distinct and more significant than those of conventional semen parameters

Overall, clinicians agree that conventional semen analysis has limited value in clinical decision-making (4,9,10,136). Routine laboratory semen analysis is subjected to marked variability due to operational and technical factors (91,117,137-140). Moreover, there is biological variability in semen parameters of same individuals as spermatozoa are mixed with and diluted by fluid secreted from accessory glands, all of which are governed by the state of testicular sperm production, epididymal transit, the activity of accessory glands as well as ejaculatory abstinence (141,142). Importantly, routine semen analysis does not assess essential biological properties of spermatozoa which are needed for fertilization and embryo development. Experience with SDF testing has shown that sperm with high DNA fragmentation can have normal motility and morphology (143), thus helping to explain the limitations of routine semen analysis as to provide robust information to distinguish fertile from infertile men (4,117,136).

In contrast, SDF testing is a more accurate indicator of male fertility status as it looks into the paternal genome. The integrity of sperm DNA is considered to be vital for normal fertilization, embryo development, successful implantation, and pregnancy success in both natural and assisted reproduction [reviewed by Agarwal *et al.* (19)]. Moreover, in a study evaluating the ability of sperm vitality to predict SDF rates, Samplaski *et al.* found that over 32% of men gain additional information from SDF testing since vitality test alone would fail to predict SDF in these patients accurately (144). The inclusion of SDF testing to the male infertility armamentarium is advantageous as the test

provides valid information that can improve the diagnostic and prognostic value of the routine seminal evaluation (117). Despite the lack of a gold standard test in male infertility, none of the available tests provide diagnostic information equivalent to that offered by SDF during the evaluation of infertile men (19,91).

SDF is a parameter with low biological variation

SDF measurement in consecutive ejaculates is associated with low biological variability. In one study involving 100 men attending an infertility clinic, two consecutive semen analyses were carried out at a median interval of 1.4 months (range: 0.5–6.9) (145). The median coefficient of variation (CV) was significantly lower (P<0.001) in SDF rates by SCSA (9.2%) than that of sperm count (43.0%), progressive motility (28.3%), and normal sperm morphology (28.3%) (145). Others have corroborated these results by showing that the biological variability is significantly less for SCSA results than conventional semen parameters in consecutive samples (146,147).

SDF is a marker of OS

Reactive oxygen species (ROS) are considered the primary cause of SDF (94,122,148,149), thus making SDF testing a candidate surrogate marker of OS. OS is common in the infertile male population and has been associated with many known and unknown infertility conditions, including varicocele, infection, advanced paternal age, heat stress, and lifestyle factors (150,151). Excessive ROS exert a detrimental effect on male fertility via multiple mechanisms including sperm membrane peroxidation, mitochondrial and nuclear DNA damage, and apoptosis. Redox processes using various pathways, including hydroxyl radical, nitric oxide, and activation of sperm caspases and endonucleases, affect not only the sperm membrane but also nuclear and mitochondrial DNA, thus explaining the higher positivity for SDF in live ejaculated sperm of infertile men than fertile counterparts (10,116,152). SDF testing can provide a common pathway to measure the effects of oxidative damage and the success of treatments whether that be varicocele surgery, antioxidants or lifestyle modification (153,154).

Weaknesses

SDF testing lacks standardization

Standardization is necessary for any diagnostic test to be used clinically (4,60,133). Achieving such a characteristic is mainly related to the nature of the test, its complexity, and subjectivity in interpretation. Moreover, accuracy (extent to which the measurement reflects the real value), and precision (reproducibility of test results) are both

important for clinicians who rely on the values provided by a laboratory test to direct the workup and counseling of the infertile male (155).

Although some methods to measure SDF suffer from high inter-laboratory variation, many efforts have been made to standardize the SCSA, TUNEL, and the SCD test (110). For instance, inter- and intra-laboratory precision, and inter- and intra-observer CV calculated for SCSA, SCD, and flow-cytometer TUNEL are extremely small (156-162) (*Table 2*). In a study using the TUNEL assay and a benchtop flow cytometer, semen specimens were evaluated in a blind fashion by two experienced observers, with results showing absolute inter- and intraobserver differences of 1.73% and 6.68% and percent inter- and intraobserver differences of 3% and 9.68% in >80% of cases, respectively (156). With the Halosperm G2 test kit, the intraobserver variability of the absolute average difference in SDF values between replicate tests was 1.02%±0.55% with a mean percent difference of 4.16% (157). In this study, the interobserver variability of SDF values between two technicians showed an absolute difference of 0.21%±0.57% and a mean percent difference of 9.56%. Furthermore, a recent study revealed no significant differences in SDF results between duplicates, with a high correlation between two independent laboratories (r=0.94) when experienced operators used the same semen samples, identical assay kit, protocol and flow cytometer settings to measure SDF by TUNEL (158).

There is a lack of criteria to which type of SDF should be recommended

In general, the methods for assessing sperm DNA damage can be grouped into three categories, namely: (I) assays that measure DNA fragmentation by incorporating DNA probes or modified nucleotides at the site of breaks; (II) assays that measure both the existing breaks and those generated after DNA denaturation; and (III) assays that indirectly measure the level of chromatin compaction, i.e., nucleus decondensation (116). While TUNEL belongs to the first group, SCSA, SCD, and Comet are examples of tests from the second group. In contrast, aniline blue and toluidine blue are methods that assess chromatin compaction (decondensation) rather than SDF (116).

Mostly, SDF refers to SS-DB or DS-DB and is predominantly associated with OS whereas sperm nucleus decondensation (SND) relates to defects in chromatin compaction (e.g., protamine mispackage via defective DNA-protein crosslinking), which are intrinsically associated with the later stages of spermatogenesis. A synergistic

Table 2 Summary of intra-laboratory and inter-laboratory correlation coefficients (r), coefficient of variation (CV), and intra- and inter-observer variation (%) for SDF assays

Method	Intra-lab (r)	Intra-lab (CV)	Inter-lab (r)	Inter-lab (CV)	Intra-observer variation (%)	Inter-observer variation (%)	Study
TUNEL; benchtop flow cytometer	0.75–0.95[6]	0.1–5.7%	0.83–0.93[7]	0.2–5.2%	NR	NR	Ribeiro *et al.*, 2017
TUNEL; benchtop flow cytometer	NR	NR	NA	NA	≤3%[4]	≤1.7%[5]	Sharma *et al.*, 2016
TUNEL; standard flow cytometry	NR	NR	NA	NA	3.2%[2]	4%[3]	Sharma *et al.*, 2010
SCD*	0.91	NR	NA	NA	1%[9]	0.21%[10]	McEvoy *et al.*, 2014
SCD	NR	NR	NA	NA	6–12%[8]	6–12%[8]	Fernandez *et al.*, 2005
SCSA	NA	NA	0.90	≤1%	NA	NA	Giwercman *et al.*, 2003
	NR	1.0–9.1%[1]	NA	NA	NA	NA	Giwercman *et al.*, 1999

*, Halosperm G2 test kit; [1], intra-assay CV varied between 1.0% and 9.1%, and the corresponding values for the inter-assay CV was 5.2% and 8.6%; [2], when absolute values were calculated, 80.0% of individual TUNEL measurement differed from the final designated values by no more than 3.2% (absolute difference); 57.1% of individual measurements in these data had a percentage difference less than 10% of the assigned value; [3], the absolute difference between an observer's designated value and the mean among 2 observers was within 4.0% in 83.3% of specimens; in 83.3% of the specimens, the percentage difference between an individual observer's designated TUNEL value and the 2 observers' average value was within 15%; [4], a single TUNEL measurement from a given observer was within that observer's average measurement by an absolute difference of 3% or less in 90% of cases; [5], the average TUNEL measurement from a given observer was within the two observers' average measurements with an absolute difference of 1.73% or less in 80 % of cases; [6], correlation between duplicate readings obtained in each laboratory (results from two participating laboratories); [7], correlation coefficient between two participating laboratories reading the same set of specimens; [8], coefficient of variation for the estimated percentage of spermatozoa with fragmented DNA; [9], the average difference in the values of SDF between the two replicates was 1.02%±0.55% (absolute variation); the average percentage difference between the two replicates for each sample was 4.16%; [10], the average difference in the values of SDF between two technicians for each sample was 0.21%±0.57% (absolute difference); the average percentage difference between the two technicians for each sample was 9.56%. SDF, sperm DNA fragmentation; TUNEL, terminal deoxyribonucleotide transferase-mediated dUTP nick-end labeling assay; SCD, sperm chromatin dispersion test; SCSA, sperm chromatin structure assay; NR, not reported; NA, not applicable; CV, coefficient of variation.

action among different effectors can contribute to the accumulation of DNA damage. As such, defective chromatin compaction can make the DNA more vulnerable to damage by ROS, and as a consequence SDF may ensue (116).

Due to these distinct assays' characteristics, the results obtained from one method do not necessarily match those provided by a different test, especially if the methods being compared assess SDF and SND (16). Notwithstanding, there seems to be a fair correlation among the three mostly commonly used SDF tests, namely, SCSA, TUNEL and SCD (133,163,164), albeit the evidence is not unequivocal (17). SDF measurement with flow cytometer has been advocated as more precise and reliable (63,78), as the technique allows thousands of cells to be analyzed in a relatively short time compared with the use of optical/fluorescence microscopy. Moreover, the ability to evaluate a large cell number is another means to control inter- and intra-laboratory variations in test results (127,156,158). At present, however,

suggesting one specific assay in preference over another does not seem to be in the best interest of clinicians and patients alike until a gold standard method is established.

Since the ideal method to measure SDF is still to be determined, any decision to consider SDF testing should take into account the limitations of testing methods and the possible benefits for clinical outcomes (117). In fact, the different type of SDF detected by each assay may be complementary to each other in distinct clinical settings (107). Nevertheless, it is essential that a reliable SDF assay with a validated threshold and performed by a skilled technician be used (10,16).

SDF lacks clear cut-off values

Although no definite threshold of DNA damage beyond which a seminal sample can be considered pathological has been agreed upon, cut-off values have been reported for SDF testing with regards to pregnancy prediction and infertility risk (112). For instance, recent data shows that

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SDF test by SCD, when used in a fertility clinic setting, has a sensitivity of 80.8% and specificity of 86.1% with the SDF cutoff of 26.1%, and a prevalence ratio of 2.84 for the occurrence of male infertility (165). Moreover, the benchtop flow cytometer TUNEL assay has been recently standardized and validated on semen samples obtained from 95 fertile controls and 261 infertile men (156). In this study, a SDF cutoff value of 16.8% was found to have a specificity of 91.6% and a positive predictive value of 91.4% in distinguishing infertile men from controls.

Along the same lines, SCD test was found to have a sensitivity of 86.2% and a negative predictive value (NPV) of 72.7% (P=0.02) in predicting successful ART treatment when the cutoff value of 25.5% was utilized (166). Likewise, a SCSA cutoff value of 30% was found to carry a significant predictive power to the likelihood of pregnancy both *in vivo* and after ART, where patients with a SDF <30% were 7.1 times [95% confidence interval (CI), 3.37–14.91] more likely to achieve a pregnancy *in vivo* and ~2.0 times (95% CI, 1.10–2.96) more likely to achieve pregnancy after ART (167). Another recent study by Rilcheva *et al.* (168) utilized the SCSA assay to investigate the influence of SDF on the pregnancy outcome of 531 couples undergoing autologous ICSI (n=416), donation ICSI (n=39) and IUI (n=71). Using a cutoff value of 27%, the authors reported a statistically significant negative relationship between SDF and pregnancy outcome with IUI (χ^2=6.87; P<0.05), and a positive relationship between SDF and pregnancy loss after IUI (*t*-test =1.58; P<0.05) and ICSI (OR =5.65; 95% CI: 4.32–7.11; P<0.05). The authors concluded that infertile men should be evaluated with SDF in addition to routine semen analysis and suggested that when the result exceeds 27%, patients should be offered ICSI at an earlier stage.

Collectively, the threshold range of 25–30% by SCSA or SCD seems valid for placing men into a statistical probability of longer time to achieve natural pregnancy, low odds of pregnancy by IUI and conventional IVF, and a higher risk of miscarriages, both natural or assisted (112,169). However, it is important is to note that having a SDF rate of 30% does not mean that 70% of remaining spermatozoa have entirely normal chromatin (72). The methods to evaluate chromatin integrity measure the percentage of cells with fragmented DNA/chromatin decondensation only and are based on the idea that the greater the fragmentation/decondensation rate, the greater the chance that the sperm population is pathological. It has been suggested that part of the remaining sperm may be already compromised as regards to DNA integrity, but not yet crossed the threshold detectable by the assay (170). High SDF probably represents the tip of an iceberg (72,127,170).

It is therefore crucial that clinicians be judicious when interpreting test results and using this information to predict reproductive outcomes. A high SDF result should be read as a general indicator of poor semen quality instead of an absolute value of abnormal sperm (57,130). Along these lines, nomograms taking into account various levels of SDF and female factors have been suggested as a means to better predict the reproductive outcomes (57). Consideration of female factors is important as the presence of SS-DB may be repaired by oocyte repair machinery (171), thereby preventing the adverse consequences of SDF. However, the oocyte has a limited capacity of repairing SDF (172) and importantly, not all types of sperm DNA breaks are repairable (173). Given the multitude of confounding factors, various cut-off values may be required to ensure satisfactory performance of the test in distinct clinical scenarios (93,127).

SDF tsting does not identify the type (nature of lesion) and site of DNA breaks

Currently, existing SDF methods are limited in revealing the kind of DNA damage and the location of break. However, as discussed above, TUNEL, SCSA, SCD test and Comet determine the presence of DNA breaks whereas aniline blue and toluidine blue the degree of chromatin compaction (decondensation) (116). None of them, however, can discriminate if the lesion affects coding or non-coding DNA domains. Since only about 1.5% of the genome encodes DNA sequences capable of producing proteins (10), it has been argued that lesions affecting non-coding sites may not be of clinical significance (33). However, the remaining genome may have specific functions ranging from various structural roles (highly repeated sequences) to direct regulation of gene expression or indirect gene expression control through epigenetic changes (174-176). Moreover, DS-DBs, especially if abundant within the nucleus, can trigger cell cycle blocking mechanisms that result in cell death (177,178). Cell death can also occur from unrepaired or erroneously repaired DNA lesions that lead to protein production deficiencies or even chromosomal abnormalities (177).

Collectively, apoptotic induction, erroneous DNA repair, and the presence of chromosomal alterations can cause cell death, and these may occur regardless of the location of the initial DNA lesions or whether or not the DNA domain has an informative capacity (10). Nonetheless, the stratification of DNA damage into testicular or post-testicular events

could help in our understanding of the pathophysiology and development of new treatment strategies (100). Although neither the amount of SDF in each cell—with the exception of Comet assay—nor the exact site of damage is quantified by SDF tests, a positive association between SDF and natural pregnancy/ART/miscarriage has been demonstrated despite the use of a wide variety of testing methods (19). Rather than being a limitation, the inability of SDF testing to assess how much DNA damage exists in each cell is advantageous given the large number of spermatozoa and the highly variable DNA integrity of each spermatozoon (130). As it stands, SDF testing reflects the quality of the DNA package in the entire semen sample, which provides an overall indication of DNA integrity and male fertility (135).

SDF testing is carried out in neat semen and may not reflect the sperm population used in ART

Although further research is needed in this area, the validity of using processed semen samples for SDF testing is currently not warranted. DNA fragmentation index (DFI) of density gradient centrifugation (DGC)-processed sperm measured by SCSA did not predict ART outcome in contrast to neat samples (103). Besides, no association between sperm SCSA DFI after swim-up and fertilization, implantation and pregnancy rates could be demonstrated in another study (179). Also, DGC has been reported to result in increased SDF especially when higher centrifugation force, longer duration and Percoll gradients were used (180,181). Furthermore, in a study investigating the dynamics of SDF by SCD and its implication on embryo development and pregnancy rate in couples undergoing ICSI, it was observed that the likelihood of pregnancy was decreased by 5.9% for every 1-unit increase in SDF observed after 12 h of incubation (182). These observations suggest that SDF results after sperm processing may increase as a function of factors such as incubation period and temperature, thus making its value questionable for pregnancy prediction in the ART setting.

Threats

Professional societies have not endorsed the use of SDF testing in daily practice

The literature is rich in systematic reviews and meta-analyses reporting on a positive association between elevated SDF and infertility and ART outcomes. However, variation in SDF assays, SDF thresholds and differences in study populations have resulted in mixed conclusions concerning the clinical utility of SDF testing (83,183).

Consequently, professional societies, such as the American Society for Reproductive Medicine (ASRM), the American Urological Association (AUA), the European Association of Urology (EAU), and the National Institute of Clinical Excellence (NICE), have not recommended SDF testing for routine clinical evaluation (74,114,184-187).

However, new evidence has been generated steadily after the publication of these guidelines. Studies reporting a significant detrimental relationship between SDF and clinical varicocele (11,187,188), unexplained infertility (189-192), and outcomes of ART (18,193,194) were published recently. Furthermore, new data emerged concerning the potential benefit of using testicular in preference over ejaculated sperm for ICSI in cases of high SDF (195-199), thus stressing the need for further updates in the clinical recommendations of the societies mentioned above. As CPGs are evolving documents, a timely review and update are highly expected.

Equally important is to consider that in addition to the sound guidance provided by CPGs, the application of a test should be weighed by the magnitude of benefit it can bring to a couple (103). Although it is unlikely that the dynamic interaction among multiple confounding factors, both in natural conception and ART, can be confirmed by a single test (109), SDF test in the clinical setting may aid in: (I) identification of the possible underlying etiology in couples who are otherwise classified as unexplained or idiopathic infertility; (II) monitoring of treatment outcomes of either empirical or new treatment modalities; (III) stratification of patients to receive more targeted treatment for SDF; and (IV) avoidance of unnecessary workup and wastage of precious time and money in unproductive treatments (93).

SDF testing is expensive and not reimbursed by insurance companies

The cost of SDF testing is approximately 170±123 USD (18), which remains an important factor limiting the routine use of SDF testing. However, cost is also a major drawback to all other fertility related therapeutic modalities and has been recognized to add significant handicap on infertile couples whose fertility treatments are usually not covered by medical insurance (200). SDF testing represents a fraction of the cost of other fertility treatments such as ART procedures or surgical varicocele ligation (112). A major aim of the CPG by Agarwal *et al.* (20) was to elucidate the circumstances where SDF testing would be most influential on treatment decisions, therefore, with a possible significant impact on the overall treatment cost.

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The use of SDF testing in the right clinical scenario can aid in recommending an ART procedure or treatment that is associated with the highest likelihood of pregnancy, thereby eliminating unnecessary cost from less successful therapeutic modalities (95). In one study evaluating the use of SDF testing and testicular sperm in preference over ejaculated sperm for ICSI in men with high SDF in semen, the number needed to treat by testicular compared to ejaculated sperm to obtain an additional live birth per fresh transfer cycles was 4.9 (95% CI: 2.8–16.8) (195). This study suggested that we could avoid one out of five oocyte retrievals to obtain an additional live birth if we used SDF testing in the aforementioned clinical scenario (92).

Besides, the SDF result can help in selecting patients who are most likely to benefit from varicocele ligation eliminating unnecessary surgery. It seems sound that any step that can potentially improve natural pregnancy or ART outcomes will likely to pose a financial benefit to both the couple and society. A more comprehensive workup of male factors by incorporation of SDF testing is certainly an attractive and economical option. The cost of SDF testing will probably be justified since the test results reflect treatment outcome (95).

Lastly, the question of whether or not doctors should ask patients to spend more money to obtain a diagnosis of the status of DNA damage in the sperm as a tool for both the patient and clinician to decide on a course of treatment should be put in the right perspective (68). Given the evidence indicating that SDF is associated with reproductive health issues in the male and the embryo, the question we as individuals and as a medical community must ultimately consider is if we are providing the best care to the infertile couple and the offspring by ignoring the "health" of the sperm (61).

Opportunities
More research has to be conducted to clarify existing gaps and refine SDF as a valid lab method
The decision about the broad applicability of a clinical test is a delicate balance among numerous factors including cost-effectiveness and patient-centeredness. Currently, there exist gaps to be filled concerning SDF testing as a laboratory method, as discussed above. However, many drawbacks perceived by infertility specialists, such as poor validation, low precision, and low accuracy seem to result from a lack of careful appreciation of the emerging

evidence concerning the enhanced performance of several test methods. The practice recommendations by Agarwal *et al.* is the first step forward to bridge the gap between research and clinical practice in promoting SDF testing. As the intended purpose of the CPG (20) was to identify the proper indications of SDF testing, meaningful refinements of its practical methods and diagnostic thresholds can be achieved further (115).

Part 2: SWOT analysis of clinical utility of SDF in specific clinical scenarios

Strengths
The CPG is timely to guide urologists and infertility specialists in requesting SDF tests in proper scenarios
Little information is available to give clinicians guidance according to which SDF testing should be requested (25). The CPG by Agarwal and colleagues (20) is the first attempt to provide this urgently needed practical orientation. It includes clear situations in which SDF testing can be used to benefit patients, with practical and easy-to-understand supportive information (24-26,30,33,54,55,57,61,69,81). The guideline also points out that SDF testing should not be regarded as the gold standard test in the male fertility assessment. Furthermore, SDF testing was not proposed as a replacement for routine semen analysis or to be used indiscriminately in all cases. Assessment of SDF should rather be a tool to provide physicians additional information that can influence their decision of how to counsel and manage their patients (89).

The four case scenarios cover the spectrum of difficult clinical decisions that most fertility specialists encounter in clinical practice. The evidence-based recommendations are extremely valuable for assessment of male subfertility and couples undergoing ART
Despite the availability of numerous studies exploring the impact of SDF on male fertility and reproductive outcomes, an understanding of the clinical utility of such an important test was still lacking. Foremost among all strengths of Agarwal *et al.*'s CPG is the scope of testing suggested (61). Although evidence is not available at this time to recommend routine screening of all men evaluated at a fertility clinic, the use of SDF testing in specific clinical scenarios is evidence-based and should be implemented in ART clinics not currently employing such assays. In brief, Agarwal *et al.* provided clear, evidence-based guidelines that

should facilitate practical implementation of SDF testing in the clinic with the objective of not only improving ART success rates, but more importantly to improve the health of the father and the offspring (54,61,66).

There is a common belief that SDF is untreatable. The guideline clarifies this issue and provides evidence-based guidance for interventions

Although the association between SDF and natural pregnancy/ART outcomes has been extensively reported, the lack of effective treatment for high SDF is a common critique of SDF testing (62,120). Despite the common belief that SDF is untreatable, objective evidence indicates that treatment options are available. Among interventions shown to improve sperm DNA integrity, varicocele repair (201) and oral antioxidant therapy can alleviate SDF and improve the chances of establishing a pregnancy (132,202-213) (*Table 3*). Moreover, modifiable lifestyle factors such as smoking, obesity, and occupational exposure have been associated with high rates of SDF making them potential targets for interventions (128,214-216). It is suggested that correction of underlying factors can alleviate SDF and potentially enable natural conception or allow the use of less complex ART methods (135). If ICSI is to be used, lower miscarriage rates are anticipated after treatment of the conditions causing SDF. For this, a clinical evaluation of the infertile male is essential to identify the causes of infertility and allow treatment of the affected men aiming at reducing SDF. Lastly, laboratory sperm selection techniques and the use of testicular sperm represent alternatives to reduce the risk of using sperm with high SDF for ICSI, with the latter being the most attractive approach given it provides the highest reduction in SDF rates and LBR by ICSI (92,195,197,217-229) (*Table 4*).

A CPG such as the one presented by Agarwal and colleagues (20) is intended to provide clinicians and other healthcare practitioners useful information to enhance the quality of care deliverable to patients (113). It is also designed to discourage potentially harmful or ineffective interventions (84). However, the complexity of the human reproductive system cannot be completely covered by any guideline. The decision to perform a particular investigation, e.g., SDF testing, and to intervene accordingly should be individualized for every couple. The management strategy depends on factors such male and female age, duration of infertility, financial issues as well as couples' willingness to pursue natural or assisted conception. The primary objective of the CPG under discussion was to translate the best evidence into practice and provide a framework of standardized care while maintaining clinical autonomy and physician judgment (84).

Weaknesses

Clinical scenario 1 (varicocele with normal/borderline semen parameters): there is no evidence that SDF alone can predict improvements in semen parameters or affect pregnancy outcomes after varicocele repair

Among infertile men with clinical varicocele, elevated SDF is observed in approximately 50% and 60% of those with normal and abnormal semen parameters, respectively (128,230). Furthermore, 70–90% of patients show improvement in SDF levels after varicocele repair (231,232). Reduction in SDF after surgery is shown to be more common in men who have a concomitant improvement in conventional semen parameters (230). Also, postoperative SDF rates are lower in men able to impregnate their partners naturally or by ART than those who are not (187,230). Notwithstanding, the presence of normal SDF levels in infertile men with clinical varicocele suggests that some patients may have scavenging mechanisms that counteract the effect of excessive ROS as causative of high DNA damage (14).

Although further studies are required, these observations provide some evidence that improvements in sperm DNA integrity after varicocele repair translate into higher pregnancy rates. However, the argument that the usefulness of SDF as a single test needs to be demonstrated is probably flawed given the interaction among multiple factors in the reproductive system (233). SDF testing in providing an assessment of the genetic content of males with varicocele should be considered complementary to conventional semen analysis. As with all diagnostic tests, care must be taken only to utilize them when the results directly affect clinical decision-making. SDF in the context of varicocele should be primarily reserved for the cases in which treatment is not warranted by itself, as depicted in *Figure 4*.

Clinical scenario 2 (unexplained infertility/recurrent pregnancy loss/IUI failure): little data is available to support the usefulness of screening for SDF

Elevated SDF (DFI >20–30%) is found in up to 45% of infertile men with unexplained infertility (128,189). Pregnancy rates are reduced among couples with unexplained infertility undergoing IUI when SDF rates are >20% (189). Furthermore, the odds of pregnancy are dramatically decreased (by 7.0 to 8.7-fold) in the general population of infertile couples subjected to IUI with high seminal SDF levels (19,234). Evidence of an association between SDF and recurrent pregnancy loss is also increasing steadily (128,190-192). SDF testing in these scenarios may help clinicians counseling the affected men to take all measures to reduce SDF, including oral antioxidant

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Table 3 Review of studies examining the effect of antioxidant therapy on sperm DNA fragmentation

Study (reference)	Supplements (daily intake)	Study design	n	Duration	SDF testing method	Result
Tunc et al. (202)	Vitamin C (100 mg), E (400 IU), selenium (26 µg), Zn (25 mg), folic acid (0.5 mg), lycopene (6 mg) and garlic oil (333 µg)	Prospective observational study	50	3 months	TUNEL, CMA3 assay	Significant decrease in SDF [pre-treatment: 22.2% (16.5–26.6%); post-treatment: 18.2% (13.4–23.1%)[†], P=0.002] and protamine packaging [pre-treatment: 69.0% (63.5–73.1%); post-treatment: 73.6% (69.3–77.5%)[†], P<0.001]. No significant improvement in semen parameters
Ménézo et al. (203)	b-carotene (18 mg), vitamins C (400 mg), E (400 mg, Zn (500 µmol) and Se (1 µmol)	Prospective observational study	58	3 months	SCSA	Significant decrease in SDF (–19.1%, P<0.0004)
Kodama et al. (204)	Vitamins C (200 mg), E (200 mg), glutathione (400 mg)	Prospective observational study for the treatment group	14	2 months	8-hydroxy-2'-deoxyguanosine	Significant improvement in sperm concentration (7±1 million/mL, P<0.05). Significant decrease in oxidative DNA damage (–0.4%±0.1%, P<0.05)
Omu et al. (205)	Group1: Zn (400 mg); group 2: Zn (400mg) + vitamin E (20 mg); group 3: Zn (400 mg) + vitamins E (20 mg) + C (10 mg)	Randomized placebo-controlled study	45 treatment (group 1: n=11; group 2: n=12; group 3: n=14; 8 placebo group)	3 months	SCSA	Improvement in SDF measures with treatment*. Significant improvement in sperm motility with the treatment group (group 1: 25%±12%; group 2: 24%±6%; group 3: 25%±9%, P<0.01)
Greco et al. (206)	Vitamin C (1,000 mg) + vitamin E (1,000 mg)	Prospective observational study	29 (patients with high SDF and prior failed ICSI)	2 months	TUNEL	Significant decrease SDF levels (–15.8%±3.6%, P<0.001). Insignificant effect on semen parameters. Significant improvement in ICSI clinical pregnancy (48.2% versus 6.9%) and implantation (19.6% versus 2.2%; P<0.01) rates compared with the pre-treatment ICSI outcomes
Greco et al. (207)	Vitamin C (1,000 mg) + vitamin E (1,000 mg)	Randomized placebo-controlled study	64 men with unexplained infertility and high SDF levels	2 months	TUNEL	Significant decrease in SDF levels (–13%±0.5%, P<0.001). Insignificant effect on semen parameters
Martinez-Soto et al. (208)	Docosahexaenoic acid (DHA) (1,500 mg)	Randomized, double blind, placebo-controlled, parallel-group study	57 (32 treatment group and 25 placebo group)	10 weeks	TUNEL	Significant decrease in SDF levels (–17.2%±2.8%, P<0.001) in treatment group vs. (11.2%±1.9%, P>0.05) in the placebo group. Insignificant effect on semen parameters

Table 3 (*continued*)

Table 3 (*continued*)

Study (reference)	Supplements (daily intake)	Study design	n	Duration	SDF testing method	Result
Fraga *et al.* (209)	Vitamin C (250 mg)	Prospective observational study	10	15 weeks	8-hydroxy-2'-deoxyguanosine	Vitamin C depletion/repletion was associated with inverse relationship with seminal vitamin C levels and oxo8dG measures (P<0.01)
Abad *et al.* (210)	L-carnitine (1,500 mg); vitamin C (60 mg); coenzyme Q10 (20 mg); vitamin E (10 mg); zinc (10 mg); vitamin (200 µg) B9 selenium (50 µg); vitamin B12 (1 µg)	Prospective observational study	20 infertile patients diagnosed with asthenoteratozoospermia	3 months	SCD measurement done following various periods of sperm storage (0, 2, 6, 8 and 24 h) at 37 °C	Significant decrease in SDF levels (at 0 h: −8.4%±6.7%, P=0.004; at 2 h: −8.07%±5.03%, P=0.003; at 6 h: −8.58%±0.81%, P=0.004; at 8 h: −9.03%±2.79%, P=0.006; at 24 h: −20.95%±8.59%, P=0.0002). Significant improvement in DDS (−1.6%±0.9%, P=0.04). Significant improvement in sperm concentration (0.1±6.47 million/mL, P=0.04), motility (6.5%±2.8%, P=0.02) and morphology (1.5%±2.4%, P=0.04)
Vani *et al.* (211)	Vitamin C (1,000 mg) 5 consecutive days in a week	Prospective comparative study	240 (120 men exposed to lead, and 120 healthy human subjects)	3 months	Comet	Significant decrease in alkaline-labile sites and mean tail length of the comet when compared to the control group (P<0.001). Significant improvement in all semen parameters (P<0.001)
Gual-Frau *et al.* (212)	L-carnitine (1,500 mg); vitamin C (60 mg); coenzyme Q10 (20 mg); vitamin E (10 mg); zinc (10 mg); vitamin B9 (200 µg), selenium (50 µg), vitamin B12 (1 µg)	Prospective observational study	20 infertile men with grade 1 varicocele	3 months	SCD	Significant decrease in SDF levels (−22.1%, P=0.02). 31.3% fewer highly degraded sperm cells (P=0.07). Significant increase in sperm concentration (P=0.04)
Piomboni *et al.* (213)	Beta-glucan (20 mg), fermented papaya (50 mg), lactoferrin (97 mg), vitamin C (30 mg), and vitamin E (5 mg)	Prospective observational study	36 men with leukocytospermia; 15 controls	3 months	Acridine orange (AO) staining	No significant decrease in SDF. Significant increase in sperm morphology (12.8%±1.3%, P<0.01) and total progressive motility (15.8%±1.1%, P<0.01). Significant reduction in leukocyte number (−1.3±0.7 million/mL, P<0.05)

†, interquartile range; *, no values were provided. SDF, sperm DNA fragmentation; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling; CMA3, chromomycin A3; SCSA, sperm chromatin structure assay; DDS, DNA degraded sperm.

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Table 4 Summary of the effect of different strategies to reduce sperm DNA fragmentation

Method	SDF relative reduction	SDF assay	Study
Short abstinence	25%	SCD	Gosálvez *et al.*, 2011
	22%	TUNEL	Agarwal *et al.*, 2016
Gradient centrifugation	22–44%*	SCD	Gosálvez *et al.*, 2011
	56.6%	SCD	Xue *et al.*, 2014
Swim-up	33.3%	SCD	Parmegiani *et al.*, 2010
	38.1%	SCD	Xue *et al.*, 2014
MACS	26.7%	TUNEL	Tsung-Hsein *et al.*, 2010
	None	TUNEL	Nadalini *et al.*, 2014
PICSI	67.9%	SCD	Parmegiani *et al.*, 2010
	None	SCSA	Rashki Ghaleno *et al.*, 2016
IMSI	78.1%	TUNEL	Hammoud *et al.*, 2013
	None	SCD	Maettner *et al.*, 2014
Testicular sperm	79.7%	SCD	Esteves *et al.*, 2015
	79.6%	TUNEL	Greco *et al.*, 2005
	66.5%	TUNEL	Moskovtsev *et al.*, 2010

*, combined with frequent ejaculation and short ejaculatory abstinence. MACS, magnetic-activated cell sorting; PICSI, 'Physiologic ICSI' with hyaluronic acid (HA) binding assay; IMSI, intracytoplasmic morphologically selected sperm injection; TUNEL, terminal deoxyribonucleotide transferase-mediated dUTP nick-end labeling assay; SCD, sperm chromatin dispersion test; SCSA, sperm chromatin structure assay.

intake and lifestyle changes, and to consider recommending IVF/ICSI as a means to overcome infertility related to poor sperm DNA integrity (*Figure 5*).

Clinical scenario 3 (IVF/ICSI failure): controversy exists regarding the predictive value of SDF testing and the association between SDF and ICSI outcomes

Most of the controversies in this scenario are due to misunderstandings which prevented proper interpretation and communication. Whereas SDF testing has been commonly utilized to distinguish couples who will or will not become pregnant naturally or with ART, studies examining test performance have used heterogeneous patient populations and outcome measures, thus complicating their interpretation (83). For SDF to be used as a valid screening test, some conditions should be met. Foremost among all is application of the test in the proper population. For instance, the positive predictive value (PPV) of SDF in the ART setting reflects the probability that a couple who tests positive fails IVF/ICSI. In contrast, the NPV is the probability that a couple who tests negative succeed. By all means, SDF testing alone is not suitable

to provide an accurate pregnancy prediction due to the multitude of factors affecting ART outcomes. Moreover, the limitations of the test will become more evident if SDF testing is applied to all comers because the PPV and NPV change with the prevalence of the condition. If the prevalence is low (e.g., men without risk factors for SDF), the PPV will be low, even if specificity and sensitivity are high (235). In contrast, if the prevalence is high (e.g., men with risk factors for SDF), the PPV will be high provided a highly specific cutoff value is chosen. As a matter of fact, it has been shown that the PPV of TUNEL concerning pregnancy in ART is only moderate whereas that of SCD is low despite the high sensitivity (77–85%) and specificity (89–91%) of both tests (83,166,236,237).

The use of SDF as a screening test for pregnancy prediction in a population at low risk for sperm DNA damage is highly problematic as couples can be erroneously categorized as having a higher or lower chance to get pregnant when in fact they have not. Because abnormal SDF levels are seen in only about 30% of men from the general IVF/ICSI population (128,167), these implications

will become evident when screening all couples to be subjected to ART, as there will be a high number of false positives results (i.e., successful pregnancy despite high SDF, thus resulting in a low PPV). Therefore, SDF testing will be most useful if applied to couples with high risk for an adverse outcome associated with SDF (e.g., men with

risk factors and older partners, and couples with repeat IVF/ICSI failures).

Equally important is the influence of the type of ART on treatment outcomes. The negative association between high SDF and pregnancy rates in conventional IVF (112,192,238), but not ICSI (239), clearly illustrate these points. Since SDF testing concerns the integrity of paternal DNA, the poorer the DNA quality, the more fragile the spermatozoon in its ability to generate a healthy embryo. Therefore, the extent of sperm journey may impact IUI and IVF/ICSI outcomes. Reducing the length of the journey to the oocyte (by conventional IVF) as well as bypassing the process of fertilization (by ICSI) can minimize the extent of sperm DNA damage (63).

Additionally, female factors, such as age and oocyte quality, can modulate the impact of SDF on IVF/ICSI outcomes (118). Results of a retrospective clinical study showed that the live birth and implantation rates during IVF/ICSI in women with reduced ovarian reserve were significantly decreased when SDF exceeded 27.3%. In contrast, clinical pregnancy, live birth, and implantation rates were not affected in women with normal ovarian reserve (194). The oocyte capability to repair, at least to some extent, DNA damage means that semen specimens with low to moderate SDF combined with high-quality oocytes may result in acceptable reproductive outcomes. Lastly, the association between high SDF and increased miscarriage after both IVF and ICSI (19,240) suggest that the outcome measured also matters. The negative impact of SDF is more often expressed later at the implantation stage and onwards (late paternal effect) (101,241,242), which may
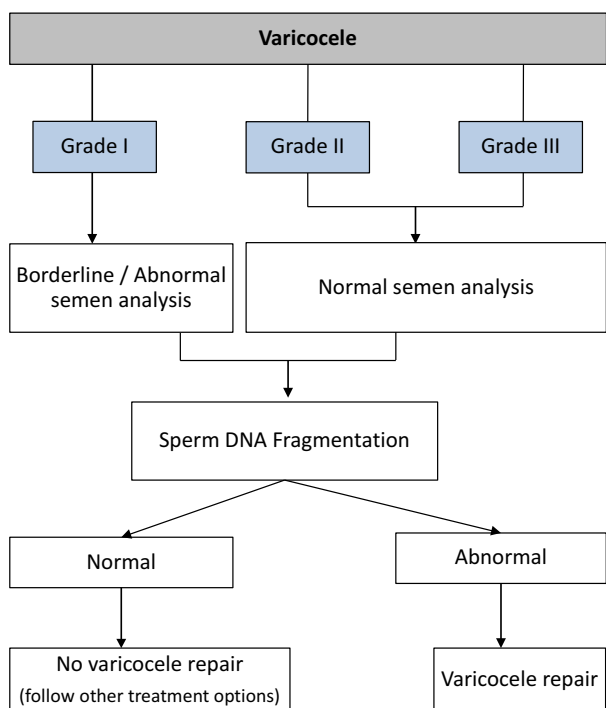

Figure 4 Algorithm for the clinical utility of sperm DNA fragmentation in patients with varicocele.
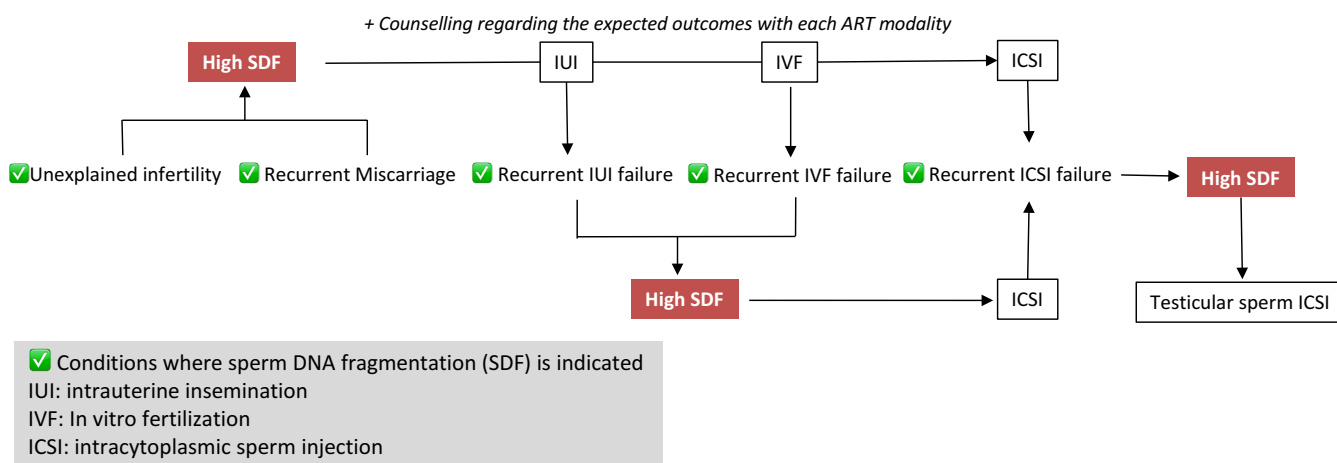

Figure 5 Algorithm for the clinical utility of sperm DNA fragmentation in assisted reproductive treatments.

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explain why the risk of miscarriage is increased in the face of high SDF whereas clinical pregnancy rates may not be compromised (195).

As far as remedies for recurrent miscarriage/repeated IVF/ICSI failure related to high SDF are concerned, SDF results may guide clinicians as well. Patients can be offered any means to decrease SDF (if a correctable cause is identified) or ICSI with testicular sperm. The latter is based on the increasing evidence indicating that testicular sperm have lower SDF levels than ejaculated sperm and result in superior ICSI outcomes (23,85,125,195,199) (*Figure 5*).

Clinical scenario 4 (borderline abnormal or normal conventional semen analysis with life-style risk factors for SDF): there is insufficient evidence that lifestyle modification will result in resolution of SDF or improve fertility outcomes

At first glance, the clinical utility of SDF seems to be least convincing in patients with lifestyle risk factors, especially when no other reversible reasons for high SDF are detected (56,90). However, the negative impact of such risk factors on SDF has been consistently reported by several studies [reviewed by Esteves *et al.* (128)]. Furthermore, recent data indicate that a healthy dietary pattern and lifestyle decrease SDF (215,216). Additionally, evidence suggests that oral antioxidant therapy improve sperm DNA integrity and translate in better changes for establishing a pregnancy using ART (132) (*Table 3*).

Although further research is warranted to confirm the role of lifestyle changes concerning sperm DNA integrity, and how these changes may translate into better reproductive outcomes, information provided by SDF testing gives solid grounds for implementing lifestyle changes as well as monitoring patient compliance in health prevention programs. Knowledge of the SDF status can be used to strengthen patient counseling and allow clinicians to provide a more realistic prognosis of every modality the couple wishes to pursue.

Overall quality of evidence leads to level C recommendation

While all CPGs include recommendations, it is the quality of the available evidence that shapes the strength of recommendations. It is true that Agarwal *et al.*'s recommendations (20) are primarily based on levels B and C evidence and that more clinical data should be attained to support their advice further. However, CPGs issued by other societies such as the AUA, EAU, and the ASRM have also synthesized their recommendations largely based on studies of moderate to low quality (5,184-186,243), thus

reflecting the limited evidence available in other areas of infertility as well. Nevertheless, the driving force of all involved in contributing guidelines, including Agarwal and his peers, is in translating the best evidence available into practice to serve as a framework for standardized care while maintaining physician autonomy. The existent shortcoming of SDF testing should not refrain physicians to take full advantage of its clinical benefits provided the data supporting that specific test be made clear to the patient.

Threats

Specialty societies and National Institutes do not recommend the use of DNA fragmentation in the routine workup of the infertile male patient

The lack of sufficient high-grade evidence in support of the application of SDF testing in the clinical scenarios discussed above is a criticism often heard (131). Moreover, despite many alternatives to treat patients with high SDF, including oral antioxidants, varicocele ligation, frequent ejaculation, and testicular sperm extraction, good clinical evidence is still lacking to support the routine use of these alternatives. As such, guidelines from various professional societies stress the lack of robust evidence to support the clinical utility of SDF testing (131). Furthermore, practices differ globally and in certain regions (e.g., UK) National Institutes for Health neither endorses varicocele treatment for infertility nor the use of SDF testing for infertility diagnosis and management (74,114). Despite that, the potential benefit of SDF testing in daily practice is increasingly being realized. Recognizing the significant efforts from researchers that have moved SDF testing from bench to clinical practice in the twenty-first century, the Society for Translational Medicine (244) has taken a step ahead and fully endorsed the CPG for SDF testing in male infertility (20,245). Also, the society mentioned above supported the publication of a journal supplement entirely dedicated to the topic in one of its official journals -Translational Andrology and Urology-, in which this paper stands, along with a concise practice recommendation for quick clinical reference (246). This initiative, in addition to the bulk of published data since previous male infertility guidelines were issued, must provide enough fuel for a timely review and update of other societies' guidelines such as the AUA, ASRM, and EAU.

The guideline is not inclusive of all clinical conditions that may benefit from using SDF testing

Case scenarios are commonly used in medical literature to report a particular clinical condition or contextualize a situation hoping for better comprehension (247,248).

Although the use of clinical scenarios to illustrate a point may not apply to a slightly different scenario (54), Agarwal and colleagues utilized this method in their CPG (20) to personalize a message giving it a clinical perspective (90). Infertile men with advanced age, diabetes, subclinical infection, and cancer, among others, may benefit from SDF testing as well so that further response to interventional treatments could be monitored (40,63).

Opportunities
More studies have to be conducted to clarify the clinical utility of SDF test results to determine the course of treatment in the presented clinical scenarios and beyond

Well-designed studies with adequate power and standard techniques will be invaluable to refine the clinical utility of SDF testing further. In this article, we scrutinized the practice recommendations and commentaries from experts concerning its utility to identify existing gaps and potential areas for translational research. Some areas for further investigation concerns: (I) the efficacy of varicocelectomy to reduce SDF rates and their impact on pregnancy outcomes; (II) the role of female age and SDF oocyte repair capacity in natural and assisted conception; (III) the clinical utility of SDF to other categories of patients, such as cancer patients exposed to chemo/radiotherapy, and diabetes; (IV) the role of preimplantation genetic screening in cases of ICSI treatment associated to high SDF; (V) cost-effectiveness of testicular sperm and other methods to reduce SDF for ICSI; and (VI) the role of lifestyle interventions to reduce SDF rates and how the effects impact on pregnancy rates.

Conclusions

Understanding the role of SDF in male infertility requires an in-depth analysis of the multiple pathophysiological processes and the theories involved, as well as the examination of different levels of scientific evidence of published studies. Agarwal and colleagues provide a reasonable proposal for integration of SDF testing in the clinic in the ICSI era, despite inherent limitations in drawing an evidence-based clinical guideline. The uniqueness of SWOT analysis offers the possibility of evaluating this CPG on the clinical utility of SDF testing based on clinical scenarios and its accompanying commentaries written by global experts in all possible angles, thus providing rich information for clinicians and researchers alike. With further refinements in SDF testing and additional supporting evidence of its clinical utility, implementation of

SDF testing in the clinic may not only increase ART success but more importantly improve the health of both fathers to be and resulting offspring.

Footnote

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