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Global Andrology Forum Clinical Guidelines on the Relevance of Sperm DNA Fragmentation in Reproductive Medicine

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Purpose: To evaluate the evidence on sperm DNA fragmentation (SDF) and its clinical applications in reproductive medicine, highlighting benefits, limitations, and guidelines for its use to assist clinicians in objective decision-making.

Materials and Methods: A multidisciplinary team of clinicians and reproductive experts from the Global Andrology Forum (GAF) reviewed the latest evidence on SDF, covering indications, testing methods, recurrent pregnancy loss, varicocele and its repair, assisted reproductive technologies (ART), treatment of associated conditions, antioxidant therapy, and sperm selection for ART. Expert statements and recommendations were developed and graded with the GRADE system using a modified Delphi process.

Results: Based on the GAF surveys, systematic reviews, and meta-analyses related to SDF, 52 experts introduced and scored 24 statements and recommendations using the GRADE system. Of these, 87.5% (21/24) achieved strong ratings, reflecting broad consensus, while 12.5% (3/24) were rated weak. The guidelines provide evidence-based recommendations for clinical scenarios, including the role of SDF in infertility, recurrent pregnancy loss, and ART outcomes.

Conclusions: While there is growing interest and evidence regarding the clinical benefit of SDF testing and its utility in managing male infertility, significant gaps in the literature limit its routine use in clinical practice. The guidelines offer a structured framework for integrating SDF testing into male infertility management, emphasizing a tailored approach based on individual clinical scenarios. Clinicians must balance the benefits and limitations of SDF testing and antioxidant treatment to optimize care in reproductive medicine. These guidelines are critical for advancing evidence-based practices in male infertility management.

Keywords: DNA fragmentation; Infertility, male; Practice guideline; Reproductive medicine; Spermatozoa

INTRODUCTION

Sperm DNA fragmentation (SDF) refers to breakages in the DNA of mature spermatozoa and is a molecular sperm abnormality that can affect fertility potential [1,2]. There has been a growing interest in SDF testing as well as its role in the evaluation and management of infertile couples. This increased popularity is reflected by a trend of more publications on the role of SDF as a diagnostic and prognostic factor in male in-

fertility, with literature supporting its use as an ancillary measure of sperm quality beyond the conventional semen parameters [3]. In addition, the 2021 World Health Organization (WHO) laboratory manual for the examination and processing of human semen included SDF as an extended semen evaluation and discussed four assays for measuring SDF [4]. Despite this interest, there remain controversies regarding SDF's utility and its implementation in clinical practice.

The Global Andrology Forum (GAF) has launched a worldwide survey regarding the clinical applications of SDF, including indications for testing, technical aspects for testing, and management approaches [5,6]. A total of 436 reproductive clinicians from 55 countries responded to the survey, providing, for the first time, insight into the current landscape of SDF implementation worldwide [6]. From the survey responses, the professional society guidelines, and published evidence, expert recommendations related to various aspects of SDF testing and treatment were devised and reviewed by 63 experts in andrology and reproductive medicine using the Delphi method to reach a consensus. These recommendations were published along with the survey responses to provide an extensive reference to clinicians worldwide on testing, treating, and counseling infertile couples with elevated SDF [5,6]. However, no recommendations were graded in this GAF-supported survey.

This article will discuss the current state of evidence on SDF and its potential applications in reproductive medicine, highlighting both benefits and limitations to allow unbiased clinical judgment and also to present clinical guidelines with the statements and recommendations, along with appropriate grading, which will be provided to guide the clinicians and researchers in an objective, clinically applicable manner.

MATERIALS AND METHODS

Clinicians and reproductive experts from the GAF reviewed contemporary evidence on SDF testing indications, methods, and therapeutic approaches in the context of male infertility. Special emphasis was placed on high-quality studies, systematic reviews and meta-analyses, professional society guidelines, and the GAF's previous global surveys on SDF, which reflect the global practice pattern of SDF use. Evidence highlighting the benefits and limitations of SDF testing and treatment is presented.

A GAF expert panel used the Delphi method [7] to develop new consensus agreements on the use of SDF testing and treatment alternatives. This panel represents clinicians, including urologists and andrologists worldwide, who are involved in the management of male infertility and have more than five years of experience in male infertility clinical practice.

To support the clinical evidence for the statements and recommendations, the level of each literature's evidence was graded based on the Oxford Centre for Evidence-based Medicine recommendations and provided in a concise format.

A total of 24 statements and recommendations were included in the survey. An invitation email with clear instructions was sent to a selected group of GAF experts, considering various ages, academic positions, geographical distributions, and subspecialties. The invitation included a description of the Delphi method, complete instructions, a link to the survey, and a copy of the manuscript. Any statements/recommendations that did not reach a score of 7/10 by 80% of the respondents were revised based on the feedback and subjected to further assessment rounds until consensus was reached for all recommendations.

The final recommendations were then graded by 52 GAF experts as "Strong" or "Weak" as per the guidelines of the GRADE working group [8], which take into account the quality of evidence, the "balance between desirable and undesirable effects," and cost. Recommendations that were rated "strong" by at least 70% of experts were accepted as "strong," while the other recommendations were graded as "weak." All panels agreed with the final list of statements and recommendations on SDF presented in this article. No ethical clearance was necessary.

RESULTS

Table 1 lists all the statements, recommendations, and expert opinions on using SDF in reproductive medicine, with the grading (strong *vs* weak) of the statements and recommendations.

Table 2 presents the results of the expert grading of the statements and recommendations on the strength of 24 statements and recommendations using the GRADE system. Out of the 24 statements and recommendations, 21 (87.5%) were considered successful,

Table 1. Statements, recommendations, and expert opinions on the use of SDF in reproductive medicine with the grading (strong *vs* weak) of the statements and recommendations

Statement category	Statements, recommendations, and expert opinions on the use of SDF in reproductive medicine	Grade of the statements and recommendations
Unexplained and idiopathic male infertility	#1. All men with unexplained or idiopathic infertility, based on a thorough negative diagnostic work-up, should be counseled to undergo SDF testing.	Strong
Recurrent pregnancy loss	#2. SDF testing may be considered in the work-up of any RPL, regardless of conventional semen parameters.	Strong
Clinical varicocele	#3. SDF testing may be considered in the work-up of a clinical varicocele, but it is not indicated for a subclinical varicocele.	Strong
	#4. SDF testing can assist in clinical decision-making in men with clinical varicoceles in the presence of normal semen parameters.	Weak
ART	#5. Elevated SDF can have detrimental impacts on ART and is associated with lower clinical pregnancy and live birth rates after IVF.	Strong
	#6. SDF testing can be considered when ART is planned due to a male factor, especially if there has been a previous failure of ART.	Strong
	#7. SDF testing is recommended after recurrent ART failure, including recurrent fertilization failure, recurrent implantation failure, or RPL after IUI, IVF, or ICSI.	Strong
	#8. In the setting of ART failure, SDF testing should be offered for men with unexplained or idiopathic infertility, men over 40 years, and men with risk factors for high SDF, given the female partner has a normal work-up.	Strong
	#9. In couples undergoing ART with elevated SDF in the male partner, additional measures to lower SDF levels may be beneficial to improve ART success.	Strong
SDF testing methods	#10. TUNEL, Comet, SCD, and SCSA are the recommended assays for measuring SDF.	Strong
	#11. There are no standard cut-off values of SDF, and every laboratory should establish its own reference values using appropriate controls and fertility outcomes.	Strong
	#12. Ordering a second confirmation test for elevated SDF is not necessary for diagnosis.	Weak
Treatment of associated conditions and risk factors	#13. SDF testing should be considered for infertile men who have risk factors for infertility such as smoking, aging, obesity, radiation exposure, chemical exposure, alcohol, and genitourinary infections.	Strong
	#14. Conservative first-line management of elevated SDF involves addressing underlying causes and risk factors, lifestyle modification strategies and reduced ejaculatory abstinence (12-24 hours) before attempting conception (natural or by ART).	Strong
	#15. Lifestyle modification strategies, including weight loss, cessation of smoking and alcohol use, treatment of genitourinary infections, and elimination of toxic exposure, are recommended for high SDF-associated male infertility, RPL, and ART failure.	Strong
Antioxidants	#16. Antioxidant treatment significantly increases spontaneous pregnancy compared to controls.	Weak
	#17. Antioxidants can be offered to men with high SDF, particularly those with risk factors known to increase oxidative stress.	Strong
	#18. The effects of antioxidant treatment on live birth and miscarriage rates have been controversial.	Strong
	#19. There is no consensus on the type, dosage, and duration of antioxidant treatment for infertile men with high SDF.	Strong
Varicocele repair	#20. Varicocele repair may significantly reduce SDF in patients with elevated levels.	Strong
	#21. Varicocele repair should be offered to infertile men with clinical varicocele and elevated SDF.	Strong
	#22. SDF testing should be ordered for men who still have persistent infertility after varicocele repair, regardless of improvement in conventional semen parameters.	Strong
Sperm selection and testicular sperm	#23. Sperm selection techniques for ART can be used in cases with elevated SDF and in the setting of ART failure.	Strong
	#24. Testicular sperm for ART are not recommended for routine use in cases with elevated SDF but may be considered in those with elevated SDF after addressing potential underlying causes and in the setting of ART failure. When used, couples should be informed that this approach is based on low-quality evidence.	Strong

SDF: sperm DNA fragmentation, IUI: intrauterine insemination, IVF: *in vitro* fertilization; ICSI: intracytoplasmic sperm injection, RPL: recurrent pregnancy loss, SRMA: systematic review and meta-analysis, SCD: sperm chromatin dispersion, SCSA: sperm chromatin structure assay, ART: assisted reproductive technologies.

Table 2. Results of expert grading on the strength of 24 statements and recommendations, using the GRADE system

Recommendation (#)	Weak (#)	Weak (%)	Strong (#)	Strong (%)
1	7	13.46	45	86.54[a]
2	7	13.46	45	86.54[a]
3	14	26.92	38	73.08[a]
4	17	33.33	34	66.67
5	8	15.38	44	84.62[a]
6	7	13.46	45	86.54[a]
7	4	7.69	48	92.31[a]
8	13	25.00	39	75.00[a]
9	3	5.88	48	94.12[a]
10	5	9.80	46	90.20[a]
11	12	23.53	39	76.47[a]
12	23	45.10	28	54.90
13	13	25.00	39	75.00[a]
14	5	9.62	47	90.38[a]
15	4	7.69	48	92.31[a]
16	35	68.63	16	31.37
17	11	21.57	40	78.43[a]
18	8	15.38	44	84.62[a]
19	3	5.77	49	94.23[a]
20	7	13.46	45	86.54[a]
21	8	15.38	44	84.62[a]
22	15	28.85	37	71.15[a]
23	14	27.45	37	72.55[a]
24	11	21.15	41	78.85[a]
Average		20.54		79.46[a]

The table shows the total number of responses for each recommendation, the number and percentage of "weak" grades, and the number and percentage of "Strong" grades.
[a]Recommendations scoring above 70%.

having received strong ratings from over 70% of the experts. The rest 3 (12.5%) were rated as "weak" supporting, with the ranges of 31.37% to 66.67% of the expert participants.

DISCUSSION

1. Indications and benefits of SDF testing

1) Unexplained and Idiopathic Male Infertility

Clinical evidence: Elevated SDF levels have been associated with 7 times higher odds of failing to achieve a natural pregnancy, with a positive predictive value of 83% [2]. In a cohort of patients with unexplained infertility and normal conventional semen parameters, using the sperm chromatin structure assay (SCSA), 26.1% had sperm DNA fragmentation index (DFI) ≥20% (normal range of <20%) [9]. Similarly, another study found that among 126 normozoospermic infertile men, using the SCSA, 15% had DFI >20%, and 5% had DFI >30% (normal range of ≤20%) [10].

Furthermore, Boeri et al. [11] found that 1 in 2 (52.7%) men with idiopathic infertility (n=792), *i.e.*, having at least one abnormality of conventional semen parameters but unknown underlying etiology, have SDF levels ≥30%, using the SCSA (normal range of <30%) [11]. Moreover, the clinical, hormonal, and semen parameters of males with idiopathic male infertility and high DFI were worse than those with normal DFI [11].

Additionally, studies have reported that high SDF levels were significantly correlated with paternal age [12-14]. Using the SCSA, Das et al [12] found that the prevalence of DFI >30% (normal range of ≤30%) was significantly higher among normozoospermic infertile men aged ≥40 years compared to those <40% (17% *vs* 3%; p<0.001). Evenson et al [13] demonstrated a more than double increase in SDF levels, using SCSA, after the age of 41.6 years in 25,455 males attending infertility clinics. Recently, Pozzi et al [14] conducted a cross-sectional study on potential predictors of elevated SDF and included 515 males with primary infertility. In their multivariate logistic regression model, paternal age ≥38 years was an independent predictor of SDF levels >30%, using the SCSA (normal range of ≤30%) (odds ratio [OR]=2.43, 95% confidential interval [95% CI] 1.71-3.46; p<0.001). The increased SDF with aging may be explained by higher oxidative stress and a decline in the apoptotic potential of spermatogenic germ cells, allowing the production of more sperm with fragmented DNA.

Therefore, these findings indicate that DFI is a potentially clinically useful marker of male fertility, as it can explain at least some cases of 'unexplained male infertility' and 'idiopathic male infertility'.

GAF Statement/Recommendation #1: All men with unexplained or idiopathic infertility, based on a thorough negative diagnostic workup, should be counseled to undergo SDF testing (Grade: Strong).

2) Recurrent pregnancy loss

Clinical evidence: SDF testing also plays a role in the evaluation of couples experiencing recurrent pregnancy loss (RPL). Several meta-analyses have been published that consistently demonstrated an association between RPL and SDF. The latest analysis by Inversetti et al

[15] included 20 studies with 1,199 RPL patients and 1,301 fertile controls and reported significantly higher SDF levels among those with RPL (mean difference 9.21, 95% CI 5.58–12.85; p<0.001). In another recent meta-analysis, conducted with 14 studies including 530 male partners of couples that experience RPL and 639 fertile controls, a significant association between RPL and SDF has been demonstrated with an average mean difference of 11.98 (95% CI 6.64–17.32; p<0.001), indicating that male partners of couples with RPL had significantly higher SDF values than the control group [16]. The American Urological Association/ the American Society for Reproductive Medicine (AUA/ASRM) and the European Association of Urology (EAU) guidelines describe a strong association between RPL and elevated SDF [17,18].

GAF Statement/Recommendation #2: SDF testing may be considered in the workup of any RPL, regardless of conventional semen parameters (Grade: Strong).

3) Clinical varicocele

Clinical evidence: Another potential benefit of SDF testing is to guide the management of infertile males with clinical varicocele. This is particularly important as patients with clinical varicocele may harbor elevated SDF levels but have normal sperm morphology [19]. A meta-analysis conducted by Wang et al [20] involving 12 studies revealed that varicocele was associated with significantly higher levels of SDF compared with controls with a mean difference of 9.84% (95% CI 9.19–10.49; p<0.001). The same meta-analysis showed that varicocele treatment significantly reduced SDF levels compared to the control group (mean difference of -3.37%; 95% CI -4.09 to -2.65; p<0.001). Regarding subclinical varicocele, there was no difference in SDF levels between infertile males with subclinical varicocele and fertile males with no varicocele [19]. Additionally, subclinical varicocele repair was not found to improve SDF levels [21]. These studies indicate that SDF testing in patients with clinical varicocele can guide the need for intervention, particularly if the patient has normal conventional semen parameters.

GAF Statement/Recommendation #3: SDF testing may be considered in the workup of a clinical varicocele, but it is not indicated for a subclinical varicocele (Grade: Strong).

GAF Statement/Recommendation #4: SDF testing can assist in clinical decision-making in men with clinical varicoceles in the presence of normal semen parameters (Grade: Weak).

4) Assisted reproductive technologies

Clinical evidence: Regarding the effects of SDF on intrauterine insemination (IUI), Bungum et al [22] reported that DFI was an independent predictor of outcomes, using the SCSA, with levels >30% associated with lower biochemical pregnancy, clinical pregnancy, and delivery rates (p<0.05 for all). In the meta-analysis by Chen et al [23], including 10 studies with 2,869 IUI cycles, a higher SDF level was significantly associated with lower pregnancy rates (risk ratio 0.34, 95% CI 0.22–0.52; p<0.001).

SDF can also have detrimental impacts on *in-vitro* fertilization (IVF) outcomes. Meta-analyses have shown that high SDF levels are associated with lower pregnancy rates and higher miscarriage rates after IVF [1,2,24]. In couples undergoing IUI and IVF with elevated SDF in the male partner, additional measures to lower SDF levels may be beneficial to improve assisted reproductive technologies (ART) success.

However, conflicting evidence also exists. The predictive value of the same assay for SDF assessment for clinical pregnancy is very heterogeneous between the studies due to study inclusion criteria, the timing of SDF testing (before or at the time of ART), patients' age and other confounding factors [25]. A study of more than 2,600 ART cycles found that different SDF levels measured by the SCSA did not influence oocyte fertilization, good-quality embryos, or clinical pregnancy rates [26]. In a meta-analysis of 23 retrospective cohort studies, the clinical pregnancy rate after ART did not differ between low and high SDF levels overall [1].

Interestingly, when analyzing IVF and intracytoplasmic sperm injection (ICSI) cycles separately, clinical pregnancy rates were significantly lower with high SDF in IVF but not ICSI [1]. Similar results were also found by Ribas-Maynou et al [24] in their meta-analysis, where high SDF did not influence clinical pregnancy in 5,467 ICSI cycles with 25 studies or live birth rates in 3,017 cycles with nine studies. Echoing these findings, using the SCSA in 2,713 infertile couples who underwent ART (a total of 5,422 cycles), the cumulative live birth rate (CLBR) in couples with high sperm DFI (≥20%) was investigated [27]. With IVF, high sperm DFI predicted a statistically significantly lower CLBR, compared to normal sperm DFI (41.6% *vs* 48.1%), while

CLBR in ICSI did not show any difference between high sperm DFI and normal sperm DFI [27]. These different outcomes may be attributed to the technique of ICSI, where oocyte repair can start earlier and where there is no overnight culture, reducing the risk of laboratory-induced damage and exposure to oxidative stress in semen. In fact, high DFI did not significantly affect the outcome of ICSI in oocyte donation cycles [28]. Therefore, if the male factor is an indication for ICSI, there is likely no benefit of SDF detection.

High sperm DFI has also recently been associated with obstetric complications following ART, including preterm birth and preeclampsia [29]. In the entire cohort of 1,594 infertile couples, using the SCSA, a DFI ≥20% was associated with increased odds of preterm birth (OR 1.4, 95% CI 1.0–2.0; p=0.03), while the OR for preeclampsia was statistically significantly increased in the group with DFI ≥20% when IVF was used as fertilization method (OR 2.2, 95% CI 1.1–4.4; p=0.02), but not in the ICSI group [29].

GAF Statement/Recommendation #5: Elevated SDF can have detrimental impacts on ART and is associated with lower clinical pregnancy and live birth rates after IVF (Grade: Strong).

GAF Statement/Recommendation #6: SDF testing can be considered when ART is planned due to a male factor, especially if there has been a previous failure of ART (Grade: Strong).

GAF Statement/Recommendation #7: SDF testing is recommended after recurrent ART failure, including recurrent fertilization failure, recurrent implantation failure, or RPL after IUI, IVF, or ICSI (Grade: Strong).

GAF Statement/Recommendation #8: In the setting of ART failure, SDF testing should be offered for men with unexplained or idiopathic infertility, men over 40 years, and men with risk factors for high SDF, given the female partner has a normal workup (Grade: Strong).

GAF Statement/Recommendation #9: In couples undergoing ART with elevated SDF in the male partner, additional measures to lower SDF levels may be beneficial to improve ART success (Grade: Strong).

5) SDF testing methods

Clinical evidence: The WHO Manual of Human Semen Analysis did mention TUNEL, Comet, sperm chromatin dispersion (SCD), and SCSA as methods to evaluate SDF [4]. Nevertheless, WHO endorsed no specific testing, and there is a lack of clinical guidance in the manual. These four assays are validated and reliable methods for measuring SDF levels but differ in their technique, with different advantages and drawbacks to each method. The method to test for SDF should take into consideration the availability of resources, personnel, and laboratory complexity of the tests. Moreover, the absence of well-defined and validated cut-offs or threshold values for specific outcomes limits the broader use of SDF testing and is considered the major handicap of SDF testing in clinical practice [30]. Ordering a second confirmation test for elevated SDF is unnecessary for diagnosis [6]. The correlations of SDF with specific fertility outcomes also vary with the type of assay, with some showing a poor predictive value of SDF for ART outcomes [31]. Lack of standardization in other aspects, such as the assay type, timing, and sperm preparation method, can cause problems with SDF interpretation, explaining the inconsistency of SDF's predictive value among studies [25].

GAF Statement/Recommendation #10: TUNEL, Comet, SCD, and SCSA are the recommended assays for measuring SDF (Grade: Strong).

GAF Statement/Recommendation #11: There are no standard cut-off values of SDF, and every laboratory should establish its own reference values using appropriate controls and fertility outcomes (Grade: Strong).

GAF Statement/Recommendation #12: Ordering a second confirmation test for elevated SDF is not necessary for diagnosis (Grade: Weak).

2. Management of elevated SDF

1) Treatment of associated conditions and risk factors

Clinical evidence: After detecting elevated SDF levels in couples experiencing adverse reproductive outcomes, it is important to consider potential management strategies. Many modalities have been investigated, beginning with those that address the underlying causes and risk factors. Some modifiable risk factors for elevated SDF include smoking, heavy alcohol consumption, obesity, diabetes mellitus, and occupational and environmental exposures [5,32]. Additionally, SDF testing may help uncover a semen infection and/or an unrecognized prolonged abstinence time [33-36]. The management will, therefore, include lifestyle modification and risk avoidance, antibiotics for genital tract

infections, weight loss, and diabetes control. Although these are typically recommended as first-line approaches, there is no strong evidence behind their application. For example, small uncontrolled clinical studies have shown the benefit of weight loss and antibiotics [34,37], while there are no studies demonstrating a benefit in avoidance of risk factors on reduction of SDF levels, such as smoking cessation. Nonetheless, as most of these interventions (*e.g.,* antibiotics, weight loss, smoking cessation, alcohol reduction) are minimally invasive and low risk, every effort should be made to minimize sperm DNA damage. Another strategy to address SDF is to reduce abstinence time. Studies have shown that a short abstinence period compared to a long abstinence time is associated with significantly lower SDF levels and improved reproductive outcomes [36,38,39].

GAF Statement/Recommendation #13: SDF testing should be considered for infertile men who have risk factors for infertility, such as smoking, aging, obesity, radiation exposure, chemical exposure, alcohol, and genitourinary infections (Grade: Strong).

GAF Statement/Recommendation #14: Conservative first-line management of elevated SDF involves addressing underlying causes and risk factors, lifestyle modification strategies, and reduced ejaculatory abstinence (12–24 hours) before attempting conception (natural or by ART) (Grade: Strong).

GAF Statement/Recommendation #15: Lifestyle modification strategies, including weight loss, cessation of smoking and alcohol use, treatment of genitourinary infections, and elimination of toxic exposure, are recommended for high SDF-associated male infertility, RPL, and ART failure (Grade: Strong).

2) Antioxidants

Clinical evidence: Given the role of oxidative stress in the pathogenesis of SDF, the effect of oral antioxidant therapy has been evaluated in this context, and studies have shown a reduction in SDF levels associated with treatment [40]. In the latest Cochrane database of 90 randomized controlled studies with 10,303 subfertile males, de Ligny and colleagues evaluated the effectiveness and safety of 20 different oral antioxidants in treating male subfertility [41]. They found that antioxidant treatment significantly increased the live birth rate with an OR of 1.43 and the clinical pregnancy rate with an OR of 1.89. In addition, they found no significant difference in miscarriage rate between antioxidant and placebo or no treatment groups. However, low-certainty evidence from these randomized controlled trials suggests that antioxidant supplementation in sub-fertile males may improve clinical pregnancy and live birth rates for couples attending fertility clinics.

Recently, an improvement in sperm parameters and spontaneous pregnancy rate has been reported after the administration of antioxidant treatment to infertile males in a systematic review and meta-analysis (SRMA) conducted with 45 randomized controlled trials, including 4,332 patients treated with antioxidants or no treatment/placebo [42]. They found that antioxidant treatment significantly increased spontaneous pregnancy compared to controls (OR=1.97, 95% CI 1.28–3.04; p<0.01). Even sperm parameters, including sperm concentration, motility, and normal sperm morphology, significantly improved after antioxidant treatment, compared to placebo or no treatment. However, this review found no significant effect of antioxidant treatment on live birth rate or miscarriage rates. For the first time in the literature and in addition to previous SRMAs, this article also showed the positive impact of antioxidant treatment on seminal total antioxidant capacity and seminal malondialdehyde acid levels compared to controls [42].

Evidence from the SRMAs suggests that antioxidant therapy for at least 3 months may improve sperm quality and conventional sperm parameters, leading to down-staging or shifting the level of ART and improving the chances of success for pregnancy [40,42]. In addition, antioxidant therapy may decrease SDF levels and increase spontaneous pregnancy and live birth rates in the presence of normal female evaluation or correctable female pathologies. Therefore, the presence of reduced SDF under antioxidant therapy would increase the chances of live birth [41].

Most evidence supporting the use of antioxidant therapy for SDF is based on uncontrolled trials, and there is no standard regimen, dose, or duration for antioxidant use for elevated SDF. Despite these considerations, 50% of reproductive clinicians always prescribe antioxidants for infertile males with elevated SDF, and 40% prescribe them depending on underlying or associated factors, such as smoking, noxious exposures, obesity, and advanced paternal age [6].

GAF Statement/Recommendation #16: Antioxidant treatment significantly increases spontaneous preg-

nancy compared to controls (Grade: Weak).

GAF Statement/Recommendation #17: Antioxidants can be offered to men with high SDF, particularly those with risk factors known to increase oxidative stress (Grade: Strong).

GAF Statement/Recommendation #18: The effects of antioxidant treatment on live births and miscarriage rates have been controversial (Grade: Strong).

GAF Statement/Recommendation #19: There is no consensus on the type, dosage, and duration of antioxidant treatment for infertile men with high SDF (Grade: Strong).

3) Varicocele repair

Clinical evidence: Varicocele repair has been shown to significantly reduce SDF in patients with elevated levels, as demonstrated by Cannarella et al [43] in their SRMA with 29 studies on infertile males with clinical varicocele who underwent varicocele repair. The pooled results showed significant reductions in SDF levels after varicocelectomy with a standardized mean difference of -1.26 ($p<0.001$). The lower SDF levels after surgery have been associated with improved pregnancy rates with both natural and assisted reproduction [44]. Similarly, in a review of 21 studies evaluating the effect of varicocelectomy on SDF, all studies reported a significant decrease in SDF rates after varicocele repair in a follow-up period ranging from 3 to 12 months [45]. Another recent meta-analysis by Lira Neto et al [46], consisting of more than 1,000 patients, demonstrated that varicocele repair decreased sperm DFI by 7.23% (95% CI, -8.86 to -5.59) in males with clinical varicocele, ranging from 2.3% to 16.3% decline in DFI among the included studies. These findings echo those of the SRMA by Qiu et al [47], who reported a 6.14% (95% CI, -6.90 to -5.37) decrease in SDF after varicocele repair.

The EAU guidelines recommend varicocelectomy in patients with elevated SDF and otherwise unexplained infertility or after failure of ARTs [18].

GAF Statement/Recommendation #20: Varicocele repair may significantly reduce SDF in patients with elevated levels (Grade: Strong).

GAF Statement/Recommendation #21: Varicocele repair should be offered to infertile men with clinical varicocele and elevated SDF (Grade: Strong).

GAF Statement/Recommendation #22: SDF testing should be ordered for men who still have persistent infertility after varicocele repair, regardless of improvement in conventional semen parameters (Grade: Strong).

4) Sperm selection and testicular sperm

Clinical evidence: Sperm selection techniques such as magnetically activated cell sorting, physiologic ICSI, and microfluidic cell sorting have also been investigated and shown to reduce high SDF [48]. However, there are no studies demonstrating fertility outcomes when they are employed in cases of elevated SDF. The use of testicular sperm for ICSI has become a commonly utilized strategy in males with high SDF, with studies reporting higher pregnancy and live birth rates with the utilization of testicular compared to ejaculated sperm [49]. The rationale for this treatment approach is that testicular sperm have lower SDF than ejaculated sperm because DNA damage may, in part, be due to a post-testicular injury [50-52]. However, the quality of evidence supporting the use of testicular sperm remains low, with no randomized controlled trials evaluating this strategy [49].

GAF Statement/Recommendation #23: Sperm selection techniques for ART can be used in cases with elevated SDF and in the setting of ART failure (Grade: Strong).

GAF Statement/Recommendation #24: Testicular sperm for ART are not recommended for routine use in cases with elevated SDF but may be considered in those with elevated SDF after addressing potential underlying causes and in the setting of ART failure. When used, couples should be informed that this approach is based on low-quality evidence (Grade: Strong).

3. Current limitations and the utility of the GAF clinical practice guidelines

SDF testing is currently not endorsed as a routine assessment of sperm by any of the medical organizations, likely because controversies continue to exist regarding its actual clinical utility [17,18]. With a wide range of mechanisms for the occurrence of DNA strand breaks in mature sperm, perhaps the underlying cause of high SDF may influence outcomes rather than the SDF itself. There is also a lack of well-designed studies and research data allowing to draw robust conclusions on SDF. This is attributed to underpowered studies, methodology flaws, and no consideration of relevant confounders such as female fertility factors. Currently, there are no high-quality studies and clinical trials on the management of elevated SDF in general. Addition-

ally, there are no strategies with a proven benefit on clinical pregnancy or live birth rates in ART if high SDF is detected.

To address the controversies and lack of guidelines from professional societies regarding the clinical utility of SDF, the GAF developed a consensus statement from a selected panel of expert reproductive clinicians. These clinical practice guidelines are presented herein and are designed to assist the busy clinician in implementing SDF testing and treatment for the appropriate patient. The clinician must weigh the pros and cons of SDF testing tailored for each case to avoid unnecessary diagnostic costs to the patient. In general, unnecessary testing should be avoided, especially if the outcome of such an assessment would not direct the treatment plan.

CONCLUSIONS

While there is growing interest and evidence regarding the clinical benefit of SDF testing and its utility in managing male infertility, major gaps in the literature remain that provide significant limitations, hampering its routine clinical implementation. These guidelines, with the current clinical practice statements and recommendations, highlight SDF's benefits and limitations, based on the GAF surveys, recent comparative, randomized studies, systematic reviews, and meta-analyses on SDF.

The current GAF guidelines will guide clinicians and researchers in implementing clear management strategies for infertile males who need SDF testing or have elevated SDF. They will also help answer practical questions for which rigorous evidence may not yet be available. In clinical reproductive medicine, the clinician must weigh the pros and cons of SDF testing and antioxidant treatment tailored for each case. Future high-quality randomized controlled trials are needed to make strong recommendations regarding the SDF in clinical reproductive medicine.

Conflict of Interest

The authors have nothing to disclose.

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Author Contribution

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REFERENCES

1. Deng C, Li T, Xie Y, Guo Y, Yang QY, Liang X, et al. Sperm DNA fragmentation index influences assisted reproductive technology outcome: a systematic review and meta-analysis combined with a retrospective cohort study. Andrologia 2019;51:e13263.
2. Zini A. Are sperm chromatin and DNA defects relevant in the clinic? Syst Biol Reprod Med 2011;57:78-85.
3. Baskaran S, Agarwal A, Panner Selvam MK, Finelli R, Robert KA, Iovine C, et al. Tracking research trends and hotspots in sperm DNA fragmentation testing for the evaluation of male infertility: a scientometric analysis. Reprod Biol Endocrinol 2019;17:110.
4. World Health Organization (WHO), Human Reproduction Programme. WHO laboratory manual for the examination and processing of human semen. 6th ed. WHO; 2021.
5. Agarwal A, Farkouh A, Saleh R, Abdel-Meguid Hamoda TA, Harraz AM, Kavoussi P, et al.; Global Andrology Forum. Controversy and consensus on indications for sperm DNA fragmentation testing in male infertility: a global survey, current guidelines, and expert recommendations. World J Mens Health 2023;41:575-602.
6. Farkouh A, Agarwal A, Hamoda TAA, Kavoussi P, Saleh R, Zini A, et al.; Global Andrology Forum. Controversy and consensus on the management of elevated sperm DNA fragmentation in male infertility: a global survey, current guidelines, and expert recommendations. World J Mens Health 2023;41:809-47.
7. Dalkey N, Helmer O. An Experimental Application of the DELPHI Method to the Use of Experts. Management Science 1963;9:458-67.
8. Guyatt GH, Oxman AD, Kunz R, Falck-Ytter Y, Vist GE, Libe-

rati A, et al.; GRADE Working Group. Going from evidence to recommendations. BMJ 2008;336:1049-51.
9. Oleszczuk K, Augustinsson L, Bayat N, Giwercman A, Bungum M. Prevalence of high DNA fragmentation index in male partners of unexplained infertile couples. Andrology 2013;1:357-60.
10. Erenpreiss J, Elzanaty S, Giwercman A. Sperm DNA damage in men from infertile couples. Asian J Androl 2008;10:786-90.
11. Boeri L, Pozzi E, Belladelli F, Corsini C, Cilio S, Bertini A, et al. One out of two idiopathic infertile men has pathologic sperm DNA fragmentation values: potential implications for clinical practice. Clin Endocrinol (Oxf) 2024;101:153-61.
12. Das M, Al-Hathal N, San-Gabriel M, Phillips S, Kadoch IJ, Bissonnette F, et al. High prevalence of isolated sperm DNA damage in infertile men with advanced paternal age. J Assist Reprod Genet 2013;30:843-8.
13. Evenson DP, Djira G, Kasperson K, Christianson J. Relationships between the age of 25,445 men attending infertility clinics and sperm chromatin structure assay (SCSA®) defined sperm DNA and chromatin integrity. Fertil Steril 2020;114:311-20.
14. Pozzi E, Fallara G, Belladelli F, Corsini C, Raffo M, Candela L, et al. Clinical parameters associated with altered sperm DNA fragmentation index among primary infertile men: Findings from a real-life cross-sectional study. Andrology 2023;11:1694-701.
15. Inversetti A, Bossi A, Cristodoro M, Larcher A, Busnelli A, Grande G, et al. Recurrent pregnancy loss: a male crucial factor-A systematic review and meta-analysis. Andrology 2025;13:130-45.
16. Tan J, Taskin O, Albert A, Bedaiwy MA. Association between sperm DNA fragmentation and idiopathic recurrent pregnancy loss: a systematic review and meta-analysis. Reprod Biomed Online 2019;38:951-60.
17. Schlegel PN, Sigman M, Collura B, De Jonge CJ, Eisenberg ML, Lamb DJ, et al. Diagnosis and treatment of infertility in men: AUA/ASRM guideline part I. J Urol 2021;205:36-43.
18. Salonia A, Bettocchi C, Capogrosso P, Carvalho J, Corona G, Dinkelman-Smit G, at al. EAU guidelines on sexual and reproductive health. European Association of Urology (EAU); 2024.
19. Ni K, Steger K, Yang H, Wang H, Hu K, Zhang T, et al. A comprehensive investigation of sperm DNA damage and oxidative stress injury in infertile patients with subclinical, normozoospermic, and astheno/oligozoospermic clinical varicocoele. Andrology 2016;4:816-24.
20. Wang YJ, Zhang RQ, Lin YJ, Zhang RG, Zhang WL. Relationship between varicocele and sperm DNA damage and the effect of varicocele repair: a meta-analysis. Reprod Biomed Online 2012;25:307-14.
21. García-Peiró A, Ribas-Maynou J, Oliver-Bonet M, Navarro J, Checa MA, Nikolaou A, et al. Multiple determinations of sperm DNA fragmentation show that varicocelectomy is not indicated for infertile patients with subclinical varicocele. Biomed Res Int 2014;2014:181396.
22. Bungum M, Humaidan P, Axmon A, Spano M, Bungum L, Erenpreiss J, et al. Sperm DNA integrity assessment in prediction of assisted reproduction technology outcome. Hum Reprod 2007;22:174-9.
23. Chen Q, Zhao JY, Xue X, Zhu GX. The association between sperm DNA fragmentation and reproductive outcomes following intrauterine insemination, a meta analysis. Reprod Toxicol 2019;86:50-5.
24. Ribas-Maynou J, Yeste M, Becerra-Tomás N, Aston KI, James ER, Salas-Huetos A. Clinical implications of sperm DNA damage in IVF and ICSI: updated systematic review and meta-analysis. Biol Rev Camb Philos Soc 2021;96:1284-300.
25. Cissen M, Wely MV, Scholten I, Mansell S, Bruin JP, Mol BW, et al. Measuring sperm DNA fragmentation and clinical outcomes of medically assisted reproduction: a systematic review and meta-analysis. PLoS One 2016;11:e0165125.
26. Yang H, Li G, Jin H, Guo Y, Sun Y. The effect of sperm DNA fragmentation index on assisted reproductive technology outcomes and its relationship with semen parameters and lifestyle. Transl Androl Urol 2019;8:356-65.
27. Malić Vončina S, Stenqvist A, Bungum M, Schyman T, Giwercman A. Sperm DNA fragmentation index and cumulative live birth rate in a cohort of 2,713 couples undergoing assisted reproduction treatment. Fertil Steril 2021;116:1483-90.
28. Antonouli S, Papatheodorou A, Panagiotidis Y, Petousis S, Prapas N, Nottola SA, et al. The impact of sperm DNA fragmentation on ICSI outcome in cases of donated oocytes. Arch Gynecol Obstet 2019;300:207-15.
29. Stenqvist A, Bungum M, Pinborg AB, Bogstad J, Englund AL, Grøndahl ML, et al. High sperm deoxyribonucleic acid fragmentation index is associated with an increased risk of preeclampsia following assisted reproduction treatment. Fertil Steril 2025;123:97-104.
30. Panner Selvam MK, Agarwal A. A systematic review on sperm DNA fragmentation in male factor infertility: laboratory assessment. Arab J Urol 2018;16:65-76.
31. Simon L, Emery BR, Carrell DT. Review: Diagnosis and impact of sperm DNA alterations in assisted reproduction. Best Pract Res Clin Obstet Gynaecol 2017;44:38-56.
32. Agarwal A, Majzoub A, Baskaran S, Panner Selvam MK, Cho CL, Henkel R, et al. Sperm DNA fragmentation: a new guide-

line for clinicians. World J Mens Health 2020;38:412-71.
33. Farahani L, Tharakan T, Yap T, Ramsay JW, Jayasena CN, Minhas S. The semen microbiome and its impact on sperm function and male fertility: a systematic review and meta-analysis. Andrology 2021;9:115-44.
34. Gallegos G, Ramos B, Santiso R, Goyanes V, Gosálvez J, Fernández JL. Sperm DNA fragmentation in infertile men with genitourinary infection by Chlamydia trachomatis and Mycoplasma. Fertil Steril 2008;90:328-34.
35. Moskovtsev SI, Lecker I, Mullen JB, Jarvi K, Willis J, White J, et al. Cause-specific treatment in patients with high sperm DNA damage resulted in significant DNA improvement. Syst Biol Reprod Med 2009;55:109-15.
36. Sørensen F, Melsen LM, Fedder J, Soltanizadeh S. The influence of male ejaculatory abstinence time on pregnancy rate, live birth rate and DNA fragmentation: a systematic review. J Clin Med 2023;12:2219.
37. Mir J, Franken D, Andrabi SW, Ashraf M, Rao K. Impact of weight loss on sperm DNA integrity in obese men. Andrologia 2018;50:e12957.
38. Agarwal A, Gupta S, Du Plessis S, Sharma R, Esteves SC, Cirenza C, et al. Abstinence time and its impact on basic and advanced semen parameters. Urology 2016;94:102-10.
39. Shen ZQ, Shi B, Wang TR, Jiao J, Shang XJ, Wu QJ, et al. Characterization of the sperm proteome and reproductive outcomes with in vitro, fertilization after a reduction in male ejaculatory abstinence period. Mol Cell Proteomics 2019;18(Suppl 1):S109-17.
40. Agarwal A, Leisegang K, Majzoub A, Henkel R, Finelli R, Panner Selvam MK, et al. Utility of antioxidants in the treatment of male infertility: clinical guidelines based on a systematic review and analysis of evidence. World J Mens Health 2021;39:233-90.
41. de Ligny W, Smits RM, Mackenzie-Proctor R, Jordan V, Fleischer K, de Bruin JP, et al. Antioxidants for male subfertility. Cochrane Database Syst Rev 2022;5:CD007411.
42. Agarwal A, Cannarella R, Saleh R, Harraz AM, Kandil H, Salvio G, et al. Impact of antioxidant therapy on natural pregnancy outcomes and semen parameters in infertile men: a systematic review and meta-analysis of randomized controlled trials. World J Mens Health 2023;41:14-48.
43. Cannarella R, Shah R, Saleh R, Boitrelle F, Hamoda TAA, Singh R, et al. Effects of varicocele repair on sperm DNA fragmentation and seminal malondialdehyde levels in infertile men with clinical varicocele: a systematic review and meta-analysis. World J Mens Health 2024;42:321-37.
44. Smit M, Romijn JC, Wildhagen MF, Veldhoven JL, Weber RF, Dohle GR. Decreased sperm DNA fragmentation after surgical varicocelectomy is associated with increased pregnancy rate. J Urol 2010;183:270-4.
45. Roque M, Esteves SC. Effect of varicocele repair on sperm DNA fragmentation: a review. Int Urol Nephrol 2018;50:583-603.
46. Lira Neto FT, Roque M, Esteves SC. Effect of varicocelectomy on sperm deoxyribonucleic acid fragmentation rates in infertile men with clinical varicocele: a systematic review and meta-analysis. Fertil Steril 2021;116:696-712.
47. Qiu D, Shi Q, Pan L. Efficacy of varicocelectomy for sperm DNA integrity improvement: a meta-analysis. Andrologia 2021;53:e13885.
48. Garrido N, Gül M, Jindal S, Vogiatzi P, Saleh R, Durairajanayagam D, et al. How to select healthy sperm for intracytoplasmic sperm injection in samples with high sperm DNA fragmentation? Panminerva Med 2023;65:148-58.
49. Khoo CC, Cayetano-Alcaraz AA, Rashid R, Tharakan T, Yap T, Sofikitis N, et al. Does testicular sperm improve intracytoplasmic sperm injection outcomes for nonazoospermic infertile men with elevated sperm DNA fragmentation? A systematic review and meta-analysis. Eur Urol Focus 2024;10:410-20.
50. Greco E, Scarselli F, Iacobelli M, Rienzi L, Ubaldi F, Ferrero S, et al. Efficient treatment of infertility due to sperm DNA damage by ICSI with testicular spermatozoa. Hum Reprod 2005;20:226-30.
51. Suganuma R, Yanagimachi R, Meistrich ML. Decline in fertility of mouse sperm with abnormal chromatin during epididymal passage as revealed by ICSI. Hum Reprod 2005;20:3101-8.
52. Esteves SC, Roque M, Bradley CK, Garrido N. Reproductive outcomes of testicular versus ejaculated sperm for intracytoplasmic sperm injection among men with high levels of DNA fragmentation in semen: systematic review and meta-analysis. Fertil Steril 2017;108:456-67.e1.