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Controversy and Consensus on Indications for Sperm DNA Fragmentation Testing in Male Infertility: A Global Survey, Current Guidelines, and Expert Recommendations

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Purpose: Sperm DNA fragmentation (SDF) testing was recently added to the sixth edition of the World Health Organization laboratory manual for the examination and processing of human semen. Many conditions and risk factors have been associated with elevated SDF; therefore, it is important to identify the population of infertile men who might benefit from this test. The purpose of this study was to investigate global practices related to indications for SDF testing, compare the relevant professional society guideline recommendations, and provide expert recommendations.

Materials and Methods: Clinicians managing male infertility were invited to take part in a global online survey on SDF clinical practices. This was conducted following the CHERRIES checklist criteria. The responses were compared to professional society guideline recommendations related to SDF and the appropriate available evidence. Expert recommendations on indications for SDF testing were then formulated, and the Delphi method was used to reach consensus.

Results: The survey was completed by 436 experts from 55 countries. Almost 75% of respondents test for SDF in all or some men with unexplained or idiopathic infertility, 39% order it routinely in the work-up of recurrent pregnancy loss (RPL), and 62.2% investigate SDF in smokers. While 47% of reproductive urologists test SDF to support the decision for varicocele repair surgery when conventional semen parameters are normal, significantly fewer general urologists (23%; p=0.008) do the same. Nearly 70% would assess SDF before assisted reproductive technologies (ART), either always or for certain conditions. Recurrent ART failure is a common indication for SDF testing. Very few society recommendations were found regarding SDF testing.

Conclusions: This article presents the largest global survey on the indications for SDF testing in infertile men, and demonstrates diverse practices. Furthermore, it highlights the paucity of professional society guideline recommendations. Expert recommendations are proposed to help guide clinicians.

Keywords: Delphi method; DNA fragmentation; Male infertility; Practice guidelines; Sperm; Survey

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INTRODUCTION

Sperm DNA fragmentation (SDF) refers to single and double-stranded DNA breaks in the mature male gamete, which can lead to impaired fertility and poor reproductive outcomes when they are elevated [1]. Various underlying pathophysiological mechanisms that impair sperm DNA integrity have been described.

During spermatogenesis, apoptosis naturally occurs to remove abnormal and excessive germ cells [2]. Failure to complete this process leads to the release of defective spermatozoa, that contain high levels of fragmented DNA, due to the action of endonuclease enzymes that cleave DNA during apoptosis [3]. During sperm maturation, chromatin is remodeled as a majority of nuclear histones are replaced with protamines, rendering the sperm chromatin compact [4]. During this process, torsional stress on sperm DNA is relieved by topoisomerase enzymes that create temporary nicks in the DNA. Failure to repair these breaks leads to the persistence of fragmented sperm DNA [3]. Furthermore, immature chromatin, which is not fully compact or is decondensed, makes sperm DNA more susceptible to damage by various insults [4]. Finally, oxidative stress (OS) can directly and indirectly lead to SDF, and is associated with various exogenous and endogenous risk factors [5]. All of these mechanisms lead to the accumulation and persistence of SDF, which cannot be adequately repaired by mature spermatozoa.

Several underlying causes and risk factors have been associated with elevated SDF. Varicocele can lead to elevated SDF by increasing reactive oxygen species (ROS) production and impairing spermatogenesis [6]. Male genital tract infections have been associated with increased SDF and impaired male fertility potential [7,8]. Medical conditions in men such as obesity, diabetes, and cancer have also been linked to high SDF levels [9]. Advanced male age can impair sperm DNA integrity as well [10]. Smoking, radiation exposure, and noxious chemical exposure, whether environmental or occupational, have all been correlated with increased SDF [9].

SDF is associated with male infertility and may be elevated in cases that are classified as unexplained or idiopathic infertility and can also lead to recurrent pregnancy loss (RPL) [11]. Elevated SDF has also been demonstrated to have a negative impact on the outcomes of assisted reproductive technologies (ART), leading to fertilization failure, poor embryonic development, failure to achieve clinical pregnancy, and miscarriage following ART pregnancies [11,12].

Over the past two decades, there has been an increase in the number of articles published related to SDF and its association with adverse male reproductive outcomes [13]. With the growing interest in this molecular sperm parameter, as well as its established importance in affecting male reproductive dynamics, the recently published sixth edition of the World Health Organization (WHO) laboratory manual for the examination and processing of human semen has described four tests that measure SDF as part of the extended semen examination [14]. The WHO manual has also stated that SDF is an important addition and a promising biomarker in the work-up of infertile men, but has failed to provide a clinical context, and does not recommend who should be tested, which test is most sensitive, and what diagnostic cut-off values should be used.

Since there are varied etiologies and associated risk factors, it is important to identify the population of infertile men who may benefit from SDF testing. Two leading andrology groups [15,16] have published two guidelines that provide indications for SDF testing to help guide clinicians. The authors of the latter two reports have supported their recommendations with vast evidence from the literature and their recommendations were unified by Agarwal et al [17] for best clinical practice. Professional society guidelines have also incorporated SDF testing in the evaluation of selected groups of infertile men. However, there are many clinical situations where there are controversies or an absence of recommendations by these professional society guidelines.

Furthermore, many questions arise as to whether SDF testing is being implemented in the appropriate clinical setting, for the appropriate patient, and at the appropriate stage of treatment. Given the many disciplines that manage couple infertility, it is also important to know whether there are differences in clinical practice between specialties in terms of requesting SDF testing during their work-up of an infertile couple. In addition, it is crucial to determine whether the current practice patterns are consistent with the available professional society guidelines, as well as the evidence provided in the literature.

Therefore, the aims of this manuscript are:

1) To investigate the global practices related to indi-

cations for SDF testing.
2) To summarize and present the professional society guidelines related to indications for SDF testing and compare them to our findings.
3) To provide expert recommendations on indications for SDF testing in infertile men based on global practices, society guidelines, and evidence available in the literature.

MATERIALS AND METHODS

This was a cross-sectional global online survey regarding the practices related to SDF testing worldwide. The questionnaire was comprehensive and covered all aspects including indications for SDF testing, technical aspects of performing SDF testing, management of elevated SDF, and barriers to incorporating SDF into clinical practice. The survey was constructed, disseminated, and analyzed in accordance with the Checklist for Reporting Results of Internet E-Surveys (CHERRIES) [18]. The checklist is provided in Supplement File 1.

1. Target population

This survey was targeted toward clinicians all over the world who may utilize SDF testing in their routine patient care and practice. Urologists, andrologists, gynecologists, reproductive endocrinologists, ART specialists, and embryologists, who actively and specifically work in the field of infertility, were included. Clinicians or researchers with no experience or knowledge of SDF were excluded.

2. Questionnaire creation & structure

A preliminary draft of survey questions was compiled by senior authors (AA, RSS, AF). This was then reviewed by 30 clinicians and expert members of the Global Andrology Forum (GAF) [19] (https://www.globalandrologyforum.com/) who routinely utilize SDF testing in their clinical practice and actively publish on SDF (AC, AH, AR, AZ, DPE, EB, ECS, EK, Fahmi B, Florence B, GC, GIR, HK, IS, MG, MM, NHVP, PKK, PB, QN, RC, RFA, RH, RS, SK, SL, SS, TH, TM, TT). These experts suggested additional clinically relevant questions, refined the questions with more precision, and ensured that the answer options were comprehensive and unambiguous. All questions and options were extensively reviewed and edited to better capture the global practices related to each aspect of SDF including the various indications for testing and treatment options available. All items included a "not applicable" option to allow completeness of the responses if the participant does not encounter such a case in their practice. The final questionnaire consisted of 64 questions divided among five sections: demographic data, indications for SDF testing, technical aspects of SDF testing, management of elevated SDF, and barriers and limitations in incorporating SDF testing into clinical practice. The first two questions were identifying information (name and email address), which were asked as a quality control measure to exclude duplicates. The online survey was seven pages long with the invitation letter on the first page, each section on an individual page of varying length and number of questions according to the respective section, and finally a page that allowed respondents to recommend other participants. The survey was constructed in a way to allow participants to move back and forth between all pages as they filled it out and they were able to change or edit responses before submitting. The final questionnaire is provided in Supplement File 2.

3. Questionnaire dissemination

This questionnaire was made available online from April 4th, 2022 to May 10th, 2022 *via* a secure platform (SelectSurvey). This ensured protection of participants' personal information. An invitation along with a secure link to complete the survey was sent by the GAF management team *via* email. The aims of the survey were explained in the invitation letter and invitees were notified that by submitting their responses, they consent to participation. The invitation letter is provided as the first page of the questionnaire in Supplement File 2. The survey was initially sent out to 260 members of GAF. On the final page of the survey, responders had the option to recommend other experts who were also eligible to take this survey. The survey was also disseminated by the GAF management team *via* email to those recommended experts, in addition to direct communication between respondents and recommended experts. Furthermore, 21 andrology and urology professional societies helped in the dissemination of the survey to their members. These societies are listed in the acknowledgments.

4. Data collection and analysis

The raw data were extracted from the SelectSurvey

platform as a CSV file. In total, 551 responses were submitted. Out of 551 responses, 115 were excluded because they were completely blank, duplicate responses, or had only answered a few questions in the demographic section. After excluding the 115 invalid responses, 436 responses were eligible for final analysis. Incomplete responses were included, provided the respondent answered some questions beyond the demographic section. The frequencies of responses in each question were calculated using MedCalc® (MedCalc Software Ltd, Ostend, Belgium). Based on the results from MedCalc®, tables and graphs for each question were created.

Question responses were described as numbers and percentages of participants for each response. For questions where multiple responses were selected, the analysis was done based on the total number of responses for that question. Subgroup analysis was performed for certain variables where the statistical significance was obtained using the chi-square or Fischer's exact test whenever appropriate. Statistical analysis and chart plotting were performed using R programming language version 4.1.2 with a p-value <0.05 considered statistically significant.

5. Society guidelines

Major professional society guidelines related to the diagnosis and management of infertile men and couples were screened for recommendations related to all aspects of SDF in the evaluation of an infertile couple. The following professional society guidelines were examined:

1) Diagnosis and Treatment of Infertility in Men: American Urological Association/American Society for Reproductive Medicine (AUA/ASRM) Guideline [20,21].
2) European Association of Urology (EAU) Guidelines on sexual and reproductive health [22,23] and the EAU Guidelines Panel on Male Sexual and Reproductive Health: A Clinical Consultation Guide on the Indications for Performing Sperm DNA Fragmentation Testing in Men with Infertility and Testicular Sperm Extraction in Nonazoospermic Men [24].
3) European Society of Human Reproduction and Embryology (ESHRE) guideline: recurrent pregnancy loss [25].
4) European Academy of Andrology (EAA) guideline: Management of oligo-astheno-teratozoospermia [26].
5) Management of male factor infertility: position statement from the Italian Society of Andrology and Sexual Medicine (SIAMS) [27].
6) Diagnosis and Treatment before Assisted Reproductive Treatments. Guideline of the German Society of Gynecology and Obstetrics (DGGG), the Austrian Society of Gynecology and Obstetrics (OEGGG), and the Swiss Society of Gynecology and Obstetrics (SGGG) [28].

6. Expert recommendations

The Delphi method was used to develop the recommendations. This method is used to collect opinions on a particular issue in order to reach a consensus by a panel of experts using a series of questionnaires [29]. Passing criteria are set for each recommendation. After the initial questionnaire, recommendations not meeting the criteria are revised and submitted for a second vote. Those that still do not meet the passing criteria after the second questionnaire are then discussed and finalized in a meeting between experts.

To reach a consensus on SDF, the initial recommendations were written by the different authors responsible for writing each section. These recommendations were made based on: (1) the survey results, (2) the professional society guideline recommendations, and (3) the evidence available in the literature. A Google survey was constructed with each initial recommendation listed and participants were invited to rate it on a scale of 1–10; with 1 indicating "strongly disagree" and 10 indicating "strongly agree". A score of 7 or more indicated acceptance of the recommendation, while a score of 1-6 indicated disagreement. A space was provided below each score to allow participants to propose an alternative recommendation if they gave a score of 1–6. A passing criterion of scoring 7 or more by >80% of participants was set. A total of 18 recommendations were included in the survey. These included seven recommendations on indications for SDF testing, ten recommendations on the management of elevated SDF, and one recommendation on technical aspects related to SDF testing. An invitation email with clear instructions was sent to a selected group of GAF experts, considering a variety in age, academic position, geographical distribution, and subspecialty. The invitation included a description of the Delphi method, complete instructions, and the link to the survey. If a recommen-

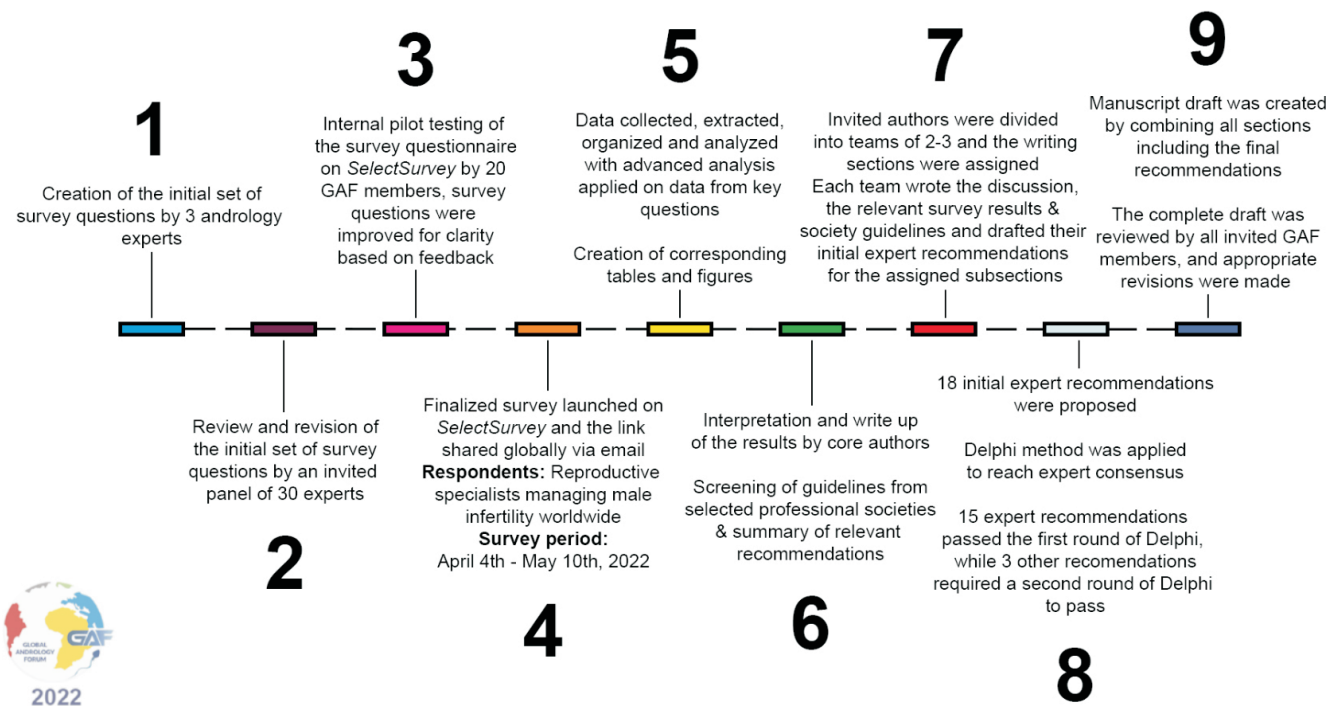


Fig. 1. Complete survey methodology. The complete survey consisted of 64 questions on SDF clinical practices divided into five sections: demographics, indications for SDF testing, technical aspects of SDF testing, management of elevated SDF, and barriers in incorporating SDF into clinical practice. A total of 18 recommendations were made as follows: seven for indications for SDF testing, ten for management of infertile men with elevated SDF, and one for technical aspects of SDF testing. Passing criteria for the Delphi method was set at >80% scoring the recommendation ≥7 in agreement. GAF: Global Andrology Forum, SDF: sperm DNA fragmentation.

dation failed to meet the criteria on the first round, a small panel of experts (AA, RSS, RS, MA, AZ, CW, KT, AF) reviewed the respondents' comments on the failed items and alternative recommendations were proposed. A second survey with revised alternative recommendations was then created.

The complete methodology is summarized in Fig. 1.

RESULTS, GUIDELINES, DISCUSSION, AND EXPERT RECOMMENDATIONS

This article presents the result of indications for SDF testing. The raw data obtained from these questions (questions 12–28) is available in Supplement File 3. Advanced analysis conducted on certain questions in this paper is available in Supplement File 4. The professional society guideline recommendations related to indications for SDF testing are also presented and discussed. Finally, expert recommendations are proposed.

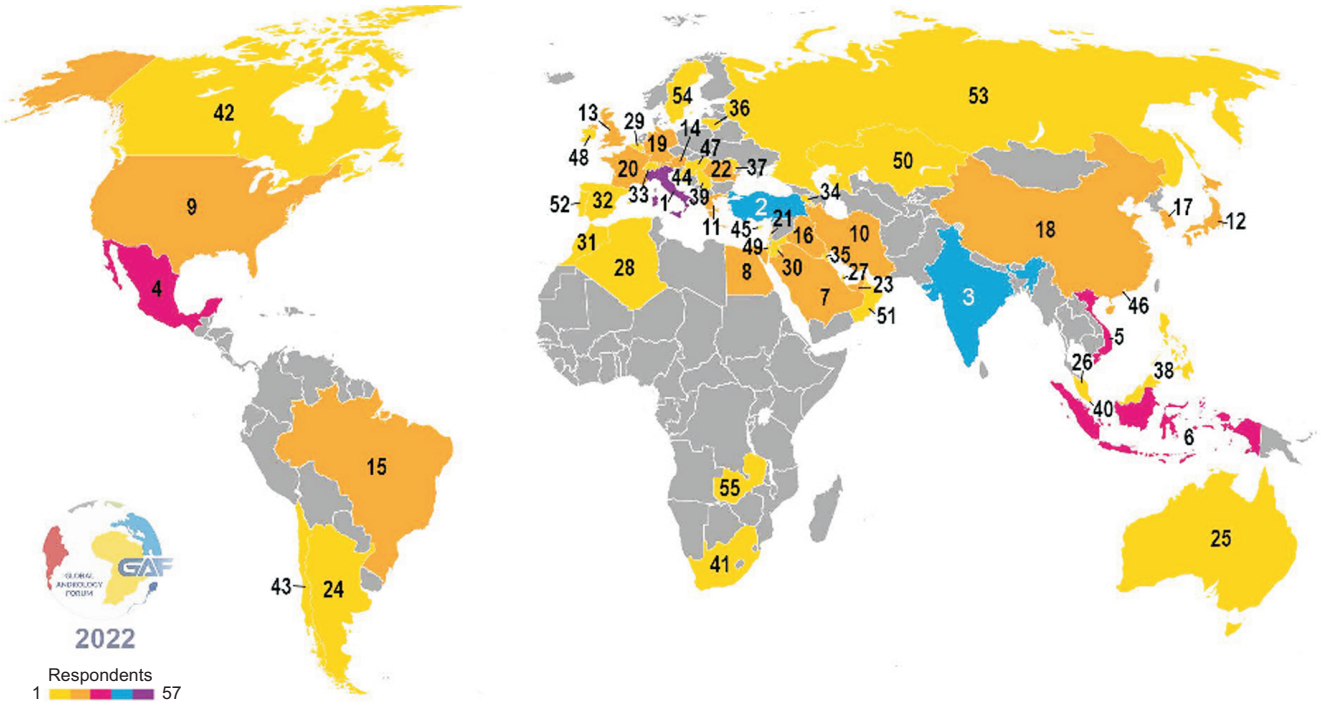
1. Participant demographics

Fig. 2 is a map representing the countries from which the respondents are from. A total of 436 reproductive specialists from 55 countries submitted the survey and whose responses were included. The largest number of respondents from a single country were from Italy (57/436, 13.1%), followed by Turkey (42/436, 9.6%), India (40/436, 9.2%), Mexico (30/436, 6.9%), and Vietnam (27/436, 6.2%).

Table 1 summarizes the questions and responses to all other demographic questions. 38.1% of participants were 35–44 years of age and 33.9% practice in an academic setting. Most respondents were general urologists (97/436, 22.2%), followed by andrologists and fellowship-trained reproductive urologists (78/436, 17.9%) (Fig. 3). More than one third (35.3%) of participants have been practicing for more than 15 years in the field of male reproduction. Almost two-thirds reported $50–200 as the cost of SDF testing in their area (Fig. 4).

2. Professional society guidelines

The recommendations made by the latest AUA/ASRM, EAU, ESHRE, EAA, SIAMS, DGGG, OEGGG, and SGGG guidelines are summarized in Table 2. Pertinent guidelines are expanded upon in the subsequent



1. Italy (57)	9. United States (15)	17. South Korea (7)	25. Australia (4)	33. Switzerland (3)	41. South Africa (2)	49. Israel (1)
2. Turkey (42)	10. Iran (13)	18. China (6)	26. Malaysia (4)	34. Armenia (2)	42. Canada (1)	50. Kazakhstan (1)
3. India (40)	11. Greece (11)	19. Germany (6)	27. Qatar (4)	35. Kuwait (2)	43. Chile (1)	51. Oman (1)
4. Mexico (30)	12. Japan (11)	20. France (5)	28. Algeria (3)	36. Lithuania (2)	44. Croatia (1)	52. Portugal (1)
5. Vietnam (27)	13. United Kingdom (9)	21. Lebanon (5)	29. Belgium (3)	37. Moldova (2)	45. Cyprus (1)	53. Russia (1)
6. Indonesia (25)	14. Austria (7)	22. Romania (5)	30. Jordan (3)	38. Philippines (2)	46. Hongkong (1)	54. Sweden (1)
7. Saudi Arabia (17)	15. Brazil (7)	23. United Arab Emirates (5)	31. Morocco (3)	39. Serbia (2)	47. Hungary (1)	55. Zambia (1)
8. Egypt (15)	16. Iraq (7)	24. Argentina (4)	32. Spain (3)	40. Singapore (2)	48. Ireland (1)	

Fig. 2. Geographical distribution of respondents. The number of respondents is shown in brackets after the name of each country. The purple color indicates the country with the greatest number of respondents (Italy; n=57), the blue color indicates the countries with the second and third highest responses (n=40 or above), the pink color indicates countries with responses between 20 and 30, the orange color represents those countries with 5–19 responses, while the yellow color indicates countries with less than 5 respondents.

subsections.

3. Results of the Delphi method

Sixty-three participants completed the questionnaire for the first round of voting. Of the seven recommendations pertaining to indications of SDF testing, six recommendations met the passing criteria. Only 73.4% of respondents gave the recommendation on "SDF testing in men with varicocele" a score ≥7. This failed recommendation was revised and submitted to a second round of voting by the same 63 experts. Forty-seven experts completed voting on the second round and the revised recommendation met passing criteria and was accepted. The participants were informed of the results, thus concluding the Delphi technique without the need for a meeting (round 3). The results are presented in the subsequent sections as "Expert Recommendations".

4. Indications for sperm DNA fragmentation testing

1) Unexplained male infertility

(1) Results

According to our survey, more than half of the participants (51.5%) would order SDF testing in some couples with unexplained infertility, while almost one-fourth of the respondents would order SDF testing in all cases. Only a minor portion of the participants believe that the results of SDF testing would not impact the treatment decision (7.4%) (Fig. 5). There is no statistical difference between the responses of urologists/andrologists compared to other specialties when ordering SDF testing in couples with unexplained infertility (p=0.1).

Table 1. Results of demographic questions

Question/option	Value
How old are you (years)?	
25–34	87 (20.0)
35–44	166 (38.1)
45–54	93 (21.3)
55–64	62 (14.2)
>65	28 (6.4)
Total	436 (100.0)
What is the nature of your employment?	
Physician, attending	275 (63.2)
Physician, fellow	22 (5.1)
Physician, resident	30 (6.9)
Advanced practice provider (physician assistant, nurse practitioner)	5 (1.1)
Reproductive biologist/embryologist	51 (11.7)
Researcher (full-time)	24 (5.5)
Other	28 (6.4)
Total	435 (100.0)
What is your area of specialization (as it relates to male infertility)?	
Fellowship-trained reproductive urology	78 (17.9)
General urology	97 (22.2)
Gynecology	23 (5.3)
Endocrinology	24 (5.5)
Clinical andrologist	87 (20.0)
Clinical laboratory	7 (1.6)
Embryology/biology	39 (8.9)
ART specialist	70 (16.1)
Primary care	1 (0.2)
Other	10 (2.3)
Total	436 (100.0)
What is your practice setting?	
Academic	148 (33.9)
Public	70 (16.1)
Private	136 (31.2)
Multiple	81 (18.6)
Other	1 (0.2)
Total	436 (100.0)
How many years have you been practicing (related to male infertility)?	
Less than 2 years	43 (9.9)
2–5 years	90 (20.7)
6–10 years	85 (19.6)
11–15 years	63 (14.5)
More than 15 years	153 (35.3)
Total	434 (100.0)
On average, how many infertile couples do you manage per week?	
<10	140 (32.6)
11–20	124 (28.8)
21–30	70 (16.3)
31–40	35 (8.1)
41–50	13 (3.0)
>50	48 (11.2)
Total	430 (100.0)
How many IVF/ICSI full cycles (= oocyte pickup) are performed in one year at your center by your entire team?	
<100	65 (15.1)
101–400	99 (23.0)
401–800	59 (13.7)
>800	72 (16.7)
I refer my IVF/ICSI couples to another center	84 (19.5)
Not applicable	52 (12.1)
Total	431 (100.0)
What is the cost of SDF testing in your area?	
Less than $50	68 (16.0)
$50–100	141 (33.1)
$100–200	132 (31.0)
$200–500	62 (14.6)
$500–1000	15 (3.5)
More than $1000	8 (1.9)
Total	426 (100.0)

Values are presented as number (%).
ART: assisted reproductive technology, ICSI: intracytoplasmic sperm injection, IVF: *in vitro* fertilization, SDF: sperm DNA fragmentation.

When considering SDF testing for men presenting with unexplained male infertility (UMI), respondents would most commonly do so after ART failure (Fig. 6). Participants who routinely requested this test at initial work-up constituted 30% of the total responses.

(2) Society guidelines

The current AUA/ASRM guideline states that SDF analysis in the initial evaluation for infertile couples is not recommended [20,21]. No explicit recommendation is made for SDF testing in unexplained infertility.

In contrast, the EAU guideline strongly recommends SDF testing to assess couples with unexplained infertility [22-24].

(3) Discussion

UMI refers to cases of male infertility, where there is no clear identifiable cause and a completely normal conventional semen analysis, imaging, and laboratory hormonal work-up in the absence of female factor infertility [30]. On reviewing epidemiologic data on the

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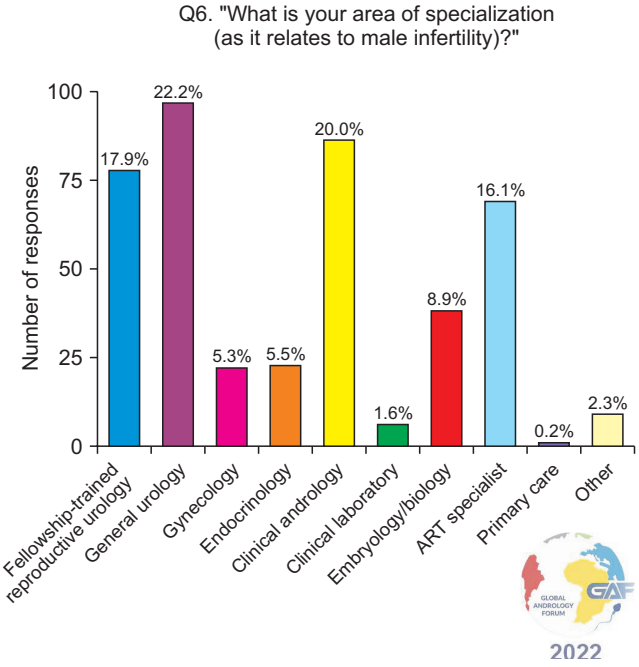

Fig. 3. Specialties of the survey respondents. ART: assisted reproductive technologies.

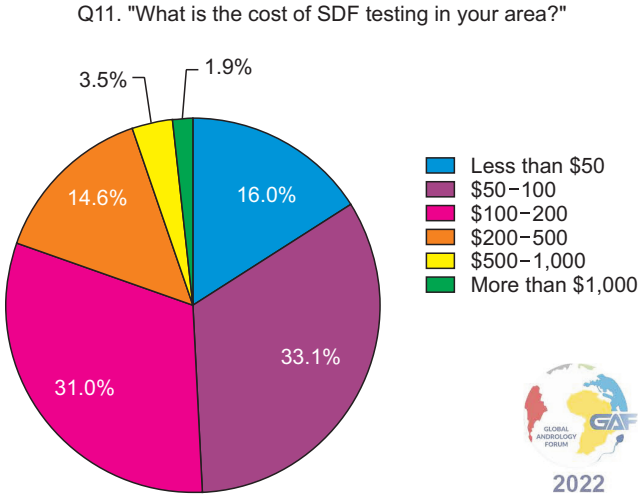

Fig. 4. Cost of sperm DNA fragmentation (SDF) testing.

prevalence of infertility, over 40% are attributed to female causes alone, 20% to male causes alone, 25% to combined causes, and 15% of couples have unexplained infertility [31]. These percentages are averaged from different epidemiological studies that report the prevalence of unexplained infertility to be between 5 and 35%, considering both male and female partners. A limitation of this classification system is that it uses conventional semen parameters to identify and classify male causes of infertility, which does not account for molecular or functional aspects of spermatozoa. Men with unexplained infertility have normal conventional semen parameters but may have high levels of SDF.

In a study that included 122 men with UMI, DNA fragmentation index (DFI) was assessed in 119 and 25% of those included had DFI levels >20%; a value which is associated with decreased fertility potential [32]. In another report, SDF levels in men with UMI were significantly higher than those in healthy fertile counterparts (40.4% *vs.* 25.7%, p=0.0004) [33]. Therefore, elevated SDF levels can explain some of these cases of male infertility, which are otherwise classified as unexplained.

Our results reveal that over 75% of respondents order SDF testing for men with UMI, whether for all cases or in certain cases. These practices are in line with the EAU guidelines that strongly recommend SDF testing in UMI, but not other society guidelines that do not discuss UMI. Investigating UMI cases for SDF can help identify a cause, which would allow clinicians to appropriately manage and counsel their patients. Furthermore, management of elevated SDF can be attempted prior to referral to ART or otherwise allow physicians to incorporate methods that may improve the success of ART with elevated SDF.

More than one-third of participants ordered SDF testing after ART failure. When a couple is referred to ART but to no avail, investigating the male partner with SDF testing may explain this ART failure and may even diagnose the underlying pathology of the infertile male partner. Therefore, clinicians may be able to properly counsel their patients and offer appropriate management.

(4) Expert recommendations

SDF testing should be considered for all men with unexplained infertility, particularly after a careful diagnostic workup and the exclusion of all the known causes of male infertility. SDF testing may be requested either (1) at diagnosis of UMI, or (2) after ART failure.

2) Idiopathic male infertility

(1) Results

In a question asked to assess the clinical practice of participants towards SDF testing in men with idiopathic male infertility (IMI), most of the responders (51.0%) would order SDF testing "in some cases". The

Table 2. Summary of recommendations by professional society guidelines on indications for SDF testing

Guidelines	AUA/ASRM	EAU	ESHRE	EAA	SIAMS	DGGG, OEGGG, and SGGG
UMI	No specific recommendation	SDF testing strongly recommended	NA	No specific recommendation	No specific recommendation	No specific recommendation
IMI	No specific recommendation	No specific recommendation	NA	SDF testing recommended in infertile men with OAT for decision regarding ART, no specific mention of IMI	No specific recommendation	No specific recommendation
RPL	Moderate recommendation for SDF testing	Strong recommendation for SDF testing	SDF testing recommended	No specific recommendation	Assessing sperm DNA is useful in RPL	No specific recommendation
Risk factors & exposures	No specific recommendation	Risk factors for elevated SDF are listed, but no specific recommendation for testing is made	NA	No specific recommendation	SDF testing beneficial in: DM, MAGI, antineoplastic treatments, exposure to toxicants	No specific recommendation
Varicocele	No specific recommendation	No specific recommendation for testing, but VR may be considered in men with elevated SDF with otherwise UMI	NA	No specific recommendation	SDF testing beneficial for varicocele patients, benefit of VR on SDF is discussed	No specific recommendation
ART planning	No specific recommendation	Strong recommendation for SDF testing in couples with RPL from ART	NA	SDF testing recommended when ART is considered (low quality evidence)	No specific recommendation	SDF testing not recommended routinely for IVF/ICSI (strong consensus)
Sperm cryopreservation	No specific recommendation	No specific recommendation	NA	No specific recommendation	No specific recommendation	No specific recommendation

ART: assisted reproductive technologies, AUA/ASRM: American Urological Association/American Society for Reproductive Medicine, DGGG, OEGGG, and SGGG: Guideline of the German Society of Gynecology and Obstetrics, the Austrian Society of Gynecology and Obstetrics, and the Swiss Society of Gynecology and Obstetrics, DM: diabetes mellitus, EAA: European Academy of Andrology, EAU: European Association of Urology, ESHRE: European Society of Human Reproduction and Embryology, ICSI: intracytoplasmic sperm injection, IMI: idiopathic male infertility, IVF: *in vitro* fertilization, MAGI: male accessory gland infections, NA: not applicable, OAT: oligo-astheno-teratozoospermia, RPL: recurrent pregnancy loss, SDF: sperm DNA fragmentation, SIAMS: Italian Society of Andrology and Sexual Medicine, UMI: unexplained male infertility, VR: varicocele repair.

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percentage of those who investigate SDF in all cases of IMI (22.4%) was slightly higher than the overall percentage of those who would never order SDF testing in patients with IMI (19.7%). Overall results are presented in Fig. 7. No statistical difference was seen when responses were compared between urologists/andrologists and other specialties when ordering SDF testing in men with IMI (p=0.4).

Concerning the timing of SDF testing for men presenting with IMI, more than 40% of the participants selected the answer "after failure of ART". As in UMI (previous section), one-third of participants would do SDF testing routinely as part of their initial workup once IMI is diagnosed. Almost the same percentage of the respondents (29.4%) would wait for the failure of empiric antioxidant or hormonal therapy. A smaller percentage of participants (22.1%), would order SDF testing before referring couples suffering from IMI to ART. The data for this question is presented in Fig. 8.

(2) Society guidelines

The current AUA/ASRM guideline does not recommend SDF analysis in the initial evaluation for infertile couples [20,21]. In the EAU guidelines, there is no specific recommendation for SDF testing in men with IMI [22-24].

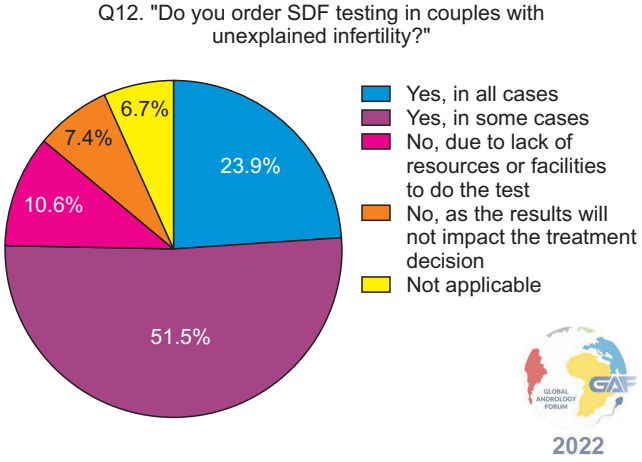

Fig. 5. Sperm DNA fragmentation (SDF) testing in couples with unexplained infertility.

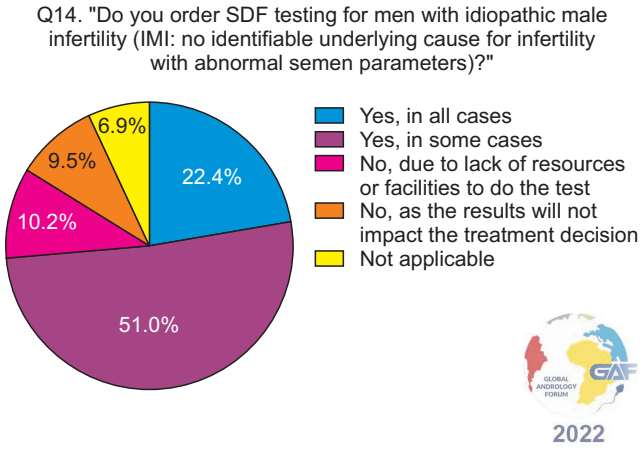

Fig. 7. Sperm DNA fragmentation (SDF) testing in idiopathic male infertility (IMI).

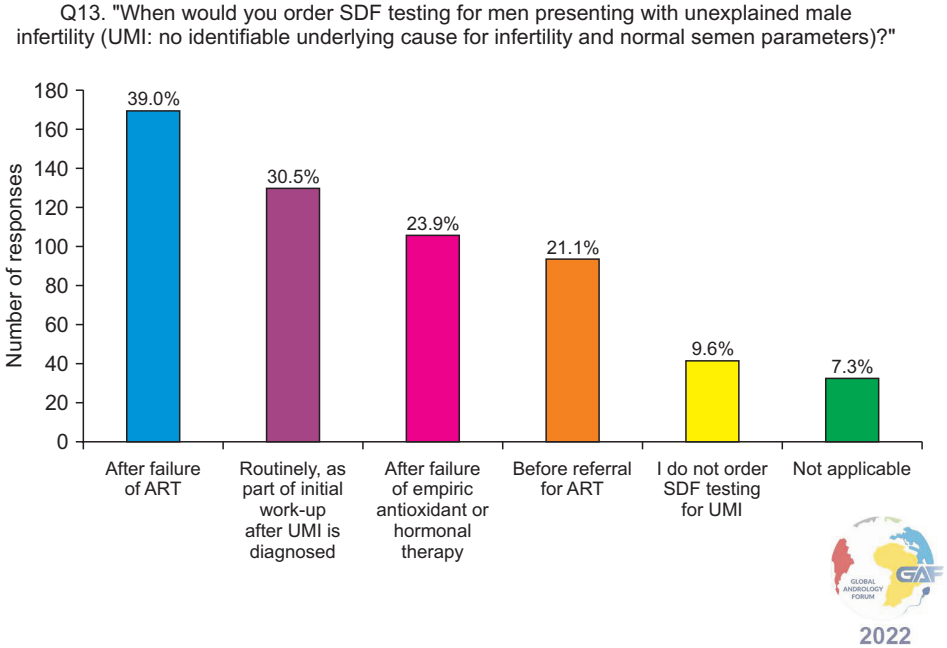

Fig. 6. Timing of sperm DNA fragmentation (SDF) testing in unexplained male infertility (UMI). Respondents were allowed to select more than one answer. The percentage for each answer was calculated by dividing the number of respondents who had selected it by the total number of respondents who had answered this question (n=436). ART: assisted reproductive technologies.

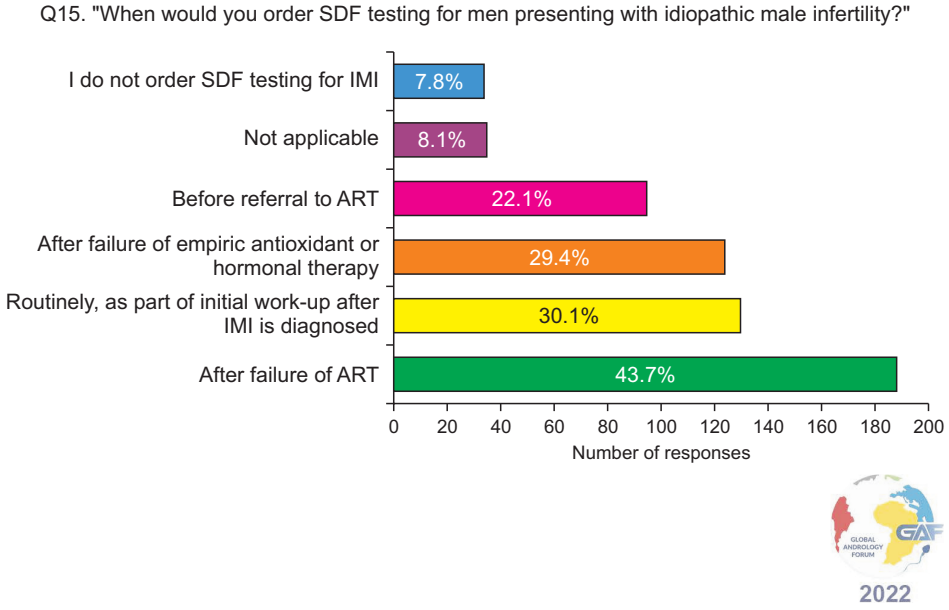

Fig. 8. Timing of sperm DNA fragmentation (SDF) testing in idiopathic male infertility (IMI). Respondents were allowed to select more than one answer. The percentage for each answer was calculated by dividing the number of respondents who had selected it by the total number of respondents who had answered this question (n=435). ART: assisted reproductive technologies.

EAA guideline makes no specific mention of IMI, however, it does recommend SDF testing in infertile men with oligo-astheno-teratozoospermia (OAT), to consider whether to refer to ART or allow additional time for spontaneous pregnancy [26].

(3) Discussion

In cases of IMI, even though a decline in semen quality as demonstrated by abnormal conventional semen parameters is well known; the exact cause for this change remains unclear. IMI is currently the leading diagnosis given to infertile men and occurs in almost 30-40% of men diagnosed with infertility without an identifiable etiology [30,34].

Studies have identified OS as the underlying mechanism through which numerous endogenous and exogenous factors could induce IMI. This condition is known as Male Oxidative Stress Infertility (MOSI), which describes infertile males with abnormal semen parameters and OS [35]. OS can in turn impair sperm DNA integrity and affect reproductive outcomes.

According to the results of the present survey, half of the participants would order SDF testing only "in some cases", while 22.4% would order it in all men with IMI. This contrasts with most society guidelines that do not make specific recommendations regarding SDF testing in this population or would recommend SDF testing after a careful diagnostic work-up in general, such as the SIAMS guidelines statement [27]. The clinical practices highlight a need for universal recommendations on performing ancillary and functional sperm tests in the diagnostic approach to infertile men who have no identifiable underlying cause, such as SDF testing (as demonstrated by this present survey), or OS testing to diagnose MOSI for example.

Concerning the timing of SDF testing, 40% request it after ART failure. This is in contrast with the EAA guidelines, which recommend SDF testing for men with OAT before ART. Several lines of evidence confirm the negative association between high SDF levels and the outcome of ART, with reduced implantation and pregnancy rates [36,37]. Thus, targeted treatment aimed to reduce the high SDF levels could lead to a better ART outcome.

(4) Expert recommendations

SDF testing should be considered for all men with idiopathic infertility, particularly after a careful diagnostic workup and the exclusion of all the known causes of male infertility. SDF testing may be requested prior to the initiation of ART.

3) Recurrent pregnancy loss

(1) Results

When asked when they would order SDF testing for a couple with RPL (2 or more miscarriages) after natural conception, and a normal female partner, almost 40% of the respondents selected "routinely, during initial work-up", whereas 30% would order SDF testing

if initial work-up is unremarkable. While almost one in five participants would order SDF testing before referring couples to ART, approximately one-fourth of the participants (22.3%) would order SDF testing after ART failure. A small minority of the respondents would not order SDF for RPL (9.4%) (Fig. 9).

(2) Society guidelines

For couples with RPL, the AUA/ASRM guideline recommends that the male partner should be evaluated with a karyotype (Expert Opinion) and SDF testing (Moderate Recommendation; Evidence Level: Grade C [20,21]).

EAU guideline recommends SDF testing be performed in the assessment of couples with RPL from natural conception (Strong recommendation [22-24]).

According to the ESHRE guideline, assessing SDF in couples with RPL can be considered for diagnostic purposes [25].

Although the SIAMS position statement does not mention any specific recommendation on SDF testing in couples with RPL, the assessment of SDF is suggested in certain cases where it would be useful to assess sperm DNA, which includes RPL [27].

(3) Discussion

RPL is defined as two or more (not necessarily consecutive) pregnancy losses by the ASRM [38]. The most recent ESHRE guideline also defines RPL as two or more pregnancy losses, excluding ectopic and molar pregnancies [25].

RPL affects approximately 5% of couples, and higher DFI has been reported in men from couples experiencing RPL following spontaneous conception [39]. Recently, Haddock et al [40] found significantly higher sperm DFI rates in male partners of women who had miscarried after spontaneous or assisted conception compared with fertile controls (33.3±0.6% *vs.* 14.9±0.7%; p<0.001), confirming the results of a previous meta-analysis in which a mean difference of 11.91% (95% CI, 4.97–18.86) was reported, with SDF being significantly higher in the RPL group [41]. This may be related to poor embryonic development that results from sperm DNA damage [37,39].

According to our survey, almost 40% of the respondents stated that they included SDF in the RPL work-up as a first-line test, regardless of basic semen parameters, while almost 30% order it in the case of normal basic semen parameters. These data are in agreement with international recommendations (AUA/ASRM, EAU, ESHRE, SIAMS), as well as the current body of evidence. Although RPL affects a couple and thorough gynecological and genetic evaluation is necessary, an appropriate andrological evaluation is also crucial and indeed SDF testing may, at least partially, explain or be a contributor to this adverse consequence.

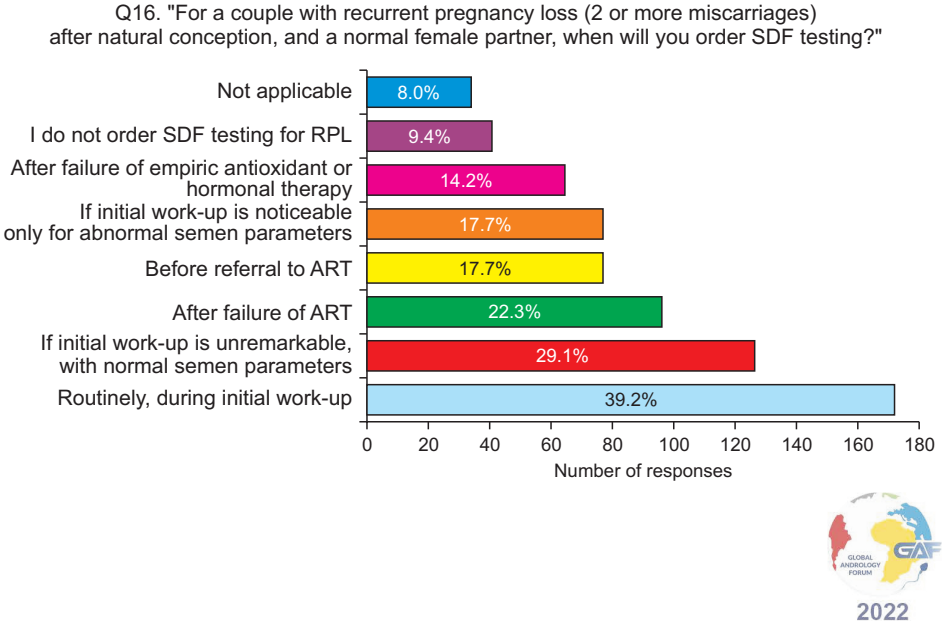

Fig. 9. Sperm DNA fragmentation (SDF) testing after recurrent pregnancy loss (RPL). Respondents were allowed to select more than one answer. The percentage for each answer was calculated by dividing the number of respondents who had selected it by the total number of respondents who had answered this question (n=436). ART: assisted reproductive technologies.

(4) Expert recommendations

SDF testing should be ordered in the work-up of any RPL regardless of conventional semen parameters.

4) Risk factors and exposures

(1) Results

When asked whether they would order SDF testing for infertile men with conditions or risk factors that may be associated with high SDF, almost two-thirds of participants (271/436, 62.2%) order SDF testing for smokers. More than 50% order SDF testing if there is a history of chemical exposure, radiation exposure, or chemotherapy. Only 13.1% do not order SDF for any of these conditions. These conditions as well as the percentage of participants who order SDF testing for them are presented in Fig. 10.

(2) Society guidelines

AUA/ASRM guideline states that the current data on most risk factors of male infertility are limited, but this is not specifically referring to SDF [20,21].

There is no specific recommendation for SDF testing in men with risk factors and exposures in the EAU guidelines [22-24]. They only list the following risk factors for increased SDF levels: varicocele, male genital tract infections, aging, cigarette smoking, chemotherapy, and ionizing radiation.

According to the SIAMS position statement, patients with RPL, diabetes, antineoplastic treatments, male genital tract infections, varicocele, or exposure to toxicants benefit from SDF testing [27]. The position statement also discusses the negative impacts that obesity and cigarette smoke have on SDF, although no specific recommendation regarding the indication of the test in such conditions is made.

(3) Discussion

SDF risk factors can be divided into clinical or environmental origins. SDF is closely related to lifestyle and endogenous factors such as aging and obesity. Exogenous factors such as tobacco use, alcohol consumption, radiation exposure, infection, and chemicals also link to an increase in SDF [16]. Some of the hypothesized pathophysiology behind elevated SDF with various insults include higher exposure to OS, atypical sperm chromatin packing, abnormal apoptosis, with extra-testicular and intra-testicular damage.

Smoking can decrease sperm count and impair motility [42], and can also adversely impact sperm chromatin integrity as demonstrated by various studies [43-45]. Obesity has also been reported to have an adverse

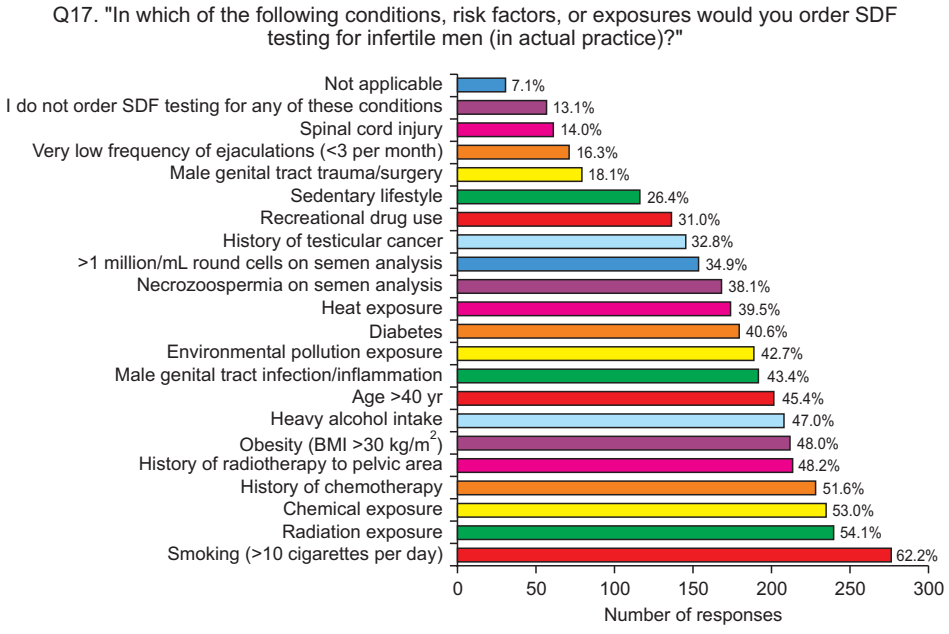

Fig. 10. Sperm DNA fragmentation (SDF) testing in men with risk factors and exposures. Respondents were allowed to select more than one answer. The percentage for each answer was calculated by dividing the number of respondents who had selected it by the total number of respondents who had answered this question (n=436). BMI: body mass index.

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effect on SDF rate [46,47]. The percentage of sperm DFI has been reported to increase with paternal aging, especially at 40 years and older [48,49]. Alcohol consumption especially in combination with smoking also showed a significant increase in SDF [50]. A recent study reported elevated SDF due to bacterial infection in infertile men with leukocytospermia [51]. Moreover, spermatogenesis can be affected by radiation, which can induce sperm DNA damage, depending on the time and dose of exposure [52]. Various chemicals and toxins are also associated with increased SDF [53-55]. For example, Irnandi et al [56] recently reported that automobile painters had significantly higher SDF compared to controls.

The results of our survey of clinical practices are in accordance with the literature on risk factors and SDF. Even though the AUA/ASRM does not explicitly mention risk factors for SDF, the EAU and SIAMS report similar clinical and environmental risk factors for increased SDF, but do not make solid recommendations for SDF testing in infertile men with such conditions. However, clinicians should be aware of these risk factors when managing a couple with infertility and the association with SDF.

Furthermore, recent evidence is revealing other factors which may be associated with SDF that were not listed or discussed, such as vitamin D deficiency [57]. There is vast room for clinical and translational research studies that explore the association between different conditions and SDF, as well as the underlying mechanisms.

(4) Expert recommendations

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. All infertile men with such risk factors and exposures should be informed that they may be at an increased risk for elevated SDF.

5) Men with varicocele

(1) Results

According to the survey results, the most common scenarios for obtaining SDF testing in infertile patients with clinical varicocele and abnormal semen parameters were after the failure of either varicocele repair (42.2%), or ART (30.4%), followed by before referral to ART (23.5%). In 18.7% of responses, SDF testing was obtained in all patients with clinical varicocele and abnormal semen parameters at the initial evaluation,

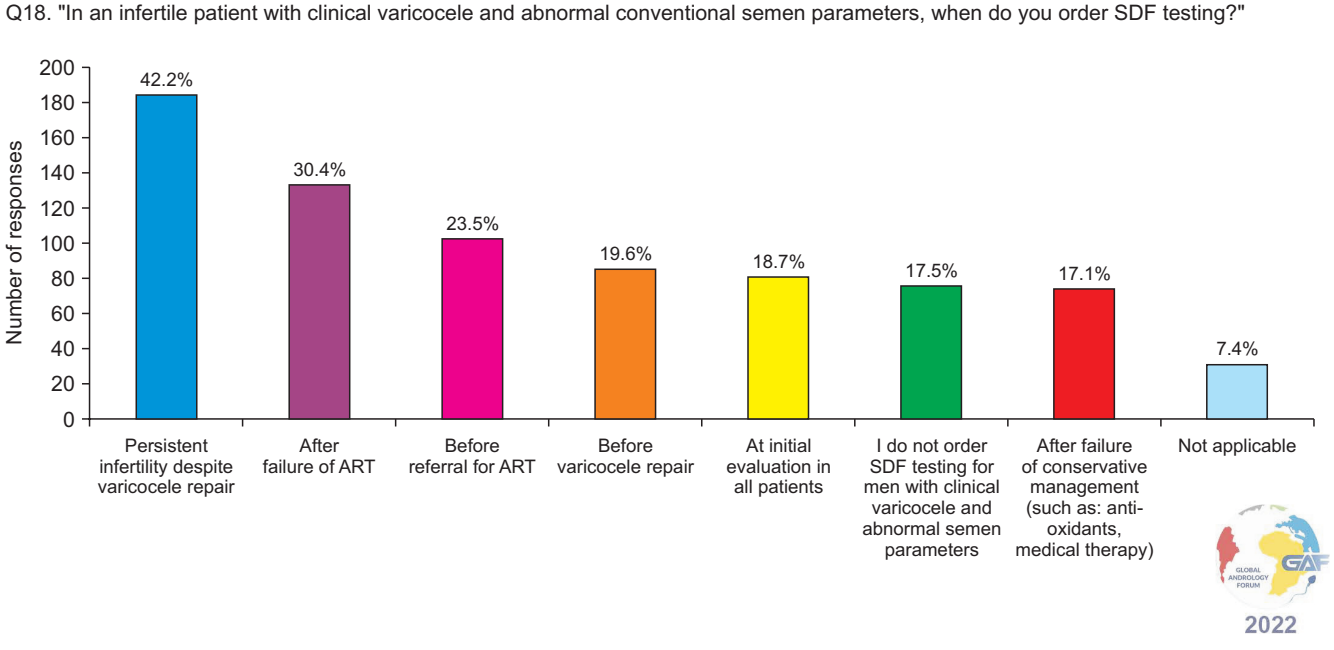

Fig. 11. Ordering sperm DNA fragmentation (SDF) testing for infertile men with clinical varicocele and normal conventional semen parameters. Respondents were allowed to select more than one answer. The percentage for each answer was calculated by dividing the number of respondents who had selected it by the total number of respondents who had answered this question (n=434). ART: assisted reproductive technologies.

while approximately the same percentage denied ordering SDF testing (Fig. 11).

In contrast, when patients had normal semen parameters, almost one-third of participants declined to order SDF testing compared to nearly one-fourth of those who accepted performing the test to support the decision for varicocele repair (Fig. 12). The responses were significantly different based on the level of training

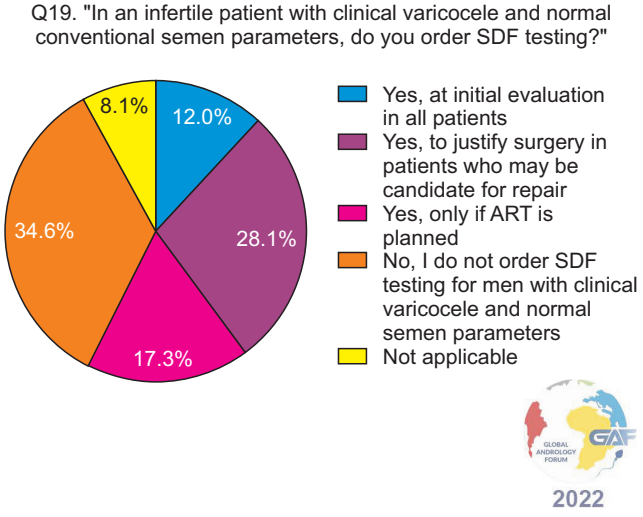

Fig. 12. Ordering sperm DNA fragmentation (SDF) testing for infertile men with clinical varicocele and normal conventional semen parameters. ART: assisted reproductive technologies.

where general urologists refused SDF testing compared to fellowship-trained andrologists (42% *vs.* 26%), while the latter group opted for performing the test to justify varicocele surgery (47% *vs.* 23%), a difference of statistical significance (p=0.008). A minority of both groups performed SDF testing at the initial evaluation (around 10% each), whilst general urologists were slightly more likely to order the test if ART is planned (15% *vs.* 10%). Fig. 13 demonstrates the responses stratified based on the level of training.

Overall, a similar response pattern was observed for patients with subclinical varicocele and abnormal semen parameters where a third of respondents did not perform SDF testing (31.7%), while 22.0% obtained the test only if ART is required, 19.7% after the failure of medical treatment, and 18.5% in all patients (Fig. 14). If the patients had normal semen parameters associated with subclinical varicocele, approximately half of the respondents (46.5%) did not request SDF testing, whereas the other half preferred obtaining the investigation for particular situations (Fig. 15).

(2) Society guidelines

AUA/ASRM guideline states that palpable clinical varicocele should be considered for surgical repair in infertile men with abnormal semen parameters, except

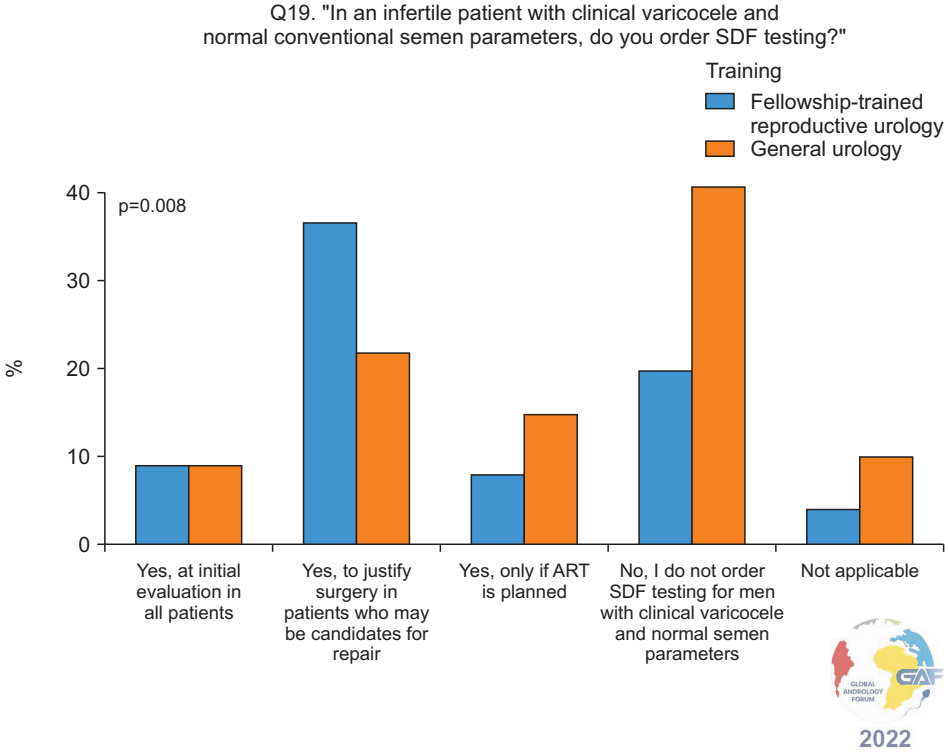

Fig. 13. Ordering sperm DNA fragmentation (SDF) testing for infertile men with clinical varicocele and normal conventional semen parameters, with responses stratified according to the level of training among urologists. Fellowship-trained reproductive urologists tend to order more SDF testing in this population of men to justify varicocele surgery compared to general urologists who are more inclined to refuse SDF testing. The differences between the practices of both groups are significant (p=0.008). ART: assisted reproductive technologies.

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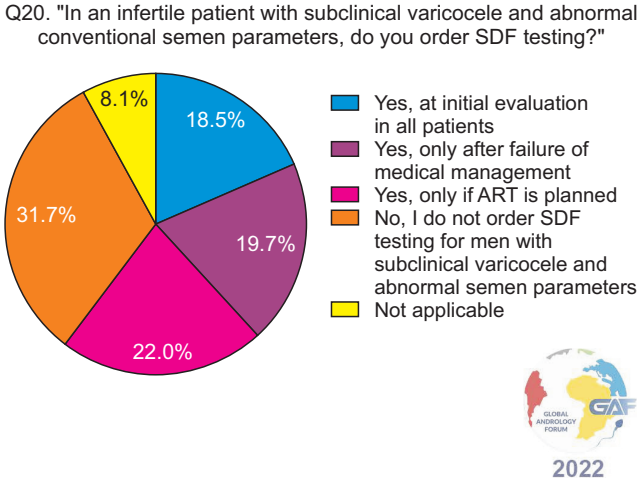

Fig. 14. Ordering sperm DNA fragmentation (SDF) testing for infertile men with subclinical varicocele and abnormal conventional semen parameters. ART: assisted reproductive technologies.

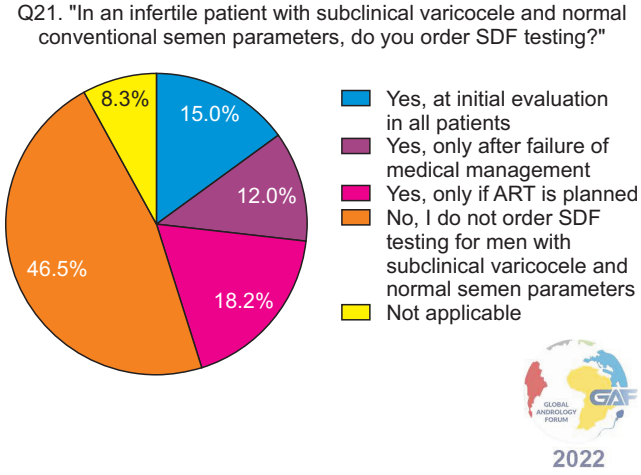

Fig. 15. Ordering sperm DNA fragmentation (SDF) testing for infertile men with subclinical varicocele and normal conventional semen parameters. ART: assisted reproductive technologies.

for azoospermia. There is no specific mention of investigating SDF within the context of varicocele [20,21].

Similarly, the EAU guideline does not state that there is a need for SDF testing for varicocele patients [22-24]. However, it states that varicocele repair may be considered in men with elevated SDF with otherwise UMI or who have failed ART, including RPL, failure of embryogenesis, and implantation. Varicocele repair may help reduce SDF and improve ART outcomes.

SIAMS guideline states that investigation of sperm DNA integrity could be helpful during the counseling of infertile couples, especially if the male partner is more susceptible to sperm DNA damage, including men with varicocele [27]. The SIAMS position statement suggests repairing varicocele in infertile couples where the male partner has abnormal semen parameters, and the female partner has normal fertility or a potentially treatable cause of infertility, and time to conception is not a concern. Although increased SDF is not listed as an indication of varicocele repair, in the evidence section, the benefits of varicocele repair on SDF are discussed.

(3) Discussion

Varicocele has been associated with high SDF levels [58], likely due to the overproduction of ROS [59]. Results of the current survey revealed that a large proportion of respondents (42%) recommend SDF testing in men with persistent infertility despite varicocele repair. 18.6% of respondents would order SDF testing during the initial evaluation of infertile patients with clinical varicocele and abnormal conventional semen parameters, and 17.5% do not order SDF testing for such patients. While one-third of respondents (34.6%) do not obtain SDF testing in infertile men with clinical varicoceles and normal conventional semen parameters, more than half of respondents would request the test for variable reasons. The practices related to SDF testing in men with clinical varicocele were very heterogenous among the respondents of this survey, which is expected given the lack of solid recommendations by the professional societies.

As compared to general urologists, reproductive urologists are significantly more likely to request SDF testing for infertile men with clinical varicocele and normal conventional semen parameters to justify varicocele repair. This finding further highlights the need for clear guidelines and recommendations for this population of infertile men.

The association between subclinical varicocele and SDF remains unclear. In a study of 60 men, SDF rates were comparable between men with clinical and subclinical varicoceles, but an improvement in SDF after varicocele repair was seen only in the subgroup of men with clinical varicoceles [60]. Interestingly, in another study, it was demonstrated that SDF values were not statistically different between infertile men with subclinical varicocele and fertile men without varicocele [61]. Therefore, more evidence is needed to elucidate the clinical value of SDF testing in men with subclinical varicocele. Despite the scarce evidence, as well as the recommendations by the professional societies against

repairing subclinical varicocele [20-23], almost half of the respondents order SDF testing for various reasons whether at initial evaluation, after the failure of medical treatment, or before ART referral. More research is needed into the value of investigating SDF for this population of infertile men.

(4) Expert recommendations

SDF testing may be considered at the initial evaluation of infertile men having clinical varicocele with normal or abnormal conventional semen parameters, and particularly if ART is planned or after the failure of medical treatment.

Performing SDF testing will assist in decision-making regarding the value of varicocele repair and may help with appropriate counseling and management of infertile men.

SDF testing should be ordered for men with clinical varicocele who still have persistent infertility after varicocele repair, regardless of improvement in conventional semen parameters.

For infertile men with subclinical varicocele, SDF testing should not be performed for this indication. SDF testing may be considered for the purpose of investigating an alternative cause of infertility rather than the subclinical varicocele itself.

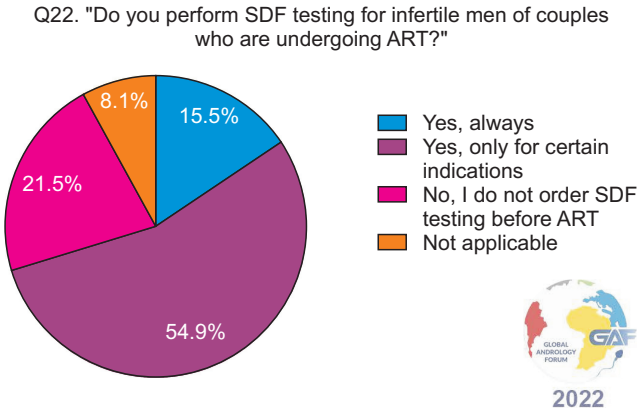

Fig. 16. Sperm DNA fragmentation (SDF) testing for infertile men before assisted reproductive technologies (ART).

6) Infertile men of couples planned for ART

(1) Results

When asked if they perform SDF testing for infertile men of couples who are undergoing ART, more than half of the respondents (54.9%) chose "yes, only for certain indications", whereas 15.5% always perform SDF testing before ART. Conversely, 93 clinicians (21.5%)

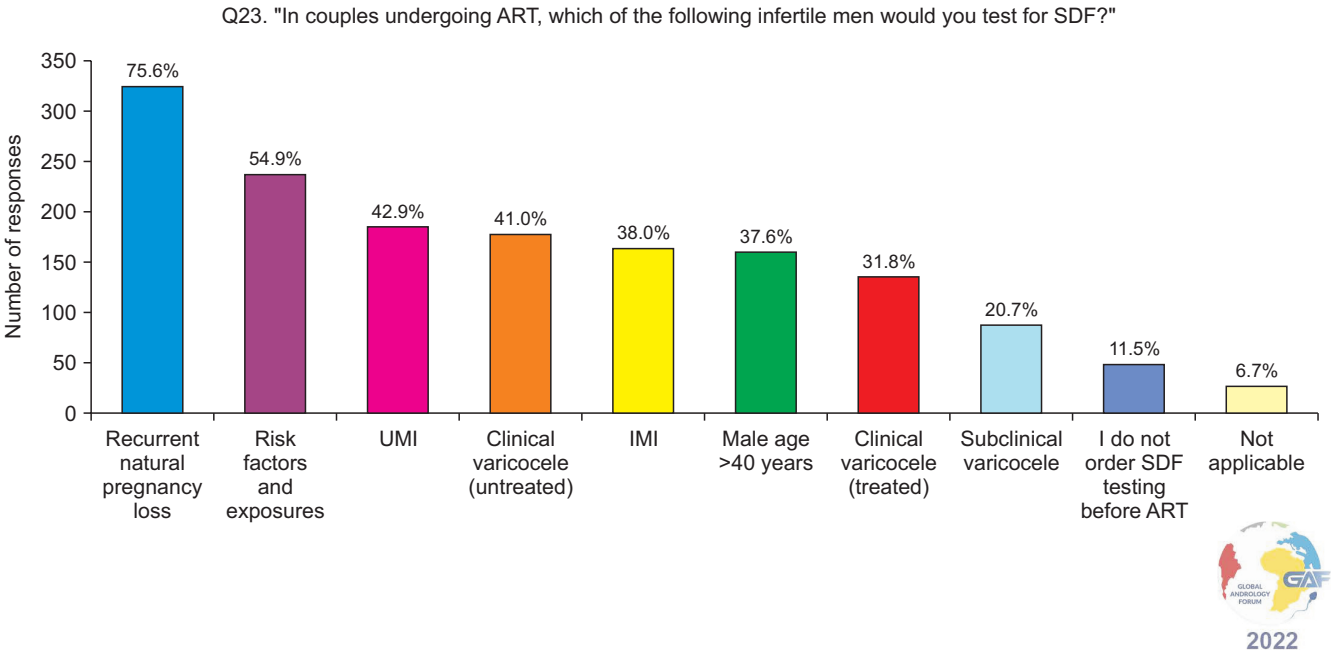

Fig. 17. Conditions before ART for which sperm DNA fragmentation (SDF) testing is ordered by respondents. Respondents were allowed to select more than one answer. The percentage for each answer was calculated by dividing the number of respondents who had selected it by the total number of respondents who had answered this question (n=434). ART: assisted reproductive technologies, IMI: idiopathic male infertility, UMI: unexplained male infertility.

answered that they never perform SDF testing before ART. Overall results are presented in Fig. 16. When responses were stratified to compare urologists/andrologists and other specialties, no significant difference was found (p=0.2). When responses were stratified based on the cost of SDF testing (less than $100 and more than $100), similarly no difference in responses was obtained (p=0.8).

In guiding the selection of infertile men from couples undergoing ART to be tested, the main determinants are recurrent natural pregnancy loss (75.6%), risk factors and exposure (54.9%), UMI (42.9%), untreated clinical varicocele (41.0%), IMI (38.0%), male age >40 years (37.6%), and treated clinical varicocele (31.8%). Complete data are shown in Fig. 17.

Finally, Table 3 presents the results of questions that ask when clinicians would order SDF testing for men of partners undergoing the different ART modalities: intrauterine insemination (IUI), *in vitro* fertilization (IVF), and intracytoplasmic sperm injection (ICSI). Interestingly, most would order SDF for recurrent miscarriage after all three methods (the highest percentage for each), followed by recurrent failure.

(2) Society guidelines

The EAU guideline recommends SDF testing be performed in the assessment of couples with RPL from ART (strong recommendation [22-24]).

The EAA recommends SDF analysis for the following scenarios: (1) When it is considered whether the couple should be referred for assisted reproduction or given additional time for trying to achieve spontaneous pregnancy; (2) When IUI is considered; (3) When standard IVF or ICSI is considered (low-quality evidence [26]).

The DGGG, OEGGG, and SGGG guidelines do not recommend routine SDF analysis for IVF/ICSI treatment (strong consensus [28]). DGGG, OEGGG, and SGGG guidelines state that SDF testing is a potentially useful clinical biomarker, but the conclusive predictive value of this test for IVF and/or ICSI treatment is still unclear.

(3) Discussion

Elevated SDF in the male partner can have a detrimental effect on the various outcomes of the different ART methods [15,62-65]. For IUI, high levels of SDF have been reported to negatively impact pregnancy and delivery rates [62]. In one study, DFI >30% by SCSA was found to predict lower pregnancy and delivery rates after IUI [66]. In another cohort, SDF rates>12% by TUNEL were associated with no pregnancy following IUI [67].

For IVF and ICSI, many studies have investigated the effect of elevated SDF on various outcomes. High sperm DNA damage was linked with lower pregnancy rates in IVF but not in ICSI cycles, whereas it was as-

Table 3. Results of ART-related questions

Question/option (more than one allowed)	Value[a]
When would you consider SDF testing for men of partners undergoing IUI? [Select all that apply]	
Recurrent miscarriage after IUI	204 (47.33)
Recurrent IUI failure	192 (44.55)
First miscarriage after IUI	84 (19.49)
Starting IUI	80 (18.56)
I do not order SDF testing before IUI	77 (17.87)
First failure of IUI (inability to achieve clinical pregnancy)	57 (13.23)
Not applicable	42 (9.74)
When would you consider SDF testing for men of partners undergoing IVF? [Select all that apply]	
Recurrent pregnancy loss after IVF	236 (54.76)
Recurrent fertilization failure after IVF	220 (51.04)
Recurrent implantation failure after IVF	203 (47.10)
First IVF failure due to failed fertilization	121 (28.07)
First IVF failure due to early pregnancy loss	118 (27.38)
Before IVF	108 (25.06)
First IVF failure due to failed implantation	98 (22.74)
I do not order SDF testing before IVF	41 (9.51)
Not applicable	33 (7.66)
When would you consider SDF testing for men of partners undergoing ICSI? [Select all that apply]	
Recurrent pregnancy loss after ICSI	240 (55.68)
Recurrent fertilization failure after ICSI	216 (50.12)
Recurrent implantation failure after ICSI	205 (47.56)
First ICSI failure due to failed fertilization	123 (28.54)
First ICSI failure due to early pregnancy loss	120 (27.84)
First ICSI failure due to failed implantation	106 (24.59)
Before ICSI	104 (24.13)
I do not order SDF testing before ICSI	47 (10.90)
Not applicable	31 (7.19)

Values are presented as number (%).
ART: assisted reproductive technology, ICSI: intracytoplasmic sperm injection, IUI: intrauterine insemination, IVF: *in vitro* fertilization, SDF: sperm DNA fragmentation.
[a]More than one option allowed. Percentage of each option chosen calculated by dividing N over the total number participants who answered the question.

sociated with higher miscarriage rates in both IVF and ICSI cycles [63,65]. A recent meta-analysis by Ribas-Ribas-Maynou et al [36] concluded that high SDF is detrimental to IVF outcomes with lower implantation rates (risk ratio [RR]=0.68; p<0.01), lower clinical pregnancy rates (RR=0.72; p=0.02), and lower but non-significant live birth rates (RR=0.48; p=0.06). Finally, it is important to note that SDF is significantly associated with increased pregnancy loss and a decrease in the good quality embryo rate after both IVF and ICSI [37].

Currently, only the EAA guidelines recommend SDF analysis for particular clinical situations in ART planning. This study found that nearly 70% of respondents order SDF analysis always or for specific indications before ART, which shows interest among clinicians in this potential type of sperm test in ART planning. Nearly three-quarters (75.58%) of clinicians recommend SDF analysis in couples with recurrent natural pregnancy loss before ART. Although evidence exists on the detrimental impact of SDF on ART, the predictive and diagnostic value of testing before ART has not been elucidated.

On the other hand, more than one-fifth (21.5%) of respondents do not order SDF testing before ART, in line with the recommendations by associations such as SIAMS, DGGG, OEGGG, and SGGG who recommend against performing routine SDF analysis prior to ART. These different practices and recommendations warrant further investigation into the role of SDF testing in different populations of infertile men, whose partners are planned for ART.

Regarding SDF testing after ART failure, results were somewhat consistent among the different ART modalities. The most common indication chosen by almost half of the surveyors was to order SDF testing after recurrent ART failure due to miscarriage (47.3% for IUI, 54.8% for IVF, and 55.7% for ICSI). These practices are in line with the EAU that recommends SDF testing for couples with RPL from ART. This was followed by recurrent ART failure due to fertilization or implantation failure, which were also selected by 40%–50% of respondents (Table 3). There are no corresponding professional society guideline recommendations on SDF testing for this population of patients experiencing ART failure.

(4) Expert recommendations

SDF testing may be beneficial for ART planning in terms of its use as a diagnostic and prognostic marker, especially if linked to known risk factors. However, its limitations should be discussed with the couples, including the lack of a standardized testing method, and the poor quality of data obtained mostly from retrospective heterogeneous cohorts.

SDF testing should be offered after ART failure for men with unexplained or idiopathic infertility, men over 40 years, and men with risk factors or exposures, given the female partner has a normal workup.

SDF testing is recommended after recurrent ART failure, including recurrent fertilization failure, recurrent implantation failure, or RPL after IUI, IVF, or ICSI.

7) Sperm cryopreservation

(1) Results

When asked whether they would order SDF testing prior to sperm cryopreservation, the majority (51.2%) do not (Fig. 18). If elevated SDF is detected prior to cryopreservation, 40.2% would treat it before cryopreservation if patient's condition allows (Fig. 19).

(2) Society guidelines

There is no specific recommendation regarding SDF testing in men undergoing sperm cryopreservation in any of the professional society guidelines.

(3) Discussion

Conventional cryopreservation of human spermatozoa involves significant physical and chemical damage

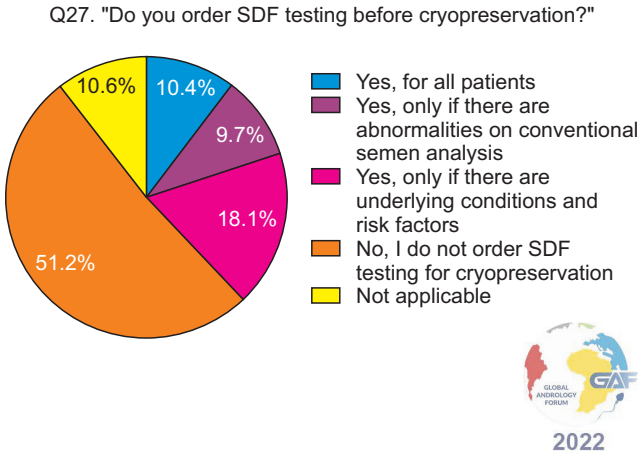

Fig. 18. Sperm DNA fragmentation (SDF) testing before sperm cryopreservation.

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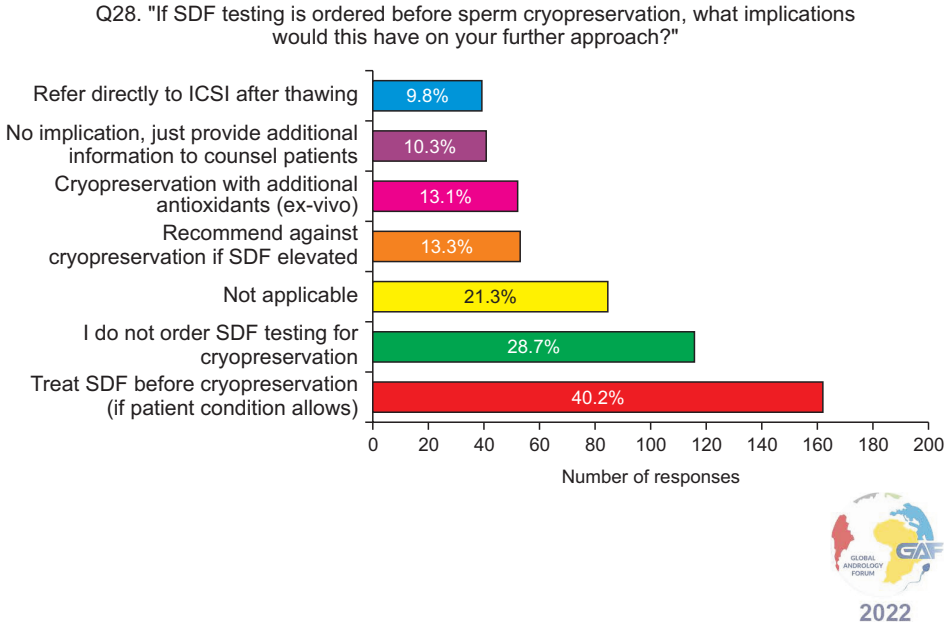

Fig. 19. Approach if elevated sperm DNA fragmentation (SDF) is found prior to cryopreservation. Respondents were allowed to select more than one answer. The percentage for each answer was calculated by dividing the number of respondents who had selected it by the total number of respondents who had answered this question (n=428). ICSI: intracytoplasmic sperm injection.

in the plasma and mitochondrial membranes, and the intracellular organelles due to increased susceptibility to lipid peroxidation, which negatively impacts sperm motility, morphology, viability, and mitochondrial function [68]. Cryopreservation-induced OS decreases sperm quality and may be attributed to damage resulting from the freezing and thawing process, including thermal shock leading to the formation of intracellular and extracellular ice crystals due to rapid cooling, cellular dehydration, and osmotic shock [69]. Besides an increased production of ROS, cryopreservation also results in mitochondrial, acrosomal, and DNA damage [70]. The increase in human SDF post-cryopreservation is well-documented to be due to cryopreservation-induced OS [71].

There are different cryopreservation methods in use, which depend on the freezing speed, cryoprotectant concentration, and temperature reduction (*e.g.*, slow/ rapid/ ultrarapid freezing, or vitrification) [70]. Sperm vitrification helps preserve motility and DNA integrity [70], while traditional sperm freezing methods may lead to impairment of DNA integrity [69,72,73].

Despite that, more than half of the respondents (51.2%) in the present study, stated that they do not test for SDF before the cryopreservation process. This response is expected as there are no specific recommendations regarding SDF testing in men undergoing sperm cryopreservation by any of the professional society guidelines, including the latest (6th) edition WHO manual [14].

However, when the respondents of the current study were asked if they would treat SDF should it be detected prior to cryopreservation, about 40% agreed that they would do so if the patient's condition permitted it. The potential benefit of testing for SDF prior to cryopreservation is that it may provide an opportunity for improvement of the patient's sperm quality prior to preservation for future use. Well-designed prospective studies are warranted to explore the utility of SDF testing in the context of sperm cryopreservation, including post-thaw SDF testing and the implications on ART outcomes.

(4) Expert recommendations

No universal recommendation can be made. SDF testing before cryopreservation should be determined on a case-by-case basis, mainly if there are underlying risks for impaired DNA integrity. If SDF is found to be elevated, appropriate measures should be taken to lower it before cryopreservation. However, if time does not permit, the patient should be counseled as to the present condition of their sample.

LIMITATIONS OF THE PRESENT STUDY

Limitations of the current survey include the sampling method; in that we were not able to capture the

response rate. Although GAF members were initially invited *via* secure email, they were able to recommend additional people and were able to graciously share this survey with their peers in the reproductive field, to enable us to capture more responses and gain more insight. Many professional societies also thankfully shared this survey with their members and many of whom posted the link on their websites.

Furthermore, the scarcity of strong evidence in the literature precluded the ability to make solid recommendations on specific aspects for each condition. Another limitation was the inability to incorporate the various demographic variables into our analysis. Due to the breadth of available data from our survey and the vast possible stratification of results, we were able to conduct advanced analysis on only a handful of questions and were able to consider only a few demographics upon which to stratify the results. Given this is a global survey, the availability of SDF testing in different countries and centers, as well as the associated costs, may considerably vary among respondents and may significantly contribute to the heterogeneity of their practices.

CONCLUDING REMARKS

This present survey reflects the actual practices of 436 clinicians from 55 countries regarding the clinical situations for which they order SDF testing as part of their diagnostic evaluation of infertile men. These

Table 4. Indications for SDF testing based on expert recommendations

Condition	Expert recommendations
UMI (normal semen parameters and no identifiable underlying cause)	SDF testing should be considered for all men with unexplained infertility, particularly after a careful diagnostic workup and the exclusion of all the known causes of male infertility. SDF testing may be requested either (1) at diagnosis of UMI, or (2) after ART failure.
IMI (abnormal semen parameters and no identifiable underlying cause)	SDF testing should be considered for all men with idiopathic infertility, particularly after a careful diagnostic workup and the exclusion of all the known causes of male infertility. SDF testing may be requested prior to initiation of ART.
Recurrent natural pregnancy loss	SDF testing should be ordered in the work-up of any RPL regardless of conventional semen parameters.
Associated conditions, risk factors, and exposures	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. All infertile men with such risk factors and exposures should be informed that they may be at an increased risk for elevated SDF.
Varicocele	SDF testing may be considered at the initial evaluation of infertile men having clinical varicocele with normal or abnormal conventional semen parameters, and particularly if ART is planned or after failure of medical treatment. Performing SDF testing will assist in decision making regarding the value of varicocele repair and may help with appropriate counselling and management of infertile men. SDF testing should be ordered for men with clinical varicocele who still have persistent infertility after varicocele repair, regardless of improvement in conventional semen parameters. For infertile men with subclinical varicocele, SDF testing should not be performed for this indication. SDF testing may be considered for the purpose of investigating an alternative cause of infertility rather than the subclinical varicocele itself.
ART planning and ART failure	SDF testing may be beneficial for ART planning in terms of its use as a diagnostic and prognostic marker, especially if linked to known risk factors. However, its limitations should be discussed with the couples, including the lack of a standardized testing method, and the poor quality of data obtained mostly from retrospective heterogeneous cohorts. SDF testing should be offered after ART failure for men with unexplained or idiopathic infertility, men over 40 years, and men with risk factors or exposures, given the female partner has a normal work-up. SDF testing is recommended after recurrent ART failure, including recurrent fertilization failure, recurrent implantation failure, or RPL after IUI, IVF, or ICSI.
Sperm cryopreservation	No universal recommendation can be made. SDF testing before cryopreservation should be determined on a case-by case basis, mainly if there are underlying risks for impaired DNA integrity. If SDF is found to be elevated, appropriate measures should be taken to lower it before cryopreservation. However, if time does not permit, the patient should be counselled as to the present condition of their sample.

ART: assisted reproductive technologies, ICSI: intracytoplasmic sperm injection, IMI: idiopathic male infertility, IUI: intrauterine insemination, IVF: *in vitro* fertilization, RPL: recurrent pregnancy loss, SDF: sperm DNA fragmentation, UMI: unexplained male infertility.

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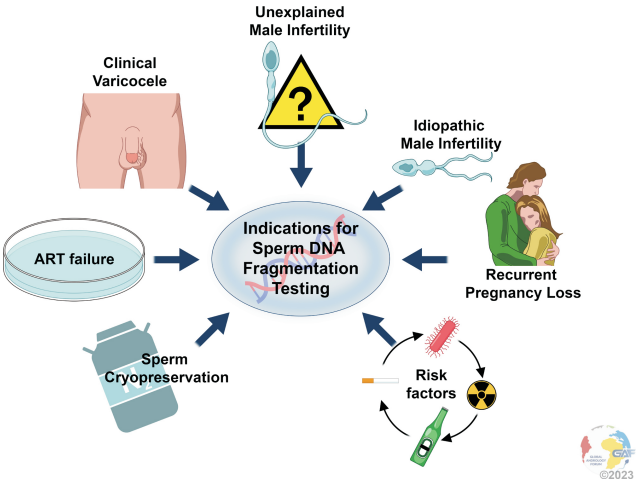

Fig. 20. Indications for sperm DNA fragmentation testing.

practices are different among the participants, and this may largely be attributed to the lack of solid recommendations by the professional society guidelines related to SDF testing as part of investigating male infertility.

Clinically, SDF testing is most useful in patients with unexplained and idiopathic infertility, RPL, clinical varicocele, and those with lifestyle or environmental risk factors. These would include males of advanced paternal age, those who smoke tobacco, abuse drugs and alcohol, or have an imbalanced diet, are obese, diabetic, are chronically exposed to ionizing radiation, environmental toxins, or heat stressors, or those who have cryptorchidism, systemic inflammation, genital infection, or cancer. Additionally, SDF testing is recommended either before or after the failure of ART and may be considered in males who opt to cryopreserve their sperm.

With the growing interest in SDF testing, there is a need to establish the appropriate clinical settings, where knowing the SDF status of the male partner would considerably alter the management approach to an infertile couple. By defining suitable indications for testing, the benefit can be conferred to as many couples as possible, while minimizing the risks of unsystematic and indiscriminate use of SDF testing in all infertile men.

This current paper demonstrates a unique and novel approach to defining these indications. Expert recommendations were initially devised using three sources of data: survey results of global practices, evidence in publications, and current professional society guidelines. Consensus was reached using the Delphi method, with a meticulous review of the recommendations by worldwide experts in the reproductive field.

The various indications for SDF testing in male infertility, based on expert recommendations, are listed in Table 4, and are summarized in Fig. 20.

Finally, there is a compelling need for universal recommendations by professional societies related to the various possible indications for SDF testing. In order to make such recommendations, high-quality evidence needs to be obtained, which can be accomplished by well-controlled studies that demonstrate the benefit of SDF testing in the evaluation of male infertility.

Conflict of Interest

The authors have nothing to disclose.

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21. Vietnam Society of Sexual Medicine (Ho Vinh Phuoc Nguyen, MD; Quang Nguyen, MD, PhD; Quang Tien Long Tran, MD; Tan V. Le, MD).

Author Contribution

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Ethics statement

Submission of the survey was voluntary for all participants invited to take the survey. Informed consent was described and obtained by all participants who agreed to submit the survey. No participant identifiers were shared with any third party. No patient information was asked or collected from any participant. Finally, no patients were involved in this study.

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