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精子 DNA 碎片检测的临床专家共识

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【摘要】 精子 DNA 碎片（sperm DNA fragmentation，SDF）在男性不育症中发生率较高。SDF 常伴发少、弱精子症，也可发生于常规精液指标正常的不育症患者。其发生机制包括精子成熟障碍、凋亡异常和氧化应激过高等。由于很多文献报道其与自然妊娠和辅助生殖技术结局存在负相关，SDF 检测已在男性不育症和辅助生殖技术中得到越来越广泛的应用。本专家共识复习和评估了 SDF 相关文献，对 SDF 的危险因素、检测方法、临床意义和处理等提出 2 项良好实践要点和 9 项推荐建议。本共识大部分推荐建议的证据和级别属于低或中等，表明仍需要进一步的研究证实临床应用 SDF 检测的价值。

【关键词】 男性不育；　精子 DNA 碎片；　辅助生殖技术
【中图分类号】 R321.1；R698+.2　　【文献标识码】 A

Clinical expert consensus on sperm DNA fragmentation detection

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【Abstract】 The incidence of sperm DNA fragmentation(SDF) is higher in male infertile patient. SDF is often associated with oligozoospermia and asthenospermia，and can also occur in infertile patients with normal conventional semen parameters. The mechanisms include abnormal spermatozoa maturation process，abnormal apoptosis，and high oxidative stress. SDF detection has been widely used in male infertility and assisted reproductive technology(ART) due to the negative correlation between SDF and the outcome of natural pregnancy and ART reported in many literatures. This expert consensus reviewed and evaluated SDF related literature，and proposed 2 good practice points and 9 recommendations on risk factors，detection methods，clinical significance and treatment of SDF. The evidence and level of the most recommendations in this consensus are low or medium，indicating that further research is still needed to confirm the value of clinical application of SDF detection.

【Key words】 Male infertility；　Sperm DNA fragmentation；　Assisted reproductive technology
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背　景

常规精液指标是目前评估男性生育力的主要手段，但研究已发现其预测男性生育力的价值有限[1]。精子 DNA 碎片（sperm DNA fragmentation，SDF）是一项新的反映精子功能的检测指标。SDF 增高常伴发少、弱精子症，也可发生在精液常规指标正常的男性不育症患者。SDF 的发生机制复杂。很多文献报道 SDF 升高与自然妊娠和辅助生殖技术结局存在负

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相关。因此，近年来 SDF 检测已广泛应用于男性不育的诊断和治疗。为规范 SDF 的临床检测和应用，中华医学会生殖医学分会制定了本临床专家共识，对 SDF 检测方法、临床意义和治疗提出推荐建议。

方　法

本共识由中华医学会生殖医学分会发起，由男科与精子库管理学组组织编写，已在国际实践指南注册平台（International Practice Guideline Registry Platform，IPGRP）国内版进行了注册（注册号为 IPGRP-2020CN122）。

本共识系统检索了 PubMed、CNKI、万方以及 Cochrane library 数据库。设计与制定步骤参考了世界卫生组织（WHO）发布的《世界卫生组织指南制定手册》、中华医学会发布的《制订/修订＜临床诊疗指南＞的基本方法和程序》和中华医学会生殖医学分会发布的《CSRM 指南共识的制定规范（2016）》。参照 GRADE 方法予以证据质量评价及推荐强度评级（表 1、表 2）。对未能给予 GRADE 但有必要予以关注的问题，根据指南制定小组专家临床经验推荐了良好实践要点（GPP）。

表 1　GRADE 证据质量评价及其定义

证据强度	说明
高质量（A）	进一步研究也不可能改变该疗效评估结果的可信度。
中等质量（B）	进一步研究很可能影响该疗效评估结果的可信度，且可能改变该评估结果。
低质量（C）	进一步研究极有可能影响该疗效评估结果的可信度，且该评估结果很可能改变。
极低质量（D）	任何疗效评估结果都很不确定。

表 2　推荐强度评级及其定义

推荐分级	说明
强推荐（1）	当明确显示干预措施利大于弊或弊大于利。
弱推荐（2）	当利弊不确定或无论质量高低的证据均显示利弊相当时。

共识内容

一、总览

本共识包含以下 11 项推荐建议：

序号	推荐建议	证据等级	推荐强度
1	男性高龄、精索静脉曲张、附性腺感染以及吸烟、酗酒、肥胖、高温等不良生活习惯可导致 SDF 水平升高。	B	2
2	TUNEL、SCD、SCSA 和碱性 Comet 试验的检测结果之间具有相关性，可用于临床检测 SDF。	B	2
3	SDF 检测可用于不明原因不育男性，有助于发现不育原因。	C	2
4	SDF 检测可用于不明原因反复流产女性患者的配偶，有助于发现反复流产的病因。	C	2
5	SDF 检测可用于精索静脉曲张结扎术前和术后检测，有助于估计手术预后。	C	2
6	SDF 可用于辅助生殖技术前检测，有助于预测治疗结果，包括临床妊娠率、流产率和活产率。	B	2
7	改善不良生活方式可作为 SDF 的基础治疗。		GPP
8	抗氧化治疗可能改善 SDF。	C	2
9	在对高 SDF 精液进行处理时，应避免精子冷冻、过高离心力、过长离心时间和长时间的 37℃下体外培养。		GPP
10	SDF 增高的临床型精索静脉曲张不育患者可通过不同的精索静脉结扎手术降低 SDF 水平。	B	2
11	对高 SDF 患者多次采用精液精子行 ICSI 治疗失败者，可采用睾丸精子行 ICSI 治疗。	C	2

二、SDF 的定义和原因

1. 定义：SDF 是指精子 DNA 完整性受损，包括精子 DNA 发生碱基错配、丢失、修饰、DNA 加合和交联、单链和双链断裂等，但主要指精子 DNA 发生单链和/或双链断裂[2]。

2. SDF 的发生机制和原因：SDF 可发生于睾丸内精子发生和成熟过程，也可发生于男性生殖道中精子运输过程。在睾丸内精子发生过程中，精子不全凋亡（abortive apoptosis）和成熟异常是导致 SDF 的主要原因[2]。氧化应激导致过高的氧自由基也可激活凋亡通路诱导睾丸内精子发生异常凋亡，也可直接导致精子运输过程中精子的脂质膜和 DNA 过氧化损伤而导致 SDF[3-6]。文献报道了可导致 SDF 增高的各种临床因素，如男性高龄、精索静脉曲张、附性腺感染

等，以及肥胖、吸烟、酗酒和高温等不良生活习惯可由于精子生精障碍和/或通过氧化应激导致 SDF[7-13]。

序号	共识建议	证据等级	推荐强度
1	男性高龄、精索静脉曲张、附性腺感染以及吸烟、酗酒、肥胖、高温等不良生活习惯可导致 SDF 水平升高。	B	2

三、SDF 的检测方法

1. 检测方法：文献报道了不同的 SDF 检测方法[14-18]，其中，精子染色质结构分析（SCSA）、精子染色质扩散法（SCD）、末端脱氧核苷酸转移酶介导的 dUTP 缺口末端标记法（TUNEL）和彗星实验（Comet）为目前最为常用的方法（表 3）。

不同检测方法的生物学意义可能不同，一些方法直接检测 DNA 断裂，其他方法实际上通过检测双链 DNA 变性为单链的敏感性以间接检测 DNA 断裂。目前检测方法的不足包括：（1）检测到的 SDF 可能不具有临床意义，如少量的单链 DNA 断裂可被另一条完整的 DNA 链修复和复制；（2）较难以检测特定的重要 DNA 位点的断裂；（3）未能检测精子 DNA 断裂的范围和程度；（4）没有公认的检测阈值。

表 3　SDF 的常用检测方法

检测方法	原理	检测碎片类型	优点	缺点
直接检测法				
TUNEL	带有荧光 dUTP 通过 TdT 结合到 ssDNA 和 dsDNA 断裂处 3'OH 端。	检测 ssDNA 和 dsDNA 断裂	敏感性和可信度高，人员操作误差小，对精子数目要求低。	室间差异大，需要不同的实验室之间统一标准。
Comet	在碱变性和中性条件下解聚精子核，行单精子电泳，断裂 DNA 形成尾部，完整 DNA 位于精子头部，不形成尾部。	碱变性下检测 ssDNA 和 dsDNA 断裂，中性条件下检测的主要为 dsDNA 断裂	敏感性和重复性特异性高，少量细胞也可采用。	主观性强，分析人员间差异较大。
ISNT	通过 DNA 聚合酶 I 将荧光标记的核苷酸结合到缺口或游离的 3'OH 端。	检测 ssDNA 断裂	简单、可靠、准确，观察者间变异小。	室间差异大，需要不同的实验室之间统一标准。
间接检测法				
SCSA	通过温和酸变性 DNA，未变性 dsDNA 结合 AO 呈绿色荧光，变性 ssDNA 结合 AO 呈红色荧光。	检测 ssDNA 和 dsDNA 断裂	敏感、可标准化、实验室间变异小，可检测大量精子细胞。	需要流式细胞仪。
SCD	精子核 DNA 用酸去浓缩，无断裂 DNA 在琼脂中形成晕环。	检测 DNA 完整性	操作简单，设备要求低。	主观性强，分析人员间差异较大。
DBD-FISH	变性 DNA 缺口后采用全基因组探针结合 ssDNA，定量检测单细胞 DNA 断裂和碱易感变性位点。	检测 ssDNA 和 dsDNA 断裂	技术可靠，可用于精子细胞特异位点检测。	过程复杂，检测费用高。

注：TUNEL：末端脱氧核苷酸转移酶介导的 dUTP 缺口末端标记法；dUTP：脱氧尿嘧啶；dsDNA：双链 DNA；ssDNA：单链 DNA；Comet：彗星实验；ISNT：原位缺口末端标记；SCSA：精子染色质结构分析；AO：吖啶橙；SCD：精子染色质扩散法；DBD-FISH：DNA 断裂检测荧光原位杂交。

2. 不同检测方法的比较：不同 SDF 检测的方法学不同，诊断特异性和敏感性有所不同，但其结果相互间仍高度相关。很多研究比较了 SCSA 和 SCD 检测精子 DNA 损伤的结果，发现两种方法间存在显著的正相关[19-22]。比较 SCSA 和 TUNEL 方法的研究也显示两者之间显著相关[23]。一项研究比较了 TUNEL 和 SCD 与 SCSA 的结果，发现相关系数 >0.866（$P<0.01$）[24]。另有研究探讨了 TUNEL、SCD、SCSA 和 Comet 试验在男性不育和对照组的检测结果，发现虽然不同方法的预测值不同，但 TUNEL、SCD、SCSA 和碱性 Comet 试验都可以预测不育，但中性 Comet 试验则不具预测作用[25]。一项 Meta 分析发现 TUNEL、SCD 和碱性 Comet 试验都可以预测男性不育，但 TUNEL 方法的预测作用较强[26]。一项评估 SDF 对 IVF 和 ICSI 临床结果影响的综述和 Meta 分析发现，不同方法

(TUNEL、SCD、SCSA 和 Comet 试验)检测的 SDF 都可预测 IVF 和 ICSI 的临床妊娠率[27]。

目前没有足够的研究证据支持和反对上述某种特定的 SDF 检测方法[28]。

3. 质量控制:与常规精液指标一样,SDF 检测结果可能在不同时间存在个体内差异。在一项对 282 名进行宫腔内人工授精(IUI)或辅助生殖技术的不育男性的精液进行 SDF 检测的结果表明,重复 SCSA 检测 SDF 的平均变异系数(CV)为 29%,其中大约 1/3 的患者在重复检测中超过阈值水平[29]。SDF 检测结果还受到检测程序和采用仪器的影响,因此严格的质量控制非常重要。

在目前没有标准化室间质控的情况下,SDF 检测实验室除按照 WHO 精液标准化程序进行精液收集和处理外,还应严格规定检测精液的冷冻保存方法及时间和相关检测设备的校准。

序号	推荐建议	证据等级	推荐强度
2	TUNEL、SCD、SCSA 和碱性 Comet 试验的检测结果之间具有相关性,可用于临床检测 SDF。	B	2

四、SDF 检测的临床意义

(一)SDF 检测的适应证

根据目前临床研究证据,以下情况存在 SDF 的风险较大,推荐进行 SDF 检测。

1. 不明原因不育:很多研究表明,排除女方因素的不明原因不育男性精液 SDF 显著高于正常生育男性[30-33]。在常规精液指标正常的男性不育患者中,大约 5%~20%的患者 SDF 显著增高[33-35]。这些研究表明,SDF 与不明原因性不育相关,检测 SDF 有助于发现不明原因性不育男性的可能不育原因。

2. 不明原因反复流产:不明原因反复流产女性的配偶 SDF 显著高于正常对照组[36-39]。一项 Meta 分析纳入了 12 项前瞻性和 2 项回顾性研究,研究分别采用 SCD、TUNEL、SCSA 和 Comet 试验检测 SDF,结果发现不明原因反复流产女性患者的配偶 SDF 比对照组高 11.8%[95%*CI*(6.64,17.32);*P*<0.001],提示高 SDF 可能是不明原因流产的原因[40]。另一项 Meta 分析纳入 15 项前瞻性研究(共 579 例反复流产女性的配偶和 434 例正常生育女性的配偶),结果发现前者 SDF 高 11.91%[95%*CI*(4.97,18.86)],也表明高 SDF 可能是反复流产的原因[41]。欧洲人类生殖和胚胎学协会(ESHRE)的 2018 年反复妊娠丢失指南[42]认为 SDF 可能是不明原因反复流产的一个原因,级别证据中度,可用于不明原因反复流产的检测。

3. 临床型精索静脉曲张:大量研究表明,临床型精索静脉曲张患者的 SDF 显著升高[12,30,43-50]。一项纳入 12 个研究的 Meta 分析表明,精索静脉曲张患者的 SDF 与对照相比高 9.84%[95%*CI*(9.19,10.49);*P*<0.001][46]。研究还表明,精索静脉结扎手术显著降低了 SDF 水平[46,51],而精索静脉结扎术后自然妊娠者的 SDF 显著低于未妊娠者[52]。一项文献复习纳入 21 项研究共 1 200 多例患者,同样发现精索静脉结扎术在术后 3~6 个月显著降低了 SDF[53]。这些研究表明,临床型精索静脉曲张可导致 SDF 升高,SDF 检测有助于临床型精索静脉曲张结扎手术的预后判断。

4. 辅助生殖治疗前检测:近年来,关于 SDF 检测对辅助生殖技术结果的预测作用有大量的研究。有关 SDF 对 IUI 结局的影响,尽管有不同的研究结论,但 2 项 Meta 分析表明高 SDF 降低了 IUI 的临床妊娠率[54-55]。一项共纳入 10 项研究的 Meta 分析研究了 SDF 对 IUI 结果的影响,结果表明高 SDF 者的临床妊娠率[*RR*=0.34,95%*CI*(0.22,0.52);*P*<0.001]和出生率[*RR*=0.14,95%*CI*(0.04,0.56);*P*<0.001]均显著下降[54]。另一项 Meta 分析也表明低 SDF 者的 IUI 临床妊娠率显著增高[*RR*=3.3,95%*CI*(1.16,9.39)][55]。

越来越多的研究表明 SDF 影响 IVF 和/或 ICSI 的临床结局。一些研究显示高 SDF 降低 IVF 和 ICSI 的临床妊娠率和活产率,并增高流产率[56-62];也有一些研究显示 SDF 仅影响 IVF[63-67]或 ICSI[68-72]的临床结局,还有一些研究的结论是 SDF 不影响 IVF 或 ICSI 的临床结局[15,72-76]。近年来有多篇系统回顾和 Meta 分析探讨了 SDF 对 IVF/ICSI 的临床妊娠率、活产率和流产率的影响[27,77-83]。其中,4 项 Meta 分析表明,高 SDF 显著降低了 IVF 的临床妊娠率[27,78-80],一项 Meta 分析发现高 SDF 显著降低了 IVF 与 ICSI 的临床妊娠率[81]。一项 Meta 分析探究了 SDF 与活产率的相关性,发现高 SDF 显著降低了 IVF 和 ICSI 的活产率[*OR*=1.17,95%*CI*(1.07,1.28);*P*=0.000 5][82]。2 项 Meta 分析研究了 SDF 对 IVF/ICSI 流产率的影响,发现高 SDF 显著增高了 IVF 和 ICSI 的流产率[79-80]。但一

项 2008 年发表的 Meta 分析发现，尽管 SDF 与 IVF 和 ICSI 临床结果显著相关[*OR*＝1.44，95%*CI*(1.03，2.03)；*P*＝0.04]，但似然比[*LR*(＋)＝1.23，*LR*(－)＝0.81]范围较小，显示临床预测价值有限[77]；一项 2016 年发表的 Meta 分析纳入了 30 项研究，发现 SDF 影响 IVF 和 ICSI 的继续妊娠率，预测敏感性为中等～好，但特异度较低，采用 TUNEL 和 Comet 试验检测的预测价值要高于 SCSA 和 SCD[83]。

综上所述，目前的证据倾向于支持 SDF 影响 IUI、IVF 和 ICSI 治疗结局的结论。

（二）SDF 检测的诊断阈值

一些研究探讨了预测辅助生殖技术临床结局的 SDF 预测值，TUNEL 法的阈值为 4%～20%，Comet 试验的阈值为 44%～56%，SCSA 法的阈值为 11.3%～30.3%，SCD 法的阈值为 17%～27.3%[67,74,82-85]。不同 SDF 检测方法的阈值不同，但即使同一检测方法也仍缺乏公认阈值。其原因可能是不同研究针对的检测人群不同。不同中心应针对其采用的 SDF 检测方法和研究人群确定诊断阈值[86]。

序号	共识建议	证据等级	推荐强度
3	SDF 检测可用于不明原因不育男性，有助于发现不育原因。	C	2
4	SDF 检测可用于不明原因反复流产女性患者的配偶，有助于发现反复流产的病因。	C	2
5	SDF 检测可用于精索静脉曲张结扎术前和术后检测，有助于估计手术预后。	C	2
6	SDF 可用于辅助生殖技术前检测，有助于预测治疗结果，包括临床妊娠率、流产率和活产率。	B	2

五、SDF 的临床处理

（一）改善不良生活方式

大量研究提示肥胖、吸烟、酗酒和高温等不良生活方式与 SDF 升高相关。初步的研究表明，减重可改善精液质量和 SDF 并增加自然妊娠机会[87-89]。但目前尚缺乏足够的临床证据表明改善不良生活方式可改善 SDF。尽管目前的临床研究证据有限，但考虑到改善不良生活方式的手段简单方便，其潜在获益大于危害和实施成本，本共识仍推荐将其作为 SDF 临床处理的基础治疗(GPP)。

（二）抗氧化治疗

抗氧化剂包括维生素 C、维生素 E、左旋肉毒碱、辅酶 Q10、N-乙酰半胱氨酸、叶酸、锌、硒、番茄红素和虾青素等。不同抗氧化剂的抗氧化作用差别显著，如体外研究表明虾青素的抗氧化作用约为维生素 E 的 100 倍[90]。

抗氧化剂是目前治疗包括 SDF 等各种精液异常的常用方法[90-91]。一些研究表明抗氧化剂治疗显著降低了 SDF[92-97]。2019 年的一项 Cochrane 分析纳入了 4 个样本量较小的随机对照研究(RCT)，发现抗氧化剂治疗与安慰剂对比，显著降低了 SDF，分析还发现不同氧化剂的作用不同[98]。目前大部分研究仍有较大设计缺陷，缺乏安慰剂对照组和双盲设计，研究统计效率较低。抗氧化治疗可能适用于精浆高氧化应激患者[99]，但尚缺乏临床证据。进一步的研究应是采用严格设计的 RCT，证明单独或组合使用不同抗氧化剂改善 SDF 的作用以及对自然妊娠和辅助生殖技术结果的影响。

（三）精索静脉曲张结扎手术

对临床型精索静脉曲张不育患者，研究表明不同的精索静脉曲张结扎手术均可降低氧化应激水平[100]，显著降低 SDF[101-104]。一项文献综述系统回顾了 21 项前瞻性研究，发现不同的精索静脉曲张结扎手术显著降低了 SDF，平均降低 8%[53]。还有研究发现精索静脉曲张结扎手术降低 SDF 并提高了自然和辅助生育的妊娠率[51]。

（四）辅助生殖技术中处理

1. 缩短取精时禁欲时间：禁欲时间与精液常规指标和 SDF 相关，禁欲时间延长虽然提高了精液量和精子总数，但降低精液精子活率，增加了 SDF[105-107]。在辅助生殖技术中，重复取精并缩短禁欲时间至 1～3 h 取精是一个简单的降低手淫获取精子 SDF 的方法[108-110]。缩短禁欲时间是否改善 IUI 临床妊娠率的研究有不同的结论[111-112]，目前也缺乏缩短禁欲时间对 IVF 和 ICSI 治疗影响的研究。最近的一项研究发现禁欲 1 d 的精子虽然 SDF 开始较低，但在体外培养 6 h 后 SDF 显著增加 20～40 倍以上[105]。

缩短禁欲时间是否会改善 IUI、IVF 或 ICSI 的治疗结局仍需要进一步的研究。

2. 减少实验室精液处理对 SDF 的影响：精子处理过程会通过增加氧自由基导致 SDF 升高，包括冷冻精子、过高离心力、过长离心时间和 37℃下长时间培养等[113-117]。有研究发现，37℃下超过 2 h 培养可导致 SDF 显著增加[114,118]。因此，对高 SDF 的精液处理应避免精子冷冻、过高的离心力、过长的离

心时间和长时间的 37℃下体外培养。

3. 精子优选方法：上游法或密度梯度离心是实验室精子处理的常用基本技术。处理后精子的活力和正常形态率会显著提高。大部分文献报道上游法和密度梯度离心可显著降低 SDF[119-122]。但有研究发现密度梯度离心并不能改善处理后的精子 SDF，甚至在部分精液标本中增高了 SDF 水平[123]。

近年来，文献报道了许多新的精子优选方法[124-126]。有报道精子微流体分选[127-128]、透明质酸结合试验[129]、运动精子细胞器形态学检查（motile sperm organelle morphology examination，MSOME）[130-132]和磁性活化细胞分选（magnetic-activated cell sorting，MACS）[132-134]都有助于筛选 SDF 低的精子。但采用这些优选后精子行辅助生殖技术治疗是否改善临床结果仍未获证实。

4. 采用睾丸精子行 ICSI：研究发现，睾丸精子的 SDF 比精液精子低[135]。基于这一发现，在一些回顾性研究和小样本的非随机前瞻配对研究中，发现对高 SDF 反复精液精子 ICSI 失败患者，采用睾丸精子行 ICSI 治疗可获得更高的临床妊娠率[136-138]。但对于伴有严重少精子症的高 SDF 患者，可能会有较大睾丸取精失败的可能。采用睾丸精子 ICSI 需要行睾丸取精手术，可能存在麻醉和手术并发症。睾丸精子还可能具有更高的染色体非整倍体率[139]。此外，睾丸生精细胞凋亡异常可能是高 SDF 的原因之一[2]，在这种情况下，睾丸精子 SDF 可能不会比精液精子低。因此，高 SDF 患者行睾丸精子 ICSI 的临床价值仍需要更多研究证实。

序号	共识建议	证据等级	推荐强度
7	改善不良生活方式可作为 SDF 的基础治疗。		GPP
8	抗氧化治疗可能改善 SDF。	C	2
9	在对高 SDF 精液进行处理时，应避免精子冷冻、过高离心力、过长离心时间和长时间的 37℃下体外培养。		GPP
10	SDF 增高的临床型精索静脉曲张不育患者可通过不同的精索静脉结扎手术降低 SDF 水平。	B	2
11	对高 SDF 患者多次采用精液精子行 ICSI 治疗失败者，可采用睾丸精子行 ICSI 治疗。	C	2

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