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OPINION

Sperm Biology

High sperm DNA fragmentation – finding a needle in the haystack: tips on selecting the best sperm for ICSI and ART

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High levels of sperm DNA fragmentation (SDF) are associated with reduced assisted reproductive technology (ART) outcomes. Currently, SDF is not included in routine clinical assessment of male partners of infertile couples, but the 6th edition of the World Health Organization (WHO) manual for semen analysis included the SDF assessment in the chapter on extended semen examinations. SDF evaluation may be indicated in several clinical conditions that have been shown to affect and increase DNA fragmentation. In cases with high SDF levels, the clinician will attempt to correct any reversible factors and may treat the patients empirically with antioxidants. If high levels of SDF persist despite empirical treatment, intracytoplasmic sperm injection (ICSI) may be indicated. During ICSI, embryologists can employ advanced selection procedures, to reduce the proportion of spermatozoa with DNA fragmentation, although no procedure can ensure that only non-DNA-fragmented spermatozoa will be selected.

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SDF consists of single- or double-strand breaks in the backbone of the nucleic acid. It may occur because of the activation of the apoptotic pathway, derangement of the process of chromatin maturation during spermatogenesis, or due to an oxidative insult during transit in the male genital tract after spermiation.[1–3] SDF levels may be elevated in infertile men[3] and elevated SDF levels have been associated with adverse reproductive outcomes in many studies.[4] Considering that routine semen analysis cannot distinguish between fertile and infertile men (with some exceptions), the 6th edition of the WHO manual for the Laboratory and Examination and Processing of Human Semen[5] included SDF in the section on extended semen examination. SDF can be measured by several methods.[5] Among the most popular assays, it is important to distinguish those that evaluate the susceptibility of DNA to damage (sperm chromatin dispersion [SCD] and sperm chromatin structure assay [SCSA]), from those that evaluate the occurrence of real breaks (terminal deoxynucleotidyl transferase dUTP nick end labeling [TUNEL] and Comet). For most of these methods, it is suggested that laboratories should establish their own thresholds.[5] This poses problems for the standardization of assays.[5] Published original research and recent meta-analyses according to the method used for measuring SDF suggest that TUNEL and Comet assays are, in general, better predictors of reproductive outcomes such as fertilization rates, embryo quality, implantation rates, miscarriage, and live birth.[6–8]

WHEN TO ORDER AN SDF TEST?

SDF should be included among the diagnostic tests for male infertility in cases where a possible risk factor is present. Several clinical conditions have been reported to be associated with an increase in SDF.[9,10] These include varicoceles, leukocytospermia, recurrent pregnancy loss, recurrent intrauterine insemination (IUI), *in vitro* fertilization (IVF) and ICSI failure, obesity, and male age (**Table 1**). Although there are substantial evidence suggesting that elevated SDF levels are associated with poorer ART outcomes, not all reproductive societies' guidelines recommend or suggest the evaluation of SDF during the assessment of infertile men.[3] This is due to the lack of a gold standard technique, variable cut-off levels, and studies with equivocal outcomes due to bias in couple selection or low-quality study design in the assessment of SDF. It should be noted that a spermatozoon with fragmented DNA may be motile, viable, and functional, and that the oocyte may have the ability to repair damaged DNA after fertilization.[11] However, the ability of the oocyte to repair damaged DNA may be impaired in aged women or ovarian pathologies affecting ovarian function.[11] Indeed, when donor oocytes from young healthy women are employed in ART, no correlation is found between SDF and reproductive outcomes.[12] Therefore, SDF should also be evaluated in couples with unexplained infertility and recurrent

miscarriages, and in cases where oocyte quality may be compromised.

HOW TO DEAL WITH ELEVATED SDF?

Clinicians can approach patients with high SDF levels utilizing the following possible strategies: (1) offer treatment for the male patient to decrease sperm DNA damage or (2) proceed with ART only using spermatozoa with low SDF selected by sperm selection techniques (*e.g.*, Z selection and microfluidic sperm sorting; **Figure 1**). The option offered also depends on the female age as it may be advisable to proceed with ART without any further delay.

The first line of treatment consists of lifestyle changes which include short abstinence, adequate rest, proper diet, weight reduction where applicable, and cessation of smoking.[13,14] Known correctable factors such as clinically significant varicoceles and infections should also be treated. Another strategy to decrease SDF is treatment with antioxidants, considering that oxidative stress is an important cause of DNA damage.[1] Some studies reported a positive effect of antioxidants on SDF;[1,3] however, only a few of them are randomized controlled trials (RCT), and a recent systematic review selecting three eligible RCT studies did not find a significant effect on SDF.[15] With regard to apoptosis which is also involved in the induction of SDF, follicle-stimulating hormone (FSH), which reduces testis apoptosis, has been shown to reduce SDF levels in hypogonadal men with low FSH levels.[16] According to the results of a recent survey promoted by the Global Andrology Forum,[17] almost 80% of clinicians recommend lifestyle modifications, while about 77% of them would prescribe empiric antioxidants. Only a minority of clinicians recommend shorter abstinence (38%) or the use of advanced sperm selection techniques (about 21%).

With regard to the sperm selection techniques, embryologists have many potential options, such as birefringent sperm assessment, physiological intracytoplasmic sperm injection (PICSI) after selection with hyaluronic acid, intracytoplasmic morphological sperm injection (IMSI), and others,[18,19] to select healthier spermatozoa for ICSI (**Figure 1** shows some of the most popular advanced sperm selection techniques). It is not easy for the embryologist to determine the most suitable, effective, and least time-consuming method among the available techniques. Most, if not all, of these techniques have been shown to select spermatozoa with low levels of SDF,[18] but none assures that only non-DNA-fragmented spermatozoa will be selected. This is expected as there are still no methods available to assess a spermatozoon for SDF without destroying it; hence, the embryologist

Table 1: Main indications for the evaluation of sperm DNA fragmentation during male infertility diagnostic process based on the current literature

Indication	*Evidence*
Varicocele	Roque and Esteves[27] 2018
Unexplained infertility	Sugihara *et al.*[28] 2022 Repalle *et al.*[29] 2022 Wang *et al.*[30] 2023
Recurrent pregnancy loss	Coughlan *et al.*[31] 2015
Recurrent ART failure	Zhao *et al.*[32] 2014
Diabetes	Facondo *et al.*[33] 2022
Inflammatory signs of the lower genital tract	Lotti *et al.*[34] 2017
Cancer and recent cancer therapies	Song *et al.*[35] 2023 Farnetani *et al.*[36] 2024
Advanced age	Gonzalez *et al.*[37] 2022
Obesity	Mahdi *et al.*[38] 2023 Samavat *et al.*[39] 2018
Occupational exposure to environmental toxicants	Giulioni *et al.*[40] 2022

ART: assisted reproductive technology

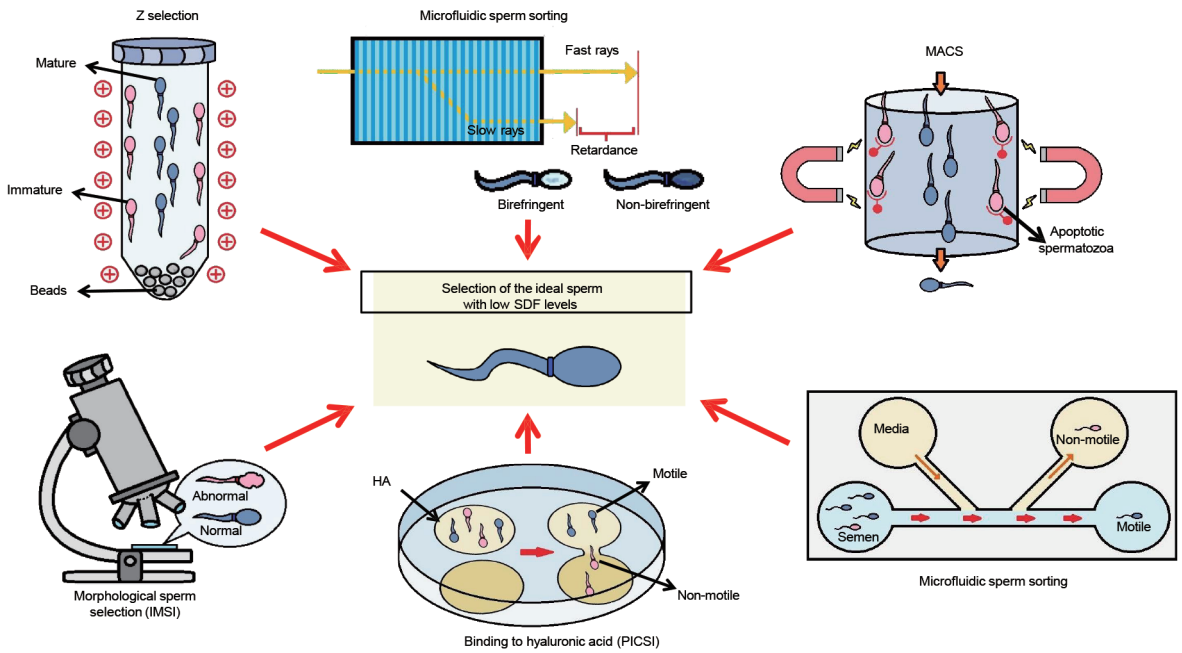

Figure 1: Sperm selection and preparation techniques demonstrated to lower SDF levels. SDF: sperm DNA fragmentation; MACS: magnetic cell sorting; IMSI: intracytoplasmic morphological sperm injection; PICSI: physiological intracytoplasmic sperm injection; HA: hyaluronic acid.

is unable to utilize a spermatozoon after testing that it is an ideal sperm. In addition, whether advanced sperm selection leads to improved ART outcomes remains controversial[20] due to the multifactorial causes of infertility. A recent study demonstrated that the use of microfluidic methods to select spermatozoa significantly increases the rate of embryo euploidy (from 25.3% to 42.9%, $P < 0.001$) in couples with previous adverse outcomes.[21] Microfluidic technology has been proposed to be more efficient compared to other sperm-sorting techniques such as magnetic cell sorting (MACS) or hyaluronic acid sperm selection.[22] Although most studies employing microfluidics show favorable outcomes, more high-quality studies are needed to confirm their routine use in clinical practice. Ideally, a robust and point-of-care method to assess viable sperm with low SDF will allow the current ejaculate produced to be used for immediate fertility treatment and avoid any inconsistencies between each ejaculate. Evidence has shown that testicular-extracted spermatozoa have significantly lower fragmentation when compared to ejaculate ones, although there is a lack of high-quality data to fully support this conclusion.[23] This has been explained by the hypothesis that spermatozoa may suffer DNA damage due to high reactive oxygen species (ROS) levels during transit along the seminal pathways.[2] Elevated SDF is frequently detected in elderly subjects which suggest that in the scrotum, the seminiferous tubules are poorly protected from microtraumas, and various factors.[24] Thus, as a man ages over the years, these factors may increase the risk of male accessory gland inflammation. In recent years, many studies, of low evidence level, have confirmed that a significantly higher cumulative live birth rate could be achieved by ICSI with testicular-extracted spermatozoa compared to ICSI with ejaculated spermatozoa when SDF is elevated. Therefore, in the selected cases, it may be worthwhile to perform testicular sperm extraction (TESE) for ICSI.[25,26]

CLINICAL CASES

To illustrate the potential of utilizing the DNA fragmentation index in sperm to improve the reproductive outcomes, here, we discuss 3 couples who were going through fertility treatments. These clinical scenarios demonstrate that assessing DNA fragmentation index in the male partners and employing the abovementioned sperm selection techniques could reduce DNA fragmentation during IVF treatments and result in higher chances of pregnancy. The clinical case results were reported under Ethical Committee of Canton Ticino, Bellinzona, Switzerland (Approval No. 2020-01580 CE 3689), which permits the use of clinical data for scientific publications.

Case 1

A 40-year-old heavy smoker, married to a 36-year-old woman, have difficulty in conceiving for 5 years. Fertility assessment of the couple revealed moderate–severe oligoasthenoteratozoospermia (OATS) only. The couple experienced a miscarriage in 2019 and the male partner underwent varicocele repair in 2020. The couple underwent four unsuccessful IUIs in 2021 and ICSI in 2022. Twelve mature (MII) oocytes were injected, and 6 fertilized, resulting in 3 obtained blastocysts that were cryopreserved. However, despite 3 single blastocyst transfers, they failed to conceive. Andrological consultation was performed for the man, which revealed normal male genitalia and a normal male hormone profile. In April 2023, a new semen analysis still demonstrated moderate OATS, with an abnormal SDF (SCD: 35%; aniline blue staining: 37%). In the following month, spermatozoa were selected with MACS, which resulted in a significant reduction in SDF (from a pretreatment SCD of 40% to a posttreatment SCD of 7%). Therefore, in the following cycle, ICSI of 6 MII oocytes was performed with MACS-selected spermatozoa, resulting in the attainment of 5 blastocysts. The first transfer of one blastocyst resulted in the delivery of a female newborn on April 11, 2024. Four blastocysts remained cryopreserved.

Case 2

A couple, with both being 36 years old, came for fertility assessment as they were unable to conceive for 3.5 years. A comprehensive andrological and fertility assessment revealed that the man had severe OATS, but with normal FSH, luteinizing hormone (LH), and total testosterone levels, normal karyotype, and normal genetic testing, whereas the female partner showed anti-Müllerian hormone (AMH) levels of 0.97 ng ml^{-1} (usually expected above 1 ng ml^{-1}) and FSH levels of 11.0 mUI ml^{-1}. In 2021, the couple underwent 2 IVF cycles which resulted in 5 blastocysts. Unfortunately, despite 5 single embryo transfers, the couple failed to conceive.

In March 2023, semen analysis with assessment of SDF was performed revealing a semen volume of 3.5 ml and still severe OATS with sperm concentration of 1.6×10^6 ml^{-1} (normal level: $>15 \times 10^6$ ml^{-1}) with total motility of 4% (normal level: ≥40%), and normal morphology of 1% (normal level: ≥4%). It is important to note that this sample had very low sperm viability (20%) with abnormal SDF (SCD: 69%; acridine orange test: 45%; aniline blue staining: 32%). Therefore, due to the very low quality of semen, which was not amenable to the current treatment strategies, the patient underwent TESE on the right testis which was arranged concurrently with his wife's IVF cycle. ICSI was performed on 9 MII oocytes with fresh TESE spermatozoa. Four blastocysts were obtained from this cycle and a single blastocyst transfer resulted in a pregnancy followed by miscarriage in August 2023. A second blastocyst transfer in November 2023 failed. A third cryopreserved blastocyst was transferred in February 2024, resulting in an ongoing pregnancy currently at 12th week of gestation. Five vials of sperm remain cryopreserved.

Case 3

A couple, both 33 years old, presented with infertility of 22 months duration. The man was obese (130 kg of weight and 186 cm of height), apparently in good health, with slight gynecomastia. Normal testes, epididymis, and vasa were noted on the clinical examination with no evidence of varicocele. Hormone profile was normal. The woman was gynecologically normal, apart from AMH levels of 0.80 ng ml^{-1} (usually expected above 1 ng ml^{-1}). Semen analyses showed severe OATS (sperm concentration: 0.2×10^6–3.0×10^6 ml^{-1}; sperm motility: 18% slow progressive). Routine genetic tests were normal. An ICSI cycle had been performed; 5 oocytes had been retrieved but only 2 were mature and injected, and without fertilization. The same semen sample had been submitted for SCD test and had showed 70% SDF. The patient was taking urofollitropin 150 IU three times per week, human chorionic gonadotropin (hCG) 2000 IU twice per week, and antioxidants and was trying to lose weight when he sought medical consultation. In May 2023, the patient was invited to provide two ejaculates in 2 h. The first semen sample revealed a volume of 5.6 ml with severe OATs with leukocytospermia: sperm concentration of 0.8×10^6 ml^{-1}; total motility of 12% slow progressive; normal morphology of 1%; leukocytes of 6.8×10^6 ml^{-1}; and high SDF as evidenced by the SCD test of 52% SDF. The second semen sample showed similar abnormalities as before: semen volume of 2.2 ml; sperm concentration of 1.3×10^6 ml^{-1}; total motility of 3% rapid progressive and 20% slow progressive; normal morphology of 1%; leukocytes of 6.4×10^6 ml^{-1}; and SCD test of

43% SDF. Leukocytospermia was considered as not significant because no other symptoms or signs suggesting male accessory gland infections were present.

In July 2023, simultaneously with his partner's oocyte pickup, the patient underwent right microsurgical testicular sperm extraction (micro-TESE) which yielded recovery of a good number of spermatozoa, apparently of good quality. Nine MII oocytes were injected with fresh spermatozoa, among these oocytes, 3 were fertilized and 2 blastocysts were obtained and cryopreserved; the remaining sperm were cryopreserved (6 vials). In September 2023, a first blastocyst transfer was performed and the pregnancy is currently ongoing at 35 weeks. The second blastocyst remains cryopreserved.

KEY POINTS

SDF may impact reproductive outcomes. Its assessment in conditions where high SDF has been demonstrated may help the diagnostic process. However, there is still no gold standard technique nor clinically acceptable cut-off levels that have been established. The embryologist may employ a sperm selection procedure demonstrated to lower SDF to improve success with ICSI.

COMMENTS

ICSI is a demanding procedure for women, and its successful outcome depends on many factors including sperm quality. Currently, spermatozoa for ICSI are selected based only on morphology and viability. An additional selection criterion for sperm would be welcome, and this could be SDF, despite its limitations. Since 2018, the European Academy of Andrology (EAA) Guidelines "suggest the addition to standard semen analysis of a sperm DNA integrity testing (if the test is available and the laboratory has significant experience) to get further information on the couple's chance of spontaneous pregnancy and assisted reproduction."[25] Similarly, the 6th edition of the WHO semen analysis manual,[5] included SDF among the extended semen evaluations to be performed in certain clinical circumstances.

Better-quality evidence will be unlikely available in the future, due to the difficulties in patient enrollment for prospective randomized trials. Therefore, the prudent clinician should apply sound judgment on existing evidence when referring couples to ICSI.[25] A preliminary assessment of SDF, in conjunction with the current protocols, may increase the take-home-baby rate of IVF.

AUTHOR CONTRIBUTIONS

EB and GMC wrote the manuscript. ZWH, BB, BF, and SR reviewed and edited the manuscript. ZWH contributed to the creation of **Figure 1**. AA coordinated the work of the authors and checked the final version. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

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REFERENCES

1 Muratori M, Tamburrino L, Marchiani S, Cambi M, Olivito B, *et al*. Investigation on the origin of sperm DNA fragmentation: role of apoptosis, immaturity and oxidative stress. *Mol Med* 2015; 21: 109–22.
2 Lotti F, Tamburrino L, Marchiani S, Maseroli E, Vitale P, *et al*. DNA fragmentation in two cytometric sperm populations: relationship with clinical and ultrasound characteristics of the male genital tract. *Asian J Androl* 2017; 19: 272–9.
3 Agarwal A, Farkouh A, Saleh R, Hamoda TA, Salvio G, *et al*. Technical aspects and clinical limitations of sperm DNA fragmentation testing in male infertility: a global survey, current guidelines, and expert recommendations. *World J Mens Health* 2024; 42: 202–15.
4 Farkouh A, Salvio G, Kuroda S, Saleh R, Vogiatzi P, *et al*. Sperm DNA integrity and male infertility: a narrative review and guide for the reproductive physicians. *Transl Androl Urol* 2022; 11: 1023–44.
5 World Health Organization. WHO Laboratory Manual for the Examination and Processing of Human Semen. 6th ed. Geneva: World Health Organization; 2021.
6 Osman A, Alsomait H, Seshadri S, El-Toukhy T, Khalaf Y. The effect of sperm DNA fragmentation on live birth rate after IVF or ICSI: a systematic review and meta-analysis. *Reprod Biomed Online* 2015; 30: 120–7.
7 Cissen M, Wely MV, Scholten I, Mansell S, Bruin JP, *et al*. Measuring sperm DNA fragmentation and clinical outcomes of medically assisted reproduction: a systematic review and meta-analysis. *PLoS One* 2016; 11: e0165125.
8 Simon L, Zini A, Dyachenko A, Ciampi A, Carrell DT. A systematic review and meta-analysis to determine the effect of sperm DNA damage on *in vitro* fertilization and intracytoplasmic sperm injection outcome. *Asian J Androl* 2017; 19: 80–90.
9 Agarwal A, Majzoub A, Esteves SC, Ko E, Ramasamy R, *et al*. Clinical utility of sperm DNA fragmentation testing: practice recommendations based on clinical scenarios. *Transl Androl Urol* 2016; 5: 935–50.
10 Muratori M, Baldi E. Some relevant points on sperm DNA fragmentation tests. *Transl Androl Urol* 2017; 6 Suppl 4: S560–3.
11 Marinaro JA. Sperm DNA fragmentation and its interaction with female factors. *Fertil Steril* 2023; 120: 715–9.
12 Hervás I, Pacheco A, Gil Julia M, Rivera-Egea R, Navarro-Gomezlechon A, *et al*. Sperm deoxyribonucleic acid fragmentation (by terminal deoxynucleotidyl transferase biotin dUTP nick end labeling assay) does not impair reproductive success measured as cumulative live birth rates per donor metaphase II oocyte used. *Fertil Steril* 2022; 118: 79–89.
13 Wright C, Milne S, Leeson H. Sperm DNA damage caused by oxidative stress: modifiable clinical, lifestyle and nutritional factors in male infertility. *Reprod Biomed Online* 2014; 28: 684–703.
14 Esteves SC, Zini A, Coward RM, Evenson DP, Gosálvez J, *et al*. Sperm DNA fragmentation testing: summary evidence and clinical practice recommendations. *Andrologia* 2021; 53: e13874.
15 Agarwal A, Cannarella R, Saleh R, Harraz AM, Kandil H, *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.
16 Santi D, Spaggiari G, Simoni M. Sperm DNA fragmentation index as a promising predictive tool for male infertility diagnosis and treatment management - meta-analyses. *Reprod Biomed Online* 2018; 37: 315–26.
17 Farkouh A, Agarwal A, Hamoda TA, Kavoussi P, Saleh R, *et al*. 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.
18 Baldini D, Ferri D, Baldini GM, Lot D, Catino A, *et al*. Sperm selection for ICSI: do we have a winner? *Cells* 2021; 10: 3566.
19 Garrido N, Gül M, Jindal S, Vogiatzi P, Saleh R, *et al*. How to select healthy sperm for intracytoplasmic sperm injection in samples with high sperm DNA fragmentation? *Panminerva Med* 2023; 65: 148–58.
20 Oseguera-López I, Ruiz-Díaz S, Ramos-Ibeas P, Pérez-Cerezales S. Novel techniques of sperm selection for improving IVF and ICSI outcomes. *Front Cell Dev Biol* 2019; 7: 1–23.
21 Kocur OM, Xie P, Cheung S, Souness S, McKnight M, *et al*. Can a sperm selection technique improve embryo ploidy? *Andrology* 2023; 11: 1605–12.
22 Lepine S, McDowell S, Searle LM, Kroon B, Glujovsky D, *et al*. Advanced sperm selection techniques for assisted reproduction. *Cochrane Database Syst Rev* 2019; 7: CD010461.
23 Khoo CC, Cayetano-Alcaraz AA, Rashid R, Tharakan T, Yap T, *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* 2023; 12: S2405–4569.
24 Lahimer M, Montjean D, Cabry R, Capelle S, Lefranc E, *et al*. Paternal age matters: association with sperm criteria's- spermatozoa DNA integrity and methylation profile. *J Clin Med* 2023; 12: 4928.
25 Mehta A, Esteves SC, Schlegel PN, Niederberger CI, Sigman M, *et al*. Use of testicular sperm in nonazoospermic males. *Fertil Steril* 2018; 109: 981–7.
26 Colpi GM, Francavilla S, Haidl G, Link K, Behre HM, *et al*. European Academy of Andrology guideline: management of oligo-astheno-teratozoospermia. *Andrology* 2018; 6: 513–24.
27 Roque M, Esteves SC. Effect of varicocele repair on sperm DNA fragmentation: a review. *Int Urol Nephrol* 2018; 50: 583–603.
28 Sugihara A, Punjabi U, Roelant E, De Neubourg D. Is there a relationship between sperm DNA fragmentation and intra-uterine insemination outcome in couples with unexplained or mild male infertility? Results from the ID-trial. *Life (Basel)* 2022; 13: 11.
29 Repalle D, Saritha KV, Bhandari S. Sperm DNA fragmentation negatively influences the cumulative live birth rate in the intracytoplasmic sperm injection cycles of couples with unexplained infertility. *Clin Exp Reprod Med* 2022; 49: 185–95.
30 Wang Q, Gu X, Chen Y, Yu M, Peng L, *et al*. The effect of sperm DNA fragmentation on *in vitro* fertilization outcomes of unexplained infertility. *Clinics (Sao*

Paulo) 2023; 78: 100261.
31 Coughlan C, Clarke H, Cutting R, Saxton J, Waite S, *et al*. Sperm DNA fragmentation, recurrent implantation failure and recurrent miscarriage. *Asian J Androl* 2015; 17: 681–5.
32 Zhao J, Zhang Q, Wang Y, Li Y. Whether sperm deoxyribonucleic acid fragmentation has an effect on pregnancy and miscarriage after *in vitro* fertilization/intracytoplasmic sperm injection: a systematic review and meta-analysis. *Fertil Steril* 2014; 102: 998–1005.
33 Facondo P, Di Lodovico E, Delbarba A, Anelli V, Pezzaioli LC, *et al*. The impact of diabetes mellitus type 1 on male fertility: systematic review and meta-analysis. *Andrology* 2022; 10: 426–40.
34 Lotti F, Tamburrino L, Marchiani S, Maseroli E, Vitale P, *et al*. DNA fragmentation in two cytometric sperm populations: relationship with clinical and ultrasound characteristics of the male genital tract. *Asian J Androl* 2017; 9: 272–9.
35 Song SH, Lee TH, Her YS, Oh M, Shin DH, *et al*. Semen quality and sperm DNA fragmentation in cancer patients undergoing sperm cryopreservation. *Investig Clin Urol* 2023; 64: 489–94.
36 Farnetani G, Vannucci M, Fino MG, Cioppi F, Rosta V, *et al*. Severe sperm DNA fragmentation may persist for up to 3 years after cytotoxic therapy in patients affected by Hodgkin lymphoma and non-Hodgkin lymphoma. *Hum Reprod* 2024; 39: 496–503.
37 Gonzalez DC, Ory J, Blachman-Braun R, Nackeeran S, Best JC, *et al*. Advanced paternal age and sperm DNA fragmentation: a systematic review. *World J Mens Health* 2022; 40: 104–15.
38 Mahdi M, Majzoub A, Elbardisi H, Arafa M, Khalafalla K, *et al*. Impact of body mass index on semen parameters and reproductive hormones among men undergoing microsurgical subinguinal varicocelectomy. *Arab J Urol* 2023; 21: 190–7.
39 Samavat J, Cantini G, Lotti F, Di Franco A, Tamburrino L, *et al*. Massive weight loss obtained by bariatric surgery affects semen quality in morbid male obesity: a preliminary prospective double-armed study. *Obes Surg* 2018; 28: 69–76.
40 Giulioni C, Maurizi V, Castellani D, Scarcella S, Skrami E, *et al*. The environmental and occupational influence of pesticides on male fertility: a systematic review of human studies. *Andrology* 2022; 10: 1250–71.