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One out of two idiopathic infertile men has pathologic sperm DNA fragmentation values: Potential implications for clinical practice

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Abstract

Objectives: To investigate the distribution of sperm DNA fragmentation (SDF) values and their association with clinical and seminal parameters in idiopathic infertile men.

Design, Patients, Measurements: Data from 3224 primary infertile men (belonging to couples having failed to conceive a pregnancy within 12 months) who underwent a thorough diagnostic work-up were analysed. A SDF value ≥ 30% (according to Sperm Chromatin Structure Assay) was considered pathologic. We excluded: (1) men with genetic abnormalities; (2) men with history of cryptorchidism; (3) men with biochemical hypogonadism; (4) men with clinical varicocele; and (5) men with other possible known aetiological factors. Descriptive statistics and logistic regression analyses were used to describe the whole cohort.

Results: Of all, 792 (23%) men with at least one abnormal WHO semen parameter but without any identified aetiologic factor for infertility, were considered as idiopathic infertile men. Of 792, 418 (52.7%) men had SDF ≥30%. Men with pathologic SDF were older (p = .02), had higher Follicle-stimulating hormone (FSH) (p = .04) but lower total testosterone (p = .03) values than those with SDF <30%. The homoeostatic model assessment index for insulin resistance (HOMA-IR) was higher in men with SDF ≥30% (p = .01). Idiopathic infertile men with SDF ≥30% presented with lower sperm concentration (p < .001) and lower progressive sperm motility (p < .01) than those with SDF < 30%. Logistic regression analysis revealed that older age (OR: 1.1, p = .02) and higher HOMA-IR score (OR: 1.8, p = .03) were associated with SDF ≥ 30%, after accounting for FSH and sperm concentration values.

Conclusions: Approximately half of infertile men categorized as idiopathic had pathologic SDF values. Idiopathic infertile men with pathologic SDF showed worse clinical, hormonal and semen parameters than those with normal SDF values. These

results suggest that including SDF testing could be clinically relevant over the real-life management work-up of infertile men.

KEYWORDS
guidelines, male infertility, semen analysis, sperm DNA fragmentation

1 | INTRODUCTION

Couples' infertility has become a considerable public health issue, affecting nearly 186 million people worldwide.[1] The infertility pandemic is reflected by epidemiological reports showing a significant decline in men's sperm count [2] and by the increasing use of assisted reproductive technologies (ARTs) in high-income countries (rising from 2.4% to 18.3% annually in Europe, the United States, Australia and New Zealand between 2012 and 2016).[3]

In infertile couples, a male factor infertility can be identified in approximately 50% of cases[4]; therefore, current guidelines on couple's infertility mandate a focused diagnostic work-up of both partners.[4–6] Over the last decades population-based data has proven that a comprehensive medical evaluation of all infertile males is of primary clinical importance to better categorize the cause of infertility, and the consequent therapeutic approach.[7]

Indeed, despite several causes have been considered in the context of male infertility, still almost 30%–40% of men report impaired sperm parameters without an identifiable cause, thus defining the condition of idiopathic male infertility.[4,8] The definition of idiopathic male infertility and its prevalence vary consistently across the literature,[9] depending on the supposed causal factors and the selected baseline diagnostic work-up. In fact, it could be the case that, after a more comprehensive and extensive diagnostic work-up, at least one underlying cause of male infertility can be identified in four out of five infertile men.[10] In this context, it is obvious that even the most sophisticated diagnostic assessments might not reveal all the possible abnormalities regarding male infertility.

As a whole, despite the significant improvements in the field,[11] the definition and the management of idiopathic male infertility are still based on routine semen analysis, which is extremely limited in predicting the actual man's fertility potential.[12,13] In this context, sperm DNA fragmentation (SDF) has increasingly gained clinical relevance in terms of male factor infertility and in predicting reproductive outcomes both under natural and ART conditions.[14] Sperm are typically protected from DNA damage by the tight compaction of DNA allowed by replacement of somatic histone proteins by protamines during spermatogenesis. This process is facilitated by topoisomerase enzymes, which create DNA breaks to reduce torsional stress and allow for histone to protamine substitution.[15,16] If these breaks are not repaired, impaired chromatin packaging may result in defective sperm maturation and sperm with persistent DNA breakage.[16] Indeed, SDF is associated with various intratesticular and post-testicular factors.[15] Intrinsic factors include defective germ cell maturation, abortive apoptosis and oxidative stress, while extrinsically, lifestyle factors, medications and environmental pollutants may contribute to DNA damage.[15,16]

Since SDF is the consequence of different insults towards the spermatozoa, such as oxidative stress which is not assessed by conventional work-up investigations for infertile men, and it has been negatively correlated with semen quality and fertilization rates in couples with idiopathic male infertility,[17] we hypothesized that the routine investigation of sperm DNA integrity could represent a major step to identify a subset of idiopathic infertile men with pathologic SDF that can deserve and follow a different work-up management compared to those with normal values.

Therefore, we aimed to investigate the prevalence of and the predictors of pathologic SDF values in a homogeneous cohort of White-European men seeking first medical attention for primary couple's infertility.

2 | METHODS

2.1 | Study population

We retrospectively analysed demographic, clinical and laboratory data from 3442 White-European primary infertile men consecutively evaluated between 2012 and 2022 at a single academic centre for couple's infertility. Infertility was defined as not conceiving a pregnancy after at least 12 months of unprotected intercourses regardless of whether or not a pregnancy ultimately occurs.[18] Patients were enroled if they were ≥18 and ≤45 years old and had pure male infertility, defined after a comprehensive diagnostic evaluation of all the female partners.[4,19] All couples were evaluated in the setting of assisted reproductive techniques.

All participants were assessed with a thorough medical and sexual history. Health-significant comorbidities were scored with the Charlson comorbidity index.[20] Likewise, weight and height were measured, calculating body mass index for each participant.[21] Testis volume was assessed using Prader's orchidometer estimation; for the specific purpose of this study we calculated the mean value between the two sides.[22] Duration of infertility and partner's age were collected in every participant.[23] Varicocele was also clinically assessed in every patient.[24]

Venous blood samples were drawn from each patient between 7 AM and 11 AM after an overnight fast. Follicle-stimulating hormone (FSH) and luteinizing hormone were measured using a heterogeneous competitive magnetic separation assay (Bayer Immuno 1 System; Bayer Corp.). Total testosterone (tT) levels were measured via a direct

chemiluminescence immunoassay (ADVIA Centaur; Siemens Medical Solutions Diagnostics), and sex hormone-binding globulin levels were measured via a solid-phase chemiluminescent immunometric assay on Immulite 2000 (Medical Systems SpA).

Glucose, measured via a glucose oxidase method (Aeroset Abbott), and insulin levels were measured for every man after a 12 h overnight fast. The homoeostasis model assessment-estimated insulin resistance (HOMA-IR) index was calculated based on glucose and insulin levels as previously reported.[25,26]

According to our internal research protocol, chromosomal analysis and genetic testing were performed in every infertile man (karyotype analysis and tests for Y-chromosome microdeletions and cystic fibrosis mutations).[27]

Participants underwent at least two consecutive semen analyses, performed 3 months apart, according to the lates edition of the WHO Laboratory Manual for the Examination and Processing of Human Semen.[13,28] Since data from 2012 to 2022 was retrospectively analysed, both the V and VI editions of the WHO manual reference criteria have been used throughout the considered time frame. As for clinical practice, we considered the absolute mean values of semen volume, sperm concentration, progressive sperm motility and normal sperm morphology between the two examinations.

Semen samples were collected in the laboratory by masturbation after a sexual abstinence of 2–5 days. Thereafter, samples were analysed within 30 min of ejaculation, in accordance with the WHO criteria.[29]

The improved Neubauer hemocytometer chamber (100-µm-deep; Brand™ Blaubrand™ Neubauer Improved Counting Chambers; Fisher Scientific) was used to calculate sperm concentration and total sperm count in the ejaculate. Sperm morphology was assessed through the following steps: preparation of a smear of semen on a slide; fixing and staining the slide (Testsimplets1Prestained Slides; Waldeck GmbH & Co. KG); examination with brightfield optics at ×1000 magnification (Nikon Eclipse E 200 Nikon; Instruments Europe B. V.) with oil immersion; and assessment of approximately 200 spermatozoa per replicate for the percentage of normal or abnormal forms. Sperm motility was assessed by mixing twice the sample, using a wet preparation of 20.7 µM deep for each replicate, by examining the slide with phase-contrast optics at ×200 magnification and by assessing approximately 200 spermatozoa per replicate for the percentage of different motile categories.

In the laboratory for semen analysis, a continuous quality assurance programme has been developed and subsequently actively kept. It relies on a quality manual containing standard operating procedures and a detailed set of instructions for the different processes and methods used in the laboratory. Internal quality control (IQC) is implemented with the inclusion of IQC materials in the laboratory's regular workload and the results for these materials are monitored using quality control charts. External quality control (EQA) is regularly performed through peer comparison and proficiency testing programmes (Italian EQA programme). The results are sent to a central facility that assesses the performance of the laboratory. Continuous training and education of the laboratory personnel is also undertaken.

The 2021 WHO reference criteria were used for classification of semen abnormalities.[28] Accordingly, oligozoospermia was defined as <16 million spermatozoa per mL; asthenozoospermia as <30% progressive motility; and teratozoospermia <4% of typical forms. Oligoasthenoteratozoospermia (OAT) was defined when all three anomalies occurred simultaneously.

SDF, measured by Sperm Chromatin Structure Assay, was requested for every participant and it was considered pathological for SDF ≥ 30%.[30] This assay measures the susceptibility of sperm nuclear DNA to in situ acid-induced DNA denaturation. Frozen seminal samples containing 1×10^6 spermatozoa were thawed, properly diluted and treated immediately with detergent solution (pH 1.2) containing 0.1% Triton X-100 (Sigma-Aldrich), 0.15 mol L^{-1} NaCl and 0.08 N HCl (Sigma-Aldrich). Spermatozoa were stained after 30 s with 0.006 mg mL^{-1} acridine orange in a phosphate citrate buffer (pH 6) (Sigma-Aldrich). Samples were stained in technical duplicate and acquired using a BD FACSLyric™ flow cytometer (BD Bioscience) with a maximum flow rate of 250 events/s to preserve hydrodynamic focusing.[31] At least 5000 spermatozoa events, gated on the basis of their morphological scatter, were recorded and their red and green fluorescence signals were plotted on a scattergram and analysed with FACSuite software (BD Bioscience). Technical duplicates must have a CV less than 10% (SDF CV < 10%); the percentage of spermatozoa with abnormal chromatin structure was represented by the SDF (%), which was calculated as the proportion of red fluorescent events on total events acquired. Internal quality procedures included the calibration of flow cytometer with the CST beads (cytometer setup and tracking beads; BD Bioscience) to assess daily instrument performance. Moreover, a frozen seminal sample with known SDF was routinely used as internal test control.

2.2 | Exclusion criteria

For the specific purpose of this study, we selected only idiopathic infertile men.[4] We considered the following definition of idiopathic male infertility; infertile men with seminal alterations but without apparent cause-effect factor, presenting with no previous history of diseases affecting fertility and normal findings on physical examination and endocrine, genetic and biochemical laboratory testing.[4] To this aim, we relied on the most common causes of male infertility according to the current EAU Guidelines, as previously reported.[8] In particular, we excluded azoospermic men, patients with known genetic diseases (any type), hypogonadal men, cases when other known causes were potential responsible factors (e.g., hormonal causes other than hypogonadism; cancer and cancer therapies; infectious conditions[32,33]; immunological disorders; cardiometabolic disorders,[34] recreational drugs and illicit substances[35,36]; and erectile or ejaculatory dysfunction) and men with clinical varicocele.[24] Of all, 792 (23.0%) men with idiopathic infertility were considered for the final analysis.

Data collection followed the principles outlined in the Declaration of Helsinki. All men signed an informed consent agreeing to share their own anonymous information for future studies. The study

was approved by the IRCCS San Raffaele Hospital Ethical Committee (Prot. 2014—Pazienti Ambulatoriali).

2.3 | Statistical methods

Distribution of data was tested graphically and with the Shapiro–Wilk test. Data are presented as medians (interquartile range) or frequencies (proportions). First, descriptive statistics was used to describe the whole cohort. Second, demographic characteristics, hormonal values and semen parameters were compared among idiopathic infertile men with normal versus pathologic SDF values with the Mann–Whitney and the χ^2 test, when appropriate. Third, univariable and multivariable logistic regression models were used to identify potential predictors of pathologic SDF in idiopathic infertile men. For completeness, linear regression models were fitted to explore clinical and seminal characteristics associated with SDF values in the same cohort.

Statistical analyses were performed using SPSS v.26 (IBM Corp.). All tests were two-sided, and the statistical significance level was determined at $p < .05$.

3 | RESULTS

Table 1 details the clinical characteristics of the entire cohort of participants and segregated according to SDF values. Of all, 418 (52.7%) idiopathic infertile men had SDF ≥ 30%.

Men with pathologic SDF were older ($p = .02$), had higher FSH ($p = .04$) but lower tT levels ($p = .03$) than those with SDF < 30% (Table 2). Median insulin values ($p = .01$) and HOMA-IR ($p = .01$) were higher in men with SDF ≥ 30% than those with normal values. Conversely, groups were similar in terms of rate of comorbidities, BMI, testicular volume and serum testosterone values. Moreover, as for semen parameters (Table 1), sperm concentration ($p < .001$) and progressive sperm motility ($p < .01$) were lower in idiopathic infertile men with SDF ≥ 30% than in men with normal values. Similarly, a higher rate of OAT was found in men with pathologic SDF than in those with SDF < 30% ($p < .001$) (Table 1).

Table 2 depicts logistic regression models testing the association between clinical, laboratory and semen predictors with pathologic SDF values. At univariable analysis, older age (OR: 1.1, $p < .01$), higher FSH values (OR: 1.1; $p = .04$), HOMA-IR (OR: 1.7; $p = .03$) and lower sperm concentration (OR: 0.9; $p < .01$) were associated with pathologic SDF. At multivariable analysis, only age (OR 1.1, $p = .02$) and higher HOMA-IR (OR 1.8; $p = .03$) were found to be independently associated with pathologic SDF in idiopathic infertile men, after accounting for FSH and sperm concentration.

Further potential associations with SDF values (continuously coded) were investigated. We confirmed that older age (β 0.8; $p < .01$), higher HOMA-IR (β 4.8; $p = .02$) and lower sperm concentration (β −0.1; $p = .02$) were associated with higher SDF values, after accounting for FSH (Table 3).

4 | DISCUSSION

Results from this cross-sectional ex post analysis revealed that almost half of primary infertile men, categorized as idiopathic according to the international guidelines definition, had pathologic SDF values. Idiopathic infertile men with pathologic SDF had worse clinical, hormonal and semen profiles compared to those with SDF < 30%. Of potential clinical relevance, current EAU Guidelines on Sexual and Reproductive health recommend to perform SDF testing throughout the assessment of couples with recurrent pregnancy loss from natural conception and ART or men with unexplained infertility.[4,17] Conversely, current findings suggest that SDF testing may help to identify idiopathic infertile men with worse clinical and seminal characteristics, derived from unknown etiologies, potentially deserving a different management work-up.

Despite continuous research and efforts over the identification of the underling causes of male infertility, idiopathic cases represent common and problematic scenarios in the everyday clinical practice.[8] For this reason, over the last decades numerous attempts have been made both in the diagnostic and the therapeutic field to better address male infertility, ranging from genetics to 'omics' technologies, oxidative stress, SDF evaluation, artificial intelligence, gene engineering and stem cell therapy.[11] Nonetheless, the definition and management of idiopathic male infertility in clinical practice still rely on (i) conventional semen analysis, which is far for being a reliable predictor of male fertility potential[12]; and (ii) the baseline diagnostic work-up of a man presenting for couple's infertility, which could actually vary according to the investigators.[37]

Indeed, it is clear that the definition of idiopathic infertility is strongly related to the quality of the baseline work-up. In this context, Ventimiglia et al. analysed a cohort of 1174 primary infertile men who underwent a thorough diagnostic work-up, including medical history, physical examination, hormonal assessment, genetic testing, semen analyses; semen and urine cultures and testis ultrasound.[10] After a comprehensive diagnostic work-up, the authors found that a causal factor for male infertility can be identified in up to 80% of cases. In our current analysis, the proportion of idiopathic male infertility (23%) is similar to the one reported by previous Authors who performed a similar thorough investigation of infertile males.[8,10] Therefore, we further support the need of a more detailed and comprehensive assessment of infertile men to better tailoring their management in the everyday clinical setting.

Of further clinical relevance we found that almost 50% of idiopathic infertile men had pathologic SDF values, despite the absence of a potential identifiable cause after an extensive investigation of all participants. Idiopathic infertile men with SDF ≥ 30% were older, had worse hormonal profile, worse markers of insulin resistance and worse semen parameters compared to those with normal SDF values. Therefore, it could be speculated that this group of men harbours clinical and metabolic mild disorders associated with alteration of DNA integrity, along with potential genetic and epigenetic semen alterations that might be responsible for higher SDF,[16] but cannot be identified by conventional semen

TABLE 1 Descriptive statistics of idiopathic infertile men according to SDF values ($N = 792$).

	Overall	Idiopathic infertile men With SDF < 30%	Idiopathic infertile men With SDF ≥ 30%	*p* Value*
No of individuals	792 (100%)	374 (47.3%)	418 (52.7%)	
Age (years)				.02
Median (IQR)	37 (33–41)	37 (35–41)	38 (35–41)	
Range	30–45	30–41	31–45	
BMI (kg/m^2)				.4
Median (IQR)	24.5 (22.8–26.1)	23.4 (22.5–25.3)	24.7 (22.8–25.7)	
Range	21.3–29.3	21.7–25.6	21.3–29.3	
CCI (score)				.6
Median (IQR)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	
Mean (SD)	0.15 (0.4)	0.1 (0.4)	0.1 (0.5)	
Range	0–1	0–1	0–1	
Mean testes volume (Prader's estimation)				.1
Median (IQR)	17 (15–22)	18 (15–22)	14 (12–21)	
Range	8–25	8–25	8–25	
Duration of infertility (months)				.4
Median (IQR)	18 (12–24)	12 (12–24)	24 (1–27)	
Range	12–84	12–24	12–84	
Partner's age (years)				.5
Median (IQR)	35 (32–38)	35 (31–37)	35 (32–39)	
Range	27–42	29–38	27–42	
Fasting glucose (mg/dL)				.1
Median (IQR)	87 (82–94)	88 (81–92)	89 (84–92)	
Range	77–106	78–95	77–106	
Insulin (mUI/mL)				.01
Median (IQR)	7.1 (5.2–9.4)	3.7 (2.8–7.3)	7.4 (5.4–8.9)	
Range	2.7–20.0	2.7–9.6	3.6–20.0	
HOMA-IR				.04
Median (IQR)	1.5 (1.0–2.1)	0.7 (0.6–1.5)	1.6 (1.0–2.1)	
Range	0.5–2.6	0.5–2.2	0.7–2.6	
tT (ng/mL)				.03
Median (IQR)	5.1 (4.1–6.0)	5.5 (4.4–6.1)	4.5 (3.4–5.6)	
Range	3.2–6.9	3.2–6.9	3.2–5.9	
FSH (mUI/mL)				.04
Median (IQR)	4.3 (2.9–6.9)	3.4 (2.3–5.5)	5.0 (2.9–9.0)	
Range	1.3–18.2	1.6–11.2	1.3–18.2	
LH (mUI/mL)				.6
Median (IQR)	3.9 (2.9–5.2)	3.0 (2.3–4.5)	2.7 (2.5–5.5)	
Range	1.7–8.8	1.7–8.8	1.7–6.3	

(Continues)

TABLE 1 (Continued)

	Overall	Idiopathic infertile men With SDF < 30%	Idiopathic infertile men With SDF ≥ 30%	*p* Value*
Prolactin (ng/mL)				.1
Median (IQR)	7.5 (6.4–11.4)	6.9 (4.9–12.6)	7.6 (6.6–12.9)	
Range	1.9–15.3	3.5–12.1	1.9–15.3	
SHBG (nmol/L)				.7
Median (IQR)	38 (26–44)	43 (36–49)	36 (29–41)	
Range	20–58	27–58	20–57	
TSH (mUI/L)				.4
Median (IQR)	1.7 (1.2–2.3)	1.6 (1.1–2.3)	2.3 (1.5–2.8)	
Range	0.7–3.3	0.7–3.1	0.7–3.3	
Semen volume (mL)				.4
Median (IQR)	3.0 (2.0–4.0)	3.1 (2.5–4.6)	3.0 (1.8–4.2)	
Range	0.5–6.0	2.4–6.0	0.5–6.0	
Sperm concentration ($\times 10^6$/mL)				<.001
Median (IQR)	17.0 (6.0–45.0)	14.5 (10.7–50.1)	5.0 (1.4–24.0)	
Range	0.1–90.0	2.0–60.2	0.1–90.0	
Progressive sperm motility (%)				<.01
Median (IQR)	23 (11–35)	16 (7–45)	15 (10–29)	
Range	0–59	0–59	0–54	
Normal sperm morphology (%)				.08
Median (IQR)	2 (1–9)	2 (1–9)	2 (1–5)	
Range	0–50	0–50	0–45	
OAT [*N* (%)]	252 (31.9)	76 (20.4)	177 (42.3)	<.001

Abbreviations: BMI, body mass index; CCI, Charlson comorbidity index; FSH, follicle-stimulating hormone; HOMA-IR, homoeostasis model assessment-estimated insulin resistance; IQR, interquartile range; LH, luteinizing hormone; OAT, oligo-asthenoteratozooserpmia; SDF, sperm DNA fragmentation; SHBG, sex hormone binding globulin; TSH, thyroid-stimulating hormone; tT, total testosterone.

*p Value according to the Mann–Whitney test and χ^2 test, as indicated.

analyses. Therefore, testing sperm DNA integrity could be important to shed light into a specific group of idiopathic infertile men with worse characteristics, that might deserve tailored therapeutic approaches compared to those with 'normal' SDF values (e.g., more aggressive and even earlier treatment or follow-up); in addition, in the future this might have implications to reduce the number of infertility cases that truly are idiopathic.

In clinical practice, different SDF assays are available. The TUNEL assay is a method of detecting single or double-stranded DNA breaks by incorporating deoxynucleotides coupled to a fluorescent marker into the sites of breaks. This technique can be used with fluorescence microscopy or flow cytometry, allowing the analysis of thousands of cells.[30] TUNEL is sensitive, reliable, with minimal interobserver variability, and can be performed on few sperm, but requires inter-laboratory standardization, and the use of expensive equipment and personnel training. The COMET assay is another direct method to evaluate SDF based on the differential migration of DNA fragments under the influence of an electric field. One unique feature of the COMET assay is its ability to distinguish between single or double-strand breaks, depending on the pH of the medium used. An alkaline comet detects both types, while a neutral comet detects double-strand breaks. The comet assay can be performed in very low sperm counts, is sensitive and reproducible, but requires an experienced observer, and suffers from high interobserver variability as well as variable protocols and thresholds.[30] The sperm chromatin dispersion (SCD) assay is an indirect method for measuring SDF that relies on the susceptibility of chromatin to denaturation after the action of an acid treatment, which occurs more when there is fragmented DNA.[30] It is a simple assay with the advantage that it does not require the use of fluorescence or any specialized equipment, and in fact, there are commercial kits available as well. The reliability of SCD has been

TABLE 2 Logistic regression models predicting pathologic SDF in idiopathic infertile men.

	Univariate model OR; *p*-value [95% CI]	Multivariate model OR; *p*-value [95% CI]
Age	1.1; <.01 [1.03–1.15]	1.1; .02 [1.01–1.21]
BMI	1.0; .3 [0.93–1.21]	
CCI	0.9; .6 [0.26–2.17]	
Testicular volume	0.8; .1 [0.91–1.15]	
FSH	1.1; .04 [1.01–1.19]	1.0; .4 [0.94–1.15]
HOMA-IR	1.7; .03 [1.04–2.35]	1.8; .03 [1.05–3.16]
Sperm concentration	0.9; <.01 [0.97–0.99]	0.9; .1 [0.97–1.01]

Abbreviations: BMI, body mass index; CCI, Charlson comorbidity index; FSH, follicle-stimulating hormone; HOMA-IR, homoeostasis model assessment-estimated insulin resistance; SDF, sperm DNA fragmentation.

TABLE 3 Linear regression models predicting SDF in idiopathic infertile men.

	UVA model *β*; *p*-value [95% CI]	MVA model *β*; *p*-value [95% CI]
Age	0.8; <.001 [0.33–1.25]	0.8; <.01 [0.22–1.47]
BMI	0.4; .6 [−0.89–1.56]	
CCI	4.1; .1 [−2.16 to 9.41]	
Testicular volume	−0.5; .1 [−1.12 to 0.09]	
FSH	1.1; <.01 [0.41–1.87]	0.6; .1 [−0.21 to 1.52]
HOMA-IR	5.3; .02 [0.89–8.81]	4.8; .02 [0.53–9.18]
Sperm concentration	−0.2; <.001 [−0.28 to −0.11]	−0.1; .02 [−0.24 to −0.02]

Abbreviations: BMI, body mass index; CCI, Charlson comorbidity index; FSH, follicle-stimulating hormone; HOMA-IR, homoeostasis model assessment-estimated insulin resistance; MVA, multivariate model; SDF, sperm DNA fragmentation; UVA, univariate model.

commended with high intra-individual agreement.[31] However, it has been criticized for high interobserver variability. The SCSA is a well-described and commonly used test with a standardized protocol for estimating DNA fragmentation index.[31] Despite the TUNEL assay appears to be the mostly used worldwide,[30] previous studies have demonstrated a high correlation between SCSA and TUNEL results.[38] For this reason, we expect that our results could also be valid in cohorts tested with the TUNEL essay.

Sperm DNA integrity has been previously found to be associated with ageing and poor semen parameters. Pozzi et al. recently analysed data from 515 primary infertile men to investigate clinical parameters associated with impaired SDF.[14] They found that infertile men older than 38 years had twofold higher risk of pathologic SDF than younger ones. Similarly, participants with lower total motile sperm count were more likely to have higher SDF values.[14] Our results, in a selected cohort of idiopathic infertile men, corroborate the association between ageing and poor semen quality with SDF.

Several studies have investigated the impact of insulin resistance in infertile men. Ventimiglia et al., for instance, analysed data from 1337 primary infertile men and found metabolic syndrome in 9.6% of participants. Infertile men with metabolic syndrome were older, had a greater number of comorbidities and lower levels of tT compared to men without metabolic alterations.[39,40] Similarly, in a study with 726 infertile men, insulin resistance was associated with lower semen quality and pathologic SDF values.[26] Despite not reaching the definition of insulin resistance (as defined by a cut-off of >2.6),[41] current findings depicted that men with higher HOMA-IR, had also higher SDF values, further underling the detrimental impact of metabolic disorders on semen quality.

This study is novel since we showed very interesting underlying abnormalities in SDF in idiopathic primary infertile men. Results from this study led us to postulate that SDF can be considered as a mirror of underling pathologic conditions that (i) are not captured by routine testing (such as oxidative stress, genetic and epigenetic diseases) or (ii) are mild in nature (insulin resistance or ageing) although they can impact on semen quality and reproductive outcomes. According to this hypothesis, infertile men with impaired SDF can be considered as having a 'more severe form of idiopathic infertility', since they might harbour various pathologic conditions responsible for DNA damage and infertility per se, even if not recognized after an extensive work-up. Thereof, this group of men might benefit from a more tailored work-up management. For instance, idiopathic infertile men with pathologic SDF might benefit suggested for hormonal or antioxidant treatment or ART with advanced sperm selection techniques.

Our study is not devoid of limitations. First, this was a single-centre study and all data was retrospectively collected, raising the possibility of selection biases. Second, we did not directly investigate the level of oxidative stress in our cohort, which can be associated with idiopathic male infertility,[42] yet not suggested by current guidelines.[4] Third, since there is a lack of consensus regarding the most adequate threshold for SDF in the context of male infertility, we adopted the 30% value which is the one commonly used in clinical practice as predictor of pregnancy outcomes in infertile couples.[32] However, our results might be different after using different cut-offs for SDF. Lastly, the lack of a control group prevented us from inquiring the strengths of causal associations.

5 | CONCLUSIONS

Findings from this cross-sectional study showed that approximately half of infertile men categorized as idiopathic after extensive baseline diagnostic work-up had pathologic SDF values. Idiopathic infertile men with pathologic SDF showed worse clinical, hormonal, metabolic and semen profiles than those with normal SDF values. These results suggest that SDF testing would help identifying idiopathic infertile

men with worse characteristics that might benefit from an even more tailored work-up protocol and a different therapeutic approach. Future studies are needed to externally validate our results.

AUTHOR CONTRIBUTIONS

Luca Boeri, Edoardo Pozzi and Andrea Salonia designed and led the study and wrote the report. Paolo Capogrosso, Federico Belladelli, Christian Corsini, Simone Cilio, Alessandro Bertini, Francesco Lanzaro, Luigi Candela, Massimiliano Raffo, Fausto Negri, Ludovica Cella, Margherita Fantin, Giuseppe Fallara, Alessia d'Arma and Francesco Montorsi took care of patients and acquired data. Luca Boeri, Andrea Salonia and Edoardo Pozzi analysed data and drafted the report.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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