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REVIEW

Sperm DNA fragmentation index as a promising predictive tool for male infertility diagnosis and treatment management – meta-analyses

BIOGRAPHY
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KEY MESSAGE
The assessment of sperm DNA fragmentation (sDF) is a promising tool to be used in clinical and research practice for both the diagnosis and the management of male infertility.

ABSTRACT
Conventional semen analyses have limitations in male infertility diagnosis and prognosis. Assessment of sperm DNA fragmentation (sDF) has been proposed to discriminate fertile from infertile men and predict FSH treatment response in infertile men, although a comprehensive evaluation of this is not available. The aims of these meta-analyses were to assess the power of sDF in male infertility diagnosis and its role in predicting FSH therapy response in infertile men. Two literature searches were conducted in MEDLINE (PubMed), Embase, the Cochrane Library, Scopus and UpToDate. First, interventional/observational clinical trials comparing fertile to infertile/subfertile men were included. Second, interventional/observational clinical trials evaluating FSH-treated infertile men were assessed. sDF levels were significantly higher in infertile men considering 28 studies ($P < 0.001$), independently of the sDF method applied. Receiver operator characteristics curves identified an sDF threshold of 20%, with sensitivity of 79% and specificity of 86%. Six studies showed significant sDF improvement of 4.24% (95% confidence interval: 0.24–8.25%) after 3 months of FSH treatment. These meta-analyses demonstrate the sDF relevance in male infertility, suggesting a higher accuracy in detecting sperm function than conventional semen parameters. Although larger prospective trials are needed, sDF represents a promising tool for clinical and research practice.

INTRODUCTION

Male infertility diagnosis includes the evaluation of conventional semen analysis, which is guided by the World Health Organization (WHO) criteria (*WHO, 2010*). However, an increasing number of studies have highlighted many difficulties and challenges inherent to this methodology. First, the quality of laboratories performing semen analyses is variable, with a generally poor adherence to the WHO guidelines and a limited reliance on quality control protocols (*Bjorndahl et al., 2016; Filimberti et al., 2013; Punjabi et al., 2016*). This lack of adherence to standardized methods significantly reduces the potential diagnostic power of this tool (*Carrell and De Jonge, 2016*). Second, conventional semen analysis applied to clinical practice does not evaluate all possible sperm quality parameters, impairing its diagnostic competence. Moreover, several studies have found an overlap of semen analysis results between fertile and infertile men (*Cooper et al., 2010; Guzick et al., 2001; Mac, 1950*). Thus, the comparison of patients' semen parameters to the normal values described for fertile men, which represents a major diagnostic step for male infertility assessment, may not clearly identify all cases of infertility (*Bjorndahl, 2011*). This represents the main challenge in the current diagnostic process of male infertility, which underestimates its real incidence, leaving 15% of infertile men with semen parameters falling within the normal ranges (*Guzick et al., 1998*). In addition, concerns about the value of conventional semen analysis also remain in the management of infertility, because there are no predictive, validated thresholds of semen parameters for assisted reproductive technology (ART) success (*van der Steeg et al., 2011; van Weert et al., 2008*). Hence, conventional semen analysis describes some features of sperm function, but does not fully address functional sperm competence. A fundamental role in determining sperm competence is currently attributed to DNA integrity, and new tests to evaluate sperm DNA fragmentation (sDF) are strongly advocated (*Shamsi et al., 2011*).

Sperm DNA integrity is continuously challenged by endogenous and exogenous factors, although different mechanisms of repairing and protecting against this damage are active in human cells (*Hoeijmakers, 2009*). This is particularly relevant in germ cells, which have to preserve DNA integrity to pass the genome to the next generation. In these cells, DNA double-strand breaks are physiologically induced during spermatogenesis and spermiogenesis to facilitate meiotic crossover and histone–protamine substitution, respectively (*Rathke et al., 2014*). Apart from this first 'physiological' DNA damage, other exogenous and endogenous factors could affect DNA integrity during sperm maturation and storage in the epididymis (*Moustafa et al., 2004; Ramos et al., 2004; Sakkas et al., 2002*). Thus, DNA integrity is constantly at risk and its assessment could be a fundamental step in the evaluation of sperm functional competence (*Lewis et al., 2008*). Hence, sperm DNA damage evaluation could be crucial for both infertility diagnosis and prediction of ART success. In the setting of IVF this evaluation plays a peculiar role because the natural selection barriers of conception are bypassed, increasing the possibility of spermatozoa with significant DNA damage transmitting the genetic aberrations to the newborn (*Host et al., 2000*). Thus, several trials have evaluated the predictive role of sperm DNA damage for either ART outcome or sperm selection.

Different assays have so far been developed and applied in research laboratories to evaluate sperm DNA damage but, despite their clinical relevance, only few laboratories have implemented them in routine semen analysis. Up to now, four methodologies have been employed: sperm chromatin structure assay (SCSA) (*Larson et al., 2000*), sperm chromatin dispersion (SCD) test (*Fernandez et al., 2003*), terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labelling (TUNEL) (*Gorczyca et al., 1993*) and the single cell gel electrophoresis (Comet) assay (*Sakkas et al., 2002*). Using these tests, several trials have demonstrated that the percentage of spermatozoa with fragmented DNA is higher in infertile compared with fertile men (*Host et al., 2000; Saleh et al., 2002*). Moreover, these studies found a similar percentage of spermatozoa with fragmented DNA in subfertile men with normal sperm parameters and in subfertile men with abnormal sperm parameters (*Host et al., 2000; Saleh et al., 2002*). These results suggest the possible role of sDF assessment in the diagnostic workup of male infertility, although a comprehensive demonstration of its clinical usefulness has not so far been achieved. On the contrary, there is extensive literature on the possible role of sDF in ART, claiming the necessity of introducing this evaluation for sperm selection (*Zhao et al., 2014*). Indeed, several meta-analyses have been designed to collect these results; however, a definitive statement has yet to be confirmed (*Li et al., 2006*). Indeed, Cissen et al. (2016) demonstrated only a limited capacity for sDF to predict ART outcome, evaluating 30 clinical trials. On the contrary, *Robinson et al. (2012)* highlighted a role for sDF in the miscarriage rate prediction during ART, considering 16 studies. Thus, sDF evaluation seems to be useful in ART, but a clear consensus is still to be reached.

In the setting of male infertility, sDF evaluation has been proposed to evaluate the efficacy of treatment with FSH. The empirical FSH administration to infertile men with endogenous FSH serum levels in the normal range (<8 IU/l) has been reported in the literature since 1991, and this therapy is available in some countries (*Acosta et al., 1991*). Two recent meta-analyses estimated an overall beneficial effect of FSH treatment of the man in couples receiving ART treatment in terms of pregnancy rate (*Attia et al., 2013b; Santi et al., 2015*). This meta-analysis in this study demonstrated that FSH administration improves some sperm parameters, such as total sperm number, and not only pregnancy rate (*Santi et al., 2015*). Many studies are limited by an important oversight when dealing with infertility treatment efficacy. In fact, if the aim of a clinical trial is the evaluation of FSH action on the gonads, the primary endpoint must be the first measurable, surely FSH-dependent outcome, i.e. sperm parameter improvement in men and oocyte number and quality in women. On the contrary, the choice of using pregnancy rate as primary endpoint is weakened by an increasing number of biases and variables, from gamete quality to the fertility status of the partner. Thus, many inconclusive studies have so far been published, mixing up male, female and couple outcomes.

The available trials dealing with sDF in male infertility are limited by the low sample size, which prevents the
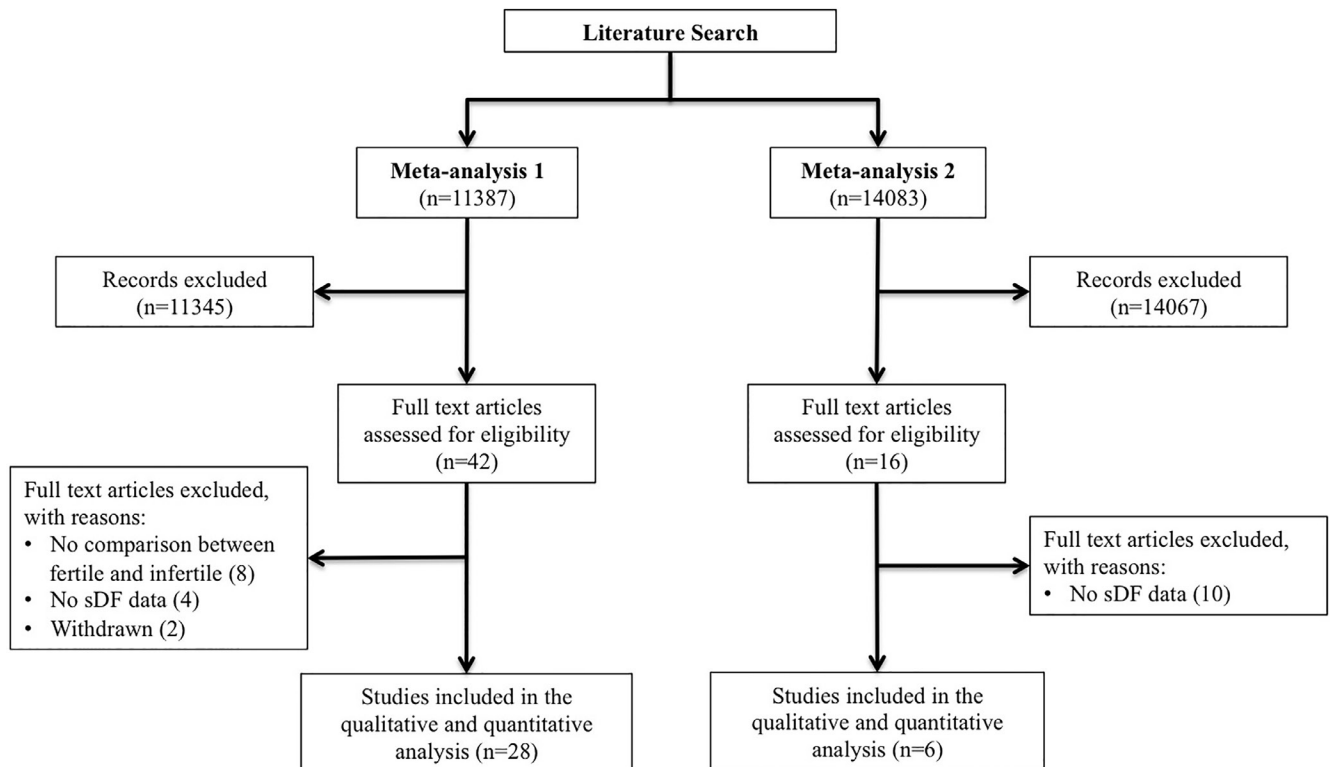
FIGURE 1 Study flow chart showing the search results for the studies included in Meta-analysis 1 comparing sperm DNA fragmentation index (sDF) in fertile and infertile men (Search 1) and in Meta-analysis 2 comparing sDF in infertile men before and after FSH administration (Search 2).

exportability of sDF in clinical practice. A meta-analytic approach could be useful to overcome this limit, increasing the number of patients evaluated. Indeed, this meta-analysis is designed to address both the sDF diagnostic power for the male infertility diagnosis, and the sDF predictive role in the assessment of FSH treatment response in infertile men.

MATERIALS AND METHODS

A meta-analysis was performed according to the Cochrane Collaboration and PRISMA statement. The meta-analysis was accepted in the International Prospective Register of Systematic Reviews (PROSPERO; registration ID 82172) prior to commencing the study, ensuring transparency and originality of the review process.

A comprehensive literature search was conductive up to December 2017 for English language articles in MEDLINE (PubMed), Embase, the Cochrane Library, Scopus and UpToDate. The literature search was performed in two steps. First, the following search was performed: (((((((((((sperm DNA fragmentation index) OR sperm DNA fragmentation) OR sDF) OR

DNA fragmentation index) OR DNA fragmentation) OR DFI) and male infertility) OR oligozoospermia) OR azoospermia) OR asthenospermia) OR teratospermia) OR asthenozoospermia) OR teratozoospermia) (Meta-analysis 1). Second, the following search was performed: (((((((((sperm DNA fragmentation index) OR sperm DNA fragmentation) OR sDF) OR DNA fragmentation index) OR DNA fragmentation) OR DFI) and FSH treatment) OR FSH administration) OR FSH therapy) (Meta-analysis 2).

Study selection and inclusion criteria
Meta-analysis 1
The first literature search evaluated all clinical trials in which fertile men were compared with either infertile or subfertile men. The diagnosis of infertility or subfertility includes all degrees of semen parameter alteration, from azoospermia to mild oligozoospermia (when total sperm count was between 10 and 20 million per ejaculate). Thus, in this first selection, both interventional and observational studies were searched and collected, without specific inclusion or exclusion criteria for the study design. Randomization and presence of controls were not considered as inclusion criteria.

Meta-analysis 2

In the second literature search, all clinical trials in which FSH was administered to infertile men, either interventional or observational, were searched. Randomization and presence of controls were not considered as inclusion criteria. Considering the type of participants, all men with a diagnosis of infertility or subfertility were considered, from mild oligozoospermia (when total sperm count was between 10 and 20 million per ejaculate) to severe oligo-astheno-teratospermia (when total sperm count was below 10 million per ejaculate, associated with alteration in sperm motility and morphology). Azoospermic men were not considered eligible. No inclusion criteria were applied for the female partner of the infertile couple. Considering the type of intervention, all studies in which the male partner was treated with any type of FSH (e.g. either recombinant or urinary-derived) and dosage were considered.

Data collection process and quality
For both literature searches, two authors (DS and GS) extracted the abstracts of all studies detected. All abstracts were evaluated for inclusion criteria

TABLE 1 CHARACTERISTICS OF STUDIES INCLUDED IN THE ANALYSIS AFTER META-ANALYSIS 1

Author	Year	sDF Method	Fertile men (CONTROL GROUP)			Infertile men (STUDY GROUP)		
			N	Age (years)	Inclusion criteria	N	Age (years)	Inclusion criteria
Zini	2001	TUNEL	7	38.3 ± 1.6	Proven fertility	33	33.1 ± 0.8	Abnormal semen analysis and varicocele
Saleh	2002	SCSA	16	31.5 ± 4.7	Healthy donors	71	33.5 ± 10.6	Abnormal semen analysis
Saleh	2003	SCSA	16		Healthy donors	23		Unexplained couple infertility
Khalili	2006	SCSA	30		Proven fertility	30		Unexplained couple infertility
de Paula	2006	TUNEL	30	33.1 ± 6.3	Normozoospermia	47	32.7 ± 9.8	Oligo-astheno-teratozoospermia
Plastira	2007	TUNEL	49	34.6 ± 5.4	Healthy donors	61	36.8 ± 6.4	Unexplained couple infertility
Nicopoullos	2008	SCSA	28		Healthy donors	56		Unexplained couple infertility
Winkle	2009	SCSA	27	33.0	Proven fertility	70	32.8	Unexplained couple infertility
Mehdi	2009	TUNEL	30	35.3 ± 5.5	Proven fertility	30	37.0 ± 6.5	Teratozoospermia
Venkatesh	2011	SCSA	50	29.4 ± 4.1	Proven fertility	100	30.7 ± 4.4	Unexplained couple infertility
Brahem	2011a	TUNEL	16	35.0	Healthy donors	79	34.6	Unexplained couple infertility
Brahem	2011b	TUNEL	30	36.8 ± 5.6	Proven fertility	70	37.0 ± 6.6	Abnormal semen analysis
Alkhayal	2013	SCSA	15		Volunteers	102		Unexplained couple infertility
Mangiarini	2013	TUNEL	18	37.0 ± 3.5	Normozoospermia	14	38.0 ± 6.0	Teratozoospermia
Chenlo	2014	TUNEL	31	29.5 ± 12.3	Healthy donors	77	37.4 ± 16.0	Unexplained couple infertility
Evgeni	2015a	SCD	78	28.0 ± 5.0	Healthy donors	539	38.0 ± 6.0	Unexplained couple infertility
Evgeni	2015b	SCD	184	38.0 ± 5.0	Normozoospermia	485	42.0 ± 6.0	Abnormal semen analysis
Garolla	2015	TUNEL	61	36.3 ± 6.3	Proven fertility	100	37.3 ± 6.2	Unexplained couple infertility
Muratori	2015	TUNEL	86	35.6 ± 12.4	Proven fertility	348	40.0 ± 28.4	Unexplained couple infertility
Ni	2016	SCSA	25	28.9 ± 3.9	Healthy donors	15	31.3 ± 3.9	Abnormal semen analysis and varicocele
Bareh	2016	TUNEL	31	35.0 ± 4.3	Proven fertility	26	35.1 ± 4.5	Unexplained recurrent pregnancy loss
Liu	2016	TUNEL	50	Range 26–38	Normozoospermia	50	Range 26–38	Asthenozoospermia
Malić Vončina	2016	TUNEL	51	36.0 ± 39.1	Proven fertility	85	34.0 ± 41.7	Unexplained couple infertility
Dorostghoal	2017	Comet	105	35.7 ± 19.4	Volunteers	112	36.5 ± 18.7	Abnormal semen analysis
Wiweko and Utami	2017	SCD	36	34.0 ± 3.2	Proven fertility	78	37.0 ± 3.5	Abnormal semen analysis
Zandieh	2017	SCD	30	Range 25–35	Proven fertility	28	Range 25–35	Unexplained couple infertility
Atig	2017	TUNEL	50	34.8 ± 5.4	Proven fertility	40	39.4 ± 5.2	Oligo-astheno-teratozoospermia
Carlini	2017	TUNEL	114	37.4 ± 4.6	Proven fertility	114	20.8 ± 8.9	Unexplained couple infertility

Comet = the single cell gel electrophoresis assay; SCD = sperm chromatin dispersion test; SCSA = sperm chromatin structure assay; TUNEL = terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labelling.

and data were extracted from each study considered eligible, with regard to study design, year of publication, and number of included/excluded subjects. Moreover, DS and GS extracted study subjects' demographics and underlying diseases, with particular attention to the inclusion criteria and the method used for sDF measurement. DS, GS and MS performed quality control checks on extracted data.

For both literature searches, the primary endpoint was the sDF evaluation. In particular, for Meta-analysis 1, the comparison of sDF between fertile and infertile/subfertile men was performed.
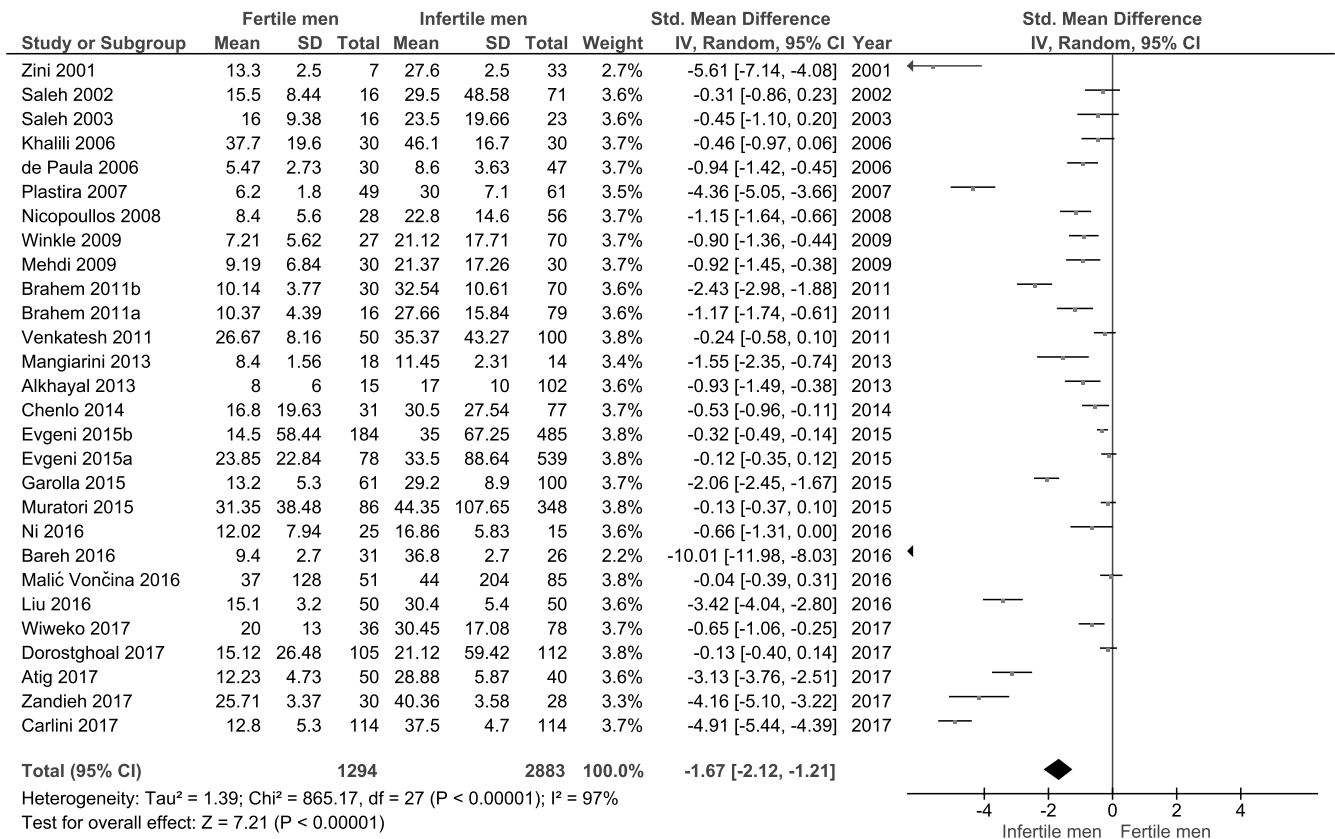
In Meta-analysis 2 the sDF changes from baseline and after FSH administration were considered.

The investigators (DS and GS) independently assessed the risk of bias for all trials using a Cochrane risk-of-bias algorithm. The following quality criteria and methodological details were evaluated for each trial included in the meta-analysis: (i) method of randomization, even if the randomization was not an inclusion criterion, (ii) concealment of allocation, (iii) presence or absence of blinding to treatment allocation, (iv) duration and type of treatment and follow-up phases,

(v) number of participants recruited, analysed or lost to follow-up, (vi) timing of trial, (vii) whether an intention to treat analysis was performed, (viii) whether a power calculation was performed, (ix) source of funding, and (x) criteria for including participants and assessing outcomes.

Data synthesis and analysis
The meta-analysis was conducted using Review Manager (RevMan) 5.3 software (Version 5.3.1 Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014). Data were combined using the fixed-effects model. The random-effects model was

Study or Subgroup	Fertile men Mean	SD	Total	Infertile men Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI	Year
Zini 2001	13.3	2.5	7	27.6	2.5	33	2.7%	-5.61 [-7.14, -4.08]	2001
Saleh 2002	15.5	8.44	16	29.5	48.58	71	3.6%	-0.31 [-0.86, 0.23]	2002
Saleh 2003	16	9.38	16	23.5	19.66	23	3.6%	-0.45 [-1.10, 0.20]	2003
Khalili 2006	37.7	19.6	30	46.1	16.7	30	3.7%	-0.46 [-0.97, 0.06]	2006
de Paula 2006	5.47	2.73	30	8.6	3.63	47	3.7%	-0.94 [-1.42, -0.45]	2006
Plastira 2007	6.2	1.8	49	30	7.1	61	3.5%	-4.36 [-5.05, -3.66]	2007
Nicopoullos 2008	8.4	5.6	28	22.8	14.6	56	3.7%	-1.15 [-1.64, -0.66]	2008
Winkle 2009	7.21	5.62	27	21.12	17.71	70	3.7%	-0.90 [-1.36, -0.44]	2009
Mehdi 2009	9.19	6.84	30	21.37	17.26	30	3.7%	-0.92 [-1.45, -0.38]	2009
Brahem 2011b	10.14	3.77	30	32.54	10.61	70	3.6%	-2.43 [-2.98, -1.88]	2011
Brahem 2011a	10.37	4.39	16	27.66	15.84	79	3.6%	-1.17 [-1.74, -0.61]	2011
Venkatesh 2011	26.67	8.16	50	35.37	43.27	100	3.8%	-0.24 [-0.58, 0.10]	2011
Mangiarini 2013	8.4	1.56	18	11.45	2.31	14	3.4%	-1.55 [-2.35, -0.74]	2013
Alkhayal 2013	8	6	15	17	10	102	3.6%	-0.93 [-1.49, -0.38]	2013
Chenlo 2014	16.8	19.63	31	30.5	27.54	77	3.7%	-0.53 [-0.96, -0.11]	2014
Evgeni 2015b	14.5	58.44	184	35	67.25	485	3.8%	-0.32 [-0.49, -0.14]	2015
Evgeni 2015a	23.85	22.84	78	33.5	88.64	539	3.8%	-0.12 [-0.35, 0.12]	2015
Garolla 2015	13.2	5.3	61	29.2	8.9	100	3.7%	-2.06 [-2.45, -1.67]	2015
Muratori 2015	31.35	38.48	86	44.35	107.65	348	3.8%	-0.13 [-0.37, 0.10]	2015
Ni 2016	12.02	7.94	25	16.86	5.83	15	3.6%	-0.66 [-1.31, 0.00]	2016
Bareh 2016	9.4	2.7	31	36.8	2.7	26	2.2%	-10.01 [-11.98, -8.03]	2016
Malić Vončina 2016	37	128	51	44	204	85	3.8%	-0.04 [-0.39, 0.31]	2016
Liu 2016	15.1	3.2	50	30.4	5.4	50	3.6%	-3.42 [-4.04, -2.80]	2016
Wiweko 2017	20	13	36	30.45	17.08	78	3.7%	-0.65 [-1.06, -0.25]	2017
Dorostghoal 2017	15.12	26.48	105	21.12	59.42	112	3.8%	-0.13 [-0.40, 0.14]	2017
Atig 2017	12.23	4.73	50	28.88	5.87	40	3.6%	-3.13 [-3.76, -2.51]	2017
Zandieh 2017	25.71	3.37	30	40.36	3.58	28	3.3%	-4.16 [-5.10, -3.22]	2017
Carlini 2017	12.8	5.3	114	37.5	4.7	114	3.7%	-4.91 [-5.44, -4.39]	2017
Total (95% CI)			**1294**			**2883**	**100.0%**	**-1.67 [-2.12, -1.21]**	

Heterogeneity: Tau² = 1.39; Chi² = 865.17, df = 27 (P < 0.00001); I² = 97%
Test for overall effect: Z = 7.21 (P < 0.00001)

FIGURE 2 Forest plot comparing sDF in fertile with infertile men (Meta-analysis 1).

used when a high heterogeneity rate was discovered. Heterogeneity among the results of different studies was examined by inspecting both the scatter in the data points and the overlap in their confidence intervals (CI), and by performing I^2 statistics. The I^2 statistic answers the question: What proportion of the observed variance reflects real differences in effect size? It is a measure of inconsistency across the findings of the studies, not a measure of the real variation across the underlying true effects. A high heterogeneity rate was considered for $I^2 > 50\%$.

Weighted mean differences (WMD) and 95% CI were estimated for the first literature search. Here, the mean sDF difference between fertile and infertile/subfertile men was reported. This analysis was further performed by subgrouping patients according to the methodology used to detect sDF. Mean difference and 95% CI were used in the second search. Here, the mean sDF difference before and after FSH treatment was reported. Moreover, the sDF difference between fertile and infertile men was evaluated both before and after FSH treatment.

Finally, the sDF diagnostic accuracy for male infertility was investigated using receiver operator characteristics (ROC) curves, considering studies found by the first literature search. Thus, in order to define the sDF threshold, which better identifies infertile men, mean sDF values were considered together with standard deviations (SD) and sample sizes. ROC cut-offs were calculated by the Youden's index through the identification of the best pair of sensitivity and specificity.

Values of $P < 0.05$ were considered statistically significant.

RESULTS

Meta-analysis 1
The first literature search found 11,387 papers (**FIGURE 1**). Forty-two potentially relevant studies were identified by considering the information given in the abstract. All trials were thoroughly appraised for eligibility in the meta-analysis and methodological quality. Fourteen studies were excluded from the final analysis because they did not fulfil the inclusion criteria (*Avendano et al., 2009; Binsaleh et al., 2015;* *Garcia-Peiro et al., 2011; Jurewicz et al., 2016; Manente et al., 2015; Omran et al., 2013; Ramzan et al., 2015; Sergerie et al., 2005; Sharma et al., 2010, 2016; Varghese et al., 2009; Wdowiak et al., 2014; Zalata et al., 2015; Zribi et al., 2011*). In particular, eight studies did not compare fertile with infertile men, four papers did not report sDF and two papers had been withdrawn. Finally, 28 studies met the inclusion criteria and were included in the final analysis (**FIGURE 1**) (*Alkhayal et al., 2013; Atig et al., 2017; Bareh et al., 2016; Brahem et al., 2011a, 2011b; Carlini et al., 2017; Dorostghoal et al., 2017; Chenlo et al., 2014; de Paula et al., 2006; Evgeni et al., 2015a, 2015b; Garolla et al., 2015; Khalili et al., 2006; Liu et al., 2016; Malić Vončina et al., 2016; Mangiarini et al., 2013; Mehdi et al., 2009; Muratori et al., 2015; Ni et al., 2016; Nicopoullos et al., 2008; Plastira et al., 2007; Saleh et al., 2002, 2003; Venkatesh et al., 2011; Winkle et al., 2009; Wiweko and Utami, 2017; Zandieh et al., 2017; Zini et al., 2001*).

Overall, 2883 infertile men (mean age 35.22 ± 4.31 years) were compared with 1294 fertile men (mean age 34.24 ± 3.03
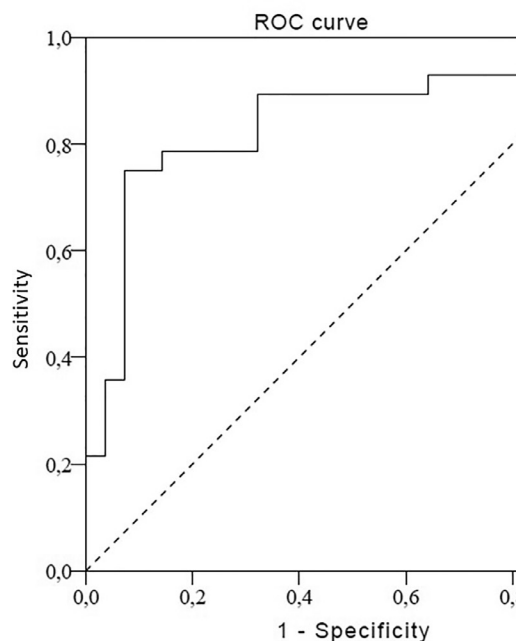
FIGURE 3 Receiver operator characteristics (ROC) curve for the identification of the best sDF threshold for the male infertility diagnosis. Area under the curve = 0.844, P < 0.001.

years). Among fertile controls, 14 studies enrolled men of proven fertility (*Atig et al., 2017; Bareh et al., 2016; Brahem et al., 2011b; Carlini et al., 2017; Garolla et al., 2015; Khalili et al., 2006; Malić Vončina et al., 2016; Mehdi et al., 2009; Muratori et al., 2015; Venkatesh et al., 2011; Winkle et al., 2009; Wiweko and Utami, 2017; Zandieh et al., 2017; Zini et al., 2001*), eight studies healthy donors (*Brahem et al., 2011a; Chenlo et al., 2014; Evgeni et al., 2015a; Ni et al., 2016; Nicopoullos et al., 2008; Plastira et al., 2007; Saleh et al., 2002, 2003*), two studies volunteers (*Alkhayal et al., 2013; Dorostghoal et al., 2017*) and four studies included men with normal semen analysis (*de Paula et al., 2006; Evgeni et al., 2015b; Liu et al., 2016; Mangiarini et al., 2013*) (**TABLE 1**). Concerning infertile men, 15 studies considered patients with unexplained couple infertility (*Alkhayal et al., 2013; Brahem et al., 2011a; Carlini et al., 2017; Chenlo et al., 2014; Evgeni et al., 2015a; Garolla et al., 2015; Khalili et al., 2006; Malić Vončina et al., 2016; Muratori et al., 2015; Nicopoullos et al., 2008; Plastira et al., 2007; Saleh et al., 2003; Venkatesh et al., 2011; Winkle et al., 2009; Zandieh et al., 2017*) and 12 studies considered men with abnormal semen analyses (*Atig et al., 2017; Brahem et al., 2011b; de Paula et al., 2006; Dorostghoal et al., 2017; Evgeni et al., 2015b; Liu et al., 2016; Mangiarini et al., 2013; Mehdi et al., 2009; Ni et al., 2016;*

Saleh et al., 2002; Wiweko and Utami, 2017; Zini et al., 2001).

Fertile controls showed significantly higher sperm number (WMD 1.41; 95% CI: 1.01–1.80, P < 0.001) and sperm motility (WMD 1.50; 95% CI: 1.07–1.93, P < 0.001) compared with infertile men (Supplementary Figures 1 and 2, respectively). The sDF index was significantly higher in infertile men than fertile controls (P < 0.001) (**FIGURE 2**). This difference remained statistically significant, also subgrouping patients according to the sDF method used, such as SCD (P = 0.004) (Supplementary Figure 3A), SCSA (P < 0.001) (Supplementary Figure 3B) and TUNEL (P < 0.001) (Supplementary Figure 3C). Moreover, the result remained significantly different when eight studies comparing men with proven fertility to men with unexplained couple infertility were considered (P = 0.003) (Supplementary Figure 4).

ROC curve analysis, performed considering all datasets and all four sDF assays (area under the curve [AUC] 0.844, P < 0.001), identified a sDF threshold of 20%, indicated by the best pair of values for the sensitivity and specificity (**FIGURE 3**). With this cut-off, the sDF diagnostic power showed a sensitivity of 79% and a specificity of 86% (**FIGURE 3**). Considering the heterogeneity of sDF

assays, a ROC curve was designed considering one methodology alone. In particular, TUNEL was used in 15 studies. The ROC curve built on this subgroup of studies confirmed the overall analysis (AUC 0.831, P = 0.002) (Supplementary Figure 5).

Meta-analysis 2
Sixteen potentially relevant studies were identified among the total 14,083 found by the literature search. Ten studies were excluded after evaluation of the entire manuscript, because they did not report sDF (*Ben-Rafael et al., 2000; Caroppo et al., 2003; Casamonti et al., 2017; Ding and Zhang, 2014; Efesoy et al., 2009; Foresta et al., 2002, 2005; Paradisi et al., 2006, 2014; Selice et al., 2011*). Finally, six studies were included in the analysis (*Colacurci et al., 2012; Garolla et al., 2014, 2017; Palomba et al., 2011; Ruvolo et al., 2013; Simoni et al., 2016*) (**FIGURE 1**).

Overall, 383 men with idiopathic infertility or with abnormal semen analyses were treated with FSH for a maximum of 3 months (**TABLE 2**). Recombinant FSH (rFSH) was used in three studies and urinary-derived FSH (uFSH) (*Colacurci et al., 2012; Ruvolo et al., 2013; Simoni et al., 2016*) in the other three (*Garolla et al., 2014, 2017; Palomba et al. 2011*) (**TABLE 2**).

FSH administration significantly improved sDF in infertile men by 4.24% (95% CI: 0.24–8.25%; P = 0.04) after 3 months of treatment (**FIGURE 4**), although no sDF differences were observed between study and control groups both before and after FSH administration (Supplementary Figures 6A and 6B). Finally, despite this significant sperm quality improvement, FSH administration did not significantly increase total sperm count after treatment (Supplementary Figure 7).

Supplementary Figure 8 reports the risk of biases registered across studies, showing an overall low risk of bias, except for the high risk of selection bias.
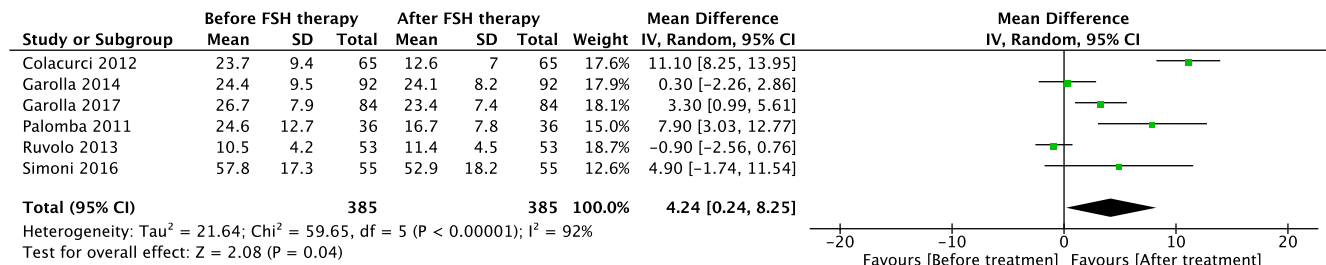
DISCUSSION

This is thought to be the first comprehensive demonstration of the clinical utility of sDF measurement for diagnosis and treatment of male infertility. In particular, the study confirms that infertile men show higher sDF levels compared with fertile men. This result suggests that sDF could be evaluated

TABLE 2 CHARACTERISTICS OF STUDIES INCLUDED IN THE ANALYSIS AFTER META-ANALYSIS 2

| | | | | | | Untreated men (CONTROL GROUP) | | | | | | | FSH-treated men (STUDY GROUP) | | | | | | |
| | | | | | | Baseline | | After 3 months | | | | | | | | Baseline | | After 3 months of treatment | |
Author	Year	Method	N	Age (years)	Inclusion criteria	sDF (%)	Total sperm count x 106/ml	sDF (%)	Total sperm count x 106/ml	N	Age (years)	Inclusion criteria	FSH	Dosage	Duration	sDF (%)	Total sperm count x 106/ml	sDF (%)	Total sperm count x 106/ml
Simoni	2016	TUNEL	/							55	34.5 ± 40.49	Idiopathic infertility	rFSH	150 UI on alternate days	90 days	57.8 ± 17.3	82.1 ± 101.9	52.9 ± 18.2	74.1 ± 96.9
Garolla	2017	TUNEL	82	Range 25–45	OS	25.5 ± 7.4	10.9 ± 8.9	26.1 ± 6.3	11.3 ± 9.3	84	Range 25–45	OS	uFSH	150 UI 3 times a week	90 days	26.7 ± 7.9	8.5 ± 7.5	23.4 ± 7.4	19.9 ± 16.1
Garolla	2014	TUNEL	82	Range 25–45	OS with maturative disturbance or normal spermatogenesis	22.9 ± 9.4	13.9 ± 7.1	23.5 ± 9.1	14.3 ± 9.3	92	Range 25–45	OS with hypo-spermatogenesis	uFSH	150 UI 3 times a week	90 days	24.4 ± 9.5	13.8 ± 7.6	24.1 ± 8.2	28.2 ± 15.9
Ruvolo	2013	TUNEL	/							53	33.6 ± 5.4	OA	rFSH	150 UI 3 times a week	90 days	10.5 ± 4.2	8.2 ± 1.2	11.4 ± 4.5	7.5 ± 2.3
Cola-curci	2012	TUNEL	64	33.6 ± 3.5	OA	22.3 ± 9.3	21.6 ± 5.8	23.9 ± 10.2	21.2 ± 5.3	65	31.6 ± 3.1	OA	rFSH	150 UI on alternate days	90 days	23.7 ± 9.4	19.8 ± 7.5	12.6 ± 7	30.4 ± 8.9
Palomba	2011	SCD	/							36	34 ± 7.3	Subnormal semen parameters	uFSH	150 UI on alternate days	90 days	24.6 ± 12.7	17.1 ± 3.2	16.7 ± 7.8	22.9 ± 5.3

OA = oligo-asthenozoospermia; OS = oligozoospermia; rFSH = recombinant FSH; sDF = sperm DNA fragmentation; uFSH = urinary-derived FSH.

Study or Subgroup	Before FSH therapy			After FSH therapy			Weight	Mean Difference IV, Random, 95% CI	Mean Difference IV, Random, 95% CI
	Mean	SD	Total	Mean	SD	Total			
Colacurci 2012	23.7	9.4	65	12.6	7	65	17.6%	11.10 [8.25, 13.95]	
Garolla 2014	24.4	9.5	92	24.1	8.2	92	17.9%	0.30 [−2.26, 2.86]	
Garolla 2017	26.7	7.9	84	23.4	7.4	84	18.1%	3.30 [0.99, 5.61]	
Palomba 2011	24.6	12.7	36	16.7	7.8	36	15.0%	7.90 [3.03, 12.77]	
Ruvolo 2013	10.5	4.2	53	11.4	4.5	53	18.7%	−0.90 [−2.56, 0.76]	
Simoni 2016	57.8	17.3	55	52.9	18.2	55	12.6%	4.90 [−1.74, 11.54]	
Total (95% CI)			385			385	100.0%	4.24 [0.24, 8.25]	

Heterogeneity: Tau² = 21.64; Chi² = 59.65, df = 5 (P < 0.00001); I² = 92%
Test for overall effect: Z = 2.08 (P = 0.04)

Favours [Before treatmen] Favours [After treatment]

FIGURE 4 Forest plot comparing sDF in infertile men before and after FSH administration (Meta-analysis 2).

as a new marker in the male infertility workup, adding additional information to conventional semen analyses. The sDF threshold of 20% indicates the presence of infertility with high sensitivity and specificity. Alongside this, sDF seems to be a useful pharmacodynamic marker of FSH treatment efficacy in infertile men. Thus, in spite of the controversies concerning its predictive value in ART, sDF seems to be a promising tool, at least in research practice, where more evidence should be sought to expand its utility in clinical practice.

Sperm DNA damage can result from five different pathogenic mechanisms (*Perrin et al., 2011; Sakkas and Alvarez, 2010*). First, DNA damage could be the result of increasing apoptosis during spermatogenesis (*Burrello et al., 2004*). Second, DNA breaks could be induced by chromatin remodelling during the process of spermiogenesis (*McPherson and Longo, 1993*). Third, oxygen radicals could lead to sperm DNA fragmentation during transport through the seminiferous tubules and the epididymis (*Ollero et al., 2001*). Fourth, endogenous caspases and endonucleases could cause DNA damage (*Banks et al., 2005*). Fifth, exogenous factors, such as radiotherapy, chemotherapy and environmental toxicants, could lead to sperm DNA damage (*O'Flaherty et al., 2008; Rubes et al., 2007*). This latter mechanism has been widely evaluated recently, resulting in increasing evidence of a negative effect of air pollutants on sperm quality (*Santi et al., 2016*). All these factors lead to DNA damage characterized by single- or double-strand breaks, which can be detected using different methodologies. Four assays are commonly employed in the literature, differing in the first phase of the procedure. In particular, SCSA, SCD and Comet methodologies are based on an initial denaturation step to detect DNA breaks, by creating acidic or alkaline

conditions (*Singh et al., 1989*), whereas TUNEL directly measures DNA breaks without denaturation (*Gorczyca et al., 1993*). Several authors have suggested that this different initial phase could be responsible for the heterogeneity of the results obtained by these assays (*Borini et al., 2006; Bungum et al., 2007*). This study demonstrates for the first time a substantial overlap among available sDF assays in the comparison between fertile and infertile men. However, this comparison considered different numbers of patients included for each method. Thus, no conclusion can be drawn in favour or against any particular methodology of sDF measurement.

The different characteristics of sDF could influence its predictive capacity, e.g. (i) type of DNA damage (single- or double-strand breaks), (ii) percentage of spermatozoa with DNA damage, (iii) extent of DNA damage in each sperm, (iv) whether DNA damage affects introns or exons, and (v) ability of the oocyte to repair sperm DNA damage during fertilization (*Sakkas and Alvarez, 2010*). These aspects leave some uncertainties on the real predictive role of sDF measurement in clinical practice. Several authors recently demonstrated that the high DNA damage rate in in-vivo and in-vitro generated embryos leads to embryo development blockage, suggesting that sDF could be a relevant factor related to late paternal age in couple infertility (*Borini et al., 2006*). Despite the fact that embryo DNA damage could similarly arise from oocyte DNA, sperm DNA damage is thought to be involved in impeding blastocyst development (*Seli et al., 2004*) and losing preimplantation embryos (*Shoukir et al., 1998*). Hence, the evaluation of sperm DNA integrity might be very important in predicting pregnancy rate and guiding IVF. However, clinical trials and meta-analyses in the setting of ART do not provide conclusive evidence about the predictive role of sDF for IVF outcomes. However, this meta-analysis

suggests that sDF measurement is at least useful to predict male fertility, using a threshold of 20%. It should be stressed, however, that differences among sDF assays limit the utility of this threshold in clinical practice. Therefore, the value of the proposed cut-off of 20% sDF in the definition of infertility must be confirmed by appropriate prospective studies. Given that, *Evenson and Wixon (2006)* suggested that fecundity starts to decrease when sDF is higher than 15%. Similarly, *Spanò et al. (2000)* suggested that the chances of fertilization are close to zero when sDF is higher than 30%. However, these trials evaluated sDF as a predictive tool for infertility management, using ART outcomes. On the contrary, this study is the first designed to detect a role for sDF in the male fertility setting, confirming a promising application for the diagnosis and management of infertile men.

In the literature, a limited number of uncontrolled trials examined potential treatments to reduce sperm DNA damage, although no therapies have been validated to manage and reduce sDF. Here, considering each study detected by the second literature search individually, a beneficial effect of FSH administration in infertile men is suggested and supported. Taking these studies together, it is clear that FSH administration did not increase the total sperm number after 3 months of treatment. This result could be explained by different mechanisms. First, the studies considered here are extremely heterogeneous in inclusion criteria. Second, the treatment scheme (i.e. FSH dosage and therapy length) is highly variable and not standardized. Moreover, it is important to highlight that the genetic pattern also seems to play a relevant role in the management of FSH treatment in male infertility. In particular, specific genotypes, regarding FSH receptor and FSHβ genes, were suggested to be possible predictors

of response to FSH administration in terms of semen parameters in male infertility (*Simoni et al., 2016*). However, the stratification of patients based on genetics is not currently possible in this meta-analytic approach because only one study assessed this factor. Here, the evaluation of sDF before and after FSH administration clearly highlights the beneficial effect of FSH, with an overall reduction of about 4.24% in sDF after treatment. This is the first comprehensive demonstration of a beneficial effect of FSH on sperm DNA integrity. A positive FSH influence on sperm DNA integrity was first suggested in 1998 (*Kamischke et al., 1998*) and there is some evidence of this effect on couple infertility (*Attia et al., 2013a; Santi et al., 2015*). It is worth noting that these results were obtained considering pregnancy rate as the primary endpoint. Here, it is shown that sDF improves after 3 months of FSH administration, suggesting that this assay could be a promising tool in the clinical evaluation of treatment response. This result needs to be confirmed by larger and properly designed clinical prospective trials, but it opens up new horizons for monitoring male infertility treatment. For instance, if exogenous antioxidant agents show a beneficial effect on sperm quality at conventional semen analysis (*Kefer et al., 2009*), some trials could be based on the combined administration of FSH and antioxidants in infertile men. In this setting, the evaluation of sDF could be useful to detect the possible beneficial role of the combined therapy.

Despite the wide implication of these results, the study shows several limitations. First, the meta-analytic approach to a clinical problem evaluates data obtained in different clinical contexts. Indeed, a high heterogeneity among studies has been detected, suggesting wide differences among patients enrolled in each study. This represents the main difficulty in collecting this data, although it is related to the intrinsic variability of this condition. The weight of study heterogeneity on the final result could be reduced by the random-effects model, but properly designed and powered trials are needed to better understand these issues, in which homogeneous patient inclusion criteria should be used. Second, a sDF threshold was detected using ROC curves. This statistical method is validated and useful in clinical

trials, although its validity remains unclear in meta-analyses. Moreover, the sDF threshold was determined by considering all studies together, irrespective of the sDF assay used. This heterogeneity in the sDF determination could limit the predictive strength of this threshold, although it is confirmed at least in the subgroup of studies using the TUNEL method, which is the most frequent approach. However, no conclusion can be drawn about the specific sDF cut-off to be used, but this threshold can be suggested for the time being, pending the need to identify a more accurate value.

In conclusion, this study suggests that sDF could be a useful tool for male infertility diagnosis and management, using a threshold of 20%. Despite the need for confirmation in future clinical trials, this result represents a starting point for the application of sDF in clinical and research practice.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.rbmo.2018.06.023.

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