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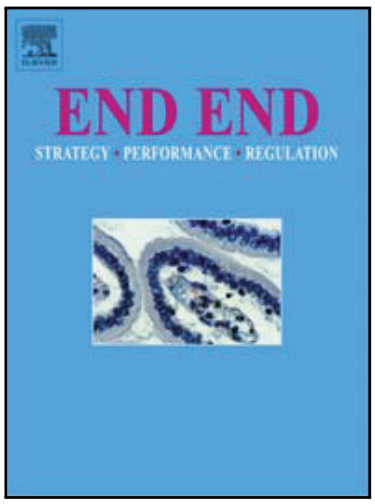

Sperm DNA fragmentation index as a promising predictive tool for male infertility diagnosis and treatment management – meta-analyses

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Short title: Sperm DNA fragmentation for male infertility

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

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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.

Keywords: FSH, male infertility, sperm DNA

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 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 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 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 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 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.

References

Acosta, A.A., Oehninger, S., Ertunc, H., Philput, C., 1991. Possible role of pure human follicle-stimulating hormone in the treatment of severe male-factor infertility by assisted reproduction: Preliminary report. Fertility and sterility. 55, 1150–6

Alkhayal, A., San Gabriel, M., Zeidan, K., Alrabeeah, K., Noel, D., Mcgraw, R., Bissonnette, F., Kadoch, I.J., Zini, A., 2013. Sperm DNA and chromatin integrity in semen samples used for intrauterine insemination. Journal of assisted reproduction and genetics. 30, 1519–24

Atig, F., Kerkeni, A., Saad, A., Ajina, M., 2017. Effects of reduced seminal enzymatic antioxidants on sperm DNA fragmentation and semen quality of tunisian infertile men. Journal of assisted reproduction and genetics. 34, 373–381

Attia, A.M., Abou-Setta, A.M., Al-Inany, H.G., 2013a. Gonadotrophins for idiopathic male factor subfertility. The Cochrane database of systematic reviews. CD005071

Attia, A.M., Abou-Setta, A.M., Al-Inany, H.G., 2013b. Gonadotrophins for idiopathic male factor subfertility. The Cochrane database of systematic reviews. 8, CD005071

Avendano, C., Franchi, A., Taylor, S., Morshedi, M., Bocca, S., Oehninger, S., 2009. Fragmentation of DNA in morphologically normal human spermatozoa. Fertility and sterility. 91, 1077–84

Banks, S., King, S.A., Irvine, D.S., Saunders, P.T., 2005. Impact of a mild scrotal heat stress on DNA integrity in murine spermatozoa. Reproduction (Cambridge, England). 129, 505–14

Bareh, G.M., Jacoby, E., Binkley, P., Chang, T.C., Schenken, R.S., Robinson, R.D., 2016. Sperm deoxyribonucleic acid fragmentation assessment in normozoospermic male partners of couples with unexplained recurrent pregnancy loss: A prospective study. Fertility and sterility. 105, 329–36 e1

Ben-Rafael, Z., Farhi, J., Feldberg, D., Bartoov, B., Kovo, M., Eltes, F., Ashkenazi, J., 2000. Follicle-stimulating hormone treatment for men with idiopathic oligoteratoasthenozoospermia before *in vitro* fertilization: The impact on sperm microstructure and fertilization potential. Fertility and sterility. 73, 24–30

Binsaleh, S., Al-Qahtani, R., Madbouly, K., Isa, A.M., Abu-Rafea, B., 2015. Evaluation of sperm DNA damage in men from infertile saudi couples. The Journal of reproductive medicine. 60, 135–40

Bjorndahl, L., 2011. What is normal semen quality? On the use and abuse of reference limits for the interpretation of semen analysis results. Human fertility (Cambridge, England). 14, 179–86

Bjorndahl, L., Barratt, C.L., Mortimer, D., Jouannet, P., 2016. 'How to count sperm properly': Checklist for acceptability of studies based on human semen analysis. Human reproduction (Oxford, England). 31, 227–32

Borini, A., Tarozzi, N., Bizzaro, D., Bonu, M.A., Fava, L., Flamigni, C., Coticchio, G., 2006. Sperm DNA fragmentation: Paternal effect on early post-implantation embryo development in art. Human reproduction (Oxford, England). 21, 2876–81

Brahem, S., Mehdi, M., Elghezal, H., Saad, A., 2011a. Detection of DNA fragmentation and meiotic segregation in human with isolated teratozoospermia. Journal of assisted reproduction and genetics. 28, 41–8

Brahem, S., Mehdi, M., Elghezal, H., Saad, A., 2011b. The effects of male ageing on semen quality, sperm DNA fragmentation and chromosomal abnormalities in an infertile population. Journal of assisted reproduction and genetics. 28, 425–32

Bungum, M., Humaidan, P., Axmon, A., Spano, M., Bungum, L., Erenpreiss, J., Giwercman, A., 2007. Sperm DNA integrity assessment in prediction of assisted reproduction technology outcome. Human reproduction (Oxford, England). 22, 174–9

Burrello, N., Arcidiacono, G., Vicari, E., Asero, P., Di Benedetto, D., De Palma, A., Romeo, R., D'agata, R., Calogero, A.E., 2004. Morphologically normal spermatozoa of patients with secretory oligo-astheno-teratozoospermia have an increased aneuploidy rate. Human reproduction (Oxford, England). 19, 2298–302

Carlini, T., Paoli, D., Pelloni, M., Faja, F., Dal Lago, A., Lombardo, F., Lenzi, A., Gandini, L., 2017. Sperm DNA fragmentation in italian couples with recurrent pregnancy loss. Reproductive biomedicine online. 34, 58–65

Caroppo, E., Niederberger, C., Vizziello, G.M., D'amato, G., 2003. Recombinant human follicle-stimulating hormone as a pretreatment for idiopathic oligoasthenoteratozoospermic patients undergoing intracytoplasmic sperm injection. Fertility and sterility. 80, 1398–403

Carrell, D.T., De Jonge, C.J., 2016. The troubling state of the semen analysis. Andrology. 4, 761–2

Casamonti, E., Vinci, S., Serra, E., Fino, M.G., Brilli, S., Lotti, F., Maggi, M., Coccia, M.E., Forti, G., Krausz, C., 2017. Short-term fsh treatment and sperm maturation: A prospective study in idiopathic infertile men. Andrology. 5, 414–422

Chenlo, P.H., Curi, S.M., Pugliese, M.N., Ariagno, J.I., Sardi-Segovia, M., Furlan, M.J., Repetto, H.E., Zeitler, E., Cohen, M., Mendeluk, G.R., 2014. Fragmentation of sperm DNA using the tunel method. Actas urologicas espanolas. 38, 608–12

Colacurci, N., Monti, M.G., Fornaro, F., Izzo, G., Izzo, P., Trotta, C., Mele, D., De Franciscis, P., 2012. Recombinant human fsh reduces sperm DNA fragmentation in men with idiopathic oligoasthenoteratozoospermia. Journal of andrology. 33, 588–93

Cooper, T.G., Noonan, E., Von Eckardstein, S., Auger, J., Baker, H.W., Behre, H.M., Haugen, T.B., Kruger, T., Wang, C., Mbizvo, M.T., Vogelsong, K.M., 2010. World health organization reference values for human semen characteristics. Human reproduction update. 16, 231–45

De Paula, T.S., Bertolla, R.P., Spaine, D.M., Cunha, M.A., Schor, N., Cedenho, A.P., 2006. Effect of cryopreservation on sperm apoptotic deoxyribonucleic acid fragmentation in patients with oligozoospermia. Fertility and sterility. 86, 597–600

Ding, X., Zhang, J., 2014. [the association study between eppin gene polymorphisms and idiopathic male infertility]. Zhonghua yu fang yi xue za zhi [Chinese journal of preventive medicine]. 48, 1072–7

Dorostghoal, M., Kazeminejad, S.R., Shahbazian, N., Pourmehdi, M., Jabbari, A., 2017. Oxidative stress status and sperm DNA fragmentation in fertile and infertile men. Andrologia. 49,

Efesoy, O., Cayan, S., Akbay, E., 2009. The efficacy of recombinant human follicle-stimulating hormone in the treatment of various types of male-factor infertility at a single university hospital. Journal of andrology. 30, 679–84

Evenson, D., Wixon, R., 2006. Meta-analysis of sperm DNA fragmentation using the sperm chromatin structure assay. Reproductive biomedicine online. 12, 466–72

Evgeni, E., Lymberopoulos, G., Gazouli, M., Asimakopoulos, B., 2015a. Conventional semen parameters and DNA fragmentation in relation to fertility status in a greek population. European journal of obstetrics, gynecology, and reproductive biology. 188, 17–23

Evgeni, E., Lymberopoulos, G., Touloupidis, S., Asimakopoulos, B., 2015b. Sperm nuclear DNA fragmentation and its association with semen quality in greek men. Andrologia. 47, 1166–74

Fernandez, J.L., Muriel, L., Rivero, M.T., Goyanes, V., Vazquez, R., Alvarez, J.G., 2003. The sperm chromatin dispersion test: A simple method for the determination of sperm DNA fragmentation. Journal of andrology. 24, 59–66

Filimberti, E., Degl'innocenti, S., Borsotti, M., Quercioli, M., Piomboni, P., Natali, I., Fino, M.G., Caglieresi, C., Criscuoli, L., Gandini, L., Biggeri, A., Maggi, M., Baldi, E., 2013. High variability in results of semen analysis in andrology laboratories in tuscany (italy): The experience of an external quality control (eqc) programme. Andrology. 1, 401–7

Foresta, C., Bettella, A., Garolla, A., Ambrosini, G., Ferlin, A., 2005. Treatment of male idiopathic infertility with recombinant human follicle-stimulating hormone: A prospective, controlled, randomized clinical study. Fertility and sterility. 84, 654–61

Foresta, C., Bettella, A., Merico, M., Garolla, A., Ferlin, A., Rossato, M., 2002. Use of recombinant human follicle-stimulating hormone in the treatment of male factor infertility. Fertility and sterility. 77, 238–44

Garcia-Peiro, A., Martinez-Heredia, J., Oliver-Bonet, M., Abad, C., Amengual, M.J., Navarro, J., Jones, C., Coward, K., Gosalvez, J., Benet, J., 2011. Protamine 1 to protamine 2 ratio correlates with dynamic aspects of DNA fragmentation in human sperm. Fertility and sterility. 95, 105–9

Garolla, A., Cosci, I., Bertoldo, A., Sartini, B., Boudjema, E., Foresta, C., 2015. DNA double strand breaks in human spermatozoa can be predictive for assisted reproductive outcome. Reproductive biomedicine online. 31, 100–7

Garolla, A., Ghezzi, M., Cosci, I., Sartini, B., Bottacin, A., Engl, B., Di Nisio, A., Foresta, C., 2017. Fsh treatment in infertile males candidate to assisted reproduction improved sperm DNA fragmentation and pregnancy rate. Endocrine. 56, 416–425

Garolla, A., Selice, R., Engl, B., Bertoldo, A., Menegazzo, M., Finos, L., Lenzi, A., Foresta, C., 2014. Spermatid count as a predictor of response to fsh therapy. Reproductive biomedicine online. 29, 102–12

Gorczyca, W., Traganos, F., Jesionowska, H., Darzynkiewicz, Z., 1993. Presence of DNA strand breaks and increased sensitivity of DNA *in situ* to denaturation in abnormal human sperm cells: Analogy to apoptosis of somatic cells. Experimental cell research. 207, 202–5

Guzick, D.S., Overstreet, J.W., Factor-Litvak, P., Brazil, C.K., Nakajima, S.T., Coutifaris, C., Carson, S.A., Cisneros, P., Steinkampf, M.P., Hill, J.A., Xu, D., Vogel, D.L., 2001. Sperm morphology, motility, and concentration in fertile and infertile men. The New England journal of medicine. 345, 1388–93

Guzick, D.S., Sullivan, M.W., Adamson, G.D., Cedars, M.I., Falk, R.J., Peterson, E.P., Steinkampf, M.P., 1998. Efficacy of treatment for unexplained infertility. Fertility and sterility. 70, 207–13

Hoeijmakers, J.H., 2009. DNA damage, ageing, and cancer. The New England journal of medicine. 361, 1475–85

Host, E., Lindenberg, S., Smidt-Jensen, S., 2000. DNA strand breaks in human spermatozoa: Correlation with fertilization *in vitro* in oligozoospermic men and in men with unexplained infertility. Acta obstetricia et gynecologica Scandinavica. 79, 189–93

Jurewicz, J., Radwan, M., Sobala, W., Radwan, P., Bochenek, M., Hanke, W., 2016. Dietary patterns and their relationship with semen quality. American journal of men's health.

Kamischke, A., Behre, H.M., Bergmann, M., Simoni, M., Schafer, T., Nieschlag, E., 1998. Recombinant human follicle stimulating hormone for treatment of male idiopathic infertility: A randomized, double-blind, placebo-controlled, clinical trial. Human reproduction (Oxford, England). 13, 596–603

Kefer, J.C., Agarwal, A., Sabanegh, E., 2009. Role of antioxidants in the treatment of male infertility. International journal of urology: official journal of the Japanese Urological Association. 16, 449–57

Khalili, M.A., Aghaie-Maybodi, F., Anvari, M., Talebi, A.R., 2006. Sperm nuclear DNA in ejaculates of fertile and infertile men: Correlation with semen parameters. Urology journal. 3, 154–9

Larson, K.L., Dejonge, C.J., Barnes, A.M., Jost, L.K., Evenson, D.P., 2000. Sperm chromatin structure assay parameters as predictors of failed pregnancy following assisted reproductive techniques. Human reproduction (Oxford, England). 15, 1717–22

Lewis, S.E., Agbaje, I., Alvarez, J., 2008. Sperm DNA tests as useful adjuncts to semen analysis. Systems biology in reproductive medicine. 54, 111–25

Li, Z., Wang, L., Cai, J., Huang, H., 2006. Correlation of sperm DNA damage with ivf and icsi outcomes: A systematic review and meta-analysis. Journal of assisted reproduction and genetics. 23, 367–76

Liu, J., Zhu, P., Wang, W.T., Li, N., Liu, X., Shen, X.F., Wang, Y.W., Li, Y., 2016. Tat-peroxiredoxin 2 fusion protein supplementation improves sperm motility and DNA integrity in sperm samples from asthenozoospermic men. The Journal of urology. 195, 706–12

Mac, L.J., 1950. The male factor in fertility and infertility; an analysis of ejaculate volume in 800 fertile men and in 600 men in infertile marriage. Fertility and sterility. 1, 347–61

Malic Voncina, S., Golob, B., Ihan, A., Kopitar, A.N., Kolbezen, M., Zorn, B., 2016. Sperm DNA fragmentation and mitochondrial membrane potential combined are better for predicting natural conception than standard sperm parameters. Fertility and sterility. 105, 637–644 e1

Manente, L., Pecoraro, S., Picillo, E., Gargiulo, U., Gargiulo, P., De Luca, A., Politano, L., 2015. Molecular evidence of apoptotic pathway activation in semen samples with high DNA fragmentation. *In vivo* (Athens, Greece). 29, 289–94

Mangiarini, A., Paffoni, A., Restelli, L., Ferrari, S., Guarneri, C., Ragni, G., Somigliana, E., 2013. Specific sperm defects are differentially correlated with DNA fragmentation in both normozoospermic and teratozoospermic subjects. Andrology. 1, 838–44

Mcpherson, S., Longo, F.J., 1993. Chromatin structure-function alterations during mammalian spermatogenesis: DNA nicking and repair in elongating spermatids. European journal of histochemistry: EJH. 37, 109–28

Mehdi, M., Khantouche, L., Ajina, M., Saad, A., 2009. Detection of DNA fragmentation in human spermatozoa: Correlation with semen parameters. Andrologia. 41, 383–6

Moustafa, M.H., Sharma, R.K., Thornton, J., Mascha, E., Abdel-Hafez, M.A., Thomas, A.J., Jr., Agarwal, A., 2004. Relationship between ros production, apoptosis and DNA denaturation in spermatozoa from patients examined for infertility. Human reproduction (Oxford, England). 19, 129–38

Muratori, M., Marchiani, S., Tamburrino, L., Cambi, M., Lotti, F., Natali, I., Filimberti, E., Noci, I., Forti, G., Maggi, M., Baldi, E., 2015. DNA fragmentation in brighter sperm predicts male fertility independently from age and semen parameters. Fertility and sterility. 104, 582–90.e4

Ni, K., Steger, K., Yang, H., Wang, H., Hu, K., Zhang, T., Chen, B., 2016. A comprehensive investigation of sperm DNA damage and oxidative stress injury in infertile patients with subclinical, normozoospermic, and astheno/oligozoospermic clinical varicocoele. Andrology. 4, 816–24

Nicopoullos, J.D., Gilling-Smith, C., Almeida, P.A., Homa, S., Norman-Taylor, J.Q., Ramsay, J.W., 2008. Sperm DNA fragmentation in subfertile men: The effect on the outcome of intracytoplasmic sperm injection and correlation with sperm variables. BJU international. 101, 1553–60

O'flaherty, C., Vaisheva, F., Hales, B.F., Chan, P., Robaire, B., 2008. Characterization of sperm chromatin quality in testicular cancer and hodgkin's lymphoma patients prior to chemotherapy. Human reproduction (Oxford, England). 23, 1044–52

Ollero, M., Gil-Guzman, E., Lopez, M.C., Sharma, R.K., Agarwal, A., Larson, K., Evenson, D., Thomas, A.J., Jr., Alvarez, J.G., 2001. Characterization of subsets of human spermatozoa at different stages of maturation: Implications in the diagnosis and treatment of male infertility. Human reproduction (Oxford, England). 16, 1912–21

Omran, H.M., Bakhiet, M., Dashti, M.G., 2013. DNA integrity is a critical molecular indicator for the assessment of male infertility. Molecular medicine reports. 7, 1631–5

Palomba, S., Falbo, A., Espinola, S., Rocca, M., Capasso, S., Cappiello, F., Zullo, F., 2011. Effects of highly purified follicle-stimulating hormone on sperm DNA damage in men with male idiopathic subfertility: A pilot study. Journal of endocrinological investigation. 34, 747–52

Paradisi, R., Busacchi, P., Seracchioli, R., Porcu, E., Venturoli, S., 2006. Effects of high doses of recombinant human follicle-stimulating hormone in the treatment of male factor infertility: Results of a pilot study. Fertility and sterility. 86, 728–31

Paradisi, R., Natali, F., Fabbri, R., Battaglia, C., Seracchioli, R., Venturoli, S., 2014. Evidence for a stimulatory role of high doses of recombinant human follicle-stimulating hormone in the treatment of male-factor infertility. Andrologia. 46, 1067–72

Perrin, A., Basinko, A., Douet-Guilbert, N., Gueganic, N., Le Bris, M.J., Amice, V., De Braekeleer, M., Morel, F., 2011. Aneuploidy and DNA fragmentation in sperm of carriers of a constitutional chromosomal abnormality. Cytogenetic and genome research. 133, 100–6

Plastira, K., Msaouel, P., Angelopoulou, R., Zanioti, K., Plastiras, A., Pothos, A., Bolaris, S., Paparisteidis, N., Mantas, D., 2007. The effects of age on DNA fragmentation, chromatin packaging and conventional semen parameters in spermatozoa of oligoasthenoteratozoospermic patients. Journal of assisted reproduction and genetics. 24, 437–43

Punjabi, U., Wyns, C., Mahmoud, A., Vernelen, K., China, B., Verheyen, G., 2016. Fifteen years of belgian experience with external quality assessment of semen analysis. Andrology. 4, 1084–1093

Ramos, L., De Boer, P., Meuleman, E.J., Braat, D.D., Wetzels, A.M., 2004. Chromatin condensation and DNA damage of human epididymal spermatozoa in obstructive azoospermia. Reproductive biomedicine online. 8, 392–7

Ramzan, M.H., Ramzan, M., Khan, M.M., Ramzan, F., Wahab, F., Khan, M.A., Jillani, M., Shah, M., 2015. Human semen quality and sperm DNA damage assessed by comet assay in clinical groups. Turkish journal of medical sciences. 45, 729–37

Rathke, C., Baarends, W.M., Awe, S., Renkawitz-Pohl, R., 2014. Chromatin dynamics during spermiogenesis. Biochimica et biophysica acta. 1839, 155–68

Robinson, L., Gallos, I.D., Conner, S.J., Rajkhowa, M., Miller, D., Lewis, S., Kirkman-Brown, J., Coomarasamy, A., 2012. The effect of sperm DNA fragmentation on miscarriage rates: A systematic review and meta-analysis. Human reproduction (Oxford, England). 27, 2908–17

Rubes, J., Selevan, S.G., Sram, R.J., Evenson, D.P., Perreault, S.D., 2007. Gstm1 genotype influences the susceptibility of men to sperm DNA damage associated with exposure to air pollution. Mutation research. 625, 20–8

Ruvolo, G., Roccheri, M.C., Brucculeri, A.M., Longobardi, S., Cittadini, E., Bosco, L., 2013. Lower sperm DNA fragmentation after r-fsh administration in functional hypogonadotrophic hypogonadism. Journal of assisted reproduction and genetics. 30, 497–503

Sakkas, D., Alvarez, J.G., 2010. Sperm DNA fragmentation: Mechanisms of origin, impact on reproductive outcome, and analysis. Fertility and sterility. 93, 1027–36

Sakkas, D., Moffatt, O., Manicardi, G.C., Mariethoz, E., Tarozzi, N., Bizzaro, D., 2002. Nature of DNA damage in ejaculated human spermatozoa and the possible involvement of apoptosis. Biology of reproduction. 66, 1061–7

Saleh, R.A., Agarwal, A., Nada, E.A., El-Tonsy, M.H., Sharma, R.K., Meyer, A., Nelson, D.R., Thomas, A.J., 2003. Negative effects of increased sperm DNA damage in relation to seminal oxidative

stress in men with idiopathic and male factor infertility. Fertility and sterility. 79 Suppl 3, 1597–605

Saleh, R.A., Agarwal, A., Nelson, D.R., Nada, E.A., El-Tonsy, M.H., Alvarez, J.G., Thomas, A.J., Jr., Sharma, R.K., 2002. Increased sperm nuclear DNA damage in normozoospermic infertile men: A prospective study. Fertility and sterility. 78, 313–8

Santi, D., Granata, A.R., Simoni, M., 2015. Fsh treatment of male idiopathic infertility improves pregnancy rate: A meta-analysis. Endocrine connections. 4, R46–58

Santi, D., Vezzani, S., Granata, A.R., Roli, L., De Santis, M.C., Ongaro, C., Donati, F., Baraldi, E., Trenti, T., Setti, M., Simoni, M., 2016. Sperm quality and environment: A retrospective, cohort study in a northern province of italy. Environmental research. 150, 144–53

Seli, E., Gardner, D.K., Schoolcraft, W.B., Moffatt, O., Sakkas, D., 2004. Extent of nuclear DNA damage in ejaculated spermatozoa impacts on blastocyst development after *in vitro* fertilization. Fertility and sterility. 82, 378–83

Selice, R., Garolla, A., Pengo, M., Caretta, N., Ferlin, A., Foresta, C., 2011. The response to fsh treatment in oligozoospermic men depends on fsh receptor gene polymorphisms. International journal of andrology. 34, 306–12

Sergerie, M., Laforest, G., Bujan, L., Bissonnette, F., Bleau, G., 2005. Sperm DNA fragmentation: Threshold value in male fertility. Human reproduction (Oxford, England). 20, 3446–51

Shamsi, M.B., Imam, S.N., Dada, R., 2011. Sperm DNA integrity assays: Diagnostic and prognostic challenges and implications in management of infertility. Journal of assisted reproduction and genetics. 28, 1073–85

Sharma, R., Ahmad, G., Esteves, S.C., Agarwal, A., 2016. Terminal deoxynucleotidyl transferase dutp nick end labelling (tunel) assay using bench top flow cytometer for evaluation of sperm DNA fragmentation in fertility laboratories: Protocol, reference values, and quality control. Journal of assisted reproduction and genetics. 33, 291–300

Sharma, R.K., Sabanegh, E., Mahfouz, R., Gupta, S., Thiyagarajan, A., Agarwal, A., 2010. Tunel as a test for sperm DNA damage in the evaluation of male infertility. Urology. 76, 1380–6

Shoukir, Y., Chardonnens, D., Campana, A., Sakkas, D., 1998. Blastocyst development from supernumerary embryos after intracytoplasmic sperm injection: A paternal influence? Human reproduction (Oxford, England). 13, 1632–7

Simoni, M., Santi, D., Negri, L., Hoffmann, I., Muratori, M., Baldi, E., Cambi, M., Marcou, M., Greither, T., Baraldi, E., Tagliavini, S., Carra, D., Lombardo, F., Gandini, L., Pallotti, F., Krausz, C., Rastrelli, G., Ferlin, A., Menegazzo, M., Pignatti, E., Linari, F., Marino, M., Benaglia, R., Levi-Setti, P.E., Behre, H.M., 2016. Treatment with human, recombinant fsh improves sperm DNA fragmentation in idiopathic infertile men depending on the fsh receptor polymorphism p.N680s: A pharmacogenetic study. Human reproduction (Oxford, England). 31, 1960–9

Singh, N.P., Danner, D.B., Tice, R.R., Mccoy, M.T., Collins, G.D., Schneider, E.L., 1989. Abundant alkali-sensitive sites in DNA of human and mouse sperm. Experimental cell research. 184, 461–70

Spano, M., Bonde, J.P., Hjollund, H.I., Kolstad, H.A., Cordelli, E., Leter, G., 2000. Sperm chromatin damage impairs human fertility. The danish first pregnancy planner study team. Fertility and sterility. 73, 43–50

Van Der Steeg, J.W., Steures, P., Eijkemans, M.J., Jd, F.H., Hompes, P.G., Kremer, J.A., Van Der Leeuw-Harmsen, L., Bossuyt, P.M., Repping, S., Silber, S.J., Mol, B.W., Van Der Veen, F., 2011. Role of semen analysis in subfertile couples. Fertility and sterility. 95, 1013–9

Van Weert, J.M., Repping, S., Van Der Steeg, J.W., Steures, P., Van Der Veen, F., Mol, B.W., 2008. A prediction model for ongoing pregnancy after *in vitro* fertilization in couples with male subfertility. The Journal of reproductive medicine. 53, 250–6

Varghese, A.C., Bragais, F.M., Mukhopadhyay, D., Kundu, S., Pal, M., Bhattacharyya, A.K., Agarwal, A., 2009. Human sperm DNA integrity in normal and abnormal semen samples and its correlation with sperm characteristics. Andrologia. 41, 207–15

Venkatesh, S., Singh, A., Shamsi, M.B., Thilagavathi, J., Kumar, R., Mitra, D.K., Dada, R., 2011. Clinical significance of sperm DNA damage threshold value in the assessment of male infertility. Reproductive sciences (Thousand Oaks, Calif.). 18, 1005–13

Wdowiak, A., Raczkiewicz, D., Stasiak, M., Bojar, I., 2014. Levels of fsh, lh and testosterone, and sperm DNA fragmentation. Neuro endocrinology letters. 35, 73–9

Who, W.H.O., 2010. Who laboratory manual for the examination and processing of human semen, 5th ed.

Winkle, T., Rosenbusch, B., Gagsteiger, F., Paiss, T., Zoller, N., 2009. The correlation between male age, sperm quality and sperm DNA fragmentation in 320 men attending a fertility centre. Journal of assisted reproduction and genetics. 26, 41–6

Wiweko, B., Utami, P., 2017. Predictive value of sperm deoxyribonucleic acid (DNA) fragmentation index in male infertility. Basic and clinical andrology. 27, 1

Zalata, A., El-Samanoudy, A.Z., Shaalan, D., El-Baiomy, Y., Mostafa, T., 2015. *In vitro* effect of cell phone radiation on motility, DNA fragmentation and clusterin gene expression in human sperm. International journal of fertility and sterility. 9, 129–36

Zandieh, Z., Vatannejad, A., Doosti, M., Zabihzadeh, S., Haddadi, M., Bajelan, L., Rashidi, B., Amanpour, S., 2017. Comparing reactive oxygen species and DNA fragmentation in semen samples of unexplained infertile and healthy fertile men. Irish journal of medical science.

Zhao, J., Zhang, Q., Wang, Y., Li, Y., 2014. 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. Fertility and sterility. 102, 998–1005 e8

Zini, A., Bielecki, R., Phang, D., Zenzes, M.T., 2001. Correlations between two markers of sperm DNA integrity, DNA denaturation and DNA fragmentation, in fertile and infertile men. Fertility and sterility. 75, 674–7

Zribi, N., Chakroun, N.F., Elleuch, H., Abdallah, F.B., Ben Hamida, A.S., Gargouri, J., Fakhfakh, F., Keskes, L.A., 2011. Sperm DNA fragmentation and oxidation are independent of malondialdheyde. Reproductive biology and endocrinology: RB and E. 9, 47

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Table 1 – Characteristics of studies included in the analysis after Meta-analysis 1.

		sDF	Fertile men (CONTROL GROUP)			Infertile men (STUDY GROUP)		
Author	Year	Method	*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.

Table 2 – Characteristics of studies included in the analysis after Meta-analysis 2.

			Untreated men (CONTROL GROUP)							FSH-treated men (STUDY GROUP)									
		sDF				Baseline		After 3 months								Baseline		After 3 months of treatment	
Author	Year	Method	*N*	Age (years)	Inclusion criteria	sDF (%)	Total sperm count x 10^6/ml	sDF (%)	Total sperm count x 10^6/ml	*N*	Age (years)	Inclusion criteria	FSH	Dosage	Duration	sDF (%)	Total sperm count x 10^6/ml	sDF (%)	Total sperm count x 10^6/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	20	TUNEL	/							53	33.6	OA	rFS	150 UI	90 day	10.	8.2 ±	11.	7.5 ±

	13										± 5.4		H	3 times a week	s	5 ± 4.2	1.2	4 ± 4.5	2.3
Colacurci	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.

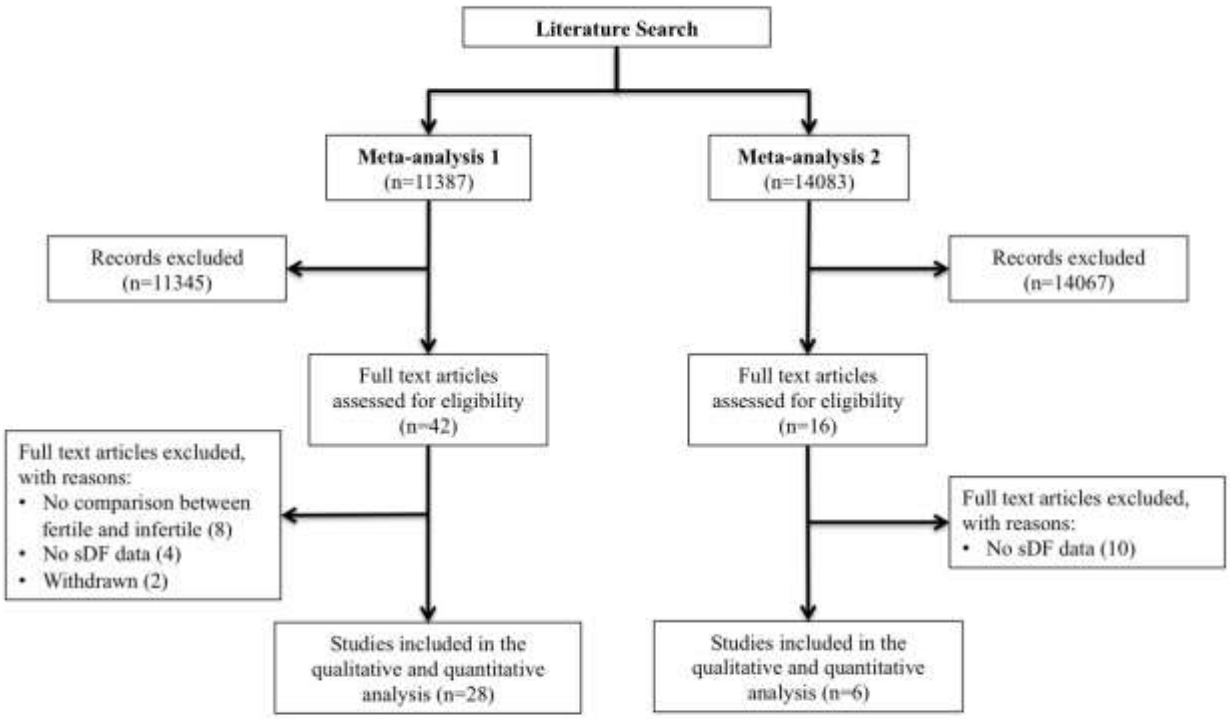

Figure 1 – Study flow chart showing the search results for the studies included in Meta-analysis 1 comparing sperm DNA fragmentation index (sDF) in fertile and infertile men (Search 1) and in Meta-analysis 2 comparing sDF in infertile men before and after FSH administration (Search 2).

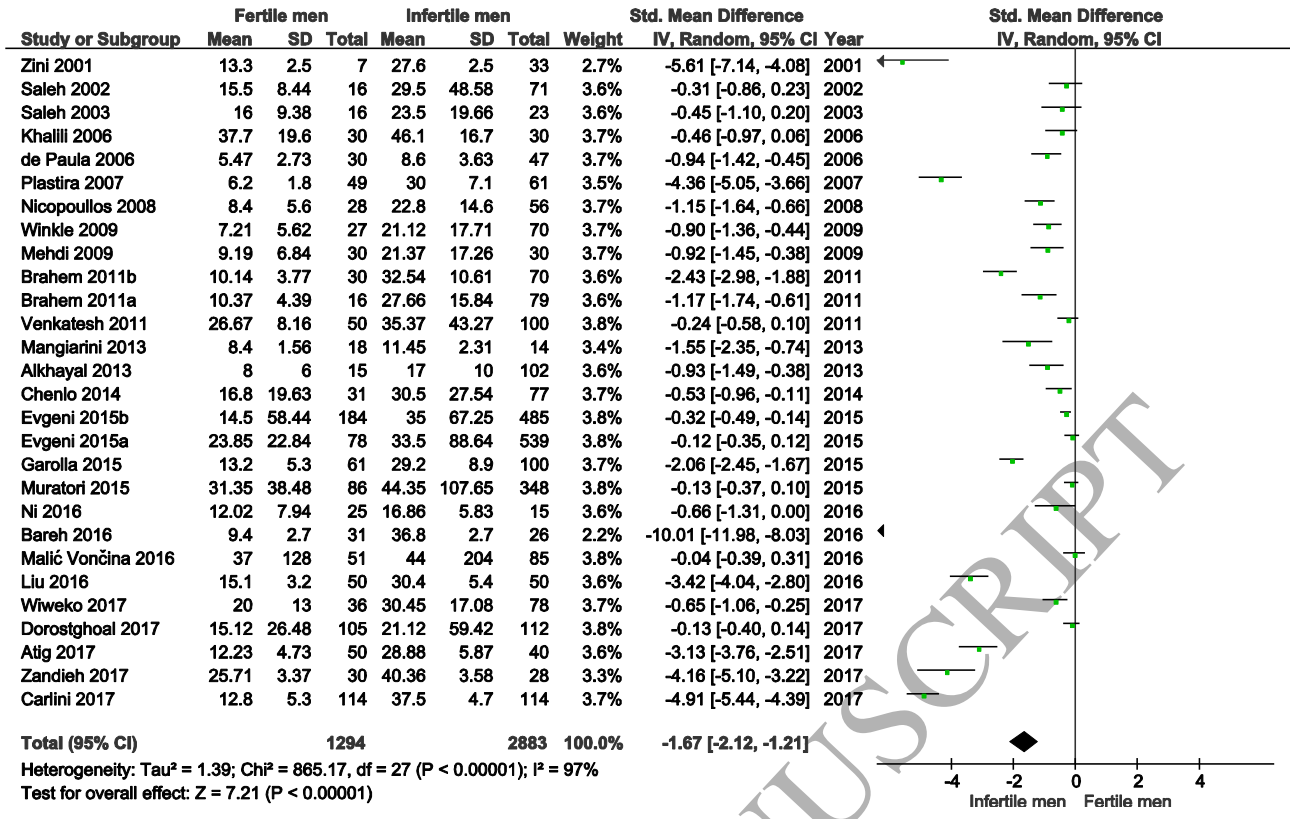

Figure 2 – Forest plot comparing sDF in fertile with infertile men (Meta-analysis 1).

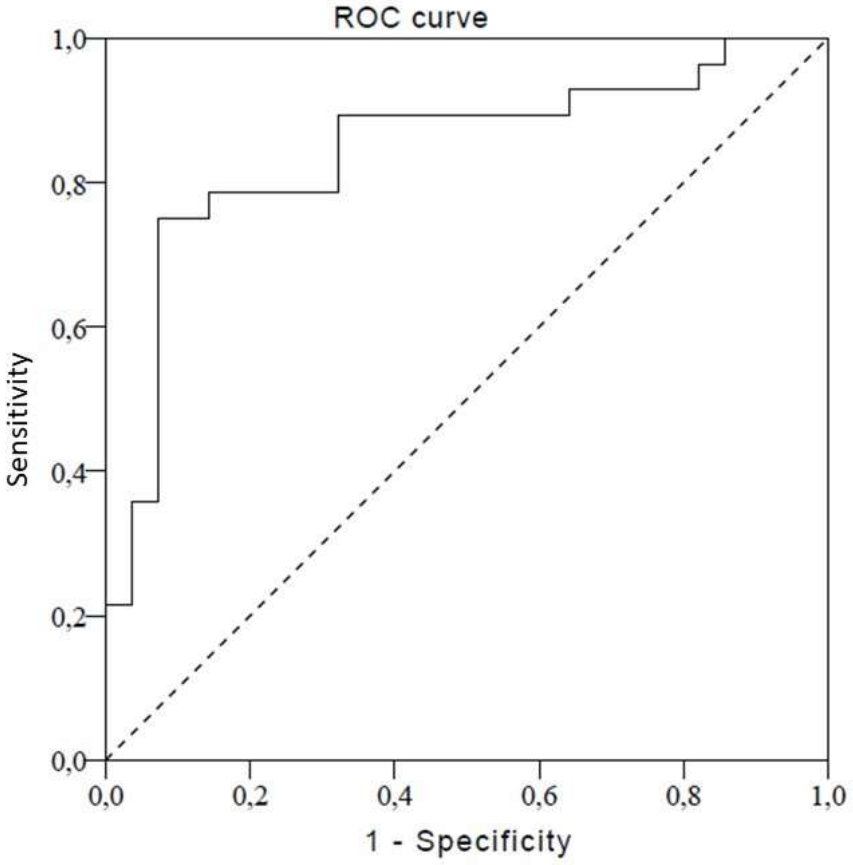

Figure 3 – Receiver operator characteristics (ROC) curve for the identification of the best sDF threshold for the male infertility diagnosis. Area under the curve = 0.844, *P* < 0.001.

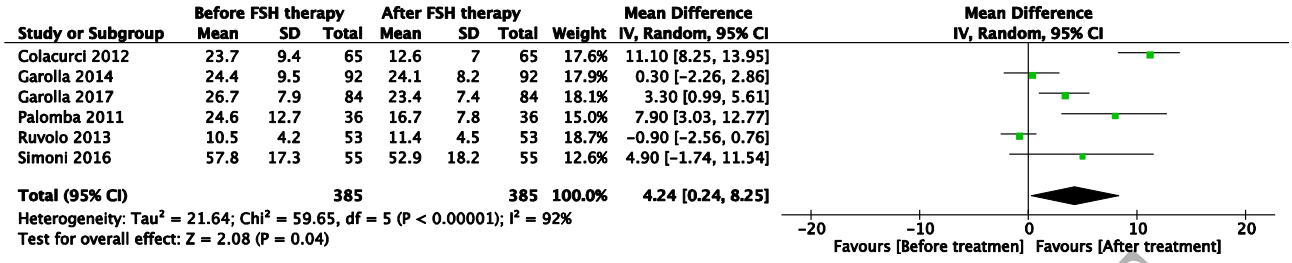

Figure 4 – Forest plot comparing sDF in infertile men before and after FSH administration (Meta-analysis 2).