ORIGINAL ARTICLE

Sperm Biology

A systematic review and meta-analysis to determine the effect of sperm DNA damage on *in vitro* fertilization and intracytoplasmic sperm injection outcome

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Sperm DNA damage is prevalent among infertile men and is known to influence natural reproduction. However, the impact of sperm DNA damage on assisted reproduction outcomes remains controversial. Here, we conducted a meta-analysis of studies on sperm DNA damage (assessed by SCSA, TUNEL, SCD, or Comet assay) and clinical pregnancy after IVF and/or ICSI treatment from MEDLINE, EMBASE, and PUBMED database searches for this analysis. We identified 41 articles (with a total of 56 studies) including 16 IVF studies, 24 ICSI studies, and 16 mixed (IVF + ICSI) studies. These studies measured DNA damage (by one of four assays: 23 SCSA, 18 TUNEL, 8 SCD, and 7 Comet) and included a total of 8068 treatment cycles (3734 IVF, 2282 ICSI, and 2052 mixed IVF + ICSI). The combined OR of 1.68 (95% CI: 1.49–1.89; *P* < 0.0001) indicates that sperm DNA damage affects clinical pregnancy following IVF and/or ICSI treatment. In addition, the combined OR estimates of IVF (16 estimates, OR = 1.65; 95% CI: 1.34–2.04; *P* < 0.0001), ICSI (24 estimates, OR = 1.31; 95% CI: 1.08–1.59; *P* = 0.0068), and mixed IVF + ICSI studies (16 estimates, OR = 2.37; 95% CI: 1.89–2.97; *P* < 0.0001) were also statistically significant. There is sufficient evidence in the existing literature suggesting that sperm DNA damage has a negative effect on clinical pregnancy following IVF and/or ICSI treatment.

INTRODUCTION

In recent years, a number of sperm-specific biomarkers have been studied to identify useful diagnostic tests of sperm function[1–4] as the conventional semen parameters are shown to have a limited diagnostic value for male fertility. To date, tests of sperm DNA integrity and sperm nuclear protein have shown potential to discriminate infertile from fertile men.[5] The integrity of sperm DNA is considered to be vital for normal fertilization, embryo development, and for successful implantation and pregnancies in both natural and assisted reproduction.[6–8] Although some studies have found some value in the use of sperm DNA tests in the evaluation of male infertility,[9–12] the true prognostic value of sperm DNA assessment to predict assisted reproductive technology (ART) outcomes remains uncertain.

The current literature on sperm DNA damage and its effect on ART outcome is still controversial. The meta-analysis by Li *et al.*[13] concluded that sperm DNA damage is detrimental to IVF clinical pregnancy rates but not with ICSI pregnancy. Another meta-analysis[14] concluded that assessment of sperm DNA damage is not strong enough to provide any clinical advantage of these assays to evaluate infertile men. The Practice

Committee of the American Society for Reproductive Medicine[15] concluded that the existing data do not support a detrimental effect of sperm DNA damage on ART outcomes. In contrast, the meta-analysis by Zini *et al.*[16] shows the negative effect of sperm DNA damage on ART outcomes and provides a clinical indication for the evaluation of sperm DNA damage before IVF or ICSI, and a rationale for further investigating the association between sperm DNA damage and pregnancy loss. A recent meta-analysis[17] strongly suggests that assays detecting sperm DNA damage should be recommended to those suffering from recurrent failure to achieve pregnancy.

The lack of agreement in the literature is partially due to the diversity of sperm DNA test methods, lack of standardized protocols, inter-laboratory variations, the use of wide ranges of threshold values, and to some extent, the limited understanding of what each of the sperm DNA assays actually measures.[7,8,18] To date, there are four widely used methods to access sperm DNA damage: the Comet assay, terminal deoxyuridine nick end labeling (TUNEL) assay, Sperm Chromatin Structure Assay (SCSA), and Sperm Chromatin Dispersion (SCD) assay.[9,19–21] The Comet and TUNEL assays detect

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DNA strand breaks while SCSA and SCD measure chromatin integrity and the susceptibility of DNA to denaturation.[7]

Each assay is known to measure different aspects of sperm DNA damage.[22,23] The ability of these assays to accurately measure the level of DNA damage depends on the technical and biological aspects of each test.[24] The alkaline Comet assay may be used to study single- or double-strand DNA breaks and measures the migration of the DNA fragments in the electric field. The intensity of the comet tail represents the amount of fragmented DNA.[25] The TUNEL assay quantifies the level of labeled nucleotide incorporated at single- and double-strand DNA breaks in a reaction catalyzed by the template-independent enzyme deoxynucleotidyl transferase.[26] The SCD assay is based on the principle that sperm with fragmented DNA fail to produce the characteristic halo following acid denaturation and removal of nuclear proteins.[27] With the SCSA, the extent of DNA damage is determined by measuring the metachromatic shift from green fluorescence to red fluorescence following acid denaturation and acridine orange staining.[28] Despite differences in the principle and methodology of these assays, the levels of DNA damage measured by these assays show some degree of correlation.[29]

Tests of sperm DNA damage appear to have some clinical utility in the evaluation of male infertility (discriminate infertile from fertile men) and correlate with conventional sperm parameters[0,30,31] while their ability to predict ART success remains limited.[7,13,14] Comparative analysis of these sperm DNA tests shows that some assays may be better predictors of ART outcomes than others.[32,33] To reach a more definitive conclusion regarding the predictive value of these assays in the context of ARTs and to further examine why there are discrepancies between the various studies, we have performed a systematic review and meta-analysis with separate subanalyses, evaluating the value of each sperm DNA test in predicting ART outcomes. Furthermore, we conducted additional subanalyses to examine the relationship between these sperm DNA tests and reproductive outcomes after different ARTs (IVF, ICSI, or mixed IVF + ICSI).

METHODS

Literature search strategy and selection criteria

We searched the following electronic databases: MEDLINE, EMBASE, and PUBMED. We did not apply any restriction on date, type of publication, or language. A computerized search was performed in April 2014 using the search strategy by combinations of search terms related to "sperm DNA damage," "sperm DNA fragmentation," "sperm DNA integrity," or "sperm DNA" along with "ART," "IVF," "ICSI," "outcome," "fertilization," "embryo," or "pregnancy," and in combinations with "Comet," "TUNEL," "SCSA," "Acridine orange," "Halo," or "SCD." Reference lists of previous meta-analyses, relevant articles, and reviews were cross-searched for additional articles. In this way, data from studies that were missed by our search criteria were identified for inclusion. Two authors (L.S. and A.Z.) independently reviewed the abstracts and papers for eligibility and discrepancies were resolved by group discussion. When it was certain from the abstract that the paper was not relevant, the paper was excluded. Authors were contacted whenever possible if full manuscript, translations, or two-by-two data table were not available. We also considered inclusion of studies that collected relevant data but were excluded from previous meta-analysis due to the inability to extract two-by-two tables.

Inclusion and exclusion criteria

Studies analyzing the relationship between sperm DNA damage and IVF or ICSI clinical pregnancy outcome were considered for inclusion in the meta-analysis if they satisfied the following criteria: (1) clinical study in human; (2) sperm DNA damage detected by the Comet, SCSA, SCD, or TUNEL assays; (3) IVF, ICSI, or mixed (IVF + ICSI) treatment methods; and (4) studies with sufficient data to construct the two-by-two table. Studies were excluded using the following criteria: (1) overlapping data or no original data; (2) conference abstracts; (3) extremely low sample size ($n < 10$); (4) testing of processed or washed sperm samples (to reduce heterogeneity of the meta-analysis); and (5) studies using slide-based acridine orange staining method as this method is deemed unreliable.[29]

Data extraction

The primary outcome measures included in the systematic review were clinical pregnancy following IVF, ICSI, and mixed IVF + ICSI treatment methods. The following information was extracted from the articles to perform the systematic analysis: author names, publication year, DNA damage assay, type of treatment, study design, sample size in each group, exclusion of important female factors (e.g., advanced age), and control of female factors (e.g., age).

For studies to be eligible for inclusion in the meta-analysis, we had to be able to construct two-by-two tables from the reported data (with pregnancy rate above and below DNA damage cutoff). The following outcomes were prerequisites for inclusion: clinical pregnancy (presence of a fetal heartbeat confirmed by ultrasound). If necessary, study authors were contacted to clarify the data. We recorded author names, publication year, patient selection, female inclusion/exclusion criteria, the treatment type (e.g., IVF, ICSI), sperm DNA assay type, cutoff point, number of cycles or patients, and number of pregnancies relative to abnormal or normal test results. From the two-by-two tables of test results, the following test properties were calculated for each study: sensitivity, specificity, positive predictive value, negative predictive value, proportion of abnormal tests, and diagnostic odds ratio (OR). In those studies using the SCSA where data with multiple cutoffs were reported, we selected the cutoff closest to the most frequently reported thresholds (e.g., %DFI at 27% or 30%).

Statistical analysis

The measure of treatment effect was the combined odds ratio of clinical pregnancy in the group with high levels of sperm DNA damage compared with the group with low levels of sperm DNA damage. The study-by-study comparisons were synthesized by a standard meta-analytic approach applied to the odds ratios (ORs) of the individual two-by-two tables.[34,35] We attributed the value 0.5 to empty cells of the two-by-two tables.[34] We tested study homogeneity depending on whether homogeneity was accepted or rejected; we used the fixed or the random effect models for meta-analysis to calculate an overall OR and its 95% CI. Q statistics was used to test between study homogeneity: homogeneity was rejected when the Q statistic $P < 0.10$. The meta-analysis was conducted using the STATA software (StataCorp LP, College Station, TX, USA).

RESULTS

Eligible studies

The extensive literature search yielded 1279 citations. Of these, 1116 were excluded from the study based on their titles and abstracts. Full texts of 163 articles were obtained as they addressed the study question, but 67 articles were excluded because they were not original research papers (**Figure 1**). Following a careful review of the 96 articles, we excluded 29 articles for the reasons shown in **Table 1**.

Study characteristics

There were 67 eligible papers for our analysis and 41 of the 67 papers were used for the meta-analysis (in these 41 papers, a two-by-two
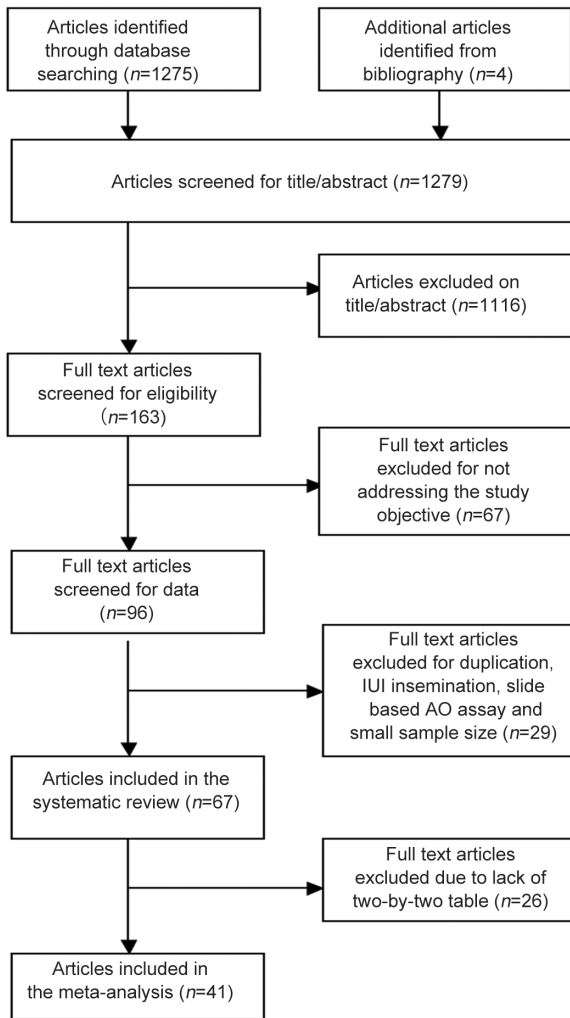
Figure 1: Flowchart for systematic review and meta-analysis.

tables could be constructed from the data). The 41 articles (with a total of 56 studies) included 16 IVF studies, 24 ICSI studies, and 16 mixed (IVF + ICSI) studies. The studies were segregated into SCSA (23 studies), TUNEL (18 studies), Comet (7 studies), and SCD (8 studies) based on the sperm DNA damage assays. The estimated odds ratio with confidence intervals and weight of the 56 studies using random-effect and fixed-effect models is presented in **Table 2**.

Heterogeneity of the studies included for the meta-analysis
The overall and subgroup combined odds ratios of clinical pregnancy are shown in **Table 3**. Assessment of the overall consistency of effects across the evaluated studies was low ($I^2 = 61\%$). When the studies were segregated based on the type of DNA damage assays, the datasets of SCSA and TUNEL assays were of moderate heterogeneity ($I^2 = 38\%$ and 44%, respectively) and the datasets for Comet and SCD assays had a high degree of heterogeneity ($I^2 = 66\%$ and 73%, respectively). Among these studies, there were differences in study design, selection of subjects, and definition of threshold values for DNA damage (for a given assay).

Meta-analysis: relationship between sperm DNA damage and clinical pregnancy after IVF and/or ICSI
In this meta-analysis, 56 studies (including 8068 ART cycles) were combined to determine the overall relationship between sperm DNA damage and clinical pregnancy outcome (**Table 4**). The diagnostic odds ratios (ORs) ranged from 0.45 to 76 (**Table 2**), and in 18 of the 56 estimates, the ORs revealed a strong detrimental effect of sperm DNA damage on clinical pregnancy outcome (**Table 2** and **Figure 2a–2c**). The Q statistic $P < 0.0001$ with an $I^2 = 60.9\%$ indicates a moderate to high degree of study heterogeneity (**Table 3**). The negative effect of sperm DNA damage on clinical pregnancy was observed using the fixed effects model where the combined OR estimates of all studies was statistically significant (56 estimates, OR = 1.68; 95% CI: 1.49–1.89, $P < 0.0001$) (**Table 3**). Similarly, the negative effect of sperm DNA damage on clinical pregnancy was also observed using the random effects model where the combined OR estimates of all studies were also statistically significant (56 estimates, OR = 1.84; 95% CI: 1.5–2.27,

Table 1: List of studies excluded from the meta-analysis (*n*=55)

Reason for exclusion	Studies
Overlapping data	Larson *et al.* 2000;[38] Bungum *et al.* 2004;[39] Henkel *et al.* 2004;[40] Simon *et al.* 2012[41]
Lack of data - low sample size	Saleh *et al.* 2003;[55] Gandini *et al.* 2004 (IVF);[56] Smit *et al.* 2010[57]
Inappropriate inclusion criteria - only patients with high DNA damage	Greco *et al.* 2005[58]
Analyzed using processed sperm	Bungum *et al.* 2008[59]
Use of assays not included in the systematic review - neutral comet assay	Chi *et al.* 2011;[42] Abu-Hassan *et al.* 2006[43]
Use of assays not included in the systematic review - *in situ* nick translation	Sakkas *et al.* 1996;[44] Tomlinson *et al.* 2001[45]
Use of acridine orange slide based staining method	Claassens *et al.* 1992;[60] Hoshi *et al.* 1996;[61] Angelopoulos *et al.* 1998;[62] Hammadeh *et al.* 2001;[63] Hammadeh *et al.* 2008;[64] Virant-Klun *et al.* 2002;[65] Duran *et al.* 1998;[66] Duran *et al.* 2002;[67] Katayose *et al.* 2003;[68] Henkel *et al.* 2004;[40] Cebesoy *et al.* 2006;[69] Zhang *et al.* 2008;[70] Jiang *et al.* 2011b;[71] Wang *et al.* 2012[72]
Treatment outcome associated with IUI insemination method	Duran *et al.* 2002;[67] Saleh *et al.* 2003;[55] Bungum *et al.* 2004;[39] Bungum *et al.* 2007;[73] Bungum *et al.* 2008;[59] Boe-Hansen *et al.* 2006;[74] Muriel *et al.* 2006b;[75] Thomson *et al.* 2011;[76] Yang *et al.* 2011;[77] Alkhayal *et al.* 2013[78]
Insufficient data to construct two-by-two table	Sun *et al.* 1997;[79] Lopes *et al.* 1998;[80] Marchetti *et al.* 2002;[81] Tomsu *et al.* 2002;[82] Lewis *et al.* 2004;[83] Nasr-Esfahan *et al.* 2005;[84] Hammadeh *et al.* 2006;[85] Hammadeh *et al.* 2008;[64] Caglar *et al.* 2007;[86] Stevanato *et al.* 2008;[87] Velez de la Calle *et al.* 2008;[88] Gu *et al.* 2009;[89] Gu *et al.* 2011;[90] Nijs *et al.* 2009;[91] Nijs *et al.* 2011;[92] Tarozzi *et al.* 2009;[93] Tavalaee *et al.* 2009;[94] Daris *et al.* 2010;[95] Kennedy *et al.* 2011;[96] Na and Li, 2011;[97] Rama Raju *et al.* 2012;[98] Sharbatoghli *et al.* 2012;[99] Pregl Breznik *et al.* 2013;[3] Lazaros *et al.* 2013;[100] Sanchez-Martin *et al.* 2013;[101] Smit *et al.* 2010[57]

IUI: intrauterine; IVF: *in vitro* fertilization

Table 2: Selected clinical and statistical characteristics of the individual studies

Study	Assay	Study design	FF inclusion	FF CTL (yes/no)	Female age	ETs	Cut-off value (%)	n	ART	OR	95% CI	%weight (fixed)	%weight (random)
Benchaib et al. 2007[102]	TUNEL	Prospective	NA	Yes	34±4	2.1±0.8	15	84	IVF	0.46	0.11–2.00	0.67	1.26
Borini et al. 2006[103]	TUNEL	Prospective	NA	No	38±4	NA	10	82	IVF	1.66	0.33–8.28	0.56	1.12
Frydman et al. 2008[104]	TUNEL	Prospective	Age <38, FSH <10	Yes	33	2.1±0.1	35	117	IVF	2.97	1.39–6.32	2.53	2.34
Henkel et al. 2003[105]	TUNEL	Prospective	NA	No	NA	NA	36.5	208	IVF	2.24	1.09–4.58	2.83	2.42
Host et al. 2000[106]	TUNEL	Prospective	NA	Yes	NA	NA	4	175	IVF	1.92	0.92–4.04	2.63	2.37
Huang et al. 2005[107]	TUNEL	Retrospective	NA	No	NA	3or4	4	217	IVF	1.30	0.66–2.56	3.19	2.5
Boe-Hansen et al. 2006[74]	SCSA	Prospective	NA	No	NA	1.6	27	139	IVF	2.43	0.28–20.83	0.31	0.74
Bungum et al. 2007[73]	SCSA	Prospective	Age <40, BMI <30, FSH <12	Yes	NA	2 median	30	388	IVF	1.24	0.69–2.26	4.1	2.66
Lin et al. 2008[108]	SCSA	Retrospective	Age <40, FSH <15	Yes	NA	NA	27	137	IVF	0.88	0.35–2.19	1.73	2.05
Speyer et al. 2010[109]	SCSA	Prospective	Age <45	No	36±4	1.97	19	192	IVF	4.51	0.99–20.48	0.63	1.22
Jiang et al. 2011a[110]	SCSA	Prospective	Age <37 years, BMI <25, FSH <10	Yes	NA	NA	30	137	IVF	3.44	1.2–10.59	1.15	1.71
Fang et al., 2011[111]	SCSA	Prospective	NA	No	NA	NA	10	111	IVF	0.84	0.39–1.79	2.49	2.33
Ni et al. 2014[112]	SCD	Prospective	Normal female	Yes	NA	NA	30	1380	IVF	1.12	0.71–1.76	6.94	2.92
Simon et al. 2011a[12]	Comet	Prospective	Normal female	Yes	NA	NA	52	70	IVF	76.0	9.06–637.57	0.32	0.75
Simon et al. 2010[113]	Comet	Prospective	NA	No	NA	NA	56	224	IVF	4.33	1.82–10.31	1.93	2.14
Simon et al. 2011b[114]	Comet	Prospective	NA	No	NA	NA	52	73	IVF	4.50	1.28–15.89	0.91	1.51
Benchaib et al. 2007[102]	TUNEL	Prospective	NA	No	33±4	2.1±0.9	15	218	ICSI	1.55	0.71–3.41	2.33	2.28
Borini et al. 2006[103]	TUNEL	Prospective	NA	No	37±5	NA	10	50	ICSI	7.36	1.67–32.44	0.66	1.25
Avendano et al. 2010[115]	TUNEL	Prospective	NA	No	34±4	2.4.0.5	17.6	36	ICSI	6.40	1.47–27.83	0.67	1.26
Henkel et al. 2003[105]	TUNEL	Prospective	NA	No	NA	NA	36.5	54	ICSI	3.67	1.12–12.00	1.03	1.62
Host et al. 2000[106]	TUNEL	Prospective	NA	Yes	NA	NA	4	61	ICSI	0.79	0.28–2.25	1.31	1.82
Huang et al. 2005[107]	TUNEL	Retrospective	NA	No	NA	3or4	4	86	ICSI	1.80	0.76–4.27	1.94	2.14
Ozmen et al. 2007[137]	TUNEL	Prospective	NA	No	NA	NA	10	42	ICSI	6.67	0.35–127.48	0.17	0.43
Boe-Hansen et al. 2006[74]	SCSA	Prospective	NA	No	NA	1.3	27	47	ICSI	0.76	0.21–2.72	0.89	1.5
Bungum et al. 2007[73]	SCSA	Prospective	NA	Yes	NA	2 median	30	223	ICSI	0.65	0.37–1.14	4.53	2.71
Gandini et al. 2004[56]	SCSA	Prospective	NA	Yes	35 median	NA	27	22	ICSI	0.50	0.09–2.81	0.49	1.02
Check et al. 2005[116]	SCSA	Prospective	NA	No	NA	NA	30	106	ICSI	1.34	0.52–3.43	1.64	2
Zini et al. 2005[117]	SCSA	Prospective	Age <40	Yes	NA	NA	30	60	ICSI	0.87	0.2–3.23	0.84	1.45
Micinski et al. 2009[118]	SCSA	Prospective	Age <38	No	31±4	NA	15	60	ICSI	3.73	0.74–18.77	0.56	1.12
Speyer et al. 2010[109]	SCSA	Prospective	Age <45	No	35±4	1.93	19	155	ICSI	1.37	0.60–3.13	2.13	2.21
Lin et al. 2008[108]	SCSA	Retrospective	Age <40, FSH <15	Yes	NA	NA	27	86	ICSI	1.21	0.45–3.23	1.5	1.93
Dar et al. 2013[119]	SCSA	Prospective	NA	No	NA	NA	15	153	ICSI	0.77	0.36–1.64	2.55	2.35
Yang et al. 2013[120]	SCSA	Prospective	NA	No	NA	NA	25	62	ICSI	2.01	0.70–5.75	1.32	1.82
Jiang et al. 2011a[110]	SCSA	Prospective	Age <37 years, BMI <25, FSH <10	Yes	NA	NA	30	50	ICSI	0.61	0.18–2.09	0.97	1.57
Nicopoullos et al. 2008[121]	SCSA	Prospective	NA	No	NA	NA	30	56	ICSI	1.00	0.26–3.92	0.78	1.38
Ni et al. 2014[112]	SCD	Prospective	Normal female	Yes	NA	NA	30	355	ICSI	0.97	0.61–1.53	6.87	2.91
Nunez-Calonge et al. 2012[122]	SCD	Prospective	Donor oocytes	Yes	NA	NA	17	70	ICSI	10.0	3.11–32.29	1.06	1.64
Gosalvez et al. 2013[123]	SCD	Prospective	NA	No	NA	NA	15.5	81	ICSI	2.39	0.74–7.70	1.06	1.64
Simon et al. 2010[113]	Comet	Prospective	NA	No	35	NA	56	127	ICSI	1.73	0.82–3.66	2.58	2.36
Simon et al. 2011b[114]	Comet	Prospective	NA	No	NA	NA	52	22	ICSI	2.67	0.42–16.82	0.43	0.93
Seli et al. 2004[124]	TUNEL	Prospective	NA	No	35±1	NA	20	49	IVF + ICSI	1.33	0.43–4.16	1.12	1.69
Esbert et al. 2011[125]	TUNEL	Prospective	NA	No	39±5	NA	15	178	IVF + ICSI	1.70	0.753–3.85	2.18	2.23
Benchaib et al. 2003[126]	TUNEL	Prospective	NA	No	33±4	NA	20	104	IVF + ICSI	5.42	0.30–97.35	0.17	0.45
Simon et al. 2014a[33]	TUNEL	Prospective	NA	No	NA	NA	10	224	IVF + ICSI	3.38	1.94–5.87	4.73	2.74

Contd...

Table 2: Contd...

Study	Assay	Study design	FF inclusion	FF CTL (yes/no)	Female age	ETs	Cut-off value (%)	n	ART	OR	95% CI	%weight (fixed)	%weight (random)
Bakos *et al.* 2007[127]	TUNEL	Prospective	NA	Yes	36±1	NA	48	113	IVF + ICSI	20.27	4.54–90.43	0.65	1.24
Larson-Cook *et al.* 2003[128]	SCSA	Retrospective	NA	Yes	32±5	3.1±0.2	27	89	IVF + ICSI	55.24	3.09–87.76	0.17	0.45
Virro *et al.* 2004[129]	SCSA	Retrospective	NA	No	NA	≤2	30	249	IVF + ICSI	2.28	1.26–4.13	4.1	2.66
Payne *et al.* 2005[130]	SCSA	Prospective	NA	Yes	34±3	3.0	27	95	IVF + ICSI	0.45	0.16–1.27	1.37	1.86
Simon *et al.* 2014a[33]	SCSA	Prospective	NA	No	NA	NA	27	96	IVF + ICSI	1.32	0.41–4.25	1.06	1.64
Guerin *et al.* 2005[131]	SCSA	Prospective	NA	No	NA	NA	30	100	IVF + ICSI	3.53	0.43–28.71	0.33	0.77
Muriel *et al.* 2006a[132]	SCD	Prospective	NA	No	37±3	1.8±0.1	18.8	84	IVF + ICSI	2.96	1.19–7.33	1.76	2.06
Lopez *et al.* 2013[133]	SCD	Prospective	NA	No	NA	NA	25.5	152	IVF + ICSI	2.54	1.09–5.91	2.03	2.18
Anifandis *et al.* 2015[134]	SCD	Prospective	NA	No	36±0	NA	35	139	IVF + ICSI	0.83	0.38–1.82	2.36	2.29
Meseguer *et al.* 2011[135]	SCD	Prospective	NA	No	38±6	NA	27.1	98	IVF + ICSI	3.58	1.55–8.26	2.07	2.19
Simon *et al.* 2014a[33]	Comet	Prospective	NA	No	NA	NA	82	229	IVF + ICSI	4.74	2.53–8.86	3.7	2.6
Morris *et al.* 2002[136]	Comet	Prospective	Age <40	No	NA	NA	NA	53	IVF + ICSI	0.92	0.27–3.10	0.98	1.57

NA: not available; TUNEL: terminal deoxyuridine nick end labeling assay; SCD: sperm chromatin dispersion assay, SCSA: sperm chromatin structure assay; FSH: follicle stimulating hormone; BMI: biometric index; IVF: *in vitro* fertilization; ICSI: Intra cytoplasmic sperm injection; ART: assisted reproductive technology; OR: odds ratio; CI: confidence interval; FF CTL: female factor controlled; ETs: embryo transfers

Table 3: Meta-analysis summary: Overall and subgroup odds ratios of studies on sperm DNA damage and pregnancy

Effect		Number of studies	Fixed effects model		Random effects model		Percentage of variation across studies I² (%)	Test of heterogeneity (Q2) P value
			OR (95% CI)	P	OR (95% CI)	P		
Overall effect		56	1.68 (1.49–1.89)	0.0000*	1.84 (1.5–2.27)	<0.0001*	60.9	<0.0001*
Sperm DNA damage assays								
SCSA		23	1.18 (0.96–1.44)	0.1115	1.22 (0.93–1.61)	0.1522	38.1	0.0338*
TUNEL		18	2.18 (1.75–2.72)	0.0000*	2.22 (1.61–3.05)	<0.0001*	43.8	0.0247*
Comet		7	3.34 (2.32–4.82)	0.0000*	3.56 (1.78–7.09)	0.0003*	65.5	0.0079*
SCD		8	1.51 (1.18–1.92)	0.0011*	1.98 (1.19–3.3)	0.0086*	72.9	0.0005*
Types of assisted treatment								
IVF		16	1.65 (1.34–2.04)	0.0000*	1.92 (1.33–2.77)	0.0005*	60.7	0.0008*
ICSI		24	1.31 (1.08–1.59)	0.0068*	1.49 (1.11–2.01)	0.0075*	48.7	0.0042*
Mixed		16	2.37 (1.89–2.97)	0.0000*	2.32 (1.54–3.5)	0.0001*	64.4	0.0002*
Assays	Types							
SCSA	IVF	6	1.32 (0.91–1.91)	0.1471	1.43 (0.86–2.37)	0.1670	35.9	0.1678
SCSA	ICSI	12	0.96 (0.72–1.27)	0.7800	0.96 (0.72–1.27)	0.7800	0.0	0.5811
SCSA	Mixed	5	1.69 (1.07–2.66)	0.0234*	1.93 (0.68–5.42)	0.2147	70.5	0.0089*
TUNEL	IVF	6	1.81 (1.29–2.55)	0.0007*	1.78 (1.2–2.65)	0.0039*	20.1	0.2822
TUNEL	ICSI	7	2.11 (1.38–3.23)	0.0005*	2.38 (1.31–4.31)	0.0042*	42.4	0.1078
TUNEL	Mixed	5	2.92 (1.95–4.38)	0.0000*	3.17 (1.45–6.94)	0.0038*	61.5	0.0344*
Comet	IVF	3	5.86 (2.97–11.53)	0.0000*	8.39 (2.16–32.55)	0.0021*	67.8	0.0448*
Comet	ICSI	2	1.84 (0.92–3.68)	0.0859	1.84 (0.92–3.68)	0.0859	0.0	0.6692
Comet	Mixed	2	3.36 (1.92–5.86)	0.0000*	2.27 (0.46–11.26)	0.3150	81.9	0.0187*
SCD	IVF	1	1.12 (0.71–1.76)	0.6405	1.12 (0.71–1.76)	0.6405	N/A	N/A
SCD	ICSI	3	1.42 (0.95–2.12)	0.0896	2.65 (0.64–10.86)	0.1770	85.9	0.0008*
SCD	Mixed	4	2.07 (1.36–3.16)	0.0007*	2.14 (1.09–4.19)	0.0272*	60.9	0.0534

*Significance at 95%. TUNEL: terminal deoxyuridine nick end labeling assay; SCD: sperm chromatin dispersion assay, SCSA: sperm chromatin structure assay; FSH: follicle stimulating hormone; IVF: *in vitro* fertilization; ICSI: Intra cytoplasmic sperm injection; CI: confidence interval; OR: odds ratio

P < 0.0001) (**Table 3**). Overall, a strong negative association between sperm DNA damage and clinical pregnancy was observed after assisted treatments.

Relationship between sperm DNA damage and clinical pregnancy: subgroup analysis by type of assisted reproduction
The relationship between sperm DNA damage (assessed by one of four different DNA damage tests: TUNEL, SCSA, SCD, and Comet assay) and clinical pregnancy was analyzed in 3734 IVF treatment cycles (16 studies), 2282 ICSI treatment cycles (24 studies), and 2052 mixed IVF + ICSI treatment cycles (16 studies) (**Table 4**). The heterogeneity (*I²* value, *Q* statistic *P* value) was moderate to high in the IVF and mixed IVF + ICSI studies (60.7%, *P* = 0.0008 and 64.4%, *P* = 0.0002, respectively) and moderate in the ICSI studies (48.7%, *P* = 0.0042) (**Table 3**). The negative effect of sperm DNA damage on clinical pregnancy was observed with the fixed effects model where

Table 4: Selected diagnostic properties of studies on sperm DNA damage and clinical pregnancy after assisted reproduction

Study	ART	Assay	RR	95% CI	Z statistics	P	Sensitivity	Specificity	NPV	PPV	Positive LH	95% CI	Negative LH	95% CI
Benchaib et al. 2007[102]	IVF	TUNEL	0.73	0.36–1.49	0.87	0386	7.14	85.71	31.58	50.00	0.50	0.13–1.85	1.08	0.92–1.28
Borini et al. 2006[103]	IVF	TUNEL	1.10	0.85–1.44	0.71	0.475	17.19	88.89	23.19	84.62	1.55	0.38–6.36	0.93	0.76–1.14
Frydman et al. 2008[104]	IVF	TUNEL	1.72	1.18–2.51	2.80	0.005	57.89	68.33	63.08	63.46	1.83	1.19–2.82	1.62	0.43–0.87
Henkel et al. 2003[105]	IVF	TUNEL	1.24	1.04–1.46	2.47	0.014	34.93	80.65	80.95	34.48	1.80	1.04–3.14	0.81	0.68–0.96
Host et al. 2000[106]	IVF	TUNEL	1.21	0.99–1.48	1.88	0.059	33.90	78.95	76.92	36.59	1.61	0.92–2.83	0.84	0.70–1.01
Huang et al. 2005[107]	IVF	TUNEL	1.15	0.81–1.63	0.80	0.425	21.21	82.50	55.93	50.00	1.21	0.70–2.09	0.96	0.84–1.09
Boe-Hansen et al. 2006[74]	IVF	SCSA	1.20	0.87–1.66	1.13	0.258	6.00	97.44	28.79	85.71	2.34	0.29–18.81	0.96	0.90–1.04
Bungum et al. 2007[73]	IVF	SCSA	1.07	0.90–1.27	0.76	0.447	16.92	85.94	33.74	70.97	1.20	0.73–2.00	0.97	1.06
Lin et al. 2008[108]	IVF	SCSA	0.93	0.57–1.53	0.27	0.785	15.15	83.10	51.30	45.45	0.90	0.42–1.93	1.02	0.88–1.18
Speyer et al. 2010[109]	IVF	SCSA	1.44	1.16–1.79	3.25	0.001	11.57	97.18	39.20	87.50	4.11	0.96–17.55	0.91	0.84–0.98
Jiang et al. 2011a[110]	IVF	SCSA	1.33	1.09–1.61	2.80	0.005	27.08	90.24	34.58	86.67	2.78	1.03–7.45	0.81	0.69–0.95
Fang et al. 2011[111]	IVF	SCSA	0.93	0.69–1.27	0.46	0.648	43.28	52.27	37.70	58.00	0.91	0.60–1.37	1.09	0.76–1.54
Ni et al. 2014[112]	IVF	SCD	1.03	0.90–1.19	0.48	0.629	7.03	93.65	32.12	70.21	1.11	0.72–1.70	0.99	0.96–1.02
Simon et al. 2011a[12]	IVF	Comet	2.82	1.7–4.68	4.04	<0.001	80.00	95.00	65.92	97.56	16.00	2.36–108.63	0.21	0.12–0.37
Simon et al. 2010[113]	IVF	Comet	1.24	1.11–1.39	3.68	<0.001	48.65	82.05	25.20	92.78	2.71	1.36–5.39	0.63	0.51–0.77
Simon et al. 2011b[114]	IVF	Comet	1.30	1.04–1.76	2.24	0.025	62.07	73.33	33.33	90.00	2.33	0.98–5.52	0.52	0.33–0.81
Benchaib et al. 2007[102]	ICSI	TUNEL	1.15	0.92–1.45	1.21	0.228	18.57	87.18	37.36	72.22	1.45	0.74–2.84	0.93	0.83–1.05
Borini et al. 2006[103]	ICSI	TUNEL	1.63	1.08–2.48	2.33	0.020	71.05	75.00	45.00	90.00	2.84	1.04–7.73	0.39	0.21–0.70
Avendano et al. 2010[115]	ICSI	TUNEL	2.29	1.08–4.86	2.15	0.032	76.19	66.67	66.67	76.19	2.29	1.07–4.86	0.36	0.15–0.83
Henkel et al. 2003[105]	ICSI	TUNEL	1.57	1.02–2.42	2.06	0.040	62.86	68.42	50.00	78.57	1.99	0.98–4.05	0.54	0.32–0.92
Host et al. 2000[106]	ICSI	TUNEL	0.91	0.61–1.36	0.45	0.652	56.76	37.50	36.00	58.33	0.91	0.60–1.38	1.15	0.61–2.18
Huang et al. 2005[107]	ICSI	TUNEL	1.36	0.85–2.16	1.29	0.196	64.29	50.00	59.46	55.10	1.29	0.89–1.86	0.71	0.43–1.18
Ozmen et al. 2007[137]	ICSI	TUNEL	1.26	0.92–1.23	1.43	0.154	25.00	90.91	29.41	88.89	2.75	0.39–19.58	0.83	0.63–1.08
Boe-Hansen et al. 2006[74]	ICSI	SCSA	0.92	0.62–1.37	0.41	0.683	36.36	57.14	27.59	66.67	0.85	0.40–1.80	1.11	0.66–1.88
Bungum et al. 2007[73]	ICSI	SCSA	0.83	0.65–1.07	1.44	0.150	28.79	61.54	37.33	52.05	0.75	0.52–1.09	1.16	0.95–1.41
Gandini et al. 2004[56]	ICSI	SCSA	0.75	0.36–1.57	0.76	0.445	38.46	44.44	33.33	50.00	0.69	0.28–1.71	1.38	0.59–3.23
Check et al. 2005[116]	ICSI	SCSA	1.09	0.83–1.44	0.64	0.53	29.17	76.47	33.77	72.42	1.24	0.61–2.52	0.93	0.73–1.18
Zini et al. 2005[117]	ICSI	SCSA	0.93	0.46–1.88	0.21	0.836	17.24	80.65	51.02	45.45	0.89	0.30–2.61	1.03	0.81–1.30
Micinski et al. 2009[118]	ICSI	SCSA	1.26	0.99–1.60	1.88	0.059	40.43	84.62	28.21	90.48	2.63	0.70–9.85	0.70	0.51–0.98
Speyer et al. 2010[109]	ICSI	SCSA	1.11	0.86–1.43	0.80	0.426	23.76	81.48	36.36	70.59	1.28	0.66–2.48	0.94	0.79–1.11
Lin et al. 2008[108]	ICSI	SCSA	1.10	0.68–1.78	0.38	0.702	26.19	77.27	52.31	52.38	1.15	0.55–2.43	0.96	0.75–1.22
Dar et al. 2013[119]	ICSI	SCSA	0.91	0.69–1.20	0.65	0.513	23.76	71.15	32.46	61.54	0.82	0.47–1.43	1.07	0.87–1.31
Yang et al. 2013[120]	ICSI	SCSA	1.40	0.86–2.27	1.35	0.177	45.16	70.97	56.41	60.87	1.56	0.79–3.05	0.77	0.51–1.14
Jiang et al. 2011a[110]	ICSI	SCSA	0.85	0.56–1.30	0.74	0.457	32.35	56.25	28.12	61.11	0.74	0.35–1.55	1.20	0.74–1.96
Nicopoullos et al. 2008[121]	ICSI	SCSA	1.00	0.51–1.98	0.00	1.000	17.86	82.14	50.00	50.00	1.00	0.33–3.08	1.00	0.78–1.28
Ni et al. 2014[112]	ICSI	SCD	0.99	0.86–1.14	0.15	0.885	39.18	60.00	30.70	68.57	0.98	0.74–1.29	1.01	0.84–1.22
Nunez-Calonge et al. 2012[122]	ICSI	SCD	4.45	1.89–10.46	3.42	<0.001	80.77	70.45	86.11	61.76	2.73	1.67–4.48	0.27	0.12–0.61
Gosalvez et al. 2013[123]	ICSI	SCD	1.60	0.92–2.78	1.65	0.099	25.00	87.76	64.18	57.14	2.04	0.78–5.33	0.85	0.68–1.07
Simon et al. 2010[113]	ICSI	Comet	1.22	0.92–1.62	1.36	0.174	67.47	45.45	42.55	70.00	1.24	0.91–1.68	0.72	0.46–1.12
Simon et al. 2011b[114]	ICSI	Comet	1.39	0.71–2.67	0.97	0.331	66.67	57.14	44.44	76.92	1.56	0.62–3.93	0.58	0.22–1.53
Seli et al. 2004[124]	Mixed	TUNEL	1.14	0.68–1.93	0.50	0.617	46.15	60.87	50.00	57.14	1.18	0.61–2.28	0.88	0.55–1.43
Esbert et al. 2011[125]	Mixed	TUNEL	1.27	0.91–1.76	1.41	0.158	19.78	87.36	51.01	62.07	1.56	0.78–3.12	0.92	0.81–1.05
Benchaib et al. 2003[126]	Mixed	TUNEL	1.17	0.93–1.48	1.32	0.186	10.98	95.65	23.16	90.00	2.52	0.34–18.91	0.93	0.83–1.04
Simon et al. 2014a[33]	Mixed	TUNEL	1.93	1.43–2.61	4.27	<0.001	59.60	69.60	68.50	60.82	1.96	1.44–2.68	0.58	0.45–0.76
Bakos et al. 2007[127]	Mixed	TUNEL	2.10	1.62–2.72	5.62	<0.001	48.53	95.56	55.13	94.29	10.92	2.76–43.26	0.54	0.42–0.68
Larson-Cook et al. 2003[128]	Mixed	SCSA	3.42	2.27–5.16	5.86	<0.001	32.26	98.31	73.42	90.91	19.03	2.55–141.92	0.69	0.54–0.88
Virro et al. 2004[129]	Mixed	SCSA	1.36	1.11–1.66	2.99	0.003	35.17	80.77	47.19	71.83	1.83	1.16–2.87	0.80	0.69–0.93
Payne et al. 2005[130]	Mixed	SCSA	0.74	0.47–1.16	1.31	0.191	15.62	70.97	28.95	52.63	0.54	0.24–1.19	1.19	0.93–1.52
Simon et al. 2014a[33]	Mixed	SCSA	1.15	0.66–1.99	0.48	0.629	15.22	88.00	53.01	53.85	1.27	0.46–3.50	0.96	0.82–1.13
Guerin et al. 2005[131]	Mixed	SCSA	1.16	0.98–1.36	1.76	0.079	18.07	94.12	19.05	93.75	3.07	0.43–21.72	0.87	0.74–1.02
Muriel et al. 2006[132]	Mixed	SCD	1.59	1.04–2.41	2.16	0.031	70.00	55.88	55.88	70.00	1.59	1.04–2.41	0.54	0.32–0.90
Lopez et al. 2013[133]	Mixed	SCD	1.79	0.99–3.21	1.94	0.053	86.57	28.24	72.73	48.74	1.21	1.01–1.42	0.48	0.24–0.95
Anifandis et al. 2014[134]	Mixed	SCD	0.96	0.79–1.16	0.47	0.641	54.29	41.18	22.58	74.03	0.92	0.66–1.29	1.11	0.71–1.75
Meseguer et al. 2011[135]	Mixed	SCD	1.79	1.20–2.66	2.87	0.004	64.15	66.67	61.22	69.39	1.92	1.22–3.05	0.54	0.36–0.81
Simon et al. 2014a[33]	Mixed	Comet	2.11	1.61–2.76	5.46	<0.001	45.00	85.27	66.67	70.67	2.11	1.61–2.76	0.65	0.53–0.78
Morris et al. 2002[136]	Mixed	Comet	0.98	0.69–1.37	0.14	0.888	57.89	40.00	27.27	70.97	0.96	0.59–1.58	1.05	0.51–2.17

TUNEL: terminal deoxyuridine nick end labeling assay; SCD: sperm chromatin dispersion assay; SCSA: sperm chromatin structure assay; FSH: follicle stimulating hormone; IVF: *in vitro* fertilization; ICSI: intra cytoplasmic sperm injection; RR: relative risk; NPV: negative predictive value; PPV: positive predictive value; LH: luteinizing hormone; ART: assisted reproductive technology; CI: confidence interval
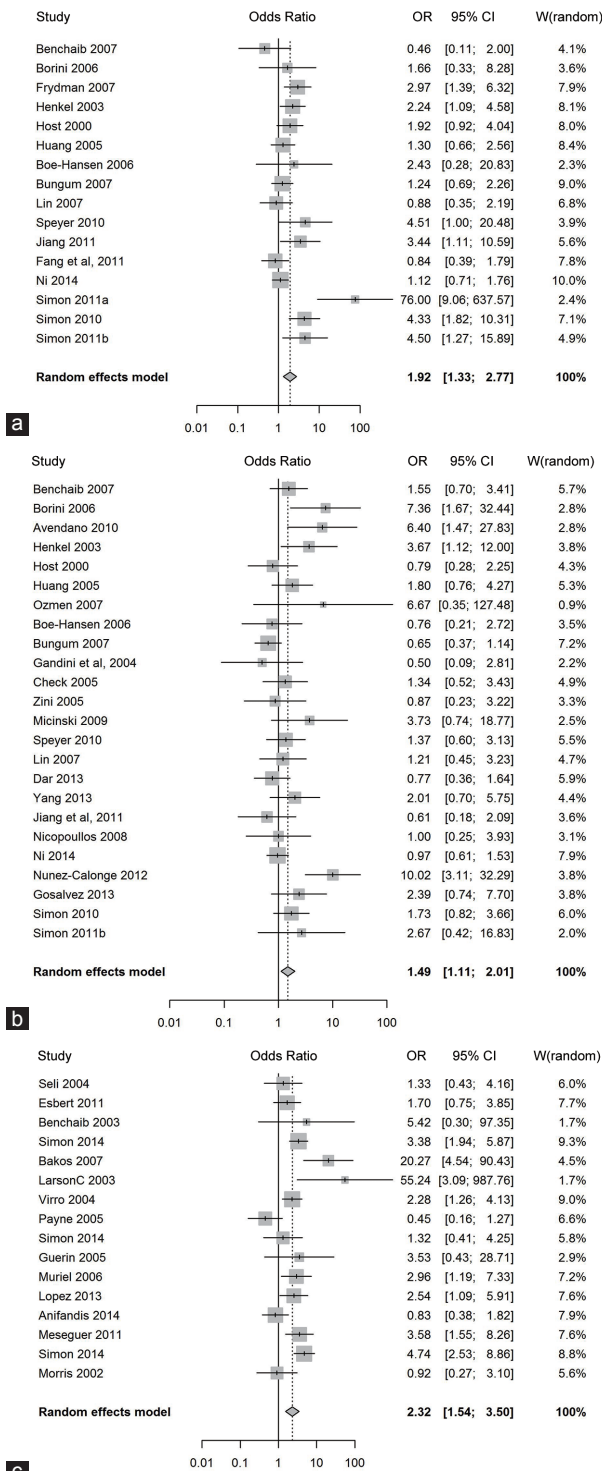
Figure 2: Forest plot of odds ratio to determine the negative effect of sperm DNA damage on clinical pregnancy outcome. (**a**) following "IVF" type of assisted reproduction, (**b**) following "ICSI" type of assisted reproduction, (**c**) following "Mixed" type of assisted reproduction.

the combined OR estimates of IVF (16 estimates, OR = 1.65; 95% CI: 1.34–2.04; $P < 0.0001$), ICSI (24 estimates, OR = 1.31; 95% CI: 1.08–1.59; $P = 0.0068$), and mixed IVF + ICSI studies (16 estimates, OR = 2.37; 95% CI: 1.89–2.97; $P < 0.0001$) were all statistically

significant. Similarly, the negative effect of sperm DNA damage on clinical pregnancy was observed using the random effects model where the combined OR estimates of IVF (16 estimates, OR = 1.92; 95% CI: 1.33–2.77; $P = 0.0005$), ICSI (24 estimates, OR = 1.49; 95% CI: 1.11–2.01; $P = 0.0075$), and mixed IVF + ICSI studies (16 estimates, OR = 2.32; 95% CI: 1.54–3.5; $P = 0.0001$) were all statistically significant. The forest plots depicting the individual ORs and random effects model combined OR estimate (with 95% CI) for the IVF, ICSI, and mixed IVF + ICSI studies are shown in **Figure 2a–2c**, respectively. Overall, a strong negative association between sperm DNA damage and clinical pregnancy was observed after IVF and/or ICSI treatments.

Relationship between sperm DNA damage and clinical pregnancy: subgroup analysis by type of sperm DNA damage assay

In this meta-analysis, data from studies using one of four commonly used sperm DNA damage measurement assays were analyzed separately using the fixed and random effect models. Of the total treatment cycles ($n = 8068$), sperm DNA damage was measured by SCSA in 2813 cycles (34.9%), SCD in 2359 cycles (29.2%), TUNEL in 2098 cycles (26.0%), and Comet in 798 cycles (9.9%) (**Table 4**). The heterogeneity (I^2 value, Q statistic P value) was moderate to high in studies using the SCD (72.9%, $P = 0.0005$) and Comet assays (65.5%, $P = 0.0079$) and low to moderate in studies using the SCSA (38.1%, $P = 0.0338$) and TUNEL assays (43.8%, $P = 0.0247$) (**Table 3**). The negative effect of sperm DNA damage on clinical pregnancy was observed using the random effects model where the combined OR estimates of TUNEL (18 estimates, OR = 2.22; 95% CI: 1.61–3.05; $P < 0.0001$), Comet (7 estimates, OR = 3.56; 95% CI: 1.78–7.09; $P = 0.0003$), and SCD studies (8 estimates, OR = 1.98; 95% CI: 1.19–3.3; $P = 0.0086$) were all statistically significant. In contrast, the random effects model, combined OR estimate of SCSA studies (23 estimates, OR = 1.22; 95% CI: 0.93–1.61; $P = 0.1522$), was not statistically significant. In summary, the combined ORs for TUNEL, Comet, and SCD studies but not SCSA studies demonstrated a negative effect of sperm DNA damage on clinical pregnancy outcome after ART.

Relationship between sperm DNA damage and clinical pregnancy: subgroup analysis by type of assisted reproduction and type of sperm DNA damage assay

In a further subgroup analysis, we examined the relationship between sperm DNA damage and clinical pregnancy by type of assisted reproduction and type of sperm DNA damage assay using the random effects model (**Table 3**). The combined OR estimates for TUNEL studies were statistically significant for all types of assisted reproduction (IVF, ICSI, and mixed IVF + ICSI studies) by the random effects model, demonstrating a negative effect of sperm DNA damage on clinical pregnancy outcome. The combined OR estimates for Comet studies were statistically significant ($P = 0.0021$) for IVF treatment type only and combined OR estimates for SCD studies were statistically significant ($P = 0.0272$) for mixed IVF + ICSI treatment type only. In contrast, the combined OR estimates for SCSA studies were not statistically significant when subgrouped according to the type of assisted reproduction. Taken together, the data show that a strong negative association between sperm DNA damage and clinical pregnancy (with a statistically significant combined OR estimate) was more consistently demonstrated in studies utilizing assays that measure sperm DNA damage directly (TUNEL and Comet assays) than in studies that measure sperm DNA damage indirectly (SCSA and SCD assay).

DISCUSSION

We conducted a systematic review and meta-analysis of studies on sperm DNA damage (measured by SCSA, TUNEL, SCD, or Comet assay) and reproductive outcome after IVF and/or ICSI. We identified 67 pertinent articles in this systematic review. In 41 of these 67 papers, there were sufficient data to construct two-by-two tables and perform a meta-analysis of studies on the relationship between sperm DNA damage and clinical pregnancy after IVF and/or ICSI. From the 41 articles, we identified 56 studies involving 8068 treatment cycles (IVF and/or ICSI), which include 16 IVF studies (3734 treatment cycles), 24 ICSI studies (2282 treatment cycles), and 16 mixed IVF + ICSI studies (2052 treatment cycles). Of the total treatment cycles (*n* = 8068), sperm DNA damage was measured by SCSA in 2813 cycles (34.9%), SCD in 2359 cycles (29.2%), TUNEL in 2098 cycles (26.0%), and Comet in 798 cycles (9.9%).

In this study, we identified an overall detrimental effect of sperm DNA damage on clinical pregnancy rate after IVF and/or ICSI (56 IVF, ICSI or mixed IVF + ICSI studies) with a combined odds ratio of 1.68 (95% CI: 1.49–1.89). This is in contrast to prior meta-analyses[13,14] and the report from the Practice Committee of the American Society for Reproductive Medicine[15] where a negative effect of sperm DNA damage on clinical pregnancy outcome was not established. A recent meta-analysis showed a negative association between sperm DNA damage and IVF pregnancy but not with ICSI outcomes.[17] Moreover, in our study, the random effects model combined OR estimates of IVF (16 estimates, OR = 1.92; 95% CI: 1.33–2.77; *P* = 0.0005), ICSI (24 estimates, OR = 1.49; 95% CI: 1.11–2.01; *P* = 0.0075), and mixed IVF + ICSI studies (16 estimates, OR = 2.32; 95% CI: 1.54–3.5; *P* = 0.0001) were all statistically significant, suggesting a detrimental effect of sperm DNA damage on ART outcome. Unlike the previous meta-analyses, where the majority of the studies evaluated sperm DNA damage by SCSA and TUNEL assays, we included more recent studies with several evaluating sperm DNA damage by SCD and Comet assay.[13,14] When we segregated our dataset according to the type of DNA damage assay, all but the SCSA studies showed a detrimental effect of sperm DNA damage on clinical pregnancy (after IVF and/or ICSI). The large dataset of SCSA studies (23 studies including 2813 ART cycles) did not show a statistically significant negative association between sperm DNA damage and clinical pregnancy. This is unlike a previous meta-analysis[36] where sperm DNA damage assessed by SCSA was positively associated with *in vivo*, IUI, and routine IVF pregnancy. We observed that studies using the SCSA and SCD assays showed a detrimental effect of sperm DNA damage on clinical pregnancy with the mixed treatment group only (mixed IVF + ICSI studies). In contrast, an analysis of studies using the TUNEL assay demonstrated the negative effect of sperm DNA damage on clinical pregnancy with all the three treatment groups (IVF, ICSI, and mixed IVF + ICSI studies), suggesting that a direct method of DNA damage measurement may be a better predictor of pregnancy outcome.[37]

Our meta-analysis has a number of strengths. We performed a comprehensive literature search using three databases (MEDLINE, EMBASE, and PUBMED) and a reference search from the bibliography of the articles, which resulted in the identification of 56 studies. Two independent investigators approved the studies to be included or excluded from the meta-analysis. This study had sufficient published data to perform a meta-analysis on various types of treatment (IVF, ICSI, and mixed) as well as different type of DNA damage assays (SCSA, TUNEL, Comet, and SCD assays). We obtained a dataset with consistent protocols by excluding four studies with overlapping data[38–41] and 18 studies with sperm DNA tests that were not one of the four

inclusion tests (SCSA, TUNEL, SCD, or alkaline Comet assay): two studies using neutral Comet assay,[42,43] two studies using *in situ* nick translation assay,[44,45] and 14 studies using slide-based acridine orange staining method.

Our meta-analysis also has several weaknesses. One of the most important weaknesses of our study is the variable and poorly controlled clinical parameters of the evaluable studies (i.e., female factors, female age, number of embryos transferred, and assay cutoff/threshold). Moreover, the meta-analysis is also weakened by virtue of the different assisted treatment types and sperm DNA damage assays. Another important factor is the high study heterogeneity (61%; *P* < 0.001). This degree of study heterogeneity together with above factors (clinical parameters, sperm DNA assays) reduces our confidence in the combined ORs.

In the past two decades, sperm DNA damage has been one of the most extensively studied sperm parameters in the hope that this test may have clinical value.[46] Conventional semen parameters are shown to diagnose male fertility to some extent, but their associations to ART outcomes are limited; therefore, a need for newer tests has been emphasized.[47] A clinically useful sperm function test should have predictive value for natural and/or ART pregnancy outcomes and provide some added value in clinical decision-making.[46,48] Although sperm DNA damage has the potential to become a useful clinical biomarker,[49,50] the predictive value of this test in the context of IVF and or ICSI remains to be defined.[51,52] Assuming that we are confident in the combined ORs derived from our meta-analysis, our data suggest that tests of sperm DNA damage may provide some predictive value in the context of IVF, ICSI, and mixed IVF + ICSI studies. An analysis of the 16 IVF studies (with a median pregnancy rate of 32%) revealed a median PPV of 79% and median NPV of 35%. Thus, in populations with an overall IVF pregnancy rate of 32%, sperm DNA damage assessment can discriminate between IVF pregnancy rates of 21% (positive test) and 35% (negative test), a notable difference in pregnancy rate of important clinical value. An analysis of the 24 ICSI studies (with a median pregnancy rate of 36%) revealed a median PPV of 64% and median NPV of 40%. Thus, in populations with an overall ICSI pregnancy rate of 36%, sperm DNA damage assessment can discriminate between ICSI pregnancy rates of 36% (positive test) and 40% (negative test), a small difference of modest clinical value. An analysis of the 16 mixed (IVF + ICSI) studies (with a median pregnancy rate of 44%) revealed a median PPV of 70% and median NPV of 50%. Thus, in populations with an overall mixed (IVF + ICSI) pregnancy rate of 44%, sperm DNA damage assessment can discriminate between mixed (IVF + ICSI) pregnancy rates of 30% (positive test) and 50% (negative test), a notable difference in pregnancy rate of important clinical value. However, it is important to exercise caution when estimating the predictive value of sperm DNA tests in the context of IVF and/or ICSI because these estimates are derived from relatively small studies (100–200 cycles), the study characteristics are heterogeneous (e.g., different assay types, sperm DNA threshold levels, and clinical parameters) and the precision of the various sperm DNA assays remains to be validated.

In spite of the large number of studies examining the relationship between sperm chromatin and DNA damage with pregnancy rate, the wide acceptance of sperm chromatin tests as part of the assessment of a man's fertility potential has met resistance. This stems from various factors, but the main factor is the lack of standardized protocols shown to provide reproducible results across a range of laboratories (i.e., unknown precision regarding reproducibility and repeatability of the various assays) and the fact that the thresholds for

many of these tests have not been validated.[15] Moreover, our limited understanding of the underlying nature of sperm DNA damage has also limited the wide acceptance of these assays.[53] For example, how can we explain that when sperm DNA damage is measured in a given population using the Comet assay, a threshold value of 82% is obtained, while using the TUNEL assay the threshold value is 10%,[33,54] yet both threshold values are associated with clinical pregnancy rates.

To date, several reports have noted that there are insufficient data to demonstrate a negative association between sperm DNA damage and reproductive outcomes after IVF and/or ICSI. In this updated systematic review and meta-analysis, we have found a modest but statistically significant detrimental effect of sperm DNA damage on clinical pregnancy rate after IVF and/or ICSI (IVF, ICSI, and mixed IVF + ICSI studies). Although the adverse effect of sperm DNA damage on clinical pregnancies was observed in all three treatment groups (IVF, ICSI, and mixed IVF + ICSI studies), this effect appears to vary according to the type of assay used to measure sperm DNA damage.

AUTHOR CONTRIBUTIONS

LS is a postdoctoral fellow and was responsible for performing collection of data, and writing the manuscript. AZ was responsible for collection of data, compelling the studies for meta-analysis and writing the manuscript. AD and AC were responsible for statistical analysis and generating the figures. DTC is the principle investigator for this study and is responsible for the design of the study and writing the manuscript. DTC is also the head of ART unit and the corresponding author for this manuscript.

COMPETING INTEREST

DTC has received no personal financial support for this work. AZ has no conflicts of interest. LS has no conflicts of interest.

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