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

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Objective: To examine whether sperm DNA fragmentation has an effect on pregnancy and miscarriage after IVF and/or intracytoplasmic sperm injection (ICSI).
Design: Systematic review and meta-analysis.
Setting: University-affiliated teaching hospital.
Patient(s): Infertility patient(s).
Intervention(s): An exhaustive electronic literature search was conducted on MEDLINE, Google Scholar, and the Cochrane Library, from database inception to October 2013. We included clinical trials that examined the influence of sperm DNA damage on pregnancy and miscarriage of IVF/ICSI.
Main Outcome Measure(s): The outcomes of interest were pregnancy rate and miscarriage rate.
Result(s): In the analysis of pregnancy, 16 cohort studies (3,106 couples) were included. Of these, 14 studies (2,756 couples, 965 pregnancies) that also mentioned miscarriage were identified in the analysis of miscarriage. Meta-analysis showed that high-level sperm DNA fragmentation has a detrimental effect on outcome of IVF/ICSI, with decreased pregnancy rate and increased miscarriage rate. The stratified analysis by type of procedure (IVF vs. ICSI) indicated that high sperm DNA damage was related to lower pregnancy rates in IVF but not in ICSI cycles, whereas it was associated with higher miscarriage rates in both IVF and ICSI cycles.
Conclusion(s): The results indicate that assays detecting sperm DNA damage should be recommended to those suffering from recurrent failure to achieve pregnancy. Selection of sperm without DNA damage for use may improve the clinical outcome of ART. The data also provide a rationale for conducting further research aimed at evaluating the underlying mechanism(s) responsible for the detrimental effect of high sperm DNA fragmentation and the potential therapy. (Fertil Steril® 2014;102: 998–1005. ©2014 by American Society for Reproductive Medicine.)
Key Words: Sperm DNA damage, sperm DNA fragmentation, pregnancy, miscarriage, assisted reproductive technology

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Approximately one of every six couples will experience infertility, and approximately 40% of these cases are male factor infertility (1, 2). Conventional semen analysis continues to be the only routine test to diagnose this condition, even though it is known that such descriptive assessments cannot discriminate between the spermatozoa of fertile and infertile men. The shifting values for normality (all normal values now lower) in the fifth edition of the World Health Organization manual compared with the previous editions may result in even fewer men being classified as

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infertile. Conventional semen analysis may show normal parameters in the presence of high levels of sperm DNA damage (3–5). The conventional sperm test has relatively low predictive value for fertility and sperm functional assessment (6).

Sperm DNA testing has been increasingly used as an adjunct to the conventional sperm parameters (7–10). Sperm DNA integrity is one of the important determinants of normal fertilization and embryo development. A number of studies have investigated the relationship between outcome of assisted reproductive technology (ART) and high DNA damage in sperm (11–20).

As a biomarker for fertility, a number of studies have shown that high sperm DNA damage has an association with numerous reproductive processes, including impaired fertilization, disrupted preimplantation embryo development, miscarriage, and birth defects in the offspring (8, 9, 21–25). Some studies indicated that sperm with DNA damage can fertilize oocytes successfully and give rise to good-grade embryos that subsequently result in early pregnancy loss (24, 26–31).

However, some studies found that sperm with DNA damage were capable of fertilizing an oocyte (32–34), because they only found a modest effect on conception rates with conventional IVF and little, if any, effect with intracytoplasmic sperm injection (ICSI) (11–17, 34–40).

Zini et al. (9) have reviewed these observations and concluded that sperm DNA damage was associated with a significantly increased risk of pregnancy loss after IVF and ICSI. Another review, by Robinson et al. (41), also analyzed the effect of sperm DNA fragmentation on miscarriage rates not only after ART but also after spontaneous conception. To date, the bulk of the data indicate that sperm DNA damage has no detectable effect on pregnancy rates after ICSI and a modest effect on pregnancy rates after conventional IVF.

In the present review we aim to evaluate further whether sperm DNA damage has an effect on pregnancy and miscarriage after IVF/ICSI by performing a systematic review and meta-analysis of the available literature.

MATERIALS AND METHODS

Identification of the Literature

The following electronic databases were searched: MEDLINE, Google Scholar, and the Cochrane Library, from inception until October 2013. The following medical subject and text words were used to search relative studies: one including terms on sperm DNA damage (human sperm DNA chromatin, human sperm DNA, human sperm DNA fragmentation, human sperm DNA damage), one including terms on outcome of ART (pregnancy, pregnancy loss, abortion, miscarriage), and the last one about reproductive techniques (in vitro fertilization, IVF, intracytoplasmic sperm injection, ICSI, assisted reproduction). These subsets were combined with "AND" to generate a subset of citations relevant to our research question. Only full articles published in English were searched. Two investigators independently reviewed the articles for eligibility, and discrepancies were resolved by group discussion.

Study Selection and Data Extraction

We selected studies that evaluated sperm DNA damage in couples undergoing IVF and/or ICSI. The primary outcome of interest was clinical pregnancy rate and/or miscarriage rate. For studies to be eligible, outcome data (with pregnancy rate and/or miscarriage rate above and below DNA damage cutoff) were extracted in 2 × 2 tables. We also recorded the treatment type, sperm DNA assay type, cutoff point, number of cycles of ART, and number of pregnancies/miscarriages relative to abnormal or normal test result. If necessary, we contacted the research author to clarify the data. Newcastle–Ottawa Quality Assessment Scales were used to evaluate the quality of the observational studies (42). Two reviewers completed the quality assessment, and any disagreements about inclusion were resolved by consensus or arbitration by a third reviewer.

Statistical Analysis

The study-by-study comparisons were synthesized by a standard meta-analytic approach applied to the relative risks (RRs) of the individual 2 × 2 tables. Heterogeneity of the exposure effects was evaluated graphically using Forest plots and statistically using the I^2 statistic to quantify heterogeneity across studies. A fixed- or random-effects model for meta-analysis was implied to calculate an overall RR and its 95% confidence interval (CI). Because the χ^2 test for heterogeneity has low power in the situation of a meta-analysis when studies have small sample size or are few in number, a P value of .10, rather than the conventional level of .05, was used to determine statistical significance. Statistical analyses were performed using RevMan 5.0 (Cochrane Collaboration).

RESULTS

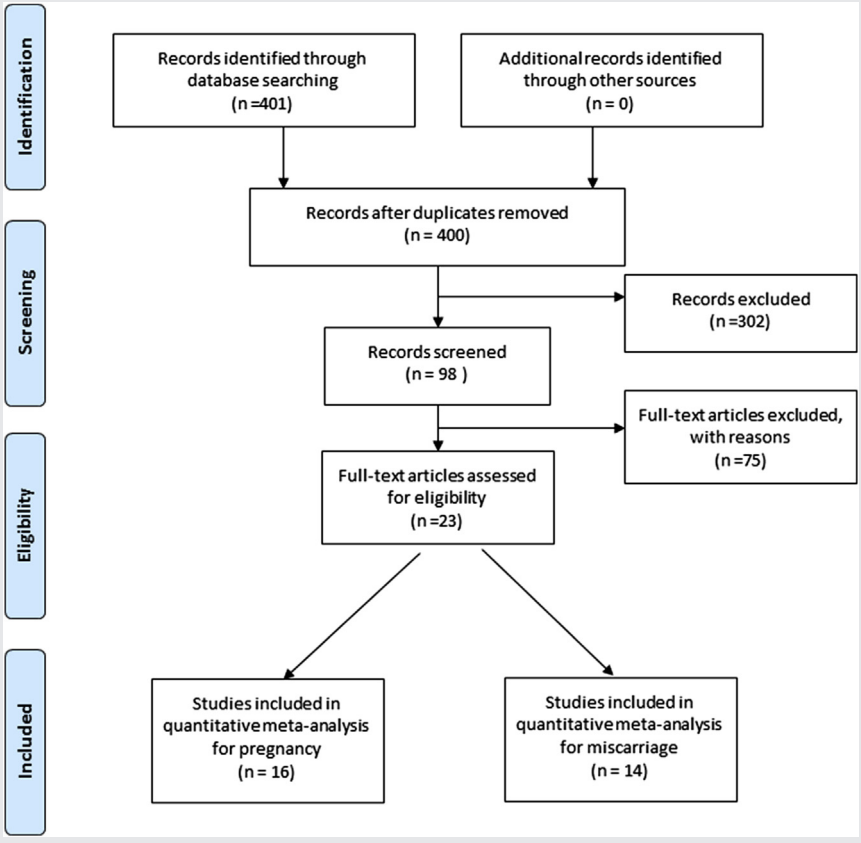
Studies Selection and Characteristics

The search strategy yielded 401 citations. Review of the titles and abstracts indicated that 302 were not relevant. Of the 99 remaining publications, 75 were excluded because neither pregnancy nor miscarriage data were reported. One study was excluded because all its data were duplicated in a later article that we have included in our meta-analysis. An additional seven articles were excluded because a 2 × 2 table could not be constructed from the data. One of the studies was later replaced by an updated report that included all of the earlier patients (Fig. 1).

The total number of eligible studies included in the review was 16 (one IVF study, four ICSI studies, nine IVF/ICSI studies, and two IVF/ICSI/IUI studies), comprising 3,106 couples and reporting pregnancy rate after ART with 1,084 pregnancies. Of these, 14 publications involving 2,756 couples also reported miscarriage rate with 965 pregnancies and 187 miscarriages.

The study characteristics are depicted in Table 1. Of these 16 articles, 14 were reportedly prospective, and 2 were retrospective and prospective. Eight of the studies evaluated the sperm DNA damage in raw semen samples: one study (43) used testicular spermatozoa, and seven studies reported DNA damage in prepared semen samples. One of the studies

FIGURE 1



Flow chart showing study selection process.

Zhao. Effect of sperm DNA fragmentation on ART outcome. Fertil Steril 2014.

evaluated couples with a history of multiple IVF failures (13). Five of these studies used the sperm chromatin structure assay (SCSA) to evaluate the sperm DNA damage; seven studies used the TUNEL assay, two studies the Comet assay, and two used acridine orange. The threshold for defining high DNA damage varied between 10% and 50% of the sperm population.

Meta-analysis

Sixteen studies were included in our meta-analysis to evaluate the effect of sperm DNA damage on pregnancy rate after ART. We found a significant decrease in pregnancy in patients with high DNA damage compared with those with low DNA damage. There was moderate statistical heterogeneity in the results, although not significant at $P<.1$ ($I^2 = 30\%$, $P=.12$). The random-effects model combined odds ratio (OR) was 0.81 (95% CI 0.70–0.95; $P=.008$) (Fig. 2).

Of these studies, 14 also evaluated the effect of sperm DNA damage on miscarriage rate. The result of the meta-analysis indicated a significantly increased miscarriage rate in patients with high sperm DNA damage. The Q statistic P value was below .1, indicating heterogeneity of the studies ($I^2 = 44\%$, $P=.04$). The random-effects model was implied, and the combined OR was 2.28 (95% CI 1.55–3.35; $P<.0001$) (Fig. 3).

When we evaluated the effect of sperm DNA damage on pregnancy rate after IVF, nine studies were included. We found a significant decrease in pregnancy in patients with high DNA damage compared with those with low DNA damage. There was good statistical heterogeneity in the results, although not significant at $P<.1$ ($I^2 = 10\%$, $P=.36$). The random-effects model combined OR was 0.66 (95% CI 0.48–0.90; $P=.008$) (Supplemental Fig. 1, available online). Ten studies were used to analyze this effect in ICSI cycles. We did not find significant decrease in pregnancy in patients with high DNA damage compared with those with low DNA damage. The Q statistic P value was $<.1$, indicating heterogeneity of the studies ($I^2 = 45\%$, $P=.06$). The random-effects model combined OR was 0.94 (95% CI 0.70–1.25; $P=.65$) (Supplemental Fig. 2, available online).

We also evaluated the effect of sperm DNA damage on miscarriage rate in IVF or ICSI cycles. Eight studies and seven studies were included in IVF and ICSI cycles, respectively. The result indicated a significantly increased miscarriage rate with high sperm DNA damage after ICSI (OR 2.68; 95% CI 1.40–5.14; $P=.003$), and there was good statistical heterogeneity in the results ($I^2 = 10\%$, $P=.36$) (Supplemental Fig. 3,

TABLE 1

Characteristics of studies of the effect of sperm DNA damage on pregnancy and miscarriage after IVF/ICSI.

Study year	First author (reference)	Type of study	Treatment	Assay	Threshold (%)	High DNA damage T	High DNA damage P	High DNA damage M	Low DNA damage T	Low DNA damage P	Low DNA damage M
2002	Morris (24)	Pro	IVF/ICSI	Comet assay	N	31	9	3	22	6	0
2004	Gandini (34)	Pro	IVF/ICSI	SCSA	27	10	5	0	24	7	0
2004	Virro (12)	Pro and Retro	IVF/ICSI	SCSA	30	57	16	N	107	50	N
2005	Check (13)	Pro	ICSI	SCSA	30	29	8	5	77	26	11
2005	Greco (43)	Pro	ICSI	TUNEL	15	18	1	1	18	8	0
2005	Zini (37)	Pro	IVF/ICSI	Acridine orange	30	11	6	2	49	25	3
2006	Borini (39)	Pro	IVF/ICSI	TUNEL	10	43	5	3	89	25	2
2006	Boe-Hansen (70)	Pro	IUI/IVF/ICSI	SCSA	27	25	7	N	161	46	N
2007	Ozmen (18)	Pro	ICSI	TUNEL	10	8	1	1	34	10	3
2007	Benchaib (14)	Pro	IVF/ICSI	TUNEL	15	44	14	5	258	92	7
2007	Bungum (15)	Pro	IUI/IVF/ICSI	TUNEL	30	201	55	14	797	242	55
2008	Frydman (16)	Pro	IVF	TUNEL	35	52	20	7	65	40	4
2008	Lin (17)	Pro and Retro	IVF/ICSI	SCSA	27	43	22	6	180	93	9
2011	Esbert (71)	Pro	IVF/ICSI	TUNEL	36	26	11	5	135	76	8
2013	Simon (19)	Pro	IVF/ICSI	Comet assay	50	192	37	5	147	49	8
2013	Dar (20)	Pro	ICSI	Acridine orange	50	39	19	7	114	53	13

Note: T = total number; P = pregnancy; M = miscarriage; Pro = prospective cohort study; Retro = retrospective cohort study; N = not mentioned.

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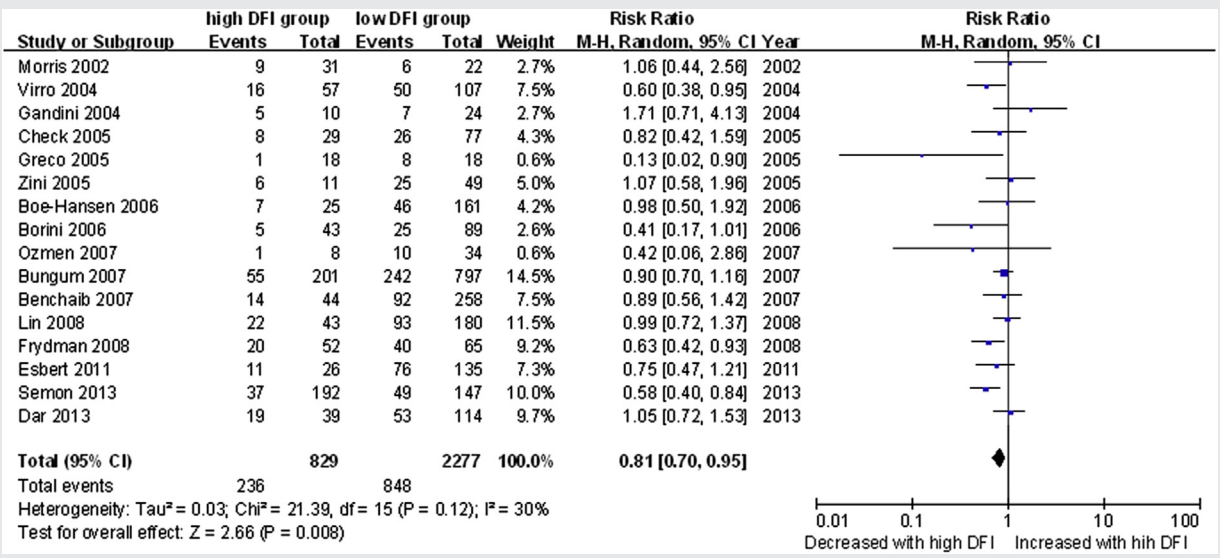
available online). No significant effect of high sperm DNA damage on miscarriage rate was found after IVF (OR 1.84; 95% CI 0.98–3.46; P=.06), with good statistical heterogeneity (I^2 = 39%, P=.15) (Supplemental Fig. 4, available online).

In addition, we presented stratified results by the technique used to assess sperm DNA integrity separately. Because the studies using Comet and acridine orange were too small (n = 2), we analyzed TUNEL and SCSA and combined the data of Comet and acridine orange as a third group. When evaluating the effect of sperm DNA fragmentation on pregnancy, seven, five, and four studies were included in the three groups, respectively. The result indicated a significantly decreased pregnancy rate with high sperm DNA damage with TUNEL (OR 0.74; 95% CI 0.58–0.94; P=.01), and there was no significant association between pregnancy rate with sperm DNA fragmentation when tested with SCSA (OR 0.89; 95% CI 0.67–1.19; P=.44) and Comet assay and acridine orange (OR 0.87; 95% CI 0.60–1.25; P=.44) (Supplemental Fig. 5, available online). There were seven, three, and four studies with TUNEL, SCSA, and Comet assay and acridine

FIGURE 2



Forest plot showing the results of meta-analysis of studies comparing the effect of high sperm DNA damage and low sperm DNA damage on pregnancy after IVF/ICIS.

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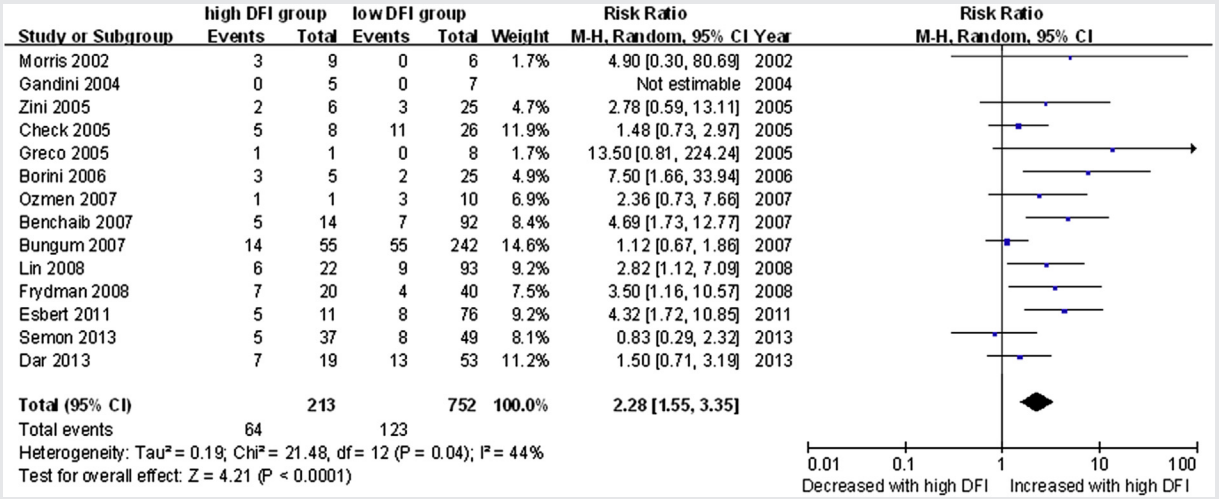
FIGURE 3



Forest plot showing the results of meta-analysis of studies comparing the effect of high sperm DNA damage and low sperm DNA damage on miscarriage after IVF/ICSI.

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orange, respectively, that were included to assess the effect of sperm DNA fragmentation on miscarriage. We found a significantly increased miscarriage rate with high sperm DNA damage with TUNEL (OR 3.23; 95% CI 1.67–6.27; $P=.0005$) and SCSA (OR 1.90; 95% CI 1.01–3.59; $P=.05$), and we did not find significant association between miscarriage rate with sperm DNA fragmentation when tested with Comet assay and acridine orange (OR 1.43; 95% CI 0.82–2.50; $P=.20$). (Supplemental Fig. 6, available online).

The studies scored well on the Newcastle–Ottawa Quality Assessment Scale (not shown). The funnel plot of meta-analysis evaluating the effect of sperm DNA damage on pregnancy rate suggests a lack of publication bias, owing to its symmetrical shape, although a small study may have been missed (Supplemental Fig. 7, available online). However, the studies showed modest publication bias when assessing the effect of sperm DNA damage on miscarriage rate (Supplemental Fig. 8, available online).

DISCUSSION

To date only two reviews (9, 41) have evaluated the association between sperm DNA damage and miscarriage with or without assisted reproduction, and the present study is to our knowledge the largest in regard to sample size, with 3,106 IVF/ICSI cycles. In the present systematic review, 16 studies and 14 studies were included to evaluate the effect of sperm DNA damage on pregnancy and miscarriage, respectively. Sperm DNA damage was statistically significantly associated with pregnancy (combined RR 0.81; 95% CI 0.70–0.95; $P=.008$) and miscarriage (combined RR 2.28; 95% CI 1.55–3.35; $P<.0001$). The two RR values demonstrated that a high frequency of sperm with elevated DNA damage (sperm DNA damage above the cutoff point) is associated with a decreased chance of pregnancy and increased chance of miscarriage. The conclusion was in agreement with the systematic reviews by Zini et al. (9) and Robinson et al. (41), but we included an additional several publications and evaluated not only miscarriage but also pregnancy after IVF/ICSI.

In the present study we also analyzed the effect of sperm DNA damage on outcome after ICSI cycles or IVF cycles. The results showed a significant decrease in pregnancy with high DNA damage after IVF cycles (OR 0.66; 95% CI 0.48–0.90; $P=.008$), whereas there was no such effect on pregnancy after ICSI cycles (OR 0.94; 95% CI 0.70–1.25; $P=.65$). When evaluating the effect on miscarriage, there was significantly increased miscarriage with high DNA damage after ICSI cycles (OR 2.68; 95% CI 1.40–5.14; $P=.003$), whereas there was no such effect after IVF cycles (OR 1.84; 95% CI 0.98–3.46; $P=.06$). Our results were consistent with the research by Sun et al. (44), who believe sperm DNA damage may be most strongly correlated with abnormal morphology and second with abnormal sperm motility. With ICSI, sperm with normal morphology are selected for injection, which theoretically should reduce the impact of DNA damage on the fertilized zygote, and clinical results should be better than for IVF samples with high DNA damage with random fertilization.

In addition, we evaluated the effect of sperm DNA damage on outcome with different techniques testing sperm DNA integrity. We analyzed TUNEL and SCSA and combined the Comet and acridine orange results, because the studies using Comet and acridine orange were small. The three subgroups showed that sperm DNA fragmentation has significant effect on pregnancy with TUNEL, whereas there was no such effect with SCSA and with combining Comet and acridine orange. When analyzing the association between sperm DNA fragmentation and miscarriage, the subgroups all showed that sperm DNA fragmentation has a significant effect on miscarriage. Such contradiction is probably due to differences

in methodology (between and within DNA tests), sensitivity of tests or specific types of DNA damage measured by each test, and patient groups. Although these tests are methodologically different, they both provide a direct evaluation of DNA damage.

A strength of systematic reviews is the improved precision of the summary RR estimates compared with the individual studies. The combined estimate in these studies indicated that sperm DNA damage has an effect on pregnancy and miscarriage after IVF/ICSI/IUI. Evaluating the effect on pregnancy, there were four studies in which the RRs were above 1 slightly, and the combined RR was below 1 (95% CI 0.70–0.95). Evaluating the effect on miscarriage, the RRs of the nine studies were greater than unity, although the number of miscarriages in some studies was small. On the other hand, a weakness of this meta-analysis is the highly variable study characteristics: different sperm DNA damage test assays, different treatment types (IVF/ICSI/IUI), and different thresholds for DNA damage were used. Female inclusion/exclusion criteria and the definition of pregnancy loss were not always clearly stated or varied.

Many studies did not use a clinically relevant cutoff level but selected the cutoff on the basis of [1] a previously reported cutoff (17, 39), [2] the median value for the study population (16), or [3] receiver operating characteristic curves (14). Thus, it may be unreasonable to exclude studies that did not use a clinically relevant cutoff level.

There was no difference in the effect of high DNA damage on miscarriage with different fertility treatments (41). However, paternal age may have a link with miscarriage. Older men ($\geq$40 years) have more double-strand DNA breaks, and sperm are incapable of DNA repair and rely on the oocyte for repair after fertilization (45). It is acknowledged that oocyte quality is strongly attributed to female age, and the innate capacity to repair sperm DNA damage may be weaker in eggs from older women ($\geq$35 years). Besides, there are a number of assays that are used to analyze DNA damage, and it is worth noting that the reported percentages of sperm with DNA damage have varied meanings between different techniques (9, 41).

The finding of an association between sperm DNA damage and clinical outcome of IVF/ICSI is consistent with the results reported in other otherwise eligible studies. Indeed, an association between sperm DNA damage and pregnancy/miscarriage has been observed in non-IVF studies. A study by Carrell et al. (29) reported that recurrent miscarriage is related to higher levels of sperm DNA damage, and Evenson et al. (46) observed that pregnancy loss rate increased significantly with sperm DNA damage. Animal studies (47–49) regarding the possible mechanism underling the association between sperm DNA damage and miscarriage showed that sperm DNA damage can lead to abnormal embryo development and impaired embryo implantation.

When interpreting these pregnancy outcome data, it is important to understand that in any population of sperm the DNA damage levels per cell are heterogeneous. Therefore, if the fertilizing sperm is randomly picked naturally or by ICSI, the detailed frequency distribution of damage levels in the population will be what affects the pregnancy outcome. One group suggests that paternal effects on early development, before the activation of the embryonic genome, are mediated by centrosome dysfunction or deficiency of oocyte-activating factors and are not associated with high frequency of sperm with DNA damage. However, increased sperm DNA damage has been associated with a "late paternal effect" during the activation of male gene expression and hence could give rise to an increased risk of miscarriage (31).

Damage to DNA in sperm can be induced by many mechanisms (50), and oxidative stress is a primary cause for DNA damage in spermatozoa (51–54). Reactive oxygen species (ROS) are principally produced by leucocytes and sperm cytoplasm (55). The spermatozoa that reflect such stress most profoundly are those morphologically abnormal cells, and they will produce less ROS than immature sperm because the latter contain more cytoplasm. Normally the amount of ROS produced is counterbalanced by endogenous antioxidant activity, but if this balance is impaired then extensive DNA damage can occur. Subfertile men seem to have lower levels of antioxidative activity than fertile men (56–58).

It is important to develop strategies that reduce sperm DNA damage in humans. Antioxidants (such as vitamins C and E, folate, zinc, selenium, carntine, and carotenoids) are scavengers of ROS, and they have been proposed as a treatment to reverse the adverse impact of high ROS concentrations on semen parameters (59–62). However, some caution should be used when using antioxidants: one study reported a >20% increase in sperm decondensation (63), and excessive levels of antioxidants can be harmful (64, 65). In addition to antioxidants, eliminating exposure to environmental toxins and reducing testicular hyperthermia may help optimize sperm DNA integrity (66, 67). Varicocele repair may also reduce sperm DNA damage, particularly in men with high levels of baseline sperm DNA damage (37, 38, 68).

To conclude, the findings of this systematic review demonstrate that sperm DNA damage has a detrimental effect on the clinical outcome (pregnancy/miscarriage) after IVF/ICSI. Although the number of events in some studies is relatively small and the study characteristics are variable, tests for DNA damage and selection of undamaged sperm should be considered as part of the diagnostic and treatment pathways for those with recurrent pregnancy failure. Selecting ICSI sperm by maturation markers such as hyaluronic acid or other zona pellucida receptors, and/or by novel noninvasive imaging techniques, could help to prevent fertilization by DNA-damaged sperm (69). Further research is required into the mechanisms responsible for and preventing DNA damage, including antioxidant therapy.

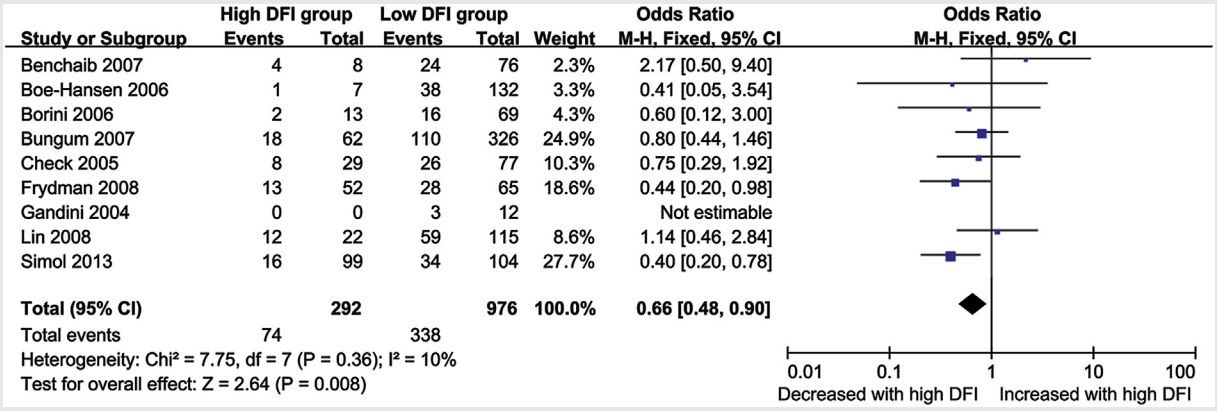
REFERENCES

1. Hull MG, Glazener CM, Kelly NJ, Conway DI, Foster PA, Hinton RA, et al. Population study of causes, treatment, and outcome of infertility. Br Med J (Clin Res Ed) 1985;291:1693–7.
2. Chung CC, Fleming R, Jamieson ME, Yates RW, Coutts JR. Randomized comparison of ovulation induction with and without intrauterine insemination in the treatment of unexplained infertility. Hum Reprod 1995;10:3139–41.
3. Simon L, Lutton D, McManus J, Lewis SE. Sperm DNA damage measured by the alkaline Comet assay as an independent predictor of male infertility and in vitro fertilization success. Fertil Steril 2011;95:652–7.

4. Simon L, Lewis SE. Sperm DNA damage or progressive motility: which one is the better predictor of fertilization in vitro? Syst Biol Reprod Med 2011;57:133–8.
5. Giwercman A, Richthoff J, Hjollund H, Bonde JP, Jepson K, Frohm B, et al. Correlation between sperm motility and sperm chromatin structure assay parameters. Fertil Steril 2003;80:1404–12.
6. Lewis SE. Is sperm evaluation useful in predicting human fertility? Reproduction 2007;134:31–40.
7. Aitken RJ, De Iuliis GN. Value of DNA integrity assays for fertility evaluation. Soc Reprod Fertil Suppl 2007;65:81–92.
8. Evenson DP, Kasperson K, Wixon RL. Analysis of sperm DNA fragmentation using flow cytometry and other techniques. Soc Reprod Fertil Suppl 2007;65:93–113.
9. Zini A, Boman JM, Belzile E, Ciampi A. Sperm DNA damage is associated with an increased risk of pregnancy loss after IVF and ICSI: systematic review and meta-analysis. Hum Reprod 2008;23:2663–8.
10. Cohen-Bacrie P, Belloc S, Menezo YJ, Clement P, Hamidi J, Benkhalifa M. Correlation between DNA damage and sperm parameters: a prospective study of 1,633 patients. Fertil Steril 2009;91:1801–5.
11. Bungum M, Humaidan P, Spano M, Jepson K, Bungum L, Giwercman A. The predictive value of sperm chromatin structure assay (SCSA) parameters for the outcome of intrauterine insemination, IVF and ICSI. Hum Reprod 2004;19:1401–8.
12. Virro MR, Larson-Cook KL, Evenson DP. Sperm chromatin structure assay (SCSA) parameters are related to fertilization, blastocyst development, and ongoing pregnancy in in vitro fertilization and intracytoplasmic sperm injection cycles. Fertil Steril 2004;81:1289–95.
13. Check JH, Graziano V, Cohen R, Krotec J, Check ML. Effect of an abnormal sperm chromatin structural assay (SCSA) on pregnancy outcome following (IVF) with ICSI in previous IVF failures. Arch Androl 2005;51:121–4.
14. Benchaib M, Lornage J, Mazoyer C, Lejeune H, Salle B, Francois GJ. Sperm deoxyribonucleic acid fragmentation as a prognostic indicator of assisted reproductive technology outcome. Fertil Steril 2007;87:93–100.
15. Bungum M, Humaidan P, Axmon A, Spano M, Bungum L, Erenpreiss J, et al. Sperm DNA integrity assessment in prediction of assisted reproduction technology outcome. Hum Reprod 2007;22:174–9.
16. Frydman N, Prisant N, Hesters L, Frydman R, Tachdjian G, Cohen-Bacrie P, et al. Adequate ovarian follicular status does not prevent the decrease in pregnancy rates associated with high sperm DNA fragmentation. Fertil Steril 2008;89:92–7.
17. Lin MH, Kuo-Kuang LR, Li SH, Lu CH, Sun FJ, Hwu YM. Sperm chromatin structure assay parameters are not related to fertilization rates, embryo quality, and pregnancy rates in in vitro fertilization and intracytoplasmic sperm injection, but might be related to spontaneous abortion rates. Fertil Steril 2008;90:352–9.
18. Ozmen B, Caglar GS, Koster F, Schopper B, Diedrich K, Al-Hasani S. Relationship between sperm DNA damage, induced acrosome reaction and viability in ICSI patients. Reprod Biomed Online 2007;15:208–14.
19. Simon L, Proutski I, Stevenson M, Jennings D, Mcmanus J, Lutton D, et al. Sperm DNA damage has a negative association with live-birth rates after IVF. Reprod Biomed Online 2013;26:68–78.
20. Dar S, Grover SA, Moskovtsev SI, Swanson S, Baratz A, Librach C. In vitro fertilization- intracytoplasmic sperm injection outcome in patients with a markedly high DNA fragmentation index (>50%). Fertil Steril 2013;100:75–80.
21. Griffin DK, Abruzzo MA, Millie EA, Sheean LA, Feingold E, Sherman SL, et al. Non-disjunction in human sperm: evidence for an effect of increasing paternal age. Hum Mol Genet 1995;4:2227–32.
22. Spano M, Bonde JP, Hjollund HI, Kolstad HA, Cordelli E, Leter G. Sperm chromatin damage impairs human fertility. The Danish First Pregnancy Planner Study Team. Fertil Steril 2000;73:43–50.
23. Sartorelli EM, Mazzucatto LF, de Pina-Neto JM. Effect of paternal age on human sperm chromosomes. Fertil Steril 2001;76:1119–23.
24. Morris ID, Ilott S, Dixon L, Brison DR. The spectrum of DNA damage in human sperm assessed by single cell gel electrophoresis (Comet assay) and its relationship to fertilization and embryo development. Hum Reprod 2002;17:990–8.
25. Carrell DT, Liu L, Peterson CM, Jones KP, Hatasaka HH, Erickson L, et al. Sperm DNA fragmentation is increased in couples with unexplained recurrent pregnancy loss. Arch Androl 2003;49:49–55.
26. Twigg JP, Irvine DS, Aitken RJ. Oxidative damage to DNA in human spermatozoa does not preclude pronucleus formation at intracytoplasmic sperm injection. Hum Reprod 1998;13:1864–71.
27. Ahmadi A, Ng SC. Developmental capacity of damaged spermatozoa. Hum Reprod 1999;14:2279–85.
28. Tomlinson MJ, Moffatt O, Manicardi GC, Bizzaro D, Afnan M, Sakkas D. Interrelationships between seminal parameters and sperm nuclear DNA damage before and after density gradient centrifugation: implications for assisted conception. Hum Reprod 2001;16:2160–5.
29. Carrell DT, Wilcox AL, Lowy L, Peterson CM, Jones KP, Erickson L, et al. Elevated sperm chromosome aneuploidy and apoptosis in patients with unexplained recurrent pregnancy loss. Obstet Gynecol 2003;101:1229–35.
30. Henkel R, Hajimohammad M, Stalf T, Hoogendijk C, Mehnert C, Menkveld R, et al. Influence of deoxyribonucleic acid damage on fertilization and pregnancy. Fertil Steril 2004;81:965–72.
31. Tesarik J, Greco E, Mendoza C. Late, but not early, paternal effect on human embryo development is related to sperm DNA fragmentation. Hum Reprod 2004;19:611–5.
32. Aitken RJ, Gordon E, Harkiss D, Twigg JP, Milne P, Jennings Z, et al. Relative impact of oxidative stress on the functional competence and genomic integrity of human spermatozoa. Biol Reprod 1998;59:1037–46.
33. Lopes S, Sun JG, Jurisicova A, Meriano J, Casper RF. Sperm deoxyribonucleic acid fragmentation is increased in poor-quality semen samples and correlates with failed fertilization in intracytoplasmic sperm injection. Fertil Steril 1998;69:528–32.
34. Gandini L, Lombardo F, Paoli D, Caruso F, Eleuteri P, Leter G, et al. Full-term pregnancies achieved with ICSI despite high levels of sperm chromatin damage. Hum Reprod 2004;19:1409–17.
35. Henkel R, Kierspel E, Hajimohammad M, Stalf T, Hoogendijk C, Mehnert C, et al. DNA fragmentation of spermatozoa and assisted reproduction technology. Reprod Biomed Online 2003;7:477–84.
36. Larson-Cook KL, Brannian JD, Hansen KA, Kasperson KM, Aamold ET, Evenson DP. Relationship between the outcomes of assisted reproductive techniques and sperm DNA fragmentation as measured by the sperm chromatin structure assay. Fertil Steril 2003;80:895–902.
37. Zini A, Blumenfeld A, Libman J, Willis J. Beneficial effect of microsurgical varicocelectomy on human sperm DNA integrity. Hum Reprod 2005;20:1018–21.
38. Zini A, Meriano J, Kader K, Jarvi K, Laskin CA, Cadesky K. Potential adverse effect of sperm DNA damage on embryo quality after ICSI. Hum Reprod 2005;20:3476–80.
39. Borini A, Tarozzi N, Bizzaro D, Bonu MA, Fava L, Flamigni C, et al. Sperm DNA fragmentation: paternal effect on early post-implantation embryo development in ART. Hum Reprod 2006;21:2876–81.
40. Collins JA, Barnhart KT, Schlegel PN. Do sperm DNA integrity tests predict pregnancy with in vitro fertilization? Fertil Steril 2008;89:823–31.
41. Robinson L, Gallos ID, Conner SJ, Rajkhowa M, Miller D, Lewis S, et al. The effect of sperm DNA fragmentation on miscarriage rates: a systematic review and meta-analysis. Hum Reprod 2012;27:2908–17.
42. Wells G, Shea B, O' Connell D. The Newcastle-Ottawa Scale (NOS) for assessing the quality of non-randomised studies in meta-analysis. In: Proceedings or the Third Symposium on Systematic Reviews Beyond the Basics. Improving Quality and Impact. July 3–5, 2000; Oxford, United Kingdom: The Ottawa Health Research Institute; 2000.
43. Greco E, Scarselli F, Iacobelli M, Rienzi L, Ubaldi F, Ferrero S, et al. Efficient treatment of infertility due to sperm DNA damage by ICSI with testicular spermatozoa. Hum Reprod 2005;20:226–30.
44. Sun JG, Jurisicova A, Casper RF. Detection of deoxyribonucleic acid fragmentation in human sperm: correlation with fertilization in vitro. Biol Reprod 1997;56:602–7.
45. de la Rochebrochard E, Thonneau P. Paternal age and maternal age are risk factors for miscarriage; results of a multicentre European study. Hum Reprod 2002;17:1649–56.

46. Evenson DP, Jost LK, Marshall D, Zinaman MJ, Clegg E, Purvis K, et al. Utility of the sperm chromatin structure assay as a diagnostic and prognostic tool in the human fertility clinic. Hum Reprod 1999;14:1039–49.
47. Ahmadi A, Ng SC. Fertilizing ability of DNA-damaged spermatozoa. J Exp Zool 1999;284:696–704.
48. Fatehi AN, Bevers MM, Schoevers E, Roelen BA, Colenbrander B, Gadella BM. DNA damage in bovine sperm does not block fertilization and early embryonic development but induces apoptosis after the first cleavages. J Androl 2006;27:176–88.
49. Perez-Crespo M, Moreira P, Pintado B, Gutierrez-Adan A. Factors from damaged sperm affect its DNA integrity and its ability to promote embryo implantation in mice. J Androl 2008;29:47–54.
50. Sakkas D, Alvarez JG. Sperm DNA fragmentation: mechanisms of origin, impact on reproductive outcome, and analysis. Fertil Steril 2010;93:1027–36.
51. Lewis SE, Agbaje IM. Using the alkaline comet assay in prognostic tests for male infertility and assisted reproductive technology outcomes. Mutagenesis 2008;23:163–70.
52. Tremellen K. Oxidative stress and male infertility—a clinical perspective. Hum Reprod Update 2008;14:243–58.
53. Agarwal A, Sharma RK, Desai NR, Prabakaran S, Tavares A, Sabanegh E. Role of oxidative stress in pathogenesis of varicocele and infertility. Urology 2009;73:461–9.
54. Kefer JC, Agarwal A, Sabanegh E. Role of antioxidants in the treatment of male infertility. Int J Urol 2009;16:449–57.
55. Aitken RJ, Harkiss D, Knox W, Paterson M, Irvine DS. A novel signal transduction cascade in capacitating human spermatozoa characterized by a redox-regulated, cAMP-mediated induction of tyrosine phosphorylation. J Cell Sci 1998;111(Pt 5):645–56.
56. Fraga CG, Motchnik PA, Wyrobek AJ, Rempel DM, Ames BN. Smoking and low antioxidant levels increase oxidative damage to sperm DNA. Mutat Res 1996;351:199–203.
57. Lewis SE, Sterling ES, Young IS, Thompson W. Comparison of individual antioxidants of sperm and seminal plasma in fertile and infertile men. Fertil Steril 1997;67:142–7.
58. Tremellen K, Miari G, Froiland D, Thompson J. A randomised control trial examining the effect of an antioxidant (Menevit) on pregnancy outcome during IVF-ICSI treatment. Aust N Z J Obstet Gynaecol 2007;47:216–21.
59. Ross C, Morriss A, Khairy M, Khalaf Y, Braude P, Coomarasamy A, et al. A systematic review of the effect of oral antioxidants on male infertility. Reprod Biomed Online 2010;20:711–23.
60. Showell MG, Brown J, Yazdani A, Stankiewicz MT, Hart RJ. Antioxidants for male subfertility. Cochrane Database Syst Rev 2011:CD007411.
61. Pang SC, Chan PJ, Lu A. Effects of pentoxifylline on sperm motility and hyperactivation in normozoospermic and normokinetic semen. Fertil Steril 1993;60:336–43.
62. Oeda T, Henkel R, Ohmori H, Schill WB. Scavenging effect of N-acetyl-L-cysteine against reactive oxygen species in human semen: a possible therapeutic modality for male factor infertility? Andrologia 1997;29:125–31.
63. Menezo YJ, Hazout A, Panteix G, Robert F, Rollet J, Cohen-Bacrie P, et al. Antioxidants to reduce sperm DNA fragmentation: an unexpected adverse effect. Reprod Biomed Online 2007;14:418–21.
64. Pintauro SJ, Bergan JG. Effects of ascorbic acid on in vitro steroidogenesis in guinea pigs. J Nutr 1982;112:584–91.
65. Donnelly ET, McClure N, Lewis SE. Antioxidant supplementation in vitro does not improve human sperm motility. Fertil Steril 1999;72:484–95.
66. Fraga CG, Motchnik PA, Shigenaga MK, Helbock HJ, Jacob RA, Ames BN. Ascorbic acid protects against endogenous oxidative DNA damage in human sperm. Proc Natl Acad Sci U S A 1991;88:11003–6.
67. Evenson D, Jost L. Sperm chromatin structure assay is useful for fertility assessment. Methods Cell Sci 2000;22:169–89.
68. Werthman P, Wixon R, Kasperson K, Evenson DP. Significant decrease in sperm deoxyribonucleic acid fragmentation after varicocelectomy. Fertil Steril 2008;90:1800–4.
69. Parmegiani L, Cognigni GE, Filicori M. Sperm selection: effect on sperm DNA quality. Adv Exp Med Biol 2014;791:151–72.
70. Boe-Hansen GB, Fedder J, Ersbøll AK, Christensen P. The sperm chromatin structure assay as a diagnostic tool in the human fertility clinic. Hum Reprod 2006;21:1576–82.
71. Esbert M, Pacheco A, Vidal F, Florensa M, Riqueros M, Ballesteros A, Garrido N, Caldero'n G. Impact of sperm DNA fragmentation on the outcome of IVF with own or donated oocytes. Reprod BioMed Online 2011;23:704–10.

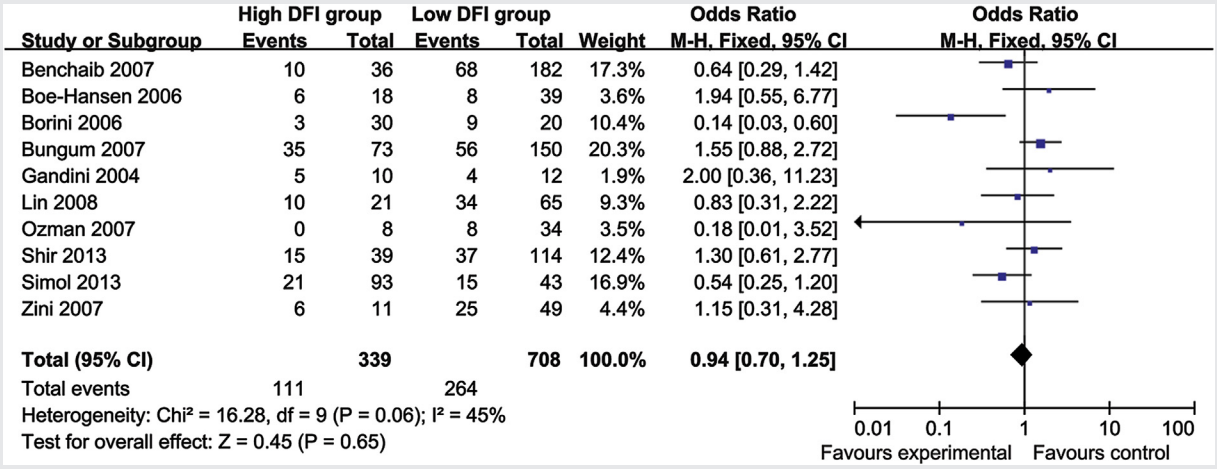
SUPPLEMENTAL FIGURE 1



Forest plot showing the results of meta-analysis of studies comparing the effect of high sperm DNA damage and low sperm DNA damage on pregnancy after IVF.

Zhao. Effect of sperm DNA fragmentation on ART outcome. Fertil Steril 2014.

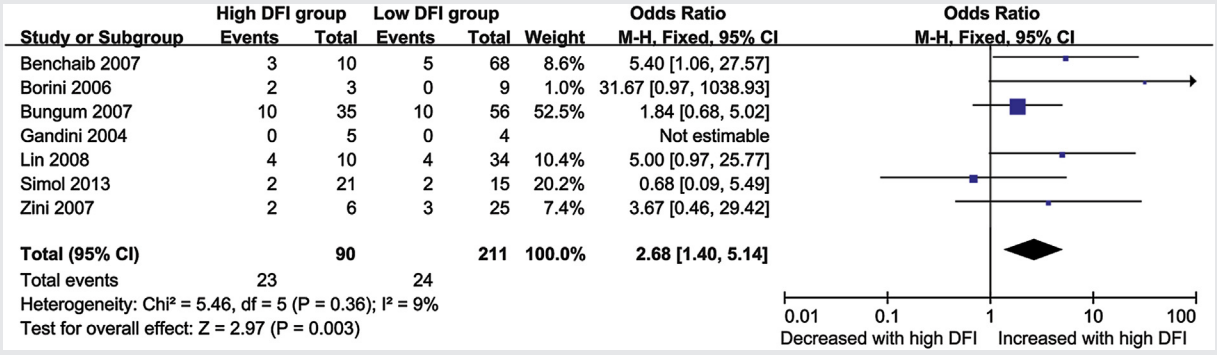
SUPPLEMENTAL FIGURE 2



Forest plot showing the results of meta-analysis of studies comparing the effect of high sperm DNA damage and low sperm DNA damage on pregnancy after ICSI.

Zhao. Effect of sperm DNA fragmentation on ART outcome. Fertil Steril 2014.

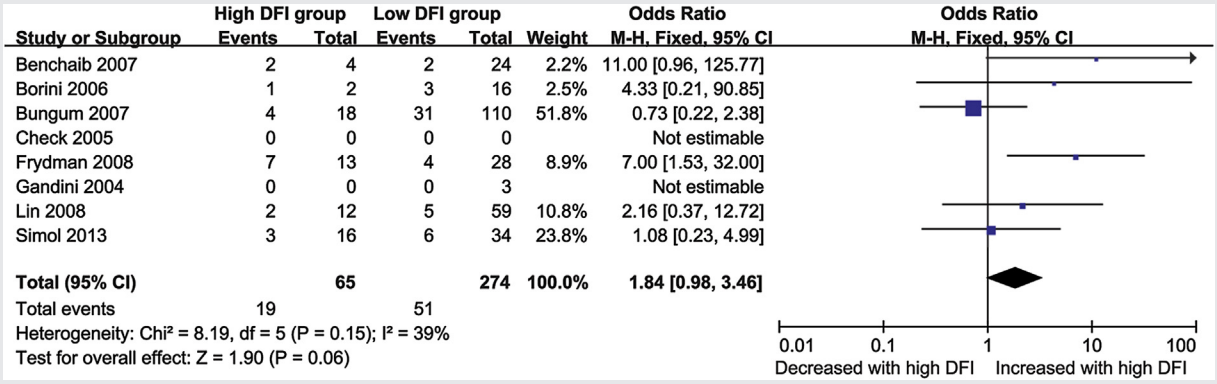
SUPPLEMENTAL FIGURE 3



Forest plot showing the results of meta-analysis of studies comparing the effect of high sperm DNA damage and low sperm DNA damage on miscarriage after ICSI.

Zhao. Effect of sperm DNA fragmentation on ART outcome. Fertil Steril 2014.

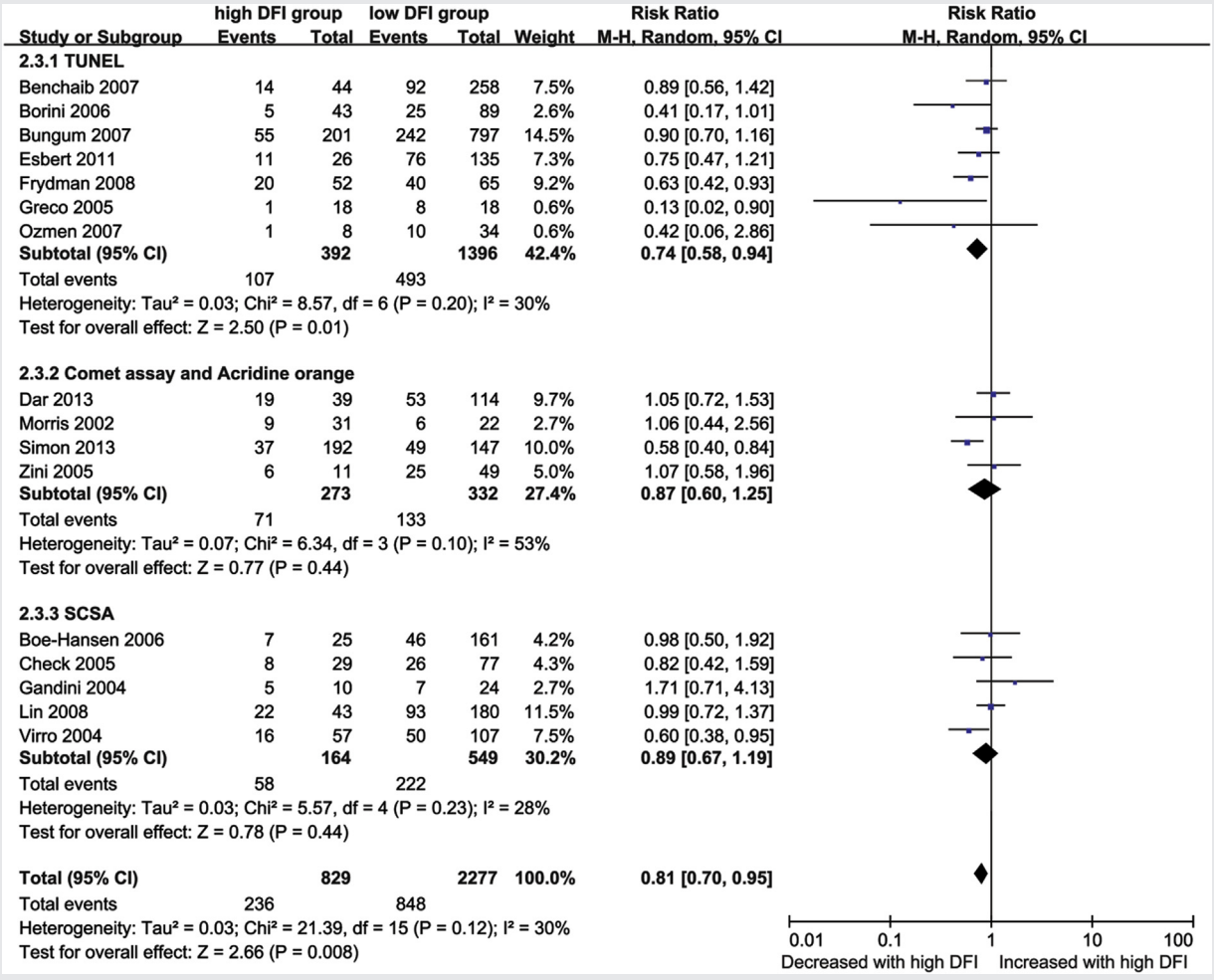
SUPPLEMENTAL FIGURE 4



Forest plot showing the results of meta-analysis of studies comparing the effect of high sperm DNA damage and low sperm DNA damage on miscarriage after IVF.

Zhao. Effect of sperm DNA fragmentation on ART outcome. Fertil Steril 2014.

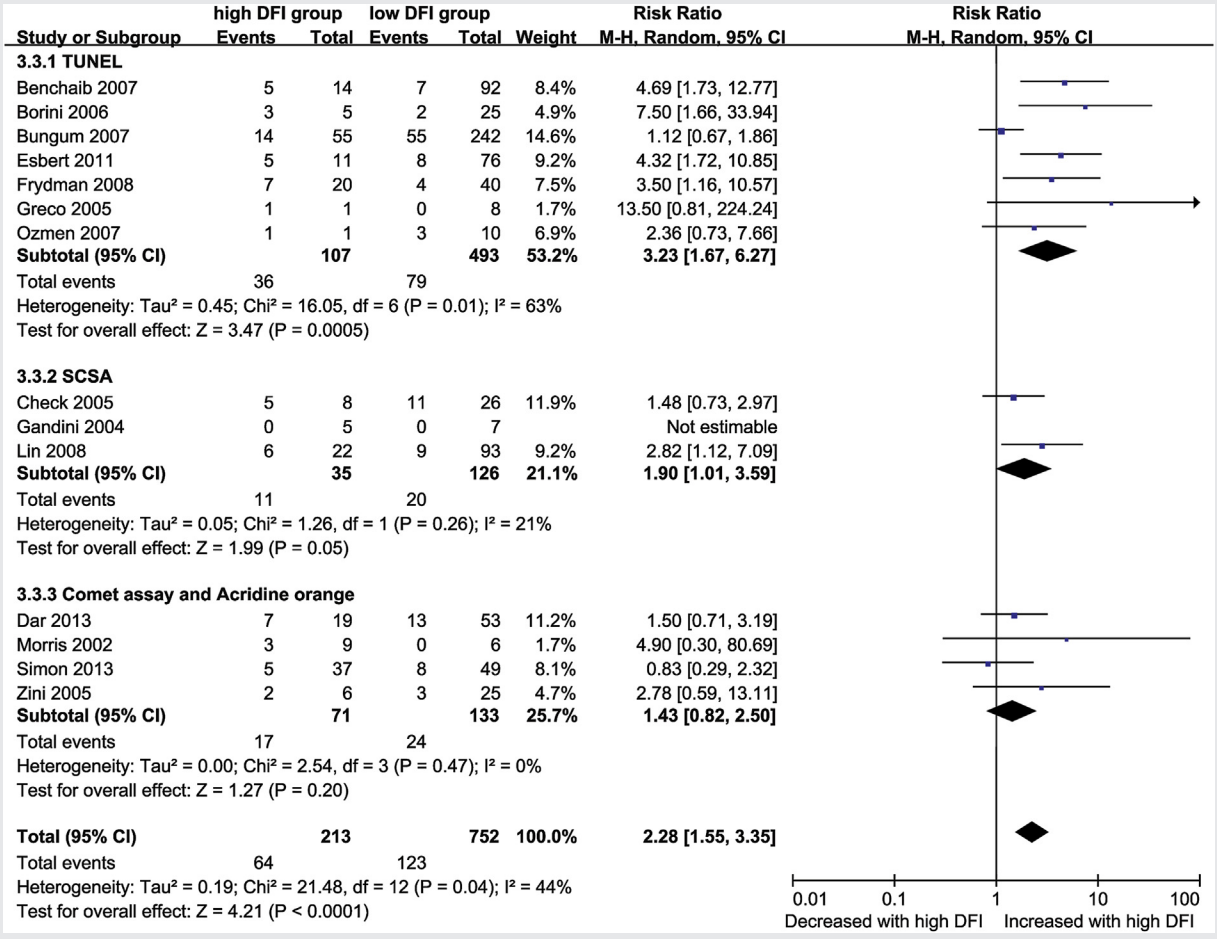
SUPPLEMENTAL FIGURE 5



Forest plot showing the results of meta-analysis of studies comparing the effect of sperm DNA damage on pregnancy, with different types of techniques testing sperm DNA fragmentation.

Zhao. Effect of sperm DNA fragmentation on ART outcome. Fertil Steril 2014.

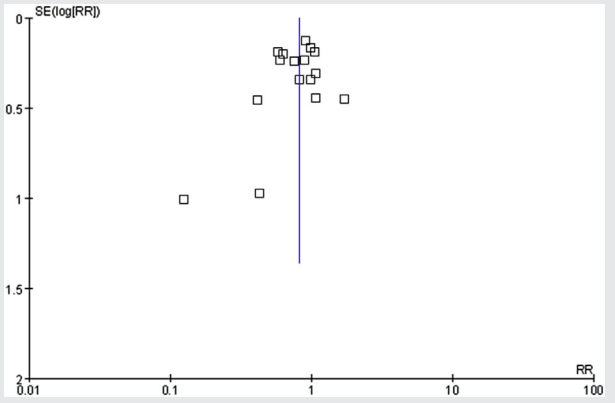
SUPPLEMENTAL FIGURE 6



Forest plot showing the results of meta-analysis of studies comparing the effect of sperm DNA damage on miscarriage, with different types of techniques testing sperm DNA fragmentation.

Zhao. Effect of sperm DNA fragmentation on ART outcome. Fertil Steril 2014.

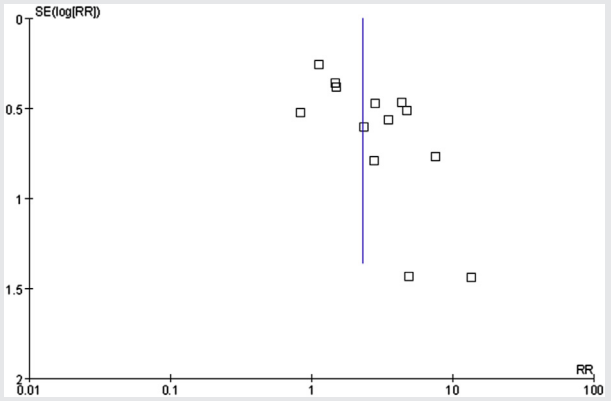
SUPPLEMENTAL FIGURE 7



Funnel plot of analysis for the effect of sperm DNA damage on pregnancy, showing the results of Eggers to assess publication bias.

Zhao. Effect of sperm DNA fragmentation on ART outcome. Fertil Steril 2014.

SUPPLEMENTAL FIGURE 8



Funnel plot of analysis for the effect of sperm DNA damage on miscarriage, showing the results of Eggers to assess publication bias.

Zhao. Effect of sperm DNA fragmentation on ART outcome. Fertil Steril 2014.