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META-ANALYSIS ***Andrology***

The effect of sperm DNA fragmentation on miscarriage rates: a systematic review and meta-analysis

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STUDY QUESTION: Is there an association between high levels of sperm DNA damage and miscarriage?

SUMMARY ANSWER: Miscarriage rates are positively correlated with sperm DNA damage levels.

WHAT IS KNOWN ALREADY: Most ejaculates contain a subpopulation of sperm with DNA damage, also referred to as DNA fragmentation, in the form of double or single-strand breaks which have been induced in the DNA prior to or following ejaculation. This DNA damage may be particularly elevated in some subfertile men, hence several studies have examined the link between sperm DNA damage levels and conception and miscarriage rates.

STUDY DESIGN, SIZE, DURATION: A systematic review and meta-analysis of studies which examined the effect of sperm DNA damage on miscarriage rates was performed. Searches were conducted on MEDLINE, EMBASE and the Cochrane Library without any language restrictions from database inception to January 2012.

PARTICIPANTS/MATERIALS, SETTING, METHODS: We used the terms 'DNA damage' or 'DNA fragmentation' combined with 'miscarriage', 'abortion' or 'pregnancy' to generate a set of relevant citations. Data extraction was performed by two reviewers. Study quality was assessed using the Newcastle–Ottawa Scale. Meta-analysis of relative risks of miscarriage was performed with a random effects model. Subgroup analyses were performed by the type of DNA damage test, whether the sperm examined were prepared or from raw semen and for pregnancies resulting from IVF or ICSI treatment.

MAIN RESULTS AND THE ROLE OF CHANCE: We identified 16 cohort studies (2969 couples), 14 of which were prospective. Eight studies used acridine orange-based assays, six the TUNEL assay and two the COMET assay. Meta-analysis showed a significant increase in miscarriage in patients with high DNA damage compared with those with low DNA damage [risk ratio (RR) = 2.16 (1.54, 3.03), $P < 0.00001$)]. A subgroup analysis showed that the miscarriage association is strongest for the TUNEL assay (RR = 3.94 (2.45, 6.32), $P < 0.00001$).

LIMITATIONS, REASONS FOR CAUTION: There is some variation in study characteristics, including the use of different assays and different thresholds for DNA damage and the definition of pregnancy loss.

WIDER IMPLICATIONS OF THE FINDINGS: The use of methods which select sperm without DNA damage for use in assisted conception treatment may reduce the risk of miscarriage. This finding indicates that assays detecting DNA damage could be considered in those suffering from recurrent pregnancy loss. Further research is necessary to study the mechanisms of DNA damage and the potential therapeutic effects of antioxidant therapy.

STUDY FUNDING/COMPETING INTEREST(S): None.

Key words: spermatozoa / DNA fragmentation / miscarriage / pregnancy loss / male infertility

Introduction

Spontaneous miscarriage occurs in ~10–15% of clinical pregnancies in the normal fertile population but the rate is known to be higher in subfertile couples (Hamamah *et al*., 1997). Sperm DNA integrity is one of the important determinants of normal fertilization and embryo development. However, sperm with DNA damage are capable of fertilizing an egg (Aitken *et al*., 1998a; Lopes *et al*., 1998; Gandini *et al*., 2004), which may explain why studies evaluating the relationship between high DNA damage and pregnancy rates have only found a modest effect on conception rates with conventional IVF and little, if any effect with ICSI (Henkel *et al*., 2003; Larson-Cook *et al*., 2003; Gandini *et al*., 2004; Virro *et al*., 2004; Check *et al*., 2005; Zini *et al*., 2005a,b; Borini *et al*., 2006; Benchaib *et al*., 2007; Bungum *et al*., 2007; Collins *et al*., 2008; Frydman *et al*., 2008; Lin *et al*., 2008). For the purposes of this paper we define sperm DNA damage as fragmentation of the sperm DNA, in the form of double or single-strand breaks which have been induced in the DNA prior to, or post, ejaculation.

One group suggests that paternal effects on early development, prior to 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 (Tesarik *et al*., 2004a).

In contrast, other studies report marked reductions in all the major early check points: fertilization, embryo quality and pregnancy rates following IVF in couples with high levels of sperm DNA damage as measured by the Comet assay (Simon *et al*., 2010, 2011, L. Simon et al., Unpublished results).

DNA damage in sperm can be induced by six main mechanisms: (i) apoptosis during spermatogenesis; (ii) strand breaks during chromatin remodelling during spermiogenesis; (iii) post-testicular DNA fragmentation induced by oxygen free radicals during transit through the male reproductive tract; (iv) DNA fragmentation induced by endogenous endonucleases; (v) DNA damage induced by radiotherapy and chemotherapy and (vi) DNA damage induced by environmental factors such as smoking and air pollution (Sakkas and Alvarez, 2010).

There are a number of assays which are used to analyse DNA damage. Some tests measure DNA damage directly, such as TdT-mediated-dUTP nick-end labelling (TUNEL) and COMET at a neutral pH. Other tests are indirect, such as the sperm chromatin structure assay (SCSA), acridine orange test, sperm chromatin dispersion (SCD test) at acid and COMET at alkaline pH. It is worth noting that when used clinically the reported percentages of sperm with DNA damage have varied meanings between different techniques: most methods score a percentage of sperm above a certain detectable damage threshold, not a percentage of DNA damaged within a given cell; the exception to this is the COMET assay which scores the percentage of DNA damage per sperm and returns an average level of damage per sperm for the population. In COMET almost all sperm in even a fertile sperm donor population are observed to have some level of detectable damage (Simon *et al*., 2011). When interpreting this miscarriage data, it is therefore important to understand that in any population of sperm the DNA damage levels per cell are heterogenous. 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.

Several studies have investigated the link between pregnancy loss and high DNA damage in sperm (Bungum *et al*., 2004; Virro *et al*., 2004; Check *et al*., 2005; Benchaib *et al*., 2007; Bungum *et al*., 2007; Frydman *et al*., 2008; Lin *et al*., 2008; Ozmen *et al*., 2007); a review by Zini *et al*. concluded that sperm DNA damage was associated with a significantly increased risk of pregnancy loss after IVF and ICSI (Zini *et al*., 2008). Zini *et al*.'s review identified seven studies, but we have an additional eight studies and have addressed the issue of DNA fragmentation in both spontaneous and assisted conception.

Our aim was to investigate the relationship between frequency of DNA damage in the sperm population and pregnancy loss in both ART and spontaneous conceptions by performing a systematic review and meta-analysis of the available literature.

Methods

Identification of the literature

The following electronic databases were searched: MEDLINE, EMBASE and the Cochrane Library from inception until January 2012. The following Medical Subject and Emtree headings and textword were used to generate two subsets of citations: one including terms on pregnancy loss and reproductive techniques (spontaneous abortion, miscarriage, pregnancy loss, infertility, IUI, IVF, ICSI and terms on sperm and DNA damage (DNA damage, DNA fragmentation, sperm and spermatozoa). These subsets were combined with 'AND' to generate a subset of citations relevant to our research question. The reference list of all recent review and primary articles were examined to identify any articles not captured by our searches. No language restrictions were placed on the searches and the searches were conducted by two independent researchers (L.R. and J.K.B.).

Study selection and data extraction

We selected studies that examined the association between sperm DNA damage levels measured in raw or prepared semen and pregnancy loss. The studies included couples conceiving spontaneously or via assisted conception in the form of IUI, IVF or ICSI. The primary outcome of interest was miscarriage rate.

Studies were selected in a two stage process. Firstly, titles and abstracts of articles from the electronic searches were scrutinized and full manuscripts of all citations that were likely to meet the selection criteria were obtained. These included abstracts which gave pregnancy rates without stating miscarriage rates as these were often found within the text. Secondly, a final decision on inclusion or exclusion of studies was made on examination of the full manuscripts. Any disagreements about inclusion were resolved by consensus or arbitration by a third reviewer (A.C.).

Two reviewers (L.R. and I.G.) completed the quality assessment using the Newcastle–Ottawa Quality Assessment Scales for observational studies (Wells, 2000; Table I). Items assessed included selection of cohorts, comparability of cohorts, assessment of outcomes and follow-up. We used an arbitrary score based on the assumption of equal weight of all items included in the Newcastle–Ottawa Scale. This was used to give a quantitative appraisal of overall quality of the individual studies. The score ranged from 0 to 9, with a score of either 0 or 1 for each item. From each study, outcome data were extracted in 2 × 2 tables.

Table I Characteristics of studies of the effect of high DNA fragmentation versus low DNA fragmentation in sperm on miscarriage rates.

Study	Type of study	Treatment	Assay	Normal range	Outcome measure
Evenson et al. (1999a) ($n = 165$)	Prospective cohort study	Spontaneous conception	SCSA	DFI <15%	Miscarriage rate
Morris et al. (2002) ($n = 60$)	Prospective cohort study	IVF, ICSI	COMET		Miscarriage rate
Gandini et al. (2004) ($n = 34$)	Prospective cohort study	IVF, ICSI	SCSA	DFI <27%	Miscarriage rate
Virro et al. (2004) ($n = 249$)	Retrospective and prospective cohort study	IVF, ICSI	SCSA	DFI <30%	Miscarriage rate
Borini et al. (2006) ($n = 132$)	Prospective cohort study	IVF, ICSI	TUNEL	DFI <10%	Miscarriage rate
Check et al. (2005) ($n = 106$)	Prospective cohort study	ICSI	SCSA	DFI <30%	Miscarriage rate
Greco et al. (2005a) ($n = 18$)	Prospective cohort study	ICSI	TUNEL	DFI <15%	Miscarriage rate
Zini et al. (2005a) ($n = 60$)	Prospective cohort study	ICSI	Acridine orange	DFI <30%	Miscarriage rate
Boe-Hansen et al. (2006) ($n = 234$)	Prospective cohort study	IVF, ICSI	SCSA	DFI <27%	Biochemical pregnancy rate
Benchaib et al. (2007) ($n = 322$)	Prospective cohort study	IVF, ICSI	TUNEL	DFI <15%	Miscarriage rate
Bungum et al. (2007) ($n = 637$)	Prospective cohort study	IUI, IVF, ICSI	SCSA	DFI <30%	Miscarriage rate
Ozmen et al. (2007) ($n = 42$)	Prospective cohort study	ICSI	TUNEL	DFI <10%	Miscarriage rate
Frydman et al. (2008) ($n = 117$)	Prospective cohort study	IVF	TUNEL	DFI <35%	Miscarriage Rate
Lin et al. (2008) ($n = 223$)	Retrospective and prospective cohort study	IVF, ICSI	SCSA	DFI <27%	Miscarriage rate
Esbert et al. (2011) ($n = 178$)	Prospective cohort study	IVF, ICSI	TUNEL	DFI <36%	Miscarriage rate
L. Simon et al., Unpublished results ($n = 392$)	Prospective cohort study	IVF, ICSI	COMET	DFI <25%	Miscarriage rate

Statistical analysis

Relative risks from individual studies were meta-analysed using a random effects model as appropriate. Heterogeneity of the exposure effects was evaluated graphically using Forest plots (Lewis and Clarke, 2001) and statistically using the I^2 statistic to quantify heterogeneity across studies (Higgins et al., 2002). Since 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 0.10 rather than the conventional level of 0.05, was used to determine statistical significance (Deeks et al., 2008). Exploration of the causes of heterogeneity was performed using variation in features of population and assays for measuring DNA damage. We subgrouped the studies according to assays that were used for the measurements of DNA damage. We also subgrouped according to the use of prepared or raw semen. To assess for publication bias we performed funnel plot analysis (Fig. 1) and assessed visually for asymmetry for the primary outcome of miscarriages (Egger et al., 1997). Statistical analyses were performed using RevMan 5.0 (Cochrane Collaboration, Oxford, UK) and Stata 9.0 (Stata Corp, TX, USA). We also subgrouped the analysis according to the treatment performed: IVF, ICSI or IVF and ICSI.

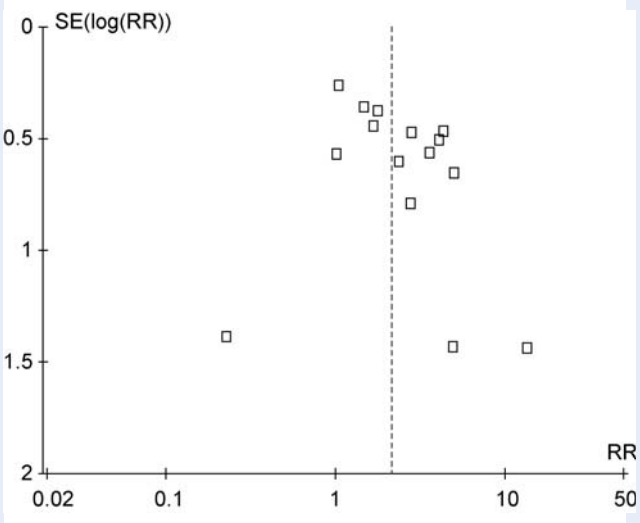

Figure 1 Funnel plot demonstrating results of the Eggers test to assess publication bias.

Results

The search strategy yielded 829 citations, all captured from electronic citations (Fig. 2). Of these, 704 publications were excluded as they did not fulfil the selection criteria. Of the 125 remaining publications, 101 were excluded as no pregnancy loss data were reported. One study was excluded (Bungum et al., 2004) as all its data were duplicated in a later paper (Bungum et al., 2007) which we have included in our meta analysis. Seven studies were excluded as they had pregnancy loss data which were incomplete or for which it not possible to construct a 2 × 2 table (Payne et al., 2005; Borini et al., 2006; Velez de la

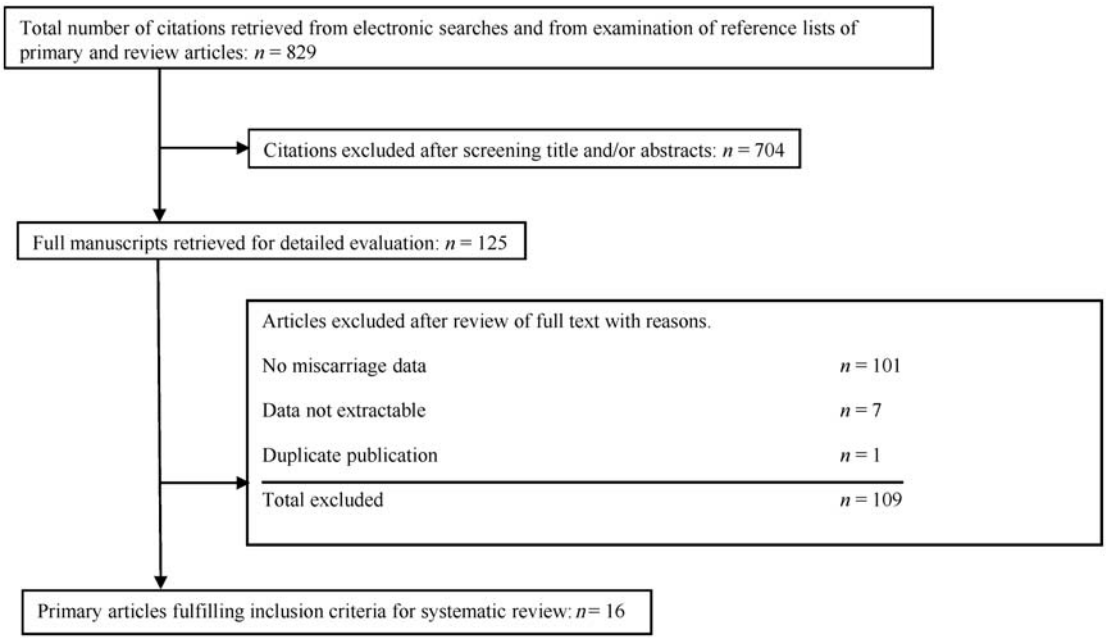

Figure 2 Flow chart showing study selection process.

Calle *et al.*, 2008; Gu *et al.*, 2009; Meseguer *et al.*, 2009; Bellver *et al.*, 2010; Simon *et al.*, 2010).

Therefore, the total number of studies included in the review was 16 (Fig. 2) comprising 2969 couples. Fourteen of the studies were prospective and two were retrospective. Eight studies used acridine orange-based assays, six the TUNEL assay and two the COMET assay. The threshold for defining high DNA damage varied between 10 and 36% of the sperm population.

The main characteristics of the 16 studies and the Newcastle–Ottawa Quality Assessment are presented in Figure 3 and Table I. Table I shows the study characteristics. The size of studies varied from 18 couples (Greco *et al.*, 2005a) to 637 couples (Bungum *et al.*, 2007). The majority of the studies ($n = 11$) included both IVF and ICSI data, with only one study having IVF data alone and one study (Evenson *et al.*, 1999b) involved miscarriage rates after spontaneous conception. Fifteen studies used miscarriage rates as an outcome measure; Boe-Hansen *et al.* used biochemical pregnancy rates (Boe-Hansen *et al.*, 2006).

The studies scored well on the Newcastle–Ottawa Quality Assessment Scale (Fig. 3). Eleven studies scored the maximum of 9 points, one study scored 8 and three studies scored 7. One study (Simon *et al.*) is unpublished. The funnel plot (Fig. 1) suggests a lack of publication bias due to its symmetrical shape, although a small study may have been missed.

Meta-analysis

Our meta-analysis included 16 studies of which 14 were ICSI papers, 11 were IVF papers and one study looked at miscarriage after spontaneous conception. These studies comprised 2969 couples with 1252 pregnancies and 225 pregnancy losses (biochemical and/or clinical pregnancy).

In our meta-analysis of the 16 studies we found a significant increase in miscarriage in patients with high DNA damage compared with those with low DNA damage [risk ratio = 2.16 (1.54, 3.03), $P < 0.00001$; Fig. 4]. There was moderate statistical heterogeneity in the results, although not significant at $P < 0.05$ ($I^2 = 34\%$, $P = 0.10$).

The TUNEL assay was used in six of the studies, SCSA or acridine orange in eight and COMET in two of them. In the subgroup meta-regression analysis, we found that the miscarriage association was the strongest for the TUNEL assay [six studies, RR = 3.94 (2.45, 6.32), $P < 0.00001$; Fig. 4], but the summary RR estimates of studies using SCSA were also significant (seven studies, RR = 1.47; 95% confidence interval (CI): 1.04, 2.09; $P = 0.03$; Fig. 3; Fig. 4). However, the summary of estimates for the studies using the COMET assay (two studies, RR = 1.43, 95% CI: 0. 4, 5.14; $P = 0.58$; Fig. 4) and the acridine orange assay (one study, RR = 2.78, 95% CI: 0.59, 13.11; $P = 0.20$; Fig. 4) did not reach significance.

We also performed subgroup analysis on treatment type but this showed no significant difference in miscarriage (data not shown).

As studies used either raw or prepared semen we subgrouped the studies accordingly (Fig. 5). Although the prepared semen group had a stronger association with high DNA damage and miscarriage (RR = 3.47, 95% CI: 2.13, 5.63; $P < 0.00001$) than the raw semen group (RR = 1.50, 95% CI: 1.11, 2.01; $P = 0.007$), both groups showed a significant association (pooled RR = 1.65 95% CI: 1.66, 2.33; $P < 0.00001$).

Discussion

Our meta-analysis has demonstrated a significant relationship between high frequency of sperm with elevated DNA damage and miscarriage. All but 2 of the 16 studies had an RR greater than unity (with the

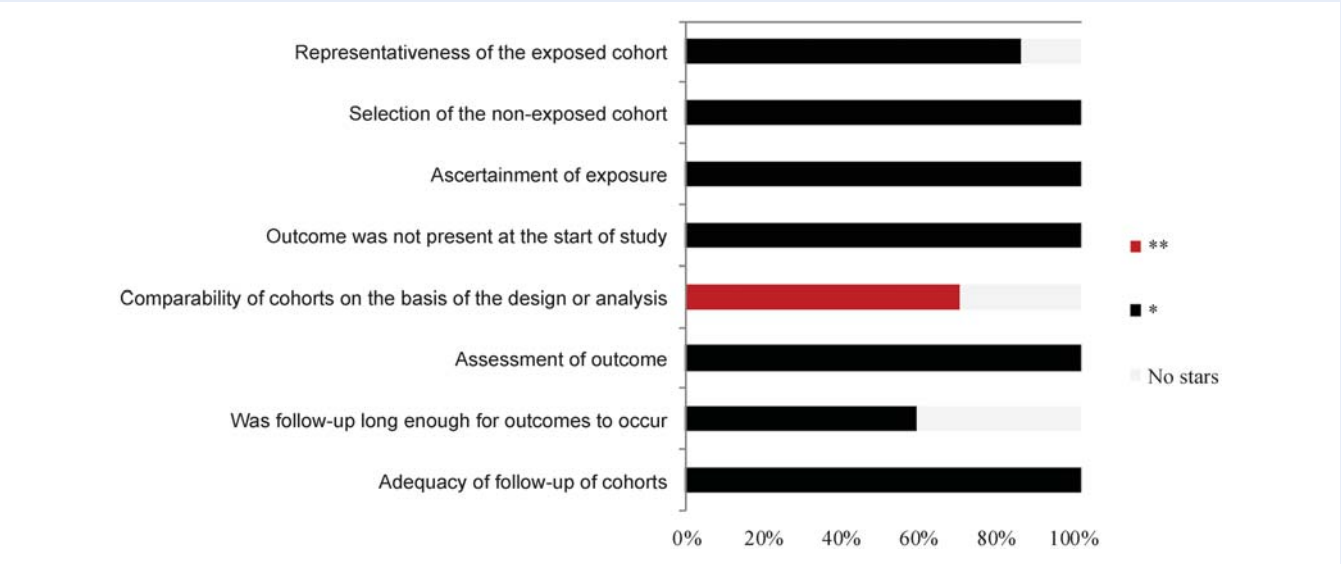

Figure 3 The Newcastle–Ottawa Quality Assessment Scales for observational studies. The maximum score that can be awarded for items in black is one star (*) and for items in red two stars (**). The 'x' axis represents the percentage of the studies where the quality item received the maximum score.

exception of Gandini *et al.*, 2004 and Boe-Hansen *et al.*, 2006), showing that DNA damage in sperm is consistently associated with pregnancy loss. We subanalysed the data with regard to the use of prepared or raw semen and found that both groups showed a significant increase in miscarriage rates with men with high rates of DNA damage in the sperm. When we analysed the different types of assays used to assess DNA damage, we found that the miscarriage association was the strongest for the TUNEL assay. This method of analysis directly quantifies DNA damage by the incorporation of labelled dUTP into single- and double-stranded DNA breaks.

The association of DNA damage in sperm with pregnancy loss is in agreement with the meta-analysis by Zini *et al.* (2008) but in our systematic review we have included an additional nine studies and we did not limit our search to IVF- and ICSI-treated patients. We included a study by Evenson *et al.* (1999a) which analysed sperm DNA damage in two groups of couples trying to conceive spontaneously: one group were presumed normally fertile and one group had suffered miscarriage. This study showed an association between miscarriage and high DNA damage and the SCSA data predicted 7 out of 18 miscarriages (39%).

One of the studies included in our review examined DNA damage and miscarriage rates from ejaculated sperm compared with testicular sperm (Greco *et al.*, 2005b). Interestingly, there was a much higher level of DNA damage in the ejaculated sperm in comparison with the testicular sperm. This may be explained by the hypothesis that most DNA damage is acquired at the post-testicular level. This study's findings are in agreement with other studies, showing that sperm with DNA damage can fertilize oocytes successfully and give rise to good grade embryos which subsequently fail to implant or result in early pregnancy loss (Twigg *et al.*, 1998; Ahmadi and Ng, 1999; Tomlinson *et al.*, 2001; Morris *et al.*, 2002; Carrell *et al.*, 2003; Henkel *et al.*, 2004; Tesarik *et al.*, 2004b).

Gross chromosomal abnormalities are present in ~70% of miscarriages investigated (Rull *et al.*, 2012) and the majority of cases are of maternal origin arising during meiosis (Hassold *et al.*, 2007). However, most miscarriages are either unrecognized or unavailable for genetic analysis. Furthermore, the random and subtle nature of the defects arising from sperm DNA damage means that any resulting miscarriages will not be included in such figures. Interestingly in women with recurrent miscarriages, the frequency of abnormal embryonic karyotypes was found to significantly decrease with the number of previous miscarriages (Ogasawara *et al.*, 2000) suggesting that in this population other factors are responsible, one of which may be sperm DNA damage.

In our meta-analysis we did not find any difference in the link between high DNA damage and miscarriage with different fertility treatments. This is in agreement with other studies (Collins *et al.*, 2008; Zini *et al.*, 2008). With ICSI, spermatozoa with significant DNA damage are more likely to fertilize the oocyte than with IVF but miscarriage can still ensue. The degree to which sperm DNA damage affects pregnancy outcome to some extent may depend on oocyte quality. Spermatozoa are incapable of DNA repair and so they rely on the oocyte for repair post-fertilization. The negative impact of high DNA fragmentation on pregnancy can be overcome by using high-quality oocytes, as shown in a study comparing standard and donor cycles (Meseguer *et al.*, 2008).

The weakness of this meta-analysis is the variation in study characteristics. Both retrospective and prospective studies have been included and studies use different assays and different thresholds for DNA damage. Female inclusion and exclusion criteria are not always clearly stated and the definition of pregnancy loss varied, i.e.: biochemical or clinical pregnancy.

Conventional semen analysis may show normal parameters in the presence of high levels of sperm DNA damage (Giwercman *et al.*, 2003; Simon *et al.*, 2011; Simon and Lewis, 2011). Some authors have questioned the benefit of the additional information (Castilla *et al.*, 2010), however, we contend that for the many couples suffering from 'unexplained' infertility who may have male factor fertility problems arising from elevated sperm DNA damage, this knowledge could be beneficial as they can plan treatment accordingly.

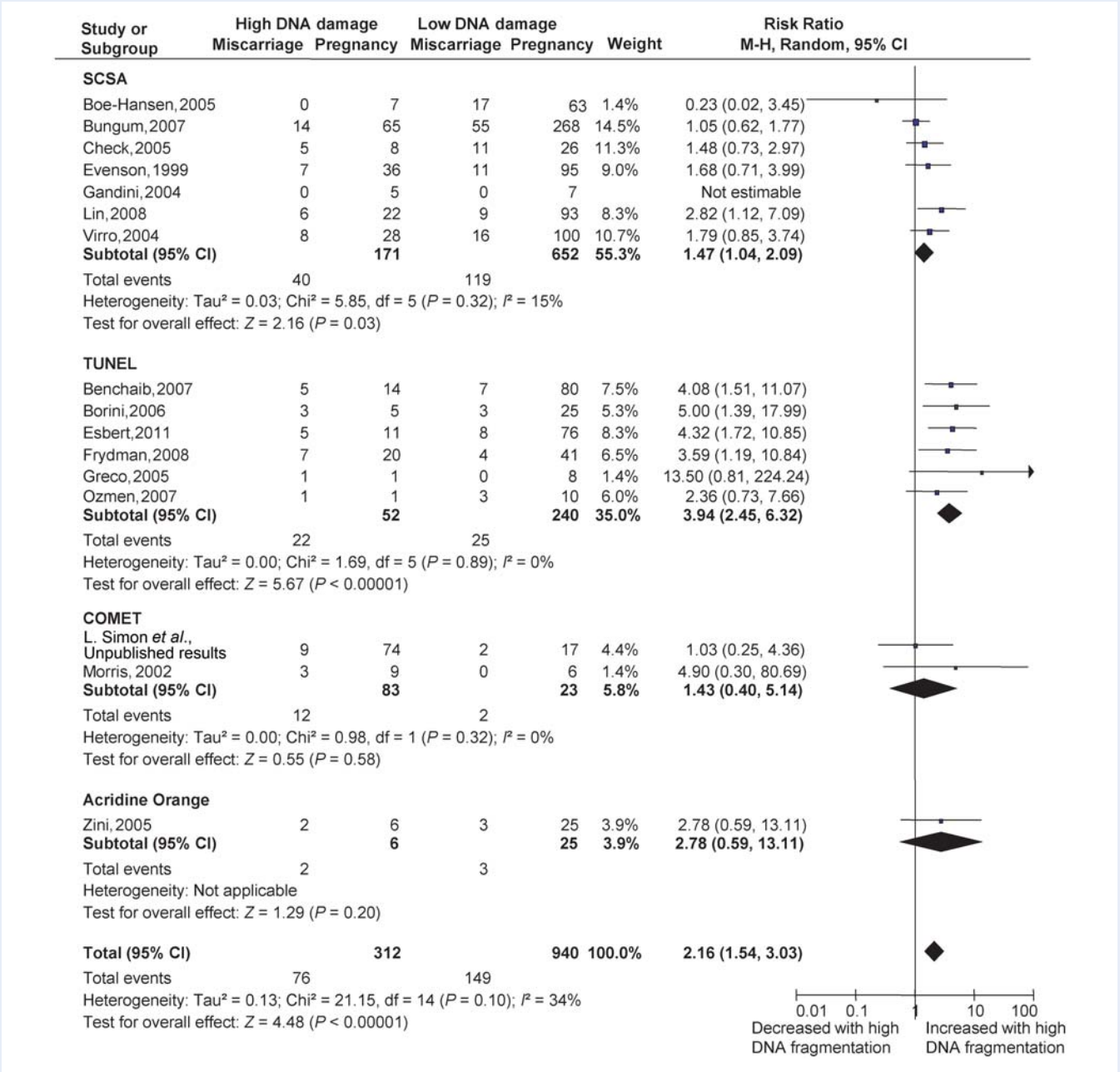

Figure 4 Forest plot showing the results of subgroup meta-analysis of assays used in studies comparing the effect of high DNA fragmentation versus low DNA fragmentation in sperm on miscarriage rates.

For those with mild male factor infertility planning to have IUI, high levels of DNA fragmentation appear to be predictive of a poor outcome and therefore knowledge of this would be helpful in treatment planning (Bungum *et al.*, 2007).

Paternal age may have a link with miscarriage as suggested by a recent study. A European multi-centre study (de la Rochebrochard and Thonneau, 2002) demonstrated a substantially higher miscarriage risk in couples where the female was ≥35 years and the male was ≥40 years compared with couples of other age combinations. A possible explanation for this may be higher levels of DNA damage in sperm of older men as these men have been shown to have more double-strand DNA breaks (Singh *et al.*, 2003). The probability of producing aneuploid offspring (Griffin *et al.*, 1995) is increased in older men and there are a higher frequency of sperm chromosome aberrations (Sartorelli *et al.*, 2001). It is also widely 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.

There is considerable evidence which points towards oxidative stress as a major factor in male infertility (Lewis and Agbaje, 2008; Tremellen, 2008; Agarwal *et al.*, 2009; Kefer *et al.*, 2009). Reactive oxygen species (ROS) are principally produced by leucocytes and sperm cytoplasm (Aitken *et al.*, 1998b). Morphologically normal

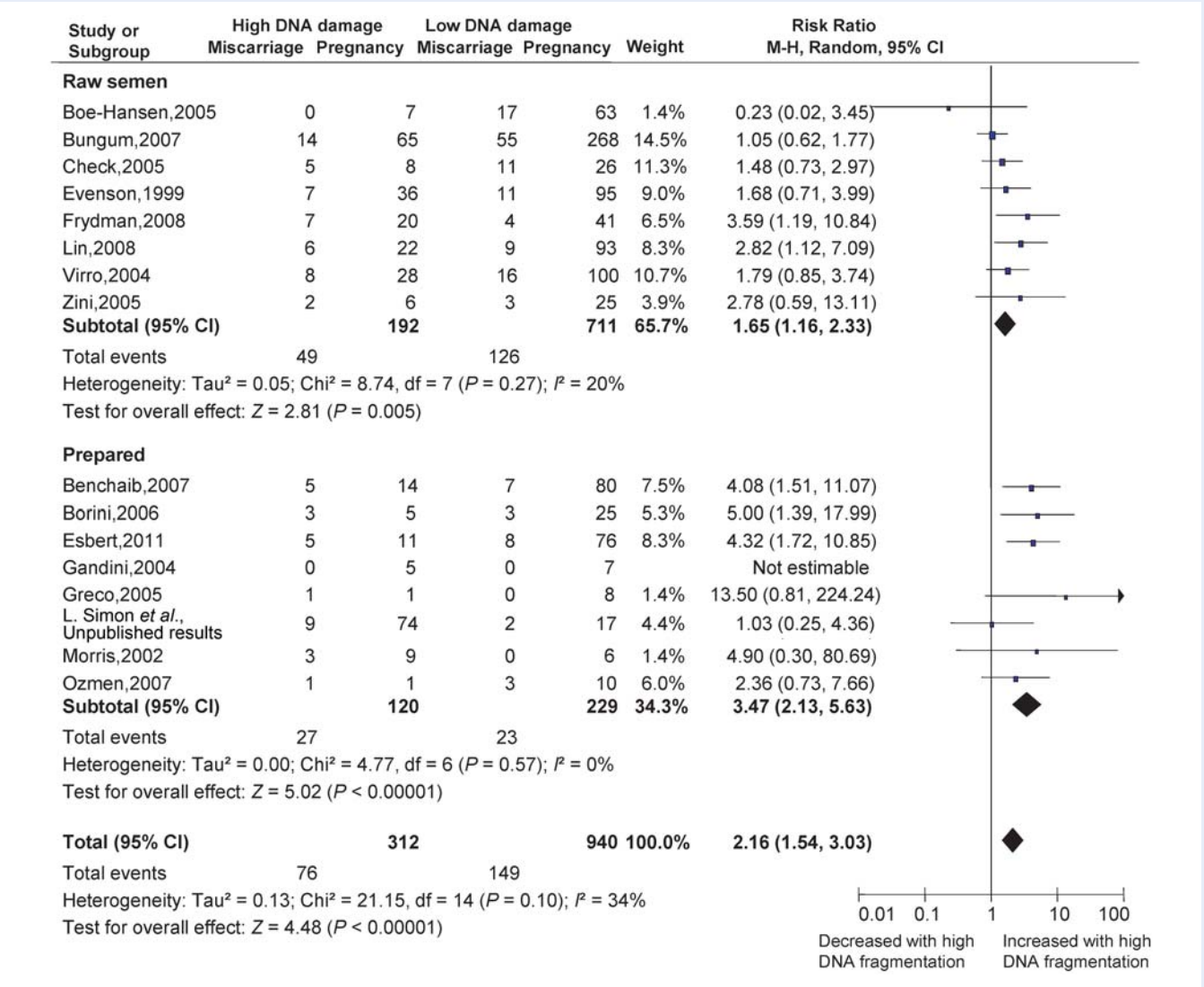

Figure 5 Forest plot showing the results of subgroup meta-analysis semen preparation used in studies comparing the effect of high DNA fragmentation versus low DNA fragmentation in sperm on miscarriage rates.

sperm will produce less ROS than immature sperm as 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 appear to have lower levels of antioxidative activity than fertile men (Fraga *et al.*, 1996; Lewis *et al.*, 1997; Tremellen *et al.*, 2007). Antioxidants (such as vitamins C and E, folate, zinc, selenium, carnitine and carotenoids) are scavengers of ROS and therefore they have been proposed as a treatment to reverse the adverse impact of high ROS concentrations on semen parameters. A recent meta-analysis (Ross *et al.*, 2010) showed an improvement in sperm motility and pregnancy rates, both spontaneous and assisted, with antioxidant use. Recently, a Cochrane Review (Showell *et al.*, 2011) showed a statistically significant increase in live birth rate and pregnancy rate with the use of antioxidants. However, only three trials reported on live birth rate and no recommendation could be made on individual antioxidants. Some studies have also suggested an improvement in sperm motility and decreased ROS production when antioxidants are added to sperm *in vitro* (Pang *et al.*, 1993; Oeda *et al.*, 1997; Okada *et al.*, 1997).

However, there should be some caution employed when using antioxidants as one study reported >20% increase in sperm decondensation (Menezo *et al.*, 2007). Perturbation of sperm chromatin structure may cause changes in paternal gene expression during preimplantation development as a result of asynchronous chromosome condensation, as well as cytoplasmic fragments in the embryo. Also excessive levels of antioxidants can be harmful; ascorbate can increase the chance of miscarriage (Pintauro and Bergan, 1982) and ascorbate and α tocopherol can, either singly or in combination, decrease sperm motility (Donnelly *et al.*, 1999). Therefore, although antioxidant therapy is promising, further research is required and its use should be employed with a degree of caution.

To conclude, the findings of this systematic review demonstrate a significant relationship between levels of DNA damage in sperm and spontaneous pregnancy loss. Moreover, the data suggest that it

should be possible to reduce such losses if sperm for injection could be non-destructively screened for DNA damage beforehand. Several promising screening methods are in development for this purpose including electrostatic/phoretic, microscopical and biochemical techniques (Said and Land, 2011). Of these, hyaluronan binding has been shown to select for sperm with strict Tygerberg criteria (Prinosilova *et al*., 2009) and a small clinical trial of hyaluronan selected sperm showed efficacy in increasing the numbers of Grade 1 embryos and live birth rates (Parmegiani *et al*., 2010). Tests for DNA damage and selection of undamaged sperm should be considered as part of the diagnostic and treatment pathways for those suffering from recurrent pregnancy loss. Further research is required into the mechanisms responsible for and preventing the DNA damage including antioxidant therapy.

Authors' roles

L.R. contributed to the study design and acquisition of data, drafted the article and revised it critically and organized the final approval of the version to be published. I.G. contributed to conception and design, acquisition of data and analysis and interpretation of data. I.G. also helped with drafting and revision of the article and the final approval of the version to be published. S.C. contributed substantially to the acquisition of data, and analysis and interpretation of the data. S.C. also helped with drafting and revision of the article and the final approval of the version to be published. D.M. contributed substantially to the interpretation of the data. D.M also helped with drafting and revision of the article and the final approval of the version to be published. M.R. contributed to the analysis and interpretation of data helped with drafting and revision of the article and the final approval of the version to be published. S.L. substantially contributed to the design of the study and analysis and interpretation of data and also helped with revising the article critically and for final approval of the version to be published. J.K.-B. substantially contributed to design of the study, acquisition of data, and analysis and interpretation of data and also helped revise the article and contributed to the final approval of the version to be published. A.C. substantially contributed to the conception and design of the study and analysis and interpretation of data and also helped with revising the article and the final approval of the version to be published.

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Conflict of interest

SEM Lewis is CEO of Lewis Fertility Testing; a spin out company from Queens University, Belfast, UK. All papers from her group were supported by peer reviewed public funding prior to the set up of the company.

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