Human Reproduction Vol.23, No.12 pp. 2663–2668, 2008 doi:10.1093/humrep/den321
Advance Access publication on August 29, 2008

Sperm DNA damage is associated with an increased risk of pregnancy loss after IVF and ICSI: systematic review and meta-analysis

Armand Zini[1,4], Jason M. Boman[1], Eric Belzile[2] and Antonio Ciampi[2,3]

[1]Division of Urology, Department of Surgery, St Mary's Hospital Center, Mary's Hospital, 3830 Lacombe Avenue, Montreal, Quebec, Canada H3T 1M5; [2]Department of Clinical Epidemiology and Community Studies, St. Mary's Hospital Center, Mary's Hospital, 3830 Lacombe Avenue, Montreal, Quebec, Canada H3T 1M5; [3]Department of Epidemiology and Biostatistics, McGill University, Montreal, Quebec, Canada

[4]Correspondence address. Fax: +1-514-734-2718; E-mail: ziniarmand@yahoo.com

BACKGROUND: Sperm DNA damage is common amongst infertile men and may adversely impact natural reproduction, IUI-assisted reproduction and to a lesser degree IVF pregnancy. The aim of this study was to examine the influence of sperm DNA damage on the risk of spontaneous pregnancy loss after IVF and ICSI. METHODS: We conducted a systematic review and meta-analysis of studies on sperm DNA damage and pregnancy loss after an IVF and/or ICSI pregnancy. RESULTS: Two by two tables were constructed and odds ratios (ORs) were derived from 11 estimates of pregnancy loss (five IVF and six ICSI studies from seven reports). These 11 studies involved 1549 cycles of treatment (808 IVF and 741 ICSI cycles) with 640 pregnancies (345 IVF and 295 ICSI) and 122 pregnancy losses. The combined OR of 2.48 (95% CI 1.52, 4.04, $P < 0.0001$) indicates that sperm DNA damage is predictive of pregnancy loss after IVF and ICSI. CONCLUSIONS: In conclusion, sperm DNA damage is associated with a significantly increased risk of pregnancy loss after IVF and ICSI. These data provide a clinical indication for the evaluation of sperm DNA damage prior to IVF or ICSI and a rationale for further investigating the association between sperm DNA damage and pregnancy loss.

Keywords: spermatozoa; DNA damage; pregnancy loss; miscarriage; male infertility; *in vitro* fertilization

Introduction

Mammalian fertilization involves the direct interaction and fusion of the sperm and oocyte, with subsequent union of male and female gamete genomes (Primakoff and Myles, 2002). Animal studies have shown that embryo development and implantation depend in part on the integrity of the sperm DNA and that there may be a threshold of sperm DNA damage (e.g. DNA fragmentation) beyond which these processes are impaired (Ahmadi and Ng, 1999). Moreover, there is also experimental evidence that sperm DNA fragmentation increases the risk of cancer development and reduces longevity in the offspring (Fernandez-Gonzalez *et al*., 2008; Perez-Crespo *et al*., 2008). However, human studies indicate that DNA-damaged spermatozoa can fertilize successfully at IVF (Gandini *et al*., 2004) and allow for normal embryo development (Bungum *et al*., 2004). These observations have raised concerns regarding the safety of using DNA-damaged sperm for IVF and have led investigators to recommend assessment of sperm DNA damage as part of assisted reproductive technology (ART) programs (Perreault *et al*., 2003).

There is now clear evidence that infertile men possess substantially more sperm DNA damage than do fertile men (Evenson *et al*., 1980; Irvine *et al*., 2000; Shen and Ong, 2000; Spano *et al*., 2000; Zini *et al*., 2001, 2002). This is clinically relevant given that infertile men (especially those with severe male-factor infertility and with poor sperm DNA integrity) will be seeking treatment with ARTs.

The influence of sperm DNA damage on ART pregnancy has been the subject of numerous studies. There is evidence to suggest that sperm DNA damage is associated with poor pregnancy rates after IUI, although there is only one valid study in this regard (Bungum *et al*., 2007). The relationship between sperm DNA damage and pregnancy after IVF and ICSI has recently been evaluated using a systematic analysis (Collins *et al*., 2008). 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 (Larson-Cook *et al*., 2003; Henkel *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; Lin *et al*., 2008; Collins *et al*., 2008; Frydman *et al*., 2008).

A number of studies have reported an increased (albeit non-significant) risk of pregnancy loss after IVF and/or ICSI. However, these observations have not been reviewed and/or analyzed in a systematic fashion (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; Lin *et al*., 2008, Frydman *et al*., 2008). As such, we sought to evaluate further the relationship between sperm DNA damage and the risk of spontaneous abortion after standard IVF and after ICSI. We carried out a systematic review of the literature and performed a meta-analysis to evaluate the influence (if any) of sperm DNA damage on pregnancy loss after IVF and after ICSI.

Methods

Search strategy and selection criteria

We searched the Medline database from 1999 to January 2008 using the following search terms: 'human sperm DNA', 'human sperm DNA damage', 'human sperm chromatin', in combination with 'pregnancy', 'pregnancy loss', 'abortion', 'miscarriage' 'assisted reproduction', '*in vitro* fertilization', 'IVF' and 'ICSI'. Additional studies were identified from the study reference lists. Only full articles published in English were searched. Two investigators (A.Z. and J.M.B.) independently reviewed the papers for eligibility and discrepancies were resolved by group discussion.

Data extraction

We selected studies that evaluated sperm DNA damage in whole or washed semen and spontaneous pregnancy loss in couples undergoing IVF and/or ICSI. For studies to be eligible, we had to be able to construct 2×2 tables from the reported data (with pregnancy loss rate above and below DNA damage cutoff). The following outcomes were pre-requisites for inclusion: biochemical pregnancy (serum hCG elevation) and biochemical pregnancy loss (i.e. loss of documented biochemical pregnancy) and/or clinical pregnancy (i.e. presence of a fetal heartbeat, confirmed by ultrasound) and clinical pregnancy loss (i.e. loss of documented clinical pregnancy). If necessary, study authors were contacted to clarify the data. We recorded the accrual type (i.e. consecutive), patient selection, female inclusion/exclusion criteria, treatment type, sperm DNA assay type, cutoff point, number of cycles or patients and number of pregnancies relative to abnormal or normal test results. From the 2×2 tables of test results, the following test properties were calculated for each study: sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), proportion of abnormal tests and diagnostic odds ratio (OR).

The studies included in the final analysis utilized one of two tests of sperm DNA damage: the sperm chromatin structure assay (SCSA) or the terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) assay. The SCSA is an objective, flow cytometry-based assay that measures the susceptibility of DNA to denaturation (under acid conditions) and provides a quantitative but indirect assessment of sperm DNA damage. The TUNEL assay is a semi-quantitative (microscopy-based) assay that provides a direct assessment of sperm DNA fragmentation by labeling DNA breaks. 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%).

Data synthesis and analysis

The measure of treatment effect was the combined odds ratio of a pregnancy loss 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 2×2 tables (Egger *et al*., 2001; Deville *et al*., 2002). We attributed the value 0.5 to empty cells of the 2×2 tables (Egger *et al*., 2001). We tested study homogeneity and depending on whether homogeneity was accepted or rejected, we used the fixed or the random effect model for meta-analysis in order to calculate an overall OR and its 95% CI. We used the Q statistics to test between study homogeneity: homogeneity was rejected when the Q statistic P-value was less than 0.10. A meta-regression was used to evaluate whether the overall conclusions were affected by the type of assisted reproduction (IVF or ICSI) (Egger *et al*., 2001). The meta-analysis was conducted using the STATA software (StataCorp LP, College Station, TX, USA).

Results

Studies selected

Of the initial 310 citations retrieved, review of the titles and abstracts indicated that 277 were not relevant. Full papers were obtained for the remaining 33 citations. After reviewing the 33 papers, 24 were excluded because spontaneous pregnancy loss was not reported. An additional paper was excluded because a 2×2 table could not be constructed from the data (Virro *et al*., 2004). One of the studies (Bungum *et al*., 2004) was later replaced by an updated report (this was verified by contacting the lead author) that included all of the earlier patients (Bungum *et al*., 2007).

Study characteristics

The seven eligible reports (with 11 studies) involved 1549 cycles of treatment (808 IVF and 741 ICSI treatment cycles) with 640 pregnancies (345 IVF and 295 ICSI) and 122 pregnancy losses. The study characteristics are depicted in Table I. Of these seven papers, five were reportedly prospective (Zini *et al*., 2005a,b; Benchaib *et al*., 2007; Bungum *et al*., 2007; Lin *et al*., 2008; Frydman *et al*., 2008) but sampling appeared to be consecutive in only two papers (Zini *et al*., 2005a,b; Check *et al*., 2005; Bungum *et al*., 2007). Sperm DNA damage was evaluated in washed semen samples in one of the papers (Borini *et al*., 2006) with all other studies reporting DNA damage in whole (unprocessed semen). One of the studies evaluated couples with a history of multiple IVF failures (Check *et al*., 2005). One paper (two studies) reported pregnancy loss per biochemical pregnancy (Bungum *et al*., 2007) and the other six papers (nine studies) reported pregnancy loss per clinical pregnancy (Check *et al*., 2005; Zini *et al*., 2005a,b; Borini *et al*., 2006; Benchaib *et al*., 2007; Lin *et al*., 2008; Frydman *et al*., 2008).

Meta-analysis

We included the seven eligible papers (five IVF and six ICSI studies) in our meta-analysis. Altogether, these 11 studies involved 1549 cycles of treatment (808 IVF and 741 ICSI cycles) with 640 pregnancies (345 IVF and 295 ICSI) and 122 pregnancy losses (per biochemical and/or clinical pregnancy).

Table I. Characteristics of studies on sperm DNA damage and pregnancy loss (PL) after IVF and IVF/ICSI.

Study	*n*	ART	Assay	Population	Study design	PL-Def	Female Dx
Check *et al*. (2005)	104	ICSI	SCSA	failed IVFx2	unspecified	per CP	unspecified
Zini *et al*. (2005a,b)	60	ICSI	SCSA	unspecified	prospective	per CP	<40
Borini *et al*. (2006)	82	IVF	TUNEL	unspecified	unspecified	per CP	unspecified
	50	ICSI	TUNEL	unspecified	unspecified	per CP	unspecified
Benchaib *et al*. (2007)	84	IVF	TUNEL	unspecified	prospective	per CP	unspecified
	218	ICSI	TUNEL	unspecified	prospective	per CP	unspecified
Lin *et al*. (2008)	137	IVF	SCSA	unspecified	prospective	per CP	<40, FSH<15
	86	ICSI	SCSA	male factor	prospective	per CP	<40, FSH<15
Bungum *et al*. (2007)	388	IVF	SCSA	female factor	prospective	per BP	<40, FSH<12
	223	ICSI	SCSA	male factor	prospective	per BP	<40, FSH<12
Frydman (2008)	117	IVF	TUNEL	unspecified	prospective	per CP	<38, FSH<10

n, number of IVF or ICSI cycles; ART, assisted reproductive technology; PL-Def, pregnancy loss definition; CP, clinical pregnancy; BP, biochemical pregnancy; Female Dx, female diagnosis; <40 or <38, <40 or <38-year-old; FSH<15 (<12, <10), Day 3 serum FSH<15 (<12, <10) IU/l.

Table II. Selected diagnostic properties of studies on sperm DNA damage and pregnancy loss (PL) after IVF and IVF/ICSI.

Study	ART	Assay	PL (%)	Abn Test* (%)	Sens	Spec	PPV	NPV
Check *et al*. (2005)	ICSI	SCSA	47	24	0.31	0.83	0.63	0.58
Zini *et al*. (2005a,b)	ICSI	SCSA	16	19	0.40	0.85	0.33	0.88
Borini *et al*. (2006)	IVF	TUNEL	6	11	0.91	0.94	0.50	0.99
	ICSI	TUNEL	25	25	0.97	0.99	0.97	0.99
Benchaib *et al*. (2007)	IVF	TUNEL	15	15	0.50	0.91	0.50	0.91
	ICSI	TUNEL	12	15	0.38	0.88	0.30	0.91
Lin *et al*. (2008)	IVF	SCSA	10	17	0.29	0.84	0.17	0.92
	ICSI	SCSA	18	23	0.50	0.83	0.40	0.88
Bungum *et al*. (2007)	IVF	SCSA	24	14	0.11	0.85	0.19	0.76
	ICSI	SCSA	19	40	0.50	0.63	0.24	0.84
Frydman (2008)	IVF	TUNEL	19	32	0.64	0.75	0.37	0.90

ART, assisted reproductive technology; Abn Test, proportion of abnormal sperm DNA test amongst documented pregnancies; PL, pregnancy loss; Sens, sensitivity; Spec, specificity; PPV, positive predictive value; NPV, negative predictive value.

The SCSA was used in six of these studies and the TUNEL assay in five. Selected diagnostic test properties for the individual studies are shown in Table II. Diagnostic odds ratios (OR) ranged from 0.73 to 2700, and in one of 11 estimates, these were statistically different from unity (see Fig. 1). The Q statistic P-value was 0.255, indicating homogeneity of the studies. The fixed effects model combined OR was 2.48 (95% CI, 1.52, 4.04; $P < 0.0001$) (Fig. 1).

In a meta-regression analysis, we found no significant difference in the OR according to treatment type (IVF or ICSI). The combined OR estimates of IVF (five estimates, OR = 2.17; 95% CI, 1.02, 4.60; $P < 0.05$) and ICSI studies (six estimates, OR = 2.73; 95% CI, 1.43, 5.20; $P < 0.01$) were both significant.

The summary OR estimates of studies using SCSA (six estimates, OR = 1.77; 95% CI, 1.01, 3.13; $P < 0.05$) and TUNEL (five estimates, OR = 7.04; 95% CI, 2.81, 17.67; $P < 0.001$) were both significant. However, the meta-regression analysis demonstrated a significant difference in the OR estimates between the TUNEL and the SCSA studies ($P = 0.012$).

We conducted a separate meta-analysis excluding the Borini *et al*. (2006) study (this is the only study that uses sperm DNA damage levels in prepared semen) based on the understanding that there is a difference in sperm DNA damage levels in whole and prepared semen, and, that the same sperm DNA damage cutoffs may not be reliable when evaluating washed semen in predicting outcome of ART (Bungum *et al*., 2008). The summary OR estimate of this sub-analysis is also significant (nine estimates, OR = 2.37; 95% CI, 1.45, 3.88; $P < 0.05$) and is not significantly different form that of the overall meta-analysis.

Discussion

In this systematic review of 11 studies (from seven papers) involving 1549 cycles of treatment (IVF or ICSI) with 640 pregnancies and 122 pregnancy losses, sperm DNA damage was statistically significantly associated with pregnancy loss (combined OR 2.48; 95% CI, 1.52, 4.04; $P < 0.0001$). An OR above one indicates that abnormal sperm DNA integrity (sperm DNA damage above the cutoff point) is associated with an increased chance of disease (i.e. pregnancy loss). Meta-regression analyses showed that test accuracy was not affected by treatment type (e.g. IVF or ICSI) but was related to the type of assay (TUNEL versus SCSA).

A strength of systematic reviews is the improved precision of the summary OR estimates compared with the individual studies. The combined estimate in the 11 studies was significantly different from unity, indicating that sperm DNA damage has an effect on pregnancy loss after IVF and ICSI. Although the number of events (pregnancy loss) per study was small, the ORs of the individual studies were all (with

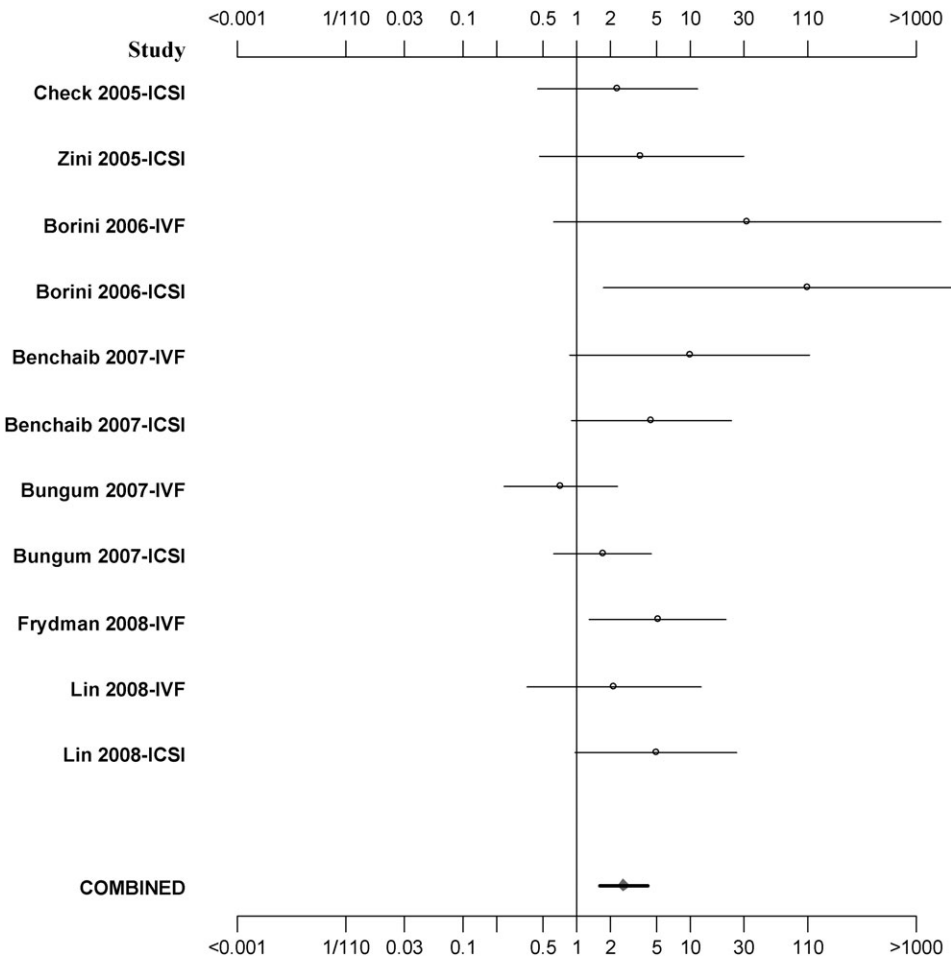

Figure 1: Forest plot depicting odds ratio (OR) and 95% confidence interval (CI) of the 11 studies and the combined OR from the meta-analysis (note: scale is logarithmic).

the exception of one study (Bungum *et al*., 2007)) greater than unity. On the other hand, a weakness of this meta-analysis is the highly variable study characteristics: data collection (prospective or retrospective), definition of pregnancy loss (biochemical or clinical), population characteristics (unselected, repeated IVF failures), female inclusion/exclusion criteria, sperm DNA test type and sperm DNA test cutoff.

In many of the studies included in the meta-analysis, a clinically relevant cutoff level was not used (i.e. the authors did not establish a normal range based on the evaluation of a fertile population). In these studies, the cutoff was selected based on (i) previously reported cutoffs (Borini *et al*., 2006; Lin *et al*., 2008), (ii) the median value for the study population (Frydman *et al*., 2008) or (iii) receiver-operating characteristic curves (Benchaib *et al*., 2007). However, it is not known whether a clinically relevant cutoff level (that is based on a fertile population) is the optimal cutoff to be used in the evaluation of pregnancy loss after IVF or ICSI. As such, it may be unreasonable (and biased) to exclude studies that did not utilize a clinically relevant cut-off level.

Using predictive values allows for a simpler (more intuitive) interpretation of the results. However, predictive values vary according to the prevalence of disease and, therefore, may vary depending on the clinical setting. An analysis of the 11 studies (with a median pregnancy loss rate of 18%), revealed a median PPV of 37% and median NPV of 90%. This means that in populations with an overall pregnancy loss of 18%, the rate of pregnancy loss is estimated at 37% when there is an abnormal test result and at 10% when the test result is normal. Thus, in this analysis, sperm DNA damage assessment provides clinically valuable information as it can discriminate between pregnancy loss rates of 37 and 10%. The effect of DNA damage on pregnancy loss should be discussed with patients prior to undergoing ART, although ultimately, this information may not alter clinical practice as couples will often proceed to ART regardless of test results.

The predictive value of sperm DNA damage assessment may vary depending on the sperm DNA test and cutoff level that is used. Indeed, the difference in the summary ORs between those studies using SCSA and TUNEL assay may reflect the impact of different types of DNA damage or different DNA damage cutoff levels in predicting pregnancy loss. A large prospective study evaluating multiple aspects of sperm DNA integrity may help corroborate the findings of this meta-analysis and identify the type of DNA damage (e.g. single or double strand DNA breaks, DNA denaturation, oxidation) associated with pregnancy loss.

The finding of an association between sperm DNA damage and pregnancy loss is consistent with the results reported in another otherwise eligible study. Indeed, Virro *et al*. (2004) also observed an increased pregnancy loss in IVF and IVF/ICSI pregnancies achieved using samples with DNA damage. However, in the Virro study (Virro *et al*., 2004), a 2×2 table could not be constructed. An association between sperm DNA damage and pregnancy loss has also been observed in non-IVF studies. Indeed, Evenson *et al*. (1999), observed an increased (albeit insignificant) risk of pregnancy loss in couples with sperm DNA damage and Carrell *et al*. (2003), reported that recurrent pregnancy loss is associated with higher levels of sperm DNA damage. Although the possible mechanism(s) that underlie the association between sperm DNA damage and pregnancy loss are not known, animal studies indicate that sperm DNA damage can lead to abnormal embryo development and impaired embryo implantation (Ahmadi and Ng, 1999; Fatehi *et al*., 2006; Perez-Crespo *et al*., 2008). The findings of this study and the data on sperm DNA damage and pregnancy rates after IVF (modest effect) and ICSI (no measurable effect) (Collins *et al*., 2008) suggest that ICSI may lead to a higher birth rate for men with sperm DNA damage.

The findings of this study (i.e. the association between sperm DNA damage and pregnancy loss) stress the importance of developing strategies to reduce sperm DNA damage in humans. Eliminating exposure to environmental toxins and reducing testicular hyperthermia may help optimize sperm DNA integrity (Fraga *et al*., 1991; Evenson and Jost, 2000). Although the data on vitamin supplementation are inconclusive, there may be some benefit in treating men with antioxidant vitamins (Greco *et al*., 2005; Silver *et al*., 2005; Menezo *et al*., 2007). Varicocele repair may also reduce sperm DNA damage, particularly, in those men with high levels of baseline sperm DNA damage (Zini *et al*., 2005a,b; Werthman *et al*., 2007).

The findings of this systematic review demonstrate an important relationship between sperm DNA damage and spontaneous pregnancy loss after IVF and IVF/ICSI. Although the number of events is relatively small and the study characteristics are variable, the data are significant enough to justify the clinical application of sperm DNA integrity tests in the context of IVF and IVF/ICSI. The data also provide a rationale for conducting further research aimed at evaluating the underlying mechanism(s) responsible for the increased pregnancy loss in couples with sperm DNA damage.

References

Ahmadi A, Ng SC. Fertilizing ability of DNA-damaged spermatozoa. *J Exp Zool* 1999;**284**:696–704.

Benchaib M, Lornage J, Mazoyer C, Lejeune H, Salle B, Francois Guerin J. Sperm deoxyribonucleic acid fragmentation as a prognostic indicator of assisted reproductive technology outcome. *Fertil Steril* 2007;**87**: 93–100.

Borini A, Tarozzi N, Bizzaro D, Bonu MA, Fava L, Flamigni C, Coticchio G. Sperm DNA fragmentation: paternal effect on early post-implantation embryo development in ART. *Hum Reprod* 2006;**21**:2876–2881.

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**:1401–1408.

Bungum M, Humaidan P, Axmon A, Spano M, Bungum L, Erenpreiss J, Giwercman A. Sperm DNA integrity assessment in prediction of assisted reproduction technology outcome. *Hum Reprod* 2007;**22**:174–179.

Bungum M, Spano M, Humaidan P, Eleuteri P, Rescia M, Giwercman A. Sperm chromatin structure assay parameters measured after density gradient centrifugation are not predictive for the outcome of ART. *Hum Reprod* 2008;**23**:4–10.

Carrell DT, Wilcox AL, Lowy L, Peterson CM, Jones KP, Erickson L, Campbell B, Branch DW, Hatasaka HH. Elevated sperm chromosome aneuploidy and apoptosis in patients with unexplained recurrent pregnancy loss. *Obstet Gynecol* 2003;**101**:1229–1235.

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**: 121–124.

Collins JA, Barnhart KT, Schlegel PN. Do sperm DNA integrity tests predict pregnancy with in vitro fertilization? *Fertil Steril* 2008;**89**: 823–831.

Deville WL, Buntinx F, Bouter LM, Montori VM, de Vet HC, van der Windt DA, Bezemer PD. Conducting systematic reviews of diagnostic studies: didactic guidelines. *BMC Med Res Methodol* 2002;**2**:9.

Egger M, Smith GD, Altman DG. *Systematic Reviews in Health Care: Meta-analysis in Context*. London: BMJ Publishing, 2001.

Evenson D, Jost L. Sperm chromatin structure assay is useful for fertility assessment. *Methods Cell Sci* 2000;**22**:169–189.

Evenson DP, Darzynkiewicz Z, Melamed MR. Relation of mammalian sperm chromatin heterogeneity to fertility. *Science* 1980;**210**:1131–1133.

Evenson DP, Jost LK, Marshall D, Zinaman MJ, Clegg E, Purvis K, de Angelis P, Claussen OP. Utility of the sperm chromatin structure assay as a diagnostic and prognostic tool in the human fertility clinic. *Hum Reprod* 1999;**14**:1039–1049.

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**:176–188.

Fernandez-Gonzalez R, Moreira PN, Perez-Crespo M, Sanchez-Martin M, Ramirez MA, Pericuesta E, Bilbao A, Bermejo-Alvarez P, Hourcade Jde D, Fonseca FR *et al.* Long-term effects of mouse intracytoplasmic sperm injection with DNA-fragmented sperm on health and behavior of adult offspring. *Biol Reprod* 2008;**78**:761–772.

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 USA* 1991;**88**:11003–11006.

Frydman N, Prisant N, Hesters L, Frydman R, Tachdjian G, Cohen-Bacrie P, Fanchin R. Adequate ovarian follicular status does not prevent the decrease in pregnancy rates associated with high sperm DNA fragmentation. *Fertil Steril* 2008;**89**:92–97.

Gandini L, Lombardo F, Paoli D, Caruso F, Eleuteri P, Leter G, Ciriminna R, Culasso F, Dondero F, Lenzi A *et al.* Full-term pregnancies achieved with ICSI despite high levels of sperm chromatin damage. *Hum Reprod* 2004;**19**:1409–1417.

Greco E, Iacobelli M, Rienzi L, Ubaldi F, Ferrero S, Tesarik J. Reduction of the incidence of sperm DNA fragmentation by oral antioxidant treatment. *J Androl* 2005;**26**:349–353.

Henkel R, Kierspel E, Hajimohammad M, Stalf T, Hoogendijk C, Mehnert C, Menkveld R, Schill WB, Kruger TF. DNA fragmentation of spermatozoa and assisted reproduction technology. *Reprod Biomed Online* 2003;**7**: 477–484.

Irvine DS, Twigg JP, Gordon EL, Fulton N, Milne PA, Aitken RJ. DNA integrity in human spermatozoa: relationships with semen quality. *J Androl* 2000;**21**:33–44.

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.

Lin MH, Kuo-Kuang Lee R, 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.

Menezo YJ, Hazout A, Panteix G, Robert F, Rollet J, Cohen-Bacrie P, Chapuis F, Clement P, Benkhalifa M. Antioxidants to reduce sperm DNA fragmentation: an unexpected adverse effect. *Reprod Biomed Online* 2007;**14**: 418–421.

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.

Perreault SD, Aitken RJ, Baker HW, Evenson DP, Huszar G, Irvine DS, Morris ID, Morris RA, Robbins WA, Sakkas D *et al.* Integrating new tests of sperm genetic integrity into semen analysis: breakout group discussion. *Adv Exp Med Biol* 2003;**518**:253–268.

Primakoff P, Myles DG. Penetration, adhesion, and fusion in mammalian sperm-egg interaction. *Science* 2002;**296**:2183–2185.

Shen H, Ong C. Detection of oxidative DNA damage in human sperm and its association with sperm function and male infertility. *Free Radic Biol Med* 2000;**28**:529–536.

Silver EW, Eskenazi B, Evenson DP, Block G, Young S, Wyrobek AJ. Effect of antioxidant intake on sperm chromatin stability in healthy nonsmoking men. *J Androl* 2005;**26**:550–556.

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.

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**:1289–1295.

Werthman P, Wixon R, Kasperson K, Evenson DP. Significant decrease in sperm deoxyribonucleic acid fragmentation after varicocelectomy. *Fertil Steril* 2007. Epub ahead of print.

Zini A, Bielecki R, Phang D, Zenzes MT. Correlations between two markers of sperm DNA integrity, DNA denaturation and DNA fragmentation, in fertile and infertile men. *Fertil Steril* 2001;**75**:674–677.

Zini A, Fischer MA, Sharir S, Shayegan B, Phang D, Jarvi K. Prevalence of abnormal sperm DNA denaturation in fertile and infertile men. *Urology* 2002;**60**:1069–1072.

Zini A, Blumenfeld A, Libman J, Willis J. Beneficial effect of microsurgical varicocelectomy on human sperm DNA integrity. *Hum Reprod* 2005a;**20**:1018–1021.

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* 2005b;**20**:3476–3480.

Submitted on June 2, 2008; resubmitted on July 2, 2008; accepted on July 24, 2008