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

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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; Benchabib et al., 2006).
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. 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. 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).
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 for heterogeneity of the studies was small, the ORs of the individual studies were all (with 95% CI, 1.01, 4.60; P < 0.05) and TUNEL studies (six 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

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

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>ART</th>
<th>Assay</th>
<th>Population</th>
<th>Study design</th>
<th>PL-Def</th>
<th>Female Dx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Check et al. (2005)</td>
<td>104</td>
<td>ICSI</td>
<td>SCSA</td>
<td>failed IVFx2</td>
<td>unspecified</td>
<td>per CP</td>
<td>unspecified</td>
</tr>
<tr>
<td>Zini et al. (2005a,b)</td>
<td>60</td>
<td>ICSI</td>
<td>SCSA</td>
<td>unspecified</td>
<td>prospective</td>
<td>per CP</td>
<td>&lt;40</td>
</tr>
<tr>
<td>Borini et al. (2006)</td>
<td>82</td>
<td>IVF</td>
<td>TUNEL</td>
<td>unspecified</td>
<td>unspecified</td>
<td>per CP</td>
<td>unspecified</td>
</tr>
<tr>
<td>Benchab et al. (2007)</td>
<td>84</td>
<td>IVF</td>
<td>TUNEL</td>
<td>unspecified</td>
<td>unspecified</td>
<td>per CP</td>
<td>unspecified</td>
</tr>
<tr>
<td>Lin et al. (2008)</td>
<td>218</td>
<td>ICSI</td>
<td>TUNEL</td>
<td>unspecified</td>
<td>prospective</td>
<td>per CP</td>
<td>unspecified</td>
</tr>
<tr>
<td>Bungum et al. (2007)</td>
<td>86</td>
<td>ICSI</td>
<td>SCSA</td>
<td>male factor</td>
<td>prospective</td>
<td>per CP</td>
<td>&lt;40, FSH&lt;15</td>
</tr>
<tr>
<td>Frydman (2008)</td>
<td>117</td>
<td>IVF</td>
<td>TUNEL</td>
<td>unspecified</td>
<td>unspecified</td>
<td>per CP</td>
<td>&lt;40, FSH&lt;10</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Study</th>
<th>ART</th>
<th>Assay</th>
<th>PL (%)</th>
<th>Abn Test* (%)</th>
<th>Sens</th>
<th>Spec</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Check et al. (2005)</td>
<td>ICSI</td>
<td>SCSA</td>
<td>47</td>
<td>24</td>
<td>0.31</td>
<td>0.83</td>
<td>0.63</td>
<td>0.58</td>
</tr>
<tr>
<td>Zini et al. (2005a,b)</td>
<td>ICSI</td>
<td>SCSA</td>
<td>16</td>
<td>19</td>
<td>0.40</td>
<td>0.85</td>
<td>0.33</td>
<td>0.88</td>
</tr>
<tr>
<td>Borini et al. (2006)</td>
<td>IVF</td>
<td>TUNEL</td>
<td>6</td>
<td>11</td>
<td>0.91</td>
<td>0.94</td>
<td>0.50</td>
<td>0.99</td>
</tr>
<tr>
<td>Benchab et al. (2007)</td>
<td>IVF</td>
<td>TUNEL</td>
<td>25</td>
<td>25</td>
<td>0.97</td>
<td>0.99</td>
<td>0.97</td>
<td>0.99</td>
</tr>
<tr>
<td>Lin et al. (2008)</td>
<td>ICSI</td>
<td>TUNEL</td>
<td>15</td>
<td>15</td>
<td>0.50</td>
<td>0.91</td>
<td>0.50</td>
<td>0.91</td>
</tr>
<tr>
<td>Bungum et al. (2007)</td>
<td>IVF</td>
<td>SCSA</td>
<td>10</td>
<td>17</td>
<td>0.29</td>
<td>0.84</td>
<td>0.17</td>
<td>0.92</td>
</tr>
<tr>
<td>Frydman (2008)</td>
<td>ICSI</td>
<td>SCSA</td>
<td>19</td>
<td>40</td>
<td>0.50</td>
<td>0.63</td>
<td>0.24</td>
<td>0.84</td>
</tr>
</tbody>
</table>

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 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 (Benchbaib 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.

**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 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 x 2 table could not be constructed. An association between sperm DNA damage and pregnancy loss has also been observed in non-IVF studies. Indeed, Eveson 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 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; Eveson 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; Menez 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


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