Chapter 8

Sperm DNA damage does not influence reproductive outcome in patients undergoing IUI or ART

Introduction

To date, the short and long term ramifications of successful fertilization and development with DNA damaged spermatozoa are unknown. 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 beyond which these processes are impaired (Ahmadi and Ng, 1999a). 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).

Sperm DNA integrity neither affects fertilization nor early embryo development since the embryonic genome is not expressed until after the second cleavage division (Braude et al., 1988; Tesarik et al., 2002). However, once the paternal genome is active, it results in poor blastocyst development, unequal cleavage (Seli et al., 2004; Tesarik et al., 2004; Tesarik, 2005), implantation failure or early fetal loss (Paul et al., 2008a, 2008b; Ahmadi and Ng, 1999b, Perez-Crespo et al., 2008). The mammalian oocyte is capable of repairing DNA damage throughout oogenesis and provides gene products that are responsible for repairing DNA damage in both parental genomes after fertilization. Small DNA damages in sperm are repaired by pre- and post
replication repair mechanisms, but large DNA damages cannot be repaired (Brandiff and Pedersen, 1981; Ashwood and Edwards, 1996), resulting in pregnancy loss, birth of offspring’s with major or minor congenital malformations, severe dysmorphogenesis or increased predisposition to certain cancers like retinoblastoma (Shamsi et al., 2008). These observations have raised concerns regarding the safety of using DNA-damaged sperm for ART and have led investigators to recommend assessment of sperm DNA damage as a part of ART programs (Perreault et al., 2003).

Sperm DNA fragmentation analysis is a potentially valuable tool to reveal the paternal origin of some unexplained repeated ICSI failures in term of fertilization and implantation failures (Tesarik et al., 2004). It may even help to decide the choice of the most efficient ART procedure (Bungum et al., 2007) to reduce the paternal negative contribution. These data provide a clinical indication for the evaluation of sperm DNA damage prior to infertility treatment and a rationale for further investigating the association between sperm DNA damage and pregnancy loss (Zini et al., 2008).

Indeed, the results of a study conducted by our own group in 7770 subjects presenting for infertility evaluation from 1993 to 2005 provided the first evidence that the quality of human semen evaluated for infertility is deteriorating in the southern part of the India over the years (Adiga et al., 2008). Based on the presence of an association between sperm DNA damage, age, standard semen parameters, presence of varicocele and alcohol consumption in infertile, Indian men (Chapter 4), it has been planned to assess the influence of sperm DNA damage on overall reproductive outcome, with specific reference to risk of adverse reproductive events.
Materials and Methods

Subjects:
Patients visiting the infertility clinic of Kasturba Medical College for fertility evaluation and treatment participated in this prospective study. This study included a total of 491 men (291 and 200 subjects in the IUI and ART categories, respectively). A detailed medical history was obtained from the participants and their partners including reproductive history and infertility evaluation. Maximal care was exerted to include patients with normal female partners i.e., normal reproductive history, normal ovulation (by follicular ultrasound study), normal luteal phase progesterone levels and tubal patency (hysterosalphingogram) in the present study. All subjects were asked to provide semen samples after 3-5 days of ejaculatory abstinence. Semen specimens were produced by masturbation directly into a sterile plastic container, in a room specially provided for this purpose and located adjacent to the laboratory.

Analysis of semen characteristics:
Routine andrological examination of the semen (volume, count, motility, morphology and vitality) has been performed according to WHO criteria (WHO, 1999). The reference values of semen variables and nomenclature for some semen variables used throughout the thesis has been presented in Chapter 3.

Terminal deoxy nucelotidyl transferase mediated nick end labeling (TUNEL) assay:
The assay was carried out according to standard protocol (Piqueras et al., 1996) provided by the manufacturer. One drop of semen sample was placed on a poly-L-lysine coated cover slip and was allowed to dry at room temperature. The cells were fixed in 4% paraformaldehyde solution for 30 minutes. The sperm membranes were permeabilized by treating with 0.2% triton-X 100 for 30 minutes (on ice) and then washed three times with PBS. The sperm cells were incubated in terminal deoxynucleotidyl transferase (TdT) and nucleotide mix (Apoalert DNA fragmentation
assay kit, Cat No. 630108, Clontech, USA) for 1 hour at 37°C in a humidified incubator. The cells were washed three times with PBS and counterstained with Propidium Iodide solution (10 µg/ml) and mounted on a glass slide. TUNEL positive cells exhibited a strong nuclear green fluorescence which was observed under fluorescence microscope (Imager-A1, Zeiss, Germany) equipped with a 490 nm excitation filter. A total of 2000 spermatozoa were assessed per sample in random fields. DNA damage was expressed as percentage of TUNEL positive spermatozoa.

**Sperm Chromatin Structure Assay (SCSA):**

Sperm DNA damage was measured by SCSA following the procedure as described (Evenson *et al.*, 1999). In the present study, as the SCSA was conducted in a different laboratory, located at a distance of approximately 350 km, the samples were fixed, followed by storage at 4°C. Owing to the same reason, samples collected over the entire study period, were analysed periodically. The sperm density in the semen was adjusted to a concentration of approximately 1-2 millions/ml and then centrifuged at 200 x g for 10 minutes. The supernatant was removed and pellet was resuspended in 200 µl of PBS. The spermatozoa were fixed in 70% chilled ethanol and stored at 4°C in cryovials until analysis. Maximal care was taken while shipping of samples to the SCSA laboratory; the samples were shipped in commercial insulated shipping containers. Small chunks of ice were first placed at the bottom of the shipping container, and then the sample box was placed in the centre of shipping box, followed by placement of ice over and around the box. On the day of analysis, an aliquot of ethanol fixed sperm was washed with TNE buffer (0.01 M Tris, 0.15 M NaCl and 1 mM EDTA) and the pellet was resuspended in 200 µl TNE buffer and treated with 0.4 ml of acid/Triton X-100 solution (0.1% Triton X-100, 0.15 M NaCl and 0.08 M HCl in double distilled water at pH 1.2). After 30 seconds, 1.2 ml of staining solution (0.15 M NaCl, 0.1 M citric acid, 0.2 M Na₂PO₄, pH 6.0 and 6 µg of Acridine Orange) was added. The samples were analyzed on flow cytometer (FACS calibur, Becton Dickinson, San Jose, CA), equipped
with an air-cooled argon ion laser. The samples were excited at 488 nm, the green and red emission was collected at 530 nm and 585 nm respectively. The SCSA quantitates metachromatic shift from green (native, double-stranded DNA) to red (acid-denatured, single-stranded DNA) fluorescence. The extent of damage caused by acid denaturation was quantified by calculating alpha t values i.e the mean channel of red fluorescence/ (mean channel of red fluorescence + mean channel of green fluorescence). The alpha t values range between 0 and 1. Higher alpha t values indicate high degree of sperm DNA damage (Evenson et al., 1999).

**ART Techniques:**

*Controlled Ovarian Hyperstimulation (COH):*

All stimulation protocols in women during this period involved down regulation with GnRH analogue starting from luteal phase of the previous cycle (long protocol) followed by gonadotropin therapy after ensuring the adequate down regulation (Estradiol levels < 50 pg / ml, endometrium <5 mm). Only recombinant follicle stimulating hormone (rFSH) was used and the dose was individualized on the basis of patient age, ovulation history, and prior response to COH (the average starting dose is 250 IU). The dose of gonadotropin was adjusted after day six on the basis of serum estradiol level and ultrasound findings. The follicular development was further monitored using ultrasonography and serum estradiol. Follicular maturation was triggered by administering human chorionic gonadotropin (hCG) (Profasi 10000 IU, Serono) when at least three follicles reached 18 mm in diameter.

*Oocyte pick up and identification:*

The oocyte pick-up was done 36 hours after the administration of hCG. Ultrasound guided, transvaginal aspiration was performed under controlled pressure. The oocytes in the follicular fluid were identified and assessed for their maturity. The oocytes were further incubated for 2-4 hours for the final maturation to occur.
**In vitro fertilization (IVF):**

Sperm concentration was adjusted to 200,000 sperm/ml/oocyte and insemination was done in 80 µl droplet. The insemination dish was incubated at 37°C for 16 hours in 5% CO₂ (humidified) under standard laboratory conditions.

**Fertilization by Intra Cytoplasmic Sperm Injection (ICSI):**

Oocytes were denuded enzymatically using hyaluranidase (Medicult, Denmark) and quality was assessed. The oocytes were transferred to 5 µl IVF medium (Medicult, Denmark) droplet and microinjection of sperm was performed using Olympus-Narishige micromanipulator system. After successful injection, eggs were washed twice in IVF medium and incubated at 37°C for 16 hours in 5% CO₂ (humidified) under standard laboratory conditions.

**Assessment of fertilization and embryonic development:**

The fertilization was assessed under inverted microscope fitted with Hoffman optics (Olympus, Japan) and successful fertilization was confirmed by the presence of two pronuclei and polar bodies. The fertilized zygotes were cultured in IVF medium droplet for two-three days. The data on speed of cleavage, incidence of fragmentation, degeneration and embryonic arrest was recorded at regular intervals.

**Pregnancies after IUI, IVF and ICSI:**

Data on successful implantation as confirmed by elevated serum beta hCG level 14 days after embryo transfer/ IUI, clinical pregnancy as confirmed by the appearance of gestational sac four week after embryo transfer, incidence of miscarriage, fetal abnormalities and delivery were collected and analyzed for their possible association with sperm DNA damage.
Statistical Analysis:

The data obtained was entered and analysed using Statistical Package for Social Sciences (SPSS 16.0). The Kruskal-Wallis test which is the non-parametric alternative to one-way analysis of variance has been used. When the number of independent groups is 2, the Mann-Whitney test has been performed. As non-parametric tests have been applied, the values as expressed as median, followed by the 25\textsuperscript{th} percentile and 75\textsuperscript{th} percentile (represented within brackets). A P value < 0.05 was considered statistically significant.
Reagents

Sigma Aldrich, St. Louis, MO, USA

Nigrosin Cat No N4754
Poly-L-Lysine Cat No P4707
Paraformaldehyde Cat No P6148
Triton X-100 Cat No T8532
Potassium chloride Cat No P5405
Tris base Cat No T4661
Citric acid Cat No C7254
Propidium iodide Cat No P4170

Himedia, India

Sodium chloride Cat No MB023
Disodium hydrogen phosphate Cat No RM1154
Potassium dihydrogen phosphate Cat No RM249
Disodium Ethylene diamine tetra acetic acid Cat No MB011
Tris Hydrochloride Cat No RM613

Polysciences, Inc, USA

Acridine Orange C.I. 46005 Cat No 04539

Merck & Co, USA

Shorr stain Cat No UN1993
Xylene Cat No 1330-20-7
Hydrochloric acid Cat No 1003172500
Ammonia solution (about 25% pure) Cat No 1336-21-6

Fisher Scientific, UK

Methanol Cat No 32407

Hayman, UK

Absolute Alcohol (Ethanol)
**BDH Chemicals, UK**

Eosin  
Cat No 3419720

**Dako, California, USA**

Dako Fluorescence Mounting Medium  
Cat No S3023

**Sisco Research Laboratories, India**

DPX Mountant for histology  
Cat No 42848
Results

In the present study, assessment of DNA damage by TUNEL assay and SCSA was carried out in a total of 491 patients undergoing various infertility treatments, followed by an attempt to understand its influence on reproductive outcome. Although maximal care has been exerted to include patients with normal female partners with normal reproductive history, normal ovulation (by follicular ultrasound study), normal luteal phase progesterone levels and tubal patency (hysterosalpingogram), this inclusion criteria has been difficult to meet owing to the inherent nature of patients visiting the University infertility clinic between the period 2006-2010. The study was not exclusive of patients with only male factor and/or idiopathic infertility and identified approximately 66.4% of patients with female factor infertility. If this variable is not accounted for in the analysis, it is impossible to identify an association between sperm DNA damage and reproductive outcome. Hence factors such as age, body mass index (BMI) of the female partner, presence or absence female factor infertility were identified as variables that could affect the reproductive outcome. As a previous study (Chapter 4) identified a trend towards increase in DNA damage with advancing age of the subject, additionally the age of the participant was also taken into consideration for data analysis.

Preliminary data analysis revealed that DNA damage measured in terms of TUNEL positive sperm and alpha T by SCSA did not follow a normal distribution. Tests for normality revealed a statistically significant deviation (P<0.001) from normality, with a standard deviation greater than the mean (Figure 8.1). It was further evident that a majority of the subjects (90.6%) had DNA damage below a threshold of 26% by TUNEL assay. Only a small proportion of the study subjects (9.4%) had DNA damage between 27 and 98% by TUNEL assay. This difference in distribution of DNA damage can account for the above observation. A similar effect was also
observed in relation to alpha T values measured by SCSA (Figure 8.1). Hence log transformation of the two variables namely TUNEL positive sperm and alpha T which are both measures of DNA damage was carried out. The data did not conform to normality even after logarithmic transformation (Figure 8.2). Further, as there was a wide variation between logarithmic TUNEL positive sperm ($\log_{\text{tps}}$), logarithmic alpha T ($\log_{\text{scsa}}$) in relation to the variables considered to affect the reproductive outcome (age of the subject, maternal age and body mass index), a definitive relationship between these variables could not be established (Figure 8.3). Subsequent to this, the data analysis reduced to assessing if DNA damage influenced reproductive outcome, taking into consideration the presence or absence of a female factor. This was achieved by the Mann-Whitney test as the number of groups for analysis reduced to two. Logically, as the DNA damage between the group with or without female factor infertility was statistically insignificant, it was assumed that the association between DNA damage and female factor infertility is negligible and therefore overlooked (Table 8.1).

In ART, no significant differences with respect to the number of mature eggs retrieved, fertilization rate, embryonic arrest at one cell stage, embryonic cleavage delay on day 2 of development or embryonic fragmentation has been observed in the present study (Table 8.2). In order to assess the overall influence of sperm DNA damage on the reproductive outcome, patients who underwent infertility treatment, either in the form of IUI or ART was grouped into two main classes – pregnant and non-pregnant. This was followed by a comparison of the median distribution of DNA damage between the two groups. Overall, the results demonstrated no difference in DNA damage between the pregnant and non-pregnant groups (Table 8.3). Although the pregnant group had a lower median distribution of sperm with DNA damage, the
difference between the groups was statistically insignificant, revealing a lack of association between sperm DNA damage and pregnancy outcome in patients who underwent IUI and ART.

Furthermore, data related to adverse reproductive outcome such as clinical abortion and neonatal death due to extreme prematurity has been collected and analysed for possible association with sperm DNA damage. Patients with no adverse reproductive outcome i.e. uneventful delivery after sperm DNA damage assessment served as an internal control group against which the above was compared. The median distribution of DNA damage by both TUNEL and SCSA among these groups was insignificant (Table 8.4).
Figure 8.1: Mean Distribution of DNA damage by TUNEL assay and SCSA does not conform to normality
Figure 8.2: Distribution of DNA damage by TUNEL assay and SCSA does not conform to normality after logarithmic transformation
Figure 8.3: Scattered Distribution of BMI, age of female partner and age of subject in relation to DNA damage by TUNEL assay and SCSA
Table 8.1 – DNA damage in presence or absence of female factor infertility

<table>
<thead>
<tr>
<th>Category</th>
<th>N / Valid percent</th>
<th>TUNEL positive sperm</th>
<th>Alpha T by SCSA</th>
<th>Logarithm of TUNEL positive sperm</th>
<th>Logarithm of Alpha T by SCSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female factor infertility</td>
<td>326 (66.4%)</td>
<td>8 (4,16)</td>
<td>0.24 (0.21,0.26)</td>
<td>2.07 (1.38, 2.77)</td>
<td>-1.39 (-1.55,-1.34)</td>
</tr>
<tr>
<td>No female factor</td>
<td>165 (33.6%)</td>
<td>9 (4,14)</td>
<td>0.25 (0.21,0.26)</td>
<td>2.19 (1.38,2.63)</td>
<td>-1.35 (-1.55,-1.34)</td>
</tr>
</tbody>
</table>

Table 8.2: Effect on early embryogenesis

<table>
<thead>
<tr>
<th>Category</th>
<th>N</th>
<th>TUNEL positive sperm (%)</th>
<th>Alpha T (SCSA)</th>
<th>Logarithm of TUNEL positive sperm</th>
<th>Logarithm of Alpha T by SCSA</th>
<th>Mature oocytes</th>
<th>Fertilization Rate (%)</th>
<th>Embryos arrested in 1 cell stage</th>
<th>Cumulative fragmentation rate (%)</th>
<th>Embryonic delay (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnant</td>
<td>45</td>
<td>9 (3.5,21.5)</td>
<td>0.23 (0.21,0.25)</td>
<td>2.19 (1.24,3.06)</td>
<td>-1.46 (-1.54,-1.35)</td>
<td>7 (5,9)</td>
<td>85.71 (63.33,100)</td>
<td>1 (1,3)</td>
<td>12.17 (10,17.5)</td>
<td>11.11</td>
</tr>
<tr>
<td>Non-pregnant</td>
<td>155</td>
<td>11 (4,17)</td>
<td>0.24 (0.20,0.26)</td>
<td>2.29 (1.38,2.83)</td>
<td>-1.41 (-1.56,-1.34)</td>
<td>6 (4,9)</td>
<td>85.71 (66.67,100)</td>
<td>1(1,3)</td>
<td>14 (10,23.5)</td>
<td>29.03</td>
</tr>
</tbody>
</table>
### Table 8.3 – DNA damage in pregnant and non-pregnant groups of infertility treatment

<table>
<thead>
<tr>
<th>Category</th>
<th>N / Valid percent</th>
<th>TUNEL positive sperm</th>
<th>Alpha T by SCSA</th>
<th>Logarithm of TUNEL positive sperm</th>
<th>Logarithm of Alpha T by SCSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not pregnant</td>
<td>388 (70.2%)</td>
<td>9 (4,15)</td>
<td>0.25 (0.21,0.26)</td>
<td>2.19 (1.38, 2.70)</td>
<td>-1.38 (-1.55,-1.34)</td>
</tr>
<tr>
<td>Pregnant</td>
<td>103 (18.6%)</td>
<td>8 (4,17)</td>
<td>0.24 (0.24,0.26)</td>
<td>2.07 (1.38,2.83)</td>
<td>-1.41 (-1.55,-1.34)</td>
</tr>
</tbody>
</table>

### Table 8.4: Sperm DNA damage and risk of miscarriage

<table>
<thead>
<tr>
<th>Category</th>
<th>N</th>
<th>TUNEL positive sperm</th>
<th>Alpha T by SCSA</th>
<th>Logarithm of TUNEL positive sperm</th>
<th>Logarithm of Alpha T by SCSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>No adverse reproductive</td>
<td>43</td>
<td>8.5 (5,19.5)</td>
<td>0.24 (0.21,0.26)</td>
<td>2.1 (1.6, 2.9)</td>
<td>-1.41 (-1.55,-1.34)</td>
</tr>
<tr>
<td>outcome</td>
<td></td>
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<tr>
<td>Adverse reproductive</td>
<td>27</td>
<td>8.5 (3,31.25)</td>
<td>0.22 (0.20,0.26)</td>
<td>2.1 (1,3.4)</td>
<td>-1.48 (-1.57,-1.34)</td>
</tr>
<tr>
<td>outcome</td>
<td></td>
<td></td>
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Discussion

The result from this study suggests that sperm DNA damage does not influence the success of IUI. Although DNA damage in the sperm is expected to induce defective fertilization and variety of damage response in the embryos, this study did not show any significant differences with respect to fertilization rate, embryonic arrest at one cell stage, embryonic cleavage delay on day 2 of development or embryonic fragmentation and pregnancy rate in ART. In addition, although no significant association between sperm DNA integrity and pregnancy and/or risk of adverse reproductive outcome has been observed, patients with pregnancy loss displayed a tendency towards increased sperm DNA damage.

Majority of the clinical studies that have examined the relationship between sperm DNA damage and reproductive outcome in the context of natural and ART pregnancies are small, heterogeneous, poorly designed and most importantly, do not control for female factors (Collins et al., 2008; Zini and Sigman, 2009). The present study therefore assumes greater clinical importance as a genuine effort has been made to understand the impact of compromised DNA integrity and reproductive outcome taking into consideration factors such as maternal age, maternal body mass index and the presence or absence of female factor infertility.

Although many tests have been developed to detect and investigate sperm DNA damage, the TUNEL and SCSA has been chosen in the present study as these assays have been widely used in clinical investigations. Further, in clinical practice, threshold values of sperm DNA damage beyond which embryo development and pregnancy may be impaired have been reported for only for the TUNEL assay (Sergerie et al., 2005) and SCSA (Evenson et al., 1999). The TUNEL
assay has been however been reported to consistently underestimate DNA damage in infertile men (Mitchell et al., 2011).

Preliminary evidence for consequences of compromised genomic integrity of the male germ line has been derived from animal studies, which have proven to be vital to our understanding of basic biological mechanisms in the context of fertilization. As DNA damage in the paternal germ line can be experimentally induced, in animal models, clear and strong associations have been shown between damage to the paternal genome and embryo development including effects on new born and subsequent generations (Auroux et al., 1990; Hales et al., 1992). Additionally, damage to sperm DNA has been clearly shown to affect fertilization rate. Exposure of male mice to acrylamide, etoposide and malphalan at post meiotic stages of spermatogenesis has been reported to result in a significant decline in the fertilization rate (Marchetti et al., 2004). Treatment of male rats with cyclophosphamide at a dose of 6 mg/kg/day for 4 weeks followed by mating with untreated females has been known to result in significant alteration in fertilization rates (Barton et al., 2005). Report of factors from damaged sperm with potential ability to affect its DNA integrity and hinder embryo implantation in murine model has been reported (Perez-Crespo et al., 2008). Additionally, injecting oocytes with spermatozoa known to contain DNA lesions leading to multigenerational effects have also been reported (Fernandez-Gonzalez et al., 2008). More recently, observations of impaired placental steroid metabolism between pregnancies conceived naturally and with ART (Collier et al, 2009) and transgenerational changes in somatic and germ line genetic integrity of first-generation offspring derived from the DNA damaged sperm in murine model (Adiga et al., 2010) have added to concerns regarding possible long-term consequences of using sperm with compromised genomic integrity.
Although similar experiments are not feasible in human beings, the above data provides a clear warning and rationale for assessment of DNA integrity in humans. In view of the above-mentioned worrisome postnatal effects observed in animal studies, the need for more studies to assess the relationship between sperm DNA damage and late reproductive outcome (pregnancy loss, delivery rate and neonatal health) that can yield high quality clinical data has been stressed (Barratt et al., 2010). Surprisingly, there are no data relating sperm DNA damage to late fetal development or post-natal health in humans (Zini et al., 2008). The present study is therefore unique as the female partners of all the study subjects have been followed until either uneventful delivery or adverse reproductive outcome such as clinical abortion or neonatal death due to extreme prematurity.

In humans, several studies suggest that sperm DNA damage is associated with lower rates of natural insemination and IUI pregnancies (Irvine et al., 2000; Morris et al., 2002; Benchaib et al., 2003). Indeed, couples in whom the husband has a high percentage of spermatozoa with DNA damage have a very low potential for natural fertility and a prolonged time to pregnancy (Evenson et al., 1999; Spano et al., 2000; Loft et al., 2003). A recent metaanalysis indicates a strong association between sperm DNA damage and failure to achieve a natural pregnancy (Evenson and Wixon, 2008). High levels of sperm DNA damage have generally been associated with lower IUI pregnancy rates (Duran et al., 2002; Muriel et al., 2006b; Bungum et al., 2004, Bungum et al., 2007).

From an ART perspective, a negative association between strand breaks in sperm DNA and fertilization rate has been reported (Sun et al., 1997; Lopes et al., 1998b). Based on a systematic review of literature, a meta-analysis highlighted that sperm DNA damage is associated with
lower natural, IUI and IVF pregnancy rates but not with ICSI pregnancy rates. Interestingly, this review which included 11 studies, involving 1549 cycles of treatment (IVF and ICSI) with 640 pregnancies and 122 pregnancy losses, pointed out that compromised DNA integrity of the sperm is associated with a significantly increased risk of pregnancy loss after IVF and ICSI (Zini et al., 2008).

While exploring the relationship between sperm DNA damage and reproductive outcome in the context of ART, it is imperative to understand the extent of DNA damage in individual spermatozoa and how different sperm processing techniques can remove DNA damaged sperm. In techniques, such as IVF or ICSI, although a high percentage of sperm in a sample may have damaged DNA, sperm processing is routinely employed and hence the possibility of selection of non-damaged sperm cannot be overruled (Sakkas and Alvarez, 2010). In this regard, both discontinuous gradient and swim-up has been reported to effectively eliminate sperm with DNA damage, allowing for embryo development (Hashimoto et al., 2008). Subsequently, the first study to compare the effects of gradient-density centrifugation and swim-up techniques on sperm apoptosis using flow cytometry also suggested that both the sperm preparation methods allow obtaining a sperm population with low percentage of apoptotic sperm (Ricci et al., 2008). Yet another interesting observation where a high DNA fragmentation index (DFI%) among spermatozoa in raw semen was related to low success after intrauterine insemination (Bungum et al., 2007) while from that of prepared sperm populations actually used for the insemination had low and normal DFI% has also been reported recently (Bungum et al., 2008). Supporting the above contention, it has been revealed that currently used methods of sperm selection are equally effective in eliminating sperm with DNA damage (Chapter 7). Therefore it is hypothesized that the use of sperm processing, and consequently, the reduced risk of using a genetically
incompetent sperm for IUI/ART could account for lack of association between sperm DNA damage, pregnancy and failure to conceive, observed in the present study.

While preimplantation embryos derived from fertilization with DNA damaged sperm have been reported to display different survival strategies (Shimura et al., 2002a, Shimura et al., 2002b; Barton et al., 2005, Toyoshima et al., 2005; Adiga et al., 2007a; 2007b), in humans, embryos derived from sperm with elevated levels of DNA fragmentation has been known to result in higher proportion of heavily fragmented embryos on Day 2 and a significantly higher number of embryos arrested at the 6-8 cell stage despite prolonged culturing (Virant-Klun et al., 2002). However, no such effects were observed in our study and overall, the results of our study are in agreement with other studies where lack of association between DNA damage in ejaculated sperm and fertilization rates in IVF and ICSI has been reported (Larson-Cook et al., 2000; Tomlinson et al., 2001; Larson-Cook et al., 2003; Henkel et al., 2004; Virro et al., 2004; Lin et al., 2008).

In this study, neither the TUNEL assay which measures DNA strand breaks nor the sperm chromatin structure assay (SCSA), which measures susceptibility to DNA denaturation in situ, was predictive adverse reproductive outcome when IUI or ART is employed. Although previous studies have reported the miscarriage rate in those who underwent assisted fertilization programs to be between 18–34% (Bulleti et al., 1996) in the present study, a miscarriage rate close to 40% has been observed, suggesting that compromised sperm DNA integrity may enhance the risk of miscarriage in the partners. It is possible that future studies with larger sample size may identify a relationship between compromised DNA integrity and increased risk of pregnancy loss after infertility treatment.
Hence, to conclude, sperm DNA damage does not appear to influence either the establishment or maintenance of a viable pregnancy in medically assisted conception (IUI) and assisted reproduction. Nevertheless, the study of sperm DNA damage is highly relevant in the era of ART, particularly ICSI, because (1) these technologies bypass the barriers of natural selection, 2) Infertile men, particularly those with severe male factor infertility, possess substantially more sperm DNA damage than do fertile men 3) Experimentally, sperm DNA damage has been shown to adversely affect embryo development, pregnancy rates and offspring health (Ahmadi and Ng, 1999b; Fernandez-Gonzalez et al., 2008; Zini et al., 2008). Owing to the limited the diagnostic and prognostic value of this one measure of sperm quality, currently, it cannot be recommended as a routine investigation to improve treatment of the infertile couple.