Fragmentation of DNA in morphologically normal human spermatozoa

Conrado Avendaño, M.Sc., Anahí Franchi, Ph.D., Steven Taylor, Ph.D., Mahmood Morshedi, Ph.D., Silvina Bocca, M.D., Ph.D., and Sergio Oehninger, M.D., Ph.D.

Department of Obstetrics and Gynecology, Jones Institute for Reproductive Medicine, Eastern Virginia Medical School, Norfolk, Virginia

Objective: To evaluate DNA fragmentation in spermatozoa with normal morphological appearance.

Design: Prospective study.

Setting: Academic tertiary center.

Patient(s): Fertile, subfertile, and infertile men were studied.

Intervention(s): Terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate–fluorescein nick-end labeling assay and morphology assessment by phase contrast in the swim-up fractions.

Main Outcome Measure(s): Simultaneous assessment of the percentage of normally shaped sperm and DNA fragmentation.

Result(s): No DNA fragmentation was found in spermatozoa with normal morphology in any of the samples from the fertile group. In only one sample from the subfertile group did we observe normally shaped sperm cells exhibiting DNA fragmentation. However, in all the samples from the infertile group, we observed normal spermatozoa with DNA fragmentation. Spermatozoa from this late group exhibited a high proportion of DNA damage.

Conclusion(s): In infertile men with moderate and severe teratozoospermia, the spermatozoa with apparently normal morphology present in the motile fractions after swim-up may have DNA fragmentation. (Fertil Steril 2009;91:1077–84. ©2009 by American Society for Reproductive Medicine.)

Key Words: DNA fragmentation, normal morphology, sperm selection, teratozoospermia

Sperm morphology has been recognized as an excellent predictor of the outcome of in vivo natural conception (1), intrauterine insemination (2) and conventional IVF therapies (3–5). In addition, the morphological normalcy of the sperm nucleus has been established as an important factor for achieving pregnancy after intracytoplasmic sperm injection (ICSI), as demonstrated recently by the use of high-magnification methods of sperm selection for microinjection (6, 7).

Ejaculated spermatozoa from infertile men reveal a variety of alterations of chromatin organization and structure, single-strand or double-strand DNA breaks, aneuploidy, and/or chromosome Y microdeletions (8, 9). Among such abnormalities, DNA damage, particularly in the form of DNA fragmentation, appears to be one of the main causes of decreased reproductive capacity of men, both in natural fertility as well as in assisted conception. Patients with oligoasthenoteratozoospermia, who more frequently require ICSI to overcome their infertility condition, have an increased sperm aneuploidy rate, despite a normal blood karyotype and increased levels of DNA fragmentation. Additional studies have shown that couples in whom pregnancy resulted in miscarriage demonstrated a trend toward poorer sperm DNA integrity, compared with fertile couples (10–14).

The prevalence of chromosomal abnormalities (de novo abnormalities) was found to be significantly higher among children conceived through ICSI than among naturally conceived children (15). Epigenetic abnormalities, such as errors in DNA methylation, have been linked to certain rare genetic diseases (Beckwith-Wiedemann and Angelman’s syndromes) and, although still rare, they are found in slightly higher numbers among children conceived through IVF-ICSI than among naturally conceived children (16).

Because the presence of a high percentage of spermatozoa with DNA damage may have a negative effect on the outcome of assisted reproductive technologies (17), the exclusion of spermatozoa with nuclear defects thus can be expected to decrease the probability of accidental injection of a DNA-damaged spermatozoon into the oocyte. Intracytoplasmic...

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Reprint requests: Sergio Oehninger, M.D., Ph.D., Department of Obstetrics and Gynecology, Jones Institute, Eastern Virginia Medical School, 601 Colley Avenue, Norfolk, Virginia 23507 (FAX: 757-446-8998; E-mail: oehninsc@evms.edu).
sperm injection bypasses natural sperm selection processes, because the embryologist subjectively chooses the spermatozoon to be injected into the oocyte on the basis of its motility and morphologic appearance. However, these selection criteria will not exclude, for example, the presence of a chromosomal abnormality, acquired during spermatogenesis as a result of an altered intratesticular environment, that may disrupt the fine-tuned mechanisms of chromosome segregation during spermatogenesis. Of particular concern is the fact that spermatozoa with normal morphology also may be aneuploid, canceling out the benefit of the careful selection of a normally shaped spermatozoon for ICSI, which is a process intended to reduce the risk of transmitting aneuploidy to the ICSI offspring (18, 19).

It is believed that if the genetic damage in the male germ is severe, embryonic development may arrest at the time that the paternal genome is switched on, resulting in a failed pregnancy (12, 17, 20). However, genetic and biological protection mechanisms do not necessarily preclude further embryonic development, because fertilization with damaged spermatozoa can result in a live-born infant (20, 21). In addition, reports regarding increased chromosomal abnormalities, minor or major birth defects, or childhood cancer suggest increased risks for babies born after ICSI (15, 17).

The findings mentioned in the previous paragraph suggest that paternal genomic alterations may compromise not only fertilization and early embryo quality but also embryo development and progression of pregnancy, resulting in spontaneous miscarriage. To date, a number of studies have highlighted the potential influence of a so-called paternal factor, but the relationship between sperm DNA integrity and early postimplantation embryo quality but also embryo development was assessed by using an HTR-IVOS semen analyzer (Hamilton Thorne Research, Beverly, MA) and were manually monitored, with fixed parameter settings (24). Motion parameters were examined after mixing the sperm suspension and loading a 5-μL aliquot into a Makler chamber (MidAtlantic Diagnostics Inc., Mt. Laurel, NJ). Sperm morphology was examined by using ×1,000 oil immersion microscopy, using strict criteria (3, 4, 25), as described elsewhere in reports from our laboratories (4, 5), after staining with STAT III Andrology Stain (MidAtlantic Diagnostics Inc.). Teratozoospermia was classified as severe (≤4% normal forms or “poor prognosis pattern”) or as moderate (5%–10% normal forms), in the same way as published elsewhere in reports from our laboratory (4, 5).

Motile spermatozoa were selected by swim-up that was performed in human tubal fluid (Irvine Scientific, Santa Ana, CA) that was supplemented with 0.2% human serum albumin (Irvine). The spermatozoa were washed twice with human tubal fluid–human serum albumin and were processed by centrifuge for 10 minutes at 300 × g. After the second wash, the supernatant was removed, and fresh human tubal fluid–human serum albumin was layered over the pellet and incubated for 60 minutes at 37°C. To retrieve the highly motile fraction, the volume from the top was removed. After the separation, the purified sperm population with high motility was resuspended in human tubal fluid–human serum albumin at a concentration of 5–10 × 10^6 spermatozoa per milliliter and was stored at −196°C without cryoprotectant until examined.

Semen samples were collected by masturbation into sterile cups after 2–4 days of sexual abstinence. The samples were allowed to liquefy for 30 minutes at room temperature, followed by assessment of semen characteristics and sperm parameters. Sperm concentration and progressive motility were assessed by using an HTR-IVOS semen analyzer (Hamilton Thorne Research, Beverly, MA) and were manually monitored, with fixed parameter settings (24). Motion parameters were examined after mixing the sperm suspension and loading a 5-μL aliquot into a Makler chamber (MidAtlantic Diagnostics Inc., Mt. Laurel, NJ). Sperm morphology was examined by using ×1,000 oil immersion microscopy, using strict criteria (3, 4, 25), as described elsewhere in reports from our laboratories (4, 5), after staining with STAT III Andrology Stain (MidAtlantic Diagnostics Inc.). Teratozoospermia was classified as severe (≤4% normal forms or “poor prognosis pattern”) or as moderate (5%–10% normal forms), in the same way as published elsewhere in reports from our laboratory (4, 5).

Materials and Methods

Subjects

This was a prospectively designed clinical study. The Institutional Review Board of Eastern Virginia Medical School approved the study, and all participants gave written informed consent. Ejaculates from 19 men were studied. The participating individuals were classified into three groups: [1] a fertile group (FER, n = 4), which included healthy male volunteers without any history of infertility problems and whose partners had conceived and delivered a child within the last 2 years; [2] a subfertile group (SF, n = 5), including men being evaluated for infertility (defined as the inability to achieve a pregnancy in a stable relationship for at least a 1-year period); and [3] an infertile group (INF, n = 10), which included patients participating in our ICSI program who had a diagnosis of male infertility associated with teratozoospermia and who had failed controlled ovarian hyperstimulation combined with intrauterine insemination therapy in the absence of female factors (23).

Sperm Preparation

Semen samples were collected by masturbation into sterile cups after 2–4 days of sexual abstinence. The samples were allowed to liquefy for 30 minutes at room temperature, followed by assessment of semen characteristics and sperm parameters. Sperm concentration and progressive motility were assessed by using an HTR-IVOS semen analyzer (Hamilton Thorne Research, Beverly, MA) and were manually monitored, with fixed parameter settings (24). Motion parameters were examined after mixing the sperm suspension and loading a 5-μL aliquot into a Makler chamber (MidAtlantic Diagnostics Inc., Mt. Laurel, NJ). Sperm morphology was examined by using ×1,000 oil immersion microscopy, using strict criteria (3, 4, 25), as described elsewhere in reports from our laboratories (4, 5), after staining with STAT III Andrology Stain (MidAtlantic Diagnostics Inc.). Teratozoospermia was classified as severe (≤4% normal forms or “poor prognosis pattern”) or as moderate (5%–10% normal forms), in the same way as published elsewhere in reports from our laboratory (4, 5).

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Samples were thawed in a 37°C water bath for 3 minutes immediately before assessment of DNA fragmentation and sperm shape (fixed wet preparation). An aliquot of approximately 25 μL was transferred to a multiwell slide (Cel-Line/Erie, Scientific Co, Portsmouth, NH) for examination of DNA fragmentation and morphological normalcy. Fragmentation of DNA and morphology were evaluated simultaneously in the same droplet and on the same sperm cell by using immunofluorescence and phase contrast, respectively. Each sample was analyzed in duplicate droplets, and the results were averaged.
Terminal Deoxynucleotidyl Transferase–Mediated Deoxyuridine Triphosphate–Fluorescein Nick-End Labeling Assay

Sperm DNA fragmentation was evaluated by TUNEL assay, using the In Situ Cell Death Detection Kit, Fluorescein (Roche Diagnostics GmbH, Mannheim, Germany). The assay uses fluorescein–dUTP to label single and double DNA strand breaks. It was performed according to the manufacturer’s instructions and as published elsewhere (13). Each sperm suspension was aliquoted in 25-μL drops on a multiwell slide, fixed with 4% paraformaldehyde, and permeabilized with 0.1% Triton X-100 in 0.1% sodium citrate. This was followed by incubation in the dark at 37°C for 1 hour in TUNEL reaction mixture containing 0.5 IU/μL of calf thymus terminal deoxynucleotidyl transferase and fluorescein–dUTP. Negative (omitting the enzyme terminal transferase) and positive (using deoxyribonuclease I, 1 U/mL for 20 minutes at room temperature) controls were performed in each experiment. Mounting Medium for Fluorescence (Vectashield; Vector Laboratories, Burlingame, CA) was added, before the evaluation, to protect the fluorescence. A total of 200 cells (100 per well) was randomly analyzed per sample, by using a Nikon Eclipse E600 (Nikon, Tokyo, Japan) microscope at ×1,000 oil-immersion objective. Each sperm cell was diagnosed as having DNA intact (no fluorescence) or as having DNA fragmentation (intense green nuclear fluorescence).

Simultaneous Examination of Normal Sperm Morphology and DNA Fragmentation

Immediately after TUNEL, and in the same droplets used for DNA fragmentation analysis, sperm morphology (fixed wet preparation without staining) was assessed in several randomly selected fields under phase-contrast microscopy using a Nikon Eclipse E600 equipped with a SPOT-RT Slider digital camera (Diagnostic Instruments, Inc., Sterling, MI) using a ×1,000 oil-immersion objective. A total of 400 cells were evaluated, in two droplets per patient. During this examination, every time a spermatozoon with normal morphology was found, the light immediately was switched to the fluorescence to determine DNA integrity. Spermatozoa were considered normal when the head had a normal shape, a symmetrical and oval head configuration, vacuoles occupying <20% of the head area, an acrosomal region comprising 40%–70% of the head area, a symmetrical insertion of the tail, and absence of midpiece or neck defects (25–27).

Statistical Analysis

Data are expressed as mean ± SD. Comparisons were performed by using the Kruskal-Wallis test. The Mann-Whitney test was used to identify significant differences between groups. P <.05 was considered statistically significant.

RESULTS

The characteristics of the ejaculates of the groups analyzed are summarized in Table 1. Sperm concentration was 41.9 × 10⁹/mL ± 18.4 × 10⁹/mL (range, 23.4–60.3 × 10⁹/mL) in the FER group, 52.5 × 10⁹/mL ± 28.2 × 10⁹/mL (range, 37.0–99.0 × 10⁹/mL) in the SF group, and 92.0 × 10⁹/mL ± 62.0 × 10⁹/mL (range, 58.0–136.0 × 10⁹/mL) in the INF group. The progressive motility was 64.2% ± 5.9% (range, 56.0–70.0%) for the FER group, 57.2% ± 21.5% (range, 24.0–77.0%) for the SF group, and 61.4% ± 16.8% (range, 41.4–95.1%) for the INF group. Results of morphology evaluation of the three groups stained with STAT III were as follows: 11.1% ± 0.6% (range, 10.5–12%) in the FER group, 4.3% ± 1.8% (range, 3.0–7.5%) in the SF group, and 3.8% ± 1.6% (range, 1.5–7.0%) in the INF group. The morphological abnormalities in all cases were predominantly in the head of the spermatozoa (severely amorphous heads), with occasional presence of vacuoles, and midpiece defects represented by cytoplasmic droplets; tail defects were present only in <5% of spermatozoa examined. There was no significant difference in sperm concentration (P >.05) and progressive motility (P >.05) between groups. The sperm morphology in the FER group was significantly higher

### Table 1

<table>
<thead>
<tr>
<th>Patient</th>
<th>Concentration (×10⁹/mL)</th>
<th>Motility (%)</th>
<th>Morphology (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FER1</td>
<td>29.0</td>
<td>56.0</td>
<td>10.5</td>
</tr>
<tr>
<td>FER2</td>
<td>23.4</td>
<td>66.0</td>
<td>12.0</td>
</tr>
<tr>
<td>FER3</td>
<td>55.1</td>
<td>65.0</td>
<td>11.0</td>
</tr>
<tr>
<td>FER4</td>
<td>60.3</td>
<td>70.0</td>
<td>11.0</td>
</tr>
<tr>
<td>SF1</td>
<td>56.0</td>
<td>51.0</td>
<td>4.0</td>
</tr>
<tr>
<td>SF2</td>
<td>37.0</td>
<td>59.0</td>
<td>3.5</td>
</tr>
<tr>
<td>SF3</td>
<td>99.0</td>
<td>77.0</td>
<td>7.5</td>
</tr>
<tr>
<td>SF4</td>
<td>44.5</td>
<td>75.0</td>
<td>3.0</td>
</tr>
<tr>
<td>SF5</td>
<td>26.0</td>
<td>24.0</td>
<td>3.5</td>
</tr>
<tr>
<td>INF1</td>
<td>82.0</td>
<td>95.1</td>
<td>3.5</td>
</tr>
<tr>
<td>INF2</td>
<td>71.0</td>
<td>63.4</td>
<td>5.0</td>
</tr>
<tr>
<td>INF3</td>
<td>58.0</td>
<td>41.4</td>
<td>2.0</td>
</tr>
<tr>
<td>INF4</td>
<td>136.0</td>
<td>60.0</td>
<td>7.0</td>
</tr>
<tr>
<td>INF5</td>
<td>97.5</td>
<td>59.0</td>
<td>4.5</td>
</tr>
<tr>
<td>INF6</td>
<td>4.0</td>
<td>63.0</td>
<td>1.5</td>
</tr>
<tr>
<td>INF7</td>
<td>32.0</td>
<td>47.0</td>
<td>3.0</td>
</tr>
<tr>
<td>INF8</td>
<td>201.5</td>
<td>52.0</td>
<td>3.0</td>
</tr>
<tr>
<td>INF9</td>
<td>174.5</td>
<td>49.0</td>
<td>4.0</td>
</tr>
<tr>
<td>INF10</td>
<td>64.0</td>
<td>84.4</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Note: Results of sperm concentration, motility and morphology in the studied ejaculates of the fertile (FER), subfertile (SF), and infertile (INF) men.

a P >.05 comparing the three groups regarding sperm concentration.
b P >.05 comparing the three groups regarding sperm motility.
c P <.05 comparing FER versus SF and FER versus INF.

Motile spermatozoa selected by swim-up were used to perform TUNEL assay under fluorescence field. Then a simultaneous assessment of normal sperm morphology and DNA fragmentation was performed under phase-contrast microscopy, switched to fluorescence, using the ×1,000 oil immersion objective.

The proportion of TUNEL-positive cells was 3.9% ± 2.9% (mean ± SD) for the FER group, 9.8% ± 5.5% for the SF group, and 21.2% ± 13.4% for the INF group. There was a statistically significant difference between the FER and INF groups (P < 0.05). These results are presented in Figure 1.

The percentages of normal sperm morphology in the fixed-wet preparations without staining, as examined under phase contrast, were as follows: FER group, 7.5% ± 0.6%; SF group, 1.3% ± 1.7%; and INF group, 1.0% ± 0.3% (P < 0.05, FER vs. INF). Next, the spermatozoa with normal morphology were examined with fluorescence for TUNEL analysis. Representative photomicrographs are shown in Figures 2 and 3. No DNA fragmentation was found in spermatozoa with normal morphology in any of the samples from the FER group. In only one sample from the SF group did we observe normal spermatozoa exhibiting fluorescence (TUNEL positive). However, in all the samples from the patients in the INF group, we observed spermatozoa with normal morphology and with DNA fragmentation, with a high proportion of TUNEL-positive cells in all these subjects (P < 0.05 in INF vs. FER). Results are summarized in Table 2.

DISCUSSION

To the best of our knowledge, this is the first study to simultaneously examine individual spermatozoa for normal morphology and DNA fragmentation. We demonstrate for the first time the presence of DNA fragmentation in spermatozoa with apparently normal morphology by using light microscopy. We examined the presence of DNA fragmentation in the elite population of swim-up–separated spermatozoa. The results demonstrated absent DNA fragmentation in normal spermatozoa from fertile men and demonstrated significantly higher levels in infertile men with moderate or severe teratozoospermia.

Further studies are needed to determine the presence of DNA fragmentation in infertile men with other sperm abnormalities, including various degrees of oligozoospermia. It is known that swim-up separation increases the population of highly motile and morphologically normal sperm (24). However, we did observe some discrepancy in the percentage of normal morphology in the raw (postliquefied) sample in air-dried and stained smears, compared with the results of the fixed-wet (unstained preparation) sample after swim-up and after TUNEL. This discrepancy can be explained by the fact that the recommended clinical procedures for evaluating human sperm morphology (air-dried and stained) can cause morphological artifacts and may not demonstrate small nuclear vacuoles (26–29). In addition, Meschede et al. (30) showed a significant difference between Papanicolaou stain (normal morphology: 31%) and wet preparations (12%). However, Oral and colleagues (31) compared Diff-Quik–stained and wet preparations for sperm morphology and did not find differences between the two methodologies.

Intracytoplasmic sperm injection is used primarily for the treatment of infertile men with very poor sperm quality, and therefore a main concern is the possible inadvertent use of DNA-damaged spermatozoa to fertilize the oocyte. Reports have suggested potential adverse consequences such as fertilization failure, early embryo death, spontaneous abortion, childhood cancer, and infertility in the offspring (17).

Previous observations that sperm DNA damage is common in infertile men, together with the concerning preliminary reports on genetic and epigenetic abnormalities in children conceived through ICSI, urged us to explore the subject of sperm DNA damage further. Deoxyribonucleic acid that possesses measurable damage (for example, DNA fragmentation) may cause misreading errors to occur during DNA replication, and this may cause de novo mutations (32). Recent data point to the fact that the integrity of the paternal genome may have a critical role in human reproductive potential. The impact of an altered paternal genome on conception may be as detrimental as the impact of an altered maternal one. However, the true effect of DNA fragmentation of mature male germ cells on conception is still largely unknown.

Several studies have shown increased levels of sperm nuclear DNA damage in infertile men with abnormal sperm parameters (concentration, motility, and morphology) (12, 17, 33). Moreover, Saleh et al. (34) showed that men with normal basic sperm parameters may have significant levels of DNA...
damage. Celik-Ozanci et al. (18) used phase-contrast microscopy and fluorescence in situ hybridization with centromeric probes for chromosomes X, Y, 10, 11, and 17 to evaluate human sperm shape and chromosomal aberrations in the same sperm cell of 15 men who presented for semen analysis. They found that sperm with normal shape could have chromosomal aberrations and concluded that sperm dimensions or shape are not reliable attributes in selection of haploid sperm for ICSI.

Burrello et al. (19) evaluated 10 patients with oligoasthenoteratozoospermia and 6 age-matched normozoospermic men. Those investigators analyzed morphology by using the same criteria recommended by the World Health Organization (27), but without Papanicolaou staining. The location of each spermatozoon was recorded by using an electronic microstage locator. Slides then were subjected to fluorescence in situ hybridization for chromosomes X, Y, 12. This study concluded that morphologically normal spermatozoa of oligoasthenoteratozoospermic patients carry an abnormal chromosomal constitution with the same frequency as that found in spermatozoa with an abnormal head shape.

By using high magnification for the morphological selection of motile spermatozoa, Berkovitz et al. (29) investigated

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**FIGURE 2**

Representative photomicrographs of the simultaneous assessment of normal sperm morphology and DNA fragmentation following swim up separation (left side: phase contrast; right side: TUNEL fluorescence). A: a normal spermatozoon (N) with DNA fragmentation; note three morphologically abnormal spermatozoa without DNA fragmentation; B: a normal spermatozoon (N) with DNA fragmentation; note several amorphous spermatozoa without DNA fragmentation; C: two normal spermatozoa (N), one of them with DNA fragmentation. Inset: photomicrograph at lower magnification showing the two normal spermatozoa in the same field.

the presence of large nuclear vacuoles and showed that ejaculates of men routinely referred for ICSI exhibited 30%–40% of spermatozoa with vacuolated nucleus. According to those investigators, in cases of spermatozoa with normal nuclear shapes but large vacuoles, the fertilization, embryonic cleavage, and implantation rates were normal; but the embryo developmental capacity in the later stages was compromised, as demonstrated by low pregnancy and high miscarriage rates. These results were supported by those of Thundathil et al. (35), who found that nuclear vacuoles in bovine sperm do not decrease fertilization rate but increase the rate of early embryonic death.

Tesarik et al. (20) analyzed the relationship between ICSI failure and sperm DNA fragmentation. Those investigators evaluated 18 infertile couples who had previously failed attempts of assisted reproductive techniques. In 8 couples, the adverse paternal effect did not produce any perceptible deterioration of zygote morphology. However, a late paternal effect was associated with an increased percentage of spermatozoa with fragmented DNA. Tesarik et al. (20) concluded that evaluation of sperm DNA integrity is useful for detecting late paternal effect, which is not associated with morphological abnormalities at the zygote and early cleavage stages.

FIGURE 3
Representative photomicrographs of the simultaneous assessment of normal sperm morphology and DNA fragmentation following swim up separation (left side: phase contrast; right side: TUNEL fluorescence). A: a normal spermatozoon (N) without DNA fragmentation; note several abnormal spermatozoa, one of them with DNA fragmentation; B: a normal (N) and an abnormal spermatozoon without DNA fragmentation; and C: an abnormal spermatozoon with DNA fragmentation.
Data from some publications have shown no relationship between sperm DNA damage and fertilization rates in ICSI (36). However, patients with low DNA fragmentation had a statistically significantly higher clinical pregnancy rate. These results may be accounted for by the fact that high DNA fragmentation does not preclude fertilization but may prevent blastocyst formation and/or successful embryo development (20, 21).

However, other investigators have concluded that DNA damage in spermatozoa is associated with reduced rate of IVF, impaired preimplantation development of the embryo, increased rates of early pregnancy loss, and poor fertility after natural or assisted conception (17). A number of theories have been advanced to explain the occurrence of sperm DNA fragmentation, including abortive apoptosis, oxidative stress associated with male genital tract infection, and defects of spermiogenesis (17, 37). The causes of sperm DNA damage appear to be multifactorial, and a solid conclusion about pathogenic mechanisms cannot yet be drawn.

It therefore is probable that the DNA damage present in a fertilizing spermatozoon will be transferred to the embryo. The fact that this damage may or not result in a given phenotype in the immediate offspring does not necessary mean, however, that the genetic disorders have not been transmitted to the progeny or that they will not become evident in future generations.

Under normal conditions, it has been speculated that the sperm DNA damage brought into the zygote effectively may be repaired by the oocyte. Shimura et al. (38) demonstrated the presence of a p53-dependent S-phase DNA damage checkpoint that could suppress DNA synthesis in both the male and female pronuclei before repair of the DNA damage. Nevertheless, it also is possible that the DNA repair capacity of the oocyte may be defective because of a high degree of DNA damage in the spermatozoon, or because of defects in the repair mechanisms themselves, as a consequence of factors such as maternal aging or in vitro culture conditions (39). In these situations, the damaged DNA could either remain unrepaired or be incorrectly repaired, leading to DNA mutations.

We conclude that [1] this is the first study to demonstrate that morphologically normal spermatozoa present in the motile sperm fractions of infertile men with moderate and severe

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**TABLE 2**

Swim up fractions: percentage of sperm with DNA fragmentation, percentage of sperm with normal morphology, and the proportion of normal sperm with DNA fragmentation.

<table>
<thead>
<tr>
<th>Patient</th>
<th>TUNEL (%)</th>
<th>Normal morphology (%)</th>
<th>Normal sperm with positive TUNEL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FER1</td>
<td>0.5 (1/200)</td>
<td>7.00 (28/400)</td>
<td>0.0 (0/28)</td>
</tr>
<tr>
<td>FER2</td>
<td>6.5 (13/200)</td>
<td>8.00 (32/400)</td>
<td>0.0 (0/32)</td>
</tr>
<tr>
<td>FER3</td>
<td>6.0 (12/200)</td>
<td>8.00 (32/400)</td>
<td>0.0 (0/32)</td>
</tr>
<tr>
<td>FER4</td>
<td>2.5 (5/200)</td>
<td>7.00 (28/400)</td>
<td>0.0 (0/28)</td>
</tr>
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<td>SF1</td>
<td>4.0 (8/200)</td>
<td>1.00 (4/400)</td>
<td>0.0 (0/4)</td>
</tr>
<tr>
<td>SF2</td>
<td>5.0 (10/200)</td>
<td>1.25 (5/400)</td>
<td>0.0 (0/5)</td>
</tr>
<tr>
<td>SF3</td>
<td>12.5 (25/200)</td>
<td>4.25 (17/400)</td>
<td>47.0 (8/17)</td>
</tr>
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<td>SF4</td>
<td>17.5 (35/200)</td>
<td>0.25 (1/400)</td>
<td>0.0 (0/1)</td>
</tr>
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<td>SF5</td>
<td>10.0 (20/200)</td>
<td>0.00 (0/400)</td>
<td>0.0 (0/0)</td>
</tr>
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<td>INF1</td>
<td>5.0 (10/200)</td>
<td>1.00 (4/400)</td>
<td>50.0 (2/4)</td>
</tr>
<tr>
<td>INF2</td>
<td>23.0 (46/200)</td>
<td>1.25 (6/400)</td>
<td>60.0 (3/5)</td>
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<tr>
<td>INF3</td>
<td>5.5 (11/200)</td>
<td>0.75 (3/400)</td>
<td>33.3 (1/3)</td>
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<td>INF4</td>
<td>27.0 (44/200)</td>
<td>1.50 (6/400)</td>
<td>50.0 (3/6)</td>
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<tr>
<td>INF5</td>
<td>26.0 (52/200)</td>
<td>0.75 (3/400)</td>
<td>66.6 (2/3)</td>
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<tr>
<td>INF6</td>
<td>38.5 (77/200)</td>
<td>0.5 (2/400)</td>
<td>50 (1/2)</td>
</tr>
<tr>
<td>INF7</td>
<td>39.5 (79/200)</td>
<td>1.75 (7/400)</td>
<td>42.9 (3/7)</td>
</tr>
<tr>
<td>INF8</td>
<td>31 (62/200)</td>
<td>1 (4/400)</td>
<td>25 (1/4)</td>
</tr>
<tr>
<td>INF9</td>
<td>10.5 (21/200)</td>
<td>1.25 (5/400)</td>
<td>40 (2/5)</td>
</tr>
<tr>
<td>INF10</td>
<td>6.5 (13/200)</td>
<td>1.25 (5/400)</td>
<td>20 (1/5)</td>
</tr>
</tbody>
</table>

Note: Results obtained from the swim up fractions of the three groups of patients. Results are expressed as: % TUNEL (number of TUNEL positive sperm cells/200 spermatozoa), % normal sperm morphology (number of normal sperm/400 spermatozoa), and the proportion of sperm with normal morphology that were TUNEL positive, calculated as the number of morphologically normal sperm being TUNEL positive/total number of spermatozoa with normal morphology.

a P < .05 FER versus INF.
b P < .05 FER versus SF and FER versus INF.
c P < .05 FER versus SF and FER versus INF.

teratozoospermia can have DNA fragmentation; [2] further investigations and larger sample sizes are necessary to evaluate the real impact of DNA fragmentation in pregnancy outcome in patients undergoing ICSI; [3] the results pose a question about the use of so-called normal morphology alone as a reliable attribute for the selection of sperm for ICSI in this group of patients; and [4] methods that allow for an accurate separation of viable sperm with intact DNA should be sought for optimizing ICSI outcome. To further improve pregnancy rates and to prevent early childhood disease, more research is necessary to investigate these important findings.

REFERENCES