Fragmentation of DNA in morphologically normal human spermatozoa

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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 observed 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 infertile 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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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 development in couples who are undergoing assisted reproductive techniques remains to be fully understood (12, 17, 20, 22).

The aims of this study were as follows: [1] to evaluate DNA integrity, as assessed by the terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate–fluorescein nick-end labeling (TUNEL) assay, in spermatozoa from fertile, subfertile, and infertile men and [2] to determine the incidence of DNA fragmentation in spermatozoa with normal morphology that were obtained from the separated fractions of highly motile sperm, because these are the cells that have a high probability of being chosen by the embryologist at the time of oocyte injection for ICSI.

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 $\times$1,000 oil immersion microscopy, using strict criteria (3, 4, 25), as described elsewhere in reports from our laboratories (4, 5), after staining with STAT III Andrology Stain (MidAtlantic Diagnostics Inc.). Teratozoospermia was classified as severe ($\leq$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 $\times$ 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 $\times$ 10^6 spermatozoa per milliliter and was stored at –196°C without cryoprotectant until examined.

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.