Relevance of testicular sperm DNA oxidation for the outcome of ovum donation cycles

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Objective: To determine the relevance of sperm DNA oxidation caused by free radicals in samples obtained via testicular biopsies by means of flow cytometry by correlating the measurements of 8-hydroxy-2′-deoxyguanosine (8-OHdG) with embryo features and pregnancy achievement.
Design: Prospective cross-sectional study.
Setting: Private University-affiliated setting.
Patient(s): Fifty-seven azoospermic patients undergoing testicular sperm extraction (TESE) were analyzed in their corresponding assisted reporductive technology cycles using ovum donation to standardize female's characteristics.
Intervention(s): None.
Main Outcome Measure(s): Quantification of the adduct 8-OHdG in testicular tissue samples, and its effect on markers of embryo quality and reproductive success, and its relevance as marker of TESE sperm quality.
Result(s): We found the status of sperm DNA oxidation to have very little clinical relevance for several parameters of embryo quality, fertilization rates, early (days 2–3) and late (days 5–6) development, and achievement of pregnancy.
Conclusion(s): The TESE obtained cells from azoospermic males do not possess a DNA oxidation status of significant importance in the success of assisted reproduction treatments, as determined by 8-OHdG measurement of each category of cell ploidy. (Fertil Steril® 2010;94:979–88. ©2010 by American Society for Reproductive Medicine.)

Key Words: Sperm DNA oxidation, TESE, ART, ovum donation

Among all the molecular factors implicated in male infertility, the greatest attention has been paid to the sperm DNA analysis (i.e., DNA fragmentation and oxidation, abnormal chromatin packaging, protamine deficiency, etc.), and the external factors compromising sperm molecular structure (1). This interest is because of the crucial function of sperm cells by which they carry the genetic information in the DNA molecule, and by which they condition conception and embryo and fetal development. Similarly, malformations and miscarriages may occur if this code is affected, consequences that have previously been associated with assisted reproduction treatments (2).

Reactive oxygen species (ROS) are derivatives of the normal aerobic metabolism, and play diverse physiologic roles such as sperm capacitation, acrosome reaction, and sperm–oocyte fusion. Abnormally high ROS levels can harm lipids, proteins, and nucleic acids structures (3), and can induce apoptosis through the expression of key genes and signalling pathways (4–6). In spermatozoa, its sensitivity depends on the balance between the total ROS amount and antioxidant defences (7).

We have previously demonstrated that glutathione peroxidase-4 activity in sperm is 10 times lower in infertile patients than in fertile donors (8), and the variations detected in sperm glutathione peroxidase system mRNAs and enzymes support the hypothesis that in vitro embryo development is conditioned by them (8, 9).

Oxidative stress is a known cause of sperm DNA damage, and our group has previously shown the implications of DNA fragmentation for assisted reproduction outcome, a correlation that occurs particularly in IVF treatment (10–12), in which sperm DNA fragmentation is weakly related with low fertilization rates and embryo quality. Marked nucleolar asynchrony is observed in zygotes from sperm samples with high DNA fragmentation levels, whereas no relation is found with pregnancy outcome in intrauterine inseminations (13). That said, the evidence in the literature regarding these

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tendencies is somewhat inconsistent (14, 15). Exposure of DNA to ROS also induces a range of damaging biochemical alterations, including crosslinking and base modifications, as well as DNA strand breakage (7, 16, 17), such as base deletion, and the formation of modified DNA bases (18). Of all of these alterations, 8-hydroxy-2′-deoxyguanosine (8-OHdG) has received most attention because of its mutagenic potential, because misreading occurs in DNA templates containing the modified base (19). This structure (8-OHdG) has previously been related to assisted reproductive technology (ART) success in ovum donation models (20).

Azoospermia is defined as the absence of sperm cells in several ejaculates, and occurs in approximately 1% of the general male population and in 10% to 15% of the infertile population (21, 22). In obstructive azoospermia (OA) the testes produce sperm normally, but the sperm cells do not reach the ejaculate because of an obstruction in the genital tract. Males with nonobstructive azoospermia (NOA) produce sperm cells under the threshold needed to be ejaculated (23). In approximately 45% to 50% of NOA cases and almost all cases of OA, motile sperm suitable for use in assisted reproduction can be found (24).

The probability of success in ART varies considerably when sperm cells are obtained through TESE procedures, but although we understand the molecular features of ejaculated sperm that lead to reproductive success, little data about the molecular characteristics of testicular sperm cells are currently available, mainly as a consequence of the low numbers of sperm available for study in these cases. Useful data are obtained with ART that permit close follow-up of embryo quality and evaluation of male infertility factors in ovum donation, because it reduces the variability within oocyte quality that female infertility may introduce (25, 26).

The aim of this study is to analyze the DNA oxidation levels in testicular cells of azoospermic males, and to determine the usefulness of the quantification of the extent of oxidative DNA damage in the different type of cells in the testis to predict intracytoplasmic sperm injection (ICSI) success, and employing the ovum donation model to standardize female characteristics.

MATERIALS AND METHODS

Institutional Approval and Informed Consent

This study was approved by the institutional review board on the use of human gametes in research at the Instituto Valenciano de Infertilidad, and it complies with the Spanish Law of Assisted Reproduction and the Spanish law of Biomedical Research. All patients signed an informed consent form approved by the Ethical Committee of this institution and based on the abovementioned laws and that of Human Rights in Research.

TESE (Testicular Sperm Extraction) and Cryopreservation

Samples were obtained from male partners of couples undergoing assisted reproduction treatments by means of TESE when absence of sperm had been confirmed in two consecutive sperm analysis, in cases where ovum donation was necessary because of severe female infertility factor to homogenize the oocyte quality. A total number of 57 TESE samples, 18 with OA and 39 with NOA, were obtained and analyzed between August 2006 and November 2007. All male patients had a normal karyotype and no microdeletions of the Y chromosome.

TESE was performed as previously described (26). In short, open testicular biopsies were performed by means of a 2% mepivacaine spermatic cord block. After opening the scrotal skin and tunica vaginalis, three small incisions were made in the tunica albuginea in different regions of each testicle, and small pieces of extruding testicular tissue were excised. Two fragments (one per testis) were embedded within Bouin's solution and were sent for histopathologic examination. The remaining fragments were taken to an adjacent laboratory for sperm retrieval. Testicular tissue was placed in 2 mL of Sperm Medium (Medicult, Jyllinge, Norway) and minced mechanically with sterile slides. The presence of sperm cells was observed under an inverted microscope at 3,400× magnification. If motile sperm were found, samples were divided into two aliquots: one was immediately frozen for later use in assisted reproduction techniques, as described elsewhere (27), whereas the other was analyzed for testicular tissue DNA oxidation, as described later.

When this initial microscopic evaluation did not reveal motile spermatozoa, the sperm suspension was transferred into a Falcon tube and centrifuged for 5 minutes at 600 × *g*. The pellet was resuspended in 0.5 mL of Sperm medium and incubated for 1 hour at 37°C and 5% CO_2. Thereafter, the presence of motile sperm was checked once again. If no sperm or only immotile spermatozoa were found after an extensive search lasting at least 2 hours, the sample was discarded (only patients presenting sperm cells within their TESE samples were included in the study). For freezing, an equal volume of Sperm Freezing Medium (Medicult) containing glycerol was added to the sperm pellet, which was then homogenized and left at room temperature for 10 minutes. Sperm samples were frozen into small tablets by leaving them on a dry ice surface for approximately 1 minute and were then transferred to prelabeled cryotubes that were subsequently plunged into liquid nitrogen tanks where they were stored until future use (28). For thawing, the pills were removed and transferred into 5 mL Falcon tubes, which were placed in an incubator at 37°C and 5% CO_2. Samples were washed again with Sperm Medium (Medicult) and centrifuged for 5 minutes at 600 × *g*. The supernatant was discarded and samples resuspended in variable amounts of medium. Thereafter, motile spermatozoa were checked again for ICSI.

Measurement of Testicular DNA Oxidation and Cell Ploidy

To measure DNA oxidation, we employed the OXI DNA assay kit (OxiDNA Assay, Calbiochem, Barcelona, Spain), which directly binds a fluorescent probe to the DNA adduct 8-OHdG, an oxidation product of the ROS attack (29). A major oxidative DNA product, 8-oxoguanine, is employed as a sensitive marker for DNA damage caused by oxygen-