Sperm selected by both birefringence and motile sperm organelle morphology examination have reduced deoxyribonucleic acid fragmentation

Andrea Garolla, M.D.,[a] Ilaria Cosci, B.Sc.,[a] Massimo Menegazzo, B.Sc.,[a] Raffaella De Palo, M.D.,[b] Guido Ambrosini, M.D.,[c] Barabara Sartini, B.Sc.,[a] Damiano Pizzol, M.D.,[a] and Carlo Foresta, Ph.D.[a]

[a] Section of Clinical Pathology and Unit for Human Reproduction Pathology, Department of Medicine, and [b] Institute of Gynecology and Obstetrics, University of Padova, Padova, Italy; and [c] Unit of Pathophysiology of Human Reproduction and Gametes Cryopreservation, Department of Gynecology and Obstetric, I University Hospital of Bari, Bari, Italy

Objective: To evaluate DNA fragmentation in single sperm selected by both birefringence and motile sperm organelle morphology examination (MSOME) with a single instrument.
Design: Prospective study.
Setting: University setting.
Patient(s): Semen samples from 33 normozoospermic subjects.
Intervention(s): Birefringence and MSOME to distinguish different categories of sperm: nonbirefringent (category A), birefringent (category B), birefringent with nuclear vacuoles (category C), and birefringent with no nuclear vacuoles (category D). From each semen sample, sperm of any category were selected and further analyzed by TUNEL test.
Main Outcome Measure(s): A total of 660 well-characterized sperm were evaluated for DNA fragmentation.
Result(s): Category A showed a low percentage of sperm with normal MSOME results (19.4%) and high prevalence of DNA fragmentation (70.3%). Category B had 81.8% normal MSOME results, and in this group 31.8% had fragmentated DNA. Category C showed 31.8% and 92.6% DNA fragmentation in sperm with small and large nuclear vacuoles, respectively. Birefringent sperm with normal MSOME results and no vacuoles showed the lowest percentage of fragmented DNA (2.8%).
Conclusion(s): Sperm selection by birefringence or MSOME alone had one-third probability to select sperm with fragmented DNA. The lowest percentage of DNA fragmentation was found in birefringent sperm with no nuclear vacuoles and normal MSOME results. We suggest combining both methods using a single microscope and selecting sperm without nuclear vacuoles to get sperm with a higher chance of having intact DNA. (Fertil Steril® 2014;101:647–52. ©2014 by American Society for Reproductive Medicine.)
Key Words: Birefringence, DNA fragmentation, male infertility, MSOME, sperm selection

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/garollaa-sperm-birefringence-msome-dna-fragmentation/

Use your smartphone to scan this QR code and connect to the discussion forum for this article now.*

* Download a free QR code scanner by searching for "QR scanner" in your smartphone's app store or app marketplace.

Infertility is clinically defined as failure of a couple to conceive after 1 year of regular sexual intercourse, and male factor is considered the sole cause in approximately 20% of cases (1, 2). Currently male infertility is evaluated through routine semen analysis, and in particular sperm concentration, count, motility, and morphology are considered. Although fertile men, as a group, have better sperm parameters than infertile men, there is significant overlap of these semen characteristics between the two groups (1, 2). In fact, approximately 15% of patients with male factor infertility have normal results on semen analysis (3), suggesting that conventional semen parameters are

Received August 9, 2013; revised October 18, 2013; accepted November 18, 2013; published online December 20, 2013.
A.G. has nothing to disclose. I.C. has nothing to disclose. M.M. has nothing to disclose. R.D.P. has nothing to disclose. G.A. has nothing to disclose. B.S. has nothing to disclose. D.P. has nothing to disclose. C.F. has nothing to disclose.
This study was supported by grants from the Italian Ministry of University and Research.
Reprint requests: Carlo Foresta, Ph.D., Section of Clinical Pathology, Unit for Human Reproduction Pathology, Department of Medicine, University of Padova, Via Gabelli 63, 35121 Padova, Italy (E-mail: carlo.foresta@unipd.it).

Fertility and Sterility® Vol. 101, No. 3, March 2014 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2013.11.029

poor predictors of reproductive outcome and that a definitive diagnosis of male infertility cannot be made just by a routine semen analysis. In light of these considerations, there exists a need for new markers of sperm quality that might better discriminate infertile from fertile men. Recently some authors investigated the role of sperm DNA fragmentation in male factor infertility (4, 5). Moreover, some studies suggested that sperm DNA fragmentation can be considered a better predictor of male fertility, pregnancy outcome, and adverse reproductive events than standard semen analysis (6, 7). In fact, sperm DNA damage has been related to impaired fertilization, disrupted preimplantation embryonic development, low rates of implantation, increased incidence of miscarriage, and high rates of morbidity in the offspring in intracytoplasmic sperm injection ICSI (8). These findings support the hypothesis that the integrity of sperm DNA is of paramount importance in the initiation and maintenance of a healthy and viable pregnancy both in natural and in assisted conception (3). Conventional sperm selection used for ICSI is based just on morphology and motility of sperm cells, with no information on their nuclear status.

During the past two decades a number of analyses have been introduced for the study of sperm DNA. However, most techniques are highly invasive and cannot be used to select sperm for ICSI use. In the last years, new and noninvasive methods of sperm evaluation and selection have been proposed to retrieve the best and living sperm. Bartoov and colleagues (9, 10) proposed motile sperm organelle morphology examination (MSOME), based on the real-time evaluation of sperm under a magnification up to ×6,300. By using this technique, some authors reported higher implantation and pregnancy rates by ICSI (11). Moreover, using the same method at a higher magnification (×13,000), our group has previously demonstrated that selected sperm had better mitochondrial function and chromatin structure and lower DNA fragmentation and aneuploidy rates (12). Furthermore, when sperm selection was performed on the basis of absence of nuclear vacuoles a further improvement of mitochondrial and nuclear status was found. Another noninvasive technique aimed to select sperm with normal DNA for ICSI use is based on the evaluation of sperm birefringence by a polarized light microscope (13). Double refraction, or birefringence, is the decomposition of a ray of light into two rays when it passes through an anisotropic material; this phenomenon is a characteristic of cells with normal nuclear organization. Many years ago some authors reported that birefringence is due to the presence of nucleoprotein filaments arranged in rods and oriented longitudinally into the sperm head, and in particular sperm cells from many different species are birefringent (14, 15). Recently, Gianaroli et al. (16) reported higher percentages of good embryos on day 3, higher implantation rates, and higher competence to progress at least beyond 16 weeks' gestation in ICSI cycles in birefringent sperm compared with conventionally selected sperm. These data suggest that sperm birefringence could indicate those cells with a better nuclear status and thus with higher fertilizing potential. In a recent study Petersen et al. (17) evaluated DNA fragmentation in birefringent sperm selected by MSOME criteria. These authors used two different microscopes to evaluate sperm by high-magnification morphology and birefringence (17). This interesting study showed that different levels of birefringence are related to DNA fragmentation. However, in that study authors did not consider nonbirefringent cells or the presence of nuclear vacuoles. In the present study we performed sperm selection based on the concurrent evaluation of birefringence and MSOME by a single instrument. This method allowed us to select some categories of cells with different characteristics that were further analyzed for DNA fragmentation.

MATERIALS AND METHODS

Patients

This study was approved by the Institutional Ethics Committee of the Hospital of Padova, Italy. Thirty-three normozoospermic, young, and healthy subjects participating in a program of fertility screening tests at our unit were recruited in the study after providing informed consent. Inclusion criteria were age ≥20 and ≤35 years, no history of cryptorchidism or varicocele, normal sperm parameters, and absence of sperm infections and antisperm antibodies.

Semen Sample Collection and Preparation

Human semen samples were obtained by masturbation after 2–5 days of sexual abstinence, in sterile containers. Samples were allowed to liquefy for 30 minutes and were examined for seminal parameters according to the World Health Organization criteria (18); 1 μL of native semen was incubated in 10-μL microdrops of PVP 7% (polyvinylpyrrolidone solution; Origio Medicult Media) in a Petri dish and covered with liquid paraffin (Origio Medicult Media) at room temperature for 15 minutes and used for further analysis. On the basis of birefringence and MSOME characteristics, we identified four categories of sperm cells: nonbirefringent (category A), birefringent (category B), birefringent with nuclear vacuoles (category C), and birefringent without nuclear vacuoles (category D). According to MSOME criteria (9), cells from category C were further distinguished on the basis of presence of large (≥4% of total nuclear area) or small (≤4% of total nuclear area) nuclear vacuoles. Finally, among sperm with no nuclear vacuoles (category D), we distinguished cells with normal and cells with altered MSOME results. Five motile sperm of any category obtained from each of 33 semen samples (165 cells for any category) were selected one by one with a micromanipulator system and placed on a slide (total 660 sperm). Selected sperm were further evaluated for DNA fragmentation by terminal deoxyribonucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labelling (TUNEL) test.

Analysis of Sperm Birefringence and MSOME Assessment by a Single Instrument

Birefringence and MSOME were assessed in previously prepared samples using an inverted microscope (Eclipse TE 2000 U; Nikon) equipped with high-power differential interference contrast optics (Nomarski) and with Hoffman contrast