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ARTICLE

Aneuploidy rates for chromosomes X/Y and 18 among preselected spermatozoa in men with severe teratospermia

Jacob Levron [a,*], Ayala Aviram-Goldring [b], Shlomit Rienstien [b], David Bider [a], Jehoshua Dor [a], Gil Raviv [c]

[a] *The In-Vitro Fertilization Unit, Department of Obstetrics and Gynecology, The Chaim Sheba Medical Center, Tel Hashomer and Sackler School of Medicine, Tel Aviv University, Israel;* [b] *The Danek Gertner Institute of Human genetics, The Chaim Sheba Medical Center, Tel Hashomer and Sackler School of Medicine, Tel Aviv University, Israel;* [c] *The Andrology Unit, The Chaim Sheba Medical Center, Tel Hashomer and Sackler School of Medicine, Tel Aviv University, Israel*
* Corresponding author. *E-mail address:* jlevron@sheba.health.gov.il (J Levron).

Jacob Levron, MD graduated *cum laude* from the Technion School of Medicine in Haifa, Israel in 1982 and has been board certified in obstetrics and gynaecology since 1990. From 1990 to 1992, he was a Fellow in the Gamete and Embryo Research Laboratory at Cornell University Medical College, New York Hospital and from 1992 to 1995 a Fellow at the Centre for Reproductive Medicine and Science of St Barnabas Medical Centre in New Jersey, USA. He is currently a senior lecturer at the Tel-Aviv University School of Medicine and a senior physician in the division of obstetrics and gynaecology at the Sheba Medical Centre in Tel Hashomer, Israel.

Abstract Eight infertile men with various degrees of oligoasthenoteratozoospermia and repeated implantation failure were selected for this study due to exceptionally high rates of sperm aneupoidy in their ejaculates. All subjects had normal physical examination, karyotype and serum FSH concentration. Prior to IVF treatment, spermatozoa was collected, processed, micromanipulated and tested for chromosomes X, Y and 18 using fluorescence in-situ hybridization. Aneupoidy rates for chromosomes X, Y and 18 were determined among sperm population selected for normal morphology using high-order magnification light microscopy. A second group of fast motile spermatozoa were collected using an intracytoplasmic sperm injection pipette from the medium–oil interface from microdroplets. The average aneuploidy rates for the three chromosomes were 7.6% (395/5182) in the sperm specimen before selection, 8.7% (116/1326) in the normal morphology selected group and 4.3% (59/1388; $P < 0.001$) in the fast motile selected group. In conclusion, high-magnification light microscopy aimed at selection of spermatozoa with normal morphology did not affect the aneuploidy rate. On the other hand, fast motile spermatozoa harboured significantly less chromosomal abnormalities ($P < 0.001$). Preselection of the most rapid sperm subpopulation for intracytoplasmic sperm injection may improve the qualities of the fertilizing spermatozoon.

KEYWORDS: ICSI, sperm aneuploidy, sperm chromosomes, sperm morphology, sperm selection

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Introduction

The fertilizing spermatozoon plays an important role in the process of fertilization and early embryonic development. Poor fertilization rates and hindered early embryonic development are often prevalent in couples with severe male factor infertility. Such performance is, by large, a result of functional and quality factors of the fertilizing spermatozoon, yet this phenomenon is poorly understood.

One of the important contributions, which can be easily evaluated, is the genetic constitution of the fertilizing spermatozoon. Patients with severe oligoasthenoteratozoospermia and high serum FSH concentration are prone to have increased incidence of sperm numerical chromosomal errors, especially involving the sex chromosomes (Levron et al., 2001). These findings are correlated with observations of the increased risk of fetal anomalies among babies born after intracytoplasmic sperm injection (ICSI) due to severe male factor infertility (Bonduelle et al., 1998; In't Veld et al., 1995).

Several studies have suggested evidence of sperm DNA breakage as an underling cause for the increased rates of fetal anomalies after ICSI. Commercial assays have been developed for detecting such patients at risk. The clinical correlation with this assay, however, is disappointing (Robinson et al., 2012). The first authors to report that preselection of normal-appearing spermatozoa prior to ICSI may improve the outcome of IVF in comparison with controls were Bartoov et al. (2002). During the last decade or so, more practical approaches have been proposed for improving IVF outcome in male factor patients. These methods rely either on light microscopy features enhanced by high-order or empty magnification or on polarized light scattering patterns for the prediction of good sperm qualities (Berkovitz et al., 2007; De Vos et al., 2003; Garolla et al., 2008; Oliveira et al., 2010).

The purpose of this study was to determine whether selecting spermatozoa under light microscopy in a wet preparation affects sperm quality in terms of ploidy. For this purpose, sperm aneuploidy rate was investigated as an objective measurable assessment method of sperm quality.

Materials and methods

Patients

Eight male factor patients with high sperm aneuploidy rate for chromosomes X, Y and 18 were included in the study. These patients were evaluated for ejaculated sperm aneuploidy rates as a part of investigation for repeated implantation failures. The selected couples for this study had seven or more consecutive IVF failures, normal karyotypes, normal female partners and various degrees and combinations of oligoasthenoteratospermia according to World Health Organization criteria (WHO, 1992). Sperm morphology was assessed according to Kruger's strict criteria (Kruger et al., 1987). The evaluation of the male partners included physical examination, ultrasound of the testes and hormonal profile evaluation. All couples had genetic counselling. The study was approved by the local IRB committee (#3869/2005, approved 4 September 2005).

Sperm preparation

The samples were collected by masturbation, allowed to liquefy and washed in Sperm Wash Medium. The pellet was separated on discontinuous Sperm Gradient Medium, washed again and resuspended in Sydney IVF Gamete Buffer to a final concentration of 1×10^6/ml. For the purpose of sperm selection, aliquots of the washed pellet were transferred either to microdroplets of handling medium (Sydney IVF Gamete Buffer) or PVP medium (Sydney IVF PVP), both under Sydney IVF Culture Oil. All media were supplied by Cook.

Microscopy

For morphology-based sperm selection, individual spermatozoa were evaluated using a Diaphot 300 Nikon inverted microscope fitted with Hoffman modulated optics (Nikon, Japan). Observation was performed using ×400 true magnification on a 21'' HD Sony screen combined with a Sony CCD camera (XC-777AP) with an empty magnifying power of ×63 (Sony, Japan). The overall magnifying power using these imaging components was ×2520. A transparent tape with silhouettes of normal-shaped sperm heads was used to aid in selecting sperm cells for analysis. The sperm nuclei of motile cells were examined from the aliquots in the PVP droplets and excluded first for vacuoles and refractive bodies, crevices, depressions and dents. Normally appearing spermatozoa were then immobilized with an ICSI pipette in order to facilitate the handling, observation and collection of individual spermatozoa. The heads were matched to fit the normal silhouettes by rotation adjustments of the CCD camera and moving the microscope table. These cells were collected using the micropipette and pooled into a clean 10% PVP microdroplet.

The group of normal spermatozoa were loaded into the ICSI pipette and transferred into a microdroplet of double-distilled water mounted on a glass slide. After the water evaporation, the slide was transferred into fixation solution of 3:1 methanol/glacial acetic acid (Sigma, St. Louis, MS, USA).

Fast motile sperm collection was performed by an ICSI pipette, which was lowered into the medium—oil interface of the aliquots in handling medium. Rapidly moving spermatozoa were loaded and transferred into a microdroplet of double distilled water on a glass slide and fixed as described above.

FISH analysis

The slides were processed for fluorescence in-situ hybridization (FISH) as described elsewhere (Levron et al., 2001). The analysis consisted of chromosomes X, Y and 18. In order to avoid overdiagnosis, sperm nuclei were considered chromosomally unbalanced only when both the sex- and autosomal-related signals were detected. Nullisomy was not taken into account as an abnormal result.

Statistical analysis

Aneuploidy rates were compared by the chi-squared test.