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Original Article

Chromosome abnormalities in embryos derived from microsurgical epididymal sperm aspiration and testicular sperm extraction

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ABSTRACT

Objective: To evaluate the patterns of chromosome abnormalities in embryos derived from intracytoplasmic sperm injection (ICSI) in microsurgical epididymal sperm aspiration (MESA) or testicular sperm extraction (TESE) in comparison to embryos that are derived from naturally ejaculated (EJAC) patients.
Materials and methods: Male partners with azoospermia who required MESA or TESE for ICSI were studied for chromosomal abnormalities. The ICSI patients with EJAC sperm served as the control group. Preimplantation genetic diagnosis (PGD) was performed by fluorescence *in situ* hybridization (FISH). Chromosome abnormalities were categorized as polyploidy, haploidy, aneuploidy, and complex abnormality (which involves more than two chromosomes). Fertilization, embryo development, and patterns of chromosome abnormalities were accessed and evaluated.
Results: There was no difference between the MESA, TESE, and EJAC patient groups in the rates of fertilization and pregnancy and the percentages of euploid embryos. In all three groups, less than one-half of the embryos for each group were normal (41 ± 31%, 48 ± 38%, and 48 ± 31% in MESA, TESA, and EJAC, respectively). Complex chromosomal abnormality was significantly more frequent in the MESA group than in the EJAC group (48.3% vs. 26.5%, respectively; $p < 0.001$). Furthermore, the overall pattern of chromosomal aneuploidy was similar among all three studied groups.
Conclusion: We suggest that MESA and TESE, followed by ICSI and PGD, appear to be acceptable approaches for treating men with severe spermatogenesis impairment.
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Introduction

The introduction of intracytoplasmic sperm injection (ICSI) has provided hope for treating men with severe spermatogenesis impairment [1]. With obstructive azoospermia, sperm can be retrieved from the blocked epididymis or from the seminiferous tubules of the testes [i.e., microsurgical epididymal sperm aspiration (MESA) or testicular sperm extraction (TESE) procedures] [2–5]. With nonobstructive azoospermia, TESE can retrieve sperm for successful fertilization in less than 60% of patients [4,6]. Generally speaking, the fertilization and pregnancy rates from ICSI, even with severe spermatogenic defects, are comparable to the rates of conventional *in vitro* fertilization [4,7].

In obstructive azoospermia, sperm are preferably retrieved from the epididymis at the first attempt because it is easier for surgeons and patients. If this fails, TESE can be pursued as an alternative. The overall fertilization, cleavage, and pregnancy rates are not significantly different between cycles that use TESE and MESA sperm sources. In addition, these rates are not affected by whether the obstruction is caused by congenital absence of the vas deferens or failed vasoepididymostomy [4].

Under physiologic conditions, sperm may survive for several weeks in the epididymis with less than 7 days of fertilization capability [8,9]. This creates a heterogeneous sperm population in the epididymis by age and spermatozoa maturity. Therefore, there may be an increase in the possibility of an overmature sperm being

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chosen for ICSI. One study on sperm DNA in obstructive azoospermia has shown that testicular sperm DNA appears to be significantly less damaged than epididymal sperm DNA [10]. These findings may explain the higher fertilization rate but lower implantation potential with epididymal sperm [11].

In nonobstructive azoospermia, sperm can only be retrieved from testicles and often present with the most unfavorable parameters. A recent study has shown that only 22% of TESE-derived embryos were normal, compared to 41.8% of embryos from ICSI cycles with ejaculated sperm [12]. These abnormalities include a doubled rate of mosaic chromosomes in embryos from TESE (53%) versus ICSI (26.5%). These findings can be explained by the fact that TESE-derived sperm from men with nonobstructive azoospermia have a higher rate of compromised or immature centrosome structures, which leads to mosaicism in the embryos [12].

Several studies report an increased incidence of chromosomal anomalies—particularly anomalies involving sex chromosomes—in ICSI cycle-derived children in comparison to the incidence in the normal newborn population [7,13–16]. In addition, the possibilities of chromosomal anomalies in infertile males who require ICSI treatment using TESE or MESA include meiotic disruption during spermatogenesis [16] and an increased frequency of aneuploidy in sperm [11,17,18].

Previous studies have revealed the correlation between severe male infertility and sperm chromosomal abnormalities. However, the rates of abnormalities in the chromosomal complement of mild oligozoospermia-derived embryos that require ICSI and sperm from MESA and TESE remain unclear; this is important when counseling patients at the time of embryo transfer in the ICSI procedure. Therefore, the aim of this study was to evaluate the rate and pattern of chromosomal abnormalities in MESA-derived and TESE-derived embryos and to compare these rates and patterns to those of ICSI-derived embryos using naturally ejaculated sperm.

Materials and methods

Patient selection

This study was approved by Institutional Review Board (IRB) of UCLA (Los Angeles, California, USA) in accordance with the Helsinki Declaration of 1975 on human experimentation. Patients with severe male factor infertility that required ICSI and who also received preimplantation genetic diagnosis (PGD) between January 2005 and April 2006 were enrolled in this study. The sources of sperm included MESA, TESE, and natural ejaculation (EJAC). For obstructive azoospermia, MESA was initially attempted to obtain viable sperm. If no motile sperm cells were detected in the epididymal sample, TESE was then performed. For nonobstructive azoospermic patients, sperm was obtained by TESE. If the ejaculates on the day of egg retrieval had less than 15 M/mL of sperm and/or a motility of less than 20%, ICSI was performed. Patients who had PGDs after EJAC/ICSI were the controls. Patients who had undergone oocyte donation were excluded from this study.

ICSI and FISH analysis

Patients undergoing long or short ovulation induction protocols in the ICSI cycle were enrolled in the study. When the dominant follicle size exceeded 18 mm in diameter, 10,000 IU of human chorionic gonadotropin (Profasi; Serono Merck Inc., Norwell, MA) was administered. Approximately 34–36 hours later, oocytes were transvaginally retrieved and cultured at 37°C in human tubal fluid that was supplemented with 5% human serum albumin in a 5% CO_2 humidified gas atmosphere. After 4 hours of incubation, ICSI was performed. The embryos were cultured for 3 days in G1.3 medium (Vitrolife Products, Englewood, CO), and then underwent blastomere biopsy for PGD. All viable embryos with at least four cells were subjected to PGD analyses. After the biopsy, the embryos were switched to G2.3 medium (Vitrolife Products, Englewood, CO) for growth to the blastocyst stage. Only embryos that were classified by PGD as chromosomally normal were transferred.

Aneuploidy screening was performed with FISH using probes primarily for chromosomes 13, 18, 21, X, and Y (Vysis Corporation, Downers Cove, MI). Chromosome abnormalities were categorized as haploidy, polyploidy, aneuploidy, and complex abnormality. These four terms were defined by the presence of one set of the tested chromosomes (i.e., haploidy); more than two sets of the tested chromosomes (i.e., polyploidy); one or two chromosomes with an abnormal number of copies (i.e., aneuploidy); and more than two chromosomes with an abnormal number of copies (i.e., complex abnormality) [19].

Statistical analysis

The proportion of abnormal embryos was computed for each patient for each type of investigated abnormality. Pair-wise comparisons were performed using the Wilcoxon–Mann–Whitney test. The Kruskal–Wallis test was used to compare heterogeneous percentages between the three studied groups. All statistical analyses were performed using the SPSS statistical package software (SPSS, Inc., Chicago, IL, USA).

Results

In total, 572 embryos that were derived from 112 ICSI cycles with natural ejaculated sperm and MESA or TESE were analyzed by FISH. There were 58 embryos in 11 cycles in the MESA group, 54 embryos in 11 cycles in the TESE group, and 460 embryos in 101 cycles in the EJAC group.

As Table 1 shows, there was no difference between these three groups in the number of retrieved eggs, number of mature oocytes, rates of fertilization, cleavage, and embryos that underwent a biopsy for PGD. There was also no difference in the pregnancy rate. Maternal age was the only variable that differed and was more advanced in the EJAC group (38.5 ± 4.3 years), compared to the maternal age in the MESA and TESE groups (33.8 ± 4.4 years and 36.5 ± 4.0 years, respectively; $p = 0.004$; Table 1).

In all three groups, less than one-half of the embryos analyzed by PGD were normal (41 ± 31%, 48 ± 38%, and 48 ± 31% in the MESA, TESA, and EJAC groups, respectively; Table 2). These rates were not statistically significant. The rates for haploidy, polyploidy, and aneuploidy were also similar among these three groups. However,

Table 1
The demographic data of the three investigated groups.

Group	MESA	TESE	EJAC
No. of cases	11	11	101
Maternal age (y)	33.8 ± 4.4*	36.5 ± 4.0	38.5 ± 4.3*
No. of retrieved oocytes	10.6 ± 6.3	12.0 ± 8.4	9.4 ± 5.7
No. of mature oocytes	8.6 ± 5.3	8.5 ± 5.6	7.4 ± 4.2
Fertilization rate (%)	75 ± 20	71 ± 27	73 ± 26
No. of embryos for PGD	58	54	460
Embryo cleavage rate (%)	87 ± 30	83 ± 24	87 ± 20
Rate of no biopsy (%)	13 ± 30	23 ± 31	9 ± 15
Rate of euploidy (%)	41 ± 31	48 ± 38	48 ± 31
Pregnancy rate, % (no./total no.)	36 (4/11)	18 (2/11)	20 (20/101)

EJAC = ejaculation; MESA = microsurgical epididymal sperm aspiration; PGD = preimplantation genetic diagnosis; TESE = testicular sperm extraction.
Some data are presented as the mean ± standard deviation.
*$p = 0.004$.

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