Original Article

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

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A B S T R A C T

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, TESE, 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.

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 vasovasostomy [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 spermatid maturity. Therefore, there may be an increase in the possibility of an overmature sperm being...
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 centromere 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% CO₂ 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 haploid, polyploid, aneuploid, and complex abnormality. These four terms were defined by the presence of one set of the tested chromosomes (i.e., haploid); more than two sets of the tested chromosomes (i.e., polyploid); one or two chromosomes with an abnormal number of copies (i.e., aneuploid); 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, TESE, 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, some data are presented as the mean ± standard deviation.*p = 0.004.

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

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.
complex abnormality was nearly twice as common in the MESA group in comparison with the EJAC group (48.3% vs. 26.5%, \( p < 0.001 \)). The rates of complex abnormalities were not statistically different between the MESA and TESE groups (Table 2).

Table 3 depicts the impact of the number of FISH probes that were studied on the PGD results. When only five probes (chromosomes 13, 18, 21, X, and Y) were used for PGD, the rates of normal chromosomes were higher (42%, 67%, and 52% in the MESA, TESE, and EJAC groups, respectively), compared with the rates when using nine probes (data not shown). When using only five probes, the rate of euploidy embryos from TESE was higher than the rate in embryos from MESA (66.7% vs. 41.9%, respectively; \( p = 0.06 \)). The haploid, polyploid, and aneuploid patterns remained similar, even when more than five probes were used. However, when five probes were used, the difference in rates of complex abnormalities was less significant among these three groups (\( p = 0.06 \)), compared with when more than five probes were used (Tables 2 and 3).

Table 4 demonstrates that the rate of aneuploidy in each studied chromosome was not different among these three groups. The rate of abnormality in sex chromosomes did not differ from the rate of autosomal chromosomes. There was no difference in the rates of abnormality between the X and the Y chromosomes (data not shown). The overall pattern of chromosomal aneuploidy was similar among all three studied groups.

### Discussion

This study demonstrates that despite the fact that the MESA and TESE procedures are performed in patients with the most severe forms of male factor infertility, the rates of fertilization, embryo cleavage, pregnancy, and euploidy are the same as those achieved using ICSI cycles that use ejaculated sperm. In addition, the rate of aneuploidy in embryos derived from MESA and TESE procedures is not any higher than the rate in EJAC-derived embryos. However, the rate of complex chromosomal abnormality is significantly higher in the MESA group.

<table>
<thead>
<tr>
<th>Study group</th>
<th>MESA</th>
<th>TESE</th>
<th>EJAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of embryos</td>
<td>58</td>
<td>54</td>
<td>460</td>
</tr>
</tbody>
</table>

There was a higher incidence of complex chromosomal abnormality in MESA-derived embryos than in TESE and EJAC embryos. This occurred with statistical significance (Table 2) and without statistical significance (Table 3). Representative of the most chaotic situation during chromosome meiosis, complex chromosomal abnormality appears to occur more often in MESA-derived embryos. The exact cause of this phenomenon is unclear, although it may partly result from the process of sperm maturation, which involves testicular sperm acquiring fertilization potential during its passage through the epididymis. Sperm chromatin is normally a highly organized, compact structure that consists of DNA and heterogeneous nucleoproteins. For a spermatozoon to be fertile, it must be able to undergo decondensation at the appropriate time in the fertilization process; however, previous studies show that the prolonged presence of the sperm in the epididymis may increase the formation of disulphide bonds in human sperm protamines [6,19]. There was a significant increase in the decondensation time for these overmature sperm, which increased their resistance to nuclear decondensation in the in vitro-stored sperm and reflected an increase in cross-linking within the sperm histones via the formation of the disulphide bonds [6]. This phenomenon may induce a higher risk of abnormal segregation processes in MESA embryos.

The effect of female age in severe male infertility on chromosomal abnormality is also a factor of great importance. Studies show that a maternal age of greater than 40 years is more relevant to increasing chromosomal abnormalities in embryos, compared with a maternal age of less than 40 years [20,21]. In this study, the mean female age was higher in the EJAC group than in the MESA and TESE groups, 38.5, 33.8, and 36.5 years, respectively. Therefore, the negative impact of maternal age on the EJAC group in this study may not be significant. The rates of normal chromosomes and pregnancy rates in the EJAC group were not statistically different from these rates in the MESA and TESE groups.

In the present study, the rates of normal chromosomes are, as expected, higher if only five-chromosome probes are used in all three groups (Tables 2 and 3). When more FISH probes are used for analyses, a higher rate of chromosomal abnormality is detected. The rate of complex abnormality was higher, whereas the rates of haploidy, polyploid, and aneuploidy remained the same. Complex chromosomal abnormalities represent the most chaotic or complicated situation in embryos that are derived in severe male infertility, particularly in the MESA and TESE groups. However, the
fertilization and pregnancy rates were not statistically different between the three groups. This interesting phenomenon may indicate that the sperm from the MESA and TESE groups have potentially more damage control in chromosome segregation. The detailed mechanism therefore requires further elucidation.

To date, an increasing number of advanced molecular genetic diagnostic technologies are available for PGD or for screening. For instance, array comparative genomic hybridization (CGH) with whole genome amplification can detect translocations and 23 pairs of chromosomes [22,23]. In the future, this technique may replace traditional FISH in preimplantation screening [24,25]. Furthermore, using blastocyst biopsy in the PGD procedure may be able to reduce misdiagnosis resulting from mosaicism because more than one cell can be examined [26]. This is particularly important in the PGD for embryos from TESE because of their high mosaic rates [12]. However, the blastocyst formation rate of the embryos derived from TESE may not be satisfactory for blastocyst biopsy using array CGH. In addition, the prevalence of nonobstructive azoospermia is low. Therefore, until the case number is sufficient for whole genome array CGH, the information provided by the present study is still useful for counseling patients at the time of embryos transfer in the ICSI procedure. In embryos with more than six blastomeres on Day 3, a biopsy of two blastomeres may nevertheless be an alternative option for couples undergoing in vitro fertilization—PGD for a chromosome rearrangement because the embryo development and clinical outcomes were similar between the biopsy of the one blastomere group and the two blastomere group [27].

In conclusion, MESA and TESE, followed by ICSI and PGD, appear to be acceptable approaches for treating men with severe spermatogenesis impairment, and exhibit clinical and laboratory results that are comparable to using ejaculated sperm. However, more FISH probes are necessary to assess the true incidence of chromosomal abnormalities that are comparable to using ejaculated sperm. However, more FISH probes are necessary to assess the true incidence of chromosomal abnormalities. Therefore, the use of epididymal and testicular spermatozoa for preimplantation sperm injection: Fertil Reprod 1999;10:2031—43.

References