The value of chromosomal analysis in oligozoospermic men

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Objective: To determine the prevalence of chromosomal abnormalities in relation to sperm concentration in subfertile oligozoospermic men.

Design: Retrospective cohort study.

Setting: Two teaching hospitals.

Patient(s): We retrospectively studied all men who received chromosomal analysis prior to intracytoplasmic sperm injection (ICSI) treatment from 2000 to 2010 in two teaching hospitals.

Intervention(s): None.

Main Outcome Measure(s): The results of chromosomal analysis and semen analysis were recorded. The frequency of abnormal karyotypes was analyzed in relation to the sperm concentration, categorized as extreme oligozoospermia (>0 to ≤1 million/mL), severe oligozoospermia (>1 to ≤5 million/mL), moderate oligozoospermia (>5 to ≤20 million/mL), or normospermia (>20 million/mL).

Result(s): Among 582 male ICSI candidates, the rates of abnormal karyotypes were 1.2% (2/162), 2.2% (5/227), and 1.5% (2/130) for men with extreme, severe, and moderate oligozoospermia, respectively. No abnormalities were present in normospermic men.

Conclusion(s): The risk of conceiving a viable child with unbalanced structural chromosomal abnormalities in men with oligozoospermia may not justify karyotyping. (Fertil Steril 2012;98:1438–42. ©2012 by American Society for Reproductive Medicine.)

Key Words: Chromosomal analysis, ICSI, oligozoospermic men, abnormal karyotype

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Subfertility is defined as the failure to conceive after 12 months of unprotected intercourse. In The Netherlands it is estimated that ~15% of couples seek specialist medical care because of subfertility (1). About 50% of these reproductive problems can be attributed at least in part to the male partner (2).

The frequency of chromosomal abnormalities in subfertile men eligible for intracytoplasmic sperm injection (ICSI) treatment is increased compared with normal fertile men, with a reported range varying from 2.0% to 7.7% (3–6). Several studies show an increase of abnormal karyotypes when sperm concentration declines (4, 6, 7). In men with nonobstructive azoospermia the prevalence of chromosomal abnormalities is even estimated to be 16% (6, 8). Men with nonobstructive azoospermia have a significantly higher risk for chromosomal abnormalities compared with nonazoospermic men (9, 10).

Since its introduction two decades ago, ICSI is widely accepted as the treatment of choice in couples with severe male factor subfertility, such as azoospermia and severe oligo-, astheno-, and/or teratozoospermia (OAT syndrome) (11). In The Netherlands alone, ICSI resulted in 17,315 ongoing pregnancies from 1996 to 2009 (12). However, concerns have been raised regarding the safety and potential risks of ICSI. The process of ICSI bypasses many steps in the normal process of fertilization, thereby eliminating parts of the natural selection, and consequently there is a small increased risk of transmitting chromosomal abnormalities to the offspring (13).

Until recently, all male ICSI candidates were offered a chromosomal analysis before the ICSI procedure. Today, the Dutch Society of Obstetrics and Gynecology recommends chromosomal analysis in men with nonobstructive azoospermia or extreme OAT syndrome (14).

However, debate remains about whether oligozoospermic men should be offered chromosomal analysis. To assess the potential value of karyotyping...
in men with oligozoospermia undergoing ICSI, we studied the prevalence of abnormal karyotypes in oligozoospermic men.

MATERIALS AND METHODS

We performed a retrospective analysis of all clinical files from couples applying for ICSI treatment at the Maxima Medical Center, Veldhoven, from 2000 to 2010 and at the Catharina Hospital, Eindhoven, from 2005 to 2010. These hospitals are both large teaching hospitals in the south of The Netherlands. Patients were included when results from semen analysis and chromosomal analysis could be drawn from the charts. An Institutional Review Board approval was not required, because this was a retrospective study.

During the study period, all male partners were screened for chromosomal abnormalities as part of routine care. The indication for ICSI was either severe male infertility or fertilization failures in previous IVF attempts. From 1996 to 2007 there was a moratorium on ICSI with surgically derived sperm (percutaneous sperm aspiration [PESA], testicular sperm extraction [TESE], microscopic epididymal sperm aspiration, and percutaneous sperm extraction) in The Netherlands. Since 2007, PESA and TESE are performed in a limited number of centers under strict conditions (15). Almost all patients presenting with azoospermia at both clinics were directly referred to one of these centers. The small number of patients presenting with azoospermia at both clinics were excluded in the present study.

Semen analyses were performed at the IVF laboratory of the Catharina Hospital. Semen specimens were produced by masturbation and collected for analysis within 1 hour after production. Sperm count, volume, and motility were evaluated in native semen. Sperm counts were performed with the use of a Mäkler or Bürker-Türk counting chamber. Semen analysis had to be performed at two separate occasions in case of oligo- or azoospermia. The sperm concentration was categorized into the following groups: extreme oligozoospermia (>0 to ≤1 million/mL), severe oligozoospermia (>1 to ≤5 million/mL), moderate oligozoospermia (>5 to ≤20 million/mL), and normozoospermia (>20 million/mL).

Chromosome analysis was performed in the Department of Clinical Genetics, Cytogenetic Laboratories in Maastricht, following standard protocol (Dutch Working Group of Human Cytogenetics). Karyotyping was performed on GTG-banded chromosome spreads obtained from lymphocyte cultures derived from peripheral blood samples. At least five metaphases were analyzed. A further 25 cells were counted to detect numeric, especially sex chromosomal, mosaicism. Chromosomes with a recurrent variant or polymorphism were considered to be normal.

The difference between the prevalence of abnormal karyotypes in the extreme, severe, and moderate oligozoospermic groups was assessed by Pearson chi-square test. A P value of ≤.05 was assumed to indicate statistical significance.

RESULTS

A total number of 853 files were studied retrospectively. We identified 582 candidate couples for ICSI treatment in which the results from semen analysis and chromosomal analysis could be drawn from the charts. The remaining 271 couples were excluded because of incomplete records. The general features of our cohort are presented in Table 1. Nine out of 582 male ICSI candidates (1.5%) were diagnosed with a chromosomal abnormality. There were three robertsonian translocations, two reciprocal translocations, two inversions, and one unbalanced translocation; also, one sex chromosomal abnormality (47,XYY syndrome) was detected (Table 2).

The frequency of chromosomal abnormalities was analyzed in relation to sperm concentration (Table 3). All abnormalities were observed in the group of men with oligozoospermia (9/519, 1.7%). In the group of extreme oligozoospermia (>0 to ≤1 million/mL; n = 162), two patients were diagnosed with an abnormal karyotype (1.2%). In patients with severe oligozoospermia (>1 to ≤5 million/mL; n = 227) there were five abnormal karyotypes (2.2%), and in the 130 men with moderate oligozoospermia (>5 to ≤20 million/mL) there were two abnormal karyotypes (1.5%). No chromosomal abnormalities were found in the group of normospermic men (n = 63). The differences between the prevalence of chromosomal abnormalities in the extreme, severe, and moderate oligozoospermic groups were not significant (P=.756).

In two couples carrying an abnormal karyotype, ICSI treatment resulted in ongoing pregnancies. In the first couple, the father was carrier of an inversion [46,XY,inv(2)(p11.2q13)]. They attempted two ICSI cycles. The second cycle resulted in an ongoing pregnancy, in which amnion puncture showed a normal female karyotype. A healthy daughter was born. Two spontaneous pregnancies followed. The second pregnancy resulted in a healthy son with an abnormal karyotype inherited by his father. The third pregnancy resulted in a healthy boy with a normal karyotype.

In the second couple, the father was carrier of an unbalanced translocation [46,XY,der(3)ins(3;5)(q26.2;?q22? q14.2)]. One ICSI cycle was performed, which resulted in an ongoing pregnancy. A healthy child was born. To our knowledge chromosomal analysis was not performed.

DISCUSSION

Men eligible for ICSI treatment are supposed to be at increased risk for genetic abnormalities (16). We found a frequency of 1.5% abnormal karyotypes among male ICSI candidates.
which is slightly higher than the frequency of abnormal karyotypes in normospermic men. The frequency of abnormal karyotypes in oligozoospermic men in our study is below the rate of chromosomal abnormalities in ICSI candidates as reported in the literature (Table 4) (3–6, 10, 17–20). The lower rate of abnormal karyotypes in our study can be explained by the exclusion of azoospermic men, whereas other studies did include azoospermic men. The frequencies of chromosomal abnormalities in the different categories of oligozoospermia in our study are similar to the frequencies reported in the literature (Table 4).

A study performed in The Netherlands found no significant difference between the frequency of chromosomal abnormalities in the different categories of oligozoospermia (10). We also did not find a significant difference in abnormal karyotypes between the different categories of oligozoospermia.

A study among phenotypically normal fertile men (sperm donors) reported a 0.37% incidence of chromosomal abnormalities (21). In Denmark, a large incidence study on chromosomal abnormalities in 34,910 newborns was performed. They found a 0.85% incidence of chromosomal abnormalities (22). There appears to be a small increased risk of chromosomal abnormalities in oligozoospermic men (1.7%) compared with newborns and sperm donors.

It is described in the literature that sex chromosomal abnormalities, mainly Klinefelter syndrome, are significantly more frequent in nonobstructive azoospermic men than in oligozoospermic men. Autosomal abnormalities are mainly found in men with oligozoospermia (6, 23). In our cohort of oligozoospermic men, the majority of abnormal karyotypes were autosomal, with the exception of one sex chromosomal abnormality (47, XYY).

Carriers of chromosomal abnormalities are supposed to be at increased risk for fertility problems, repeated miscarriages, and birth of viable offspring with unbalanced chromosomal abnormalities. The main reason for chromosomal analysis in male ICSI candidates is to prevent the birth of viable offspring with unbalanced chromosomal abnormalities.

A study reported that the chance of having a healthy child was not significantly different between carrier couples of a structural chromosomal abnormality and noncarrier couples in natural pregnancies (24). The risk of viable offspring with an unbalanced chromosome abnormality in carrier couples of a structural chromosomal abnormality was estimated to be 0.7% (4 out of 550 spontaneous pregnancies). Because ICSI possibly imposes extra risks of transmitting a genetic abnormality, the estimated risk of conceiving a chromosomally unbalanced child in a carrier of a chromosomal abnormality as listed above might be slightly higher with ICSI. In the present cohort, only one couple transmitted the chromosomal abnormality to a child, and that child was the result of a spontaneous pregnancy.

Chromosomal analysis and genetic counseling are relatively costly and time consuming. Karyotyping oligozoospermic men in the light of ICSI adds another 700 euros to a relatively costly treatment. The benefits and costs of karyotyping should be weighed. Interesting in this discussion is the further reproductive decision of couples in which the man is

### Table 2

**Type of chromosome aberrations in nine male patients undergoing intracytoplasmic sperm injection.**

<table>
<thead>
<tr>
<th>Karyotype</th>
<th>Sperm concentration (million/mL)</th>
<th>Sperm category</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Autosomal aberrations</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reciprocal translocations</td>
<td>46,XY,t(12;15)(q22;q214)</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>46,XY,t(4;16)(q12;q22)</td>
<td>3.11</td>
</tr>
<tr>
<td>Robertsonian translocations</td>
<td>45,XY,der(13;14)(q10;q10)</td>
<td>1.50</td>
</tr>
<tr>
<td></td>
<td>45,XY,der(13;14)(q10;q10)</td>
<td>1.60</td>
</tr>
<tr>
<td></td>
<td>45,XY,der(13;14)(q10;q10)</td>
<td>11.90</td>
</tr>
<tr>
<td>Inversions</td>
<td>46,XY,inv(6p;7q33;9q13)</td>
<td>1.70</td>
</tr>
<tr>
<td></td>
<td>46,XY,inv(2q11.2q13)</td>
<td>14.00</td>
</tr>
<tr>
<td>Unbalanced translocation</td>
<td>46,XY,der(3ins;5;q26.2?q22?q14.2)</td>
<td>1.00</td>
</tr>
<tr>
<td>Sex chromosomal aberration</td>
<td>47,XY</td>
<td>1.20</td>
</tr>
</tbody>
</table>


### Table 3

**Prevalence of abnormal karyotypes in relation to sperm concentration.**

<table>
<thead>
<tr>
<th>Sperm disorder</th>
<th>Men investigated (n)</th>
<th>Abnormal karyotypes (n)</th>
<th>Abnormal karyotypes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extreme oligozoospermia</td>
<td>162</td>
<td>2</td>
<td>1.2</td>
</tr>
<tr>
<td>Severe oligozoospermia</td>
<td>227</td>
<td>5</td>
<td>2.2</td>
</tr>
<tr>
<td>Moderate oligozoospermia</td>
<td>130</td>
<td>2</td>
<td>1.5</td>
</tr>
<tr>
<td>Normospermia</td>
<td>63</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total ICSI candidates</td>
<td>582</td>
<td>9</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Note: ICSI = intracytoplasmic sperm injection.

a carrier of a chromosomal abnormality. The impact of the detection of a chromosome abnormality in male candidates for ICSI treatment has been investigated (25). After extensive genetic counseling in a group of 75 couples with a chromosome abnormality in the male, 56% went through with ICSI treatment, 31% decided to refrain from further treatment, and 13% were still in doubt. When treatment resulted in a pregnancy, most couples did not want to put the pregnancy at risk with prenatal diagnosis. In another study, none of the couples (n = 74) diagnosed with a chromosomal abnormality decided to withdraw from the fertility treatment, even after extensive genetic counseling (5). In our study, all of the nine couples diagnosed with a chromosomal abnormality continued with ICSI treatment. In two couples, ICSI treatment was successful, each resulting in a healthy child.

Most couples dealing with fertility problems have faced many ups and downs along the way. They have great desire to conceive a child of their own. An ICSI pregnancy is very precious for them. Because amniocentesis carries a 0.6% risk of fetal loss (26) and the risk of a chromosomally unbalanced fetus is small, the decision to refrain from prenatal diagnosis is understandable. The knowledge of carrying a chromosomal abnormality does not seem to influence reproductive decisions to a great extent.

As doctors, we have to inform patients in the most optimal way. Chromosomal analysis can provide an explanation of the subfertility and help the patient to understand his fertility problems. However, this is the case in just a small percentage of men.

**CONCLUSION**

In the present study there was no significant difference in the prevalence of chromosomal abnormalities among the three different categories of oligozoospermia. The prevalence of chromosomal abnormalities found in the group of oligozoospermic men was similar to the prevalence found in recent literature. Chromosomal analysis is relatively costly and time consuming. It is our opinion that the small risk of conceiving a viable chromosomally unbalanced child in oligozoospermic men may not justify screening oligozoospermic men for chromosomal abnormalities.

**REFERENCES**


