Structural abnormalities of autosomal chromosomes in the male partner do not influence fertilization and early development of embryos after intracytoplasmic sperm injection

Atsumi Yoshida1,4, Yasuhisa Araki1, Mitsuhiro Motoyama1, Sang-Yong Kim1, Hiro Sung1, Shigeo Araki3, Kazukiyo Miura2 and Masafumi Shirai2

1The Institute of Advanced Medical Technology Central Clinic, Yakushiji 3154, Minamikawachi, Kawachi-gun, Tochigi-ken, 329-04, 2First Department of Urology, Toho University School of Medicine, 6-11-1, Omorinshii, Ota-Ku, Tokyo 143 and 3Department of Obstetrics and Gynecology, Jichi Medical School, Yakushiji, Minamikawachi, Kawachi-gun, Tochigi-ken, 329-04, Japan
4To whom correspondence should be addressed: First Department of Urology, Toho University School of Medicine, 6-11-1, Omorinshii, Ota-Ku, Tokyo 143, Japan

The objective of this study was to determine whether or not abnormalities in the autosomal structure of the male partner have any influence on fertilization and early embryo development after intracytoplasmic sperm injection (ICSI). Thirty-seven couples in whom the male partners were examined by the same andrologist were included in this study. Six couples (group I) in whom the male partner possessed autosomal structural abnormality underwent seven ICSI cycles. As a control group, 31 couples (group II) in which the male partner was karyotypically normal underwent 41 ICSI cycles. Although the normal fertilization rates seen in group I were significantly higher than those in group II (P < 0.05), there was no significant difference in the cleavage rates between the two groups. We did not perform the analysis of the female partner’s chromosomes, but we surmise that structural autosomal abnormalities in the male partner do not adversely influence fertilization at ICSI and early development of embryos.

Key words: chromosome abnormality/fertilization/genetic counselling/intracytoplasmic sperm injection/male infertility

Introduction

Intracytoplasmic sperm injection (ICSI) was developed in 1992 for the treatment of intractable male infertility (Palermo et al., 1992). Using ICSI, Nagy et al. (1995) reported normal mean fertilization rates despite high concentrations of antisperm antibody-bound spermatozoa. Similar results were achieved by injecting round-headed spermatozoa lacking acrosomal membranes and acrosine contents (Lundin et al., 1994) as well as spermatozoa collected by microsurgical epididymal sperm aspiration or testicular sperm extraction in cases of obstructive azoospermia, necrozoospermia and even non-obstructive azoospermia (Schoysman et al., 1993; Tournaye et al., 1994; Devroey et al. 1995; Tournaye et al., 1996). Because of its ability to overcome many such barriers which limit other techniques such as in-vitro fertilization (IVF) and gamete intra-Fallopian transfer (GIFT), ICSI has become recognized as a versatile and revolutionary form of severe infertility treatment.

However, early surveys of infertile males revealed an incidence of major chromosome abnormality that ranged from 2.2 to 14.3% (Laurent et al., 1973; Chandley et al., 1979; Matsuda et al., 1992). Very recently, Pandiyan and Jequier (1996) reported that karyotyping of the white blood cells of 1210 men who showed abnormal semenograms according to World Health Organization criteria revealed 44 (3.6%) individuals with either autosomal or sex chromosomal aberrations. Moreover, Nagafuchi et al. (1993) indicated that 13% of males with azoospermia whose Y chromosomes are cytogenetically normal, have been observed to have deletions of the azoospermic factor. Anguiano et al. (1992) showed that 64% of males with congenital bilateral absence of the vas deferens had at least one detectable cystic fibrosis gene mutation. Additionally, Moosani et al. (1995) reported that 46,XY males with oligozoospermia, asthenozoospermia, and/or teratozoospermia had a high incidence of numerical and structural chromosome abnormalities, based on sperm karyotyping.

Retief et al. (1984) reported that major chromosome abnormalities were observed in 5.1% of oligozoospermic subjects with sperm counts <10×10⁶/ml. Moreover, Bourrouillou et al. (1985) reported major chromosome abnormalities in 6.9% of the males in an oligozoospermic group with a sperm concentration <10×10⁶/ml. Autosomal abnormalities, which were more frequent than sex chromosome abnormalities, were most often structural abnormalities. It may therefore be possible to conduct ICSI using a spermatozoon from a male partner with an autosomal structural abnormality.

In this report, we present a comparison of various clinical features, which include fertilization rates, embryo cleavage rates and pregnancy rates by ICSI, between a group of couples in which the male partner possesses a structural autosomal abnormality and one in which the male partner has a normal karyotype.

Materials and methods

Thirty-seven couples in whom the male partners were examined by the same andrologist were included in this study, before performing ICSI at the Institute of Advanced Medical Technology Central Clinic. Most of the couples had already undergone repeated intrauterine insemination and IVF attempts without success. In all cases, infertility was attributed to the male partner, with no apparent dysfunction among the female partners. Chromosome analysis was performed for each of the male partners prior to ICSI. However, chromosome
analysis was not performed on the female partner because infertility in every case was attributed to male infertility. In cases where the karyotype of the male partner was found to be abnormal, it was then necessary to refer the patients to genetic counselling, including detailed explanations of the risks concerning fetal chromosome abnormality and the importance of prenatal diagnosis. Karyotyping was conducted by analysing G-banded metaphase using the peripheral blood lymphocyte culture technique (Seabright, 1971). Fluorescent in-situ hybridization (FISH) using DNA probes, Q-banding and C-banding were combined in certain cases, for the purposes of eliminating the existence of mosaicism, and confirming the presence of the Y chromosome and the number of centromeres.

Semen analysis was performed on samples obtained by masturbation at the hospital after 5 days of sexual abstinence according to methods recommended by the World Health Organization (WHO, 1992). However, the sperm morphological evaluation was based on the strict criteria established by Kruger et al. (1988). Testicular volume was measured using an orchidometer. The sperm hormone levels of follicle stimulating hormone (FSH) in the male partners was measured by immunoradiometric assay and compared with normal values, which range between 1.8 and 13.6 mIU/ml.

Ovarian stimulation was performed as previously described by Sayama et al. (1996). A combination of pure FSH/human menopausal gonadotrophin (HMG) with a long gonadotrophin-releasing hormone analogue (GnRHa) (Suprecur; Hoechst Japan, Tokyo) was used for ovarian stimulation. Our standard stimulation protocol involved 3 days of 300 IU of pure FSH (HMG-NIKKEN; Nikkenkagaku, Tokyo or Fertinorm P; Serono Japan, Tokyo) injections followed by 3 days of combined 150 IU of pure FSH and 150 IU of HMG (Humegon; Nihon Organon, Tokyo) injections and finally 3 days of 300 IU HMG injections. Oocytes were retrieved under vaginal ultrasonic guidance 34–36 h after administration of 10 000 IU of human chorionic gonadotrophin (HCG; Mochida, Mochida Seiyaku, Tokyo). The oocytes were cultured at 37°C in human tubal fluid medium (HTF) supplemented with 10% heat-inactivated patient serum under mixed gas (5% O₂; 5% CO₂; 90% N₂) (Quinn et al., 1985). Motile spermatozoa were obtained from freshly ejaculated spermatozoa mainly by the swim-up method or a continuous-step Percoll gradient treatment (Kaneko et al., 1987).

Cumulus cells were removed using the needle of a tuberculin syringe. The corona radiata cells were then removed by incubation for 30 s in a modified human tubal fluid (m-HTF) (Quinn et al., 1985) with 60 IU hyaluronidase/ml (Type VIII, specific activity 320 IU/mg; Sigma Chemical Co., St Louis, MO, USA). ICSI was performed according to the procedure described by Van Steirteghem et al. (1993) with one slight modification. In this study, only motile spermatozoa with normal appearance were selected from the concentrated sperm pellet for microinjection. After injection, the oocytes were washed and stored in HTF with 10% heat-inactivated patient serum at 37°C under mixed gas (5% O₂; 5% CO₂; 90% N₂). At 16–18 h after ICSI, oocytes were observed for survival and fertilization. The number and attributes of any polar bodies and pronuclei were recorded. Embryo cleavage and quality were evaluated at 49–52 h after ICSI. Approximately 49–52 h after ICSI, three or four good quality cleaved embryos in 5 μl of the female partner’s inactivated serum were transferred to the uterus using an embryo transfer catheter (KPETS; W.A.Cook, Queensland, Australia).

Progesterone 50 mg (Luteum; Teikokuzuki, Tokyo, Japan) on days 1, 2 and 3 after oocyte retrieval (day 0) and 200 IU of HCG on day 2, 4 and 6 were administered i.m. In addition, 250 mg of hydroxyprogesterone caproate (Proluton Depot; Nihon Shering, Osaka, Japan) and 5 mg of oestradiol dipropionate (Ovahormon Depot; Teikokuzuki, Tokyo, Japan) were given on day 6. However, for cases in which the occurrence of ovarian hyperstimulation syndrome was anticipated due to an excessive response of the ovaries to gonadotrophin stimulation, 200 mg of progesterone was administered twice a day using vaginal suppositories instead of HCG. Pregnancy was detected by measuring urine HCG concentration 14 days after oocyte retrieval.

Statistical analysis was performed on a Macintosh computer using the Student’s t-test, the Welch’s t-test and χ² analysis. A probability of P < 0.05 was considered a statistically significant difference.

Results

Six cases (group I) had some form of autosomal structural abnormality which consisted of two cases of Robertsonian translocation, three cases of reciprocal translocation and one case of reciprocal translocation with an inversion of chromosome 9 as a normal variant. Among the two cases of Robertsonian translocation, one was 45,X,Y,der(13;14)(q10; q10), while one case was 45,X,Y,der(14;15)(q10;q10). Each of the four reciprocal translocations was different. The control group of 31 cases (group II) all exhibited a normal karyotype of 46,XY.

A comparison between the clinical features and the results of ICSI in group I and those in group II is shown in Table I. There were no significant differences in the duration of infertility, and the ages of the male and female partners between the two groups (duration of infertility; Student’s t-test P = 0.788, male partner’s age; Student’s t-test P = 0.386, female partner’s age; Student’s t-test P = 0.317). There were no significant differences in semen volume, the sperm concentration, sperm progressive motility and sperm morphology between the two groups (semen volume; Student’s t-test P = 0.387, sperm density; Welch’s t-test P = 0.184, sperm progressive motility; Student’s t-test P = 0.857, sperm morphology; Student’s t-test P = 0.337).

Group I underwent seven ICSI cycles, while Group II underwent 41 ICSI cycles. Fifty-nine oocytes were injected in group I and 445 oocytes were injected in group II. The normal fertilization rate for metaphase II oocytes was significantly higher in group I, as compared to that of group II (χ² analysis P < 0.05). There was no significant difference in the rate of cleaved embryos per normal fertilized oocyte between the two groups (χ² analysis). There were no significant differences in the pregnancy rate and the delivery rate between the two groups (χ² analysis).

Table II gives an overview of seven ICSI cycles of six couples in group I. Three of the male partners had a past history of surgical treatment of varicocele on the left side. All of the male partners had normal serum FSH values and did not have atrophic testicles.

In the two ICSI cycles of patients with the Robertsonian translocations, all resulted in pregnancy, while none of the five ICSI cycles of patients with reciprocal translocation cycles resulted in pregnancy. Pregnancy was detected in the two Robertsonian translocation cycles (cycle 1 and 2) by measuring urine HCG concentration 14 days after oocyte retrieval. Cycle 1 ended in abortion in the fourth week of pregnancy as indicated by the absence of a gestational sac. Although urinary HCG concentration was 1600 IU/l in the fifth week of
pregnancy (day 39) of cycle 2, this cycle terminated in abortion in the sixth week of pregnancy as indicated by the absence of a gestational sac.

**Discussion**

In the present study, we demonstrated that while the normal fertilization rate was significantly higher in the group of couples in which the male partner possessed an autosomal structural abnormality, there was no significant difference in the rate of embryo cleavage between the autosomal structural abnormality group (Group I) and the normal karyotype group (Group II). Recently, Testart et al. (1996) performed chromosome analysis of both partners of 261 couples, before performing ICSI. They showed that there was no difference in fertilization rates among couples with an autosomal chromosome abnormality and normal karyotype and the rates of clinical pregnancy per ICSI attempt were 25.0 and 20.6% respectively. In contrast to Testart et al. (1996), we did not perform chromosome analysis of the female partner, since infertility in our sample was due to male factors, and we surmised that abnormalities of the male partner’s autosomal chromosome structure do not adversely influence fertilization and early development of embryos. We may speculate that this lack of adverse influence lies in the fact that homologous chromosomes do not pair as they do during a meiotic division of gametogenesis.

Somma et al. (1991) reported contradictory results from animal research, however. They investigated the participation of chromosomally abnormal gametes in fertilization using direct cytogenetic analysis of meiotic cells and in-vivo fertilized ova in Chinese hamsters with reciprocal translocations. They suggest that male gametes with specific chromosomal abnormalities, such as nullisomy for certain chromosome segments, may either have functional disadvantages during fertilization or fail to develop into mature spermatozoa. Moreover, Xian et al. (1992) demonstrated the influence of genome content on fertilizing ability in animal research, suggesting that the

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Table I. Comparison of clinical features and results of ICSI between male partner’s autosomal structural abnormality group (group I) and male partner’s normal karyotype group (group II).

| Karyotype | Serum FSH (mIU/ml) | Testicular volume (Right) (ml) | Operative history | Material age (years) | Serum volume (ml) | Sperm concentration (×10^6/ml) | Sperm progressive motility (%) | No. of metaphase II oocytes injected | No. of normally fertilized oocytes | No. of cleaved embryos | Results of ICSI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Group I | Male partner’s autosomal structural abnormal group | Group II | Male partner’s normal karyotype group | P-value |
| Duration of infertility^a (months) | 64.3 ± 20.8 | 60.6 ± 32.1 | NS^b,c |
| Male partner’s age^a (years) | 33.3 ± 3.4 | 35.1 ± 4.6 | NS^b,c |
| Female partner’s age^a (years) | 31.0 ± 2.6 | 32.4 ± 3.2 | NS^b,c |
| Semen volume^a (ml) | 5.2 ± 1.9 | 4.6 ± 1.7 | NS^b,c |
| Sperm concentration^a (×10^6/ml) | 11.7 ± 12.4 | 20.6 ± 28.7 | NS^b,d |
| Sperm progressive motility (%) | 30.4 ± 15.0 | 29.0 ± 19.5 | NS^b,c |
| Sperm normal morphology^a (%) | 2.4 ± 3.1 | 5.6 ± 5.4 | NS^b,c |
| Total number of metaphase II oocytes injected | 59 | 445 | | |
| Normal fertilization rate (%) per metaphase II oocyte | 57.6 (34/59) | 42.9 (191/445) | <0.05^e |
| Embryo cleavage rate (%) per normalized oocyte | 4.2 (1/23) | 7.4 (1/14) | | |
| Pregnancy rate per cycle (%) | 28.6 (27/95) | 31.0 (28/91) | | |
| Delivery rate per cycle (%) | 0 (0/7) | 2.4 (2/94) | | |

^aMean ± SD.
^bNS = not significant.
^cStudent’s t-test.
^dWelch’s t-test.
^eχ² analysis.

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Table II. Overview of seven ICSI cycles in six couples in which male partner exhibited an autosomal structural abnormality.

<table>
<thead>
<tr>
<th>Couple Cycle</th>
<th>Male partner’s characteristics</th>
<th>Results of ICSI</th>
</tr>
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<tbody>
<tr>
<td>Karyotype</td>
<td>Serum FSH (mIU/ml)</td>
<td>Testicular volume (Right) (ml)</td>
</tr>
<tr>
<td>Group I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>45,XYdel(13)(q14q14)</td>
<td>7.0</td>
</tr>
<tr>
<td>2</td>
<td>45,XYdel(14)(q14q14)</td>
<td>7.3</td>
</tr>
<tr>
<td>3</td>
<td>45,XYdel(17)(q17q17)</td>
<td>6.9</td>
</tr>
<tr>
<td>4</td>
<td>46,XY(24)(p22q22)</td>
<td>4.2</td>
</tr>
<tr>
<td>5</td>
<td>46,XY(22)(p22q22)</td>
<td>5.0</td>
</tr>
<tr>
<td>6</td>
<td>46,XY[12]21q21.q21; p11.21.1</td>
<td>3.5</td>
</tr>
</tbody>
</table>

^aAn inversion of chromosome 9 is considered to be a normal variant.

FSH = follicle stimulating hormone.
fertilizing ability of mouse spermatozoa is directly related to partial deletion of Y chromosome.

In this present human study, however, abnormalities in the autosomal structure of the male partner do not influence fertilization or early embryonic development. One possible explanation of these conflicting results between this study and those utilizing animal research may lie in the fact that Sonta et al. (1991) performed in-vivo fertilization and Xian et al. (1992) performed IVF while we performed ICSI as a fertilization method. If the results of semen analysis indicate the presence of motile spermatozoa which is possible for IVF, IVF should be the first choice because natural sperm selection occurs in this method as opposed to ICSI, which bypasses sperm selection.

One of the causes of recurrent abortions is chromosome abnormality. In approximately 5% of these couples, one of the partners was a carrier of a balanced translocation, reciprocal translocation (two-thirds of cases) or Robertsonian translocation (1/3 of cases) (Campana, M., Serra, A. and Neri, G. (1986). The frequency of these translocations is twice as high among the female partners. For example, among pregnancies in which one partner had a Robertsonian translocation, the frequency of unbalanced karyotypes observed by chionic villus sampling was 20.8% (Mikkelsen, 1985) while the frequency seen by amniocentesis was 9.0% (Daniel, A., Hook, EB.,  Wulf, G. (1989). As indicated by the data on amniocentesis, the risk of giving birth to a fetus with an unbalanced karyotype for couples in which the male partner possesses the Robertsonian translocation (2.6%) is lower than if the female partner possesses the Robertsonian translocation (13.0%). These data strongly indicate a need for selection against chromosomally abnormal spermatozoa. The two Robertsonian translocation cycles which resulted in pregnancy were aborted at the fourth and sixth weeks respectively. We surmise that pregnancy loss due to Robertsonian translocation occurs after the fourth week of pregnancy, although the sample size is too small to be conclusive regarding this point.

According to reports dealing with the analysis of the ejaculated sperm complement from males with reciprocal and Robertsonian translocation, in-vitro fertilization of golden hamster oocytes resulted in an average frequency of spermatozoa with unbalanced karyotypes of 54.0% for cases of reciprocal translocation and 13.7% for those with Robertsonian translocation (Martin et al., 1992, 1993). However, because only spermatozoa which have the ability to fertilize golden hamster oocytes were analysed, the frequency of spermatozoa with unbalanced karyotypes seen in IVF may not be equal to the frequency of the spermatozoa with unbalanced karyotypes that occur in ICSI. Prior to performing ICSI, we suggest the investigation of the incidence of unbalanced type spermatozoa after swim-up or Percoll gradient treatment for couples in which the male partner possesses an autosomal structural abnormality. This investigation may be performed using fluorescent in-situ hybridization of interphase spermatozoa (Rousseaux et al., 1995) or a technique in which the chromosome constitution of human spermatozoa can be determined by injecting individual spermatozoa into mouse oocytes (Lee et al., 1996). Moreover, since there is a high likelihood of yielding an embryo with chromosome abnormalities, the possibility of an early loss must be considered when making any decisions concerning the number of embryo transfers that should be performed for a patient undergoing IVF–embryo transfer (Yoshida et al., 1996).

Testart et al. (1996) reported cases of ICSI in which a male or female partner had a balanced autosomal chromosome abnormality. Among seven fetuses which they retrieved, five had a balanced autosomal translocation, while two had a normal karyotype. Furthermore, Baschat et al. (1996) reported a case of ICSI in which a male subject had a karyotype exhibiting a translocation between the Y chromosome and chromosome 22. This case resulted in a twin pregnancy, and subsequent amniocentesis at 14 weeks of gestation revealed that although one fetus had a normal female karyotype, the other had an abnormal male karyotype with the derivative translocation between the Y chromosome and chromosome 22. In the event that a couple with an autosomal chromosome abnormality becomes pregnant, the resulting child may also be born with a chromosomal abnormality. The expectant female should therefore undergo a villus biopsy and amniocentesis for prenatal diagnosis. Furthermore, the couple should be informed that there is a risk of transmitting defects responsible for infertility (i.e. the azoospermic factor gene).

In conclusion, when male factor infertility is presumed to be caused by structural chromosomal abnormalities, the fertilization rate by ICSI is increased over the control group of male factor fertility of unknown cause and no significant difference is observed during early embryo development between the groups. Although chromosome analysis of the female partners was not performed in this study, we therefore conclude that autosomal structural abnormalities in male partners do not adversely influence fertilization and early embryonic development following ICSI.

References
Male autosomal structural abnormality and ICSI


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