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Chromosome studies in 1792 males prior to intra-cytoplasmic sperm injection: the Dutch experience

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The chance of a male with severe oligozoospermia or azoospermia achieving a pregnancy has undergone a revolutionary increase with the introduction of the intracytoplasmic sperm injection technique (ICSI). However, since ICSI circumvents part of the natural sperm selection mechanisms, the possible transmission of genetic defects to the offspring is a major concern. Cytogenetic analysis is a relatively simple technique to identify at least the carriers of a chromosomal aberration before starting the ICSI procedure. In order to assess the frequency of chromosomal aberrations in male ICSI candidates, we have performed a nationwide cytogenetic study. Of the 1792 males examined, 72 (4.0%) revealed a chromosomal aberration, and one individual even had two. Numerical sex chromosomal aberrations and Robertsonian translocations predominated, followed by reciprocal translocations, inversions and supernumerary marker chromosomes. The different implications, in case a chromosomal aberration is encountered prior to ICSI, are discussed.

Keywords: ICSI; chromosomal aberration; male infertility; oligozoospermia; azoospermia; genetic counselling

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Introduction

Forty years ago, it was already clear that certain chromosomal abnormalities in males cosegregate with infertility.[1] Since then, many studies have been conducted to determine the chromosomal contribution to male infertility; most of these show a correlation between a declining sperm count and an increasing frequency of chromosomal abnormalities.[2]

Until recently, the chances of a male with a severe oligozoospermia or azoospermia achieving a pregnancy was low to negligible. The introduction of intracytoplasmic sperm injection (ICSI) has brought about a revolutionary change, and currently pregnancy rates up to 35% per cycle have been achieved.[3] However, since ICSI circumvents part of the natural selection mechanism, transmission of genetic defects to the offspring is a major concern. It is therefore important to identify at least the males with a chromosomal cause of infertility prior to the ICSI procedure, because only then can one offer genetic counselling and discuss prenatal diagnosis before an eventual pregnancy. This is all the more so since the presence of chromosomal aberrations in offspring born after an ICSI procedure has been reported.[4] In The Netherlands, this has led to advice from the Dutch Society of Obstetrics and Gynaecology to karyotype all male ICSI candidates prior to the ICSI procedure.[5]

We present here the results of a nationwide cytogenetic study performed on a large cohort of males prior to the ICSI treatment, in order to assess the frequency of chromosomal aberrations in these males. In addition, we discuss the implications of the various subgroups of chromosomal aberrations encountered.

Material and Methods

All male candidates for ICSI in The Netherlands were included in our study. The indication for ICSI was azoospermia, severe oligoasthenoteratozoospermia (OAT; less than 5 million spermatozoa with group A and B motility per ejaculate, according to the standard of the World Health Organization), or total fertilisation failure in two previous *in vitro* fertilisation cycles. The different types of indication for ICSI were documented in more than half of the total cohort (Table 1).

All males were offered chromosomal analysis in one of the ten cytogenetic laboratories in The Netherlands, following a standardised protocol (Dutch Working Group on Human Cytogenetics). According to this protocol, cytogenetic studies were performed on GTG-banded chromosomes using lymphocyte cultures derived from peripheral blood samples. From every individual five cells were fully analysed and two karyotyped. A further 25 cells were counted to detect numerical, especially sex chromosomal, mosaicism. All chromosomes with a so called 'variant' or a polymorphism were considered normal.[6]

In cases where a structural chromosome aberration was discovered, cytogenetic analysis was offered to family members of the index patient to examine whether the aberration was inherited or the result of a *de novo* event.

Results

A total of 1792 males ICSI candidates were karyotyped. The exact indication for ICSI could be retrieved in 968 of the cases (Table 1). Seventy-two of the 1792 males showed a chromosomal aberration (4.0%), and one individual even had two – inv(5)(p13.1q13.1) and der(13;14)(q10;q10)). All aberrations were categorised as either sex chromosomal or autosomal. Of the 30 cases of sex chromosome aberrations, 24 were numerical and six were structural rearrangements involving the Y chromosome (see Table 2).

Among the 42 males with an autosomal aberration (including the one with two aberrations) were 18 Robertsonian translocations, 14 reciprocal translocations, seven inversions and four supernumerary marker chromosomes (see Table 3). Of the inversions five were pericentric (breakpoints on opposite sites of the centromere) and two were paracentric (both breakpoints on one arm of the chromosome).

The most frequent aberration overall was the well known Robertsonian translocation between chromosomes 13 and 14 (14 times).

A total of 48 structural abnormalities were encountered, of which 17 were familial, three occurred *de novo*, and of 26 the familial status was not then known. In two the familial status was not tested (see Table 2 and Table 3).

Table 1 Indications for ICSI treatment in a subset of the investigated men with the numbers of chromosomal analyses and abnormal results

	Number analysed	*Number abnormal*	*Percentage*
Azoospermia	62	4	6.5
Oligoasthenoteratozoospermia	865	30[a]	3.5
Total fertilisation failure[b]	41	0	–
Total	968	34	3.5

[a]One patient showed two chromosomal abnormalities.
[b]After two or more IVF procedures.

Table 2 Sexchromosomal aberrations in male ICSI candidates and their origin

Karyotype	*Number*	*Origin*
47,XXY	7	not tested
46,XY/47,XXY	3	not tested
45,X/46,XY/47,XXY	1	not tested
46,XY/47,XXY/48,XXXY	1	not tested
47,XYY	6	not tested
46,XY/47,XYY	1	not tested
45,X/46,XY	5	not tested
46,X,t(Y;16)(q11.2;q24)	1	*de novo*
46,X,der(Y),t(Y;?)(q11.2;?)	1	unknown
46,X,del(Y)(q11.2)/46,XY	1	not tested
45,X/46,X,t(Y;?)	1	unknown
46,X,Yps	1	familial
46,X,Yqs	1	unknown
Total number of sex-chromosome aberrations	30	

Discussion

In the present study, 4.0% of the karyotyped male ICSI candidates showed a chromosomal aberration. Since the introduction of ICSI, only few comparable studies on a relatively small number of males have been performed, which report aberrant findings of 4.2–12%.[4,7,8] A similar cytogenetic study in couples referred for IVF or gamete intrafallopian transfer revealed a chromosomal abnormality in only 0.8% of the males.[9] However, the incidence of karyotypic abnormalities among infertile males is highly dependant on selection criteria (eg inclusion of low levels of sex chromosome mosaicism) and the definition of 'infertility'. As has been shown in other studies, we found that the frequency of chromosomal aberrations is inversely correlate to the sperm count (Table 1). Reported frequencies of chromosomal aberrations range from 2.2% in subfertile males to 6.0% in males with oligozoospermia and 19.6% in azoospermic males.[2,10]

The relatively low frequency of chromosomal abnormalities in the present cohort of male ICSI candidates may have two reasons.

Table 3 Structural autosomal aberrations in male ICSI candidates and their origin

Karyotype	*Number*	*Origin*
45,XY,der(13;13)(q10;q10)/46,XY, der(13;13)(q10;q10),der(13;13)(p10;p10)	1	*de novo*
45,XY,der(13;14)(q10;q10)	13	4 familial and 9 unknown
45,XY,der(13;15)(q10;q10)	1	unknown
45,XY,der(14;21)(q10;q10)	1	unknown
45,XY,der(15;22)(q10;q10)	1	familial
46,XY,t(1;18)(q12;q23)	1	unknown
46,XY,t(3;6)(p14.2;p25.1)	1	unknown
46,XY,t(3;12)(p26.1;p13)	2[a]	familial
46,XY,t(3;14)(p10;q10)	1	unknown
46,XY,t(4;16)(q31.1;q22)	1	familial
45,XY,–8,–22,+dic(8;22)(p23.3;p11.1)	1	unknown
46,XY,dup(8)(p23.1p23.1)	1	familial
46,XY,t(9;20)(p22;p13)/46,XY	1	not tested
46,XY,t(9;22)(p11.1;p11.1)	1	familial
46,XY,t(10;12)(q22.1;p13.1)	1	familial
46,XY,t(10;13)(q23.2;q13)	1	unknown
46,XY,t(11;19)(p15.5;p13)	1	*de novo*
46,XY,t(15;17)(q22;p13)	1	familial
46,XY,inv(3)(p13p26)	1	familial
45,XY,inv(5)(p13.1q13.1), t(13;14)(q10;q10)	1[b]	unknown
46,XY,inv(7)(q22q31)	1	familial
46,XY,inv(10)(p11.2q21.2)	2[c]	familial
46,XY,inv(10)(p13q22.3)	1	unknown
46,XY,inv(13)(q12.1q14.1)	1	familial
46,XY\47,XY,+mar	1	unknown
47,XY,+idic(15)(pter–>q11)	2	unknown
47,XY,+inv dup(22)(pter–>q11)	1	unknown
Total number of structural autosome aberrations	42[b]	

[a]Two brothers, who were analysed independently.
[b]One male with two chromosomal aberrations.
[c]Two males, who were analysed independently in different centres.

1. The relatively low number of males with azoospermia (due to a moratorium in The Netherlands on using surgically retrieved sperm for ICSI[5]).
2. One of the indications for ICSI treatment was 'total fertilisation failure' after *in vitro* fertilisation (about 4% of this cohort), which implied that males with normospermia were included as well. For these males, the frequency of chromosomal aberrations is expected to be very low.[2]

Sex Chromosomal Aberrations and their Consequences

Of the numerical sex chromosomal aberrations, 47,XXY including mosaics were the most frequent (Table 2 and Table 4). Men with Klinefelter syndrome (47,XXY) are usually infertile with azoospermia or, where a normal 46,XY cell line is also present, severe oligozoospermia.[11]

A 47,XYY karyotype was found in six males and a mosaic 47,XYY/46,XY in one. This frequency of 0.4% is about four times higher than was expected from studies in the normal newborn male population.[12] Most males with 47,XYY are reported to be normally fertile, and although associations between 47,XYY and infertility have been described in some cases, the relatively high frequency of males with a 47,XYY karyotype in this cohort is quite remarkable finding.[11,13]

In five males a 45,X/46,XY mosaicism was detected. Individuals with a 45,X/46,XY karyotype have a phenotypic spectrum ranging from a normal male to a female with gonadal dysgenesis.[14] Prenatally diagnosed cases indicate that by far the most cases reveal a normal male phenotype.[15] Unfortunately, prospective studies on 45,X/46,XY males and their fertility status have not yet been undertaken.

It is of importance to estimate the chances of transmitting a numerical sex chromosome abnormality to the offspring, since it has been shown that primordial 47,XXY and 47,XYY germ cells are able to complete a full meiotic cell cycle. Theoretically, males with three sex chromosomes may carry two sex chromosomes instead of one, in up to 50% of their spermatozoa. However, based on spontaneously achieved pregnancies of 47,XYY males and 47,XXX females, it has been estimated that the risk of having offspring with three sex chromosomes is probably less than 1%.[16] This relatively low risk could be due to natural selection. Unfortunately, since ICSI partially precludes natural selection, the risks for offspring resulting from ICSI are unknown. Counting sex chromosomes in spermatozoa by means of molecular cytogenetic studies like fluorescence *in situ* hybridisation (FISH) could give a clue to some extent in these cases. Preliminary data of FISH analysis on sperm of males with three sex chromosomes shows a small but significant increased frequency of hyperploid sperm as compared to the normal sperm (see for review Gutenbach and others).[17–20]

In this study there were six cases of a structural Y-chromosome aberration (Table 2), an extremely rare occurrence in the normal newborn male population. In four cases we were unable to study the inheritance pattern of the aberration. The translocation (Y;16) appeared to have arisen *de novo*, and the Y chromosome with satellites on the short arm was inherited from the father. Satellited Y chromosomes, which are

Table 4 Incidence of chromosomal aberrations in male ICSI candidates and in the normal newborn (male)[a] population

Chromosomal aberrations	*Incidence/1000 male ICSI candidates*	*Incidence/1000 normal newborn (male)[a] population*
Sex chromosomal aberrations	16.7	2.8
47,XXY (and mosaics)	6.7	1.6[b]
47,XYY (and mosaics)	4.0	1.1[b]
45,X/46,XY	2.7	<0.1[b]
Structural Y-chromosome aberrations	3.3	<0.1[b]
Autosomal aberrations[c]	24.0	4.6
Robertsonian translocations[c]	10.1	1.4[d]
Reciprocal translocations	7.8	2.0[d]
Inversions[c]	3.9	0.6[d]
Supernumerary marker chromosomes	2.2	0.6[d]
All aberrations[c]	40.7	7.4

[a]For the sex chromosomal aberrations incidence for the male newborn population is given as appropriate.
[b]Evans JA, *et al*[12].
[c]One male had two aberrations.
[d]Rooney DE and Czepulkowski[27].

the result of a translocation between the short arm of an acrocentric chromosome and the Y chromosome, are not exceptional but may be associated with male infertility.[21,22] Unfortunately, large (prospective) studies on males carrying a satellite-bearing Y chromosome, with respect to reproduction, are not available.

In three of the six Y-chromosomal aberrations the breakpoint was located in band Yq11.2 which is the presumed location of the azoospermia factors a, b and c, implying that one or more of the *AZF* genes, which play a role in spermatogenesis, could be truncated or deleted by the cytogenetic rearrangement.[23] Unfortunately, no material was available in all three cases for molecular studies.

The presence of a Y-autosome translocation, as in the case of some autosomal translocations (see later), leads to the failure of spermatogenesis.[24] At present it is not known whether (some of the) primordial germ cells of infertile males carrying a Y-autosome translocation can complete a full meiotic cell cycle at all. However, if this is indeed possible, the question of genetic risk is no longer an academic issue in this ICSI era. Again, cytogenetic sperm analysis could be very helpful in assessing risk figures for such carriers when performing ICSI.[25]

Autosomal Aberrations and their Consequences

Robertsonian translocations between two acrocentric chromosomes are by far the most frequent structural autosomal aberrations (Table 3 and Table 4), with fusions between chromosomes 13 and 14 predominating in this group. One male was a mosaic with two abnormal cell lines, both containing a Robertsonian translocation between two chromosomes 13 (see Table 3). In this case, all gametes are expected to be aneuploid because either a chromosome 13 is lacking (nullisomy) or the translocation is present (disomy for chromosome 13). Therefore, ICSI is not an option for this male![26] Of the 14 reciprocal translocations encountered (Table 3 and Table 4), two proved to have exactly the same breakpoints in a chromosome 3 and 12. These were detected independently in two brothers, both were candidates for ICSI in two different centres. The mother and another infertile brother were also carriers of the translocation.

Of the seven inversions encountered (Table 3 and Table 4), one male had two chromosomal aberrations – an inversion in chromosome 5 and a Robertsonian translocation between a chromosome 13 and 14. Two males from two different centres appeared to carry exactly the same inversion in one of their chromosomes 10 (Table 3). In these cases preliminary data do not point to any close relationship.

Supernumerary marker chromosomes were found in four males (Table 3 and Table 4). In three of the cases the exact origin of the marker could be identified as duplication of the short arms of chromosome 15 and 22, respectively. Most of the supernumerary marker chromosomes that are present in normal healthy individuals are thought to be derived from short arms of acrocentric chromosomes. Such markers are regarded as harmless with no discernible increased risk for offspring.[19] However, we as well as others have found an excess of such marker chromosomes in infertile males.[8,10]

The frequency of Robertsonian translocations, reciprocal translocations and inversions in this study is also higher than that in the normal newborn population (see Table 4).[27] This implies a causal relation between the presence of these chromosomal aberrations and male infertility. Interference of structural chromosome aberrations with the sex vesicle (a compartment in the nucleus specially formed for the pairing of the sex chromosomes) during meiosis has been suggested as the cause of the disturbance of spermatogenesis.[2,28,29] It remains a mystery, however, why some male carriers have fertility problems, while other males, even within the same family, appear to be normally fertile. The risk of inheriting an autosomal rearrangement which is accompanied with infertility, from a healthy fertile carrier, is not known. It is also not possible to estimate this risk for infertile male carriers who are candidates for ICSI.

During meiosis, all translocations and inversions may give rise to gametes with an unbalanced karyotype, resulting in a higher risk of abortion, stillbirth or a child with congenital anomalies and/or mental retardation. In case of such a (familial) chromosomal rearrangement, estimates of risks following natural procreation are based on family history and analogous aberrations reported in literature.[30] At present, these risks for ICSI pregnancies are not exactly known because we do not know the effect of ICSI, which bypasses part of the natural selection mechanism.[4] As stated earlier, cytogenetic sperm studies by FISH analysis to detect numerical aberrations and/or by ICSI on human sperm in mouse or hamster oocytes to obtain a complete karyotype could be very helpful in assessing these risk figures.[20,25] For the time being, it seems reasonable to assume that these risks must be at least the same as for

normal fertile carriers of a balanced autosomal aberration. Fortunately, the presence of unbalanced karyotypes in the foetus can in most cases be detected by means of prenatal diagnosis.

In general, it is important to document whether chromosomal aberrations in infertile males are '*de novo*' or inherited. In case a structural chromosomal aberration is familial and co-segregates with male infertility, this might pinpoint a chromosomal region harbouring one or more genes involved in spermatogenesis. Therefore, all structural chromosomal aberrations involved in male infertility should be recorded. This could provide important clues for the localisation and identification of genes involved in male (in)fertility.

In conclusion, in view of the fact that chromosomal aberrations are encountered five times more frequently in male ICSI candidates than in the normal population, cytogenetic analysis of the males prior to ICSI is essential. Although much has to be learned about the genetic risks for carriers of chromosomal aberrations following ICSI, it is very important to offer genetic counselling if a chromosomal aberration is detected. However, one has to bear in mind that the risks for offspring following natural procreation are not directly applicable to the offspring resulting from ICSI.

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