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Intracytoplasmic sperm injection in infertile patients with structural chromosome abnormalities

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In the present study we investigated the results of cytogenetic analysis in male and female patients included in an intracytoplasmic sperm injection (ICSI) programme for severe male infertility as well as in conceptuses resulting from these ICSI treatments. In the 261 couples treated, 11 male (4.2%) and three female (1.2%) abnormal karyotypes were found, all consisting of structural chromosome anomalies. Chromosomal translocation exhibited the highest frequency (eight males and two females), and there were also three cases of chromosomal inversion (two males and one female) and one male with one additional marker chromosome. There was no difference in fertilization rates among couples with abnormal (n = 14) and normal (n = 147) cytogenetic results, and the rates of clinical pregnancy per ICSI attempt were 25.0% (5/20) and 20.6% (78/378) respectively. In pregnancies obtained in couples with normal karyotypes, all of the 108 fetuses were free of chromosomal abnormalities. Among the eight fetuses from couples with chromosome structural anomalies, three out of five and two out of three inherited the cytogenetic defects found in their father or mother respectively. In this series of 83 ICSI pregnancies there were no chromosomal abnormalities other than those inherited from the parents. These findings suggest that normal pregnancy rates can be obtained by ICSI in cases of chromosomal translocation in couples with severe male infertility. However, until further evaluations of available data can be performed, cytogenetic analysis must be conducted prior to ICSI in men with low sperm counts, and genetic counselling must include prenatal diagnosis for all growing conceptuses.

Key words: chromosome abnormalities/fertilization rate/ICSI/karyotype

Introduction

Since the first success of in-vitro fertilization (IVF) by intracytoplasmic sperm injection (ICSI, Palermo *et al.*, 1992) there has been a substantial decrease in the number of patients requesting artificial insemination with donor spermatozoa, and efforts have been concentrated on using the semen of males suffering severe infertility. Among such infertile men there are cases with genetic pathology, since low sperm count or azoospermia has been shown to be associated with a 10-fold increase in chromosomal anomalies compared with the general male population (Retief *et al.*, 1984). Thus, infertility treatment with ICSI concerns a population of men with a particular risk of carrying chromosomal aberrations or structural anomalies. Although prenatal karyotypes and prospective follow-up of children born after ICSI have not indicated an increase in congenital abnormalities compared with 'classical' IVF (Bonduelle *et al.*, 1994, 1996), more information is needed on the heritability of chromosomal defects. In addition to the isolated case of a man with mosaic Klinefelter's syndrome (Harari *et al.*, 1995), two cases of abnormal karyotypes in men undergoing ICSI were recently reported but only one resulting pregnancy was analysed (Baschat *et al.*, 1996). We present here our results of cytogenetic analysis prior to ICSI in cases of severe male infertility, and details are given of success rates and prenatal karyotypes in couples where one parent had a structural chromosome anomaly.

Materials and methods

During the years 1994–1995, ICSI procedures in our IVF centre were preceded by chromosomal analysis in male and female partners according to the recommendations of our Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale

Most couples requesting ICSI treatments had already undergone unsuccessful attempts at insemination, either with semen from the husband or a donor, and IVF. Infertility was attributed to poor semen quality in all of the 261 couples included in the present study.

Cytogenetic analysis

Prior to commencement of ICSI, cytogenetic studies were performed on lymphocytes collected from the peripheral blood of both partners of all 261 couples. The standard techniques used Giemsa standard and GTG-banded chromosomes (Verma and Babu, 1989) In the case of chromosomal abnormality in a patient, genetic counselling was proposed before any ICSI attempt. Prenatal karyotyping was systematically performed at 15–19 weeks in all ICSI pregnancies.

Ovarian stimulation and oocyte collection

Following initial pituitary suppression with a gonadotrophin-releasing hormone agonist, ovarian stimulation was achieved by administration of human menopausal gonadotrophin. According to sonographic (leading follicle size >17 mm) and hormonal criteria (oestradiol concentration), oocyte maturation was induced by administration of human chorionic gonadotrophin (10 000 IU), and oocytes were collected under vaginal ultrasound-guided puncture 36 h later.

Sperm evaluation and preparation

Semen produced by male candidates for ICSI was assessed according to World Health Organization procedures and only men in whom spermatozoa were found were included in this study. On the day of ICSI, spermatozoa prepared by Percoll gradient centrifugation were suspended in culture medium (SPM; Medi-Cult, Copenhagen, Denmark).

ICSI procedures

Microinjection needles and holding pipettes were prepared as described previously (Tesarik *et al.*, 1994), and sperm injection was performed with the use of Narishige manipulators mounted on either an Olympus IMT-2 or a Leica DMIRB inverted microscope

Embryo culture and transfer

Culture of gametes and embryos was done in B2 medium (BioMérieux, Marcy L'Etoile, France) or in IVF medium (Medi-Cult) under 5% O_2 at 37°C. Two days after ICSI, two or three embryos were usually transferred into the patient's uterus and supernumerary embryos were frozen. In this study, only results obtained with fresh embryo transfers were taken into account.

Statistical analysis

Comparisons of the ages of the patients, semen characteristics, ovarian responses, fertilization rates, pregnancy rates and fetal karyotypes were made between couples demonstrating a chromosomal defect and couples with normal karyotypes. Means (± standard error) were compared by using Student's *t*-test, and proportions were compared with the χ^2 test.

Results

Cytogenetic analysis performed on 261 couples found structural anomalies in 14 couples, i.e. 11 men (4.2%) and three women (1.2%). Chromosomal translocation was detected in eight men and two women, and chromosome inversion in two men and one woman (Table I). In addition a 47,XY, mar male had one additional marker chromosome. There were also three cases of chromosome 9 inversion (one male and two females) considered to be a variant and not analysed in this study.

Sperm analysis revealed no differences between semen samples of infertile male ICSI patients, with or without chromosome defects (Table II). There were <40% males with sperm count of $>5\times10^6$/ml. Results of ICSI were similar in couples with an abnormal karyotype in the male or in the female partner to those in which both partners had a normal karyotype (Table III). Fertilization rates (53.3–64.6%) were similar and transfer of embryos led to a similar number of fetuses (12.5–16.1%) per transferred embryos, regardless of the chromosomal status of the parents (Table IV). Although the 108 fetuses resulting from ICSI procedures in couples with normal karyotypes were all free of structural anomalies, three out of five (60%) and two out of three (67%) fetuses from couples with male or female chromosomal defects respectively were carriers of a chromosomal defect. However, the cytogenetic features in these five fetuses were all strictly inherited from their parents (Table V) and the four pregnancies (three single and one twin) resulted in the birth of five babies with normal paediatric outcome at 6 months.

Discussion

Compared with a rate of nearly 1% of karyotype abnormalities in the general male population, the frequencies of chromosomal aberrations were 2.2% in a non-selected group of males with subfertility (Chandley *et al.*, 1975) and 7–14% in cases of low sperm count or azoospermia (Retief *et al.*, 1984). Baschat

Table I. Chromosomal abnormalities detected in 261 couples attempting intracytoplasmic sperm injection

Male abnormalities (n = 11)	Female abnormalities (n = 3)
46,XY, t(13,14)	
45,XY, t(13,14)	46,XX, t(3,9) (q13;q34)
45,XY, t(13,14)	46,XX, t(8,12) (p11,q13)
45,XY, t(13,14)	46,XX, inv14 (q23;q32)
45,XY, t(13;14)	
45,XY, t(13,14)	
46,XY, t(3;20) (q13;q13)	
46,XY, t(15,19) (p12;p11)	
46,XY, inv2 (p11,q13)	
46,XY, inv2 (p11;q13)	
47,XY + mar bisat	

Table II. Sperm analysis in ICSI patients according to cytogenetic analysis Values in parentheses are percentage of patients studied

Chromosome analysis	Total	Number of men with		
		$<5\times10^6$ spermatozoa/ml	<30% normal forms	<20% motile cells
Abnormal	11	7 (63 6)	4 (36 4)	5 (45 4)
Normal	250	155 (62.0)	106 (42 4)	96 (38 4)

Table III. Fertilization in intracytoplasmic sperm injection (ICSI) cycles according to cytogenetic analysis. Values are mean ± SE

Chromosomal abnormality	No of couples	Patient age (years)	No. of ICSI cycles	No. of oocytes		No of embryos (% of oocytes)
				Total	Injected	
In men	11	33.5 ± 1 9	14	12.0 ± 3 2	9.8 ± 2 7	6.3 ± 1.5 (64.3)
In women	3	34.3 ± 2.9	6	22.3 ± 10.7	15.0 ± 4 0	8.0 ± 3 6 (53.3)
None	247	34.2 ± 0 3 (male) 33.1 ± 0 2 (female)	378	13.5 ± 0.4	9.9 ± 0.3	6.4 ± 0.2 (64 6)

Table IV. Results of embryo transfers according to cytogenetic analysis

Chromosome abnormality	No of couples	No of embryo transfers	Transferred embryos		No. of pregnancies		No of fetuses	
			Total	Mean ± SD	Total	Clinical (% of transfers)	Total (% of embryos)	Carriers
In men	11	13	31	2.4 ± 0.3	7	4 (30.8)	5 (16.1)	3[a]
In women	3	5	16	3.2 ± 0.7	1	1 (20.0)	2 (12.5)	2[b]
None	247	272	680	2.5 ± 0.1	102	78 (28.7)	108 (15.9)	0

[a]Two males with paternal translocation (13,14) and one female with chromosome 2 inversion.
[b]Two male twins inherited maternal chromosome 14 inversion

Table V. Pregnancies and fetal karyotypes after intracytoplasmic sperm injection (ICSI) in patients with chromosome abnormalities

Patient karyotypes[a]	No. of ICSI attempts	No of pregnancies		No of fetuses	
		Total	Clinical	Total	Carriers
46,XY, t(13,14)	2	1	0		
45,XY, t(13,14)	7	4	3	4	2
46,XY, t(3,20)	1	0			
46,XY, t(15,19)	1	1	0		
46,XY, inv2	2	1	1	1	1
47,XY + mar	1	0			
46,XX, t(3,9)	3	0			
46,XX, t(8,12)	2	0			
46,XX, inv14	1	1	1	2	2

[a]See Table I for cytogenetic details

et al. (1996) reported two cases of translocation, i.e. 45,XY, t(21;22) and 46,X, t(22;Y), in 32 men (6.4%) prior to ICSI. In our study of infertile males with very low sperm counts (i.e. a majority had $<5\times10^6$ spermatozoa/ml) 4.2% had structural anomalies and aneuploïdy was never observed. There were also three chromosomal defects in female patients (1.2%). Each of these three women had a male partner with a normal karyotype, but in all cases the husband's sperm count was $<10\times10^6$/ml. An additional structurally abnormal chromosome was found in one male (47XY, mar). We postulate that such a bisatellized marker could be implicated in male infertility since we have found it with high frequency (five out of 800) in men karyotyped for severe oligoasthenoteratozoospermy (E.Gautier *et al.*, unpublished data).

Robertsonian translocation of chromosomes 13 and 14 was the abnormality found in one-half of our male patients with chromosomal defects (six out of 11), as expected from other studies in infertile males (Johannisson *et al.*, 1993). Sperm karyotyping after heterospecific fertilization of zona-free hamster oocytes (Pellestor *et al.*, 1987) showed 93% alternate segregation, with 39 normal and 33 balanced out of 78 spermatozoa studied from a male with a 13;14 translocation. As ICSI does not allow selection of spermatozoa with a normal karyotype, either by the operator or by the oocyte, there should be the same inheritance of translocation in zygotes as in sperm cells. In our short series of four fetuses from three fathers with t(13;14), there were two normal and two balanced karyotypes. This proportion needs to be confirmed with further cases to support the hypothesis of a similar viability for normal and balanced zygotes.

The other three fetuses were all carriers of a parental chromosome inversion either from the father (inv2) or the mother (inv14). Since three ICSI attempts involving these patients with chromosomal inversions resulted in two viable pregnancies, it may be that such structural abnormalities have no deleterious effect on either the ability of spermatozoa to fertilize when ICSI is used, or the induction of normal development.

Fluorescence in-situ hybridization has indicated an increased frequency of disomy (chromosome 1 and gonosomes) in spermatozoa from idiopathic infertile men with normal karyotypes (Moosani *et al.*, 1995). In such cases, infertility was considered as arising from the non-viable chromosomal constitution of the zygotes. This situation, very different from that occurring in men with chromosomal abnormalities, is no longer relevant in men with normal karyotypes when infertility may be counteracted by ICSI. We confirm previous observations (Baschat *et al.*, 1996; Bonduelle *et al.*, 1996) that pregnancy can be achieved by ICSI using spermatozoa from an infertile male partner with a chromosomal translocation. In our limited series, the biological and clinical results of ICSI procedures were not different from those obtained in patients with normal karyotypes. All of the 108 fetuses obtained in chromosomally normal couples were free of any cytogenetic defect. Although a high risk for chromosome abnormalities, mostly on sex chromosomes, has been reported in ICSI fetuses (In't Veld *et al.*, 1995), larger series of patients (Bonduelle *et al.*, 1996) found this risk to be ~1.0%. At the initial stage of ICSI treatment, karyotyping of both partners has been performed as a general measure of risk evaluation (Meschede *et al.*, 1995). The rationale for chromosomal analysis of the female partner prior to ICSI has never been clearly established, and this local legal obligation no longer prevents the rapid expansion of the use of ICSI in France. However, male karyotyping has to be carried out in cases of severe semen deficiency because of the association between low sperm count and chromosomal anomalies, as confirmed in our study Genetic counselling is necessary to inform the couple of the risk of an abnormal fetus in cases where the father's karyotype is abnormal. The risk of pathology causing infertility in offspring born following ICSI procedures is not in doubt, even in cases of a normal father's karyotype. To limit the genetic risk to infertility, aneuploidies resulting from either paternal mosaicism (Persson *et al.*, 1996) or abnormal chromosome pairing during spermatogenesis (Martin, 1996) should be detected by karyotyping all conceptuses.

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