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CASE REPORT

Intracytoplasmic sperm injection (ICSI) and chromosomally abnormal spermatozoa

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An infertile couple was referred for intracytoplasmic sperm injection (ICSI) because of primary infertility and oligoasthenoteratozoospermia (OAT) in the male. It was observed that although the sperm cells presented with an unusual head size and multiple tails they were able to fertilize the oocytes after ICSI. Subsequent molecular cytogenetic analysis demonstrated de-novo chromosome abnormalities in virtually all sperm cells with 40% diploidy and 24% triploidy in addition to aneuploidy for the sex chromosomes.
Key words: intracytoplasmic sperm injection/chromosome abnormality/spermatozoa/oligozoospermia

Introduction

Infertile oligozoospermic patients often display an enhanced frequency of phenotypically abnormal sperm cells. Although such cells have been successfully used in intracytoplasmic sperm injection (ICSI), controversy exists over whether sperm phenotype abnormalities are indicative of genotype abnormalities (Engel *et al.*, 1996). We present a case history of an oligoasthenoteratozoospermic (OAT) patient with multiple phenotypic abnormalities in sperm cells and show that these cells are chromosomally abnormal.

Case report

An infertile couple first visited the in-vitro fertilization (IVF) clinic in Utrecht in 1992. During fertility work-up, semen analyses were performed and an OAT was found. Three consecutive semen samples showed <2% normal sperm cells, 10% cells with enlarged sperm heads and various other head abnormalities in the remaining cells (World Health Organization, 1987). IVF was not advised because the chances on fertilization were considered to be very low. The couple returned in 1995 and were scheduled for ICSI. During the ICSI procedure it was noted that all available spermatozoa were now macrocephalic. Semen analysis showed a sperm concentration of 15×10^6 spermatozoa/ml, a progressive motility of 20%, 100% macrocephalic sperm heads (head area 25.4 $\pm$ 4.6 μm^2 compared with 10.4 μm^2 in controls; mean $\pm$ SD; $P < 0.001$), one to three tails per cell, and absence of the acrosomal cap. After oocyte retrieval 15 oocytes in the metaphase II stage were injected. Four oocytes displayed two pronuclei 18 h after injection and three embryos developed. The possibility of chromosome abnormalities in the macrocephalic sperm cells and the genetic risks for an ICSI-derived pregnancy were discussed with the couple who opted for continuation of the procedure and two embryos were replaced at the 6-cell stage. The procedure did not result in a pregnancy.

Because of the abnormal semen characteristics, the 31 year old male was further investigated. A testis biopsy was performed and a reduced spermatogenesis was observed resulting in a Johnson score of 6. A family history revealed that the patient was part of an East Mediterranean family consisting of three brothers and four sisters; one brother was normally fertile but the fertility status of the other siblings was unknown. The patient's hormone concentrations were in the normal range [testosterone 20 nmol/l; follicle stimulating hormone (FSH) 6.0 IU/l; luteinizing hormone (LH) 3.8 IU/l]) and a normal 46,XY karyotype was found in all 200 peripheral lymphocytes examined.

Interphase fluorescent in-situ hybridization (FISH) was performed using probes for the chromosomes 18, X and Y in a three-colour and for chromosome 1 in a single-colour hybridization protocol. The multi-colour FISH analysis for two sex chromosomes and an autosome allowed the distinction between aneuploidy for the sex chromosomes and variations in the ploidy status in individual sperm cells (Blanco *et al.*, 1996). Probes included satellite probes for the centromeric regions for chromosomes X and 18; a satellite III probe for the subcentromeric region of chromosome 1, and a satellite III probe for the heterochromatic region Yq12. All probes were directly labelled with fluorophores and obtained commercially (Vysis Inc, Downers Grove, IL, USA). Sperm cells were fixed in Carnoy's fixative and brought onto coated glass slides (Vectabond; Vector Laboratories, Burlingame, CA, USA). Decondensation was obtained by a 5 min incubation in a 1 M Tris–HCl solution containing 25 mM dithiothreitol (DTT) at pH 9.5 (Martini *et al.*, 1995). Dehydrated slides were denatured at 75°C in 70% formamide/2× sodium chloride/sodium citrate (SSC) and hybridized overnight at 37°C. The post-hybridization

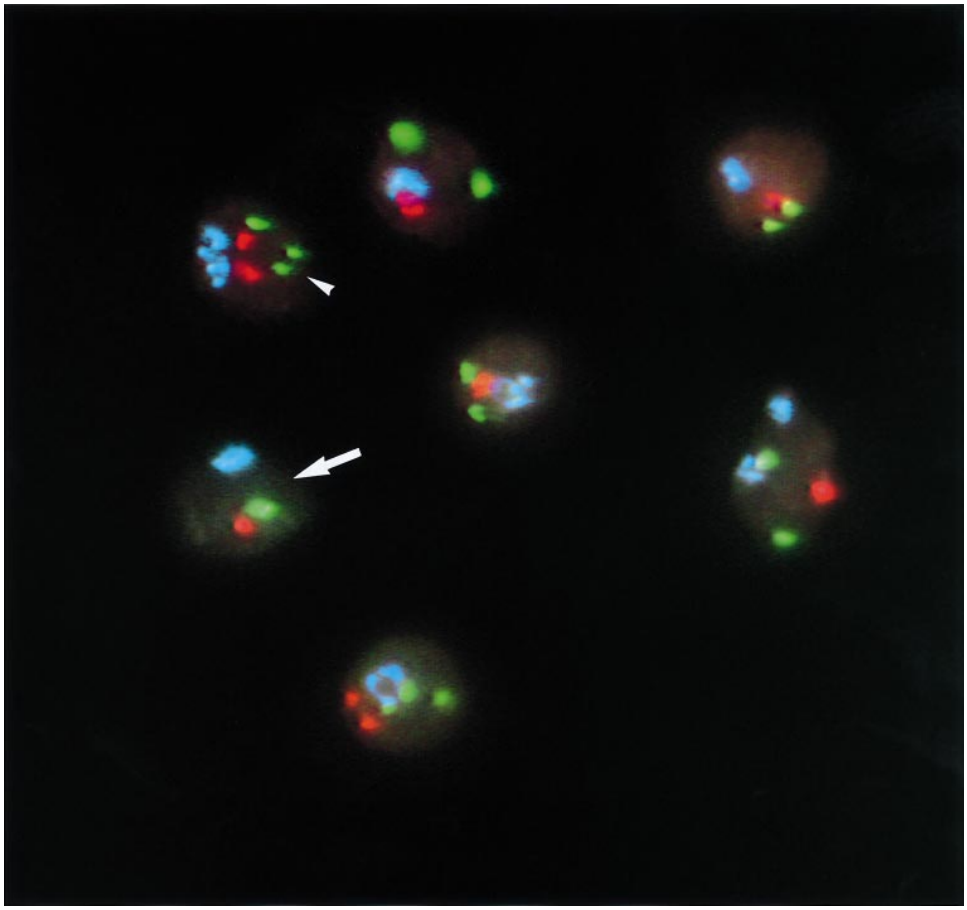

Figure 1. Fluorescence in-situ hybridization with probes for chromosome 18 (green), X (red) and Y (blue) on spermatozoa of an infertile patient referred for intracytoplasmic sperm injection showing a signal distribution compatible with hyperhaploid (18,X,Y = arrow) and polyploid (18,18,18,X,X,Y = arrowhead) spermatozoa (magnification ×1000).

Table I. Three-colour fluorescence in-situ hybridization (FISH) for chromosomes 18, X and Y on sperm cells of an infertile male

FISH signals	Patient readings	Control readings (range; n = 3)
–,X	0.1	0.8–1.2
–,Y	4.3	1.2–3.8
18,–	0.6	0.3–1.1
18,X	0.4	47.4–49.9
18,Y	1.2	44.5–47.6
18,XY	13.8	0.1–0.5
18,XXY	3.7	0
18,18,XY	27.6	0.1–0.9
18,18,XYY	2.3	0
18,18,XXY	7.9	0
18,18,18,XY	4.3	0
18,18,18,XXY	10.7	0
18,18,18,XY	8.1	0
18,18,18,18,XY	2.4	0
18,18,18,18,XXY	2.7	0
Other	10.3	1.2–2.5

Results are expressed as percentage of hybridizing sperm cells with a particular chromosome composition in 1000 interphase nuclei per case. Patient data are based on the analysis of two separate semen samples (500 cells per sample) showing similar signal distributions.

Table II. Single-colour fluorescence in-situ hybridization (FISH) for chromosome 1 on sperm cells of an infertile male

FISH signals	Patient readings	Control readings
1	35.3	99.6
1,1	43.1	0.4
1,1,1	19.0	0
1,1,1,1	2.1	0

Results are expressed as percentage of hybridizing cells with a particular number of chromosome 1 signals in interphase nuclei of the patient (n = 232) or a normal control (n = 522).

wash was performed in 0.4× SSC/0.3% NP-40 at 73°C for 2 min. Slides were examined using a triple filterblock (Leitz, Wetzlar, Germany), allowing the simultaneous visualization of red, green and blue fluorophores, and photographed using MacProbe 2.5 image analysis software (Perceptive Scientific Instruments Inc, Chester, UK). Hybridization signals were scored in the patient and in a series of controls with normal sperm characteristics (World Health Organization, 1987). Hybridization efficiency was 89% in the patient and 97–99% in the controls. Only sperm cells with well defined boundaries were scored; signals in a particular fluorescent colour were considered to be multiple when separated by at least one signal diameter.

In the patient, FISH on interphase nuclei resulted in a signal distribution compatible with the virtual absence of normal haploid cells (<2%) and the presence of diploid (40%) and

triploid (24%) sperm cells and hyperhaploid spermatozoa aneuploid for the sex chromosomes (22%) (Figure 1; Table I). Single colour FISH for chromosome 1 confirmed the presence of diploid and triploid sperm cells (Table II). Most spermatozoa (93%) were found to display both X and Y chromosomal signals suggesting that malsegregation of the sex chromosomes and defects at the level of meiosis I were part of the underlying process. As polyploidy was also involved it could not be excluded that defects were not limited to meiosis I but also involved meiosis II and/or pre-existing chromosome anomalies in a gonadal mosaic form. The couple was counselled against any future application of ICSI.

Discussion

Idiopathic infertile men have an increased risk for constitutional chromosome aberrations (Chandley, 1979; De Braekeleer and Dao, 1991), interstitial deletions of the Y chromosome (Reijo *et al.*, 1996) and an increased frequency of sperm cells disomic for the sex chromosomes (Moosani *et al.*, 1995). In addition, oligozoospermic patients often display increased frequencies of phenotypically abnormal spermatozoa.

Little information is available with respect to the correlation of sperm phenotype and genetic constitution in such patients. The present case, with phenotypic and chromosomal abnormalities in virtually all sperm cells, may represent an extreme example not reported before in humans and matched only by reports of diploidy in some strains of experimental animals (Levy and Burgoyne, 1986). However, early reports using densitometric techniques have indicated the presence of low percentages of diploid cells in both man and experimental animals (Carothers and Beatty, 1975). Such cells were found to present an enlarged sperm head and multiple tails (Beatty and Fechheimer, 1972), thus closely resembling the macrocephalic sperm heads in the present case. Experiments in mice have suggested that morphologically abnormal spermatozoa are less likely to cross the utero–tubal junction (Krzanowska, 1974). The avoidance of selection barriers by ICSI, the relatively high frequency of morphologically abnormal spermatozoa in oligozoospermic males, and the present case history, raise some concern about the use of morphologically abnormal cells in ICSI. Studies using the human sperm–hamster oocyte fusion model to analyse the human sperm karyotype have indicated that no significant relationship exists between the frequencies of chromosomally and morphologically abnormal spermatozoa (Martin and Rademaker, 1988). However, such studies may be biased by an altered fertilizing capability of aberrant spermatozoa and may underestimate the percentage of chromosome abnormalities. Recently, a new technique has been described in which human sperm cells are injected into mouse oocytes (Lee *et al.*, 1996). Preliminary results have indicated that some morphological abnormalities in sperm heads are associated with chromosome defects but no increase was reported for spermatozoa with enlarged heads. Additional studies, including FISH, for the detection of specific chromosome abnormalities, should be performed on phenotypically abnormal sperm cells in a large population of infertile males to estimate the genetic risks involved. If possible, such data should be related to the incidence of chromosomally abnormal embryos in both preimplantation and postimplantation stadia. Meanwhile, it seems advisable not to use macrocephalic, multi-tailed and acrosome-less spermatozoa for injection purposes without proper genetic screening.

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References

Beatty, R.A. and Fechheimer, N.S. (1972) Diploid spermatozoa in rabbit semen and their experimental separation from haploid spermatozoa. *Biol. Reprod.*, **7,** 267–277.

Blanco, J., Egozcue, J. and Vidal, F. (1996) Incidence of chromosome 21 disomy in human spermatozoa as determined by fluorescent in-situ hybridization. *Hum. Reprod.*, **11,** 722–726.

Carothers, A.D. and Beatty, R.A. (1975) The recognition and incidence of haploid and polyploid spermatozoa in man, rabbit and mouse. *J. Reprod. Fertil.*, **44,** 487–500.

Chandley, A.C. (1979) The chromosomal basis of human infertility. *Br. Med. Bull.*, **35,** 181–186.

De Braekeleer, M. and Dao, T.N. (1991) Cytogenetic studies in male infertility: a review. *Hum. Reprod.*, **6,** 245–250.

Engel, W., Murphy, D. and Schmid, M. (1996) Are these genetic risks associated with microassisted reproduction? *Hum. Reprod.*, **11,** 2359–2370.

Krzanowska, H. (1974) The passage of abnormal spermatozoa through the uterotubal junction of the mouse. *J. Reprod. Fertil.*, **38,** 81–90.

Lee, J.R., Kamiguchi, Y. and Yanagimachi, R. (1996) Analysis of chromosome constitution of human spermatozoa with normal and aberrant head morphologies after injection into mouse oocytes. *Hum. Reprod.*, **9,** 1942–1946.

Levy, E.R. and Burgoyne, P.S. (1986) Diploid spermatids: a manifestation of spermatogenic impairment in XOSxr and T31 H/+ male mice. *Cytogenet. Cell Genet.*, **42,** 159–163.

Martin, R.H. and Rademaker, A. (1988) The relationship between sperm chromosomal abnormalities and sperm morphology in humans. *Mutat. Res.*, **207,** 159–164.

Martini, E., Speel, E.J.M., Geraedts, J.P.M. *et al*. (1995) Application of different in-situ hybridisation detection methods for human sperm analysis. *Hum. Reprod*., **10,** 855–861.

Moosani, N., Pattinson, H.A., Carter, M.D. *et al.* (1995) Chromosomal analysis of sperm from men with idiopathic infertility using sperm karyotyping and fluorescence *in situ* hybridisation. *Fertil. Steril.*, **64,** 811–817.

Reijo, R., Alagappan, R.K., Patrizio, P. and Page, D.C. (1996) Severe oligozoospermia resulting from deletions of azoospermia factor gene on Y chromosome. *Lancet*, **347,** 1290–1293.

World Health Organization (1987) *Laboratory Manual for the Examination of Human Semen and Semen–Cervical Mucus Interaction.* Cambridge University Press, Cambridge, UK.

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