Article

Prevalence of chromosome defects in azoospermic and oligoastheno-teratozoospermic South Indian infertile men attending an infertility clinic

Dr Lalji Singh

Dr Lalji Singh obtained his PhD (Cytogenetics) from Banaras Hindu University, Varanasi, India. He was a post-doctoral Research Fellow and Research Associate at the University of Edinburgh, UK from 1974 to 1987. He is currently Director of the Centre for Cellular and Molecular Biology (CCMB), Hyderabad, India. He has pioneered the use of DNA fingerprinting technology for forensic science in India, leading to its acceptance as a source of evidence by the Indian judiciary and the setting up of a new institute, the Centre for DNA Fingerprinting and Diagnostics at Hyderabad. His research interests are on the molecular basis of sex determination, human genetic diversity and wildlife conservation. His awards include the CSIR Technology award in 1992 and New Millennium plaque of honour for 2001–2002. He is a fellow of the Third World Academy of Sciences, Trieste. He has published more than 100 research papers in highly reputed national and international journals. He has delivered more than 100 invited lectures in India and abroad and has played an important role in the Indian uptake of the DNA fingerprinting technology developed by his group in CCMB.

K Lakshmi Rao[1], K Arvind Babu[1], MK Kanakavalli[1], VV Padmalatha[1], Mamata Deenadayal[2], Lalji Singh[1,3]
[1]Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad-500007;[2] Infertility Institute and Research Centre, St Mary's Road, Secunderabad 500003, India
[3]Correspondence: Tel: +91 40 27160789; Fax: +91 40 27160252; e-mail: lalji@ccmb.res.in

Abstract

The prevalence of chromosomal abnormalities in azoospermic and oligoastheno-teratozoospermic infertile men of South Indian origin undergoing assisted reproductive technologies was evaluated. In addition, the study aimed to investigate new abnormal karyotypes involving autosomes in azoospermia and sex chromosomes in oligoastheno-teratozoospermic individuals that are supposed to be rare. Metaphase chromosomes of 744 infertile men, including 272 men with azoospermia and 472 men with oligoastheno-teratozoospermia (OAT), were analysed using Giemsa–trypsin–Giemsa banding and fluorescence in-situ hybridization (FISH) wherever necessary. Chromosomal abnormalities were observed in 59 (7.9%) individuals of the total studied population. Among these, 30 out of 272 (11.0%) azoospermic men and 29 out of 472 (6.1%) infertile men with OAT showed chromosomal abnormalities. A strong and statistically significant association (OR = 1.89; $P = 0.0235$) of chromosomal abnormalities and sex chromosome abnormalities (OR = 4.29; $P = 0.001$) with azoospermia when compared with OAT was observed. In addition, six autosomal abnormalities associated with azoospermia and two abnormalities involving Y chromosome, which include a novel karyotype (mos 46,XY/51,XYYYYYY) in OAT individuals, were detected.

Keywords: *azoospermia, chromosome abnormalities, ICSI, karyotype, male infertility, oligoastheno-teratozoospermia*

Introduction

Although infertility affects about 5% of the male population, its cause in most cases is uncertain (Yong *et al*., 2000). Male infertility has been associated with several genetic and non-genetic conditions, whereas idiopathic spermatogenic disorder accounts for more than 50% (Namiki, 2000). From the genetic point of view, infertility patients seeking assisted reproduction have to be classified as a high risk group. In these patients, the prevalence of numerical chromosomal abnormalities is around 10% and structural chromosomal abnormalities is around 1%, compared with 0.85 and 0.1% in the general population (Gruber *et al*., 2003). Genetic abnormalities were identified in men with unexplained azoospermia and oligozoospermia, including numerical and structural chromosome abnormalities (Chandley, 1998). A review of the literature on somatic chromosome investigations in infertile males has shown that 13.7% of azoospermic males and 4.6% of oligozoospermic males have an abnormal karyotype (Van Assche *et al*., 1996). In a different study (Kalantari *et al*., 2003), the frequency of abnormal karyotypes among patients with azoospermia was 10% and the most frequent anomaly was 47,XXY (8.6%).

It is known that usually sex chromosome abnormalities are seen in azoospermic individuals, whereas autosomal or autosome-sex chromosome abnormalities are considered rare (Van Assche *et al.,* 1996; Kalantari *et al.,* 2003). Therefore, this paper aimed to investigate and evaluate the prevalence of chromosomal abnormalities in azoospermic and oligoasthenoteratozoospermic (OAT) infertile men of South Indian origin undergoing assisted reproductive technologies. In addition, the study was intended to discover new abnormal karyotypes, especially focusing on those abnormalities involving autosomes in azoospermia and sex chromosomes in oligoasthenoteratozoospermic individuals that are supposed to be uncommon. This study provides information on those regions (genes) of autosomes that may have an important role to play in azoospermic male infertility.

Materials and methods

Patient selection and clinical evaluation

A total of 744 men of South Indian origin presenting with infertility and seeking help through assisted reproduction were recruited at the Infertility Institute and Research Centre, Hyderabad, India. Twenty-five normal fertile men of the same origin with a sperm count >30 × 10^6per ml were considered as controls. All infertile men in age groups ranging from 22 to 46 years (mean, 32.55), who were referred for evaluation of infertility and technically met the definition of infertility (1 year of unprotected intercourse and not leading to conception), were enrolled in the study, regardless of the fertility status of their partners. These men were subjected to comprehensive questionnaires related to their medical, surgical, sexual and family history, lifestyle habits (such as smoking, alcohol use, and drug use) and exposure to gonadotoxins (such as drugs used in cancer chemotherapy). A thorough physical examination was also included as an assessment of secondary sexual characteristics. Every individual provided a minimum of two semen specimens, each after sexual abstinence extending from 2 to 5 days. These specimens were evaluated on the basis of the criteria of World Health Organization (WHO, 2002). Informed written consent was obtained from each subject. The Institutional Review Board of Centre for Cellular and Molecular Biology, Hyderabad, approved this study.

On the basis of the sperm count, all the infertile men were categorized accordingly as azoospermia (absence of spermatozoa) and oligoasthenoteratozoospermia (OAT: abnormal sperm morphology and motility). Individuals with oligozoospermia (severe – sperm count <5 million per ml; mild – sperm count <20 × 10^6 per ml) were considered as non-azoospermic and included the OAT group for the present study. Decreased sperm motility was determined by using antisperm antibodies. In addition, blood samples were obtained for DNA extraction and serum for measurement of testosterone, prolactin and FSH by radioimmunoassay. After thorough clinical evaluation, a respective diagnosis of infertility was given to each individual accordingly. Those men with clinical evidence of obstructive azoospermia were excluded from the study. Semen analysis, hormone profile, and histology of the testicular biopsy performed during testicular sperm extraction were reviewed on charts.

Cytogenetic analysis

Chromosomal analysis was performed on phytohaemagglutinin (PHA)-stimulated peripheral lymphocyte cultures using standard cytogenetic methods (Benn and Perle, 1992; Gosden *et al*., 1992). Twenty to 30 metaphases were analysed per individual and in cases of suspected mosaicism, the number of metaphases was increased to a total of 100 for analysis. Resolution of the 400-band stage was considered as a minimum; for a more detailed structural analysis 550–700 band stage was preferred.

Fluorescence in-situ hybridization and microscopy

Fluorescence in-situ hybridization (FISH) experiments were performed on metaphase spreads derived from patient's lymphocytes, wherever it was necessary to confirm the Giemsa–trypsin–Giemsa (GTG) banding results, according to the Vysis (Naperville, IL, USA) protocol. Hybridizations were performed using Spectrum Green (Vysis) DNA probes. Slides were counterstained with 20 µl of 4–6 diamino-2-phenylinodole (DAPI) (10 g/ml). Bright light and fluorescence microscopy was performed with a Zeiss Axioscope microscope (Zeiss, Jena, Germany). A dual filter set (Vysis) for simultaneous detection of DAPI and Spectrum Green was used to visualize green signals. The images for GTG-banding were captured and processed using the Cytovision 2.72 automated karyotyping system (Applied Imaging, Santa Barbara, CA, USA). Each slide was scored by at least three observers. The FISH images were captured and processed by Axiovision 3.0 (Carl Zeiss GmbH, Jena, Germany).

Results

On the basis of semen analysis, 272 (36.6%), individuals were categorized as azoospermic and 472 (63.4%) individuals as OAT (**Table 1**). Semen profiles, hormone concentrations and testicular morphology showed considerable variations in men in whom genetic defects were observed.

Chromosomal abnormalities were detected in 59 (7.9%) individuals out of 744 infertile men. Chromosomal investigations in 272 infertile men with azoospermia showed 30 (11.0%) subjects with chromosomal aberrations, most of them involving sex chromosomes. Major autosomal anomalies were detected in 29 (6.1%) individuals with idiopathic OAT. A strong and statistically significant association (OR = 1.89; CI = 1.07–3.35; P = 0.0235) of chromosomal abnormalities with azoospermia was observed when compared with OAT. A list of abnormal karyotypes detected in 272 azoospermic individuals is presented in **Table 2**, which shows six (2.2%) autosomal abnormalities associated with azoospermia and 24 (8.8%) sex chromosome abnormalities. The abnormal karyotypes observed in 474 individuals with OAT are given in **Table 3**, where 27 (5.7%) autosomal abnormalities and two (0.4%) abnormalities involving the Y chromosome are identified in OAT individuals. These include one rare and novel karyotype (mos 46,XY/51,XYYYYYY) associated with OAT. Frequencies of chromosomal abnormalities in azoospermia and OAT individuals classified on the basis of structural, numerical and mosaics are presented in **Table 4**.

Table 1. Chromosomal abnormalities in 744 infertile men with azoospermia and oligoastheno-teratozoospermia.

Type of infertility	*No. of individuals* n *(%)*	*Chromosomal abnormalities (%)*	*Odds ratio*	*Confidence limits*	P-*value*
Azoospermia	272 (36.6)	30 (11.0)	1.89	1.07–3.35	0.0235[a]
Autosomal abnormalities		6 (2.2)			
Sex chromosome abnormalities		24 (8.8)	4.29	1.67–13.02	0.0011[b]
Oligoastheno-teratozoospermia	472 (63.4)	29 (6.1)			
Autosomal abnormalities		27 (5.7)	14.26	3.54–124.2	0.0000[c]
Sex chromosome abnormalities		2 (0.4)			
Total	744	59 (7.9)			
Autosomal abnormalities		33 (4.4)	1.28	0.73–2.26	NS[d]
Sex chromosome abnormalities		26 (3.5)			

[a]Association of chromosomal abnormalities with azoospermia.
[b]Association of sex chromosome abnormalities with azoospermia.
[c]Association of autosomal abnormalities with oligoasthenoteratozoospermia.
[d]Not significant, i.e. no association between autosomal and sex chromosome abnormalities in total study population.

Table 2. Chromosomal abnormalities in 272 infertile men with azoospermia.

Karyotype	*No. of cases*
Autosomal abnormalities	
46,XY inv(9)(p21q13)	1
46,XY del(4)(p12→p14), 46,XY del(4)(pter→p12::p14→qter)	1
45,XY der(13;14)(q10;q10), 45,XY der(13;14)(13qter→13q10::14q10→14qter)	1
46,XY add (13)(p13)	1
46,XY t(1:19)(p31.1;p13.3)	1
46,XY t(12;15)(q28;p13)	1
Sex chromosome abnormalities	
46,XY t(X;15)(q28;q22)	1
46,XY del(Yq)	1
46,XY?dup(Yq)	3
47,XXY	12
47,XYY	4
49,XXYYY	1
mos 46,XY/47XY,+mar	1
mos 46,XY/47,XXY	1
Total	30

Table 3. Chromosomal abnormalities in 474 infertile men with oligoastheno-teratozoospermia.

Karyotype	*No. of cases*
Autosomal abnormalities	
46,XY add(14)(p13)	2
46,XY add(21)(p13)	4
46,XY add(21)(p13), 46,XY add(21)(pter→p13::?)	1
45,XY der(13;14)(q10;q10), 45,XY der(13;14)(13qter→13q10::14q10→14qter)	1
46,XY del (13p)	2
46,XY del(21)(p11.2→p13)	1
46,XY del(7)(q32→qter)	1
46,XY ins(1;?)(q12:?), 46,XY,ins(1;?)(pter→q11::?::q12→qter)	1
46,XY ins(9;?)(p13.1;?)	1
46,XY inv(2)(q11.2q13)	1
46,XY inv(22)(p11.2;q13.1), 46,XY inv(22)(pter?p11.2::q13.2→q11.2::q11.2→qter)	1
46,XY inv(22)(p13)	3
46,XY inv(9)	1
46,XY inv(9)(p12q13)	1
46,XY inv(9)(p13q12)	1
46,XY inv(9)(p21q13)	1
46,XY t(1;10)(p32;p15)	1
46,XY t(15;21)(q22.1;p11.2)	1
46,XY t(4;15)(q33;p11)	1
46,XY t(6;15)(q10;q10)	1
Sex chromosome abnormalities	
46,XY,?dup(Yq)	1
mos 46,XY/51,XYYYYYY	1
Total	29

Table 4. Different types of chromosomal abnormalities observed in 744 infertile men. OAT = oligoastheno-teratozoospermia.

Type of abnormality	*No. with azoospermia (%)*	*No. with OAT (%)*	*Total no. (%)*
Structural	11 (36.6)	28 (95.6)	39 (66.1)
Numerical	17 (56.6)	0 (0)	17 (28.8)
Mosaics	2 (6.6)	1 (3.4)	3 (5.1)
Total	30	29	59

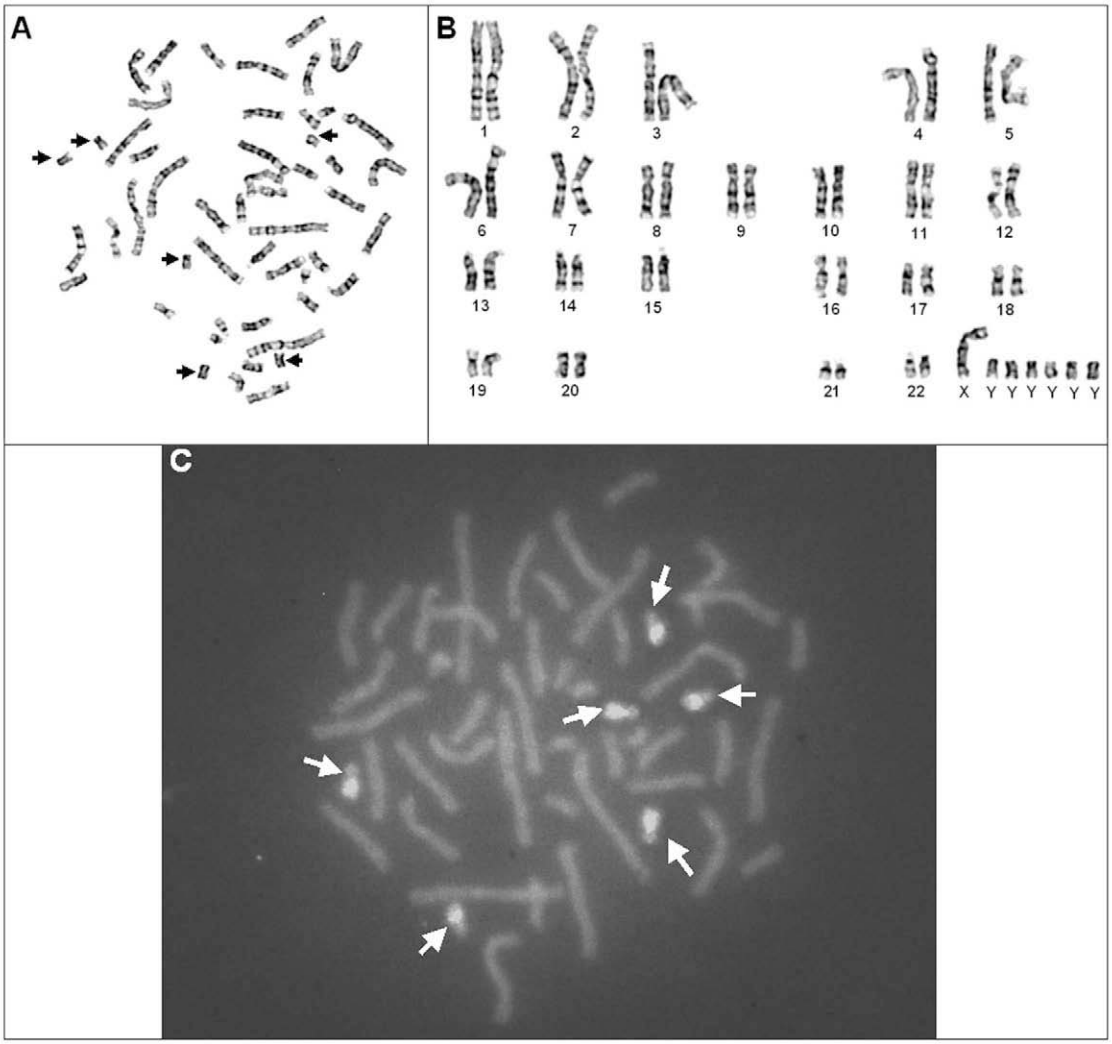

Figure 1. (**A**) and (**B**) Giemsa–trypsin–Giemsa (GTG) banding results in metaphase spread and karyotype (51,XYYYYYY cell line) of mos 46,XY/51,XYYYYYY karyotype associated with oligoastheno-teratozoospermia showing six Y chromosomes. (**C**) Fluorescence in-situ hybridization (FISH) of metaphase spread with the Vysis Y specific whole chromosome painting (WCP) DNA probe, which hybridizes the Y chromosome, indicated by arrows.

Table 5. Comparison of previous cytogenetic studies with the present study on infertile men. IVF = in-vitro fertilization, ICSI = intracytoplasmic sperm injection.

Previous studies	*Type of treatment*	*Males karyotyped* n	*Abnormal karyotypes* n *(%)*
Lange *et al*. (1993)	IVF	72	2 (2.8)
Baschat *et al*. (1996b)	ICSI	32	2 (6.2)
Mau *et al*. (1997)	ICSI	150	18 (12.0)
Peschka *et al*. (1996)	ICSI	200	6 (3.0)
Meschede *et al*. (1998)	ICSI	432	9 (2.1)
Testart *et al*. (1996)	ICSI	261	11 (4.2)
Cruger *et al*. (2003)	ICSI	392	18 (4.6)
Duzcan *et al*. (2003)	ICSI	176	9 (5)
All above studies (average)	-	1715	75 (4.3)
Present study	ICSI	744	59 (7.9)

Discussion

In a previous study (Rao *et al*., 2004), an association of genetic abnormalities (including Y chromosome microdeletions) with varicocoele related male infertility was reported when compared with idiopathic male infertility. In the same study, 2% azoospermic individuals showed Y chromosome microdeletions. Infertile individuals with OAT as clinical diagnosis rarely show Y chromosome abnormalities; therefore, the present study was restricted to screen chromosomal abnormalities only excluding the screening of Y chromosome microdeletions.

The present study shows a statistically significant association (P = 0.028) of chromosomal abnormalities in azoospermic individuals compared with OAT individuals from South India. The rate of chromosomal anomalies detected in the patient cohort was 7.9% and no significant difference was observed between the frequencies of autosomal abnormalities (4.4%) and sex chromosome abnormalities (3.5%). For a clinically useful interpretation, these crude figures were broken down by the type of anomalies detected and type of clinical abnormality; unsurprisingly, a large and statistically significant variation in frequencies was then found (**Table 1**).

According to Van Assche *et al*. (1996), 13.7% of azoospermic males and 4.6% of oligozoospermic males have an abnormal karyotype. When these results were compared with the present study, the frequency of abnormal karyotypes in azoospermic males was a little less (11%), while it was more (6.1%) in OAT individuals. Kalantari *et al*. (2003) reported the most frequent anomaly as 47,XXY (Klinefelter syndrome), found in 8.57% of azoospermic patients, which is considerably higher, and nearly twice as high as the 4.41% found in the present study.

A large difference in the frequencies of chromosomal abnormalities in azoospermia and OAT individuals was observed when classified on the basis of structural, numerical and mosaic types (**Table 4**). Except for one, all the chromosomal abnormalities in OAT individuals were structural abnormalities (95.6%) and only two numerical abnormalities associated with OAT were found. In contrast to this, azoospermic individuals showed more numerical abnormalities (56.6%).

A major objective of this study was to detect the contribution of autosomal abnormalities in azoospermia and sex chromosomal abnormalities in OAT. Here, six autosomal abnormalities associated with azoospermia were detected. Since, all the six abnormalities (involving chromosomes 1, 4, 9, 12, 13, 14, 15 and 19) resulted in azoospermia, these regions (**Table 2**) may have an important role to play in the production of spermatozoa in men. Therefore, further fine mapping of these regions may yield the data related to the genes on autosomes involved in azoospermia. In addition, two Y chromosome abnormalities (duplication and mosaic) associated with OAT were detected. Interestingly, this novel mosaicism (**Figure 1**) had a 46,XY cell line (62/100 metaphases) and 51,XYYYYYY cell line (38/100 metaphases) shown in **Figure 1**. The OAT condition of this patient may be a consequence of a possible mosaicism of his spermatogonial cells. In such cases, an elevated sperm aneuploidy rate has shown to be associated with a greater risk of pregnancy failure and some patients are still able to achieve a pregnancy with an increased risk of generating an aneuploid offspring (Calogero *et al*., 2004).

Data from previous similar studies (**Table 5**) were collected to investigate and compare the global frequency of chromosomal abnormalities in infertile men undergoing assisted reproduction with the present study. From the collected data, out of 1715 infertile men karyotyped, 75 (4.3%) showed chromosome defects, which is comparatively less than in the present study (7.9%). This shows the importance of considering the genetic defects as a factor for male infertility, especially in Indian subjects. For example, if a man with a specific chromosome defect had a child through ICSI, then it is more likely for the child to have the same type of defect. It should be emphasized that in such cases, if the pregnancy succeeds, a defect has mistakenly been allowed to pass to the next generation. Hence, a cytogenetic test is essential to detect these defects and should be offered to all men with

azoospermia and OAT who are thinking of fathering a child through intracytoplasmic sperm injection (ICSI).

In conclusion, a near 2-fold risk of chromosomal defects was observed in infertile men with azoospermia when compared with OAT individuals in a South Indian population. In general, numerical and sex chromosome abnormalities were detected in men with azoospermia, while structural and autosomal abnormalities were observed associated with OAT. With rapid advances in assisted reproductive technologies, it is gradually becoming accepted practice to use spermatozoa from men with infertility for ICSI and IVF purposes. Therefore, chromosome diagnosis for men with non-obstructive azoospermia needs to be more focused on OAT individuals considering assisted reproduction.

Acknowledgements

The study was supported by the grant from the Council for Scientific and Industrial Research and the Department of Science and Technology, New Delhi. The authors are thankful to Ms DV Jayanthi of the Infertility Institute and Research Centre, Secunderabad, for her help in patient recruitment.

References

Baschat AA, Kupker W, al Hasani S *et al*. 1996 Results of cytogenetic analysis in men with severe subfertility prior to intracytoplasmic sperm injection. *Human Reproduction* **11**, 330–333.

Benn PA, Perle MA 1992 Chromosome staining and banding techniques. In: Ronney DE, Czepulkowski BH (eds) *Human Cytogenetics. A Practical Approach*, vol. 1, *Constitutional Analysis*. IRL Press, Oxford, pp. 91–118.

Calogero AE 2003 Sperm aneuploidy in infertile men. *Reproductive BioMedicine Online* **6,** 310–317.

Chandley AC 1998 Chromosome anomalies and Y chromosome microdeletions as causal factors in male infertility. *Human Reproduction* **13** (Suppl. 1), 45–50.

Cruger DG, Agerholm I, Byriel L *et al*. 2003 Genetic analysis of males from intracytoplasmic sperm injection couples. *Clinical Genetics* **64,** 198–203.

Duzcan F, Atmaca M, Cetin GO *et al*. 2003 Cytogenetic studies in patients with reproductive failure. *Acta Obstetricia et Gynecologicia Scandinavica* **82**, 53–56.

Gosden CM, Davidson C, Robertson M 1992 Lymphocyte culture. In Rooney DE, Czepulkowski BH (eds) *Human Cytogenetics. A Practical Approach*. Vol. 1: *Constitutional Analysis*. IRL Press, Oxford, pp. 31–54.

Gruber CJ, Hengstschlager M, Leipold H *et al*. 2003 Genetic factors in assisted reproduction. *Wiener klinische Wochenschrift* **115**, 805–811.

Kalantari P, Sepehri H, Behjati F *et al*. 2003 Chromosomal studies of male infertility. *Genetika* **39**, 423–426.

Lange R, Johannson G, Engel W 1993 Chromosome studies in in-vitro fertilization patients. *Human Reproduction* **8**, 572–574.

Mau UA, Backert IT, Kaiser P *et al*. 1997 Chromosomal findings in 150 couples referred for genetic counselling prior to intracytoplasmic sperm injection. *Human Reproduction* **12**, 930–937.

Meschede D, Lemcke B, Exeler JR *et al*. 1998 Chromosome abnormalities in 447 couples undergoing intracytoplasmic sperm injection – prevalence, types, sex distribution and reproductive relevance. *Human Reproduction* **13**, 576–582.

Namiki M 2000 Genetic aspects of male infertility. *World Journal of Surgery* **24**, 1176–1179.

Peschka B, Schwanitz G, Van der Ven K *et al*. 1996 Type and frequency of constitutional chromosome aberrations in couples undergoing ICSI. *Human Reproduction* **11,** 224–225.

Rao L, Babu A, Kanakavalli M *et al*. 2004 Chromosomal abnormalities and y chromosome microdeletions in infertile men with varicocele and idiopathic infertility of South Indian origin. *Journal of Andrology* **25**, 147–153.

Testart J, Gautier E, Brami C *et al*. 1996 Intracytoplasmic sperm injection in infertile patients with structural chromosome abnormalities. *Human Reproduction* **11**, 2609–2612.

Van Assche E, Bonduelle M, Tournaye H *et al*. 1996 Cytogenetics of infertile men. *Human Reproduction* **11** (Suppl. 4), 24–26.

World Health Organization 2002 *Laboratory Manual for the Examination of Human Semen and Semen–Cervical Mucus Interaction*, 4th edn. Cambridge University Press, Cambridge, UK.

Yong EL, Lim J, Wang Q *et al*. 2000 Genetics of male infertility: role of androgen receptor mutations and Y-microdeletions. *Annals of Academy of Medicine Singapore*. **29**, 396–400.

Received 25 November 2004; refereed 8 December 2004; accepted 21 January 2005.