ORIGINAL RESEARCH

CYTOGENETIC ANALYSIS IN INFERTILE MALES WITH SPERM ANOMALIES

Ebru Önalan Etem, Hüseyin Yüce, Deniz Erol, Şükriye Derya Deveci, Gülay Güleç Ceylan, Halit Elyas

Fırat Üniversitesi, Tıp Fakültesi, Tıbbi Biyoloji ve Genetik, Elazığ, Türkiye

ABSTRACT

Objective: In a half of all childless partnerships the infertility is caused by the male. Chromosomal abnormalities are more prevalent in infertile men compared to fertile men. Chromosomal abnormalities are known to be associated with spermatogenetic failure. The present study investigates the frequency and types of major chromosomal abnormalities by using standard cytogenetic methods in infertile men with sperm anomalies.

Materials and Methods: A total of 214 infertile males (138 were azoospermic, 76 oligospermic) were studied for the cytogenetic evaluation. Chromosomal analysis of peripheral blood lymphocytes was performed according to standard protocols.

Results: Of the 214 infertile men, 24 (11.2%) had a chromosomal abnormality in the form of a Klinefelter syndrome/variant (16/24; 7.5%), XYY syndrome (1/24; 0.5%), XX male syndrome (1/24; 0.5%), 45,X, mar(Y) (1/24; 0.5%), 46,XX, inv(Y)(p11q11) (1/24; 0.5%), 46,XY, der(1)t(1;5)(p33;qter) (1/24; 0.5%), 46,XY, t(15;15) (1/24; 0.5%) or 46,XY,t(14;21) (1/24; 0.5%).

Conclusions: This study shows that chromosomal anomalies were found in 11.2% of the infertile men. The potential risk of transmitting these genetic disorders to offspring provides a rationale for screening infertile men prior to intra cytoplasmic sperm injection (ICSI). In addition, genetic screening and counseling should be offered to infertile patients routinely.

Keywords: Infertility, Chromosome, Cytogenetic, Azoospermia, Oligoospermia

SPERM ANOMALİSİ GÖSTEREN ERKEKLERDE SİTOGENETİK ANALİZLER

ÖZET

Amaç: Erkek infertilitesi çocuk sahibi olamayan çiftlerin yarısından sorumludur. Kromozomal abnormaliteler fertil erkeklerle karşılaştırıldığında infertil erkeklerde daha sıktır. Kromozomal anomalilerin spermotogenezde başarısızlığa neden olarak erkek infertilitesine neden olduğu bilinmektedir. Çalışmada sperm anomalisi gösteren infertil erkeklerde major kromozomal anomalilerin tipleri ve sıklığının araştırılması amaçlanmıştır.

Gereç ve Yöntem: Toplam 214 (138 azospermik, 76 oligospermik) infertil erkek bireye sitogenetik inceleme yapıldı. Tüm hastaların periferik kan lenfositlerinin kromozomal analizleri sdandart yöntemlere göre yapıldı.

Bulgular: Toplam 214 infertil erkeğin 24 (%11.2)'ünde klinifelter sendromu (16/24; %7.5), XYY sendromu (1/24; %0.5), XX erkek sendromu (1/24; %0.5), 45,X, mar (Y) (1/24; %0.5), 46,XX, inv(Y)(p11q11) (1/24; %0.5), 46,XY, der(1)t(1;5)(p33;qter) (1/24; %0.5), 46,XY, t(15;15) (1/24; %0.5) ve 46,XY,t(14;21) (1/24; %0.5) kromozomal anomalileri tespit edildi.

Sonuçlar: Bu çalışma infertil erkeklerde kromozomal anomalilerin sıklığı %11.2 olduğunu göstermektedir. Bu genetik bozuklukların yeni nesillere aktarılmasındaki potansiyel risk infertil erkeklerin ICSI'dan önce taranması için bir sebep oluşturmaktadır. Ayrıca, genetik tarama ve danışmanın infertil hastalara rutin olarak yapılması gerekmektedir.

Anahtar Kelimeler: İnfertilite, Kromozom,Sitogenetik, Azospermi, Oligospermi

İletişim Bilgileri:
Ebru Önalan Etem, M.D.
Fırat Üniversitesi, Tıp Fakültesi, Tıbbi Biyoloji ve Genetik, Elazığ, Türkiye
e-mail: ebruetem@gmail.com

Marmara Medical Journal 2009;22(3);217-224

INTRODUCTION

Infertility affects about 15 per cent of all couples attempting pregnancy, with a male-factor identified in approximately half of the cases[1]. Numerous factors contribute to male infertility, genetic factors including chromosomal abnormalities and genetic syndromes cause gene defects, and other factors include the hormonal milieu, genital infections, chemical and physical agents. infection, varicose, spermatic duct obstruction, antisperm antibodies, cryptorchidism, retrograde ejaculation, systemic diseases, testicular cancer, testicular trauma, etc. Male infertility can also be caused by a variety of other factors, apart from these, and in 30–40% of male infertile cases that are referred to as idiopathic, a genetic abnormality is suspected[2].

The examination of male infertility should be complex, including a detailed history, physical examination, semen analysis, hormonal screening, and chromosomal and genetic analysis of somatic cells[3]. The fact that chromosomal abnormalities are increased in infertile men relative to fertile men is well established. Most studies report a wide range of frequencies of chromosomal abnormalities, from 2.2% to 10.3%, due to different cytogenetic procedures and case inclusion criteria[1]. In cases of non-obstructive azoospermia, there is a 15% risk of an associated chromosome abnormality including both aneuploidies and structural rearrangements[4]. Nevertheless, all of them point to an increasing percentage of chromosomal abnormalities concomitant with a decreasing sperm count. In addition, the nature of chromosomal abnormalities differs depending on whether a patient has oligoospermia or azoospermia. An early mutational event in the stem cells could produce structural rearrangements (translocations, inversions, or small deletions) during spermatogenesis, persisting through mitotic and meiotic divisions to the mature sperm stage[1].

The main purpose of this study was the investigation of the possible cytogenetic causes of azoospermia and oligozoospermia among infertile Turkish men. The prevalence and types of cytogenetic abnormalities were analyzed using standard cytogenetic methods.

MATERIAL AND METHOD

Patients

The study was conducted retrospectively according to the records of the patients referred to the Department of Medical Biology and Genetics at Fırat University. From January 1998 to August 2009, 214 infertile Turkish men were enrolled in the study. Among these 214 men, 138 had azoospermia and 76 had oligoospermia. The average age was 33, ranging from 18 to 51 years. A complete semen analysis was performed in all patients according to the guidelines of the World Health Organization (1999). Semen was collected by masturbation at the laboratory after 3–5 days of sexual abstinence, and examined as soon as liquefied. Cases were classified into groups using sperm counts. Azoospermia was defined as the total absence of sperm cells and oligozoospermia was defined as a sperm cell count of less than 5×106 cells/ml in seminal liquid.

Cytogenetic Analysis

Chromosomal analysis of peripheral blood lymphocytes was performed according to standard protocols[5]. Peripheral blood (2 ml) was collected in heparin vacutainers (Becton Dickinson, USA). For every subject whole blood (0.5 ml) cultures were set up in 5 ml Roswell Park Memorial Institute (RPMI) 1640 media (GIBCO BRL, USA) containing 15% fetal calf serum (Biological Industries, KBH, Israel), antibiotic mixture and phytohemagglutinin P (DIFCO Lab, USA) for 72 h. Chromosome preparations were obtained from lymphocyte cultures and analyzed after Giemsa-Trypsin-Giemsa (GTG) -banding[6]. In all cases, at least 20 metaphases were analyzed. In cases of suspected mosaicism, 50 cells were counted. The karyotypes were interpreted using the recommendation of the International System for Human Cytogenetic Nomenclature[7].

Fluorescence in situ hybrizidation (FISH) Analysis

FISH for 46,XX and 47,XYY male patients, to exclude mosaicism was performed on lymphocyte metaphase spreads using the Y centromere-specific DNA probe: CEP Y alpha-satellite spectrum orange (32-130025) (Vysis, Illinois, USA). It was also performed using the X centromere and sex-determining region Y gene (SRY)-specific DNA probe: LSI SRY Yp11.3 spectrum orange/CEP X spectrum green (32-191007) (Vysis, Illinois, USA). The Y centromere-specific DNA single color probe was labeled with biotin and detected by FITC avidin. The chromosomal DNA was then counterstained with propidium iodide (PI). FISH using the locus specific identifier (LSI) SRY/CEP X DNA dual color probe was performed following the manufacturer's instructions (VYSIS) and chromosomal DNA was counterstained with 4',6-diamidino-2-phenylindole (DAPI). Statistical analysis was carried out by the Statistical Package for Social Science for Windows, version 11.0 (SPSS; Chicago, IL, USA). The unpaired t-test, Mann-Whitney U-test and Chi-squared test were used. $P < 0.05$ was considered significant.

RESULTS

Among the 214 infertile men studied, 24 showed some kind of constitutional chromosomal abnormality corresponding to a frequency of 11.2%. The frequency of abnormalities was 13.7% in the cases of azoospermia, and 6.5% in men with oligoospermia (Table III). Numerical and structural chromosomal abnormalities, which were detected in 24 patients, are summarized in Table I. Patients with Klinefelter Syndrome had azoospermia. The frequency of autosomal chromosome anomalies detected in the present study was 1.9% (4/214 patients), one patient who was a t(15;15) carrier was azoospermic (138/1), other translocation carriers were oligoospermic (3/76). There was a statistically significant difference in the autosomal translocation carrier between oligoospermic and azoopermic infertile male goups ($p<0.05$).

Polymorphisms were detected in 25 (11,6%) patients (Table II). Abnormality in the heterochromatin region of the Y chromosome and inv(9) was the most frequently identified polymorphism in 10/214 (4.6%) and 9/214 (4.2%) in infertile men, respectively.

For patients with a 47,XYY karyotype mosaicism was shown by FISH in Y chromosome content: 47,XYY (76%)/46,XY (24%). Hybridization with the Y centromere-SRY specific DNA dual probe in 46,XX male patients was positive, ruling out any hidden mosaicism with a Y-bearing cell line in peripheral blood cells.

Table I: Chromosomal abnormalities in azoospermic and oligospermic men.

Chromosomal Finding	Total (n=214)
46,XX male	0.5 % (1)
Numerical	
47,XXY	7.5 % (16)
47,XYY	0.5 % (1)
45,X, mar(Y)	0.5 % (1)
Structural	
Inversion	
46,XX,inv(Y)(p11q11)	0.5 % (1)
Translocation	
46,XY,der(1)t(1;5)(p33;qter)	0.5 % (1)
46,XY,t(15;15)	0.5 % (1)
46,XY,t(14;21)	0.5 % (1)
46,XY,t(9;15)(q21.1:q11.1)	0.5% (1)
	11.2 % (24)

Table II: Chromosomal polymorphisms

Chromosomal polymorphism	Frequency
46,XY, inv(9)	4.2 % (9)
46,XY, 9qh+	0.5 % (1)
46,XY,16qh+	0.5 % (1)
46,XY,Yqh(-)	1.8 % (4)
46,XY, Yqh(+)	4.6 % (10)
	11.6 % (25)

Table III: The cytogenetic findings in the literature

Author	Patient Number/chromosomal frequencies	Azoospermia	Oligospermia	Cytogenetic Abnormalities Structural	Numerical	Frequencies
Vincert et al (8)	2651/204	111/792 (14%)	93/1859 (5%)	73	131	7.69%
Zuffardi and Tiepolo et al (9)	2542/215	-	-	40	175	8.6%
Chandley et al (10)	2372/51	-	-	33	18	2.1%
Clementini et al (11)	2078/42	-	-	6	36	2.02%
Tuerlings et al (12)	1792/62	-	-	6	24	3.45%
Nakamura et al (13)	1790/225	-	-	64	126	12.5%
Yoshida et al (14)	1007/65	-	-	24	41	6.5%
Koulischer et al (15)	1000/33	-	-	6	27	3.3%
Salahshourifar et al (16)	874/136	106/444 (23.8%)	11/175 (6.2%)	20	116	15.5%
Şamlı et al (17)	819/52	42/383 (10.9%)	10/436 (2.2%)	13	39	5.9%
Mohammed et al (18)	289/23	-	-	3	20	7.9%
Akgul et al (19)	179/18	15/86 (17.4%)	5/73 (6.85%)	2	16	11.74%
Vutyavanic et al (20)	130/6	-	-	2	4	4,6%
Nagvankar et al (21)	88/9	6/42 (%14.3)	3/46 (%6.5)	5	4	10.2%
Balkan et al (22)	80/9	-	-	2	7	11.2%
Our study	**214/24**	**19/138 (13.7%)**	**5/76 (6.5%)**	**6**	**18**	**11.2%**
Total	**17.905/1174**	**299/1885 (15.8%)**	**127/2665 (4.7%)**	**305(1.7%)**	**802 (4.4%)**	**6.5 %**

DISCUSSION

Male infertility may be caused by a variety of chromosomal abnormalities, including abnormalities in the sex chromosomes and autosomes, gain or loss of an entire single chromosome resulting in aneuploidy or structural abnormalities, as in balanced and unbalanced tranlocations. The frequency of an abnormal karyotype in this study was within the previously reported range of 2.2–14.3% for infertile men (Table III) [8-22]. The incidence of cytogenetic abnormalities has been estimated at 5.8% in infertile men and only 0.5% in the normal population[1]. Possible explanations for the divergent frequencies of chromosomal abnormalities in infertile males may be populational, geographical, environmental or genetic heterogeneities, methodological detection problems (expecially for minor chromosomal abnormalities), patients' inclusion criteria or various chromosomal abnormality frequencies including the absence or the presence of chromosomal polymorphisms.

In the total population, aneuploidy (10.8%) was the most frequent chromosome-related cause among infertile males. The most common abnormality was Klinefelter's syndrome (16/24), which was in agreement with a previous study by Foresta et al.[23]. Men with a 47,XYY karyotype are generally fertile, but they are seen more frequently in infertile populations. There have been a few reports of 47,XYY syndromes in azoospermic males as in our study[23,24]. Since many 47,XYY men have normal semen parameters, the severe oligoospermia observed in these men may indicate more perturbations during meiotic pairing, subsequent loss of germ cells and the production of aneuploid sperm[24].

The clinical features of male sex reversal syndrome patients are azoospermia associated with one or more of the following: abnormal external genitalia, gynecomastia, short stature, and pelvic cyst[25]. Males with a 46, XX karyotype were mainly found in the group of azoospermic males (Table I). Most XX males originate from a crossing over between Xp and Yp during paternal meiosis, so that the SRY gene is translocated on the X chromosome. The SRY gene is present in this case (SRY+ XX males), but such patients have azoospermia.

A relationship between balanced autosomal translocations and infertility has been reported among severely oligozoospermic and azoospermic men[26-29]. In our study, reciprocal translocations t(1;5), t(9;15) and t(14;21) were seen in oligoospermic males and t(15;15) was seen in one azoospermic male. The exact mechanism by which chromosomal anomalies induce infertility is not clear. Sperm karyotyping studies of 37 reciprocal translocation heterozygotes have shown that 19–77% of spermatozoa are unbalanced[29]. When delineating the genetic basis of male infertility, it is very important to emphasize that about 50% of all translocations found in sterile men involved an acrocentric chromosome, which implicates their role in male hypofertility[30]. Guichaoua et al. emphasized the correlation between the involvement of the acrocentric chromosome in infertile translocation carriers and the severity of the spermatogenic defect[31]. It has been hypothesized that balanced translocations interfere with normal chromosome pairing and segregation at meiosis I, thus providing a potential for formation of unbalanced gametes and subsequent unbalanced abnormal offspring[32]. Another hypothesis is based on the assumption of potential autosomal genes involved in male gametogenesis that might be deregulated by chromosome breakpoints. The relation between chromosomal breakpoints and male infertility has been investigated, and it has been found that there is a nonrandom distribution of breakpoints associated with infertility[32,33]. The presence of abnormally distributed chromatin interferes with meiotic division and thus reduces sperm production. Spermatozoa bearing abnormal chromosomes may cause abnormal embryonic development, which can in turn, cause early pregnancy loss[26]. Further research in this direction is necessary. Vincent et al., reported that autosomal structural anomalies (Table III) were encountered primarily in severe

oligoospermia[8]. Our study confirms this finding because of detected three autosomal translocation in oligospermic males.

Common cytogenetic polymorphisms detected by G banding are considered as heteromorphisms and include heterochromatin regions of chromosomes 1, 9, 16 and Y[34]. The role of chromosome heteromorphisms in infertility has been studied previously[35-37]. Şahin et al., reported that the chromosomal polymorphisms frequency is 7.9% in infertile males. We found that the polymorphism frequency is 11.6%[38]. The occurrence of long Y (Yqh+) and short Y (Yqh-) in our study was 4,6% and 1,8% respectively. These frequencies were remarkably close to the frequencies of 4.4 and 1.6 per cent reported in literature[39,40]. Inv (9) is commonly seen in normal humans and the frequency has been estimated to be 1 to 3% in the general population[41]. As the frequency of inv(9) (4.2%) in infertile men was similar to that in the general population, these inversions definitely have role in the development of infertility especially in cases with de novo inversions. We advise parent's karyotyping for inv(9) carriers because the determination of unbalanced chromosomal content is important for the detection of de novo or familial inv(9) carriers.The contribution of variants to alter the carrier's fertility is still a controversial topic and further studies are required to understand this.

Among numerous etiologic factors, genetic factors play a primary role in male infertility. The creation of a specific model for the interpretation of male infertility data may lead to different results. For example, many patients with azoospermia show no chromozomomal abnormality because they may have vas deferens aplasia, which is often the result of a gene defect. However, the gene defect is invisible on a karyotype and requires a genetic diagnosis and counseling. It is clear that there are many genetic factors leading to infertility such as microdeletions of chromosome Y, some mutations of the cystic fibrosis transmembrane conductance regulator (CFTR) gene, mutations in Sry-related transcription factor (SOX9), Kallmann syndrome (KALIG1) and A-kinase anchor proteins (AKAP82) etc., but cytogenetic examinations should be made prior to molecular studies[42].

In conclusion, cytogenetic investigations in infertile men undoubtedly confirm previous reports in spite of differences in the incidence of chromosomal abnormalities in literature and they point to a risk of chromosomal abnormalities that is 20-fold higher in patients with severe oligoospermia or nonobstructive azoospermia, than in the general population. Consequently, high resolution chromosome preparations are crucial for a group with low sperm quality to detect complicated rearrangements. Therefore, genetic testing and counselling can provide support for patterns of inheritance, recurrence risks, natural history of diseases, increased risk for birth defects and genetic testing options when planning a pregnancy in patients with abnormal karyotypes. These patients can be advised as regards in vitro fertilization (IVF) and genetic screening of embryos in relation to assisted reproductive techniques.

Acknowledgement
The authors express their heartfelt gratitude to the staff and members of the Department of Medical Biology and Genetic for their assistance in various experiments, other organizational aspects of this study and Firat University Hospital. We are grateful to all volunteers who participated in this study.

REFERENCES

1. Bhasin S, de Kretser DM, Baker HW. Clinical review 64: Pathophysiology and natural history of male infertility. J Clin Endocrinol Metab 1994; 79 : 1525-1529.
2. Griffin DK, Finch KA. The genetic and cytogenetic basis of male infertility. Hum Fertil (Camb). 2005 Mar;8:19-26
3. Van Assch E, Bonduelle M, Tournaye H, et al. Cytogenetics of infertile men. Hum Reprod 1996;11:1–26.
4. Hook EB. Chromosomal abnormalities: prevalence, risks and recurrence. In Prenatal Diagnosis and Screening. Edinburgh: Churchill Livingstone 1992; 351-392.
5. Moorhead PS, Nowell PC, Mellman WJ, et al. Chromosome preparations of leukocytes cultured from human peripheral blood. Exp Cell Res 1960; 20: 613-616.

6. Seabright M. A rapid banding technique for human chromosomes. Lancet 1971; 2: 971-972.
7. Shaffer LG, Tommerup N. An International System for Human Cytogenetic Nomenclature. Karger , Farmington. 2005.
8. Vincent MC, Daudin M, De MP, et al. Cytogenetic investigations of mini review: infertile men with low sperm counts: A 25-Year Experience. J Androl 2002;23:18-22.
9. Zuffardi O, Tiepolo, L. Frequencies and types of chromosome abnormalities associated with human male infertility. In: Crosignani PG and B.L. Rubsin BL, eds. Genetic Control of Gamete Production and Function. Serono Clinical Colloquia on Reproduction III. Academic Press and Guine and Stratton, London, UK. 1982; 261-273.
10. Chandley AC. The chromosomal basis of human infertility. Br Med Bull 1979; 35: 181-186.
11. Clementini E, Palka C, Iezzi I, Stuppia L, Guanciali-Franchi P, Tiboni GM. Prevalence of chromosomal abnormalities in 2078 infertile couples referred for assisted reproductive techniques. Hum Reprod 2005; 20:437-442.
12. Tuerlings JH, de France HF, Hamers A, et al. Chromosome studies in 1792 males prior to intra-cytoplasmic sperm injection: the Dutch experience. Eur J Hum Genet 1998; 6:194-200.
13. Nakamura Y, Kitamura M, Nishimura K, et al. Chromosomal variants among 1790 infertile men. Int J Urol 2001; 8:49-52.
14. Yoshida A, Tamayama T, Nagao K, et al. A cytogenetic survey of 1007 infertile males. Contracept Fertil Sex 1995; 23: 103a.
15. Koulischer L, Schoysman R. Chromosomes and human infertility. I. Mitotic and meiotic chromosome studies in 202 consecutive male patients. Clin Genet 1974; 5: 116-126.
16. Salahshourifar I, Gilani MAS, Masoudi NS, Gourabi H. Chromosomal abnormalities in Iranian infertile males who are candidates for assissted reproductive techniques. IJFS 2007;1: 75-79.
17. Şamlı H, Solak M, İmirzalioğlu N, Şamlı MM. Nonobstruktif azoospermik ve siddetli oligozoospermik erkeklerde saptanan kromozomal anomaliler. Kocatepe Tıp Dergisi 2005; 6:7-11.
18. Mohammed F, Al-Yatama F, Al-Bader M, Tayel SM, Gouda S,Naguib KK. Primary male infertility in Kuwait: a cytogenetic and molecular study of 289 infertile Kuwaiti patients. Andrologia 2007;39:87–92.
19. Akgul M, Ozkinay F, Ercal D, et al. Cytogenetic abnormalities in 179 cases with male infertility in Western Region of Turkey: Report and review. J Assist Reprod Genet 2009; 26:119–122.
20. Vutyavanich T, Piromlertamorn W, Sirirungsi W, Sirisukkasem S. Frequency of Y chromosome microdeletions and chromosomal abnormalities in infertile Thai men with oligozoospermia and azoospermia. Asian J Androl 2007;9:68–75.
21. Nagvenkar P, Desai K, Hinduja I, Zaveri K. Chromosomal studies in infertile men with oligozoospermia and non-obstructive azoospermia. Indian J Med Res 2005;122:34–42.
22. Balkan M, Tekes S, Gedik A. Cytogenetic and Y chromosome microdeletion screening studies in infertile males with oligozoospermia and azoospermia in Southeast Turkey. J Assist Reprod Genet 2008; 25:559–565.
23. Foresta C, Garolla A, Bartoloni L, Bettella A, Ferlin A. Genetic abnormalities among severely oligospermic men who are candidates for intracytoplasmic sperm injection. J Clin Endocrinol Metab 2005; 90: 152–156.
24. El-Dahtory F, Elsheikha HM. Male infertility related to an aberrant karyotype, 47,XYY: four case reports. Cases J 2009 8;2: 28.
25. Hackstein JH, Hochstenbach R and Pearson PL. Towards an understanding of the genetics of human male infertility: lessons from flies. Trends Genet 2000; 16: 565-572.
26. Pandiyan N, Jequier AM. Mitotic chromosomal anomalies among 1210 infertile men. Hum Reprod 1996; 11:2604–2608.
27. Vicdan A, Vicdan K, Gunalp S, et al. Genetic aspects of human male infertility: the frequency of chromosomal abnormalities and Y chromosome microdeletions in severe male factor infertility. Eur J Obstet Gynecol Reprod Biol 2004; 117: 49–54.
28. Hellani A, Al-Hassan S, Iqbal M, Coskun S. Y chromosome microdeletions in infertile men with idiopathic oligo-or azoospermia. J Exp Clin Assist Reprod 2006; 30:1–6.
29. Martin RH. Cytogenetic determinants of male fertility. Hum Reprod Update 2008; 14:379–390.
30. Gabriel-Robez O, Ratomponirina C, Dutrillaux B, Carre-Pigeon F, Rumpler Y. Meiotic association between the XY chromosomes and the autosomal quadrivalent of a reciprocal translocation in two infertile men, 46,XY,t(19;22) and 46,XY,t(17;21). Cytogenet Cell Genet 1986;43:154–160.
31. Guichaoua MR, Quack B, Speed RM, Noel B, Chandley AC, Luciani JM. Infertility in human males with autosomal translocations: meiotic study of a 14;22 Robertsonian translocation. Hum Genet 1990;86: 162–166.
32. Zhou-Cun A, Yang Y, Zhang SZ, Zhang W, Lin L. Chromosomal abnormality and Y chromosome microdeletion in Chinese patients with azoospermia or severe oligozoospermia. Yi Chuan Xue Bao 2006; 33:111–116.
33. Bache I, Van Assche E, Cingoz S, et al. An excess of chromosome 1 breakpoints in male infertility. Eur J Med Genet. 2004;12:993–1000.
34. Brothman AR, Schneider NR, Saikevych I, et al. Cytogenetics Resource Committee, College of American Pathologists/American College of Medical Genetics. Cytogenetic heteromorphisms: Survey results and reporting practices of Giemsa-band regions that we have pondered for years. Arch Pathol Lab Med 2006;130:947–949.
35. Cortés-Gutiérrez EI, Cerda-Flores RM, Dávila-Rodríguez MI, Hernández-Herrera R, Vargas-Villarreal J, Leal-Garza CH. Chromosomal abnormalities and polymorphisms in Mexican infertile men. Arch Androl 2004;50:261–265.
36. Nakamura Y, Kitamura M, Nishimura K, et al. Chromosomal variants among 1790 infertile men. Int J Urol 2001;8:49–52.
37. Yakin K, Balaban B, Urman B. Is there a possible correlation between chromosomal variants and spermatogenesis? Int J Urol 2005;12:984–989.
38. Sahin FI, Yilmaz Z, Yuregir OO, Bulakbasi T, Ozer O, Zeyneloglu HB. Chromosome heteromorphisms: an impact on infertility. J Assist Reprod Genet 2008; 25:191–195.

39. Abramsson L, Beckman G, Duchek M, Nordenson I. Chromosomal aberrations and male infertility. J Urol 1982; 128 : 52-53.
40. Retief AE, Van Zyl JA, Menkveld R, Fox MR, Kotze GM, Brusnicky J. Chromosome studies in 496 infertile males with a sperm count below 10 million/ml. Hum Genet 1984; 66 : 162-164.
41. Rao BV, Kerketta L, Korgaonkar S, Ghosh K Pericentric inversion of chromosome 9[inv(9)(p12q13)]: Its association with genetic diseases. Indian J Hum Genet 2006; 12: 129-132.
42. Shah K, Sivapalan G, Gibbons N, Tempest H, Griffin DK. The genetic basis of infertility. Reproduction 2003;126:13-25.