

Chromosomal Abnormality in Men with Impaired Spermatogenesis

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Abstract

Background: Chromosomal abnormalities and Y chromosome microdeletions are regarded as two most frequent genetic causes associated with failure of spermatogenesis in the Caucasian population.

Materials and Methods: To investigate the distribution of genetic defects in the Romanian population with azoospermia or severe oligozoospermia, karyotype analysis by G-banding was carried out in 850 idiopathic infertile men and in 49 fertile men with one or more children. Screening for microdeletions in the azoospermia factor (AZF) region of Y chromosome was performed by multiplex polymerase chain reaction (PCR) on a group of 67 patients with no detectable chromosomal abnormality. The results of the two groups were compared by a two-tailed Fisher's exact test.

Results: In our study chromosomal abnormalities were observed in 12.70% and 8.16% of infertile and fertile individuals respectively.

Conclusion: Our data suggests that infertile men with severe azoospermia have higher incidences of genetic defects than fertile men and also patients from any other group. Infertile men with normal sperm present a higher rate of polymorphic variants. It is important to know whether there is a genetic cause of male infertility before patients are subjected to intracytoplasmic sperm injection (ICSI) or testicular sperm extraction (TESE)/ICSI treatment.

Keywords: Chromosomal Abnormality, Chromosome Microdeletion, Male Infertility, Azoospermia, Oligozoospermia

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Introduction

Male infertility is a common and severe health problem affecting 7% of populations (1). Infertility not only affects one's ability to have children, but also has emotional, psychological, familial and social effects. Despite the prevalence and significance of this health problem, resources and attention have not been sufficiently focused on this important issue. The most common causes of male infertility are abnormal

sperm delivery, chromosomal abnormalities, defective pituitary gland function, infections of the male accessory glands and overexposure to certain environmental factors (2).

Most men presenting with infertility are found to have idiopathic oligo-astheno-teratozoospermia (OAT) (3). The etiology of male infertility is unknown in approximately one third of the patients. The unaccountable forms of

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male infertility may be caused by several factors, such as chronic stress, endocrine disruption due to environmental pollution and genetic abnormalities (4-6). There is genetic predisposition to these pathologies such as varicocele (increased scrotal temperature) (1). Also, environmental factors are implicated in the onset and progression of male infertility (7).

Of the 7% of men suffering from infertility, 40% have idiopathic infertility. The cause of infertility in these men seems to be due to underlying genetic abnormalities (1). Chromosomal abnormalities are one of the major causes of human infertility as they interfere with spermatogenesis. Study of human chromosomes plays a key role in diagnosis, prognosis and monitoring of chromosomal abnormalities.

In infertile males, abnormal karyotype is more frequent than in the general population (8). Approximately 8 to 15% of men diagnosed with oligo or azoospermia present microdeletion on the long arm of chromosome Y, which by loss of specific DNA segments, vital genes needed for sperm production may be lost (9).

Male factor problems commonly manifest through an alteration of one or more semen parameters and the following terminology are used to describe these changes:

- *Azoospermia*—spermatozoa cannot be found in a semen sample.
- *Aspermia*—no semen sample was ejaculated.
- *Necrozoospermia*—all spermatozoa in the semen sample are dead.
- *Globozoospermia*—none of the spermatozoa have an acrosomal cap.
- *Oligozoospermia*—the concentration of spermatozoa is less than 20 million/ml of the ejaculate.
- *Asthenozoospermia*—less than 50% of spermatozoa in the semen sample exhibit forward motility.
- *Teratozoospermia*—the proportion of spermatozoa with normal shape is less than 30% (10).

At least 15 gene families, that are involved in spermatogenesis, are found on the long arm of chromosome Y (Yq) (11-14). Among infertile patients with azoospermia and oligozoospermia, Y chromosome microdeletions have been identified as causal factors. Y chromosome microdeletions are grouped in three main regions (AZFa, AZFb and AZFc) on the Yq arm (15).

Materials and Methods

From 2007 to April 2011, 850 infertile men (including 350 patients with oligo-asthenoteratozoospermia, 150 patients with severe oligozoospermia, 100 patients with azoospermia, 100 patients with teratozoospermia and 150 with normal semen analysis) who were referred to the Department of Reproductive Medicine at the Life Memorial Hospital of Bucharest in Romania were investigated for this retrospective study. 49 normozoospermic male donors with normal semen parameters (sperm count $>20 \times 10^6/\text{ml}$, progressive motility $>50\%$ and normal morphology $>30\%$) and proven fertility (with one or more children) were included as controls. All patients were initially evaluated by an andrologist and conventional diagnostic work-up including patient's history, genital examination, ultrasonography and hormone analyses were performed. None of them had any history of childhood disease, environmental exposure, radiation exposure or prescription drug usage that could account for their infertility. The median age of patients was 35.4 years (range 26-50 years).

Patients had been referred for chromosomal analysis, fluorescence in situ hybridization (FISH) for detection of Yp chromosome microdeletions and polymerase chain reaction (PCR) for 15 sites of AZF region on Y chromosome. In this prospective study we investigated infertile men prior to intracytoplasmic sperm injection (ICSI) treatment. Informed consent was obtained from the patients and controls prior to collection of heparinized blood samples.

Karyotype analysis was performed on peripheral blood lymphocytes. After 72 hours culture, the cells were harvested, hypotonised and fixed using 3:1 methanol: acetic acid. The metaphases were spread on slides. At least 10 metaphases were analyzed for each case and chromosomal abnormalities were reported according to the recommendations of the International System for Chromosome Nomenclature (ISCN 2009) (16, 17).

FISH was performed for 629 cases (using commercially available Vysis (Abbott) FISH probes which are complementary to the region of interest

on a particular chromosome) (18).

PCR was performed to screen the microdeletions in the AZF region of the Y chromosome. Genomic DNA was extracted according to standard procedure from peripheral blood samples (19). Each patient was analysed for the presence of sequence tagged sites (STS) in the AZFa, AZFb and AZFc regions (Table 1) (20). The STS probes used were sY84 and sY86 (AZFa), sY127 and sY134

(AZFb), sY254 and sY255 (AZFc), and SRY and ZFX/ZFY (controls).

This original study was approved by The Ethics Committee of Life Memorial Hospital Statistical Analysis: The results of the two groups were compared by a two-tailed Fisher's exact test and calculated online using Graph Pad (<http://www.graphpad.com/quickcalcs/contingency1.cfm>).

Table 1: Primer sequences of the sequence-tagged-sites (STSs) used in the detection of AZF loci (AZFa, AZFb and AZFc) and SRY

| STS | Sequence 5'-3' | Locus | Size (bp) |
|---------|-------------------------------|-------|-----------|
| sY86-F | GTG ACA CAC AGA CTA TGC TTC | AZFa | 320 |
| sY86-R | ACA CAC AGA GGG ACA ACC CT | | |
| sY127-F | GGC TCA CAA ACG AAA AGA AA | AZFb | 274 |
| sY127-R | CTG CAG GCA GTA ATA AGG GA | | |
| sY254-F | GGG TGT TAC CAG AAG GCA AA | AZFc | 400 |
| sY254-R | GAA CCG TAT CTA CCA AAG CAG C | | |
| sY14-F | GAA TAT TCC CGC TCT CCG GA | SRY | 495 |
| sY14-R | GCT GGT GCT CCA TTC TTG AG | | |
| sY84-F | AGA AGG GTC TGA AAG CAG GT | AZFa | 326 |
| sY84-R | GCC TAC TAC CTG GAG GCT TC | | |
| sY134-F | GTC TGC CTC ACC ATA AAA CG | AZFb | 301 |
| sY134-R | ACC ACT GCC AAA ACT TTC AA | | |
| sY255-F | GTT ACA GGA TTC GGC GTG AT | AZFc | 126 |
| sY255-R | CTC GTC ATG TGC AGC CAC | | |
| sY14-F | GAA TAT TCC CGC TCT CCG GA | SRY | 495 |
| sY14-R | GCT GGT GCT CCA TTC TTG AG | | |

Results

Karyotyping was carried out in 850 infertile men with impaired spermatogenesis. As shown in table 2, a total of 108 patients (12.70%) had chromosomal abnormalities: 6 azoospermic patients with Klinefelter's syndrome (47,XXY), 2 patients with 47,XYY syndrome (confirmed by FISH) (Fig 1) and 100 patients with structural chromosomal abnormality [of which 77 patients (71.3%) had polymorphic variants]. Only 2 out of 67 patients tested (3%) exhibited deletions on the long arm of Y chromosome, one of them being azoospermic and another oligozoospermic. In the control group we found 4 individuals with polymorphic variants: one case with an inversion on chromosome 9, two cases with chromosome heteromorphisms qh+ and one with a fragile site on chromosome 21 (Table 3). Types of chromosomal aberrations detected in men with infertility problems are presented in table 4. The most common chromosomal variants observed in infertile men are chromosomal polymorphisms (71.30%) (Table 5).

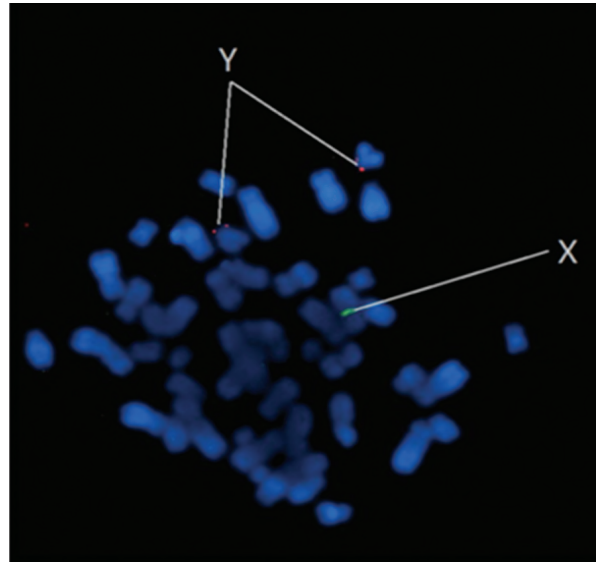


Fig 1: Isochromosom Y. Specific pattern of signals: an X chromosome specific signal (CEPX) and two signals characteristic of Y chromosome (SRY LSI). Result: 47,XYY. ish(CEPXX1) (SRYx2)

Table 2: Chromosomal abnormalities in infertile men

| Normal karyotype | Infertile male No./% | Fertile male No./% |
|------------------|----------------------|--------------------|
| CA | 108 (12.70%) | 4 (8.16%) |
| Numerical CA | 8 (7.41%) | - |
| Structural CA | 100 (92.59%) | 4 (8.16%) |

Screening of AZF microdeletions was carried out in the 67 patients including 46 infertile patients with azoospermia and 21 infertile patients with severe oligozoospermia with normal karyotype. In our study 2 of the 67 patients tested (3%) showed a Yq11 microdeletion, involving the AZFc locus, 1 of them being azospermic and 1 oligospermic and no patients presented microdeletions on Yp chromosome (out of 504 patients). No deletion in AZF region was found in fertile controls.

Table 3: Chromosomal abnormality in studied groups

| Patients | Autosomal abnormalities | Sex abnormalities | Polymorphic variants | Total abnormalities (autosomal + Sex chromosome) | Total |
|-------------------------------|-------------------------|-------------------|----------------------|--|--------------|
| Infertile male (n=850) | 20 (2.35%) | 11 (1.29%) | 77 (9.06%) | 31 (3.65) | 108 (12.71%) |
| OAT (n=350) | 5 (1.43%) | 1 (0.29%) | 10 (2.86%) | 6 (1.71%) | 16 (4.57%) |
| Azoospermia (n=100) | 8 (8%) | 7 (7%) | 15 (15%) | 15 (15%) | 30 (30%) |
| Oligozoospermia (n=150) | 2 (1.33%) | 1 (0.67%) | 5 (3.33%) | 3 (2%) | 8 (5.33%) |
| Teratozoospermia (n=100) | 1 (1%) | - | 11 (11%) | 1 (1%) | 12 (12%) |
| Normal semen analysis (n=150) | 4 (2.67%) | 2 (1.33%) | 36 (24%) | 6 (4%) | 42 (28%) |
| Fertile male (n=49) | - | - | 4 (8.16%) | - | 4 (8.16%) |

Table 4: Numerical and structural abnormalities in infertile men

| Chromosomal aberrations | | Karyotype | No. of cases | Frequency % | |
|---------------------------------|----------------|--------------------------------------|---------------------|-------------|-------|
| Structural abnormalities | Inversion | 46,XY,inv(9)(p11q13) | 22 | 2.59% | |
| | | 46,XY,inv(9)(p11q12) | 3 | 0.35% | |
| | | 46,XY,inv(3)(p11q11.2)inv(9)(p11q13) | 1 | 0.12% | |
| | | 46,XY,inv(1)(q23p13) | 1 | 0.12% | |
| | | 46,XY,inv(1)(q13p31) | 1 | 0.12% | |
| | | 46,XY,inv(10)(p11.2q21) | 1 | 0.12% | |
| | | 46,XY,inv(5)(pterq13) | 1 | 0.12% | |
| | | Deletion | 46,X,delY(q11.2) | 1 | 0.12% |
| | 46,X,delY(q12) | | 1 | 0.12% | |
| | Translocation | 46,XY,t(1;19)(p13;13.3) | 1 | 0.12% | |
| | | 46,XY,t(3;13)(p21;p11.2) | 1 | 0.12% | |
| | | 46,XY,t(1;9)(q11;p13) | 2 | 0.24% | |
| | | 45,XY,t(13;14)(q10q10) | 5 | 0.59% | |
| | | 45,XY,t(14;15)(q10q10) | 1 | 0.12% | |
| | | 46,XY,t(9;10)(q12q26) | 1 | 0.12% | |
| | | 46,XY,t(9;3)(q32q28) | 1 | 0.12% | |
| | | 46,XY,t(1;4)(q43q13) | 1 | 0.12% | |
| | | 46,XY,t(7;8)(q31.1q24) | 1 | 0.12% | |
| | | 46,XY,t(3;6)(q28;q13) | 1 | 0.12% | |
| | | Numerical abnormalities | Klinefelter Syndrom | 47,XXY | 6 |
| 47,XXY | | | | 2 | 0.24% |
| Syndrom XX | 46,XX | | 1 | 0.12% | |

Table 5: Chromosomal polymorphisms in infertile men

| Chromosomal polymorphic variations | Karyotype | No. of cases | Frequency % |
|------------------------------------|---------------|--------------|-------------|
| Heteromorphisms qh+ | 46,XY,1qh+ | 10 | 9.26% |
| | 46,XY,9qh+ | 9 | 8.33% |
| | 46,XY,16qh+ | 2 | 1.85% |
| | 46,X Yqh+ | 3 | 2.78% |
| Fragile sites | 46,XY,fra(17) | 7 | 6.48% |
| | 46,XY,fra(16) | 4 | 3.70% |
| pseudo satellites | 46,XY,14ps+ | 5 | 4.63% |
| | 46,XY,15ps+ | 2 | 1.85% |
| | 46,XY,21ps+ | 2 | 1.85% |
| | 46,XY,22ps+ | 8 | 7.41% |

Discussion

This study was designed to explore the implication of chromosomal abnormalities (CA) in male infertility. In our study constitutional chromosomal abnormalities were identified in 12.70% (71.3% polymorphic variants) of infertile patients and 8.16% (100% polymorphic variants) in the control group. Structural chromosomal abnormalities were present in a high proportion of men with infertility problems. In fertile controls, structural abnormality was detected in 4 cases only and no deletion in AZF region was found.

We identified 6 cases of Klinefelter' syndrome, which is reported to be the most frequent chromosomal aberration causing azoospermia in men followed by Yq deletions (21). The prevalence of Klinefelter' syndrome among infertile men is very high, up to 5% in severe oligozoospermia and 10% in azoospermia (22). We identified Klinefelter' syndrome in 6% of patients with azoospermia but it was absent in all other groups. The incidence of sex chromosome aneuploidies was statistically significantly different for azoospermia group versus any other group (7% vs. at most 1.33%, $p=0.018$).

The incidence of chromosomal abnormalities in patients with azoospermia was significantly higher than that in patients of all other groups (15% vs. at

most 4% for patients with normal semen) which was similar to other studies (21, 23). There was no significant difference in the incidence of autosomal abnormalities between infertile patients with OAT (1.43%), oligozoospermia (1.33%) and teratozoospermia (1%).

We identified a very high prevalence of polymorphic variants in infertile men with normal spermogram (24%). This prevalence is statistically higher than for any other group (24% vs. at most 15%, $p=0.001$). This might suggest a role for polymorphic variants in human fertility.

The most common reported clinical diagnosis among patients with inversion of chromosome 9 is azoospermia (24). This finding is similar to the present study. Pericentric inversion of chromosome 9, inv(9) (p11q12)/inv(9) (p11q13) is a common chromosomal rearrangement and some cytogeneticists consider it as a normal variant, generally without phenotypic effects (25). In this study, the inversion of chromosome 9 was found in 23.15% of patients with chromosomal abnormalities. Other chromosomal variants with a high incidence were 1qh + (9.26%), 9 qh + (8.33%) and 22ps + (7.41%). The least common polymorphic variation found in infertile couples usually occurred in the paracentric heterochromatin on the

long arms of chromosomes 16 (16qh + 1.85%), the short-arm regions of D and G group chromosomes (15ps + 1.85% and 21ps + 1.85%), and the distal heterochromatin of the Y chromosome (Yqh + 2.78%). In addition to Y chromosomal heteromorphism, other heteromorphisms related to infertility have also been described: heteromorphisms shown by short-arm regions of D and G group chromosomes and heteromorphisms shown by paracentric long-arm regions of chromosomes 1, 9 and 16, and inv(9) (26-28).

The exact mechanism by which chromosomal abnormalities induce infertility is still not completely understood. Some authors suggest that presence of abnormal chromatin interferes with meiotic division and affects sperm production (29). Genetic abnormalities which affect spermatogenesis may cause abnormal embryonic development, which in turn can lead to recurrent miscarriages (24, 30, 31). The infertile patients with normal karyotype, were further investigated for the presence of microdeletions on the short arm of chromosome Y and in the three AZF loci on Yq11. Azoospermia, severe oligozoospermia and oligozoospermia are characterized by complete absence of sperm in semen, a sperm cell count $<5 \times 10^6$ cells/ml, and a sperm cell count $>5 \times 10^6$ and $<20 \times 10^6$ cells/ml respectively. Microdeletions on the short arm of chromosome Y and in the three AZF loci on Yq11 cause severe testiculopathies and infertility in 2% of cases (12, 32-35). The prevalence of Yq microdeletions increases to 15-20% of cases in males affected by severe oligozoospermia or non-obstructive azoospermia (15, 35-38). We found no significant difference in the frequencies of AZF microdeletions between patients with azoospermia and those with severe oligozoospermia. Only 2 infertile men were diagnosed with microdeletions of the AZF region. No microdeletions were detected in either AZFa, AZFb or AZFd. In one male subject we found a 46,XX karyotype (FISH analysis revealed an SRY signal on the short arm of the chromosome X (XX male syndrome) and only 2 males who presented karyotype 46,X,delY(q11.2) on cytogenetic analysis.

Infertile men have a higher risk of constitutional chromosomal rearrangements which may be the cause of infertility (39).

However, because of the limited size of the control group in the present study, the actual frequency

of chromosome abnormalities needs to be investigated in a further study with a larger control group.

Conclusion

The incidence of chromosome abnormalities in patients with azoospermia is higher than in any other groups both when autosomal aberrations or sex chromosome aneuploidies are compared. Klinefelter's syndrome was the most frequent chromosomal aberration in azoospermic men. This association has been previously reported as such.

On the other hand, normospermic men carried the most frequent aberration (polymorphic variants) which was 1.6 times more frequent than in azoospermic men and 3 times more frequent than in fertile men.

The high rate of chromosomal abnormalities among infertile men strongly suggests the need for cytogenetic analysis and detection of Y chromosome microdeletions prior to the application of assisted reproduction techniques.

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References

1. Douglas TC. The genetics of male infertility. New Jersey: Humana Press; 2007; 99.
2. Rowe PJ, Comhaire FH, Hargreave TB, Mellows HJ. WHO manual for the standardized investigation and diagnosis of the infertile couple. Geneva: Cambridge University Press; 2000; 14-15.
3. Dohle GR, Colpi GM, Hargreave TB, Papp GK, Jungwirth A, Weidner W, et al. EAU guidelines on male infertility. Eur Urol. 2005; 48(5): 703-711.
4. Schlegel PN, Pavlovich CP. Male infertility treatment options: a cost-effectiveness analysis. JCOM. 1997; 4: 27-32.
5. Khademi A, Safdarian L, Alleyassin A, Agha Hosseini M, Hamed EA, Saeidabadi HS, et al. The effect of L-carnitine on sperm parameters in patients candidate for Intracytoplasmic Sperm Injection. IJRM. 2004; 2(2): 65-69.
6. Crosignani PG, Collins J, Cooke ID, Diczfalusy E, Rubin B. Recommendations of the ESHRE workshop on 'Unexplained Infertility'. Anacapri, August 28-9, 1992. Hum Reprod. 1993; 8(6): 977-980.
7. Warhaus S. Fertility demystified: a self-teaching guide. 1st ed. New York: The McGraw-Hill Companies; 2007; 19-22.
8. Sarrate Z, Blanco J, Anton E, Egozcue S, Egozcue J, Vidal F. FISH studies of chromosome abnormalities in germ cells and its relevance in reproductive counseling.

- Asian J Androl. 2005; 7(3): 227-236.
9. Silber SJ. The Y chromosome in the era of intracytoplasmic sperm injection: a personal review. *Fertil Steril*. 2011; 95(8): 2439-2448
 10. Rowe PJ, Comhaire FH, Hargreave TB, Mellows HJ. WHO manual for the standardized investigation and diagnosis of the infertile couple. Geneva: Cambridge University Press; 1993; 1-39.
 11. Ma K, Inglis JD, Sharkey A, Bickmore WA, Hill RE, Prosser EJ, et al. A Y chromosome gene family with RNA-binding protein homology: candidates for the azoospermia factor AZF controlling human spermatogenesis. *Cell*. 1993; 75(7): 1287-1295.
 12. Reijo R, Lee TY, Salo P, Alagappan R, Brown LG, Rosenberg M, et al. Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. *Nat Genet*. 1995; 10(4): 383-393.
 13. Jones MH, Furlong RA, Burkin H, Chalmers IJ, Brown GM, Khwaja O, et al. The *Drosophila* developmental gene fat facets has a human homologue in Xp11.4 which escapes X-inactivation and has related sequences on Yq11.2. *Hum Mol Genet*. 1996; 5(11): 1695-1701.
 14. Lahn BT, Page DC. Functional coherence of the human Y chromosome. *Science*. 1997; 278(5338): 675-680.
 15. Vogt PH, Edelmann A, Kirsch S, Henegariu O, Hirschmann P, Kiesewetter F, et al. Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. *Hum Mol Genet*. 1996; 5(7): 933-943.
 16. Shaffer LG, Slovak ML, Campbell LJ. An international system for human cytogenetic nomenclature. Basel: S. Karger; 2009.
 17. Babu A, Verma RS. Characterization of human chromosomal constitutive heterochromatin. *Can J Genet Cytol*. 1986; 28(5): 631-644.
 18. Kievits T, Devilee P, Wiegant J, Wapenaar MC, Cornelisse CJ, van Ommen GJ, et al. Direct nonradioactive in situ hybridization of somatic cell hybrid DNA to human lymphocyte chromosomes. *Cytometry*. 1990; 11(1): 105-109.
 19. Simoni M, Bakker E, Eurlings MC, Matthijs G, Moro E, Müller CR, et al. Laboratory guidelines for molecular diagnosis of Y-chromosomal microdeletions. *Int J Androl*. 1999; 22(5): 292-299.
 20. Alkhalaf M, Al-Shoumer K. Cytogenetic abnormalities and azoospermia factor (AZF) microdeletions in infertile men from Kuwait. *J Mol Genet Med*. 2010; 4: 232-234.
 21. Van Assche E, Bonduelle M, Tournaye H, Joris H, Verheyen G, Devroey P, et al. Cytogenetics of infertile men. *Hum Reprod*. 1996; 11 Suppl 4: 1-24.
 22. Poongothai J, Gopenath TS, Manonayaki S. Genetics of human male infertility. *Singapore Med J*. 2009; 50(4): 336-347.
 23. Zhou-Cun A, Yuan Y, Si-Zhong Z, Wei Z, Li L. Chromosomal abnormality and Y chromosome microdeletion in Chinese patients with azoospermia or severe oligozoospermia. *Yi Chuan Xue Bao*. 2006; 33(2): 111-116.
 24. Nagvenkar P, Desai K, Hinduja I, Zaveri K. Chromosomal studies in infertile men with oligozoospermia & non-obstructive azoospermia. *Indian J Med Res*. 2005; 122(1): 34-42.
 25. Ait-Allah AS, Ming PML, Salem HT, Reece EA. The clinical importance of pericentric inversion of chromosome 9 in prenatal diagnosis. *J Matern Fetal Invest*. 1997; 7(3): 126-128.
 26. Madon PF, Anthalye AS, Parikh FR. Polymorphic variants on chromosomes probably play a significant role in infertility. *Reprod Biomed Online*. 2005; 11(6): 726-732.
 27. Sahin F, Yilmaz Z, Yuregir OO, Bulakbasi T, Ozer O, Zeyneloglu HB. Chromosome heteromorphisms: an impact on infertility. *J Assist Reprod Genet*. 2008; 25(5): 191-195.
 28. Minocherhomji S, Anthalye AS, Madon PF, Kulkarni D, Uttamchandani SA, Parikh FR. A case-control study identifying chromosomal polymorphic variations as forms of epigenetic alterations associated with the infertility phenotype. *Fertil Steril*. 2009; 92: 88-95.
 29. Etem EO, Yüce H, Erol D, Devenci ŞD, Ceylan GG, Elyas H. Cytogenetic analysis in infertile males with sperm anomalies. *Marmara Med J*. 2009; 22(3): 217-224.
 30. Evans JA, Canning N, Hunter AG, Martsof JT, Ray M, Thompson DR, et al. A cytogenetic survey of 14,069 newborn infants. III. an analysis of the significance and cytologic behavior of the Robertsonian and reciprocal translocations. *Cytogenet Cell Genet*. 1978; 20(1-6): 96-123.
 31. Pandiyan N, Jequier AM. Mitotic chromosomal anomalies among, 1210 infertile men. *Hum Reprod*. 1996; 11(12): 2604-2608.
 32. Najmabadi H, Huang V, Yen P, Subbarao MN, Bhasin D, Banaag L, et al. Substantial prevalence of microdeletions of the Y chromosome in infertile men with idiopathic azoospermia and oligozoospermia detected using a sequence-tagged site-based mapping strategy. *J Clin Endocrinol Metab*. 1996; 81(4): 1347-1352.
 33. Qureshi SJ, Ross AR, Ma K, Cooke HJ, Intyre MA, Chandley AC, et al. Polymerase chain reaction screening for Y chromosome microdeletions: a first step towards the diagnosis of genetically-determined spermatogenic failure in men. *Mol Hum Reprod*. 1996; 2(10): 775-779.
 34. Reijo R, Alagappan RK, Patrizio P, Page DC. Severe oligozoospermia resulting from deletions of azoospermia factor gene on Y chromosome. *Lancet*. 1996; 347(9011): 1290-1293.
 35. Pryor JL, Kent-First M, Muallem A, Van Bergen AH, Nolten WE, Meisner L, et al. Microdeletions in the Y chromosome of infertile men. *N Engl J Med*. 1997; 336(8): 534-539.
 36. Foresta C, Ferlin A, Garolla A, Rossato M, Barbaux S, De Bortoli A. Y chromosome deletions in idiopathic severe testicular pathologies. *J Clin Endocrinol Metab*. 1997; 82(4): 1075-1080.
 37. Simoni M. Molecular diagnosis of Y chromosome microdeletions in Europe: state-of-the-art and quality control. *Hum Reprod*. 2001; 16(3): 402-409.
 38. Foresta C, Ferlin A, Garolla A, Moro E, Pistorello M, Barbaux S, et al. High frequency of well-defined Y chromosome deletions in idiopathic Sertoli cell-only syndrome. *Hum Reprod*. 1998; 13(2): 302-307.
 39. 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(2): 437-442.