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Prevalence of Y chromosome deletions in a Brazilian population of nonobstructive azoospermic and severely oligozoospermic men

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Abstract

We determined the prevalence of Y chromosome deletions in a population of 60 Brazilian nonobstructive azoospermic and severely oligozoospermic men. PCR-based screening of microdeletions was performed on lymphocyte DNA for the presence of 14 sequence-tagged sites (STS) located in the azoospermic factor (AZF) on the Yq chromosome. All STS were amplified efficiently in samples from 12 fertile men tested, but failed to be amplified in samples from fertile women, indicating the specificity of PCR conditions for Yq screening. Overall, 4 of the 60 infertile patients tested (6.7%) exhibited deletion of the Y chromosome, 2 of them being severely oligozoospermic patients (P10 and P32) and 2 azoospermic men (patients P47 and P57). Patients P47 and P57 presented larger deletions in the AZFa, AZFb and AZFc subregions, with apparent loss of Yq material evidenced by karyotype analysis. Patients P10 and P32 presented deletions confined to the AZFc region, involving the DAZ locus. Male relatives of patients P10 and P32 had no Y chromosome deletions and presented a normal karyotype, suggesting a *de novo* status of the deletions found. Our data add to the growing literature showing that microdeletions of the Y chromosome can be the cause of male idiopathic infertility.

Key words
- Y chromosome
- Microdeletions
- Severe oligozoospermia
- Azoospermia
- Male infertility
- Azoospermia factor
- AZF region

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Introduction

The existence of a gene, or a gene complex, associated with normal spermatogenesis was first postulated on the basis of a cytogenetic analysis that showed macroscopic deletions of the distal part of the Y chromosome (Yq11) in men with azoospermia (1). This gene or gene cluster was defined as azoospermia factor (AZF). Subsequent PCR-based screening studies used to amplify sequence-tagged sites (STS) spanning the Y chromosome (2,3) facilitated the detection of small interstitial deletions (i.e., microdeletions) of Yq11 not only in azoospermic (4-6), but also in severely oligozoospermic men (7,8). Since these deletions were variable in both extent and location, the AZF region was divided into three nonoverlapping subregions (AZFa, AZFb and AZFc) located from the

proximal to the distal part of Yq (6). Recent genetic studies have shown that several genes or gene families located in AZF regions are specifically expressed in the testis and could therefore be considered as AZF candidate genes for infertility, such as the RBM (RNA-binding motif) and the DAZ (deleted in azoospermia) gene families (for reviews, see Refs. 9-11). Recently, a fourth AZF subregion (AZFd) between AZFb and AZFc has been proposed (12). Men with deletions located exclusively in the AZFd subregion present mild oligozoospermia or even normal sperm counts associated with normal sperm morphology (12), but a candidate gene has not yet been found in this region.

Over the past few years, screening tests for detecting microdeletions on the long arm of the Y chromosome have established the distribution and characteristics of the deletions among different groups of infertile male patients (for reviews, see Refs. 9-11). Data from PCR studies have demonstrated that the prevalence of Y chromosome microdeletions among men with nonobstructive idiopathic azoospermia or severe oligozoospermia ranges from 3 to 55.5% (5-8,12-22), the higher frequencies being detected in cases of severely impaired spermatogenesis (11). A number of factors have been implicated in the wide variation of Y deletion frequencies reported such as patient selection criteria, experimental designs, environmental influences and ethnic variations (22-27). In the present paper, we report the prevalence of Y chromosome deletions in a Brazilian population of nonobstructive azoospermic and severely oligozoospermic men attending our university-based outpatient clinic.

Material and Methods

Patients

DNA samples from 60 patients referred to the Section of Human Reproduction, Gynecology and Urology Division, Universidade Federal de São Paulo-Escola Paulista de Medicina (UNIFESP-EPM) and who gave written informed consent to be submitted to genetic testing were screened for Yq chromosome microdeletions. In addition, 12 fertile men and 2 fertile women were tested as positive and negative controls, respectively. The Institutional Ethics Committee at UNIFESP-EPM approved the study design. Clinical evidence of obstructive azoospermia, or a history of mumps, injury and cryptorchidism were not observed in the selected patients. However, varicocele was not excluded because in this situation the cause of infertility is largely unknown and an associated Y chromosome deletion cannot be excluded (11). The testis volume was measured with a Prader orchidometer. Semen samples were obtained on two different occasions, with each collection being performed after a 3-day period of sexual abstinence and separated by a 3-week interval. A complete semen analysis was performed according to World Health Organization guidelines (28). The diagnosis of azoospermia was established by pellet analysis after semen centrifugation (1,000 *g*, 20 min). Of the 60 patients included in this study (mean age: 32 years; range 24-49 years), 29 were diagnosed with nonobstructive azoospermia and 31 with severe oligozoospermia ($<5 \times 10^6$ spermatozoa/ml). First-degree male relatives of the men diagnosed with Y chromosome deletions were asked to withdraw blood for DNA analysis in order to determine if the deletion was *de novo*.

Plasma hormone levels

The plasma concentrations of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were measured by immunoassay using an IM$^{®}_{X}$ ^{system} kit (Abbott Laboratories, Abbott Park, IL, USA), while plasma testosterone levels were determined by radioimmunoassay using a Coat-A-Count® Testosterone Total kit (Diagnostic Products

Co., Los Angeles, CA, USA) according to manufacturer instructions. Normal reference ranges for men are FSH 1-12 mIU/ml, LH 2-12 mIU/ml and testosterone 262-1593 ng/dl.

DNA isolation and Y chromosome analysis by PCR

To screen for microdeletions in the Y chromosome by PCR, genomic DNA was isolated from lymphocyte nuclei according to standard protocols. Each individual was analyzed for the presence of 12 STS on Yq spread over intervals 5 and 6 in the following subregions: AZFa (sY82, sY84, sY86), AZFb (sY117, sY142, sY143), AZFc (sY147, sY149, sY254, sY255, sY283, sY158). The STS sY160 in the heterochromatin region outside AZF regions was also analyzed. The STS sY14, which is localized in Yp, was used as a positive control for the presence of Y-specific DNA. All STS primers, shown in Table 1, have been previously described (2,3,5,7). PCR analysis was performed on 25 µl of reaction volume containing genomic DNA (100 ng), deoxyribonucleotides (0.1 mmol/l each), *Taq* polymerase (1.25 U), and oligonucleotide primers (1 µmol/l each) at a final concentration of 1 x PCR buffer (20 mmol/l Tris-HCl, pH 8.4, 50 mmol/l KCl, 3 mmol/l $MgCl_2$). Primers sY117, sY143, sY283, and sY160 were amplified in the presence of 6 mmol/l $MgCl_2$. Amplifications were obtained with an Idaho Rapidcycler (Idaho Technologies, Idaho Falls, ID, USA) under the following conditions: initial 2 min of denaturation at 94ºC followed by 35 sequential cycles, each including 30 s at 94ºC, 40 s at 58ºC, and 20-60 s at 72ºC. The PCR products were separated on 2.5% agarose gels by electrophoresis, stained with ethidium bromide, and photographed. During optimization of the PCR conditions, genomic DNA from fertile men and women served as normal and Y-deleted controls, respectively. The authenticity of the PCR products was confirmed by direct nucleotide sequencing performed with an ABI PRISM™ 377 automated sequencer (Applied Biosystems, Foster City, CA, USA) and the BigDye™ Terminator Sequencing kit (Applied Biosystems).

Cytogenetic evaluation

Chromosome analysis was carried out on peripheral lymphocytes with G-banding staining by the method of Moorhead et al.

Table 1. Sequence-tagged sites (STS) and primer sequences used for Y chromosome microdeletion analysis.

STS	Primer sequence	Size (base pairs)
sY14	Forward 5′ GAATATTCCCGCTCTCCGGA 3′ Reverse 5′ GCTGGTGCTCCATTCTTGAG 3′	472
sY82	Forward 5′ ATCCTGCCCTTCTGAATCTC 3′ Reverse 5′ CAGTGTCCACTGATGGATGA 3′	264
sY84	Forward 5′ AGAAGGGTCTGAAAGCAGGT 3′ Reverse 5′ GCCTACTACCTGGAGGCTTC 3′	320
sY86	Forward 5′ GTGACACACAGACTATGCTTC 3′ Reverse 5′ ACACACAGAGGGACAACCCT 3′	326
sY117	Forward 5′ GTTGGTTCCATGCTCCATAC 3′ Reverse 5′ CAGGGAGAGAGCCTTTTACC 3′	452
sY142	Forward 5′ AGCTTCTATTCGAGGGCTTC 3′ Reverse 5′ CTCTCTGCAATCCCTGACAT 3′	196
sY143	Forward 5′ GCAGGATGAGAAGCAGGTAG 3′ Reverse 5′ CCGTGTGCTGGAGACTAATC 3′	311
sY147	Forward 5′ TTTCTCGTTTGATGATCCTAG 3′ Reverse 5′ TTAATATGAGAATGAGAACAGATGT 3′	100
sY149	Forward 5′ TGTCACACTGCCCTAATCCT 3′ Reverse 5′ TGGTCATGACAAAAGACGAA 3′	132
sY254	Forward 5′ GGGTGTTACCAGAAGGCAAA 3′ Reverse 5′ GAACCGTATCTACCAAAGCAGC 3′	350
sY255	Forward 5′ GTTACAGGATTCGGCGTGAT 3′ Reverse 5′ CTCGTCATGTGCAGCCAC 3′	126
sY283	Forward 5′ CAGTGATACACTCGCACTTGTGTA 3′ Reverse 5′ GTTATTTGAAAAGCTACACGGG 3′	497
sY158	Forward 5′ CTCAGAAGTCCTCCTAATAGTTCC 3′ Reverse 5′ ACAGTGGTTTGTAGCGGGTA 3′	231
sY160	Forward 5′ TACGGGTCTCGAATGGAATA 3′ Reverse 5′ TCATTGCATTCCTTTCCATT 3′	236

The size of the expected amplified DNA product is indicated for each STS.

(29). Karyotypes were obtained in the Department of Genetics, UNIFESP-EPM. On average, 20-30 metaphases per patient were analyzed.

Results

Deletion of a specific locus on Yq11 was indicated by the failure of amplification in three independent PCR experiments. The repeated experiments were performed on new samples of DNA extracted from a different blood collection. No microdeletions were observed in any of the fertile men tested. Overall, 4 of the 60 infertile patients tested (6.7%) exhibited microdeletions of the Y chromosome, 2 of them being severely oligozoospermic patients (P10 and P32) and 2 azoospermic men (patients P47 and P57). The severely oligozoospermic patients P10 and P32 failed to amplify STS sY254, sY255 and sY283, which amplify parts of the DAZ gene in the AZFc region. Azoospermic patients presented larger deletions. Deletions of STS located in the AZFa (sY84, sY86) and AZFc regions (sY147, sY254, sY283, sY158) were found in patient P47, while deletions in the AZFb (sY142) and AZFc regions (sY254, sY255, sY283, sY158) were found in patient P57. Patients P47 and P57 did not amplify sY160, an STS localized in the Y heterochromatin outside the AZF regions. Patients P10 and P32 had microdeletions that could not be detected by classical cytogenetic analysis, both presenting a morphologically normal Y chromosome. Patients P47 and P57, on the other hand, presented a 46,XY (delY)(q11) karyotype. A schematic representation of the deletions detected is given in Figure 1.

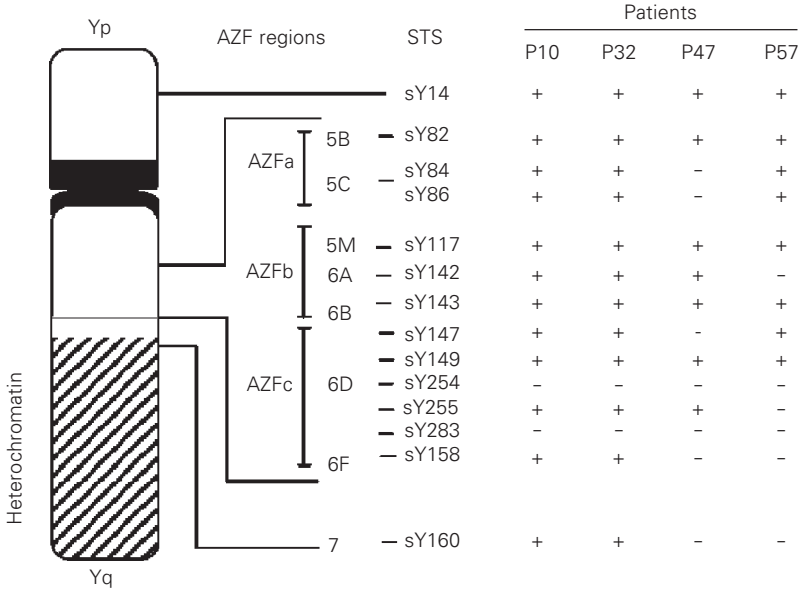

Figure 1. Schematic diagram of the human Y chromosome, including AZF regions (AZFa, AZFb and AZFc) and deletion intervals 5, 6 and 7, as defined by Vogt et al. (6). The list of sequence-tagged sites (STS) screened by PCR and their localization along the Y chromosome is shown. The presence (+) or absence (–) of STS amplification, observed in patients P10, P32, P47 and P57, is also indicated.

Table 1 summarizes the clinical and hormonal characteristics of the 4 patients who presented Yq microdeletions in the PCR-based studies. Testicular volume was normal for patients P10 and P47, but reduced for patients P32 and P57. All 4 patients carrying Y chromosome deletions presented elevated plasma FSH concentrations. Plasma LH and testosterone levels were within the normal reference range, except for azoospermic patient P57 who presented elevated LH and reduced testosterone concentration in plasma.

PCR-based screening and karyotype analysis of the Y chromosome of the father of patient P47 and the brothers of patients P32 and P57 were normal. No relative of patient P10 was available for these tests.

Discussion

In the present study, 4 of the 60 Brazilian infertile patients diagnosed with nonobstructive azoospermia or severe oligozoospermia exhibited deletions of the Y chromosome (6.7%). Of the 4 infertile men detected with deletions of the Y chromosome, 2 were nonobstructive azoospermic men and 2 were severely oligozoospermic men. Although the literature indicates a higher frequency of deletion in azoospermic than in oligozoosper-

mic patients (16,22,24), the incidence of Y chromosome deletions in the present study was similar in the azoospermic (2/29, 6.8%) and the severely oligozoospermic patient groups (2/31, 6.4%). A study on Brazilian infertile men referred from different infertility clinics has been recently reported (30). Among the 65 idiopathic cases of infertility studied, 6 (9.2%) microdeletions were detected, 3 in the AZFb region and 3 in the AZFc region. In another study, Carrara (31) observed Y microdeletions restricted to the AZFc region in 4.3% (3/70) of Brazilian infertile men studied. Thus, taken together, the results indicate that the prevalence of Y chromosome microdeletions in Brazilian infertile men lies at the lower end of the 3-55.5% range reported by the literature in populations of infertile men (5-8,11-22).

Patients P47 and P57, presenting non-obstructive azoospermia, had several non-contiguous microdeletions, as already suggested by karyotype analysis [46,XY (delY) (q11)]. The severely oligozoospermic patients P10 and P32, on the other hand, presented microdeletions confined to the AZFc region, specifically involving the DAZ locus. Since testicular histology was not available, a precise genotype/phenotype correlation was not possible in the present study. It is important to emphasize, however, that all these 4 patients had in common the presence of Y chromosome microdeletions confined to the AZFc regions, specifically in the DAZ locus, where most Y chromosome deletions are reported (5,11). Furthermore, the STS-PCR screening and karyotype analysis of the father of patient P47 and the brothers of patients P32 and P57 did not reveal Y chromosome microdeletions, confirming that Yq deletions were not paternally inherited, but rather occurred *de novo* during paternal germ cell differentiation or during an early stage of the fertilized egg (7,14,32).

Although all 60 patients analyzed in the present study were diagnosed as idiopathic infertile men, the presence of concomitant varicocele was not excluded during the clinical trial because Y chromosome deletions can coexist with this pathological condition (11,16,25,33) and has been defined as idiopathic by others (34). Both severely oligozoospermic patients (P10 and P32) carrying Y chromosome deletions in the present study had bilateral varicocele. These results are important for the clinical evaluation of these patients since it has been suggested that men with microdeletions and varicocele do not respond to varicocelectomy (35).

Since Y chromosome inheritance is always of paternal origin, men with partial somatic Y chromosome deletions are likely to have sons with similar genetic defects after treatment with assisted reproduction (36-38). Screening for Y deletions would be indicated in patients undergoing intracytoplasmic sperm injection (ICSI) programs and counseling on the risks of transmitting Y microdeletions and other Y chromosomal abnormalities to the offspring should be provided. Routine screening of Y microdeletions in all male patients before ICSI treatment is an important prerequisite for their appropriate counseling.

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References

1. Tiepolo L & Zuffardi O (1976). Localization of factors controlling spermatogenesis in the nonfluorescent portion of the human Y chromosome long arm. *Human Genetics*, 34: 119-124.
2. Foote S, Vollrath D, Hilton A & Page DC (1992). The human Y chromosome: overlapping DNA clones spanning the euchromatic region. *Science*, 258: 60-66.
3. Vollrath D, Foote S, Hilton A, Brown LG, Beer-Romero P, Bogan JS & Page DC (1992). The human Y chromosome: a 43 interval map based on naturally occurring deletions. *Science*, 258: 52-59.
4. Ma K, Sharkey A, Kirsch S, Vogt P, Keil R, Hargreave TB, McBeath S

& Chandley AC (1992). Towards the molecular localisation of the AZF locus: mapping of microdeletions in azoospermic men within 14 subintervals of interval 6 of the human Y chromosome. *Human Molecular Genetics*, 1: 29-33.
5. Reijo R, Lee TY, Salo P et al. (1995). Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. *Nature Genetics*, 10: 383-393.
6. Vogt PH, Edelmann A, Kirsch S et al. (1996). Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. *Human Molecular Genetics*, 5: 933-943.
7. Reijo R, Alagappan RK, Patrizio P & Page DC (1996). Severe oligozoospermia resulting from deletions of azoospermia factor gene on Y chromosome. *Lancet*, 347: 1290-1293.
8. Qureshi SJ, Ross AR, Ma K, Cooke HJ, McIntyre AM, Chandley AC & Hargreave TB (1996). Polymerase chain reaction screening for Y chromosome microdeletions: a first step towards the diagnosis of genetically-determined spermatogenic failure in men. *Molecular Human Reproduction*, 2: 775-779.
9. Pera RAR (2000). The DAZ gene family and germ-cell development. In: Goldeberg E (Editor), *Proceedings of the XVth Testis Workshop on the Testis: From Stem Cell to Sperm Function. Serono Symposia*. Springer, Norwell, MA, USA, 213-225.
10. Liow SL, Yong EL & Ng SC (2001). Prognostic value of Y deletion analysis. *Human Reproduction*, 16: 9-12.
11. Foresta C, Moro E & Ferlin A (2001). Y chromosome microdeletions and alterations of spermatogenesis. *Endocrine Reviews*, 22: 226-239.
12. Kent-First MG, Muallen A, Shultz J et al. (1999). Defining regions of the Y-chromosome responsible for male infertility and identification of a fourth AZF region (AZFd) by Y chromosome microdeletion detection. *Molecular Reproduction and Development*, 53: 27-41.
13. Kobayashi K, Mizuno K, Hida A et al. (1994). PCR analysis of the Y chromosome arm in azoospermic patients: evidence for a second locus required for spermatogenesis. *Human Molecular Genetics*, 3: 1965-1967.
14. Najmabadi H, Huang V, Yen P et al. (1996). 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. *Journal of Clinical Endocrinology and Metabolism*, 81: 1347-1352.
15. Stuppia L, Mastroprimiano G, Calabrese G, Peila R, Tenaglia R & Palka G (1996). Microdeletions in interval 6 of the Y chromosome detected by STS-PCR in 6 of 33 patients with idiopathic oligo- or azoospermia. *Cytogenetics and Cell Genetics*, 72: 155-158.
16. Pryor JL, Kent-First M, Muallen A, Van Bergen AH, Nolten WE, Meisner L & Roberts KP (1997). Microdeletions of the Y chromosome of infertile men. *New England Journal of Medicine*, 336: 534-539.
17. Foresta C, Ferlin A, Garolla A, Rossato M, Barbaux S & De Bortoli A (1997). Y-chromosome deletions in idiopathic severe testiculopathies. *Journal of Clinical Endocrinology and Metabolism*, 82: 1075-1080.
18. Foresta C, Ferlin A, Garolla A, Moro E, Pistorello M, Barbaux S & Rossato M (1998). High frequency of well-defined Y-chromosome deletions in idiopathic Sertoli cell-only syndrome. *Human Reproduction*, 13: 302-307.
19. Ferlin A, Moro E, Garolla A & Foresta C (1999). Human male infertility and Y chromosome deletions: role of the AZF-candidate genes, DAZ, RBM and DFFRY. *Human Reproduction*, 14: 1710-1716.
20. Kleiman SE, Yogev L, Gamzu R, Hauser R, Botchan A, Lessing JB, Paz G & Yavetz H (1999). Genetic evaluation of infertile men. *Human Reproduction*, 14: 33-38.
21. Kerr NJ, Zhang J, Sin FY, Benny P & Sin IL (2000). Frequency of microdeletions in the azoospermia factor region of the Y-chromosome of New Zealand men. *New Zealand Medical Journal*, 113: 468-470.
22. Maurer B, Gromoll J, Simoni M & Nieschlag E (2001). Prevalence of Y chromosome microdeletions in infertile men who consulted a tertiary care medical centre: the Münster experience. *Andrologia*, 33: 27-33.
23. Girardi SK, Mielnik A & Schiegel N (1997). Submicroscopic deletions in the Y chromosome of infertile men. *Human Reproduction*, 12: 1635-1641.
24. Liow SL, Ghadessy FJ, Ng SC & Yong EL (1998). Y chromosome microdeletions, in azoospermic or near-azoospermia subjects, are located in the AZFc (DAZ) subregion. *Molecular Human Reproduction*, 4: 763-768.
25. Simoni M, Kamischke A & Nieschlag E (1998). Current status of the molecular diagnosis of Y chromosome microdeletions in the work-up of male infertility. *Human Reproduction*, 13: 1764-1768.
26. Calogero AE, Garofalo MR & D'Agata R (1999). Factors influencing the variable incidence of Y chromosome microdeletions in infertile patients. *Human Reproduction*, 14: 275.
27. Tse JY, Yeung WSB, Lau EYL, Ng EHY, So WWK & Ho PC (2000). Deletions within the azoospermia factor subregions of the Y chromosome in Hong Kong Chinese men with severe male-factor infertility: controlled clinical study. *Hong Kong Medical Journal*, 6: 143-146.
28. WHO (1992). *World Health Organization Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction*. 3rd edn. The Press Syndicate of the University of Cambridge, Cambridge, UK.
29. Moorhead PS, Nowle BS, Mellman WJ, Battits DM & Hunger-Forte DA (1960). Chromosome preparation of leucocyte culture from human peripheral blood. *Experimental Cell Research*, 20: 613-616.
30. Pieri PC, Pereira DH, Glina S, Hallak J, McElreavey K & Moreira-Filho CA (2002). A cost-effective screening test for detecting AZF microdeletions of the human Y chromosome. *Genetic Testing*, 6: 185-194.
31. Carrara RCV (2000). Estudo citogenético e molecular da esterilidade masculina humana. Doctoral thesis, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil.
32. Edwards RG & Bishop CE (1997). On the origin and frequency of Y chromosome deletions responsible for severe male infertility. *Molecular Human Reproduction*, 3: 549-554.
33. Simoni M, Gromoll J, Dworniczak B et al. (1997). Screening for deletions of the Y chromosome involving the DAZ (deleted in azoospermia) gene in azoospermia and severe oligozoospermia. *Fertility and Sterility*, 67: 542-547.
34. Krausz C, Bussani-Mastellone C, Granchi S, McElreavey K, Scarselli G & Forti G (1999). Screening for microdeletions of Y chromosome genes in patients undergoing intracytoplasmic sperm injection. *Human Reproduction*, 14: 1717-1721.
35. Cayan S, Lee D, Black LD, Reijo-Pera RA & Turek PJ (2001). Response to varicocelectomy in oligospermic men with and without defined genetic infertility. *Urology*, 57: 530-535.
36. Kent-First MG, Kol S, Muallem A, Ofir R, Manor D, Blazer S, First N & Itskovitz-Eldor J (1996). The incidence and possible relevance of Y-linked microdeletions in babies born after intracytoplasmic sperm injection and their infertile fathers. *Molecular Human Reproduction*, 2: 943-950.

37. Jiang MC, Lien Y-R, Chen S-U, Ko T-M, Ho H-N & Yang Y-S (1999). Transmission of *de novo* mutations of the deleted in azoospermia genes from a severely oligozoospermic male to a son via intracytoplasmic sperm injection. *Fertility and Sterility*, 71: 1029-1032.

38. Oates RD, Silber S, Brown LG & Page DC (2002). Clinical characterization of 42 oligospermic or azoospermic men with microdeletion of the AZFc region of the Y chromosome, and of 18 children conceived via ICSI. *Human Reproduction*, 17: 2813-2824.