Tracking microdeletions of the AZF region in a patrilineal line of infertile men

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ABSTRACT. Male infertility is considered to be a difficult-to-treat condition because it is not a single entity, but rather reflects a variety of different pathologic conditions, thus making it difficult to use a single treatment strategy. Structural alterations in the Y chromosome have been the principal factor responsible for male infertility. We examined 26 family members of 13 patients with male infertility who showed deletions in the AZF region. In family 1, the father and a brother did not show microdeletions. However, a son showed a microdeletion in AZFa (sY84) and an azoospermic sperm analysis, but another son had a microdeletion in AZFa (sY84) and AZFb (sY127) and a normal sperm analysis. The father of family 2, with severe oligozoospermia, had a microdeletion in the AZFa region (sY84) and his son, conceived by intracytoplasmic sperm injection, also showed the same microdeletion. In the other families, only the men with an altered sperm analysis had a microdeletion. It is possible that in family 1, the father and brother who did not show microdeletions in this study, could have microdeletions in regions upstream or downstream of the one analyzed. The treatment with intracytoplasmic sperm injection can result in vertical transmission of microdeletions of the AZF region and can also cause the expansion of a *de novo* mutation. This finding reinforces the necessity of an

investigation of microdeletions of the Y chromosome in individuals who are candidates for assisted reproduction, as well as genetic counciling and follow-up.

Key words: Male infertility; Y chromosome; Vertical transmission

INTRODUCTION

About 8 to 15% of couples have physiopathologic problems with reproductive fertility, which is only considered when a period of attempts at pregnancy exceed a year, without the use of contraceptive methods (Niederberger and Meacham, 2003). The main causes of this disorder for men are associated with various factors, among them are genetic, physiopathologic and anatomopathologic abnormalities, intense and prolonged physical exercises, aging, drugs, and even excessive time of sexual abstinence (Pasqualotto, 2007).

In almost 20% of cases in which male infertility cannot be attributed to any other cause, the role of genetic alterations is being increasingly scrutinized (Simoni et al., 2004). This form of infertility can be classified as a genetic disorder, where structural chromosomal alterations, acquired or congenital, have been one of the main etiologies (Stankiewicz and Lupski, 2002).

Traditionally, male infertility is considered to be a condition that is difficult to treat, which is due to the fact it is not a single entity. Rather, it reflects a variety of different pathologic conditions, making it difficult to implement a single treatment strategy. On the other hand, intense technologic and therapeutic advances appear to contribute increasingly to the greater success of treatment (Pasqualotto, 2007).

Among the techniques that aid in the process of human reproduction (techniques of assisted reproduction), *in vitro* fertilization and intracytoplasmic sperm injection (ICSI) are noted for their high complexity (Lee et al., 2006).

According to Tamanini (2004), ICSI consists in the choice of better sperm, based on criteria regarding morphology, number and motility, where it is the more viable alternative for the majority of couples who have alterations in the Y chromosome. According to Chang et al. (1999), the utilization of techniques of assisted reproduction can result in pregnancy, but with the risk of subsequent transmission of infertility to male progeny (Lee et al., 2006).

Structural alterations in the Y chromosome have been the principal reason for male infertility (Vogt, 2004). Van Landuyt et al. (2000) found that the AZF region (Yq11) possesses genes involved in the regulation of spermatogenesis, which was first described in cytogenetic studies by Tiepolo and Zuffardi (1976).

This same AZF region was divided into three non-overlapping subregions called AZFa, AZFb and AZFc with size estimates of 1, 1.5 and 3 Mb, respectively (Briton-Jones and Haines, 2000; Gatta et. al., 2002), where a fourth subdivision was recently suggested and called AZFd (Foresta et al., 2001).

Interstitial microdeletions in AZF represent the etiologic factor in 10 to 15% of idiopathic cases of azoospermia and severe oligozoospermia (Athalye et al., 2004; Dada et al., 2004), where they are found in 38 and 23% of men with infertility, respectively (Raicu et al., 2003).

Despite a significant percentage of infertile individuals with microdeletions in the Y chromosome do not have children (by natural means of reproduction), some studies speculate that there is the possibility of the transmission of a predisposition to infertility from the father

to the son due to, for example, deletion events occurring more frequently associated with certain types of Y chromosome (Foresta et al., 2005).

According to Kihaile et al. (2005), the processes involved in patterns of heredity associated with gametogenesis, characterized by paternal transmission of the Y chromosome to the male progeny, i.e., vertical transmission, are very related to molecular alterations in the AZF region of this chromosome.

The objective of the present study was to track the mutation in AZF of the Y chromosome in the patrilineality of individuals with male infertility.

MATERIAL AND METHODS

Based on the results of Arruda et al. (2007), family members of 13 patients with male infertility who showed microdeletions in the AZF region were investigated. Of these patients, 7 (53.6%) had a mutation only in AZFa, 2 (15.2%) only in AZFb, 2 (15.2%) in AZFb as well as AZFa, 1 (8%) in AZFc as well as AZFa, and finally, 1 (8%) with a microdeletion in AZFa-b-c. These patients were submitted to a sperm analysis to determine the number of sperm ejaculated, revealing a proportion of 61.5% (N = 8) azoospermic individuals and 38.5% (N = 5) with severe oligozoospermia.

Twenty-six relatives of these patients, all males who were obligatorily linked by patrilineality, were analyzed at the Núcleo de Pesquisas Replicon of the Universidade Católica de Goiás, for triage of possible vertical transmission with regard to alterations of the Y chromosome. Peripheral blood samples were obtained after volunteers signed an informed consent form to participate. The study was approved by the CEP/UCG (#150/2004).

Genomic DNA was extracted utilizing the GFX Kit (Amersham Pharmacia Biotech, USA). Polymerase chain reaction (PCR) was performed according to the protocol proposed by Arruda et al. (2007) for analysis of the AZF region of the Y chromosome. Three subregions were analyzed: AZFa, AZFb and AZFc, where sequence tagged site (STS) primers were used. As positive control, fertile men with naturally conceived children were used. The SRY gene and ZFX/Y gene were used as Y chromosome markers for human genomic DNA. These STS primers were suggested by the European Academy of Andrology, which are able to detect 90% of the deletions in the loci of AZF.

The conditions for thermocycling were specific for each subregion, utilizing a GeneAmp PCR system 9700 thermocycler (Perkin-Elmer, USA). For analysis of the products obtained by PCR, the material amplified was submitted to electrophoresis on 1.5% agarose gels and stained with 5 µg/mL ethidium bromide. A visual record of the gels was made with the help of a video-documentation system (Image Master VDS® - Amersham Pharmacia Biotech, USA). STS was considered to be absent after three repetitions with negative results.

RESULTS

In family 1, the proband, who was produced by natural conception, had a microdeletion in the AZFa region (sY84) and an azoospermic sperm analysis based on a previous study (Arruda et al., 2007). In the present study, the father and a brother, who showed a normal and severe oligozoospermic sperm analysis, respectively, did not show a microdeletion in the AZF region. However, another brother, with a normal sperm analysis, had besides the deletion in AZFa (sY84) also one in AZFb (sY127) (Table 1).

Table 1. Clinical data of the probands and family members analyzed and the AZF regions with deletions in the families 1 to 6.

Family	Age (years)	Individual	Sperm analysis	AZFa		AZFb		AZFc	
				sY84	sY86	sY127	sY134	sY254	sY255
F-1	26	Proband	Azoospermia	-	+	+	+	+	+
	58	Father	Normal	+	+	+	+	+	+
	33	Brother	Normal	-	+	-	+	+	+
	30	Brother	Severe oligozoospermia	+	+	+	+	+	+
F-2	48	Proband	Severe oligozoospermia	-	+	+	+	+	+
	1	Son	ND	-	+	+	+	+	+
F-3	41	Proband	Azoospermia	+	-	+	+	+	+
	66	Father	Normal	+	+	+	+	+	+
F-4	29	Proband	Azoospermia	-	-	+	-	+	+
	58	Father	Normal	+	+	+	+	+	+
	26	Brother	Normal	+	+	+	+	+	+
F-5	35	Proband	Severe oligozoospermia	-	+	+	+	+	+
	69	Father	Normal	+	+	+	+	+	+
F-6	40	Proband	Azoospermia	-	+	+	+	+	+
	72	Father	Normal	+	+	+	+	+	+
	36	Brother	Normal	+	+	+	+	+	+
	10	Nephew	ND	+	+	+	+	+	+
	5	Nephew	ND	+	+	+	+	+	+

ND = not determined. Sequence-tagged site markers: (-) deleted; (+) present.

In family 2, the father, with severe oligozoospermia, showed a microdeletion in the AZFa region (sY84) and his son, conceived by ICSI, also showed the same microdeletion, thereby indicating vertical transmission of this microdeletion in this process of assisted reproducion (Table 1).

However, in families 3 and 5, the probands revealed in a previous study (Arruda et al., 2007) a microdeletion in the AZFa region (sY86 and sY84, respectively), showing azoospermia and severe oligozoospermia. However, in the present study, their fathers had no microdeletions in the subregions studied (Table 1).

In families 4 and 6, the probands were azoospermic, where the first showed microdeletions in the AZFa and AZFb regions (sY84, sY86 and sY134), while the second had a microdeletion only in AZFa (sY84). The father and a brother of the proband of family 4, as well as the father, a brother and two nephews of the proband of family 6, did not show microdeletions in the AZF region (Table 1).

It was not possible to collect a biological sample from the father of the probands of families 7 to 13, because some had already died and others lived very far from the city where the proband resided.

Based on a previous study (Arruda et al., 2007), the probands of families 7 and 12 had microdeletions in the AZFa region (sY84, sY86; sY84) and in the AZFb region (sY127, sY134; sY127) as well, but the proband of family 12 also had a microdeletion in AZFc (sY254). On the other hand, the proband in family 10 showed alterations in AZFa (sY86) and AZFc (sY254), and the proband in family 11 showed alterations in AZFb (sY134). The present study also revealed that the probands of families 7 and 10 are azoospermic, while in families 11 and 12 they are

severe oligozoospermic. In the brothers of the proband of family 7, no microdeletion was found, this being the same result obtained in two brothers of the proband of family 10, in the brother of the proband of family 11 and in three brothers of the proband of family 12 (Table 2).

Table 2. Clinical data of the probands and family members analyzed and the AZF regions with deletions in the families 7 to 13.

Family	Age (years)	Individual	Sperm analysis	AZFa		AZFb		AZFc	
				sY84	sY86	sY127	sY134	sY254	sY255
F-7	46	Proband	Azoospermia	-	-	-	-	+	+
	40	Brother	Normal	+	+	+	+	+	+
F-8	30	Proband	Azoospermia	-	+	+	+	+	+
	40	Brother	Normal	+	+	+	+	+	+
	10	Nephew	ND	+	+	+	+	+	+
F-9	29	Proband	Azoospermia	+	-	+	+	+	+
	18	Brother	Normal	+	+	+	+	+	+
	43	Uncle	Normal	+	+	+	+	+	+
F-10	28	Proband	Azoospermia	+	-	+	+	-	+
	26	Brother	Normal	+	+	+	+	+	+
	24	Brother	Normal	+	+	+	+	+	+
F-11	49	Proband	Severe oligozoospermia	+	+	+	-	+	+
	50	Brother	Normal	+	+	+	+	+	+
F-12	35	Proband	Severe oligozoospermia	-	+	-	+	-	+
	58	Brother	Normal	+	+	+	+	+	+
	43	Brother	Normal	+	+	+	+	+	+
	33	Brother	Normal	+	+	+	+	+	+
F-13	35	Proband	Severe oligozoospermia	+	+	+	-	+	+
	12	Nephew	ND	+	+	+	+	+	+

ND = not determined. Sequence-tagged site markers: (-) deleted; (+) present.

In families 8 and 9, both probands were found to be azoospermic and showed microdeletions in the AZFa region (sY84 and sY86, respectively). In this study, a tracking was carried out in 1st, 2nd and 3rd degree relatives of the probands, but no microdeletion was demonstrated. The brother and nephew of the proband of family 8 showed no microdeletions in AZF, as in the brother and uncle of the other proband studied, where no genetic alteration whatsoever was found (Table 2).

Finally, based on the data analyzed in a previous study (Arruda et al., 2007), it was observed that in family 13 the proband is severe oligozoospermic and has a microdeletion in the AZFb region (sY134). In the present study, it was shown that the nephew, 3rd degree relative of the proband, did not have mutations in the subregions analyzed (Table 2).

DISCUSSION

The Y chromosome provides an opportunity to study mutations in the genome with clinical importance. This haploid chromosome is unable to recombine during meiosis, where it provides for patrilineal inheritance modified only by mutations. Thus, this situation facilitates studies of genomic rearrangements, such as the duplications and deletions of regions or intrachromosomal gene conversion between paralogous repetitions (Bosch and Jobling, 2003), where gene conversion between sequences of the Y chromosome represents a form of recombination exclusive for

this chromosome (Rozen et al., 2003). The study of STS present in the Y chromosome makes it possible to detect the molecular mechanisms that act on the hotspots of this region, which are probable facilitators of the occurrence of rearrangements (Kamp et al., 2001).

Some epigenetic mechanisms, such as the insertion of exogenous DNA in the genome, can result in the rejection or retention of a sequence in rearrangements (duplication or deletion) or in silencing of genes of the host genome due to methylation of cytosines in promotor regions. On the other hand, DNA hypomethylation can be responsible for the transcription of endogenous retroviruses (HERV) (Januchowski et al., 2004).

According to Lee et al. (2006), treatments with ICSI for infertile men can lead to vertical transmission of microdeletions in the AZF region. This therapy can also cause expansion of the *de novo* mutation, as observed by Cram et al. (2000). This use of ICSI increases the probability of an abnormal sperm to be selected for fertilization, and consequently, anomalies at different levels of paternal genomic organization can affect reproductive potential and the results of techniques of assisted reproduction. Recent evidence suggests that sperm that contains microdeletions in the Y chromosome can be associated with fertilization, embryogenesis or impaired fetal development (Hatzissevastou-Loukidou et al., 2006). Due to ICSI techniques being used generally in patients with microdeletions in the Y chromosome, thereby leading to considerable risk of passing the deletion on to the progeny (Cram et al., 2006), appropriate genetic counseling followed by detailed family history and specific molecular and cytogenetic analyses are recommended.

Based on the results obtained, in family 1, the father and a brother of the proband did not show a microdeletion in the region studied, but could meanwhile have microdeletions in the regions upstream or downstream of that analyzed. Therefore, it cannot be stated that these two individuals are not carrriers of microdeletions, since in this circumstance, the method utilized could have not been precise enough, thereby suggesting a sequencing of all the AZF region for a better scrutinizing analysis. If this possibility were to be confirmed, it would show that the region is not essential for fertility, since the father had three sons by natural conception (Foresta et al., 2001; Gatta et al., 2002; Kühnert et al., 2004; Samli et al., 2006).

Along this line, it is believed that in the other two sons, carriers of the AZF mutation, there is a greater suceptibility of increasing the microdeleted region. Thus, the possible microdeletion of the father, even though not detected, could have been expanded during gametogenesis, giving rise to sons with different genotypic and phenotypic profiles (Gatta et al., 2002). This hypothesis could explain the fact that one son had a microdeletion in AZFa and the other in both AZFa and AZFb, because in the second there could have been greater expansion than in the first son. It was even seen that the son who had AZFa and AZFb deleted also showed a normal sperm analysis, which would explain a worsening prognosis with age.

In a second hypothesis, the phenomenon of a *de novo* mutation could clarify this situation, because these individuals could have possibly been exposed to mutagenic sources or even to sporadic processes, causing alterations in their genetic constitution, including microdeletions in the genome, where phenotypes are manifested that are not yet recorded (Amos et al., 2003; Kühnert et al., 2004). It is possible that these factors can be linked to epigenetic events, where clarifications could be provided by a more specific study based on mRNA and proteins formed by these genes of this locus.

The phenomenon of vertical transmission was observed in family 2, where the father who carried a microdeletion in AZFa, transmitted it to his son through ICSI, a process of assisted reproduction (Chang et al., 1999; Cram et al., 2000; Lee et al., 2006). This finding rein-

forces the necessity of an investigation of microdeletions in the Y chromosome in individuals who are candidates for assisted reproduction, as well as genetic counseling and follow-up.

In families 3 to 13, vertical transmission was not observed for the regions studied, but the occurrence of a *de novo* mutation in the probands was likely, since other family members did not show any microdeletion in this region.

Genomic rearrangements (deletions and duplications) are equally probable of occurring during meiosis, and the duplications in AZFa also support the idea that men who carry them can have children who are carriers as well (Lupski, 2003). The complete deletion of the AZFa region causes male infertility manifested as the absence of germ cells (Sertoli cell-only syndrome). The spermatogenesis-specific phenotype of the deletion in AZFa can be consequently based on the dosage of the expression of the testis-specific genes USP9Y and DBY (Dada et al., 2004).

Microdeletions are the product of reciprocal recombinations, and duplications have been reported as important causes of genetic diseases (Huh et al., 2006). Unequal exchanges between repetitions in homologous chromosomes (unequal chromosome exchange) or in sister chromatids (unequal sister chromatid exchange) are mechanisms proposed as probable causes of these rearrangements (Blanco et al., 2000).

The large number of cell divisions in spermatogenesis and therefore of replications of DNA increases the chances of mutation (Jobling and Tyler-Smith, 2003). Mutations in the microsatellite markers of the Y chromosome can be broad or completely intra-alellic and this confirms the widely held notion that replication slippage of DNA polymerase is possibly the underlying mechanism. The mutations can emerge from unequal exchanges between sister chromatids or by slippage in replication, which is facilitated by the secondary structure of the repetitions (Jobling and Tyler-Smith, 2003).

Singh et al. (2006) reported a case of increase in microdeletion that passed from father to son and suggested that some deletions do not lead to infertility, but make the Y chromosome more vulnerable to other deletions that could lead to infertility. Patsalis et al. (2002) suggested that for men with microdeletions in AZF and who utilizes ICSI, pre-implantation diagnostics should be offered to reduce the possibility of anomalies. Various cases of infertility are of genetic origin, and these genetic defects can be transmitted to later generations. Besides, many authors have observed that the microdeletions in Yq can lead to progressive worsening of sperm production and that with time oligozoospermic men can become azoospermic (Dada et al., 2004; Choi et al., 2004). Consequently, it is recommended that men with microdeletions acquired by natural transmission or by ICSI be submitted to andrologic examinations in puberty and have their sperm cryopreserved before possible decline in number with age (Georgiou et al., 2006). The genetic consequences of the transmission of microdeletions necessitate a precise evaluation of the function of various genes on the Y chromosome, especially those that involve the AZF region and that are responsible for male infertility.

Because microdeletions in AZF are transmitted from father to male progeny, the genetic evaluation of microdeletions is recommended for men with non-obstructive azoospermia or oligozoospermic individuals. In addition, large microdeletions of the Yq tip can cause chromosomal instability and can be responsible for chromosomal rearrangements or even loss of the Y chromosome. Studies of testicular mosaicism of chromosomal deletions on Y, expansion of the microdeletion in the progeny and the familial basis of deletions on the Y chromosome represent the target of various investigations, but the results are still inconsistent (Larcher, 2007).

The relation of deletions of the Y chromosome and other genetic lesions to male infertility will continue to be an area of intense interest due to the wide use of ICSI for the resolution of male infertility. It is important that these investigations are translated rapidly and appropriately into clinical practice, and that couples in need of assisted reproduction are given essential information to make this crucial decision in their life.

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REFERENCES

Amos CI, Shete S, Chen J and Yu RK (2003). Positional identification of microdeletions with genetic markers. *Hum. Hered.* 56: 107-118.

Arruda JT, Bordin BM, Santos PR, Mesquita WE, et al. (2007). Y chromosome microdeletions in Brazilian fertility clinic patients. *Genet. Mol. Res.* 6: 461-469.

Athalye AS, Madon PF, Naik NJ, Naik DJ, et al. (2004). A study of Y chromosome microdeletions in infertile Indian males. *Int. J. Hum. Genet.* 4: 179-185.

Blanco P, Shlumukova M, Sargent CA, Jobling MA, et al. (2000). Divergent outcomes of intrachromosomal recombination on the human Y chromosome: male infertility and recurrent polymorphism. *J. Med. Genet.* 37: 752-758.

Bosch E and Jobling MA (2003). Duplications of the AZFa region of the human Y chromosome are mediated by homologous recombination between HERVs and are compatible with male fertility. *Hum. Mol. Genet.* 12: 341-347.

Briton-Jones C and Haines CJ (2000). Microdeletions on the long arm of the Y chromosome and their association with male-factor infertility. *Hong Kong Med. J.* 6: 184-189.

Chang PL, Sauer MV and Brown S (1999). Y chromosome microdeletion in a father and his four infertile sons. *Hum. Reprod.* 14: 2689-2694.

Choi JM, Chung P, Veeck L, Mielnik A, et al. (2004). AZF microdeletions of the Y chromosome and *in vitro* fertilization outcome. *Fertil. Steril.* 81: 337-341.

Cram DS, Ma K, Bhasin S, Arias J, et al. (2000). Y chromosome analysis of infertile men and their sons conceived through intracytoplasmic sperm injection: vertical transmission of deletions and rarity of *de novo* deletions. *Fertil. Steril.* 74: 909-915.

Cram DS, Osborne E and McLachlan RI (2006). Y chromosome microdeletions: implications for assisted conception. *Med. J. Aust.* 185: 433-434.

Dada R, Gupta NP and Kucheria K (2004). Yq microdeletions - azoospermia factor candidate genes and spermatogenic arrest. *J. Biomol. Tech.* 15: 176-183.

Foresta C, Moro E and Ferlin A (2001). Y chromosome microdeletions and alterations of spermatogenesis. *Endocr. Rev.* 22: 226-239.

Foresta C, Garolla A, Bartoloni L, Bettella A, et al. (2005). Genetic abnormalities among severely oligospermic men who are candidates for intracytoplasmic sperm injection. *J. Clin. Endocrinol. Metab.* 90: 152-156.

Gatta V, Stuppia L, Calabrese G, Morizio E, et al. (2002). A new case of Yq microdeletion transmitted from a normal father to two infertile sons. *J. Med. Genet.* 39: E27.

Georgiou I, Syrrou M, Pardalidis N, Karakitsios K, et al. (2006). Genetic and epigenetic risks of intracytoplasmic sperm injection method. *Asian J. Androl.* 8: 643-673.

Hatzissevastou-Loukidou H, Kalemi TG, Lambropoulos AF, Nikita M, et al. (2006). Characterization of a *de novo* balanced 1;Y translocation in a phenotypically normal twin male infant. *Fertil. Steril.* 85: 1059.e5-7.

Huh JW, Kim DS, Ha HS, Kim TH, et al. (2006). Formation of a new solo-LTR of the human endogenous retrovirus H family in human chromosome 21. *Mol. Cells* 22: 360-363.

Januchowski R, Breborowicz AK, Ofori H, Jedrzejczak P, et al. (2004). Detection of a short CCR5 messenger RNA isoform in human spermatozoa. *J. Androl.* 25: 757-760.

Jobling MA and Tyler-Smith C (2003). The human Y chromosome: an evolutionary marker comes of age. *Nat. Rev. Genet.* 4: 598-612.

Kamp C, Huellen K, Fernandes S, Sousa M, et al. (2001). High deletion frequency of the complete AZFa sequence in men with Sertoli-cell-only syndrome. *Mol. Hum. Reprod.* 7: 987-994.

Kihaile PE, Yasui A and Shuto Y (2005). Prospective assessment of Y-chromosome microdeletions and reproductive outcomes among infertile couples of Japanese and African origin. *J. Exp. Clin. Assist. Reprod.* 2: 9.

Kühnert B, Gromoll J, Kostova E, Tschanter P, et al. (2004). Case report: natural transmission of an AZFc Y-chromosomal microdeletion from father to his sons. *Hum. Reprod.* 19: 886-888.

Larcher V (2007). The health of children conceived by assisted reproduction technologies. *Arch. Dis. Child* 92: 668-669.

Lee SH, Ahn SY, Lee KW, Kwack K, et al. (2006). Intracytoplasmic sperm injection may lead to vertical transmission, expansion, and *de novo* occurrence of Y-chromosome microdeletions in male fetuses. *Fertil. Steril.* 85: 1512-1515.

Lupski JR (2003). 2002 Curt Stern Award Address. Genomic disorders recombination-based disease resulting from genomic architecture. *Am. J. Hum. Genet.* 72: 246-252.

Niederberger CS and Meacham RB (2003). Male infertility. *Rev. Urol.* 5: 200-203.

Pasqualotto FF (2007). Investigation and assisted reproduction in the treatment of male infertility. *Rev. Bras. Ginecol. Obstet.* 29: 103-112.

Patsalis PC, Sismani C, Quintana-Murci L, Taleb-Bekkouche F, et al. (2002). Effects of transmission of Y chromosome AZFc deletions. *Lancet* 360: 1222-1224.

Raicu F, Popa L, Apostol P, Cimponeriu D, et al. (2003). Screening for microdeletions in human Y chromosome - AZF candidate genes and male infertility. *J. Cell Mol. Med.* 7: 43-48.

Rozen S, Skaletsky H, Marszalek JD, Minx PJ, et al. (2003). Abundant gene conversion between arms of palindromes in human and ape Y chromosomes. *Nature* 423: 873-876.

Samli H, Murat SM and Solak M (2006). Natural transmission of AZFb Y-chromosomal microdeletion from father to his three sons. *Arch. Androl.* 52: 423-426.

Simoni M, Bakker E and Krausz C (2004). EAA/EMQN best practice guidelines for molecular diagnosis of Y-chromosomal microdeletions. State of the art 2004. *Int. J. Androl.* 27: 240-249.

Singh AR, Vrtel R, Vodicka R, Dhaifalah I, et al. (2006). Genetic factors in male infertility and their implications. *Int. J. Hum. Genet.* 6: 163-169.

Stankiewicz P and Lupski JR (2002). Genome architecture, rearrangements and genomic disorders. *Trends Genet.* 18: 74-82.

Tamanini M (2004). Novas tecnologias reprodutivas conceptivas: bioética e controvérsias. *Rev. Estud. Fem.* 12: 73-107.

Tiepolo L and Zuffardi O (1976). Localization of factors controlling spermatogenesis in the nonfluorescent portion of the human Y chromosome long arm. *Hum. Genet.* 34: 119-124.

Van Landuyt L, Lissens W, Stouffs K, Tournaye H, et al. (2000). Validation of a simple Yq deletion screening programme in an ICSI candidate population. *Mol. Hum. Reprod.* 6: 291-297.

Vogt PH (2004). Molecular genetics of human male infertility: from genes to new therapeutic perspectives. *Curr. Pharm. Des.* 10: 471-500.