Review

Genetics of Azoospermia: Current Knowledge, Clinical Implications, and Future Directions. Part II
Y Chromosome Microdeletions

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Introduction: We reviewed the most recent advances in the genetics of male infertility focusing on Y chromosome microdeletions.

Materials and Methods: We searched the literature using the PubMed and skimmed articles published from January 1998 to October 2007. The keywords were the Y chromosome, microdeletions, male infertility, and azoospermia factor (AZF). The full texts of the relevant articles and their bibliographic information were reviewed and a total of 78 articles were used.

Results: Three regions in the long arm of the Y chromosome, known as AZFa, AZFb, and AZFc, are involved in the most frequent patterns of Y chromosome microdeletions. These regions contain a high density of genes that are thought to be responsible for impaired spermatogenesis. In 2003, the Y chromosome sequence was mapped and microdeletions are now classified according to the palindromic structure of the euchromatin that is composed of a series of repeat units called amplicons. Although it has been shown that the AZFb and AZFc are overlapping regions, the classical AZF regions are still used to describe the deletions in clinical practice.

Conclusion: Y chromosome microdeletions are the most common genetic cause of male infertility and screening for these microdeletions in azoospermic or severely oligospermic men should be standard. Detection of various subtypes of these deletions has a prognostic value in predicting potential success of testicular sperm retrieval for assisted reproduction. Men with azoospermia and AZFc deletions may have retrievable sperm in their testes. However, they will transmit the deletions to their male offspring by intracytoplasmic sperm injection.

Keywords: male infertility, azoospermia, genetic diseases, chromosome aberrations, Y chromosome, intracytoplasmic sperm injection

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INTRODUCTION

One in 20 men suffers from male infertility, and pure male-factor infertility comprises approximately one-third of all infertilities. A great proportion of these patients have primary spermatogenesis failure with a genetic cause.[1] With the advent of accurate diagnostic tools and recent knowledge of the Y chromosome map, the genetic aberrations responsible for infertility are more easily recognized. Moreover, men with azoospermia or severe oligospermia caused by some of these genetic defects can undergo sperm retrieval techniques and potentially father their own children. Thus, a definite diagnosis of the causal factors of spermatogenesis impairment can determine the therapeutic approaches and predict success rate of the treatment. On the other hand, since the use of testicular sperm extraction (TESE) and intracytoplasmic sperm injection (ICSI) can bypass the natural selection of intact spermatozoa, there have been consistent concerns about

the possibility of transmitting genetic disorders to the offspring.[2] The scenario is further complicated by the presence of a Y chromosome microdeletion (YCM). These structural genetic abnormalities form various genotypes that result in diverse unpredictable phenotypes, warranting further elucidation of their role in infertility and their influence on the assisted reproductive technologies (ART) outcomes.

Y chromosome microdeletions are the most frequently observed structural abnormalities in the male-specific region of the Y chromosome,[2] and of primary spermatogenesis failures, 15% are related to at least 6 known major YCM patterns.[1] Interestingly, these microdeletions have been reported to occur in fertile men, as well.[3,4] Microdeletions are present in 5% to 10% of infertile men.[5] Specifically, they have been reported in 2% to 3% of the candidates for ICSI, 6% to 16% of azoospermic men, and 4% to 5.8% of those with severe oligospermia.[2,6] Limited studies in the Middle East have been done; YCMs were reported in 3.2% of men with idiopathic azoospermia or oligospermia in Saudi Arabia, in 3.3% of those in Turkey, and in 2.6% in Kuwait.[7-9] In Iran, studies on small numbers of patients showed that 5% to 24.2% of infertile men with idiopathic severe spermatogenesis impairment had these genetic aberrations.[10-12]

Deletions in the Y chromosome are mostly de novo.[13] However, several cases of natural transmission of the microdeletion have been reported to date.[14-19] Since Tiepolo and Zuffardi reported cytologically detectable deletions of the proximal Yq in azoospermic men,[20] a tremendous amount of research has been done to scrutinize the mechanism of developing and characteristics of these deletions. In 1996, Vogt and colleagues identified 3 recurrently deleted regions in Yq11. These were termed the *azoospermia factor* (AZF), and the 3 regions were named as AZFa, AZFb, and AZFc.[21] Our understanding of these regions, however, has been revolutionized by recent sequencing of the Y chromosome and determining the breakpoints of the deletions. It is now hypothesized that most of the AZF microdeletions are generated by intrachromosomal homologous rearrangements of the genetic material by crossing over (*recombination*) occurring between a series of repeated sequence blocks that have nearly identical structures.[22]

Notwithstanding the large body of information gained on the Y chromosome during the last decade, it is still not possible to attribute spermatogenic function to definite genes, because each of the deletions usually removes multiple genes.[18] Consequently, it is not clear whether the resulted phenotype is caused by the loss of all genes in a region or by disruption of a major gene whose expression alone is responsible for spermatogenesis.[5] Furthermore, the known patterns of deletions are variable in details and preclude clear classification of men with a specific type of deletion.[23] It should be added that there is no association between the length of the deletion and the semen quality or the testicular histology.[2] Despite these challenges, the current knowledge provides us with a helpful view of the genetic causes of azoospermia that can be utilized in practice. This article is the second part of the review we performed on the genetics of male infertility. In part I, genetic causes of male infertility in karyotypic abnormalities, obstructive azoospermia, and idiopathic hypogonadotropic hypogonadism were discussed.[24] In this review, we report the latest findings about YCMs and discuss their clinical implications.

To update our previous article published in 1997 on the subject,[25] we performed an extensive search on the PubMed for the relevant articles that appeared from 1998 to October 2007. The keywords were Y chromosome, microdeletion, male infertility, and AZF. Other specific words were researched during the study if needed. We reviewed 99 papers and their bibliographic information; of these, 78 with the most relevant and valid information were included in the final analysis.

Y CHROMOSOME STRUCTURE

Since the early 20th century, in which the Y chromosome used to be known as a *genetic wasteland*, revolutionary changes have been made in our knowledge of this chromosome.[22] Currently, we know that the Y chromosome is functional for spermatogenesis and is, at the same time, polymorphic.[26] Accordingly, multiple Y chromosomes have developed during human evolution distinguished now by a rooted pedigree of at least 153 Y chromosome haplogroups around the world.[26] Generally, of the 60 Mb length of the Y chromosome, 3 Mb belongs to pseudoautosomal

regions and 57 Mb to a nonrecombining region that contains heterochromatic and euchromatic regions (Figure 1). The euchromatin embraces most of the known genes in the Y chromosome.

In the primary attempts to map the Y chromosome, Vollrath and colleagues subdivided Yq11, the region in which they found deletions, into 23 intervals termed 5A to 5Q and 6A to 6F.[28] Vogt and coworkers established another sequence-tagged site deletion map dividing Yq11 into 25 intervals of D1 to D25 (Figure 1).[29] Today, the Y chromosome has been sequenced completely and its genomic sequence is available (http://www.ensembl.org/homo-sapiens/mapveiw?chr=y). By sequencing the Y chromosome in 2003, Skaletsky and colleagues proposed a new model for analysis of the male-specific region of the Y chromosome.[22] They showed that the male-specific region of the Y chromosome comprises 95% of the Y chromosome length, and that it is a mosaic of heterochromatic and euchromatic sequences. Heterochromatin is located among repeated genes, gene families, and palindromic motifs.[2] The euchromatic DNA sequences on the Y is about 23 Mb including 8 Mb on the short arm and 14.5 Mb on the long arm.[22] There are 3 classes of euchromatic sequences (Figure 2): those transposed from the X chromosome during the process of the evolution of the Y (*X-transposed*), those somewhat similar to sequence information from the X chromosome (*X-degenerate*), and those repeated units across the proximal short arm of the Yp and across most of the Yq (*amplicons*).[22]

The X-transposed regions, with a combined length of 3.4 Mb, are almost identical to the DNA sequences in

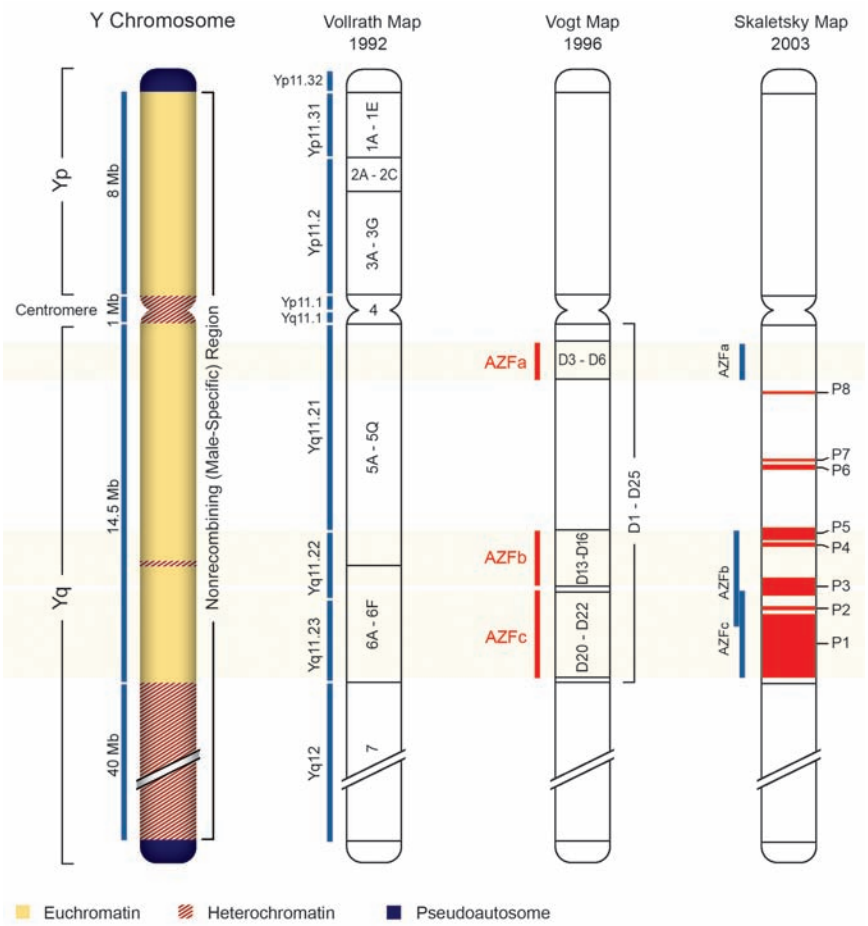

Figure 1. Pseudoautosomal and nonrecombining regions on the Y chromosome.[27] The nonrecombining region is 57 Mb in length and encompasses euchromatic regions that harbor almost all recognized genes responsible for spermatogenesis. The heterochromatin consists of 3 regions: a large part of the distal Yq, the centromere, and a newly discovered very small region within the euchromatic region of the Yq.[22] Mapping of this region has evolved in the recent decade. Vollrath and colleagues introduced their map in 1992.[28] Later in 1996, Vogt and colleagues proposed D1 to D25 in which the AZF regions were identified.[21] In the latest model by Skaletsky and associates, massive palindromic regions (P1 to P8) are introduced and it has been found that the AZFb and AZFc overlap.[22]

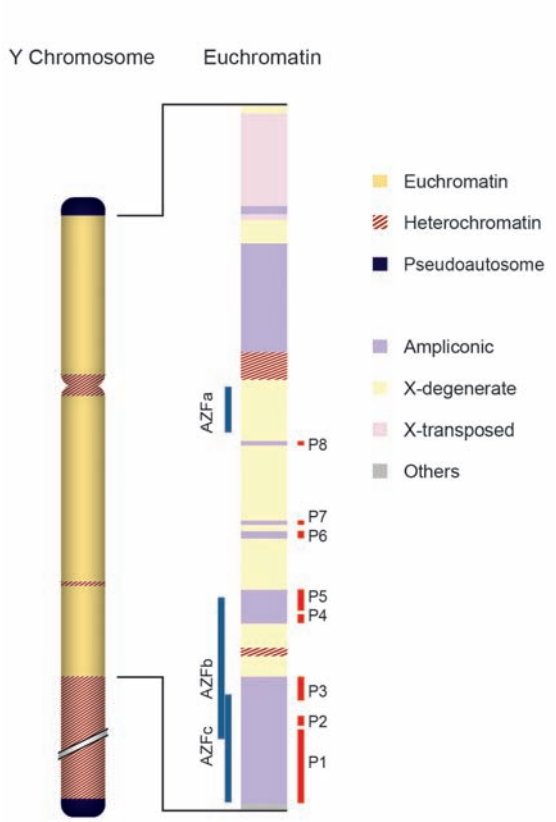

Figure 2. Three classes of the euchromatin and their relation to the AZF regions are depicted. There are 7 ampliconic, 8 X-degenerate, and 2 X-transposed regions. The AZFa is located in the X-degenerate region. All the protein-encoding genes in the AZFc are in the ampliconic regions, but the genes in the AZFb are located in both amplicons and X-degenerate region.[22]

the Xq21. The X-transposed sequences are the result of a massive X-to-Y transposition that had occurred about 3 million years ago, after the divergence of the human and chimpanzee lineages. Within the X-transposed segments, only 2 protein-encoding genes have been identified (*TGIF2LY* and *PCDH11Y*).[22] The X-degenerate regions, with a combined length of 8.5 Mb, are dotted with single-copy genes or pseudogenes that are mostly expressed ubiquitously (i.e. expressed in multiple organs in the body and not confined to a specific tissue). These genes are about 60% and 90% similar to their X-linked homologues and are thought to be relics of ancient autosomal chromosomes from which the X and Y chromosomes originated. The sex-determining gene (*SRY*) is located in this region. The *SRY* gene expresses a transcription factor that switches on the genes that direct the development of male structures in the embryo. The genes recognized in the AZFa (*DBY* and *USP9Y*) are also located in the X-degenerate region.[22,26]

The most sophisticated regions of the Y chromosome are the unique ampliconic regions in the euchromatin that are 10.5 Mb long overall. Amplicons are families of units composed of nucleotide sequences that are markedly similar to each other.[2] They are located in 7 segments that are scattered across the euchromatin in the long arm and proximal short arm of the Y chromosome. Amplicons harbor the highest density of the Y chromosome genes that are exclusively expressed in the testes. Genes related to the AZFb and AZFc are located in the ampliconic regions.[26]

The array of the amplicons forms 8 palindromes (P1 to P8) that are the most pronounced structural features of the ampliconic region (Figure 3). Each

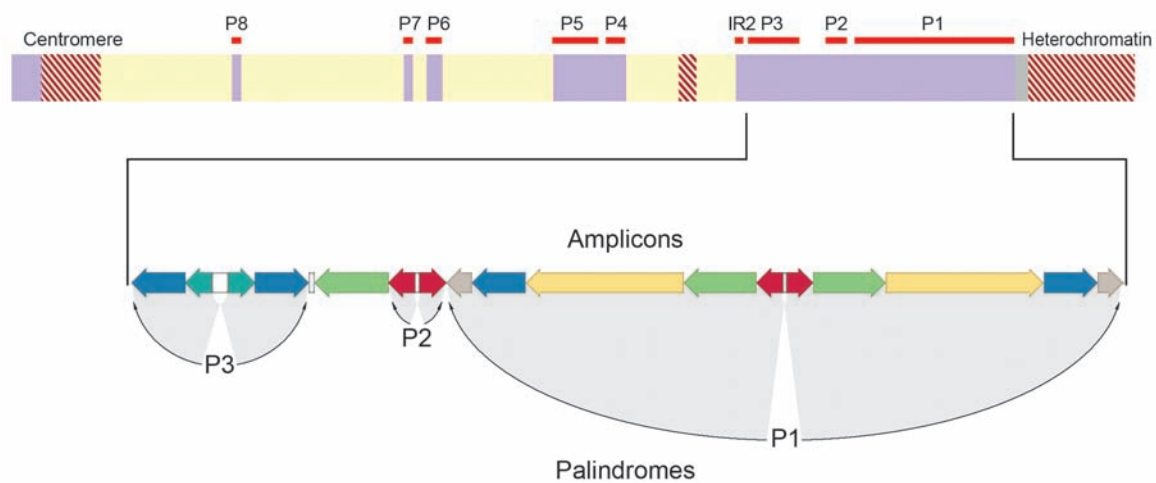

Figure 3. Palindromic structure of the Y chromosome. As an example, the structure of P1 to P3 is shown. Each palindrome consists of a set of amplicons that is repeated reversely. Thus, the palindrome reads the same in either direction. In addition to the palindromes, there are 5 sets of amplicons that are widely spaced inverted repeats (IRs) with small lengths.[22]

palindrome is comprised of 2 groups of amplicons with similar but inverted arrangements. In other words, a palindrome is a DNA sequence containing different amplicons which has a twin along the chromosome that read the same in a reverse direction.[1] Most of the recognized genes that are deleted in infertile men are located in the palindromic regions of the Yq.

GENES ON Y CHROMOSOME

To date, 122 genes and 110 pseudogenes have been identified in the Y chromosome (available from http://www.gdb.org/gdbreports/genebychromosome.y.alpha.html, last updated, December 2, 2007). However, the exact role of these genes in spermatogenesis is not elucidated because microdeletions that cause spermatogenesis impairment usually include more than 1 gene, so that the role of each deleted gene cannot be specified. Some genes have been considered to have a major part in spermatogenesis, but in most cases, reports of deletions in fertile or subfertile men have questioned their specific function. So far, only 1 isolated Yq gene mutation has been reported that leads to spermatogenesis failure.[30,31]

The abovementioned impediments have confined research on YCMs to identification of the deleted regions and the group of genes they usually harbor. Defining the classical AZF regions was the primary step. However, the newly identified breakpoints for deletions along the male-specific region of the Y chromosome do not necessarily conform to the AZF pattern. In addition, it has been shown that the AZFb and AZFc are overlapping regions.[32] Nonetheless, microdeletions are still described in relation to their location in the 3 classical AZF regions.[2,5,33,34]

AZOOSPERMIA FACTOR

In 1996, Vogt and colleagues conducted a large collaborative study and screened 370 men with idiopathic azoospermia or severe oligospermia for submicroscopic deletions in the Yq. Thirteen of these men had microdeletions mapping to 3 different regions designated, from proximal to distal, as AZFa, AZFb, and AZFc.[21] There are at least 14 protein-encoding Y gene families in the AZF loci (Table 1).[26] Deletions of these genes occur as 6 classical types of Yq deletions: AZFa, AZFb, AZFc, AZFbc,

Table 1. Protein-Encoding Gene Families on the Azoospermia Factor Regions[23,26,32]

Gene Symbol	Gene Name	Number of Copies	Expression	Location	AZF Location	Number of Deleted Copies			
						Complete AZFa	Complete AZFb (P5/Proximal P1)	AZFbc (P5/distal P1)	AZFc*
USP9Y	Ubiquitin Specific Protease 9 Y	1	Ubiquitous	X-Degenerate	AZFa	1	0	0	0
DBY	Dead Body Y	1	Ubiquitous	X-Degenerate	AZFa	1	0	0	0
RBMY	RNA-Binding Motif Y-Linked	6	Only Testis	Amplicons	AZFb	0	6	6	0
HSFY	Heat-shock Transcription Factor Y	2	Testis, Kidney	Amplicons	AZFb	0	2	2	0
PRY	PTP-BL Reloated Y	2	Only Testis	Amplicons	AZFb	0	2	2	0
XKRY	X-Kell Blood Group Precursor Related Y	2	Only Testis	Amplicons	AZFb	0	1	1	0
RPS4Y2	Ribosomal Protein S4 Y Linked 2	1	Testis, Prostate	X-Degenerate	AZFb	0	1	1	0
SMCY	Selected Mouse C DNA Y	1	Ubiquitous	X-Degenerate	AZFb	0	1	1	0
EIF1AY	Essential Initiation Translation Factor 1A Y	1	Ubiquitous	X-Degenerate	AZFb	0	1	1	0
CDY	Chromodomain Y	4	Only Testis	Amplicons	AZFb and AZFc†	0	2	2	0 to 2
DAZ	Deleted in Azoospermia	4	Only Testis	Amplicons	AZFc	0	2	4	0 to 4
BPY2	Basic Protein Y 2	3	Only testis	Amplicons	AZFc	0	1	3	0 to 3
CSPG4LY	Chondroitin sulfate proteoglycan 4 Like Y	2	Only Testis	Amplicons	AZFc	0	0	2	0 to 2
GOLGA2LY	Golgi Autoantigen, Golgin Subfamily a2 Like Y	2	Only Testis	Amplicons	AZFc	0	0	2	0 to 2

*Complete and partial AZFc deletions usually show variations in the deletions of the genes.
†*CDY1* is located in the AZFc and *CDY2*, in the AZFb. One copy of the *CDY1* is in the overlapped region of the AZFc with AZFb.

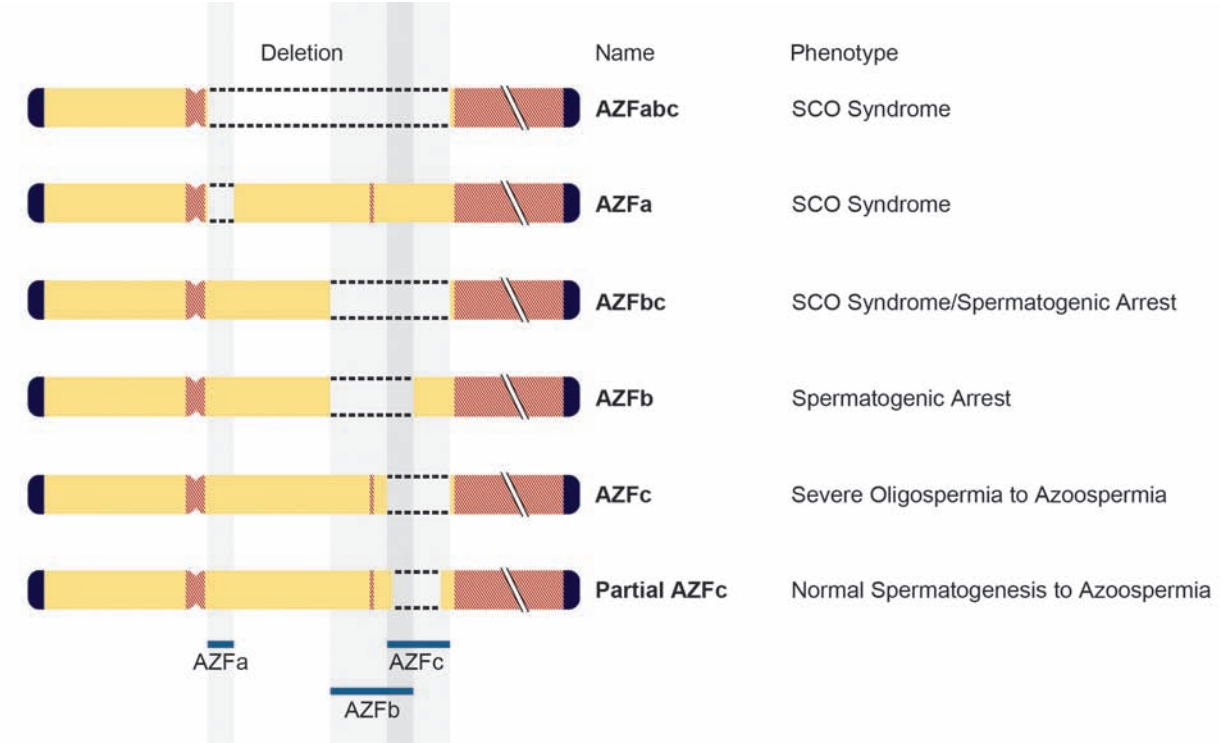

Figure 4. Six types of AZF microdeletions and the resulted phenotypes are shown. The most common deletion patterns are the AZFc and partial AZFc deletions. The partial deletions in the AZFc have several forms with different phenotypes in each population. Partial AZFa and AZFb are the other conditions that are rare.[1,2] SCO indicates Sertoli cell-only.

AZFabc, and partial AZFc (Figure 4).[1] The most common deletions are in the AZFc and AZFb. Partial and complete AZFc deletions are seen in 60% of the YCMs, and the AZFb is the deletion site of about 16% of AZF deletions in infertile men.[11] In total, 35% of the deletions are AZFb, AZFbc, or AZFabc.[1] Only 2% to 5% of the deletions are seen in the AZFa region.[11,18] Omrani and coworkers in Northwestern Iran showed that 24 out of 99 patients with azoospermia or severe oligospermia (24.2%) had microdeletions in the AZF region, but no microdeletions were found in fertile men. The deletions comprised the AZFc (87.5%) and AZFb (29.2%) regions.[10] Their relatively high frequency of YCMs is yet to be confirmed by studies on larger samples and newer diagnostic instruments. In a study on 247 Saudi men with idiopathic azoospermia or oligospermia, 3.2% had YCM, consisted of 6 in the AZFc, 1 in the AZFb, and 1 in both AZFa and AZFc.[7]

AZFa

Complete deletion of AZFa is associated with azoospermia and no foci of testicular spermatozoa.[2,18] AZFa region harbors 2 protein-encoding genes of *USP9Y*, and *DBY* (recently called *DDX3Y*) that are involved in deletions. They are both located in the X-degenerate region of euchromatin and have homologous genes on the X chromosome.

The dead box Y gene (*DBY*) encodes a putative RNA helicase.[34] Foresta and colleagues showed a major role of *DBY* in the AZFa region in spermatogenesis.[35] The ubiquity-specific protease 9Y gene (*USP9Y*, previously known as *DFFRY*) encodes a protease involved in the regulation of protein metabolism.[34] This gene is the only one in the AZF region that has been found to be deleted in isolation; its deletion was associated with severe oligospermia and azoospermia in the 2 reported cases, the histology of both of which was indicative of hypospermatogenesis.[30,31] However, Krausz and colleagues in 2006 reported the first case of AZFa partial deletion involving *USP9Y* that was transmitted naturally from a father to his son; isolated deletion of the *USP9Y* was found in 2 generations of 2 families. They concluded that *USP9Y* might have a fine-tuning role (rather than an essential role) that improves efficiency in spermatogenesis.[18]

AZFb

Complete deletion of AZFb is associated with azoospermia and no foci of testicular spermatozoa.[11] The known protein-encoding genes in this region that are associated with spermatogenesis are *EIF1AY*, *RPS4Y2*, and *SMCY* that are located in X-degenerate euchromatin, and *HSFY*, *XKRY*, *PRY*, and *RBMY* that are in the ampliconic regions (Table 1). The first proposed gene responsible for AZFb deletions was the *RBMY*.[21] The *RBMY* gene family encodes testis-specific RNA binding proteins that are exclusively expressed in the germ cells.[36] There are 6 copies of this gene family in the AZFb.[22] Ferlin and colleagues questioned the essential role of this gene in spermatogenesis; they found severe spermatogenesis failure in men with a partial AZFb deletion that had removed *SMCY*, *EIF1AY*, *RPS4Y2*, and *HSFY*, but not the *RBMY*.[37] Heat shock transcription factor, Y-linked gene (*HSFY*) is a newly discovered gene involved in YCM that encodes a protein similar to those regulated by the heat shock factor family. The HSFY protein is expressed in Sertoli cells and spermatogenic cells and it has been shown that in mammalian tastes, heat shock proteins have a role in spermatogenesis.[38] Shinka and colleagues reported the predominant expression of *HSFY* in the testes and deletion of *HSFY* along with *RBMY* in 2 azoospermic men.[38] Sato and colleagues found that the expression of *HSFY* was altered in men with Sertoli cell-only (SCO) syndrome and maturation arrest.[39] Another gene on which some researchers have focused is the *EIF1AY* that ubiquitously encodes an essential translation initiation factor.[27] Kleiman and colleagues showed that the absence of expression of *EIF1AY* might contribute to azoospermia.[34] They also studied the expression of the *PRY*, another gene in the AZFb, and found that its absence of expression resulted in testes without germ cells.[34]

AZFc

The AZFc is a 4.5-Mb region of the euchromatin and its complete deletion is one of the most frequent causes of male infertility.[40] Partial deletion of AZFc is another frequent pattern. Recently, Zhang and coworkers found partial AZFc deletions in the pedigrees of complete AZFc deletion carriers and concluded that partial deletions of AZFc could increase the risk of complete AZFc deletion.[40]

The role of these deletions in spermatogenesis is controversial. Spermatozoa can be found in the ejaculate or the testicular tissue of 50% of men with AZFc microdeletions.[2] Fertility may occur in the presence of partial AZFc deletions with various lengths; several cases of fathering children have been reported, but in all of them, the AZFc deletions are transmitted to the male offspring, and interestingly, the sons have phenotypes not necessarily similar to their fathers.[14,16,17,19]

The AZFc contains 8 gene families including *BPY2*, *CDY*, *DAZ*, *CSPG4LY*, *GOLGAZLY*, *TTY3.1*, *TTY4.1*, and *TTY7.1*, the 5 former of which are protein-encoding genes that are thought to be associated with spermatogenesis (Table 1).[26] There are 3 copies of the *BPY2*, 2 copies of the *CDY1*, and 4 copies of the *DAZ*. The first recognized gene in the AZFc was DAZ which was described in 1995 by Reijo and colleagues.[41] The *DAZ* gene belongs to a gene family including *BOULE* and *DAZL* autosomal single-copy genes.[2] This gene encodes RNA-binding proteins that are exclusively expressed in the germ cells.[34] Copies of *DAZ* in a Y chromosome are almost identical.[42] The 2 clusters of these genes are inverted pairs of *DAZ1/DAZ2* and *DAZ3/DAZ4*.[43] Deletion of each member of *DAZ* may have different effects.[44] Deletions in *DAZ2*, *DAZ3*, and *DAZ4* copies are found in both fertile and infertile men and are described as familial variants inherited from father to son.[45] However, *DAZ1/DAZ2* deletions were reported to be restricted only to infertile men.[45] Expression of *DAZ1* seems to be essential for spermatogenesis, but a recent case of fertile man with *DAZ1* deletion has been reported.[45,46]

The chromodomain Y gene (*CDY1*) encodes a protein involved in DNA remodeling.[34] Kleiman and colleagues showed that *CDY1* transcripts correlate with complete spermatogenesis.[47] The 2 copies of *CDY1* (known as *CDY1a* and *CDY1b*) are located in the AZFc; however, one copy is in a region that is now shown to have an overlap with AZFb. Thus, AZFb and AZFbc deletions may remove one copy of *CDY1*.[32] Two other copies of the *CDY* gene family (*CDY2*) are located in the AZFb.

AZFd

In 1999, Kent-First and colleagues described a

fourth AZF region between the AZFb and AZFc, termed the *AZFd*,[4] which was associated with mild oligospermia or abnormal sperm morphology.[4,48] Later, Cram and colleagues described AZFcd deletions in candidates for ICSI.[49] Muslumanoglu and colleagues reported that three-fourth of their cases of AZF deletions had AZFd deletions.[48] In patients with SCO syndrome, deletion of a single locus in the AZFd, as well as an AZFc deletion, was noted. This locus (SY152) is located proximal to the AZFc and one of the *DAZ* copies.

Despite the initial excitement about this discovery, the existence of the AZFd region was seriously questioned. Noordam and colleagues discussed that the deletions in these single loci can be a polymorphism instead of "disease-causing deletions.[50]" Moreover, according to the new models, the AZFb overlaps the proximal AZFc and there is no distinct area between these regions.[32] The AZFd sequence-tagged sites are in fact within the AZFc and are deleted in some types of partial AZFc deletions. The current consensus expert opinion is that AZFd *does not exist* and that the initial reporting of the whole concept was the result of significant technical flaws. Currently, AZFd is not considered in clinical practice.

NEW ASPECTS OF Y CHROMOSOME MICRODELETIONS

In the past few years, the molecular mechanism of YCM was recognized to be derived from the homologous recombination between identical sequence blocks. This resulted in assays of the YCMs according to new patterns that did not completely correspond to the classical AZF regions. In 2001, Kuroda-Kawaguchi and colleagues determined the complete nucleotide sequence of AZFc and proposed the structure of 6 families of massive repeat units (amplicons) that constitute a complex of 3 palindromes.[42] Later in 2002, Repping and colleagues further investigated the AZFb and AZFbc deletions and determined their breakpoints.[32] The Y chromosome was mapped by Skaletsky and colleagues in 2003.[22] This led to the researchers' attention being focused on the homologous recombinations between the amplicons, especially in partial AZFc deletions. The AZFa, however, was an exception: the X-degenerate region of the euchromatin was involved and deletions could not be explained by breakpoints between the amplicons. In 2000, Kamp and colleagues found that the proximal breakpoints of the AZFa were located in a long retroviral sequence block and the distal breakpoints in a homologous *HERV15* sequence block.[51] They assumed that intrachromosomal recombination events between the two homologous retroviral sequence blocks in the proximal Yq11 are probably the causative agents for most of the AZFa microdeletions observed in men with SCO syndrome. A mean value of 792 kb was estimated for their molecular lengths.[51]

Studying the AZFc was a trigger to the introduction of the palindromic structure of the Y chromosome. Kuroda-Kawaguchi and colleagues sequenced the entire AZFc region and found 6 distinct families of amplicons ranging from 115 kb to 678 kb in length (named after colors: yellow, green, blue, turquoise, gray, and red).[42] Members of each amplicon family are nearly identical and each of these occurs 2 to 4 times along the euchromatin (Figure 5). Together, they account for 93% of the AZFc and contain *RBMY*, *PRY*, *BPY2*, *DAZ*, *CDY1*, *CSPG4LY*, and *GOLGA2LY* genes.[42] The AZFc is particularly susceptible to deletions because its structure is completely composed of the amplicons.[44]

According to the ampliconic sequences, the classical complete AZFc deletion encompasses a 3.5-Mb totally ampliconic region between 2 blue amplicons (b2 and b4) that occurs by homologous recombination (b2/b4 recombination).[2] Other potential recombinations were then studied for explanation of partial deletions. Repping and colleagues described a partial deletion in the AZFc termed gr/gr in infertile men (one of the g1/g2, r1/r2, or r2/r4 deletion patterns that remove half of the AZFc). The gr/gr deletion was associated with varying degrees of spermatogenesis failure.[23] Yen hypothesized a b1/b3 recombination as a potential mechanism of partial AZFc deletion that removes the proximal portion of the AZFc.[52] Its role in infertility is not known yet, since it has been found in a small number of fertile and infertile men.[23,54] In 2004, Repping and colleagues described b2/b3 recombination (also called g1/g3), a 1.8-Mb deletion that removes half of the AZFc region, including 12 members of 8 testis-specific gene families (Figure 5).[53] This deletion was also identified by

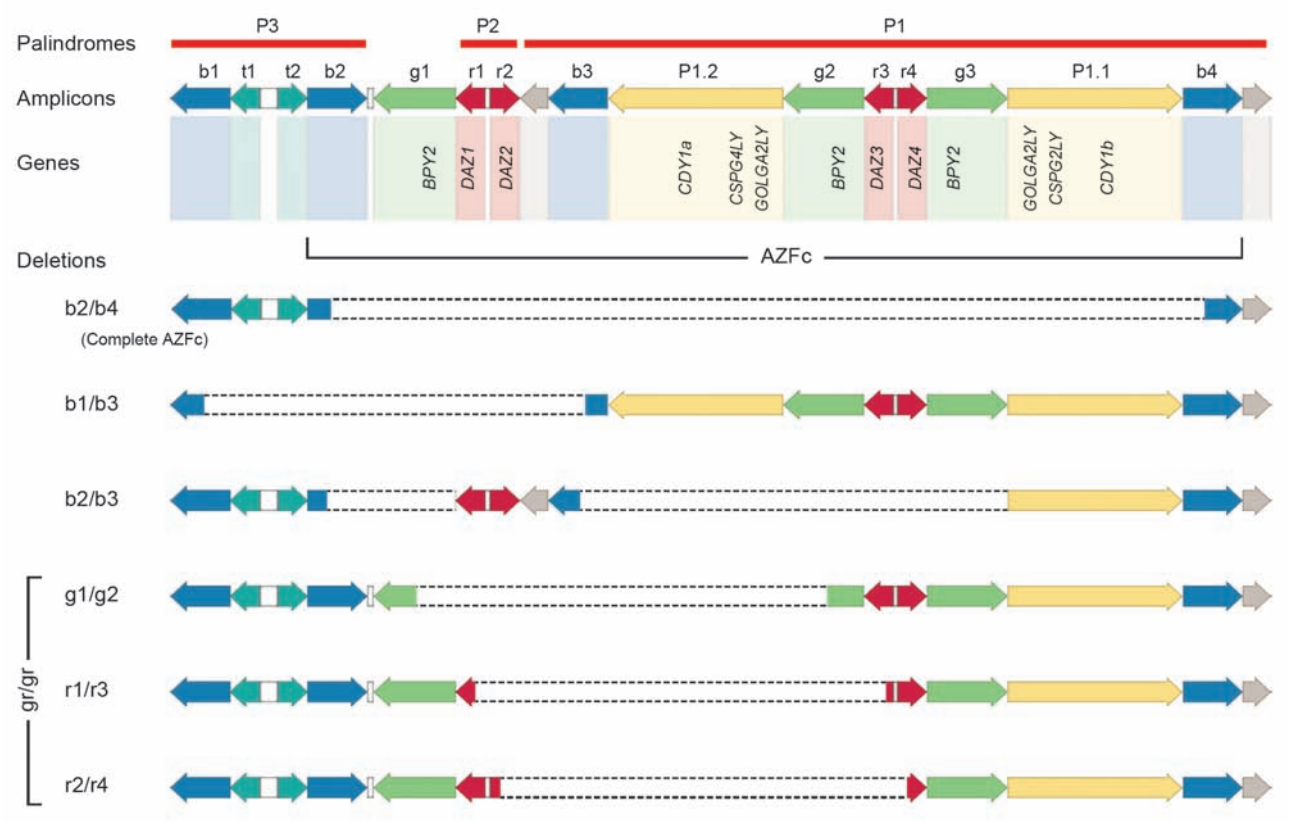

Figure 5. The amplicons and palindromes in the AZFc in relation to the protein-encoding genes are demonstrated. Below, the common types of deletions (partial and complete) are seen. The b2/b4 recombination removes all the AZFc. The gr/gr deletion appears with various patterns (g1/g2, r1/r3, and r2/r4) that remove different sets of genes.[23,42,52,53]

Fernandes and coworkers in a separate publication.[55] The roles of partial AZFc deletions (gr/gr and b2/b3) in spermatogenesis failure are controversial.[40,44]

The AZFb and AZFbc deletions were studied in 2002 by Repping and colleagues,[32] one year after the introduction of the palindromic structure of the AZFc by Kuroda-Kawaguchi and colleagues.[42]

Repping and coworkers found that AZFb deletions were extended from palindrome P5 to the proximal arm of palindrome P1, which is 1.5 Mb within the AZFc.[32] The AZFbc deletions were extended from P5 to the distal arm of P1 (Figure 6). The P5/proximal P1 deletion (AZFb) encompasses up to 6.2 Mb and removes 32 genes and transcripts

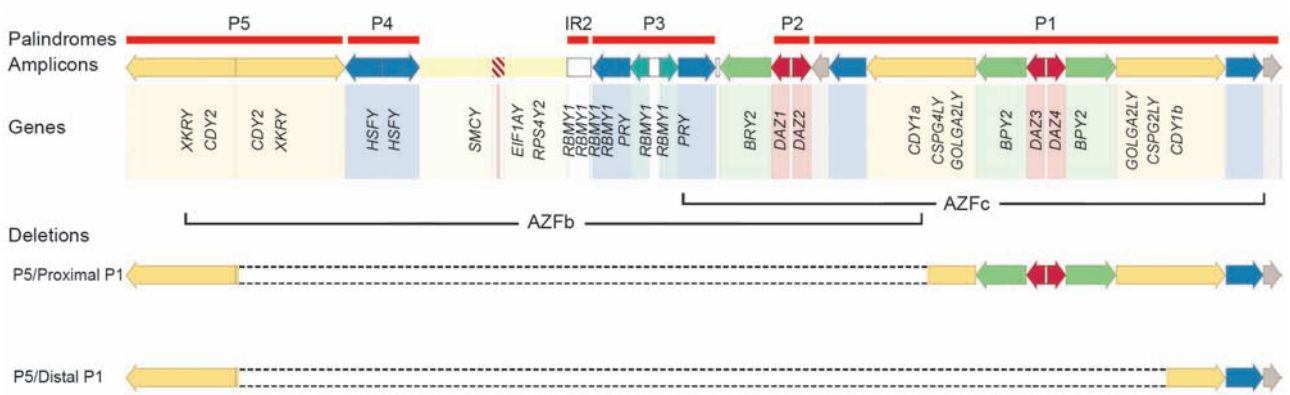

Figure 6. The AZFb and AZFbc deletions are now described as P5/proximal P1 and P5/distal P1 deletions, respectively. The AZFb deletion removes 6.2 Mb of the Yq, and the AZFbc, 7.7 Mb of this region. The deleted genes in each pattern are demonstrated.[32]

and the P5/distal P1 (AZFbc) is 7.7 Mb, removing 42 genes and transcripts (Table 1).[32] Accordingly, all the protein-encoding genes associated with spermatogenesis in the AZFb and AZFc are included in the P5/distal P1 deletion and all except *CSPG4LY* and *GOLGA2LY* are involved in the P5/proximal P1 (complete AZFb) deletion. These 2 deletions are massive, removing one-fourth to one-third of the euchromatin of the Y chromosome, and cause azoospermia.

GENOTYPE-PHNOTYPE ASSOCIATIONS

In clinical practice, information about the AZF deletions has a predictive role for ART outcomes. Hopps and associates demonstrated that men with AZFa, AZFb, and AZFbc have no possibility of sperm retrieval through TESE, while isolated AZFc is associated with successful TESE in 75% of the cases.[56] In concert with their findings, Krausz and colleagues demonstrated that AZFc deletions are associated with sperm retrieval in half of the cases, while in complete AZFa and AZFb deletions, the probability of finding mature spermatozoa by TESE is virtually nil.[57] However, it should be noted that partial deletions in the AZFa and AZFb, although extremely rare, have been reported with natural transmission of the deletions to the offspring.[15,18] In effect, complete AZFa and AZFb deletions are known to correspond to the SCO syndrome and spermatogenic arrest, respectively, while partial AZFb or AZFc and complete or partial AZFc deletions lead to variable phenotypes from hypospermatogenesis to the SCO syndrome.[2]

Two possible explanations for genotype-phenotype dissociation in YCMs are the markers and techniques used to identify the deletions and the reportedly progressive regression of the germinal epithelium over time in men with these deletions.[11] The progressive nature of spermatogenesis failure has been reported by some authors,[11,14] indicating that partial deletions may cause subfertility that progresses to azoospermia over time.[58,59] However, Oates and coworkers found a fluctuation, but not decrease, in sperm count during a 7-year period in 42 men with AZFc deletions.[60]

One other factor is the key to interpret conflicting results of the influence of partial AZFc deletions: the Y chromosome lineage on which the deletion has arisen.[43] Y chromosome lineage or haplotype is a monophyletic group of Y chromosomes defined by slowly mutating binary markers. Some haplotypes are confined to particular populations. In Europe for instance, there are 5 or 6 major Y chromosome haplogroups. Thus, the influence of AZFc partial deletions should be assessed based on the ethnic groups and their genetic characteristics of the Y chromosome.[43] The gr/gr and b2/b3 partial AZFc deletions have been studied in different haplogroups. Surprisingly, although the b2/b3 deletion removes *DAZ3/DAZ4* and *BPY2.2/BPY2.3*, it is not associated with spermatogenesis failure as it is seen in a large population of men in the Northern Europe.[55] Likewise, in East Asian populations, b2/b3 deletion was not linked with infertility, suggesting a polymorphism with limited or no effect on fertility.[33,40] However, the gr/gr deletions may have a limited effect on fertility in some specific Y chromosome haplogroups.[43] It has been proposed that the gr/gr deletion has occurred multiple times during human evolution and the fertility status of individuals carrying the gr/gr deletion is unknown.[43] In Eastern Asians, the gr/gr deletions are seen in 8% to 10% of men.[44,61] Zhang and colleagues reported that this deletion did not render an increased risk of infertility,[61] but they later found a gr/gr deletion in the pedigrees of complete AZFc deletion carriers and concluded that partial deletions of AZFc could increase the risk of complete AZFc deletion.[40] In an Australian population, the gr/gr deletion was associated with infertility (but not with the severity of spermatogenesis impairment) and it was even more frequent than complete AZFc deletion.[62] On the other hand, although Giachini and colleagues found an association of the gr/gr deletion with infertility in Italian men, they reported that cryptorchidism and varicocele were also present in 3 out of 7 men with this deletion.[63] As an explanation of these controversial results, a secondary duplication of b2/b4 was found by Repping and colleagues among gr/gr deletion cases that might rescue the phenotype.[43] The *DAZ* gene family is the main protein-encoding gene family that may have a role in these deletions. Repping and colleagues introduced 3 types of gr/gr deletions, not all of which included the *DAZ1/DAZ2* cluster or the *DAZ3/DAZ4* cluster.[23] Later, Machev and colleagues found 4 types of gr/gr deletions and showed that only deletions containing *DAZ3/DAZ4*

plus *CDY1a* were linked with infertility.[46]

DIAGNOSIS AND TREATMENT

In younger patients who are diagnosed early in their fertile years, progressive decrease in testicular spermatogenic activity over time is an indication for potential cryopreservation of ejaculated spermatozoa to avoid invasive techniques in the future.[2,11,57] Otherwise, ART/ICSI, combined with sperm retrieval techniques such as TESE in selected azoospermic men, can be a treatment of choice for infertile men with YCM. As mentioned above, some YCMs remove the chance of successful ART, while patients with other types of deletions such as AZFc deletions may have retrievable sperm in their testes.[56,57] Of note, rare cases of successful ICSI have been reported in patients with partial AZFb deletions.[6] Successful ART/ICSI in men with YCM and subsequent fertilization and childbirth has been reported frequently. However, van Golde and colleagues reported that although successful pregnancy and childbirth are readily achievable in YCM men, fertilization rate by ICSI in men with AZFc deletions was significantly lower than that in other ICSI candidates.[64]

Intracytoplasmic sperm injection is associated with some risks for the offspring if the father harbors Y chromosome aberrations. Only 2% to 3% of the ICSI candidates harbor Y microdeletions. [2] However, it is estimated that if one-half of all azoospermic men were to undergo ICSI, the incidence of male infertility would double within seven generations, a great proportion of which would be due to Y deletions transmitted to the sons.[65] Hence, the main issue of concern is that men with YCM who have intratesticular spermatozoa will almost certainly pass the deletion to male offspring through ART/ICSI.[2] In practice, several cases of AZFc deletion transmissions by ICSI have been reported.[49,66,67] Also, Katagiri and colleagues have reported sperm retrieval and fathering of a son with identical deletion in a man with partial AZFb deletion.[6] It has been reported that ICSI per se is not a risk factor for generation of Yq deletions.[1] On the other hand, some reports indicate that the incidence of chromosomal abnormalities after ICSI, including de novo deletions, is higher in the offspring of men with genetic aberrations compared to the general male population.[68,69] For the first time, Kent-First and colleagues evaluated ICSI-conceived sons for Y microdeletions in 1999. They found 1 boy with a de novo deletion while his father did not have any deletions.[4] Furthermore, although microdeletions seem to be stable when inherited by ICSI,[70] Lee and colleagues reported vertical transmission of AZF deletions in 4 fetuses conceived by ICSI, in 2 of which the deletion was expanded compared to that in the fathers.[68]

Second, although no other abnormality in the ICSI-conceived sons of fathers with AZF deletions is reported, it may still be too early to reach any conclusions. Although the data suggest that there are no health implications other than infertility associated with this type of vertical transmission, it is important to remember that the first generation of babies with YCM has not yet reached maturity.[71]

Third, new techniques bypass the natural selection of spermatozoa and may, at least theoretically, allow entry of poor-quality sperm into the reproductive process.[72] Van Golde and colleagues found a poorer embryo quality in ICSI-conceived offspring of men with AZFc deletions.[64] They hypothesized that AZFc deletions may cause impairment of the spermatozoa quality or may adversely affect sperm function in the fertilization process. This lowers the chance of conceiving boys, as supported by their ICSI data.[64]

Fourth, the relationship of YCMs and other genetic lesions to male infertility continues to be an area of concern and should be considered in studies on the risks of ICSI.[1] Rucker and coworkers showed that of 17 candidates for TESE who had YCM, 5 had additional karyotypic abnormalities.[72] Patsalis and colleagues suggested that there might be a potential risk of chromosomal aneuploidy for male offspring born to fathers with YCM.[73] Siffroi and colleagues reported that a significant fraction of spermatozoa from men with YCM are nullisomic for sex chromosomes, indicating a potential risk for the offspring to develop 45,X Turner syndrome or other abnormalities.[74] Also, in 46,XY/45,X mosaic patients with sexual ambiguity a high incidence of AZFc deletions can be found.[11]

Finally, Dewan and colleagues found that AZFc microdeletions were significantly more frequent in

Table 2. Summary of Risks Associated With Intracytoplasmic Sperm Injection for Treatment of Men With Y Chromosome Microdeletions

Level	Risks
TESE	Failure with some AZF deletions[56,57]
ICSI	Unsuccessful fertilization[64] Selection of poor-quality spermatozoa[72]
Pregnancy	Poor embryo quality[64] Pregnancy loss[75]
Offspring	Transmission of Y microdeletion of father[2,65-67] De novo abnormalities Y microdeletions[4] Expanded Y microdeletion of father[68] Karyotypic anomalies (nullisomy/trisomy)[11,72-74] Yet unknown abnormalities in adulthood[71]

men from couples with recurrent pregnancy loss than in fertile and infertile men. These men had 3 or more microdeletions. The authors suggested that the proximal AZFc region might play an important role in maintaining gestation.[75] Table 2 summarizes the risks of ICSI for men with YCM.

The omnipotence of TESE/ICSI has reduced the need for seeking the etiology of spermatogenesis failure, while pretreatment diagnosis can result in a more appropriate knowledge-based therapy. Regarding the risks depicted above, testing for Y chromosome microdeletions is an important factor in counseling before ICSI.[71] Long-term follow-up studies of ICSI-induced offspring are recommended for ICSI candidates .[73] Also, in men with hypospermatogenesis caused by YCMs, transfer of 45,X embryos may occur through ICSI; therefore, systematic screening should be emphasized.[73]

Today, most andrology and infertility centers routinely offer Y chromosome testing to men with severe spermatogenesis failure, especially before ART treatment.[1] The criteria to perform YCM analysis and the laboratory methods used play an important role. In addition, practical issues might alter the indications because of problems related to the availability of technical expertise, prohibitive costs, and lack of insurance coverage.[76] Overall, screening is definitely suggested for men with sperm count of 1×10^6/mL or less,[69] but many experts suggest 5×10^6/mL as the cutoff point, since 10.5% of patients with a sperm count less than 5×10^6/mL may harbor microdeletions; this is also the criterion for chromosome analysis.[27,70,77] Concerning the laboratory methods, the sequence-tagged sites and their number to be screened are the important factors to determine the accuracy of screening protocol.[78] Recently, high-resolution microarrays for chromosome screening and microchip devices for electrophoresis have been developed.[1] These devices require small amounts of DNA and little time for analysis,[79] and when combined by multiplex polymerase-chain reaction assays, they can be useful for detection of deletions in the AZF.[79]

In case Y microdeletions are discovered in the male infertility workup, the decision to proceed with ICSI is tied to the certain knowledge that male offspring will be infertile by definition. Interestingly, Giltay and colleagues showed that more than half of the patients who tested positive for chromosomal aberrations decided to go ahead with ICSI.[80] A thorough genetic consultation should be offered and the physician should confirm the couples' understanding of the potential risks to their child. In such cases, sex selection by preimplantation genetic diagnosis assays and female embryo selection is an option for some couples.

CONFLICT OF INTEREST

None declared.

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