Review Article on Genetic Causes and Management of Male Infertility

Histology and sperm retrieval among men with Y chromosome microdeletions

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Abstract: In this review of Y chromosome microdeletions, azoospermia factor (AZF) deletion subtypes, histological features and microTESE sperm retrieval rates are summarized after a systematic literature review. PubMed was searched and papers were identified using Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Approximately half of infertile couples have a male factor contributing to their infertility. One of the most common genetic etiologies are Y chromosome microdeletions. Men with Y chromosome microdeletions may have rare sperm available in the ejaculate or undergo surgical sperm retrieval and subsequent intracytoplasmic sperm injection to produce offspring. Azoospermia or severe oligozoospermia are the most common semen analysis findings found in men with Y chromosome microdeletions, associated with impaired spermatogenesis. Men with complete deletions of azoospermia factor a, b, or a combination of any loci have severely impaired spermatogenesis and are nearly always azoospermic with no sperm retrievable from the testis. Deletions of the azoospermia factor c or d often have sperm production and the highest likelihood of a successful sperm retrieval. In men with AZFc deletions, histologically, 46% of men demonstrate Sertoli cell only syndrome on biopsy, whereas 38.2% have maturation arrest and 15.7% have hypospermatogenesis. The microTESE sperm retrieval rates in AZFc-deleted men range from 13-100% based on the 32 studies analyzed, with a mean sperm retrieval rate of 47%.

Keywords: Y chromosome microdeletion; microTESE; sperm retrieval; testis biopsy; male infertility; azoospermia factor; azoospermia

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Introduction

Fifteen to twenty percent of couples globally report infertility issues and 20% to 70% of these cases have male factors contributing (1). The most severe form of male infertility is termed azoospermia, where no sperm are identified in semen. Azoospermia can be further divided into obstructive azoospermia (OA), as a result of an obstruction in the ejaculatory pathway or non-obstructive azoospermia (NOA), as a result of defective spermatogenesis (2). Genetic causes of NOA include sex chromosomal abnormalities, Y chromosome microdeletions (YCM), gene copy number variations (CNVs) and mutations in a variety of different genes (3,4).

The Y chromosome is one of the smallest chromosomes in the human genome (5). Structurally, the Y chromosome is composed of a short (Yp) and a long arm (Yq) with a rich assortment of repetitive elements that render it

highly unstable and prone to internal recombination with subsequent segmental deletions (5,6). Functionally, genes on the Y chromosome have been recognized to drive gonadal differentiation and testicular development to create the male phenotype (7).

The Y chromosome was first suspected to be involved in azoospermia in the 1970s, when Tiepolo & Zuffardi (8) identified deletions in Yq of patients with an otherwise normal karyotype. Vogt (9) reviews the work that continued into the 1990s, summarizing that researchers using technology that ranged from fluorescent tagging to polymerase chain reaction (PCR), found deletions that commonly spanned regions within the long arm of the Y chromosome. This region was later termed the azoospermic factor (AZF) locus. Vollrath *et al.* (10) and Vogt *et al.* (11) separately used sequence tagged sites (STS) and PCR analyses to assemble a map of the AZF locus (10,11). The AZF locus was then classified into four gene regions (AZFa, AZFb, AZFc, AZFd) that were believed to contain spermatogenesis genes involved in male infertility (12). It has subsequently been proposed by some investigators that deletions of the AZFd region (resulting from *gr/gr* recombination) are within the AZFc region and may not be clinically relevant (13). Clinically, screening for YCM is conducted using multiplex PCR to search for the presence of STS in the AZF locus (9,14). YCM normally results in men being severely oligozoospermic or azoospermic.

Depending on the AZF-deleted region, biopsies of the testes generally reveal different histological features. These include: Sertoli Cell Only syndrome (SCO), maturation arrest (MA), and hypospermatogenesis (HS) (15). The severity of infertility is greatest among men with SCO, followed by MA and then HS. It is common for different regions of the testes of these men to have variable histologic patterns, so a simple biopsy of the testis that samples very little testicular tissue, may not reflect the overall function of the testis and fail to capture spermatogenic heterogeneity.

Treatment for men with YCM and severe oligozoospermia often relies on in vitro fertilization coupled with intracytoplasmic sperm injection (ICSI). However, among men with azoospermia, surgical sperm retrieval is necessary through testicular extraction of sperm (TESE), or microdissection testicular sperm retrieval (microTESE), first reported in 1999 (16).

In this review, Y chromosome microdeletion subtypes and its associated histology and effects on testicular sperm extraction in men with YCM are summarized.

Methods

The search engine PubMed was used to identify publications published between January 1997 – May 21, 2019 addressing Y chromosome microdeletions and surgical sperm retrieval. Search terms included: “y chromosome microdeletion”, “male infertility”, “micro tese”, “microdissection testicular sperm extraction”, “microtese”, “micro-tese”, “hormone therapy”, “gr/gr”, “AZFa”, “AZFb” and “AZFc”. The search term “y chromosome microdeletion” and the Boolean operator “AND” were used to search for articles with the following search terms: “hormone therapy”, “fertilization”, “offspring”, “sperm retrieval”, “therapy”. Search restrictions included the English language and full text availability. A total of 600 articles were identified, and 560 articles remained after removing duplications. Abstracts were screened for pertinent information. An additional 25 articles were identified outside of the search. Overall, 585 papers were screened, and 97 articles were included in this review. Inclusion and exclusion processes are represented graphically below using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) in *Figure 1* (17,18).

Y chromosome microdeletions

Depending on geographic and ethnic background and selection criteria for patients in studies, the reported prevalence of Y chromosome microdeletions in azoospermic and oligozoospermic men vary from 3.2% to 29.4% (*Table 1*), with the global prevalence reported as 7% among azoospermic and severely oligozoospermic men (4). Within the AZF regions, patients may have partial or complete deletions that can result in a range of different clinical phenotypes. In general, YCM men present with significantly higher FSH levels than fertile men and exhibit lower, though not statistically significant, LH levels, testosterone levels and testicular volume (19-22,50). Semen analysis and testicular histology differ depending on the AZF region that is deleted and whether the deletion is a complete or partial deletion. *Table 1* outlines the proportion of deletion of each region, sperm profile and histological distribution.

Complete vs. partial deletions

Since men with partial deletions may have a variable pattern of genetic profiles, it is clinically important for clinicians to

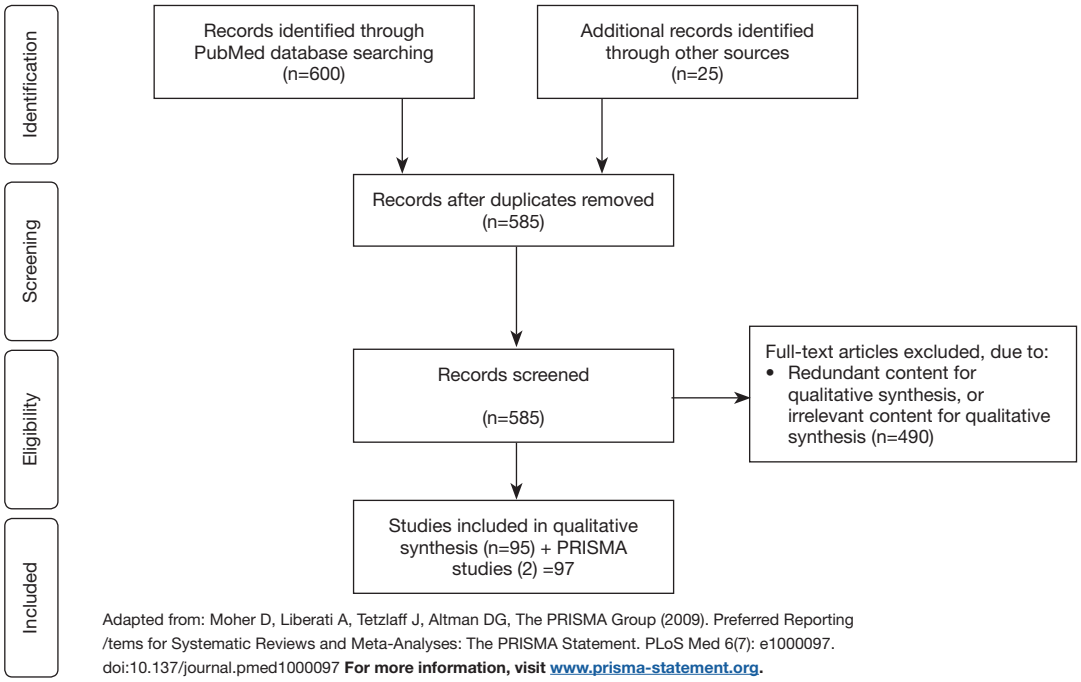

Figure 1 PRISMA flow diagram describing inclusion and exclusion process.

Table 1 Y chromosome microdeletion types, the proportion of each deletion type and their respective sperm count and histology profiles

Deletion (complete or partial)	Mean frequency of AZF deletions (%) (from references 12,19-26)	Sperm count (from references 12,19-26)	Histology, n [%]			Total n	Reference
			SCO	MA	HS		
AZFa	5.3	Azoospermia—severe oligozoospermia	35 [69]	15 [29]	1 [2]	51	(12,21,24,27-35)
AZFb	10.8	Azoospermia—severe oligozoospermia	20 [38]	25 [48]	7 [13]	52	(12,21,23,28,29,31,33,36-39)
AZFc	57.2	Azoospermia—oligozo-ospermia	82 [46]	68 [38]	28 [16]	178	(12,21-23,28-36,39-44)
AZFd	N/A	Azoospermia—normozo-ospermia	4 [50]	4 [50]	–	8	(12,37,43,45)
AZFab	1.2[¶]	N/A	1 [100]	–	–	1	(29)
AZFbc (&AZFbdc)	13.5	Azoospermia—severe oligozoospermia	26 [72]	9 [25]	1 [3]	36	(21,23,28,29,31,33,36,37,39, 40,43)
AZFbd	N/A	N/A	–	2 [100]	–	2	(37)
AZFcd	N/A	N/A	–	2 [67]	1 [33]	3	(37,45)
AZFabc	7.0	Azoospermia	11 [79]	3 [21]	–	14	(21,23,29,30,32-34,36,40,43)
Partial AZFc deletions		Azoospermia—normozo-ospermia					(44,46-49)
Gr/gr			11 [61]	7 [39]	–	18	
B1/b3			8 [73]	3 [27]	–	11	
B2/b3			2 [100]	–	–	2	

[¶]From (12) Sequence Tagged Sites (STSs). SCO, Sertoli cell only syndrome; MA, maturation arrest (did not differentiate between subtype of maturation arrest); HS, hypospermatogenesis.

remember that the most reliable data on clinical value for YCM deletions comes from those patients with complete deletions of the AZFa, b, or c regions. Data from men with complete deletions cannot be used to predict the clinical activity or testicular function of men with partial deletions. Hence, careful genetic characterization is critical of YCM-deleted men clinically.

AZFa

The frequency of complete or partial AZFa deletions among YCM men with oligozoospermia and azoospermia has been reported to range from 1.7% to 15.4%, with the mean frequency calculated to be 5.3% (*Table 1*). The AZFa region spans more than 1 megabyte (mb) on the Y chromosome and has a nonrepetitive structure with a low deletion frequency (51,52). This locus contains the three genes *USP9Y* (or *DFFRY*), *DDX3Y* and *UTY*, though only *USP9Y* and *DDX3Y* are believed to be involved in spermatogenesis (51,53,54).

Studies have shown that men with complete AZFa deletions typically have a pure SCO histology on testis biopsy (*Table 1*). Based on twelve articles with a combined n of 51, 68.6% (35/51) are SCO, 29.4% (15/51) are MA and 2.0% (1/51) are HS (*Table 1*). Blagosklonova *et al.* (27) conducted a retrospective study using archived testicular biopsies and found that AZFa-deleted specimens exhibited a combination of reduced tubular diameter, normal to thickened tunica propria, normal to increased intertubular space, hyperplastic Leydig cells and spermatogenic arrest or SCO. Later studies revealed that complete AZFa deletions, involving both USP9Y and DDX3Y, typically lead to the SCO phenotype whilst partial deletions, involving one of USP9Y or DDX3Y, lead to hypospermatogenesis (HS) or maturation arrest (MA) (21,27,45,55). Furthermore, AZFa-deleted men typically present with azoospermia (23,24,56). Indeed, it is likely that an AZFa-deleted man with sperm reported in the ejaculate may have only a partial AZFa deletions, since it is these men who can present with cryptozoospermia or oligozoospermia (21,24).

AZFb

AZFb microdeletions are more common than AZFa microdeletions, appearing in 3.5% to 20% of YCM patients, with a mean frequency of 11% (*Table 1*). Structurally, the AZFb region spans approximately 3.2 mb (57). Although the region is organized as a single copy sequence, it contains numerous palindromic sequences of large direct and inverted repeats, leading to different interpretations of where AZFb ends and AZFc begins (52,57). The region contains several different families of genes: 6 copies of *RBMY1* and *RBMY2*; single copies of *EIF1AY*, *RPS4Y2*, *KDM5D*, *HSFY*, *PRY1*, and *PRY2* (27,46,58,59). The RBMY1 and 2 proteins are related to RNA splicing (46,60) and it is believed that RBMY1 is important in RNA processing during spermatogenesis because the protein localizes in the nuclei of testicular germ cells and Sertoli cells (61). Plotton *et al.* (58) demonstrated that co-conservation of at least two *RBMY1* and *DAZ* (AZFc gene) is sufficient for preserving spermatogenesis. Krausz & Casamonti (46) summarize that *EIF1AY*, *RPS4Y2*, *KDM5D* are involved in post-transcriptional or epigenetic control. Next, *HSFY* encodes for a heat shock protein that was implicated in male infertility because two of the three transcripts are testis-specific and the gene is typically deleted in YCM (46,62,63). Stahl *et al.* (64) discovered that HSFY mRNA expression is elevated in NOA patients with successful microTESE and is reduced in AZFb-deleted patients (64,65). However, another group suggests HSFY's impact on infertility is not as deleterious and is simply a time-dependent effect that only manifests in older men (66). Kichine *et al.* (66), with a sample size of four patients, discovered the HSFY-deleted Y chromosome had been transmitted through generations and thus, HSFY's contribution to infertility likely to be minimal. Lastly, the AZFb locus contains 2 copies of *PRY*, of which two copies also exist in the AZFc locus (59). Patients with deleted *PRY1* and *PRY2* genes present with azoospermia in testis biopsies (59).

Complete AZFb deletions result in azoospermia. Histologically, MA and SCO phenotypes are most commonly observed (12,36). Based on eleven articles, with an *n* of 52, 38.5% (20/52) are SCO, 48.1% (25/52) are MA and 13.5% (7/52) are HS (*Table 1*). As a result, microTESE to date has not been successful in classic complete AZFb deletions. However, there are reported cases where patients that had a partial or non-classical AZFb microdeletion were conducive to spermatogenesis and fertilization were successful (28,67).

Again, it is critical to remember that the clinical relevance of AZFb deletions refers only to those men with complete deletions of AZFb (or deletions of AZFb & c regions); men with partial deletions of AZFb may present with cryptozoospermia (12,21,23,56).

AZFc

The AZFc locus is the most commonly deleted, clinically relevant, region of the Y chromosome that is deleted in YCM, with a mean frequency of 57% (*Table 1*). The region spans 4.5 mb, with six distinct families of amplicons, containing at least seven gene families, and palindromic sequences (52,68). Since the AZFc locus is rich in amplicons and palindromic sequences, it is particularly susceptible to structural rearrangements like deletions and duplications via non-homologous recombination (46,69), explaining why AZFc deletions are so frequent. The gene families present in this locus include *DAZ*, *BPY2*, *CDY1*, *CSPG4LY*, *GOLGA2LY*, *TTTY3* and *TTTY4* (68). AZFc is also suspected to overlap with a limited segment of AZFb deletions and thus contains RBMY genes as well (52,68). It is believed that these genes play a role in fertility. The AZFc locus contains four copies of *DAZ* that are involved in spermatogenesis. The *DAZ* gene family encodes for RNA-binding proteins and is involved in meiosis (70). It appears that combinations of deletions of *DAZ* does not always preclude spermatogenesis. Fernandes *et al.* (71) report *DAZ1* and *DAZ2* co-deletion was the cause of five cases of severe oligozoospermia and they later report that partial deletions of AZFc involving *DAZ3* and *DAZ4* co-deletions are found in fertile normozoospermic men (72,73). This observation supports our prior comments that partial deletions of AZFc cannot be compared to clinical observations for complete AZFc deletions. Complete AZFc deletions are proposed to be a result of homologous recombination between b2 and b4 within the Y chromosome (68).

The phenotype for AZFc YCM varies significantly, depending on which genes are deleted. The sperm profile ranges from azoospermia to oligozoospermia (*Table 1*). In most studies, it has been reported that azoospermia is proportionately more common than severe oligozoospermia in AZFc-deleted men and that azoospermia did not necessarily preclude the presence of sperm during biopsy or testicular sperm extraction (23,40,56). Certainly, studies may inherently select for more severe cases of male infertility, skewing the proportion of azoospermic men to oligozoospermic men. Liu & Jiang (25) proposed that patients initially are oligozoospermic, then gradually progress to azoospermia because they found that younger patients tend to have significantly greater sperm counts than older patients. However, this is not clear considering that Oates *et al.* (41) did not find that sperm producing capability declined over time.

Men with AZFc microdeletions typically have a combination of SCO, maturation arrest and hypospermatogenesis regions within the testis (*Table 1*). From nineteen studies, with a total n of 178, 46% (82/178) had a most advanced histologic pattern of SCO, 38% (68/178) had MA and 16% (28/178) had HS (*Table 1*). Based on the patient cohort, the distribution of histological features can differ significantly. For instance, Ferlin *et al.* (21) reported that 72% of the patients in their study had some foci of hypospermatogenesis; however, based on the literature consulted for *Table 1*, there are more reported cases of SCO and maturation arrest than hypospermatogenesis. Again, in our experience, these men often had mixed histologic patterns of activity in different regions of the same testis, despite the observation that the cause of low sperm production was a uniform genetic abnormality. It is worthwhile to remember that a predominant pattern of SCO histology does not reflect an absence of sperm in other regions of the testis. Oates *et al.* (41) found that 6/14 men with predominantly SCO histology had sperm present and 6/9 with purely SCO histology had sperm present. Furthermore, patients may present with a combination of histological features. Silber *et al.* (42) report 2/7 AZFc-deleted patients who had a combination of MA and SCO while Brandell *et al.* (36) report two patients who had SCO in one testis and MA or HS in the other testis.

It has been suggested that AZFc microdeletions are associated with partial AZFc deletions and Y haplogroups. In a Chinese study, Zhang *et al.* (47) discovered several haplogroups (N*, N1 and Q1) had a high proportion of AZFc microdeletions. Furthermore, participants in these haplogroups were more likely to have partial AZFc deletions and in one pedigree, a complete AZFc deletion descended from gr/gr deletions, suggesting partial deletions may predispose to complete broader deletions in later generations, although documentation of such expanded deletions is rare and anecdotal at this time (47). This study, however, had a limited number of participants within each deletion subtype binned into haplogroups. Another, more recent study found no significant effect of haplogroups on YCM (26). Further studies are warranted to determine if haplogroups are predictive of YCM.

AZFbc

The prevalence of AZFbc microdeletions range from 2.3% to 20% of infertile men, with a mean frequency of 13% (*Table 1*). All patients with AZFbc microdeletions present

with azoospermia (23,56). Histological presentation varies depending on the size of the deletion and which genes are deleted. Most frequently, patients present with SCO (75%), MA (21%) and HS (3.6%) (21,23,28,29,36,37,40,43).

AZFabc

AZFabc microdeletion is the most extensive region of YCM. Researchers have reported that AZFabc-deleted patients are all azoospermic, and exhibit SCO histologically (21,23,36,56). Prognosis for surgical retrieval of sperm is zero to date.

MicroTESE and histology

The complete deletion of a specific Y chromosome region has important value in predicting the sperm retrieval rate (SRR) in men with azoospermia. Prior to proceeding with surgical sperm extraction, it is critically important to perform a detailed semen analysis, with extended analysis of the centrifuged semen sample (74). As discussed, studies of men with AZFc deletions have reported an SRR ranging from 13% to 100% (*Table 2*). Of the reported testicular histopathologies amongst AZFc-deleted men (n=178), 28 (16%), 68 (38%), and 82 (46%) men had HS, MA, and SCO, respectively. Among AZFa, AZFb, and AZFc loci, hypospermatogenesis was the most common amongst the AZFc microdeletion (16%), followed by AZFb (13%) and AZFa (2%) (*Table 1*). However, these numbers should be interpreted with caution because older studies used PCR and STS techniques to characterize AZF deletions, and therefore did not discriminate between complete and partial AZF deletions; thus, these histology results likely reflect a mixture of complete and partial AZF deletions.

Sperm retrieval

Thirty-two studies involving men with Y chromosome microdeletions who underwent any surgical sperm retrieval procedure are summarized in *Table 2*.

AZFa

Men with complete and pure AZFa deletions are solely azoospermic and sperm has not been retrieved by any method (*Tables 1,2*). Surprisingly, a case report describing a man with a complete AZFa deletion, partial AZFb and AZFc deletions had sperm in his ejaculate (92). This is remarkable as no other comparable cases were identified in our literature search. The presence of outlier studies that report finding sperm in men with genetic abnormalities that are not verified by other laboratories may reflect a distortion of published literature and should be interpreted with caution. In all other studies of those undergoing sperm retrieval attempts, all failed (*Table 2*).

AZFb

Men with complete AZFb microdeletions have been azoospermic (*Tables 1,2*). For the exceptional AZFb-deleted men with a successful sperm extraction procedure, SR via mTESE was higher than conventional TESE or TESA (testicular sperm aspiration). The overall SRR for men with complete AZFb deletions was 0/30 (0%) (*Table 2*). This extremely poor prognosis is fitting with published studies that recommends men with complete AZFb deletions should not be offered mTESE (56,93-95). One group, Zhang *et al.* (23) report a SRR of 3/11 (27%) using mTESE in men with AZFb deletions, with two resulting in pregnancy; however, it is difficult to determine whether study participants had a complete or partial AZFb deletion. Previous literature reports that the AZFb locus is proximally defined by sY108 and distally characterized by sY134 or sY135 (57) however Zhang *et al.* (23) defined the AZFb deletions using sY127 and sY134 marker. Thus, the reported SRR of 27% was more likely conducted in men with partial AZFb deletions. In men with partial AZFb deletions, sperm extraction attempts were successful (*Table 2*). The discrepancy in sperm retrieval success in complete *vs.* partial AZFb deletions underscore the importance of correct identification of the size of deletion in the AZFb locus in order to more precisely approximate the chances of a successful sperm retrieval.

AZFc

Studies of men with YCM, when based on patients who require surgical sperm, may overemphasize the prevalence of azoospermia (*vs.* oligozoospermia). According to *Table 1* and *2*, men with either partial or complete AZFc microdeletions are typically azoospermic, though severe oligozoospermia is also common. Based on the 32 studies in *Table 2*, the proportion of men with partial or complete AZFc microdeletions that are azoospermic and oligozoospermic were 67% and 33%, respectively. When considering men referred for genetic testing, independent

Table 2 Sperm retrieval rates stratified by AZF deletion subtypes, collected from 32 studies

Author	Year	n	AZF subtype	Histology	Sperm profile (AZOY[†], SOLGY[‡])	Adjuvant therapy	Sperm retrieval fraction [%]	Procedure
Silber *et al.* (42)	1998	10	AZFc	N/A	AZOY	N/A	5/10 [50]	TESE
Kleiman *et al.* (75)	1999	1	AZFc	MA and Leydig cell hyperplasia	AZOY	N/A	2/2 [100]	Multiple sample TESE
Page *et al.* (76)	1999	1	AZFc	N/A	AZOY	N/A	1/1 [100]	TESE
Peterlin *et al.* (77)	2002	1	AZFa	SCOS	AZOY	N/A	0/1 [0]	TESE
		3	AZFc	2 MA, germinal hypoplasia (successful sperm retrievals); 1 SCOS (unsuccessful sperm retrieval)	AZOY	N/A	2/3 [67]	TESE
		2	AZFabc	SCOS	AZOY	N/A	0/2 [0]	TESE
Oates *et al.* (41)	2002	42	AZFc	SCOS +/– MA	16 SOLGY; 14 AZOY with sperm in testis; 7 AZOY w/no sperm; 5 AZOY w/no TESE	N/A	14/21 [67]	TESE & mTESE mix
Hopps *et al.* (56)	2003	3	AZFa	N/A	AZOY	N/A	0/1 [0] mTESE; 0/2 [0] biopsy	mTESE or TESE
		9	AZFb	N/A	AZOY	N/A	0/6 [0] mTESE; 0/3 [0] biopsy	
		32	AZFc	N/A	SOLGY to AZOY	N/A	9/12 [75] mTESE; 9/20 [45] biopsy	
		10	AZFbc	N/A	AZOY	N/A	0/6 [0] mTESE; 0/4 [0] biopsy	
		4	AZFabc	N/A	AZOY	N/A	0/3 [0] mTESE; 0/1 [0] biopsy	
Kihaile *et al.* (30)	2004	1	AZFa	SCOS	AZOY	N/A	0/1 [0]	TESE
		2	Partial AZFc (ΔsY202, ΔsY243)	1 SCOS; 2 SCOS	AZOY	N/A	0/2 [0]	
		2	AZFc (except sY158 and sY159	MA	AZOY	N/A	2/2 [100]	
		1	AZFabc	SCOS	AZOY	N/A	0/1 [0]	
Tsujimura *et al.* (43)	2004	1	AZFabc	SCOS	AZOY	N/A	0/1 [0]	mTESE
		1	AZFbc	SCOS	AZOY	N/A	0/1 [0]	
		1	AZFc	SCOS	AZOY	N/A	0/1 [0]	
		3	AZFd	SCOS or MA	AZOY	N/A	2/3 [67]	
Schlegel *et al.* (78)	2004	1	AZFb	N/A	AZOY	N/A	0/1 [0]	mTESE
		1	AZFabc	N/A	AZOY	N/A	0/1 [0]	
		1	AZFbc**	N/A	AZOY	N/A	0/1 [0]	
		3	AZFc[¶]	N/A	AZOY	N/A	2/3 [67]	
Choi *et al.* (31)	2004	1	AZFa	SCOS	N/A	N/A	0/1 [0]	mTESE
		1	AZFb	MA	N/A	N/A	0/1 [0]	
		1	AZFb (partial)	HS	N/A	N/A	1/1 [100]	
		2	AZFbc	SCOS or MA	N/A	N/A	0/2 [0]	
		7	AZFc	SCOS, SCOS/MA or MA	N/A	N/A	6/7 [86]	
Kihaile *et al.* (32)	2005	1	AZFa	SCOS	AZOY	N/A	0/1 [0]	TESE
		2	AZFc	MA	AZOY	N/A	2/2 [100]	
		1	AZFabc	SCOS	AZOY	N/A	0/1 [0]	
Stouffs *et al.* (33)	2005	1	AZFa	SCOS	AZOY	N/A	0/1 [0]	TESE
		3	AZFb	MA	2 AZOY; 1 SOLGY	N/A	0/3 [0]	
		17	AZFc	HS to MA and SCOS	N/A	N/A	10/17 [59]	

Table 2 (*continued*)

Table 2 (*continued*)

Author	Year	n	AZF subtype	Histology	Sperm profile (AZOY†, SOLGY‡)	Adjuvant therapy	Sperm retrieval fraction [%]	Procedure
		5	AZFbc	3 SCOS; 1 MA	AZOY	N/A	0/5 [0]	
		2	AZFabc	SCOS	AZOY	N/A	0/2 [0]	
Stahl *et al.* (79)	2010	2	AZFa	N/A	AZOY	N/A	0/2 [0]	mTESE
		7	AZFb	N/A	AZOY	N/A	0/7 [0]	
		7	AZFbc	N/A	AZOY	N/A	0/7 [0]	
		4	AZFc	N/A	SOLGY to AZOY	N/A	15/21 [71]	
		21	AZFabc	N/A	AZOY	N/A	0/4 [0]	
Gambera *et al.* (80)	2010	1	AZFc	HS	AZOY	N/A	1/1 [100]	mTESE
Kilic *et al.* (81)	2010	1	ΔsY127, ΔsY134 (partial AZFb); Mosaicsm 45X (5%)/46XY (95%) by cytogenetic analysis	SCOS, MA	AZOY	N/A	1/1 [100]	TESE
		1	ΔsY127, ΔsY134 (partial AZFb); ΔsY254, ΔsY255 (partial AZFc); 45,X(45%)/46,XY(55%) mosaicism	N/A	AZOY	N/A	0/1 [0]	
Stahl *et al.* (13)	2011	22	Partial AZFc—gr/gr	N/A	AZOY to SOLGY	N/A	14/22 [64]	mTESE
Kalsi *et al.* (82)	2012	1	AZFc	MA	AZOY	N/A	1/1 [100]	mTESE
Zhang *et al.* (23)	2013	8 (only 8/12 received TESA)	AZFb	MA	AZOY	N/A	0/8 [0]	TESA
		11	AZFb	MA	AZOY	N/A	3/11 [27]	mTESE
		16	AZFc	SCOS or MA or HS	26% SOLGY; 74% AZOY	N/A	0/16 [0]	TESA
		50	AZFc	SCOS or MA or HS	26% SOLGY; 74% AZOY	N/A	11/40 [28]	mTESE
Choi *et al.* (83)	2013	21	AZFc	N/A	AZOY	N/A	8/21 [38]	TESE
		9	AZFbc	N/A	AZOY	N/A	0/9 [0]	
Ando *et al.* (84)	2013	4	N/A	N/A	AZOY	N/A	1/4 [25]	mTESE
Bonarriba *et al.* (85)	2013	2	AZFab	N/A	AZOY	N/A	0/2 [0]	mTESE
		3	AZFc	N/A	AZOY	N/A	1/3 [33]	
Gallego *et al.* (34)	2014	2	AZFa	1 SCOS; biopsy not performed on rest	AZOY	N/A	0/1 [0]	TESE
		5	AZFc	1 SCOS; biopsy not performed on rest	4 AZOY; 1 SOLGY	N/A	0/1 [0]	
		1	AZFbc	N/A	AZOY	N/A	Not performed	N/A
		2	AZFabc	1 MA; biopsy not performed on rest	AZOY	N/A	0/1 [0]	TESE
Lo Giacco *et al.* (35)	2014	1	AZFa	SCOS	AZOY	N/A	0/1 [0]	TESE
		18	AZFc	4 complete SCOS, 2 80-90% SCOS, 1 L testicle only; 1 HS, one 10% HS; one 10% sclero hialynosis; one with R mixed atrophy with no mature spermatids	11 AZOY; 7 SOLGY	N/A	1/8 [13]	
		2	AZFc (terminal deletion)	1 HS; 1 R 50% sclero hialynosis, 50% SCOS; L SCOS	AZOY	N/A	0/2 [0]	
		2	AZFbc	N/A	AZOY	N/A	Not performed	N/A
		4	AZFabc	N/A	N/A	N/A	Not performed	

Table 2 (*continued*)

Table 2 (*continued*)

Author	Year	n	AZF subtype	Histology	Sperm profile (AZOY†, SOLGY‡)	Adjuvant therapy	Sperm retrieval fraction [%]	Procedure
Cetinkaya *et al.* (86)	2015	1	AZFb	N/A	AZOY	N/A	0/1 [0]	mTESE
		5	AZFc	N/A	AZOY	N/A	1/5 [20]	
		1	AZFac	N/A	AZOY	N/A	0/1 [0]	
		2	AZFbc	N/A	AZOY	N/A	0/2 [0]	
		1	AZFabc	N/A	AZOY	N/A	0/1 [0]	
Schwarzer *et al.* (39)	2016	1	AZFb	1 MA	N/A	N/A	0/1 [0]	Conventional multilocular TESE or mTESE (not stated)
		20	AZFc	11 SCOS; 5 MA; 4 mixed atrophy	N/A	N/A	2/8 [25] conventional multilocular TESE; 8/12 [67] mTESE	conventional multilocular TESE; mTESE
		2	AZFbc	1 SCOS; 1 MA; 4 mixed atrophy	N/A	N/A	0/2 [0]	Conventional multilocular TESE or mTESE (not stated)
		2	AZFc + other chromosomal disorder	2 SCOS	N/A	N/A	0/2 [0]	Conventional multilocular TESE or mTESE (not stated)
Mascarenhas *et al.* (87)	2016	5	AZFb	N/A	AZOY	N/A	0/4 [0]	TESA
		5	AZFc	N/A	2 SOLGY; 3 AZOY	N/A	1/1 AZOY [100] TESA; 1/1 AZOY [100] mTESE	TESA; mTESE
		2	AZFbc	N/A	AZOY	N/A	0/2 [0]	TESA
		1	AZFabc	N/A	AZOY	N/A	0/1 [0]	TESA
Ko *et al.* (88)	2016	2	ΔAZFbc; (46XY/45X (33:1) and 45,X/46,X,idic(Y)(q11.2) (12:18)	N/A	AZOY	N/A	0/2 [0]	TESE or mTESE (not stated)
		1	ΔAZFabc; 46,X,der(Y).ish i(Y)(p10)(pter++,SRY++)	N/A	AZOY	N/A	0/1 [0]	TESE or mTESE
		6	AZFc	N/A	AZOY	N/A	3/6 [50]	TESE or mTESE
Bahmanimehr *et al.* (20)	2018	1	AZFabc	N/A	AZOY	N/A	1/1 [100]	TESE
Sabbaghian *et al.* (89)	2018	96 (# who attempted mTESE)	AZFc	Of 103 patients (some did not attempt mTESE): 69/103 (67) SCOS, 27/103 (26) MA, remaining histopathology not stated	AZOY or SOLGY	N/A	42/96 [44]	mTESE
Klami *et al.* (90)	2018	7	AZFc	N/A	AZOY	Men with low testosterone were treated with aromatase inhibitor (n=26), clomiphene citrate (n=5), tamoxifen (n=11) or human chorionic gonadotropin (n=6) for four to six months prior to the operation in order to reach normal serum testosterone levels	4/7 [57]	mTESE
Miraghazadeh *et al.* (91)	2019	11	gr/gr (partial AZFc)	N/A	AZOY	N/A	7/11 [64]	mTESE
		5	b2/b3 (partial AZFc)	N/A	AZOY	N/A	2/5 [40]	
Johnson *et al.* (19)	2019	1	AZFa	N/A	AZOY	N/A	N/A	mTESE
		1	AZFa (partial)	N/A	AZOY	N/A	N/A	
		4	AZFb	MA	AZOY	N/A	0/3 [0]	
		44	AZFc	N/A	12 SOLGY; 32 AZOY	N/A	7/21 [33]	
		8	AZFbc	N/A	AZOY	N/A	N/A	

†azoospermia with a Y-chromosome microdeletion (0 sperm/cc); ‡severe oligozoospermia with a Y-chromosome microdeletion (sperm concentration >0 – <5×10^6 sperm/cc); **One patient from this cohort received varicocele repair; ¶One patient from this cohort received varicocele repair.

of semen parameters, we have found that more men had sperm in the ejaculate than not (70% oligozoospermic *vs.* 30% azoospermic). As demonstrated in *Table 2*, men with AZFc microdeletions have the most favourable chances of successful sperm retrieval compared to men with any other type of deletion in the AZF region of the Yq. The SRR for AZFc-deleted men are reported to be between 13% to 100%, with a mean of 47% across the 32 studies reviewed, though some studies also report failure to retrieve any sperm at all (*Table 2*). Concordant with published literature, mTESE had a higher SRR than conventional TESE or TESA in men with YCM (*Table 2*).

It is critically important to remember that the results of microTESE, and other sperm retrieval procedures, are dependent on the extent of tissue searched for sperm. Even microTESE procedures may vary substantially from center to center or amongst surgeons. The more extensive the procedure, the higher the chance of finding rare sites of sperm production.

Multiple AZF Deletions (AZFabc, AZFab, AZFbc)

Men with multiple AZF deletions that attempted any sperm retrieval procedure are listed in *Table 2*. Apart from one azoospermic man with an AZFabc deletion (20), all surgical sperm retrieval attempts failed (*Table 2*). Given these findings, we conclude that testicular sperm extraction attempts in men with combination deletions is very unlikely to be successful.

Adjuvant therapy

Men with severe infertility often attempt empiric medical therapy (EMT) in order to improve sperm production through enhancing endogenous testosterone production, and therefore supporting spermatogenesis. Empiric medical therapy may include the use of hormone altering agents such as human chorionic gonadotropin (hCG), aromatase inhibitors (testolactone, letrozole and anastrozole), selective estrogen receptor modifiers (clomiphene, tamoxifen), and antioxidant supplementation. A number of studies have attempted to optimize sperm production in men who are expected to undergo mTESE. Unfortunately, Level I evidence has not been produced to determine the value, if any, of medical therapy prior to attempted sperm retrieval.

In *Table 2*, only 7 men were treated prior to their operation. According to the retrospective study, men with low testosterone were treated with aromatase inhibitor, clomiphene citrate, tamoxifen, or hCG for four to six months prior to their operations to restore their serum testosterone concentration (90). However, the SRR was no different between the medically treated versus the untreated men, though the participants were not limited to men with YCM.

Data on the effects of EMT on SRR among men with YCM was absent in our literature search. However, upon an additional search, we identified a case report of a normogonadotropic azoospermic man with an AZFc deletion (96). Upon semen analyses at 2-week intervals, complete absence of sperm was concluded. A diagnostic testicular biopsy displayed maturation arrest at the spermatocyte stage. The patient underwent recombinant follicle-stimulating hormone (FSH) treatment for 6 months and subsequent semen analyses at 15-day intervals were performed. In the ejaculate, 0.001×10^6 and 0.002×10^6 were found in the entire first and second semen samples, respectively. The patient then underwent successful ICSI resulting in the delivery of two healthy girls. Unfortunately, it is not clear if a repeat semen analysis with a more thorough evaluation of the centrifuged specimen prior to receiving recombinant FSH treatment would have also demonstrated rare sperm, as reported by Ron-El *et al.*, given this study discovered occasional sperm cells after meticulous microscopic investigation amongst patients set for TESE (74). Studies on the effects of EMT on SRR among men with NOA who have failed prior sperm retrieval attempts is more extensive, though the results of the data are not clear, as these studies have not evaluated the relative effects of repeat sperm retrieval alone *vs.* sperm retrieval repeat with EMT (97). According to this review of preoperative patient optimization for mTESE, adjuvant therapy prior to mTESE for men with YCM and NOA may be used but insufficient data exists to determine if a positive effect on spermatogenesis occurs.

Conclusion

In this review, YCM and its subtypes, microTESE results, ICSI results and ART sequelae in offspring were summarized. In conclusion, YCM typically reflect deletion of discrete, predictable gene regions of the Y chromosome that severely impact male fertility. Deletions can be single- or multi-locus deletions and lead to distinct clinical and histological phenotypes. AZFa, AZFb and multi-locus deletions have the most dramatic adverse effects on spermatogenesis. As a result, sperm are rarely, if ever,

retrievable. AZFc deletions are the most benign, resulting in a combination of histologic testicular abnormalities resulting in severe oligospermia or azoospermia. Careful analysis for rare sperm in the ejaculate is critical for men with AZFc deletions. The sperm retrieval rate for AZFc ranges between 13% to 100%; for azoospermic AZFc-deleted men, a careful and detailed microTESE procedure is critical to obtain optimal sperm retrieval results.

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