

Clinical diagnostic testing for the cytogenetic and molecular causes of male infertility: the Mayo Clinic experience

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Abstract

Purpose Approximately 8% of couples attempting to conceive are infertile and male infertility accounts for approximately 50% of infertility among couples. Up to 25% of males with non-obstructive infertility have chromosomal abnormalities and/or microdeletions of the long arm of the Y-chromosome. These are detected by conventional chromosome and Y-microdeletion analysis. In this study, we reviewed the results of testing performed in the Mayo Clinic Cytogenetics and Molecular Genetics Laboratories and compared our findings with previously published reports.

Methods This study includes 2,242 chromosome studies from males ≥ 18 years of age referred for infertility between 1989 and 2000 and 2,749 Y-deletion molecular studies performed between 2002 and 2009.

Results 14.3% of infertile males tested by karyotyping had abnormalities identified. These include: (258) 47,XXY and

variants consistent with Klinefelter syndrome, (3) combined 47,XXY and balanced autosomal rearrangements, (9) 47, XYY, (9) Y-deletions, (7) 46,XX males, (32) balanced rearrangements, and (1) unbalanced rearrangement. 3.6% of males tested for Y-microdeletion analysis had abnormalities identified, 90% of which included a deletion of the AZFc region.

Conclusions This study highlights the need of males suffering from non-obstructive infertility to have laboratory genetic testing performed. An abnormal finding can have significant consequences to assisted reproductive techniques and fertility treatment, and provide a firm diagnosis to couples with longstanding infertility.

Keywords Male infertility · Y-microdeletion · Chromosome · Klinefelter syndrome · AZF · Karyotype · Azoospermia · Oligozoospermia

Capsule This report describes the results of a retrospective survey of all karyotyping and Y-microdeletion testing performed on infertile males at the Mayo Clinic.

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Introduction

Infertility is defined as a couple's failure to conceive after having sexual intercourse without contraception for 1–2 years [1]. It is estimated that 8% [2] to 24% [3] of couples suffer from infertility, and male factors are the cause in approximately half of these cases [1]. Male infertility can be attributed to several factors including medication and illegal drug side effects, exposures to toxic compounds, erectile dysfunction, cystic fibrosis, endocrine disorders, varicocele, systemic diseases, and anatomic malformations [3]. In addition, it has been known for several decades that cytogenetic and molecular abnormalities affect male fertility including autosomal abnormalities, rearrangements and microdeletions of the Y-chromosome, and sex chromosome aberrations [4, 5].

Many studies describing chromosome abnormalities in unselected infertile males, as well as selected males with azoospermia (no sperm production), oligozoospermia (reduced sperm production) have been published. Several of these studies are listed in Table 1 [6–13]. Although these studies have different inclusion criteria, a meta-analysis was performed to generate cumulative frequencies (Table 1). The cumulative frequency of autosomal abnormalities in these studies of infertile males is 3.5%, compared to 0.42% of people within the general population that are known to have balanced or unbalanced autosomal rearrangement [14]. 1.7% of infertile males are reported to have rearrangements of sex chromosomes by karyotyping, compared to the 0.10% among the general male population [14]. In addition, the most common chromosomal abnormality reported is 47,XXY (cumulative 4.9%), which is associated

with Klinefelter Syndrome. This is a large proportion considering the frequency of 47,XXY in the general male population is 0.11% [15]. It is also important to note that several studies excluded from the meta-analysis reported the frequency of a karyotype consistent with Klinefelter syndrome can be greater than 10% in oligozoospermic males [16–18].

Submicroscopic deletions (i.e. small deletions not detectable by karyotyping) of the Y-chromosome (Y-microdeletions) are also associated with male infertility. The azoospermia factor (AZF) region of the long arm of the Y-chromosome was identified in infertile males and was originally postulated to be the region associated with spermatogenesis [19]. It was later shown that the AZF region actually comprises 3 different regions and deletions of any individual or multiple AZF region(s) (AZFa, AZFb, and AZFc) can lead to reduced or abnormal sperm production or azoospermia [20]. Several published studies have analyzed the frequency of the various Y-microdeletions in selected and unselected infertile male populations [7, 12, 13, 21–24]. Similar to the cytogenetic data, a meta-analysis of these studies was performed without taking into account inclusion criteria, and the cumulative frequency of Y-microdeletions among infertile males was determined to be 3.5% (Table 2). The AZFc region is consistently reported as the most commonly disrupted region in infertile males [25], and candidate genes in that region includes the *DAZ* (deleted in azoospermia) genes [26]. The *DAZ* gene cluster comprises several functional copies in the AZFc region [27]. There are also other candidate genes on the Y-chromosome in the AZF regions that are associated with infertility, and several are found in multiple functional copies [27]. The male infertility phenotype and its severity is

Table 1 Comparison of previously published studies of the frequency of cytogenetic abnormalities in infertile males. The cumulative totals do not include the data of this study (bottom row)

Study	Number of participants	46,XY (%)	47,XXY + Variants (%)	Other sex chromosome abnormalities (%)	Autosomal abnormalities (%)
Vincent et al. [6]	2651	2447 (92.3%)	95 (3.6%)	36 (1.4%)	73 (2.8%)
Kumtepe et al. [7]	1935	1694 (87.5)	154 (8.0%)	37 (1.9%)	45 (2.3%)
Nakamura et al. [8]	1790	1565 (87.4%)	64 (3.6%)	35 (2.0%)	126 (7.0%)
Elghezal et al. [9]	1000	865 (86.5%)	70 (7%)	21 (2.1%)	44 (4.1%)
Yatsenko et al. [10]	668	613 (91.7%)	27 (4.0%)	12 (1.8%)	16 (2.4%)
Bertini et al. [11]	435	412 (94.7%)	13 (3.0%)	5 (1.1%)	5 (1.1%)
Ng et al. [12]	295	253 (85.8%)	9 (3.1%)	8 (2.7%)	8 (2.7%)
Mohammed et al. [13]	289	266 (92.0%)	16 (5.5%)	6 (2.1%)	1 (0.3%)
Cummulative totals from previously published studies	9063	7915 (87.3%)	448 (4.9%)	160 (1.8%)	318 (3.5%)
Current study	2242	1923 (85.7%)	261 ^a (11.6%)	26 ^b (1.2%)	36 ^{ab} (1.7%)

^a 3 cases had 47,XXY with an autosomal abnormality and were therefore counted in both columns

^b 1 case had a balanced translocation between an autosome (chromosome 19) and the X chromosome and was therefore counted in both columns

Table 2 Comparison of previously published studies of the frequency of Y-microdeletions in infertile males. The cumulative totals do not include the data of this study (bottom row)

Study	Number of participants	Y-microdeletions detected (AZFa, AZFb, AZFc, combined)	% of Y-microdeletion positive patients
Ferlin et al. [21]	3073	99	3.2%
Kumtepe et al. [7]	1935	105	5.4%
Maurer et al. [22]	1470	19	1.3%
Abid et al. [23]	300	6	2.0%
Ng et al. [12]	295	19	6.4%
Mohammed et al. [13]	289	7	2.4%
Peterlin et al. [24]	226	10	4.4%
Cummulative totals from previously published studies	7588	265	3.5%
Current study	2749	100	3.6%

thought to be a result of the deletion of a combination of genes and gene copies [28].

This report describes the experience of the Mayo Clinic in testing infertile males for chromosomal abnormalities and Y-microdeletions. Karyotype analysis was performed in the Cytogenetics Laboratory on 2,242 males from 1998 to 2009, and 320 were identified with a chromosome abnormality. From 2002 to 2009, PCR based Y-microdeletion analysis was performed in the Molecular Genetics Laboratory on 2,749 males and 100 with a deletion of an AZF region(s) were detected.

Methods

The work reported herein was approved by the Mayo Clinic IRB. Two independent retrospective surveys were performed to investigate the distribution of cytogenetic and molecular causes of male infertility.

Meta-analysis

PubMed was queried using the following terms in several combinations: cytogenetic, male infertility, y-microdeletion, karyotype, AZF, and Klinefelter. Available manuscripts published in the year 2000 or after were included in the analysis.

Cytogenetic analysis

The first data set includes 2,242 male patients over the age of 18 referred for infertility, azoospermia, oligospermia, or phenotypic features related to Klinefelter syndrome. Patients younger than 18 years old referred to rule out Klinefelter syndrome were excluded. 62 (2.8%) of the 2,242 were Mayo Clinic patients. Chromosome analysis

was performed in the Cytogenetics Laboratory on peripheral blood samples using standard culture and harvest methods and GTL (Giemsa-Trypsin-Leishman) banding techniques [29].

Molecular analysis

The second data set includes 2,749 male patients tested to rule out male infertility by Y-chromosome microdeletions in the Molecular Genetics Laboratory. 75 (2.7%) of the 2,749 were Mayo Clinic patients. Y-microdeletion studies were performed using a multiplex PCR amplification based method described by Simoni et al. [30]. In brief, DNA was extracted using standard techniques followed by two separate multiplexed PCR reactions. The first reaction targeted sY254 (DAZ gene; AZFc), sY86 (AZFa), and sY127 (AZFb), while the second reaction targeted sY84 (AZFa), sY134 (AZFb), and sY255 (DAZ gene; AZFc). Primers targeting *HBB* (β -globin; Forward: ACTGGG CATGTGGAGACAGAGA, Reverse: TGTTTCCCATTC TAAACTGTAC) were also used as an amplification control for each reaction. Following PCR, amplicons were separated and detected by gel electrophoresis. Loss of both markers for any one or more of the AZF regions (AZFa, AZFb, AZFc) was consistent with a Y-microdeletion. If only one of the two markers was deleted within a region, an additional set of markers (sY82 and sY98 for AZFa, sY135 and sY152 for AZFb, or sY156 and OX7 for AZFc) were used to verify the deletion within that AZF region [28]. The patient was considered to have Y-microdeletion if a deletion was detected in at least one marker within the verification marker set that flanked the deletion detected by the original marker set. If no deletions were noted using the verification set of markers, the results were interpreted as equivocal for that patient.

Table 3 Distribution of cytogenetic abnormalities

Cytogenetic finding	# of Affected individuals	% of Total
46,XY (Normal)	1923	85.7%
47,XXY and Variants	258	11.5%
47,XXY + Balanced rearrangement	3	0.1%
47,XXY	9	0.4%
Y-Deletions	9	0.4%
46,XX Male	7	0.3%
Balanced rearrangements	32	1.4%
Unbalanced rearrangements	1	<0.1%
Total specimens 2242		

Results

Cytogenetic analysis

As summarized in Table 3, 1,923 (85.7%) of the 2,242 patients tested to rule out cytogenetic causes of male infertility had a 46,XY karyotype, and 319 had abnormal karyotypes including: (258) 47,XXY and variants consistent

with Klinefelter syndrome, (3) combined 47,XXY and balanced autosomal rearrangements, (9) 47,XXY, (9) Y-deletions, (7) 46,XX males, (32) balanced rearrangements, and (1) unbalanced rearrangement. Sex chromosome abnormalities accounted for 287 or 90% of all abnormal karyotypes detected (Table 4). The most frequent sex chromosome abnormality observed was 47,XXY or variant karyotype (mosaic 47,XXY/46,XY 48,XXYY, 48,XXXY,

Table 4 Composition of sex chromosomal abnormalities

Karyotype	# of Patients with abnormality	Classification for Table 3
47,XXY	228	47,XXY + Variant
Mosaic 47,XXY	24	47,XXY + Variant
48,XXXY	2	47,XXY + Variant
48,XXYY	3	47,XXY + Variant
47,XY,+i(X)(q10)	1	47,XXY + Variant
47,XXY,inv(14)(q11.2q32.1)[23]/47,XXY[7] ^a	1	47,XXY + Balanced Rearrangement
47,XXY,t(2;11)(q21.3;q21)[27]/46,XY,t(2;11)(q21.3;q21)[3] ^a	1	47,XXY + Balanced Rearrangement
46,XXY,der(13;14)(q10;q10) ^a	1	47,XXY + Balanced Rearrangement
47,XXY	9	47,XXY
45,X[4]/46,X,idel(Y)(q11.2) [46]	1	Y- Deletions
45,X[6]/46,X,idel(Y)(q11.1) [24]	1	Y- Deletions
46,X,del(Y)(q11.2)	1	Y- Deletions
46,X,i(Y)(p10)	1	Y- Deletions
46,X,idel(Y)(q11.23)[1]/46,XY[29]	1	Y- Deletions
46,X,psu idel(Y)(q11.2)	1	Y- Deletions
46,X,-Y,+mar.ish r(Y)(p13q11.2)	1	Y- Deletions
45,X,-Y[15]/46,X,idel(Y)(q11.2)[15]	1	Y- Deletions
46,X,psu idel(Y)(q11.2)[25]/45,X[5]	1	Y- Deletions
46,X,add(X)(p22.3).ish der(X)t(X;Y)(p22.3;p11.3)(SRY+,DXZ1+)	1	XX Male
46,X,add(X)(p22.3).ish der(X)t(X;Y)(p22.3;p11.2)(DXZ1+,SRY+)	1	XX Male
46,X,add(X)(p22.3).ish der(X)t(X;Y)(p22.3;p11.2)(SRY+)	1	XX Male
46,X,der(X)t(X;Y)(p22.3;p11.2).ish der(X)t(X;Y)(SRY+,DXZ1+)	1	XX Male
46,XX	3	XX Male
46,Y,t(X;19)(p21.2;q13.3) ^a	1	Balanced rearrangement

^a Reported in Tables 4 and 5

Table 5 Composition of autosomal rearrangements

Karyotype	Balanced (B)/Unbalanced (U)	Reason for referral
45,XY,dic(18;20)(p11.2;p13)[14]/46,XY[19]	B	infertility
46,XY,der(14)ins(14)(q22q24.3q32.1)inv(14)(p13q22)[7]/46,XY[23]	B	male infertility, unspecified
46,XY,ins(3;4)(q22.2;q21.3q31.3)inv(4)(q23q31.1)	B	infertility
46,XY,inv(1)(p13.1q21.1)	B	oligospermia
46,XY,inv(1)(p13q21)	B	infertility
46,XY,inv(1)(q23.1q42.1)	B	oligospermia
46,XY,inv(2)(p23q23)	B	male infertility
46,XY,inv(2)(p25.1q23.3)	B	infertility
46,XY,inv(3)(p11.2p23)	B	r/o Klinefelter
46,XY,inv(5)(p13.3q13.3)	B	infertility
46,XY,inv(9)(q32q34.13)	B	male infertility
46,XY,t(1;11)(q23;p15.5)	B	Infertility, low sperm count
46,XY,t(1;21)(p10;q10)	B	infertility
46,XY,t(1;7)(p22.3;p15.3)	B	Oligospermia
46,XY,t(12;18)(q13.3;q11.2)	B	r/o Klinefelter
46,XY,t(13;17)(q12;p11.2)	B	infertility
46,XY,t(13;22)(q12.1;p11.2)	B	male infertility
46,XY,t(14;21)(q22;q22.3)	B	r/o Klinefelter
46,XY,t(15;16)(q22.1;q24)	B	oligospermia
46,XY,t(2;5)(p15;p13.3)	B	severe male infertility factor
46,XY,t(3;12)(p24.2;q15)	B	infertility
46,XY,t(5;10)(q35;q11.2)	B	infertility
46,XY,t(5;20)(p13.3;p11.23)	B	male infertility
46,XY,t(8;17)(q24.3;q21.1)	B	oligospermia
46,XY,t(8;19)(p23.3;p12)	B	normal volume azospermia
46,XY,t(9;10)(q21.2;p11.2)	B	r/o Klinefelter
45,XY,der(13;14)(q10;q10)	B	familial infertility
45,XY,der(13;14)(q10;q10)	B	infertility, r/o Klinefelter
45,XY,der(13;14)(q10;q10)	B	oligospermia
45,XY,der(14;21)(q10;q10)	B	Infertility and asthenospermia
45,XY,der(15;15)(q10;q10)	B	infertility, oligospermia
46,Y,t(X;19)(p21.2;q13.3) ^a	B	infertility, unspecified origin
47,XXY,inv(14)(q11.2q32.1)[23]/47,XXY[7] ^a	B	r/o Klinefelter
47,XXY,t(2;11)(q21.3;q21)[27]/46,XY,t(2;11)(q21.3;q21)[3] ^a	B	primary infertility
46,XXY,der(13;14)(q10;q10) ^a	B	r/o Klinefelter
46,XY,del(6)(q13q23)[3]/46,XY[30]	U	r/o Klinefelter

^a Reported in Tables 4 and 5

and 47,XY,+i(X)(q10)), and is consistent with Klinefelter Syndrome. Of the autosomal abnormalities (Table 5), balanced rearrangements were identified in 35 males, including 7 with balanced Robertsonian translocations, 1 translocation between chromosome 19 and the X chromosome, and 3 in a 47,XXY background. An unbalanced rearrangement was identified in only 1 patient; a low level mosaic 6q deletion was detected in 3 of 30 metaphase cells (Table 5).

Molecular Y-microdeletion analysis

Similar to the karyotyping data, the overwhelming majority of the males tested were normal. Of the 2,749 males tested for Y-microdeletion, 2,637 (96%) had no deletion detected. Results from twelve patients (0.4%) were interpreted as equivocal since only 1 of the 4 tested markers were deleted within a single AZF region. Results were equivocal since the apparent deletion of

only a single marker could be a PCR artifact due to a polymorphism under a primer or it could represent a small deletion. The frequencies of the various deletions detected are summarized in Table 6. In brief, Y-microdeletions were identified in 100 patients (4%). Of these, 90 had isolated AZFc or compound deletions involving AZFc and other AZF regions (64 with AZFc deletion only; 17 with AZFb and AZFc deletions; and 9 with AZFa, AZFb, and AZFc deletions). Interestingly, only 10 patients (0.4%) had isolated deletions of the AZFa and AZFb regions, with an equal distribution of each.

Discussion

This report aims to summarize the frequencies of cytogenetic and molecular causes of male infertility identified in two large reference laboratories at the Mayo Clinic, the Cytogenetics Laboratory and the Molecular Genetics Laboratory. The patient sample volumes in the two laboratories are among the largest in the United States and therefore supply valuable information on the frequencies of abnormalities among an unselected population of patients undergoing male infertility testing.

In the general male population, Klinefelter syndrome is the most frequent sex chromosomal abnormality, with a reported frequency of 0.11% [15]. Prior studies of chromosomal abnormalities in infertile males reported a karyotype consistent with Klinefelter syndrome at a frequency of 4.9%, making it the most abundant cytogenetic abnormality identified in infertile males as well (Table 1). As summarized in Table 3, 2,242 males over the age of 18 had chromosomes analyzed by standard karyotyping [29] to rule out cytogenetic causes of infertility, oligozoospermia or azoospermia, or for phenotypic features consistent with Klinefelter syndrome. 319 (14%) had abnormal karyotypes, and 90% of those were 47,XXY or a variant karyotype (mosaic 47,XXY/46,XY, 48,XXYY, 48,XXXYY, 47,XXY combined with an autosomal rearrangement, and 47,XY,+i(X)(q10)) consistent with Klinefelter syndrome, which accounts for 11.4% of the study population (Table 3). This frequency (11.4%) is over 100 times the frequency in general male population (0.11%) and 2.3 times the cumulative frequency in infertile males, although this cumulative frequency is based on a meta-analysis of several studies with different inclusion criteria (Table 1).

The divergence between the frequency of 47,XXY karyotype consistent with Klinefelter syndrome in this study and that of other cytogenetic studies in infertile males described in Table 1 is likely attributable to a combination of several factors. The first involves the criteria for selection of publications for the meta-analysis. The studies listed in Table 1 include unselected infertile

males, azoospermic males, and oligozoospermic males. However, the frequency of 47,XXY karyotype consistent with Klinefelter syndrome in solely oligozoospermic males has been reported to be between 10 and 15% [16–18]. If ordering physicians are aware of this high probability of 47,XXY in males with low sperm counts but not complete lack of sperm they may be more likely to order karyotyping on these patients. Another source of variation involves the inclusion criteria set forth in this retrospective analysis. All patients older than 18 years old, tested by karyotyping in our laboratory, with the reason for referral including the word Klinefelter, were included in this study. By using the cutoff of 18 years old we attempted to eliminate patients that were not being investigated for male infertility, but it may not have been completely effective. Without the clinical information to correlate these findings, this could not be further investigated. The final source of the higher frequency of karyotypes consistent with Klinefelter syndrome in this population is the different patient populations of a large reference lab compared to the setting that almost all other studies discussed herein were performed, a fertility clinic. A reference lab is more likely to test samples from patients before they are referred to a fertility clinic, some of which are ordered by general practitioners. However, the fertility clinic generally orders chromosome analyses on patients who have been referred by a non-specialist, so some of the infertile male patients with more obvious signs of Klinefelter syndrome (gynecomastia, atrophied testes, etc.) may have already been selected out of that population.

Table 6 Composition of Y-microdeletions

Category	# of Patients (%)	Interpretation
No deletions	2637 (95.9%)	No Y- Microdeletions
1 Probe deleted ^a	12 (0.4%)	Equivocal ^a
1 AZF region deleted	74 (2.7%)	Y-Microdeletion Detected
AZFa	5	
AZFb	5	
AZFc	64	
2 AZF regions deleted	17 (0.6%)	
AZFb and AZFc	17	
3 AZF regions deleted	9 (0.3%)	
AZFa, AZFb, and AZFc	9	
	Total 2749	

^a Two separate PCR amplifications (Probes) are set up for each AZF region (AZFa, AZFb, AZFc). If there is a deletion of only 1 of the 2 markers within a region, an additional set of markers are tested to verify the deletion. These results represent samples with a single deletion using the first set of markers that did not have a deletion with the verification set of markers; and could therefore represent either a small deletion or PCR artifact due to a polymorphism under a primer

Other than 47,XXY and variants consistent with Klinefelter syndrome, 7 patients were found to have a 46,XX karyotype, and 9 patients had deletions in the Y-chromosome (Table 3). These non-Klinefelter syndrome sex chromosome abnormalities are expected to have a significant impact on male fertility since many male fertility factors are located on the Y-chromosome. Interestingly, balanced chromosomal rearrangements were identified in 35 patients (1.6%). Depending on the type of balanced rearrangement, the effect on male fertility could have different etiologies. For example, Robertsonian translocations, which were found in 6 males (Table 5), have been shown to interfere with the XY bivalent forming a trivalent that also predisposes the cell to abnormal meiotic recombination or failure of sperm development [31]. An additional patient was found to have an unbalanced abnormality (Table 5), a 6q deletion observed in 3 of 30 metaphase cells. This could have an effect on fertility if the abnormal fraction is enriched in germ cells, but without clinical information, this is difficult to investigate further.

In contrast to the karyotyping, Y-microdeletion analysis has one possible reason for referral, male infertility. Since 2002, Y-microdeletion analysis has been performed in our laboratory on 2,749 patients (Table 6). The Y-microdeletion assay uses a PCR amplification strategy to identify deletions in the AZF (azoospermia factor) regions, AZFa, AZFb, and AZFc. Deletions were detected in 100 patients (4%). Of the 100 patients with a deletion of the Y-chromosome, 90 (90%) involved deletions of the AZFc region. 64 of the 90 patients (71.1%) had an isolated AZFc deletion, 17 patients (18.9%) had a combined AZFb and AZFc deletion, and 9 patients (10%) had a combined deletion of AZFa, AZFb, and AZFc. This finding supports the notion that DAZ (deleted in azoospermia) has a prominent role in male fertility and is consistent with prior reports [7, 12, 13, 21–24] showing that AZFc as the most frequently deleted AZF region in infertile males (Table 2). However, there is large variation in the literature that reflects the patient population tested. Some reports include only azoospermic males, some include azoospermic and severely oligozoospermic males, and others, like this study, include an unselected population of infertile males. An additional 5 patients had isolated deletions of the AZFa region and 5 patients had isolated deletions of the AZFb region. Deletions in these areas have also been associated with male infertility, although not as frequently, nor consistently, as the AZFc deletions [21, 27].

In conclusion, this work describes one of the largest studies of male infertility and includes the results of cytogenetic analysis of 2,242 males and molecular Y-microdeletion analysis of 2,749 males. When comparing this study to prior reports from smaller studies, the

frequencies of abnormalities are similar (Tables 1 and 2). There are a few exceptions, such as the higher proportion of Klinefelter syndrome patients in the group tested by karyotyping, which may be due to the inclusion of studies in the meta-analysis (higher frequency of azoospermic males in this study), the inclusion criteria of the patient retrospective analysis (reason for referral including rule out Klinefelter syndrome), and the patient population tested, (i.e., a large reference laboratory versus a fertility clinic). This retrospective study demonstrates that a large number of infertile males tested by cytogenetic karyotyping (14.3%) and molecular Y-microdeletion analysis (3.6%) have abnormalities identified. This information can direct fertility treatment options and ultimately aide the couple to conceive a child.

References

- Dohle GR, Colpi GM, Hargreave TB, Papp GK, Jungwirth A, Weidner W. EAU guidelines on male infertility. *Eur Urol*. 2005;48:703–11.
- Mosher WD, Bachrach CA. Understanding U.S. fertility: continuity and change in the National Survey of Family Growth, 1988–1995. *Fam Plann Perspect*. 1996;28:4–12.
- Health NCCfWsaCs. Fertility: assessment and treatment for people with fertility problems. London: Royal College of Obstetricians and Gynaecologists Press; 2004.
- Balkan M, Tekes S, Gedik A. Cytogenetic and Y chromosome microdeletion screening studies in infertile males with Oligozoospermia and Azoospermia in Southeast Turkey. *J Assist Reprod Genet*. 2008;25:559–65.
- Quilter CR, Svennevik EC, Serhal P, Ralph D, Bahadur G, Stanhope R, et al. Cytogenetic and Y chromosome microdeletion screening of a random group of infertile males. *Fertil Steril*. 2003;79:301–7.
- Vincent MC, Daudin M, De MP, Massat G, Mieuisset R, Pontonnier F, et al. Cytogenetic investigations of infertile men with low sperm counts: a 25-year experience. *J Androl*. 2002;23:18–22. discussion 44–5.
- Kumtepe Y, Beyazyurek C, Cinar C, Ozbey I, Ozkan S, Cetinkaya K, et al. A genetic survey of 1935 Turkish men with severe male factor infertility. *Reprod Biomed Online*. 2009;18:465–74.
- Nakamura Y, Kitamura M, Nishimura K, Koga M, Kondoh N, Takeyama M, et al. Chromosomal variants among 1790 infertile men. *Int J Urol*. 2001;8:49–52.
- Elghezal H, Hidar S, Braham R, Denguezli W, Ajina M, Saad A. Chromosome abnormalities in one thousand infertile males with nonobstructive sperm disorders. *Fertil Steril*. 2006;86:1792–5.
- Yatsenko AN, Yatsenko SA, Weedin JW, Lawrence AE, Patel A, Peacock S, et al. Comprehensive 5-year study of cytogenetic aberrations in 668 infertile men. *J Urol*. 2010;183:1636–42.
- Bertini V, Simi P, Valetto A. Cytogenetic study of 435 subfertile men: incidence and clinical features. *J Reprod Med*. 2006;51:15–20.
- Ng PP, Tang MH, Lau ET, Ng LK, Ng EH, Tam PC, et al. Chromosomal anomalies and Y-microdeletions among Chinese subfertile men in Hong Kong. *Hong Kong Med J*. 2009;15:31–8.
- Mohammed F, Al-Yatama F, Al-Bader M, Tayel SM, Gouda S, Naguib KK. Primary male infertility in Kuwait: a cytogenetic and molecular study of 289 infertile Kuwaiti patients. *Andrologia*. 2007;39:87–92.

14. Forabosco A, Percesepe A, Santucci S. Incidence of non-age-dependent chromosomal abnormalities: a population-based study on 88965 amniocenteses. *Eur J Hum Genet.* 2009;17:897–903.
15. Hamerton JL, Ray M, Abbott J, Williamson C, Ducasse GC. Chromosome studies in a neonatal population. *Can Med Assoc J.* 1972;106:776–9.
16. Van Assche E, Bonduelle M, Tournaye H, Joris H, Verheyen G, Devroey P, et al. Cytogenetics of infertile men. *Hum Reprod (Oxford, England).* 1996;11 Suppl 4:1–24. discussion 5–6.
17. McLachlan RI, O'Bryan MK. Clinical Review#: state of the art for genetic testing of infertile men. *J Clin Endocrinol Metab.* 2010;95:1013–24.
18. Sharlip ID, Jarow JP, Belker AM, Lipshultz LI, Sigman M, Thomas AJ, et al. Best practice policies for male infertility. *Fertil Steril.* 2002;77:873–82.
19. Tiepolo L, Zuffardi O. Localization of factors controlling spermatogenesis in the nonfluorescent portion of the human Y chromosome long arm. *Hum Genet.* 1976;34:119–24.
20. Vogt PH, Edelmann A, Kirsch S, Henegariu O, Hirschmann P, Kiesewetter F, et al. Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. *Hum Mol Genet.* 1996;5:933–43.
21. Ferlin A, Arredi B, Speltra E, Cazzadore C, Selice R, Garolla A, et al. Molecular and clinical characterization of Y chromosome microdeletions in infertile men: a 10-year experience in Italy. *J Clin Endocrinol Metab.* 2007;92:762–70.
22. Maurer B, Gromoll J, Simoni M, Nieschlag E. Prevalence of Y chromosome microdeletions in infertile men who consulted a tertiary care medical centre: the Munster experience. *Andrologia.* 2001;33:27–33.
23. Abid S, Maitra A, Meherji P, Patel Z, Kadam S, Shah J, et al. Clinical and laboratory evaluation of idiopathic male infertility in a secondary referral center in India. *J Clin Lab Anal.* 2008;22:29–38.
24. Peterlin B, Kunej T, Sinkovec J, Gligorievska N, Zorn B. Screening for Y chromosome microdeletions in 226 Slovenian subfertile men. *Hum Reprod (Oxford, England).* 2002;17:17–24.
25. Foresta C, Moro E, Ferlin A. Y chromosome microdeletions and alterations of spermatogenesis. *Endocr Rev.* 2001;22:226–39.
26. Reijo R, Lee TY, Salo P, Alagappan R, Brown LG, Rosenberg M, et al. Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. *Nat Genet.* 1995;10:383–93.
27. De Kretser DM, Baker HW. Infertility in men: recent advances and continuing controversies. *J Clin Endocrinol Metab.* 1999;84:3443–50.
28. Ravel C, Chantot-Bastaraud S, El Houate B, Rouba H, Legendre M, Lorenc D, et al. Y-chromosome AZFc structural architecture and relationship to male fertility. *Fertil Steril.* 2009;92:1924–33.
29. Van Dyke D, Wiktor, A. Clinical cytogenetics. In: McClatchey K, editor. *Clinical laboratory medicine: Williams and Wilkens*; 2002. p. 589–635.
30. Simoni M, Bakker E, Eurlings MC, Matthijs G, Moro E, Muller CR, et al. Laboratory guidelines for molecular diagnosis of Y-chromosomal microdeletions. *Int J Androl.* 1999;22:292–9.
31. Johannisson R, Schwinger E, Wolff HH, vom Ende V, Lohrs U. The effect of 13;14 Robertsonian translocations on germ-cell differentiation in infertile males. *Cytogenet Cell Genet.* 1993;63:151–5.