available at www.sciencedirect.com
journal homepage: www.europeanurology.com

European Association of Urology

Platinum Priority – Review – Andrology – Editor's Choice

Editorial by Christian F.S. Jensen, Mikkel Fode, Peter B. Østergren and Jens Sønksen on pp. 637–638 of this issue

The Prevalence of Y-chromosome Microdeletions in Oligozoospermic Men: A Systematic Review and Meta-analysis of European and North American Studies

Taylor P. Kohn [a,*], *Jaden R. Kohn* [b], *Ryan C. Owen* [c], *R. Matthew Coward* [d,e]

[a] *The James Buchanan Brady Urological Institute and Department of Urology, Johns Hopkins University School of Medicine, Baltimore, MD, USA;* [b] *Department of Gynecology and Obstetrics, Johns Hopkins University School of Medicine, Baltimore, MD, USA;* [c] *Department of Urology, Texas Tech University Health Sciences Center School of Medicine, Lubbock, TX, USA;* [d] *Department of Urology, University of North Caroline School of Medicine, Chapel Hill, NC, USA;* [e] *UNC Fertility LLC, Raleigh, NC, USA*

Article info

Article history:
Accepted July 16, 2019

Associate Editor:
Dr. Maarten Albersen

Statistical Editor:
Melissa Assel

Keywords:
Semen analysis
Y chromosome
Gene deletion
Meta-analysis
Genetics
Infertility

Abstract

Context: European and North American guidelines recommend Y-chromosome microdeletion (YCM) screening in azoospermic and oligozoospermic men with sperm concentrations of <5 million sperm/ml; however, numerous studies have suggested that YCMs are rare when sperm concentrations are >1 million sperm/ml.
Objective: We systematically reviewed and meta-analyzed European and North American studies to determine the prevalence of a complete YCM in oligozoospermic men with sperm concentrations of >0–1, >1–5, and >5–20 million sperm/ml, and to determine whether 1 or 5 million sperm/ml is the most appropriate sperm concentration threshold for YCM screening.
Evidence acquisition: A systematic review of MEDLINE, EMBASE, Cochrane Library, and ClinicalTrials.gov was performed for studies assessing the prevalence of a complete YCM in oligozoospermic men in European and North American studies.
Evidence synthesis: Thirty-seven studies were identified during a systematic review (n = 12 492 oligozoospermic men). All complete YCMs in oligozoospermic men were *AZFc* microdeletions. Eighteen studies contained data conducive to meta-analysis (n = 10 866 men). Comparing the pooled estimated prevalence by sperm concentration, complete YCMs were significantly more common in men with sperm concentrations of >0–1 million sperm/ml (5.0% [95% confidence interval {CI}: 3.6–6.8%]) versus >1–5 million sperm/ml (0.8% [95% CI: 0.5–1.3%], $p < 0.001$). YCMs were similar in men with sperm concentrations of >1–5 and >5–20 million sperm/ml (0.8% [95% CI: 0.5–1.3%] vs 0.5% [95% CI: 0.2–0.9%], p = 0.14).
Conclusions: In Europe and North America, the majority of YCMs occur in men with sperm concentrations of ≤1 million sperm/ml, with <1% identified in men with >1 million sperm/ml. Male infertility guidelines for North America and Europe should reconsider the sperm concentration screening thresholds to recommend testing for YCMs only for men with sperm concentrations of <1 million sperm/ml.
Patient summary: Complete Y-chromosome microdeletions (YCMs) are rare in men with >1 million sperm/ml. Routine screening for YCMs should occur only if sperm concentration is ≤1 million sperm/ml.

* Corresponding author. James Buchanan Brady Urological Institute, The Johns Hopkins Medical Institutions, 600 N. Wolfe Street/Marburg 134, Baltimore, MD 21287, USA.
E-mail address: tpkohn@jhmi.edu (T.P. Kohn).

https://doi.org/10.1016/j.eururo.2019.07.033
0302-2838/

1. Introduction

In 1976, Tiepolo and Zuffardi [1] identified regions of the Y chromosome responsible for controlling spermatogenesis. Microdeletions in these subregions (deemed Azoospermia Factor genes—*AZFa*, *AZFb*, and *AZFc*—also known as Y-chromosome microdeletions [YCMs]) result in an azoospermic or oligozoospermic phenotype [2]. Since this time, numerous studies around the world have been performed to determine the frequency of YCMs in azoospermic and oligozoospermic men [3–39]. Early YCM prevalence studies categorized men as either "severely oligozoospermic" (>0–5 million sperm/ml) or "oligozoospermic" (>5–20 million sperm/ml) [28]. The current guidelines from the European Association of Urology (EAU) and the American Society for Reproductive Medicine report the prevalence of YCMs in severely oligozoospermic men as 3–7% and ~5%, respectively. Both guidelines recommend screening men for YCMs if sperm concentrations are <5 million sperm/ml [40,41]. However, no study has ever definitively determined that 5 million sperm/ml was the ideal threshold, as most studies have used varying definitions of oligozoospermia and severe oligozoospermia when reporting YCM prevalence.

Over the past decade, there has been speculation about whether the sperm concentration screening threshold of 5 million sperm/ml for YCM is too broad. Lo Giacco et al [20], the European Academy of Andrology (EAA), and the European Molecular Genetics Quality Network (EMQN) have theorized that a threshold of 2 million sperm/ml may be more appropriate [42]. More recently, Johnson et al [14] found in their UK population that if only men with sperm concentrations of <0.5 million sperm/ml were to be screened for YCMs, this would identify all complete YCMs and save the health care system £55 000 over the course of their study.

To determine the prevalence of YCMs in oligozoospermic men and to compare sperm concentrations thresholds of 1 and 5 million sperm/ml, we performed a systematic review and meta-analysis.

2. Evidence acquisition

2.1. Study design

The protocol for this systematic review and meta-analysis was based on the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) [43] and was prospectively registered in the PROSPERO database (CRD42017068394, available online at www.crd.york.ac.uk/prospero). The meta-analysis was performed in accordance with the Meta-analysis of Observational Studies in Epidemiology guidelines.

2.2. Literature search

A comprehensive review of the literature was performed by systematically searching MEDLINE, EMBASE, Cochrane Library, and ClinicalTrials.gov from database inception through February 2019, to identify studies reporting the prevalence of complete YCMs in men who had performed a semen analysis and subsequently were found to be oligozoospermic. Search terms were related to YCMs without language restriction. Full details of the search strategy are provided in the Supplementary material. Additional articles were identified by assessing the reference lists from select articles, searching relevant conference abstracts, and speaking to experts in the field. When studies did not provide complete information for analysis, study investigators were contacted.

2.3. Study selection

A study was included in the systematic review if it (1) reported the prevalence of YCMs in men who were found to be oligozoospermic on semen analysis, (2) assessed at least two sequence tag sites (STSs) per *AZF* region to prevent polymerase chain reaction (PCR) artifact that could result in false positives, (3) employed appropriate internal positive and negative controls to ensure accurate reporting, (4) reported and defined a complete YCM or presented all YCMs for each STS, (5) utilized a multiplex PCR for the analysis of YCMs as recommended by EAA/EMQN guidelines to prevent false positives, and (6) took place in a clinic located in Europe or North America. This study was limited to Europe and North America to decrease study heterogeneity—as geographic variation has been demonstrated to be associated with differing prevalence of YCMs [34]. To be included in the meta-analysis, the above criteria for inclusion in the systematic review must be satisfied; in addition, the studies were required to report the prevalence of YCMs in sperm concentrations of >0–≤1 million sperm/ml and/or >1–5 million sperm/ml, as well as to test, at a minimum, the STSs sY254 and sY255 of the *AZFc* region—the two STSs recommended by the EAA/EMQN and demonstrated to identify >95% of clinically relevant deletions [42]. For the meta-analysis, a complete YCM was defined by the absence of both sY254 and sY255. YCMs were confirmed by authors by viewing either the deidentified individual patient deletion data or confirming the protocol by which laboratories determine a partial versus a complete YCM.

A study was excluded if (1) it did not report semen concentrations; (2) it did not report the prevalence of YCMs; (3) it reported only the prevalence of partial YCM; (4) it was a case-control study that did not assess the association of YCM with infertility, but assessed the association between YCM and other urological conditions, such as cryptorchidism, testicular torsion, recurrent pregnancy loss, hypospadias, testicular cancer, etc.; or (5) a clear definition of complete YCM could not be elucidated or the study did not present YCM for individual STSs. Only complete YCMs were included in this manuscript, as the association between impaired sperm concentrations and partial YCMs, such as gr/gr, b2/b3, and b1/b3, is weakly correlated. No general agreement has been reached regarding routine testing for partial YCM deletions [42].

Only published articles were included in analysis. If authors did not provide complete information in published

manuscripts, authors were contacted for further information. When several studies involved the same study population, the most comprehensive one was used. Two trained reviewers (T.P.K. and J.R.K.) screened titles and abstracts, and then reviewed the full text of selected studies to assess eligibility. Discrepancies were resolved by discussion and adjudication of a third reviewer (R.M.C.). A flow diagram for study selection is given in Fig. 1.

2.4. Data collection

The following information was extracted independently by two trained investigators (T.P.K. and J.R.K.) using a standardized form. For each YCM group (>0–≤1 million sperm/ml; >1–5 million sperm/ml; and >5–20 million sperm/ml), we extracted individual study inclusion criteria, geographic location of the study, STSs used, number of oligozoospermic men screened for complete YCM, number of oligozoospermic men with complete YCM, date of publication, and protocol used for defining complete YCM. Discrepancies were resolved by discussion and adjudication of a third reviewer (R.M.C.).

2.5. Quality assessment

Risk of bias was assessed by two trained investigators (T.P.K. and J.R.K.) using the Newcastle-Ottawa Scale; the nine domains are described in the Supplementary material. Discrepancies were resolved by discussion and adjudication by a third reviewer (R.M.C.).

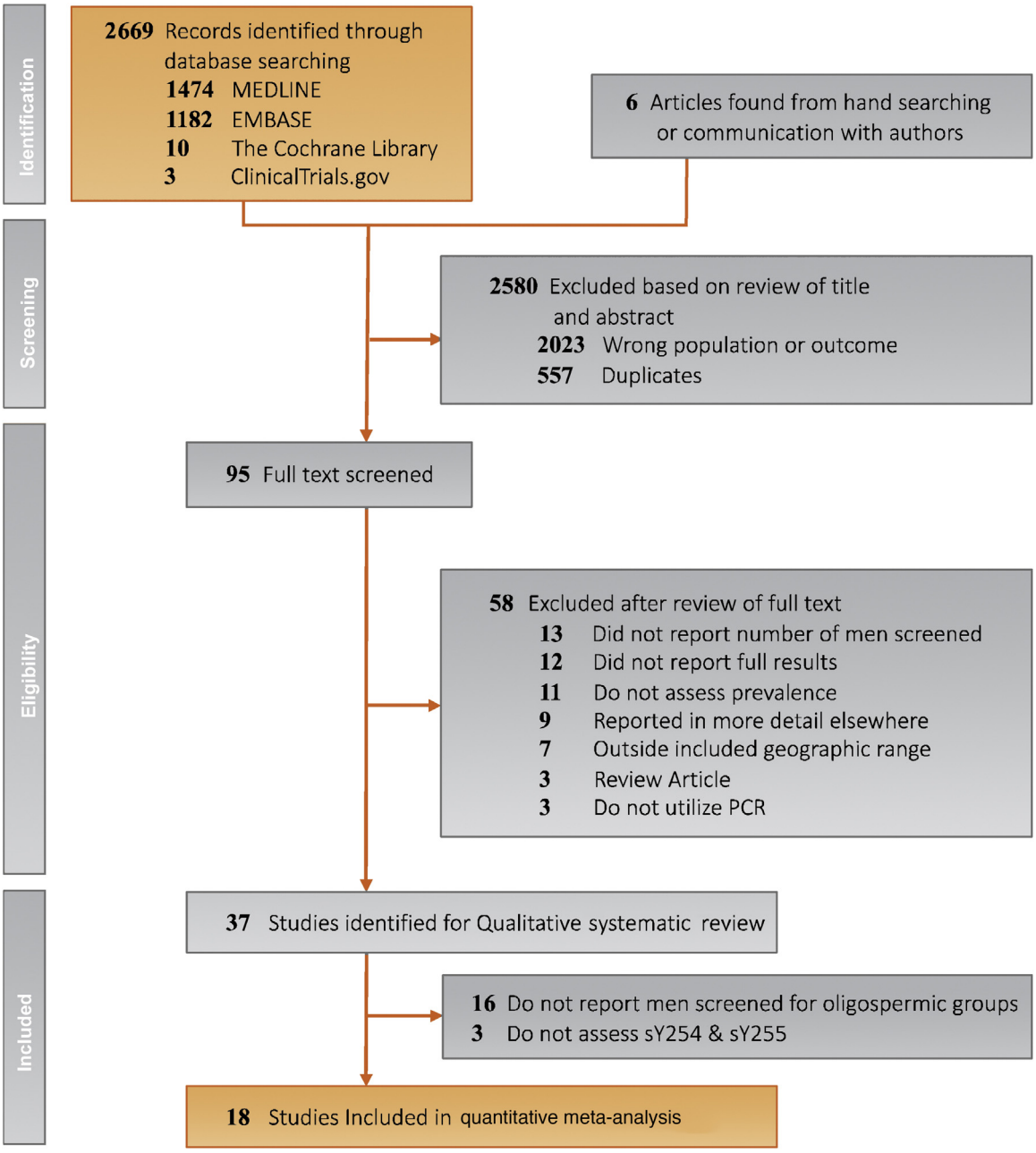

Fig. 1 – Flow diagram for study selection. PCR = polymerase chain reaction.

2.6. Data synthesis

A meta-analysis was performed using the random-effect model with between-study heterogeneity measured by Cochrane's Q tests and the I^2 statistic [44], utilizing the package *metaprop* in R 3.4.1 (R Foundation for Statistical Computing, Vienna, Austria). Statistical tests were two sided and used a significance threshold of $p < 0.05$.

Our primary outcome was the prevalence of YCM (rate of complete YCM per oligozoospermic male screened for complete YCM) among men with >0–≤1, >1–5, and >5–20 million sperm/ml.

A subgroup analysis was performed using a stratified meta-analysis to compare the prevalence of a complete YCM between studies that utilized the EAA/EMQN criteria for defining a complete YCM and studies that used an internal laboratory protocol to define a complete YCM, in order to determine whether there was a significant difference between the STSs suggested by the EAA/EMQN and the definitions of other reputable laboratories. A subgroup analysis was also performed to determine whether North American studies differed significantly from European studies.

The influence of individual studies was examined by serial exclusion in a sensitivity analysis. Publication bias was investigated using a funnel plot and Egger's test [45].

2.7. Cost-benefit analysis

A cost-savings analysis was performed to estimate the savings if the screening threshold was changed from 5 to 1 million sperm/ml. The cost per test was obtained from a 2018 publication by Johnson et al [14]—£190, €220, or $247. Currency conversions were based off exchange rates at the time of publication.

3. Evidence synthesis

3.1. Study characteristics

3.1.1. Qualitative systematic review

A total of 37 studies were identified during the systematic review with 12 492 oligozoospermic men (Table 1). All deletions in oligozoospermic men were *AZFc* microdeletions. A total of 261 YCMs were identified in oligozoospermic men. Of the YCMs identified, 92.8% (242/261) were found in oligozoospermic men with sperm concentrations of >0–≤1 million sperm/ml, 5.7% (15/261) were found in men with >1–5 million sperm/ml, and only 1.5% (4/261) were found in men with >5–20 million sperm/ml.

3.1.2. Quantitative meta-analysis

Data for meta-analysis were extracted from 18 studies involving 10 866 men. Of these, all 18 studies reported the prevalence of complete YCMs in oligozoospermic men with sperm concentrations of >0–≤1 million sperm/ml, 16 studies reported prevalence in men with >1–5 million sperm/ml, and 13 studies reported prevalence in men with >5–20 million sperm/ml (these men were used as a comparison group). Sixteen studies were reported from a European academic center and two from a North American center. All studies utilized a cross-sectional study design. For all studies, the risk of bias was low, as the majority of studies were graded to be very good or good (Supplementary Table 2).

The median number of oligozoospermic men per study screened for YCMs was 375 (range: 38–2448 men). The median number of oligozoospermic men per study screened for YCMs with sperm concentrations of >0–≤1 million sperm/ml was 146 (range: 10–1069), that of men with >1–5 million sperm/ml was 139 (range: 16–561), and that of men with >5–20 million sperm/ml was 90 (range: 20–1076).

3.2. Cumulative incidence of YCMs in oligozoospermic men

Fig. 2 illustrates the results of the meta-analysis for the prevalence of complete YCMs, stratified by the thresholds of 1 and 5 million sperm/ml. The cumulative incidence of complete YCMs in men with sperm concentrations of >0–≤1 million sperm/ml was 5.0% (95% confidence interval [CI]: 3.6–6.8%; heterogeneity: $I^2 = 75\%$, $p < 0.01$). The cumulative incidence of complete YCMs in men with >1–5 million sperm/ml was 0.8% (95% CI: 0.5–1.3%; heterogeneity: $I^2 = 0.0\%$, $p = 0.6$). There was a significant between-group difference when the incidence in these two groups was compared (5.0% vs 0.8%, $p < 0.0001$). The cumulative incidence of complete YCMs in men with sperm concentrations of >5–20 million sperm/ml was 0.5% (95% CI: 0.2–0.9%; heterogeneity: $I^2 = 0.0\%$, $p = 0.8$). When this group was compared with men with sperm concentrations of >1–5 million sperm/ml, no significant between-group difference was evident (0.8% vs 0.5%, $p = 0.1$).

3.3. Analysis by study-level characteristics to address heterogeneity

To determine whether the continent of study was a source of heterogeneity in this study, we performed a stratified meta-analysis. For men with >0–≤1 million sperm/ml, there was no significant difference in the prevalence of complete YCMs by geographic location (North American studies 4.1% vs European studies 4.9%, $p = 0.9$; heterogeneity: $I^2 = 88\%$, $p < 0.01$ and $I^2 = 71\%$, $p < 0.01$, respectively). For men with >1–5 million sperm/ml, there was again no difference when comparing geographic locations (North American studies 0.6% vs European studies 0.8%, $p = 0.6$; heterogeneity: $I^2 = 0.0\%$, $p = 0.8$ and $I^2 = 2.0\%$, $p = 0.4$, respectively). There was also no significant difference when using the EAA/EMQN protocol versus individual laboratory protocol; in men with >0–≤1 million sperm/ml, studies using EAA/EMQN protocol had a YCM prevalence of 4.3%, while studies using internal laboratory protocol had a prevalence of 5.7% ($p = 0.4$; heterogeneity: $I^2 = 85\%$, $p < 0.01$ and $I^2 = 59\%$, $p < 0.01$, respectively). Moreover, no significant difference was seen between protocols for men with >1–5 million sperm/ml (EAA/EMQN protocol 0.4% vs internal laboratory protocol 1.0%, $p = 0.2$; heterogeneity: $I^2 = 36\%$, $p = 0.2$ and $I^2 = 0.0\%$, $p = 0.9$, respectively).

Table 1 – Characteristics of included YCM studies.

	Authors/year	Year	Inclusion criteria	Level of data	Country	Sequence tag sites AZFc	Criteria for identifying complete YCM	>0–1 million sperm/ml Complete YCM	>0–1 million sperm/ml Men screened	>1–5 million sperm/ml Complete YCM	>1–5 million sperm/ml Men screened	>5–20 million sperm/ml Complete YCM	>5–20 million sperm/ml Men screened
Studies included in quantitative meta-analysis	Johnson (2019) [14]	2018	Consecutive oligospermic infertile men who had a genetic analysis	Confirmed criteria (EAA/EMQN)	UK	**sY254, sY255**	EAA/EMQN criteria	12	279	0	236	0	405
	Goncalves (2017) [11]	2017	Infertile oligospermic men	Confirmed criteria and individual STSs	Portugal	sY1197, sY1192, BPY2, sY152, **sY254, sY 255**, DAZ1, sY1291, CDY1, sY157, sY1201, and sY1206	EAA/EMQN criteria	46	736	0	342	0	186
	Olesen (2017) [24]	2017	Infertile men whose partner was undergoing IVF or ICSI	Confirmed criteria (EAA/EMQN)	Denmark	**sY254, sY255**	EAA/EMQN criteria	3	240	0	230	0	149
	Punab (2017) [29]	2017	Oligospermic infertile men undergoing evaluation	Confirmed criteria (EAA/EMQN)	Estonia	**sY254, sY255**	EAA/EMQN criteria	11	130	1	360	3	813
	Lo Giacco (2014) [20]	2014	Consecutive oligospermic infertile men undergoing evaluation	Confirmed criteria (EAA/EMQN)	Spain	**sY254, sY255**	EAA/EMQN criteria	2	154	0	123	0	61
	Stahl (2010) [35]	2010	Consecutive severely oligospermic	Confirmed criteria	New York, USA	sY153, Fr15-11pr, Y6HP52pr, sY147, sY149, **sY254, sY255**, sY157, sY158	Partial deletions in AZFc region were considered nondeletions	26	257	1	155	–	–
	Yatsenko (2010) [39]	2010	Severely oligospermic men evaluated for infertility	Confirmed individual STSs	Texas, USA	sY149, **sY254, sY255**, sY243, sY269	Deletion of a minimum of sY254, sY255 for complete AZFc, deletion of all regional STSs for AZFa and AZFb	2	157	1	214	1	85
	Simoni (2008) [34]	2007	Men presenting for infertility	Confirmed criteria (EAA/EMQN)	Germany	**sY254, sY255**	EAA/EMQN criteria	25	1069	1	506	–	–
	Ferlin (2007) [9]	2007	Consecutive oligospermic infertile men undergoing evaluation	Confirmed criteria	Italy	**sY254, sY255**, sY1291, sY1125, sY1190, sY1206, sY1201, sY159, sY160	AZFc required deletion of sY1192, sY1191, sY254, sY255, sY1291, sY1190, sY1206	45	811	6	561	0	1076
	Chernykh (2006) [6]	2006	Men from infertile married couple with pathozoospermia	Confirmed criteria (EAA/EMQN)	European Russia	**sY254, sY255**	EAA/EMQN criteria	15	145	2	64	0	240
	Medica (2005) [23]	2005	Male partners of infertile couples attending the outpatient infertility clinic	Confirmed criteria (EAA/EMQN)	Croatia	**sY254, sY255**	EAA/EMQN criteria	1	10	0	17	0	25
	Cruger (2003) [7]	2003	Men referred for ICSI	Deletions reported in results	Denmark	**sY254, sY255**	Deletion of all regional STSs	1	47	0	92	0	90
	Loginova (2003) [21]	2003	Men presenting for infertility	Deletions reported in results	European Russia	sY153, sY149, **sY254, sY255, sY269, sY202, sY158**	Undefined by authors *deletion of sY254, sY255 applied*	4	23	0	16	0	20
	Dohle (2002) [8]	2002	Infertile couple requesting ICSI with sperm concentration 1 000 000 sperm/ml	Deletions reported in results	The Netherlands	**sY254, sY255**	Deletion of all regional STSs	5	113	–	–	–	–
	Bor (2002) [4]	2002	Men in couples who were candidates for ICSI due to male factor infertility	All STS deletions report in figure	Denmark	sY144, sY150, sY152, sY153, sY155, **sY255, sY254**, SPGY, sY277, sY243, sY269, sY158, sY272, sY273, sY166	Undefined by authors *deletion of sY254, sY255 applied*	2	147	0	94	0	81
	van der Ven (1997) [37]	1997	Men in a couple undergoing in vitro fertilization	All STS deletions report in figure	Germany	sY147, sY149, **sY254, sY255**, sY157	Undefined by authors *deletion of sY254, sY255 applied*	1	32	0	47	0	79
	Kremer (1997) [19]	1997	Couple requesting ICSI, infertile >12 mo, sperm concentration 1 000 000 sperm/ml	Deletions reported in results	The Netherlands	**sY254, sY255**	Deletion of all regional STSs	7	111	–	–	–	–
	Qureshi (1996) [30]	1996	Men from a large infertility group with oligospermia	All STS deletions report in figure	UK	sY147, sY233, sY232, sY149, **sY254, sY255**, sY202, sY158	Undefined by authors *deletion of sY254, sY255 applied*	2	21	0	17	–	–
	Kovacheva (2018) [16]	2018	Infertile men referred for assisted reproductive technologies	Deletions reported in results	Bulgaria	**sY254, sY255**	EAA/EMQN criteria	0	46 [a]	0	46 [a]	–	–

Table 1 (*Continued*)

	Authors/year	Year	Inclusion criteria	Level of data	Country	Sequence tag sites: AZFc	Criteria for identifying complete YCM	>0–1 million sperm/ml: Complete YCM	>0–1 million sperm/ml: Men screened	>1–5 million sperm/ml: Complete YCM	>1–5 million sperm/ml: Men screened	>5–20 million sperm/ml: Complete YCM	>5–20 million sperm/ml: Men screened
Studies included in Qualitative Systematic Review	Khurana (2014) [15]	2014	Men seeking fertility evaluation with sperm concentration <2.5 million/ml	Deletions reported in results	Ohio, USA	sY152, sY158, **sY254, sY255**	Deletion of all regional STSs	0	205 [a]	0	205 [a]	–	–
	Tzschach (2001) [36]	2001	Men who presented with oligo- or azoospermia to an infertility center	Deletions reported in results	Germany	sY147, sY152, sY148, **sY254**, sY153, sY158	Deletion of all regional STSs	0	64 [a]	0	64 [a]	–	–
	Plaseski (2006) [27]	2006	Infertile/subfertile males attending an andrology outpatient clinic	All STS deletions report in figure	Macedonia	**sY254, sY255**	Deletion of all regional STSs	1	52 [a]	0	52 [a]	0	27
	Raicu (2003) [31]	2003	Men referred for fertility evaluation	All STS deletions report in figure	Romania	**sY254, sY255**	Deletion of all regional STSs	2	6	0	19 [b]	–	–
	Calleja Macías (2003) [5]	2003	Men with oligospermia presenting for clinical treatment	All STS deletions report in table	Mexico	sY242, sY239, sY208, **sY254, sY255**, sY157	Undefined by authors *deletion of sY254, sY255 applied*	0	16 [a]	0	16 [a]	–	–
	Gruber (2003) [12]	2003	Men seeking assisted reproductive treatments at a fertility center	Deletions reported in results	Austria	sY242, sY239, sY208, **sY254, sY255**, sY157	Deletion of all regional STSs	0	154 [a]	0	154 [a]	0	115
	Ioulianos (2002) [13]	2002	Patients presenting with infertility and sperm concentration <2 million sperm/ml	All STS deletions report in figure	Cyprus	sY152, **sY254, sY255**, sY157, sY158	Undefined by authors *deletion of sY254, sY255 applied*	0	48 [a]	0	48 [a]	–	–
	Peterlin (2002) [26]	2002	Infertile men attending an infertility clinic	All STS deletions report in figure	Slovenia	sY153, sY152, sY155, sY147, sY156, sY149, **sY254**, sY157, sY202, sY243	Deletion of all regional STSs	2	70	0	16	0	48
	Krausz (2001) [18]	2001	Consecutive patients seeking ICSI treatment	Deletions reported in results	France	sY145, sY147, sY152, BPY2, **sY254**	Deletion of all regional STSs	3	52	0	30	0	49
	Friel (2001) [10]	2001	Men attending a fertility clinic	All STS deletions report in Table	Ireland	sY220, sY245, sY242, sY262, sY257, **sY254, sY255**, sY272, sY273, sY269, sY243	Deletion of all regional STSs	0	7 [a]	0	7 [a]	–	–
	Aho (2001) [3]	2001	Oligospermic men who underwent evaluation of male factor infertility	All STS deletions report in figure	Finland	sY153, Y6HP52pr, sY147, sY146, sY149, **sY254, sY255**	EAA/EMQN criteria	10	133 [a]	2	133 [a]	–	–
	Martinez (2000) [22]	2000	Men with severe spermatogenic failure	All STS deletions report in figure	Spain	sY147, sY149, sY157	Deletion of all regional STSs	1	71 [a]	0	71 [a]	–	–
	Van Landuyt (2000) [38]	2000	Male infertility patients with normal karyotype & unexplained oligozoospermia	All STS deletions report in figure	Belgium	sY153, sY152, FR15-11, Y6HP52, sY147, sY262, sY232, sY239, sY204, sY149, **sY254, sY255**, sY267, sY243, sY269, sY202, sY157, sY158	Undefined by authors *deletion of sY254, sY255 applied*	5	121 [a]	0	121 [a]	–	–
	Seifer (1999) [33]	1999	Infertile oligospermic men	All STS deletions report in figure	France	sY153, sY147, sY149, **sY254, sY255**, sY158, sY160	Undefined by authors *deletion of sY254, sY255 applied*	3	32 [a]	0	32 [a]	–	–
	Oliva (1998) [25]	1998	Men at an infertility clinic and who were referred to genetic counseling prior to ICSI	All STS deletions report in figure	Spain	sY154, **sY254**, sY269, sY202, sY158, sY160	Deletion of all regional STSs	1	136 [a]	1	136 [a]	–	–
	Krausz (1999) [17]	1998	Males presenting with infertility associated with reduced sperm counts	All STS deletions report in figure	France	sY153, sY152, sY155, sY154, sY147, sY149, sY208, sY207, **sY254, sY255**, sY247, sY158	Undefined by authors *deletion of sY254, sY255 applied*	1	71 [a]	0	71 [a]	–	–
	Pryor (1997) [28]	1997	Consecutive oligospermic infertile men undergoing evaluation	All STS deletions report in figure	Minnesota, USA	sY147, sY154, sY240, sY245, sY203, sY242, sY148, sY262, sY221, sY233, sY232, sY239, sY257, sY249, sY156, sY224, sY231, sY294, sY201, sY149, sY206, sY208, **sY254, sY255**, sY267, sY248, sY269, sY202, sY247, sY157, sY158, sY159	Undefined by authors *deletion of sY254, sY255 applied*	1	30 [a]	0	30 [a]	0	42
	Reijo (1996) [32]	1996	Patients presenting with infertility and sperm concentration <1 million sperm/ml	All STS deletions report in figure	Massachusetts, USA	sY142, sY143, **sY254**, sY283, sY277	Deletion of all regional STSs	2	35	–	–	–	–

Sequence tag sites required for inclusion in quantitative meta-analysis (sY254 & sY255) are bolded.

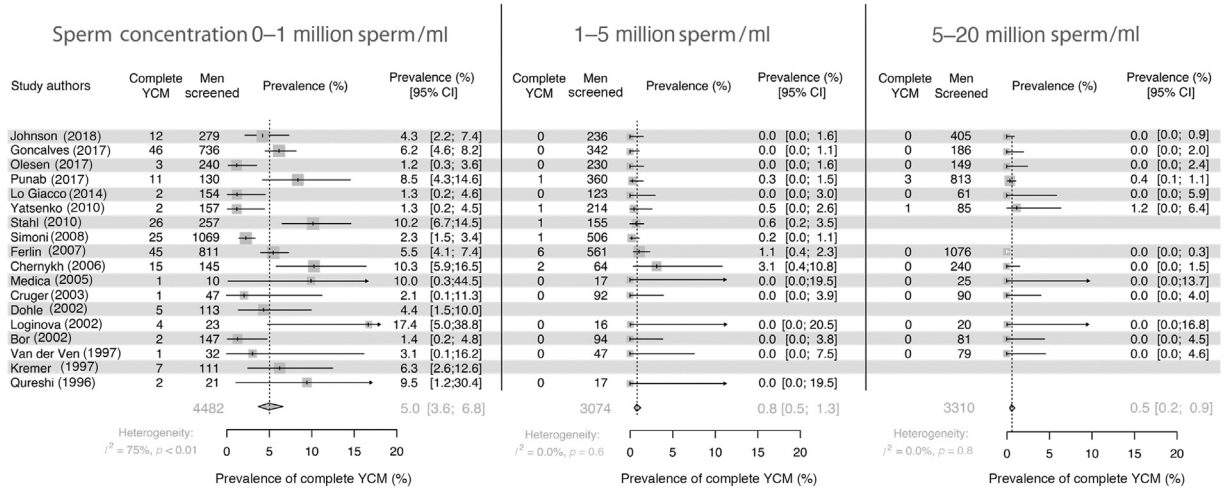

Fig. 2 – Forest plot assessing cumulative incidence of complete YCM. CI = confidence interval; YCM = Y-chromosome microdeletion.

3.4. *Sensitivity analysis*

For the >1–5 and the >5–20 million sperm/ml groups, sensitivity analysis demonstrated that no individual study affected the rate of complete YCMs by more than 0.1%. In these same two groups, omission of no study increased I^2 heterogeneity beyond 0.0%. When assessing men with >0–1 million sperm/ml, no study affected the rate of complete YCMs by more than 0.5% (Supplementary Table 2).

3.5. *Assessment of publication bias*

Funnel plots revealed mild asymmetry for studies assessing sperm concentrations of >0–1 million sperm/ml, but no asymmetry for studies assessing sperm concentrations of >1–5 or >5–20 million sperm/ml, suggesting that the pooled estimates in these groups were not biased secondary to small study effects (Fig. 3). The Egger et al regression asymmetry test supported this, as studies assessing >0–1 million sperm/ml approached but did not meet an asymmetric distribution (z = –1.8, p = 0.07) [45]. Additionally, no asymmetry was detected in studies assessing the >1–5 million sperm/ml (z = –1.2, p = 0.2) or >5–20 million sperm/ml (z = 0.09, p = 0.9) groups.

3.6. *Cost-benefit analysis*

A total of 7756 oligozoospermic men included in this meta-analysis were tested who had sperm concentrations of >0–5 million sperm/ml. Testing for all these men cost approximately €1 706 320 ($1 915 732 or £1 473 640). If a threshold of 1 million sperm/ml was applied to screen patients for YCM testing, only 4482 men would have received testing—this would result in a 57.8% savings or €986 040 ($1 107 054, or £851 580).

4. Conclusions

Our systematic review and meta-analysis of 37 cross-sectional studies with 12 492 oligozoospermic men demonstrates that all YCMs in oligozoospermic men were AZFc deletions. Overall, the prevalence of YCMs was significantly higher in men with >0–≤1 million sperm/ml (5.0%) than in men with >1–5 million sperm/ml (0.8%) or >5–20 million sperm/ml (0.5%).

While the prevalence of complete YCMs in men with nonobstructive azoospermia is consistently in the 8–15% range, the literature reports a wide range (0–44%) for severely oligozoospermic men (>0–5 million sperm/ml)

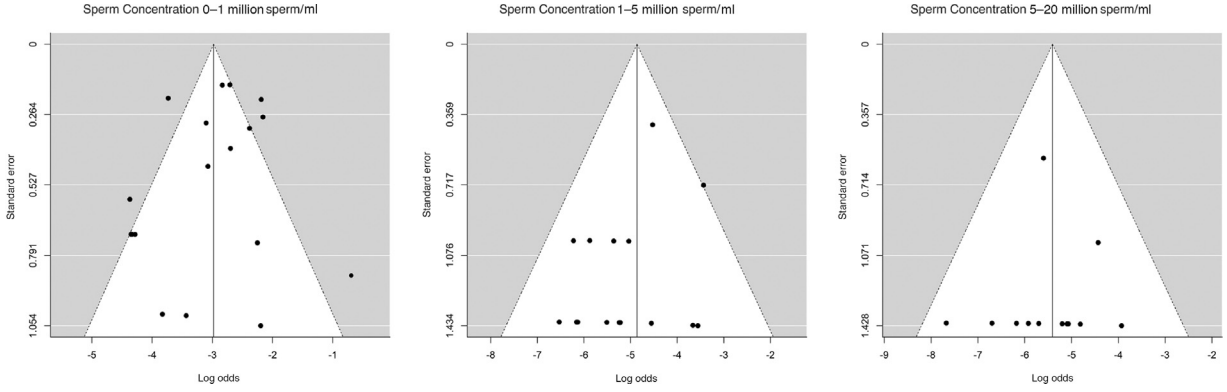

Fig. 3 – Funnel plot to assess publication bias/small study effects.

[34]. This wide range is multifactorial. Some studies use only one STS to diagnosis a YCM, when at least two STSs are key to prevent false positives. Some studies report a partial YCM as a complete YCM; additionally, early studies did not differentiate between partial and complete YCMs. Some studies combine azoospermic and oligozoospermic men, such as a meta-analysis of YCMs in Iranian men, which found that 12% of men with sperm concentration of <5 million sperm/ml were found to have a YCM [46]. Some studies use simplex PCR, which results in a higher rate of false positives that do not occur with the modern multiplex PCR. Geographic location can also result in variation [42]. In order to account for these factors and decrease heterogeneity in our study, we had four important considerations. First, we required testing of at least two STSs for each *AZF* region to be included in the systematic review, and, to be included in the meta-analysis, studies had to assess sY254 and sY255 at a minimum, which are the two STSs recommended for screening *AZFc* deletions. Second, we examined the STS results for each patient using the original deidentified or published data, or we confirmed the process by which each primary study laboratory determined a "partial" versus a complete YCM. Third, we only included studies that used a multiplex PCR. Lastly, we focused on Europe and North America to minimize potential impact of geographic variability. These study design factors lend greater strength to our study and support the validity of our point estimates.

In oligozoospermic men with YCMs, it is possible for couples to achieve pregnancy naturally, and YCMs do not appear to significantly impact fetal development after conception [42]. However, male offspring will inherit these YCMs from their fathers and may be infertile as well; thus, genetic counseling is warranted when YCMs are identified [42]. Couples may opt to undergo in vitro fertilization with preimplantation genetic testing for aneuploidy (PGT-A) to select for female progeny for embryo transfer or use donor sperm, preventing inheritance of these microdeletions. Therefore, screening for YCMs has both clinical and ethical implications for patients and their families.

Determining thresholds for genetic screening must weigh these implications with the monetary and psychological costs of screening. Genetic screening continues to increase in frequency, and several known gene mutations exist that, when identified, can affect clinical decision making in reproductive medicine and elsewhere. *BRCA1/2* is perhaps the most well known of these genes; however, not everyone with a family history of breast cancer is recommended to undergo screening [47]. The European Society for Medical Oncology (ESMO) recommends screening only when the family history is deemed "high risk." While several questionnaires are recommended to stratify the level of risk, the ESMO recommends screening only if there is >10% likelihood of identifying a *BRCA1* or a *BRCA2* mutation [47]. Within the field of reproductive medicine, noninvasive prenatal testing using cell-free DNA for the screening of trisomies 13, 18, and 21 is recommended only for screening when women are deemed "high risk" for a fetus with aneuploidy [48]. In studies assessing the prevalence of trisomies in populations deemed high risk, authors have reported pretest probability of having a trisomy pregnancy of 6.1% [49]. Noninvasive prenatal testing is not recommend in low-risk groups, which have a pretest probability of fetal aneuploidy of significantly <6% [48]. Thus, even when intervention is possible after each of these diagnoses—even as drastic as recommending a mastectomy and an oophorectomy in the case of *BRCA* mutations [47]—screening is not universally recommended for every person potentially at risk. As a comparison, in this study, we demonstrate that only 0.8% of men with sperm concentrations of >1–5 million sperm/ml had a complete YCM—this is substantially lower than the generally accepted risk thresholds for screening for many other disorders. Thus, even though an intervention such as PGT-A is possible after identifying a YCM, the prevalence of YCMs in men with >1 million sperm/ml may not merit screening.

One common argument for a more inclusive YCM screening threshold is that a greater degree of interobserver variation exists when lower sperm concentrations are present. Studies have demonstrated interobserver coefficients of variation as high as 30% when analyzing low sperm concentrations [50]. Thus, for a man with a sperm concentration of 0.8 million sperm/ml, the standard deviation would be 0.24 million sperm/ml. Such variation could result in incorrect classification of men who may merit screening into a group that does not receive screening for YCMs, if thresholds were lowered to 1 million sperm/ml. However, this was not seen in our analysis. If individuals were incorrectly classified due to interobserver variation, moderate variation in the prevalence of YCMs would be expected within the 1–5 million sperm/ml group, but the degree of calculated heterogeneity (I^2) was 0%, indicating low statistical variability between studies. Overall, the prevalence of YCMs in men with 1–5 million sperm/ml fell within a relatively narrow range, from 0% to 3.1%. One possible explanation for this low statistical heterogeneity in the range of 1–5 million sperm/ml is that most studies have identified the vast majority (84–91%) of YCMs in azoospermic or oligozoospermic men with sperm concentrations of <0.1 million sperm/ml [14,20]. Thus, even with interobserver variation in semen concentration, the impact of misclassification of a man with a complete YCM would be unlikely, as the majority of these men are well below the 1 million sperm/ml threshold. Nor does it appear that repeat semen analyses result in variation either, as Simoni et al [34] followed patients with YCMs with multiple semen analyses over 2–5 yr and demonstrated only minor variations in semen parameters that never went above the 1 million sperm/ml threshold. While our study and the current guidelines assess a static sperm concentration threshold, variation in sperm concentration across multiple semen analyses, while rare, may result in concentrations above and below the threshold. While included studies that assessed multiple semen analyses used median semen concentrations to determine whether testing was appropriate, ultimately the choice for testing for YCMs in a man with varying sperm concentrations between semen analyses must be weighed by each clinician.

Finally, the EAU Male Infertility guidelines provide some insight into the prevalence of YCMs that merit screening [41]. The committee cited the YCM prevalence to be 8–12% in azoospermic men, 3–7% in oligozoospermic men (>0–−5 million sperm/ml), and 0.7% in men with sperm concentrations of >5 million sperm/ml. The committee concluded that, given the low rate of YCMs in men with sperm concentrations of >5 million sperm/ml, screening is not recommended above this threshold. Herein, we demonstrate that the prevalence of YCMs in men with sperm concentrations of >1–5 million sperm/ml is similarly rare, with a prevalence of 0.8% YCMs. Therefore, using the same logic as the EAU Male Infertility Guideline Committee, men with >1–5 million sperm/ml should also not be screened for YCMs.

The present comprehensive meta-analysis has several notable strengths. This is the largest study and the first meta-analysis to assess thresholds at which oligozoospermic men ought to be considered for screening for YCMs, and we assessed more than 10 000 European and North American oligozoospermic men. Additionally, as sperm concentration and geographic location have consistently been shown to affect the prevalence of YCMs, we assess both of these variables. Finally, as there is large variation in STSs used in various studies, we applied a consistent definition requiring studies to assess both sY254 and sY255 and defining a complete *AZFc* deletion as both of these STSs.

Our study has several potential weaknesses. Very few studies reported the prevalence of YCMs by ethnicity; rather, the majority of studies simply report the country of study. Thus, although it is assumed that the location of the clinic may roughly correlate with regional ethnicity, these data are not currently known. Despite controlling for the majority of known sources of heterogeneity, significant heterogeneity was seen in the >0–1 million sperm/ml group, likely due to further acceleration of rates of YCMs as sperm concentrations approach azoospermia. While assessing multiple thresholds between >0 and 1 million sperm/ml would have been ideal, the vast majority of the literature report only thresholds of 1, 5, and 20 million sperm/ml. Our strict definition of a complete YCM resulted in the exclusion of several notable studies from the meta-analysis, but the majority of men identified by the systematic review were able to be included (10 866 of 12 492 oligozoospermic men, 87%).

This meta-analysis demonstrates that complete YCMs of the *AZFc* region are rare (0.8% prevalence) in men with sperm concentrations of >1 million sperm/ml, with most complete YCMs in oligozoospermic men being identified in men with ≤1 million sperm/ml (5.0% prevalence). Subsequent iterations of male infertility guidelines for North American and European populations should therefore consider lowering the screening threshold for YCMs in these populations to severely oligozoospermic men with sperm concentrations of <1 million sperm/ml.

Author contributions: Taylor P. Kohn had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: All authors.
Acquisition of data: T.P. Kohn, J.R. Kohn.
Analysis and interpretation of data: All authors.
Drafting of the manuscript: All authors.
Critical revision of the manuscript for important intellectual content: T.P. Kohn, J.R. Kohn, Coward.
Statistical analysis: T.P. Kohn.
Obtaining funding: None.
Administrative, technical, or material support: All authors.
Supervision: Coward.
Other: None.

Financial disclosures: Taylor P. Kohn certifies that all conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript (eg, employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: None.

***Funding/Support and role of the sponsor*:** None.

Acknowledgments: We extend our deepest gratitude to all authors who provided additional information in regard to data from their publication or provided expert opinion for study design or interpretation, including Dr. Susana Fernandes, Dr. Amin Herati, Dr. Mark Johnson, Dr. Dolores Lamb, Dr. Larry Lipshultz, Dr. Premal Patel, Dr. David Ralph, Dr. Ranjith Ramasamy, and Dr. Mario Sousa.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.eururo.2019.07.033.

References

[1] 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.
[2] Vogt P, Chandley AC, Hargreave TB, Keil R, Ma K, Sharkey A. Microdeletions in interval 6 of the Y chromosome of males with idiopathic sterility point to disruption of AZF, a human spermatogenesis gene. Hum Genet 1992;89:491–6.
[3] Aho M, Harkonen K, Suikkari AM, Juvonen V, Anttila L, Lahdetie J. Y-chromosomal microdeletions among infertile Finnish men. Acta Obstet Gynecol Scand 2001;80:652–6.
[4] Bor P, Hindkjaer J, Kolvraa S, Ingerslev HJ. Y-chromosome microdeletions and cytogenetic findings in unselected ICSI candidates at a Danish fertility clinic. J Assist Reprod Genet 2002;19:224–31.
[5] Calleja Macías IE, Martínez Garza SG, Gallegos Rivas MC, et al. Y chromosome microdeletion identification in patients with male infertility. Ginecol Obstet Mexico 2003;71:25–31.
[6] Chernykh VB, Chukhrova AL, Beskorovainaia TS, et al. Types of Y chromosome deletions and their frequency in infertile men. Genetika 2006;42:1130–6.
[7] Cruger DG, Agerholm I, Byriel L, Fedder J, Bruun-Petersen G. Genetic analysis of males from intracytoplasmic sperm injection couples. Clin Genet 2003;64:198–203.
[8] Dohle GR, Halley DJ, Van Hemel JO, et al. Genetic risk factors in infertile men with severe oligozoospermia and azoospermia. Hum Reprod 2002;17:13–6.
[9] Ferlin A, Arredi B, Speltra E, 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.

[10] Friel A, Houghton JA, Maher M, et al. Molecular detection of Y chromosome microdeletions: an Irish study. Int J Androl 2001;24:31–6.
[11] Goncalves C, Cunha M, Rocha E, et al. Y-chromosome microdeletions in nonobstructive azoospermia and severe oligozoospermia. Asian J Androl 2017;19:338–45.
[12] Gruber CJ, Hengstschlager M, Wieser F, et al. Absence of microdeletions in the azoospermia-factor region of the Y-chromosome in Viennese men seeking assisted reproduction. Wiener Klin Wochenschr 2003;115:831–4.
[13] Ioulianos A, Sismani C, Fouroucklas N, Patroclou T, Sergiou C, Patsalis PC. A nation-based population screening for azoospermia factor deletions in Greek-Cypriot patients with severe spermatogenic failure and normal fertile controls, using a specific study and experimental design. Int J Androl 2002;25:153–8.
[14] Johnson M, Raheem A, De Luca F, et al. An analysis of the frequency of Y-chromosome microdeletions and the determination of a threshold sperm concentration for genetic testing in infertile men. BJU Int 2019;123:367–72.
[15] Khurana KK, Baker K, Gao T, Sabanegh ES. The economic burden of genetic tests for the infertile male: A pilot algorithm to improve test predictive value. J Urol 2014;191:1066–71.
[16] Kovacheva K, Kotsev R, Konova E, Rilcheva V, Kamburova Z, Simeonova M. Chromosomal abnormalities and Y chromosome microdeletions in Bulgarian male with azoospermia or severe oligospermia. JIMAB Annu Proceeding (Sci Papers) 2018;24:2217–22.
[17] Krausz C, Bussani-Mastellone C, Granchi S, McElreavey K, Scarselli G, Forti G. Screening for microdeletions of Y chromosome genes in patients undergoing intracytoplasmic sperm injection. Hum Reprod 1999;14:1717–21.
[18] Krausz C, Rajpert-De Meyts E, Frydelund-Larsen L, Quintana-Murci L, McElreavey K, Skakkebaek NE. Double-blind Y chromosome microdeletion analysis in men with known sperm parameters and reproductive hormone profiles: microdeletions are specific for spermatogenic failure. J Clin Endocrinol Metab 2001;86:2638–42.
[19] Kremer JA, Tuerlings JH, Meuleman EJ, et al. Microdeletions of the Y chromosome and intracytoplasmic sperm injection: from gene to clinic. Hum Reprod 1997;12:687–91.
[20] Lo Giacco D, Chianese C, Sánchez-Curbelo J, et al. Clinical relevance of Y-linked CNV screening in male infertility: new insights based on the 8-year experience of a diagnostic genetic laboratory. Eur J Hum Genet 2014;22:754–61.
[21] Loginova JA, Nagornaya II, Shlykova SA, et al. Molecular genetic analysis of Y-chromosome microdeletions in men with severe spermatogenetic defects. Mol Biol 2003;37:74–80.
[22] Martinez MC, Bernabe MJ, Gomez E, et al. Screening for AZF deletion in a large series of severely impaired spermatogenesis patients. J Androl 2000;21:651–5.
[23] Medica I, Gligorievska N, Prenc M, Peterlin B. Y microdeletions in the Istria county. Croatia Asian J Androl 2005;7:213–6.
[24] Olesen IA, Andersson AM, Aksglaede L, et al. Clinical, genetic, biochemical, and testicular biopsy findings among 1,213 men evaluated for infertility. Fertil Steril 2017;107(74), e7.
[25] Oliva R, Margarit E, Ballescá JL, et al. Prevalence of Y chromosome microdeletions in oligospermic and azoospermic candidates for intracytoplasmic sperm injection. Fertil Steril 1998;70:506–10.
[26] Peterlin B, Kunej T, Sinkovec J, Gligorievska N, Zorn B. Screening for Y chromosome microdeletions in 226 Slovenian subfertile men. Hum Reprod 2002;17:17–24.
[27] Plaseski T, Novevski P, Kocevska B, Dimitrovski C, Efremov GD, Plaseska-Karanfilska D. AZF deletions in infertile men from the Republic of Macedonia. Prilozi 2006;27:5–16.
[28] Pryor JL, Kent-First M, Muallem A, et al. Microdeletions in the Y chromosome of infertile men. N Engl J Med 1997;336:534–9.
[29] Punab M, Poolamets O, Paju P, et al. Causes of male infertility: a 9-year prospective monocentre study on 1737 patients with reduced total sperm counts. Hum Reprod 2017;32:18–31.
[30] Qureshi SJ, Ross AR, Ma K, et al. Polymerase chain reaction screening for Y chromosome microdeletions: a first step towards the diagnosis of genetically-determined spermatogenic failure in men. Mol Hum Reprod 1996;2:775–9.
[31] Raicu F, Popa L, Apostol P, et al. Screening for microdeletions in human Y chromosome—AZF candidate genes and male infertility. J Cell Mol Med 2003;7:43–8.
[32] Reijo R, Alagappan RK, Patrizio P, Page DC. Severe oligozoospermia resulting from deletions of azoospermia factor gene on Y chromosome. Lancet 1996;347:1290–3.
[33] Seifer I, Amat S, Delgado-Viscogliosi P, Boucher D, Bignon YJ. Screening for microdeletions on the long arm of chromosome Y in 53 infertile men. Int J Androl 1999;22:148–54.
[34] Simoni M, Tuttelmann F, Gromoll J, Nieschlag E. Clinical consequences of microdeletions of the Y chromosome: the extended Munster experience. Reprod Biomed Online 2008;16:289–303.
[35] Stahl PJ, Masson P, Mielnik A, Marean MB, Schlegel PN, Paduch DA. A decade of experience emphasizes that testing for Y microdeletions is essential in American men with azoospermia and severe oligozoospermia. Fertil Steril 2010;94:1753–6.
[36] Tzschach A, Thamm B, Imthurn B, et al. Absence of Yq microdeletions in infertile men. Arch Androl 2001;47:167–71.
[37] van der Ven K, Montag M, Peschka B, et al. Combined cytogenetic and Y chromosome microdeletion screening in males undergoing intracytoplasmic sperm injection. Mol Hum Reprod 1997;3:699–704.
[38] Van Landuyt L, Lissens W, Stouffs K, Tournaye H, Liebaers I, Van Steirteghem A. Validation of a simple Yq deletion screening programme in an ICSI candidate population. Mol Hum Reprod 2000;6:291–7.
[39] Yatsenko AN, Yatsenko SA, Weedin JW, et al. Comprehensive 5-year study of cytogenetic aberrations in 668 infertile men. J Urol 2010;183:1636–42.
[40] Practice Committee of the American Society for Reproductive Medicine. Diagnostic evaluation of the infertile male: a committee opinion. Fertil Steril 2015;103:e18–25.
[41] Jungwirth A, Diemer T, Kopa Z, Krausz C, Minhas S, Tournaye H. EAU guidelines on male infertility 2018. European Association of Urology guidelines 2018 edition. Arnhem, The Netherlands: European Association of Urology Guidelines Office; 2018.
[42] Krausz C, Hoefsloot L, Simoni M, Tuttelmann F. EAA/EMQN best practice guidelines for molecular diagnosis of Y-chromosomal microdeletions: state-of-the-art 2013. Andrology 2014;2:5–19.
[43] Shamseer L, Moher D, Clarke M, et al. Preferred Reporting Items for Systematic Review and Meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ 2015;350:g7647.
[44] Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med 2002;21:1539–58.
[45] Sterne JA, Juni P, Schulz KF, Altman DG, Bartlett C, Egger M. Statistical methods for assessing the influence of study characteristics on treatment effects in 'meta-epidemiological' research. Stat Med 2002;21:1513–24.
[46] Yousefi-Razin E, Nasiri MJ, Omrani MD. Frequency of Y chromosome microdeletions among Iranian infertile men with azoospermia and severe oligozoospermia: a meta-analysis. J Reprod Infertil 2016;17:208–12.
[47] Paluch-Shimon S, Cardoso F, Sessa C, et al. Prevention and screening in BRCA mutation carriers and other breast/ovarian hereditary cancer syndromes: ESMO clinical practice guidelines for cancer prevention and screening. Ann Oncol 2016;27:v103–10.

[48] Dondorp W, de Wert G, Bombard Y, et al. Non-invasive prenatal testing for aneuploidy and beyond: challenges of responsible innovation in prenatal screening. Eur J Hum Genet 2015;23:1592.

[49] Palomaki GE, Deciu C, Kloza EM, et al. DNA sequencing of maternal plasma reliably identifies trisomy 18 and trisomy 13 as well as Down syndrome: an international collaborative study. Genet Med 2012;14:296–305.

[50] Auger J, Eustache F, Ducot B, et al. Intra- and inter-individual variability in human sperm concentration, motility and vitality assessment during a workshop involving ten laboratories. Hum Reprod 2000;15:2360–8.