Original Article

Prevalence and clinical considerations of Y chromosome microdeletions in azoospermic and oligozoopsermic infertile men from Al Madinah Al Munawarah, Saudi Arabia

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ABSTRACT

الأهداف: يظهر الحذف الجزئي للكروموسوم Y على شكل تشوهات في إنتاج الحيوانات المنوية والتي قد تؤدي إلى العقم عند الذكور. يُظهر انتشار الحذف الجزئي في كروموسوم Y لدى الرجال المصابين بالعقم عدم تجانس كبير في دراسات مختلفة. مع ملاحظة أن الحذف الجزئي لكروموسوم Y في السكان العرب في المملكة العربية السعودية لم تتم دراسته بشكل جيد للغاية، لذلك أجريت هذه الدراسة.

المنهجية: قمنا بفحص 97 يعانون من العقم من منطقة المدينة المنورة في المملكة العربية السعودية من فبراير 2022 إلى مارس 2024. وتم تحليل الحمض النووي الجينومي للدم بحثًا عن 8 علامات STS للكروموسوم Y عن طريق تفاعل البوليميراز المتسلسل.

النتائج: وجدنا الحذف الدقيق في 3 رجال يعانون من العقم، مما يشير إلى انتشار 3.1%. تم حذف sY254 وsY255 من علامات STS المقابلة لمناطق AZFc في هؤلاء الرجال. لم يلاحظ أي حذف في أي علامات اخرى ل STS تم التحقيق فيها في هذه الدراسة.

الخلاصة: النتائج التي توصلنا إليها للانتشار في السكان العرب في منطقة المدينة المنورة مماثلة لدراسات أخرى في المملكة العربية السعودية، ومع ذلك، لوحظ تباين كبير في انتشار الحذف الجزئي لكروموسوم Y في السكان العرب في بلدان الشرق الأوسط الأخرى. الحذف الجزئي لكروموسوم Y له قيمة تنبؤية كبيرة، لأنه يشير إلى الملف المنوي، واحتمال نجاح إجراءات ART مثل TESE وإدراك المخاطر المحتملة للانتقال الرأسي للحذف الجزئي من الأب إلى الابن في المرضى الذين يختارون ART. مع هذه الاعتبارات، نعيد التأكيد على الحاجة إلى الفحص الجيني للحذف الدقيق لكروموسوم Y لدى الرجال المصابين بالعقم الذين يعانون من قلة الحيوانات المنوية أو انعدامها.

Objectives: To characterize the potential role of Y-chromosome microdeletion (YCM) as a genetic cause for infertility in the Arab population from the Al Madinah Al Munawarah.

Methods: We screened 97 infertile men from Al Madinah Al Munawarah, from February 2022 to March 2024. Genomic blood DNA was analyzed for 8 sequence tagged site (STS) markers of Y chromosome by multiplex polymerase chain reaction.

Results: We found microdeletions in 3 infertile men, indicating a prevalence of 3.1%. The STS markers sY254 and sY255 corresponding to AZFc regions were deleted in these men. No deletion was observed in any other STS markers investigated in this study.

Conclusion: Our findings for prevalence in Arab population of Al Madinah Al Munawarah is comparable to other studies from Saudi Arabia. However, large variance in the prevalence of YCM in the Arab population of other Middle Eastern countries is reportedly observed. The YCM has significant prognostic value, since it indicates the spermatogenic profile, the success probability of assisted reproduction technique (ART) procedures as testicular sperm extraction and apprise of potential risk of vertical transmission of microdeletion from father to son in patients opting for ART. With these considerations, we re-emphasize the need for genetic screening of YCM in azoo- and oligozoospermic infertile men.

Keywords: Y chromosome microdeletions, male infertility, azoospermia, oligozoospermia, STS markers

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Infertility affects 8-12% couples in reproductive age group and in 50% cases, the male factor is the primary or contributory cause.[1] Semen analysis is the cornerstone of diagnostic evaluation in male infertility, with sperm parameters, providing a gross index of fertility status. Absence of sperm in the ejaculate (namely, non obstructive azoospermia [NOA]) which is attributed to impaired spermatogenesis, affects a large proportion of infertile men. During spermatogenesis a complex cascade of genes is involved in successive mitotic, meiotic, and post-meiotic phases to finally produce fully functional sperms.[1,2] Any genetic aberration that could potentially disrupt the numerous molecular pathways of spermatogenesis may manifest as infertility.[2]

Genes for spermatogenesis are located on Y-chromosome, therefore accurate genetic composition of Y-chromosome is extremely critical for efficient spermatogenesis. Among the wide variety of genetic defects such as chromosomal abnormalities, aneuploidies, and single gene defects on Y-chromosome, the genomic loss of Y-chromosome regions termed as Y-chromosome microdeletion (YCM) represents an important aetiology among infertile men. These YCM mainly occur in the azoospermia factor (AZF) regions spanning the Y-chromosome.[2] Since the AZF region is rich in genes critical for spermatogenesis, their deletions may lead to severe oligozoospermia or non-obstructive azoospermia.[3]

The AZF deletions based on their breakpoints have been subcategorized predominantly into AZFa, AZFb, and AZFc regions. These deletions are the genetic predictors of spermatogenic condition and are the second most common genetic cause of male infertility.[4] Distinct histopathological phenotypes are correlated with the locus of microdeletion, ranging from Sertoli cell-only syndrome (SCOS) (complete absence of germ cells) in AZFa microdeletions to maturation arrest at meiosis in AZFb microdeletions and hypospermatogenesis in AZFc microdeletions.[4,5]

The YCM assay is one of the most commonly used genetic test for infertile men and molecular diagnosis of these microdeletions is now an established clinical investigation in the workup of severe male infertility. Several international bodies as European Andrology Association, European Molecular Genetics Quality Network (EAA/EMQN), and American Urological Association have established guidelines for the diagnostic protocols for YCM screening.[4,6]

Globally, numerous studies have been accomplished on different population groups, ethnicities and patient settings. Studies on the worldwide pattern of infertility have shown that Middle East is among the global regions with the highest prevalence of infertility.[7] Although, infertility is a serious health issue in Saudi Arabia with an incidence of 19%, and approximately half a million couples need assisted conception, the YCM have so far been investigated only in very few studies from Saudi Arabia.[8,9] In view of the lacunae of data and the effect of YCM on fertility, the current research was carried out to characterize the potential role of YCM as a genetic cause for infertility in the Arab population from the Al Madinah Al Munawarah.

Methods. In this study we selectively enrolled 97 men with primary infertility having azoospermia or severe oligozoospermia (<5 million/ml) from the infertile men undergoing treatment in infertility clinics of Al Madinah Al Munawarah. Of these 97 men, 47 were azoospermic and 50 were severely oligozoospermic. These patients were ethnically Arabs from Al Madinah Al Munawarah. The investigated men were morphologically normal with no gross dysmorphic abnormalities or structural defects of the urogenital/reproductive system. Men with history of surgical intervention of genital/reproductive tract obstruction, with systemic infection of urogenital or reproductive tract or with varicocele/hydrocele/cryptorchidism/bilateral absence of vas deferens were excluded. Written informed consent was obtained from study participants. The study was carried out in accordance with the Declaration of Helsinki and approved by the ethics committee of Taibah University, A Madinah Al Munawarah, Saudi Arabia. The study was carried out between February 2022 and March 2024.

Semen analysis was carried out twice with a 3-5 day abstinence between each sample to confirm infertility as per WHO criteria.[10] Patients with less than 5×10^6 sperm/mL sperm concentration were considered severely oligozoospermic and patients with no sperm in their ejaculates were considered azoospermic. The absence of sperms was confirmed by centrifugating the sample.

The YCM assay was carried out on genomic DNA extracted from the peripheral venous blood of patients using the commercially available kit (QIAmp DNA Blood Mini Kit Catalogue No 51106). We carried out

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multiplex polymerase chain reaction (PCR) for sequence tagged site (STS) markers spanning the AZFa, AZFb, and AZFc regions on the Y-chromosome. The STS are unique genomic sequences usually 200-300 bases long, whose exact sequence is unique in the whole genome. They are helpful in PCR screening of many marker genes including the AZF genes for YCM assay.[11] Two internal controls- sY14 or SRY (gender determining region on the short arm of Y-chromosome) and ZFX/Y loci were also used (Table 1). For external quality controls we used fertile healthy male DNA (positive control), female DNA (negative control), water (substituted for DNA), and blank (no DNA). The protocol used was in accordance with the recommendations of EAA/EMQN guidelines.[4]

The selected 8 markers for YCM assay were carried out in 3 multiplexes. Multiplex 1 covered sY84, sY127, sY254, and sY255, while multiplex 2 and 3 covered sY14, sY86 and sY 134, ZFX/Y markers (Figure 1). The multiplex PCR was carried out in 50-microliter reaction volume using 100 ng of genomic DNA (Table 2). Electrophoresis was carried out using1X TBE buffer at a voltage of 115V for 90 minutes. The documentation was carried out using the gel agarose documentation system. Each experiment was carried out at least twice. In case of a deletion, the result was confirmed by a simplex PCR.

Results. In this study 97 infertile men were screened for YCM. The female partners of these men had no identifiable abnormality such as amenorrhea, polycystic ovary, or tubal blockage for infertility, thereby confirming that infertility in the enrolled subjects was due to male factor only. These infertile men had abnormal seminogram pertaining to the absence of sperm (azoospermia) or sperm less than 5 million/ml (severe oligozoospermia). Among the 97 subjects studied, only 3 men had microdeletion in the AZFc region as indicated by non-amplification of sY255, and sY254 marker (Figure 2). Two patients with YCM were azoospermic and one had severe oligozoospermia. The other investigated STS markers amplified well and therefore did not show any genomic loss by microdeletion in these patients. The deletions observed in multiplex PCR were further confirmed by simplex PCR. In the remaining 94 patients, no microdeletion for the STS markers investigated were observed. In this study the observed prevalence (3/97) of microdeletion was 3.1%. During the experiments, the internal controls (SRY and ZFX/Y) and external controls (fertile male DNA) amplified while female DNA, water, and blank did not show any amplification, indicating the expected quality control standards.

Cytogenetic abnormality was also reported in one of the 3 patients with microdeletion from our study. This patient (CG-Y79-23) had a 46,X, del(Y)(q11.223q12) chromosomal complement. The proband's brother also had muscular dystrophy. This individual also had a 4-year-old son conceived by testicular sperm extraction (TESE) and intracytoplasmic sperm injection (ICSI).

Table 1 - Sequence tagged site markers used for Y-chromosome microdeletion-assay with their corresponding regions and product size.

STS	Region	Product size (Bp)
sY84	AZFa	326
sY86	AZFa	318
sY127	AZFb	274
sY134	AZFb	301
sY254	AZFc	380
sY255	AZFc	123
sY14	--	472
ZFX/Y	--	495

STS: sequence tagged site

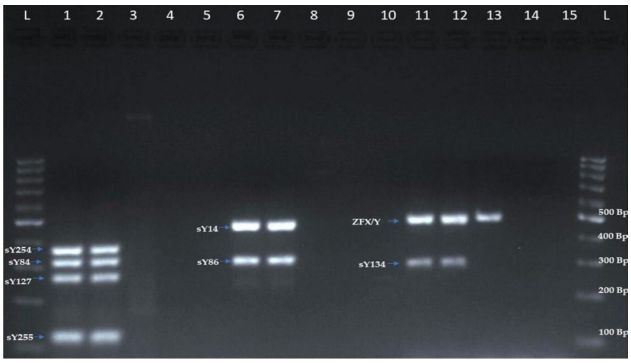

Figure 1 - Agarose gel electrophoresis image showing sequence tagged site markers for Multiplex 1, 2, and 3. Multiplex 1 [sY254 (380 bp), sY 127 (274 bp), sY255 (123 bp), sY84 (326 bp)]: Lane 1- infertile male with no deletion, Lane 2-control fertile men, Lane 3- female, Lane 4- water, Lane 5- blank. Multiplex 2 [sY86 (318 bp), sY14 (472 bp)]: Lane 6- infertile male with no deletion, Lane 7- control fertile men, Lane 8- female, Lane 9- water, Lane 10-blank.Multiplex 3 [sY134 (301 bp), ZFX/Y (495 bp)]: Lane 11- - infertile male with no deletion, Lane 12- control fertile men,Lane 13- female, Lane 14- water, Lane 15- blank.L- 100 Bp Ladder.

Discussion. In this study, we screened YCM in infertile men from Al Madinah Al Munawarah. Out of the 97 infertile men, we observed YCM, in 3 men, suggesting a prevalence of 3.1%. Our findings are comparable with the previous studies on YCM in the Saudi Arabian population (Table 3). To the best of our

Table 2 - Polymerase chain reaction conditions for multiplex polymerase chain reaction used for Y-chromosome microdeletion-assay.

Step 1	Step 2 (4 cycles)			Step 3 (3 cycles)			Step 4 (3 cycles)			Step 4 (25 cycles)			Step 5 (one cycle)
94°C	94°C	61°C	72°C	94°C	59°C	72°C	94°C	57°C	72°C	94°C	55°C	72°C	72°C
2 min	one min	30 sec	one min	one min	30 sec	one min	one min	30 sec	one min	one min	30 sec	one min	7 min
°C: degrees Celieus, Min: minute, Sec: second													

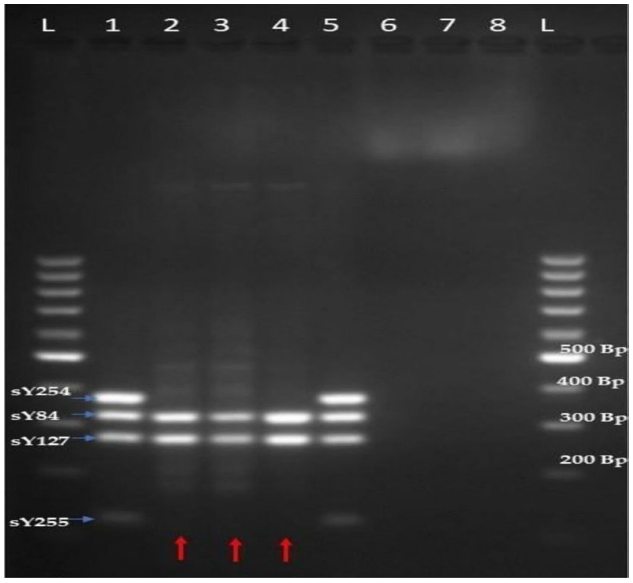

Figure 2 - Agarose gel electrophoresis image showing Y-chromosome microdeletion in sY255 and sY254 (indicated by red arrow) in the patients. Lane 1: infertile man with no deletion, Lane 2,3,4: infertile men with deletions. Lane 5: fertile man, Lane 6: female, Lane 7: water, and Lane 8: blank, L: 100 Bp ladder.

knowledge, there are only 4 previous research studies that present an estimated prevalence of YCM among the Saudi population albeit from different regions of Saudi Arabia. Three studies used STS screening by PCR to detect microdeletions, while in one study YCM were identified by whole exome sequencing (WES).[12-15]

Among the studies reported from Saudi Arabia, Beg et al[12] reported a prevalence of 2.3% (namely, 2 cases of AZFb,c microdeletion among 88 infertile men). In another study, Hellani et al[14] observed a prevalence of 3.2%, with 8 patients having microdeletions (AZFc [6 patients], AZFb [one patient], AZFa and AZFc both [one patient]) out of 247 infertile men. Hellani et al[13] investigated 133 infertile men, of which 4 men with microdeletions (3%) in various AZF regions were observed. Alhathal et al[15] identified YCM during WES and reported YCM in 4 infertile men (2.5%). These studies from the Saudi Arabian population presented a prevalence range of 2.27-3.2%. The microdeletion prevalence from our study (3.1%) is closest to 3% and 3.2% as reported by Hellani et al.[13,14] Of these studies from Saudi Arabia, except Hellani et al,[13] all the studies including ours, investigated peripheral blood DNA from azoo- and oligozoospermic infertile men. However, Hellani et al[14] investigated sperm DNA from 80 teratozoospermic infertile men and 53 normozoospermic men (anonymous donors). Three key aspects that influence the comparative analysis of studies from Saudi Arabia with Hellani et al[13] are: i) Inclusion of normozoospermic infertile men. Microdeletions are defined by spermatogenic failure and the prevalence of microdeletions in normozoospermic infertile men is extremely low (less than 1%).[16] ii) Analyzing sperm DNA rather than blood DNA. Higher frequency of YCM is reported in sperm DNA as compared to that in blood DNA.[17,18] iii) Investigating infertile men with sperm morphology defects rather than most widely studied infertile men with sperm production abnormalities.

In addition to these 4 research studies, a recently published retrospective review by Alzahrani et al[19] has presented the prevalence of YCM in azoospermic cases from previous studies of Saudi Arabia. As per the interpretations of their study, the average prevalence of 2.2% (9 YCM cases of 408 NOA patients), suggest a low-reported condition stressing the need for further emphasis on genetic evaluation in male infertility. The construal of these reports from Saudi Arabia, affirm that YCM are an important genetic etiology of infertility in Saudi men and therefore their evaluation should be included in the work-up of male infertility particularly for azoo- and oligozoospermic men.

The reported presence and prevalence of YCM display vast heterogeneity among different ethnicities, populations, and geographical regions. Our study population is ethnically Arab and geographically belongs to the western region of Saudi Arabia. A number of previous studies have also reported their findings on YCM in the Arab populations of different Middle Eastern countries (Table 4). Qatar and Lebanon have comparatively lower prevalence of 1.11 and 2.48 as compared to 3.09 observed by us in the Arab population of Saudi Arabia.[20,21] In contrast, the Arab population of Iraq (40.7%), Iran (24.2%), and Egypt (20.4%) are reported to have a very high prevalence of YCM in their infertile men.[22-24] These significant large variations

Table 3 - Previous studies investigating Y chromosome microdeletions from Saudi Arabia.

Patients with microdeletion	Total patients	Type of microdeletion							%	Reference
		AZFa	AZFb	AZFc	AZFa,c	AZFb,c	AZFa,c	AZFa,b,c		
2	88	-	-	-	-	2	-	-	2.27	[12]
4	80	-	-	-	1	1	-	2	3.0	13
8	247	-	1	6	1	-	-	-	3.2	14
7	285	-	-	5	-	1	-	1	2.45	15
3	97	-	-	3	-	-	-	-	3.09	Present study

Table 4 - Prevalence of Y chromosome microdeletion in infertile men of different Middle Eastern countries.

Countries	Total no. of patients	Number of patients with microdeletions	Prevalences	Patients	Refs.
Qatar	179	2	1.11	Azoospermic and oligozoospermic	20
Lebanon	241	6	2.5	Azoospermic and oligozoospermic	21
Iraq	103	42	40.7	Azoospermic and oligozoospermic	22
Iran	99	24	24.2	Azoospermic and oligozoospermic	23
Egypt	54	11	20.4	Azoospermic	24
Tunisia	84	8	9.52	Azoospermic and oligozoospermic	25
Middle East, Syria, Iraq, Palestine, Lebanon, Jordan, Saudi Arabia, Qatar, and the United Arab Emirates	880	66	7.5	Azoospermic	26
Tunisia	146	10	6.85	Azoospermic and oligozoospermic	27
Iran	950	152	12.1	Azoospermic and oligozoospermic	28
Jordan	142	7	5	Azoospermic	29

in prevalence (1.11-40.7%) amongst the ethnically Arab infertile men, from different Middle Eastern countries, suggest regional differences in the pattern of microdeletion from the same ethnic population.

It is worthwhile to mention, that the Arab population from the Middle-East predominantly belongs to the J-haplo group of Y-chromosome, followed by G, L, H that are found in lesser proportions.[30,31] The J-haplo group is reported to be less susceptible to YCM due to its molecular characteristics.[32,33] Variation in the haplotype composition of the Arab population investigated in these studies from the Middle East could also be one of the potential reasons for differences in the prevalence of YCM. Other probable factors that could have affected the prevalence are differences in patient selection criterion, sample bias, sample size, and environmental/occupational factors of the analyzed population in these studies.

The STS markers, sY254 and sY255 found deleted in our 3 patients, lie within the DAZ gene, and corresponds to the AZFc region of Y-chromosome. No microdeletions in AZFa or AZFb were observed by us. Deletions in AZFc are the most frequently reported microdeletions, among all the AZF regions. They account for 60-80% of total cases of microdeletions.[4,34] Fortunately, although AZFc microdeletion is most prominent, it is also associated with highest sperm retrieval rates (50-80%) during TESE.[4,34] As already known and proven, that AZF genes are critical for successful spermatogenesis, the presence of 2 additional analogues of DAZ gene, referred as DAZL (Daz-like) located on chromosome 3, is proposed to compensate for sperm production loss in cases with AZFc microdeletion.[4] These autosomal DAZL gene copies may "salvage" spermatogenesis in patients with only AZFc deletion and therefore, facilitate spermatogenesis to the bare minimum, where rare sperms can be retrieved during TESE.[34-36] Therefore, prior information of a microdeletion, in particular for AZFc region, revealed by YCM assay, may assist in clinical decision-making for TESE and for downstream recommendations of ART.

During the diagnostic workup, one infertile man (CG-Y79-23) with AZFc microdeletion in our study, was found to have a chromosomal abnormality. He was reported to have 46,X, del(Y)(q11.223q12) chromosomal complement. Cytogenetic findings suggest terminal deletion of the whole heterochromatin region of the Y-chromosome along with a proximal adjacent euchromatin segment q11.223 which contains the AZFc gene. Therefore, our results for the YCM assay are consistent with cytogenetic findings and clinically corelates with sperm production defect.

Family history of the patient (CG-Y79-23) revealed that the proband had a brother with muscular dystrophy. Another interesting observation from the medical record was that this patient, already had a 4-year-old boy, conceived by TESE-ICSI. However, the latest TESE reports of the patient suggest absence of any kind of germ cells in the TESE aspirate. The latest testicular biopsy also suggests Sertoli cells only with Leydig cell hyperplasia. This disappearance of the germ cells (in testicular tissue) in our patient, could be due to progressive decline in spermatogenesis with time, which has also been reported earlier, in patients with AZF microdeletion, in particular with AZFc microdeletion.[37] As a result of this phenomenon, an oligozoospermic infertile man may become azoospermic with time. Generally, in such cases, infertile men with AZF microdeletions are advised to cryopreserve their germ cells retrieved by TESE.

Another clinically relevant concern that arises in such situations is the vertical transmission of YCM from father to son.[37] In a case report by Chang et al,[37] an identical AZFc deletion was observed in a father and his 4 infertile sons conceived by natural conception. However, at the time of analysis the father was found to be azoospermic, suggesting that father had some degree of fertility, which progressively reduced with time and ultimately there was a complete loss of germ cells in him. Unfortunately, the above-discussed infertile patient (CG-Y79-23) from our study did not consent for sample collection from his TESE-ICSI conceived son and therefore the status of YCM in the son could not be analyzed.

Inferences from previous studies, indicate an ambiguity in the correlation between hormonal levels and YCM. Kim et al[38] and Zhang et al,[39] reported no significant differences in the levels of follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T), or estradiol (E2) between infertile men with and without Y-microdeletions. On the contrary, Abid et al[40] reported that infertile men with YCM have significantly low testosterone, FSH, and elevated LH levels as compared to those without YCM.

Study strengths & limitations. In this study, we could not analyze the hormonal associations due to inaccessibility of hormonal data in the majority of our study population. However, considering the aforementioned, lack of clear and convincing relationship between hormonal levels and YCM, this limitation does not implicate on any findings or interpretations of our study. This study presents the prevalence of YCM in the ethnically Arab infertile men, from Al Madinah Al Munawarah region of western Saudi Arabia. It also discusses a comparative account of previously published data from Saudi Arabia and also with some studies from other Middle Eastern countries. The quintessence of this study is: i) YCM are present in a considerable percentage of azoo- and oligozoospermic men in Saudi Arabia; ii) Considering the prognostic value of YCM, particularly in patients with azoo- or oligozoospermia and also in men opting for TESE, the genetic testing for AZF deletions should be part of the standard diagnostic workup of infertility; and iii) Intra-ethnic differences in haplo types are probably an important contributory to the variation of prevalence in different studies from Middle Eastern countries. Therefore, larger studies from Middle East and particularly from Saudi Arabia should be carried out to understand the association of YCM and Y-chromosome haplo type(s) in the Arab population.

In conclusion, the prevalence of YCM in infertile men from Al Madinah Al Munawarah is comparable with other studies from Saudi Arabia. However, the comparison with prevalence from the Arabian population of other Middle Eastern countries reflects inconsistency due to several variable factors. Our findings reinforce the need and necessity of YCM screening in azoo- and oligozoospermic infertile males, especially in those opting for ART procedures.

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