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Diagnostic Screening for Microdeletion Frequency in the AZF-region of Y- Chromosome among the Emirati Infertile Males

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Abstract

A total of 154 Emirati infertile patients were included in this study of which a cohort of 18 infertile males undergoing fertility counseling were recruited from Dubai Fertility Center (DFC) and University Hospital of Sharjah (UHS), in addition to136 azoospermic Emirati infertile patients who performed AZF-deletion screening as counselled and recorded by DFC during the period of January 2011 and January 2021. Diagnostic screening was conducted to investigate the frequency of microdeletion in the Azoospermia Factor (AZFa, AZFb, AZFc, AZFd) gene cluster of the Y-chromosome among Emirati infertile male patients using the Sequences-Tagged-Sites (STS)-Multiplex PCR diagnostic technology. The frequency of the AZF microdeletions was 2.2% as detected in three patients with azoospermia (n= 3/136). There were two complete deletions in the AZFc region as shown by deletion of the two STS; SY254 (380 bp) and SY255 (124 bp). Our findings are similar to those reported in regional and global range, which appeared to vary across genealogical-lineages in different geographical landscape. This article is the first to profile AZF microdeletions in Emirati Azospermic males, offering valuable insights into male infertility genomics worldwide. The novelty and importance of the findings are especially relevant for improved healthcare and male infertility in Gulf countries due to shared tribal genetic lineage. This highlights the importance of the screening for the AZF-deletion for male infertility diagnosis for better management and treatments in the fertility clinics and hospitals in the UAE.

Keywords AZF microdeletion, Y-chromosome microdeletion, azoospermia, spermatogenesis, male-infertility, Emirati-males

1. Introduction

Recently, there has been worldwide growing interest on diagnostic screening of the microdeletions on the AZF region within the human Y chromosome (Yq11.23), which have been used as genetic marker, playing a fundamental role in male infertility. The AZF region, as coding for testes-specific functions, is divided into four subsidiary subregions, namely AZFa, AZFb, AZFc, and AZFd, all of which are associated in different stages of spermatogenesis process. This makes the human Y chromosome essential in reproductive biology ensuring continuity of human species. More importantly, the problem relies on the susceptibility of the AZF region to microdeletions leading to severe male infertility. The genomic structure of AZF region consists of palindromic and ampliconic sequences with high degree of homology, which make the region unstable, inducing breakage or stalling of the replication fork or even DNA double-strand break (DSB) (Xu and Pang, 2022). Such processes also involve activation of different types of repair mechanisms as Non-Allelic Homologous Recombination (NAHR) and Non-Homologous End Joining (NHEJ), which by their activation AZF microdeletions are promoted (Chang *et al.,* 2017; Cejka and Symington, 2021; Witherspoon *et al.,* 2021). Accordingly, microdeletions within the AZF gene cluster, recognized as the most common structural chromosomal abnormality, primarily cause a severe reduction in sperm quantity (azoospermia) rather than other sperm deformities (Witherspoon *et al.,* 2021; Rabinowitz *et al.,* 2021). In fact, a major challenge upon the infertility clinical practices is that the infertile males with AZF microdeletions usually appear phenotypically normal; for this reason, implementing of AZF screening is crucial in diagnosis and treatment of male infertility.

Moreover, the Emirati population is characterized by unique homogeneity with a highly conserved gene pool, owing to the high rates of consanguineous and endogamous unions (Al-Gazali and Hamamy, 2014; Elliott *et al.,* 2022). In a previous study, we reported the chromosomal abnormalities and clinical conditions associated with male infertility among Emiratis (Ebrahim and Mahasneh, 2022). However, to date and in comparison with other parts of the world, the microdeletion on the AZF gene cluster among Emirati infertile males has not been studied before. Therefore, the aim of this study was to investigate and genetically screen for the frequency of the microdeletions in the gene cluster AZFa, AZFb, AZFc and AZFd within the infertile male patients of the Emiratis. The utilization of the latest powerful technology of STS Multiplex-PCR analysis provides a novel approach to screen for most vulnerable sites of the AZF subregions using the international standard of the World Health organization and as reported worldwide by several researchers according to the recommendations of European

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Academy of Andrology/European Molecular Genetics Quality Network (EAA/EMQN) (WHO, 2021; Krausz *et al.,* 2023). Here, we present, for the first time, the microdeletion profiling of the AZF gene cluster among Emirati infertile males with a comparative discussion in comparison with other regional and global infertile male populations.

2. Materials and Methods

2.1. Subjects and Ethics

A total of 154 Emirati infertile patients were recruited in this study, compromising of a cohort of 136 male patients with azoospermia who performed AZF-deletion screening as counselled and recorded by DFC during the period of January 2011 and January 2021 in addition to 18 infertile males undergoing fertility counseling between April 2021 and January 2022 recruited from DFC and UHS. These are:

I. Dubai Fertility Center (DFC), Dubai, UAE:

Out of cohort of 18, 15 patients were recruited from DFC, of which 11 patients were azoospermic (61.1%; n=11/18;), four patients were non-azoospermic that involved three cases of severe oligoasthenoteratozoospermia (OAT) (16.6%; n= 3/18), and one case of asthenoteratozoospermia (5.5%; n=1/18). Furthermore, due to difficulty in blood collection and to obtain a clear picture of type and frequency of AZF deletion among Emiratis, we extended our study to cover the recorded data of the patients that referred to DFC for counseling as a 10-years retrospective cross-sectional study which included 136 patients. This part of the study was approved by Postgraduate committee of University of Sharjah (session 9; dated 3/2/2020), University Student and Resident Research Committee, Dubai Health Authority (Reference USRRC09-29/PG/2020) and Dubai Scientific Research Ethics Committee, Dubai Health Authority (Reference DSREC-SR-09/2020_03).

II. Sharjah Fertility Center in University Hospital of Sharjah (UHS), Sharjah, UAE:

Out of cohort of 18, three patients (non-azoospermic) were recruited from Sharjah Fertility Center in UHS, of which two cases showed severe oligozoospermia (11.1%; n=2/18), and one case showed oligozoospermia (5.5%; n=1/18). This part of the study was approved by Postgraduate committee of University of Sharjah (session 9; dated 3/2/2020) and University Hospital of Sharjah (Reference UHS-HERC-072-16092021).

2.2. DNA Extraction

Peripheral blood was collected from the 18 participants according to venipuncture method and transferred in cool packs within couple of hours to Molecular Biology Laboratory in University of Sharjah. Genomic DNA was extracted from blood lymphocytes using the DNeasy Blood & Tissue kit according to manufacturer's instructions. The purity and quantity of the eluted gDNA were estimated using NanoDrop™ One Microvolume UV-Vis spectrophotometer.

2.3. Diagnostic AZF Microdeletion Screening Protocol

Genomic DNA of each sample was screened for AZF microdeletions using 20 set of STS markers (table 1) as provided by the latest adapted method of Y Chromosome Deletion Detection system, version 2.0 kit (MD1531 Promega, USA), according the manufacturer's recommendation and EAA/EMQN (Krausz et al., 2023).

Amplification condition was based on the following program: 94 °C for 2 minutes, then 94°C for 1 minute (denaturation phase), 57°C for 30 seconds (annealing phase), 72°C for 1 minute (35cycles) (extension phase) followed by a final extension 72 °C for 5 minutes for multiplex master mix A, B, C and E, whereas for the multiplex master mix D, the annealing temperature was reduced to 56°C for 30 second. The generated electrophoretic bands were analyzed using Gel Doc EZ Imager and Image-Lab software (BIO-RAD, USA).

Table 1. The 20 set of STS markers used to screen for Y chromosome microdeletions as provided by Promega kit.

No.	region	STS	Locus	Size of Product (bp)
1.	AZFa	SY81	DYS271	209
		SY182	KAL-Y	125
		SY86	DYS148	232
		SY84	DYS273	177
2.	AZFb	SY121	DYS212	190
		SY124	DYS215	109
		SY127	DYS218	274
		SY128	DYS219	228
		SY130	DYS221	173
		SY133	DYS223	177
		SY134	DYS224	303
3.	AZFc	SY157	DYS240	290
		SY208	DAZ	140
		SY242	DAZ	233
		SY254	DAZ	380
		SY255	DAZ	124
4.	AZFd	SY145	DYF51S1	143
		SY152	DYS236	125
5.	SRY	SY14	SRY	400

2.4. Retrospective Cross Sectional Chart Review for AZF deletion

Retrospective cross sectional chart review for the 10-year period of study (2011–2021) was conducted depending on the medical reports of 136 Emirati infertile male patients who were azoospermic and conducted AZF deletion screening between January 2011 and January 2021 in DFC.

3. Results

3.1. Demographics and Characteristics of the Male Patients

The age of the 18 patients ranged from 24 to 52 years old, with an average of 32.2 years old and a median of 32 years, whereas the age of the 136 patients ranged from 24 to 66 years old with average of 38.0 years old and median of 37 years. All of the patients were of Emirati nationals from different geographical cities across the UAE.

3.2. Diagnostic Screening of the AZF-region among Emirati Infertile Male Patients

The frequency of AZF deletion was 2.2% (n=3) among the group of 136 azoospermic male patients of which complete AZFc deletion accounted for all deletions 100% (n=3/3). The deleted sequences and their size on the AZFc subregion were: SY254 (380bp) and SY255 (124 bp). The hormonal profiling and histological finding of the three unrelated azoospermic males with complete AZFc deletion and normal karyotype are given in Table 2. On the other hand, the cohort of 18 samples with normal karyotype had no AZF deletion as the tested Multiplex-PCR results are shown in Figure 1, 2 and 3.

Table 2. The hormonal profiling and histological finding of the three unrelated azoospermic patients with complete AZFc deletion as recorded by DFC.

Patient #	Testosterone (350.0-865.0) ng/dl	(LH) (1.7-8.6) mIU/ml	(FSH) (1.5-12.4) mIU/ml	Histological findings
#1	467.7	4.7	11.57	Varicocele
#2	346.7	6.2	16.47	Sertoli Cell Only syndrome and Varicocele
#3	538.6	10.7	17.22	Sertoli Cell Only syndrome

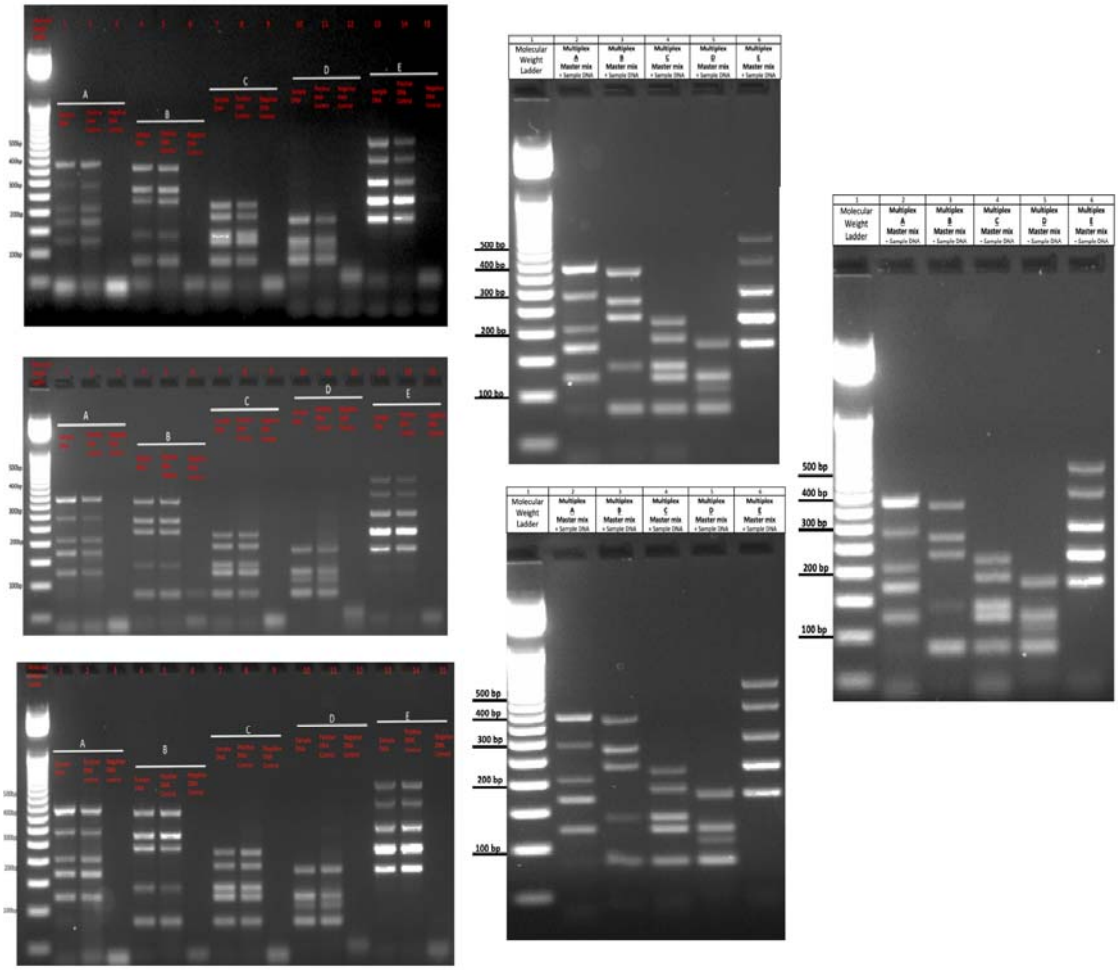
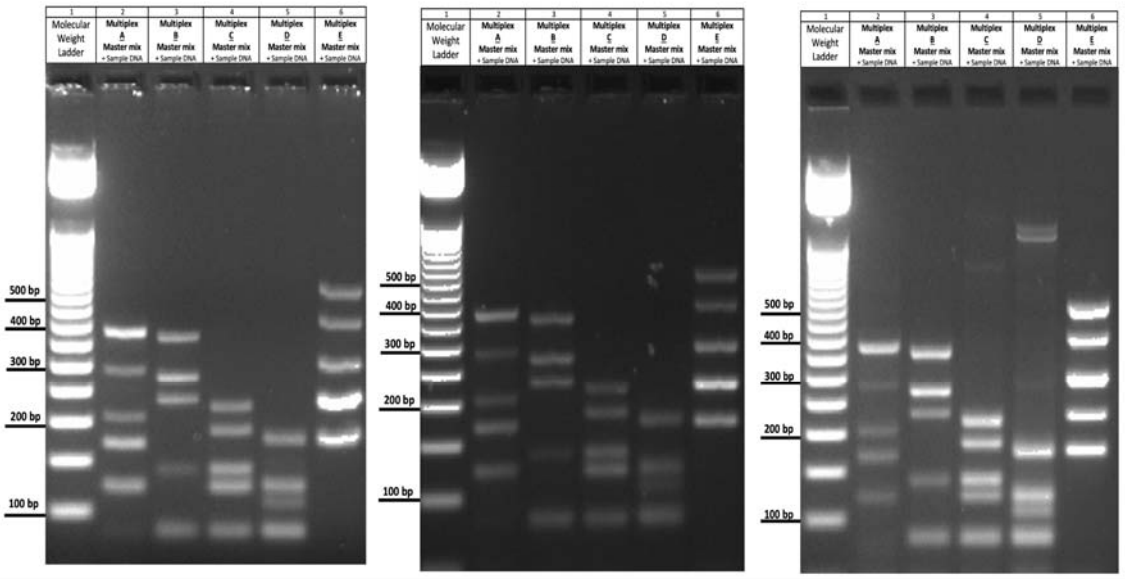
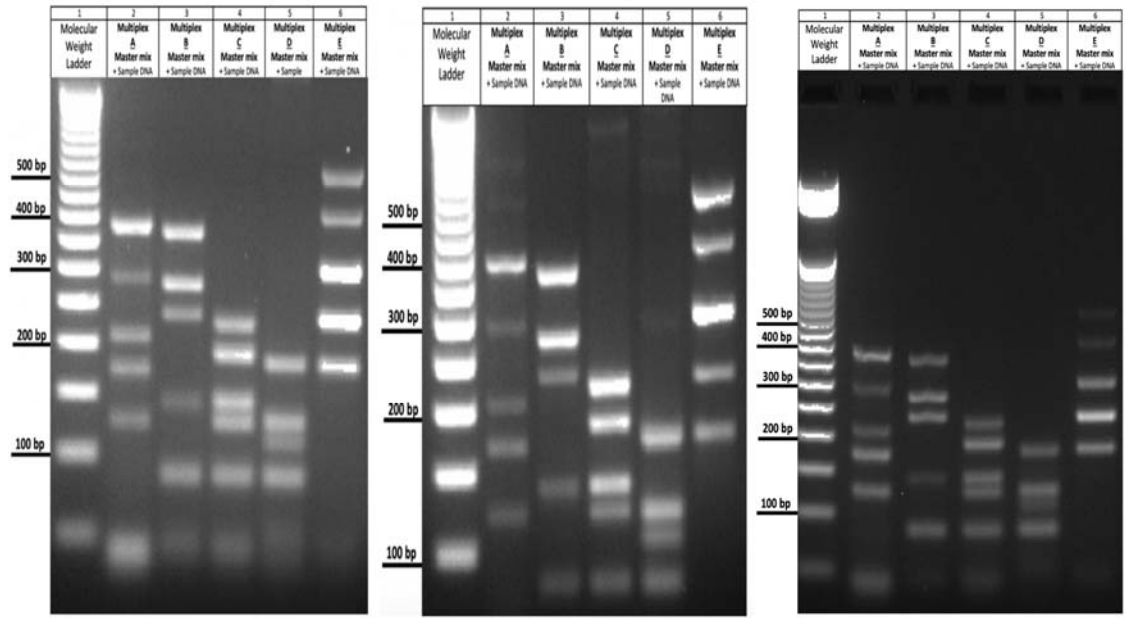


Figure 1. Diagnostic screening for the Y-chromosome showing no deletions in six selected tested patients with azoospermia. Lanes 1, 4, 7, 10 and 13 show the sample DNA with multiplex master mix; Lanes 2, 5, 8, 11 and 14 show the positive control (fertile male genomic DNA provided by Promega kit) with the multiplex master mix; Lanes 3, 6, 9, 12 and 15 shows the negative control (no DNA) with multiplex master mix.



(2.1) (2.2) (2.3)

Figure 2. Diagnostic screening for the Y-chromosome showing no deletions in three selected tested patients with severe OAT (2.1)(2.2) and asthenoteratozoospermia (2.3).



(3.1) (3.2) (3.3)

Figure 3. Diagnostic screening for the Y-chromosome showing no deletions three tested patients with severe oligozoospermia (3.1)(3.2) and oligozoospermia (3.3).

4. Discussion

4.1. Diagnostic Screening of AZF microdeletions among Emirati Infertile Male Patients

The novelty and importance of the article relies on being the first study to determine the profiling of the AZF microdeletions among Emirati Azospermic males which, therefore, underlines its significance not only across Emirates but worldwide on understanding the genomics of male infertility for a better healthcare treatment and management in the fertility clinics and hospitals. Besides, understanding infertility genetics is crucial for tailored treatments and better outcomes in fertility clinics. Our results reveal that the frequency (2.2%) of AZF microdeletions among Emirati azoospermic males is in consistence with several reported studies among Arab and non-Arab populations as it falls within the global range (Table 3). The variation among different studies is explained due to several factors such as influence of Y-haplogroups and genealogical-lineages, geographical

location, selection of inclusion criteria, protocol inconsistency and possible methodological errors. Our results showed that the AZFc microdeletion was the only type of deletion. In fact, AZFc deletion is reported as most frequent type among the AZF deletions as per different reported studies (Lahoz Alonso *et al.,* 2023; Arumugam *et al.* 2021; Akbarzadeh Khiavi *et al.,* 2020; Batiha *et al.,* 2018; Chabchoub *et al.,* 2019). This has been explained due to the genetic makeup of the AZFc region, making it more prone to deletions comparing to the rest of AZF regions (Witherspoon *et al.*, 2021; Xu and Pang, 2022).

Table 3. Comparison of frequency of AZF microdeletions among selected Arabic and global populations.

No	Population block landscape	Population	AZF microdeletions frequency (%)	Reference
1.	Gulf Council Arabs gene pool	United Arab Emirates	2.2%	The present study
		Saudi Arabia	2.27%	(Beg *et al.,* 2019)
		Qatar	1.11%	(Arafa *et al.,* 2018)
		Kuwait	2.6%	(Mohammed *et al.,* 2007)
2.	Fertile crescent Arabs gene pool	Jordan	4.93%	(Batiha *et al.,* 2018)
		Iraq	61.33%	(Al-Qaisi *et al.,* 2020)
		Iraq	47.8%	(Al-Janabi *et al.,* 2020)
		Lebanon	2.5%	(Degheili *et al.,* 2022)
		Syria	28.4%	(Al-Achkar *et al.,* 2013)
3.	North Africa Arabs gene pool	Tunisia	9.5%	(Chabchoub *et al.,* 2019)
		Morocco	9%	(Rochdi *et al.,* 2023)
		Sudan	58.8%	(Elsaid *et al.,* 2021)
		Algeria	1.3%	(Chellat *et al.,* 2013)
4.	Asia gene pool	India	2%	(Arumugam *et al.,* 2021)
		Iranian Azeri Turkish	32.05%	(Akbarzadeh Khiavi *et al.,* 2020)
		Turkey	5.5%	(Yavas *et al.,* 2023)
		Japan	6.79%	(Iijima *et al.,* 2020)
		Korea	14.1%	(Lee *et al.,* 2024)
		Bengal	16.1%	(Dutta *et al.,* 2022)
		China	3.31%	(Chen *et al.,* 2023)
		Mongolia	2.66%	(Damdinsuren *et al.,* 2022
5.	Europe gene pool	United Kingdom	4%	(Johnson *et al.,* 2019)
		Portugal	4.6%	(Pinho *et al.,* 2020)
		Spain	3.88%	(Lahoz Alonso *et al.,* 2023)

4.2. AZF Microdeletions among Emirati Infertile Males in Comparison with the that of the Gulf Cooperation Council (GCC) Arab Populations

Our study represents the most recent investigation on AZF deletion in the GCC region. The present findings have significant importance for the males in the Gulf countries as populations belongs more or less to close tribal genetic lineage. For instance, our results align with the reported data from other GCC populations such as Saudi Arabia, Qatar and Kuwait, which have high possibility in sharing gene pools with Emirati population. The range of the AZF microdeletions among males of GCC populations varies between minimum of 1.11% for Qatar to the maximum of 2.6% for Kuwait (Table 3 ; block 1). Additionally, to our knowledge, the most recent GCC study conducted by Beg *et al.* (2019), reported two cases (2.27%) of AZF microdeletions concerning AZFb+c deletions among a cohort of 88 infertile males in Western Saudi Arabia.

4.3. AZF Microdeletions among Emirati Infertile Males in Comparison with the that of the Fertile Arab Crescent Countries Populations

The range of the AZF microdeletions among males of Fertile Crescent Arab populations varies between minimum of 2.5% for Lebanon to the maximum of 61.33% for Iraq (Table 3; block 2). Batiha *et al.* (2018) presented frequency of 4.93% for the AZF microdeletions among 142 Jordanian azoospermic males, as the most frequent deletion was AZFc deletions (4.2%), followed by the AZFa deletions (2.1%) and then AZFb deletions (1.4%). On the other hand, Al-Janabi *et al.* (2020) reported higher frequency (47.8%) upon screening of 90 Iraqi infertile males in which AZFb microdeletion was most common (33.3%) followed by AZFc microdeletions (23%).

4.4. AZF Microdeletions among Emirati males in Comparison with the that of the African-Arab Populations

The range of the AZF microdeletions among males of African Arab populations varies between minimum of 1.3% for Algeria to the maximum of 58.8% for Sudan

(Table 3; block 3). Chabchoub *et al.* (2019) presented 9.5% of Tunisian infertile males with AZF microdeletions of which AZFc deletion was most common followed by AZFbc deletion and AZFb deletion.

4.5. AZF Microdeletions among Emirati Infertile Males in Comparison with the that of the Asian Populations

The range of the AZF microdeletions among males of Asian populations varies between minimum of 2% for India to the maximum of 32.05% for Iranian Azeri Turkish cohort (Table 3; block 4). Akbarzadeh Khiavi *et al.* (2020) reported frequency of 32.05% for AZF microdeletions among 100 Iranian Azeri Turkish infertile males as AZFc deletion was identified as most common deletion. On the other hand, Arumugam *et al.* (2021) conducted cross sectional study including 100 Indian infertile males, of which two cases (2%) were detected with AZF microdeletions concerning AZFc deletion only.

4.6. AZF Microdeletions among Emirati Infertile Males in Comparison with that of the European Populations

The range of the AZF microdeletions among males of European Populations varies between minimum of 3.88% for Spain to the maximum of 4.6% for Portugal (Table 3; block 5). Lahoz Alonso *et al.* (2023) reported frequency of 3.88% for AZF microdeletions among 644 Spanish infertile men of which AZFc deletion was identified as most common deletion.

5. Conclusions

In conclusion, this study sheds light on the frequency and profile of AZF microdeletions among Emirati infertile males, providing crucial insights into the genetic basis of male infertility in the region. The frequency of AZF microdeletions among Emirati infertile males falls within the regional and the global ranges as identification of only complete AZFc deletions underscores the necessity for comprehensive genetic screening in male infertility diagnostics. Comprehensive understanding of infertility genetics is crucial for improving outcomes of the patients. Therefore, according to the importance of our results in the Emirati healthcare services and clinical practice, we strongly recommend implementing the AZF diagnostic screening for the early prognosis, diagnosis, and treatment of male infertility.

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