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To cite this article: Haitham Elbardisi, Ahmad Majzoub, Sami Al Said, Khalid Al Rumaihi, Walid El Ansari, Alia Alattar & Mohamed Arafa (2018) Geographical differences in semen characteristics of 13 892 infertile men, Arab Journal of Urology, 16:1, 3-9, DOI: [10.1016/j.aju.2017.11.018](https://doi.org/10.1016/j.aju.2017.11.018)

To link to this article: <https://doi.org/10.1016/j.aju.2017.11.018>



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Published online: 18 Mar 2019.



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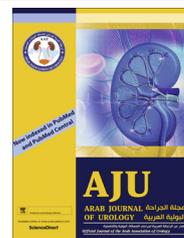
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EPIDEMIOLOGY  
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# Geographical differences in semen characteristics of 13 892 infertile men



Haitham Elbardisi<sup>a,\*</sup>, Ahmad Majzoub<sup>a</sup>, Sami Al Said<sup>a</sup>, Khalid Al Rumaihi<sup>a</sup>,  
Walid El Ansari<sup>b,c,d</sup>, Alia Alattar<sup>e</sup>, Mohamed Arafa<sup>a,f</sup>

<sup>a</sup> Department of Urology, Hamad Medical Corporation, Doha, Qatar

<sup>b</sup> Department of Surgery, Hamad General Hospital, Doha, Qatar

<sup>c</sup> College of Medicine, Qatar University, Doha, Qatar

<sup>d</sup> School of Health and Education, University of Skövde, Skövde, Sweden

<sup>e</sup> Department of Obstetrics and Gynecology, Hamad Medical Corporation, Qatar

<sup>f</sup> Department of Andrology, Cairo University, Cairo, Egypt

Received 22 August 2017, Received in revised form 11 November 2017, Accepted 27 November 2017

Available online 2 February 2018

## KEYWORDS

Male infertility;  
MENA;  
Semen quality;  
Sperm DNA fragmentation

## ABBREVIATIONS

ABF, abnormal sperm forms;  
MENA, Middle East and North Africa;  
PMot, progressive motility;  
SDF, sperm DNA fragmentation

**Abstract Objective:** To assess the relationship between geographical differences and all semen parameters, across 13,892 infertile men of 84 diverse nationalities, recruited at a specialised tertiary hospital that represents the main healthcare provider in Qatar. Male infertility is an important and global public health problem. Despite this, there is a significant scarcity of epidemiological male infertility and semen analysis research in the Middle East and North Africa (MENA) region, as well as geographical comparisons with other parts of the world.

**Patients and methods:** Retrospective study of semen findings of 13 892 infertile men assessed at the Male Infertility Unit at Hamad Medical Corporation, in Qatar between January 2012 and August 2015. Based on country of origin, patients were categorised into those from the MENA region ( $n = 8799$ ) and non-MENA patients ( $n = 5093$ ). The two groups were compared across demographic features and semen characteristics: age, sperm volume, sperm total motility, sperm progressive motility (PMot), abnormal sperm forms (ABF), and sperm DNA fragmentation (SDF).

**Results:** The whole sample's mean (SD) age was 35.7 (0.7) years, sperm concentration was  $32.3 (0.25) \times 10^6$  sperm/mL, total motility was 45.4 (0.2)%, sperm PMot was 25.1 (0.2)%, and ABF was 79.9 (0.2)%. Overall, 841 patients had azoospermia

\* Corresponding author at: Hamad Medical Corporation, P.O. Box 3050, Doha, Qatar.

E-mail address: [helbardisi@hamad.qa](mailto:helbardisi@hamad.qa) (H. Elbardisi).

Peer review under responsibility of Arab Association of Urology.



(6.05%), 3231 had oligospermia (23.3%), 4239 had asthenospermia (30.5%) and 6772 had teratospermia (48.7%). SDF (1050 patients) was abnormal in 333 patients (31.7%). MENA patients were significantly younger than their non-MENA counterparts and had a greater semen volume. Non-MENA patients had significantly higher sperm counts, total motility and PMot, and lower ABF. SDF showed no statistical difference between the two groups. MENA patients had significantly higher prevalence of oligospermia, asthenospermia, and teratospermia; and lower prevalence of normal sperm concentration, normal motility, and normal morphology. Throughout the 4 years of the study, MENA patients constantly had significantly lower sperm counts; generally lower sperm total motility percentage and generally lower quality sperm morphology. We compared patients by age ( $\leq 40$  and  $> 40$  years): in the patients aged  $\leq 40$  years, the same results as for the overall study were reproduced; in the  $> 40$ -years group, the same results were reproduced with the exception of morphology, which was not significantly different between the MENA and non-MENA patients.

**Conclusion:** Semen quality is generally lower in male infertility patients from the MENA region compared to non-MENA regions.

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## Introduction

The absence of conception over a period of 1 year in couples who are engaged in regular unprotected sexual intercourse indicates infertility. Infertility is a worldwide public health concern, affecting 15% of all couples of reproductive age; and male causes, including reduced semen quality, are solely responsible for  $\sim 25\%$  of these [1]. When infertility is suspected, couples usually undergo standard investigations including ovulation and tubal patency tests for women, and semen analysis for men. When the test results return normal, the couples are diagnosed with unexplained infertility, which is prevalent in 22–28% of the general population [2].

In cases of male infertility, a wide range of factors has been examined to assess their associations with semen parameters, including sperm motility, density, and morphology. For instance, demographic features e.g. age play an important role in male infertility. As men grow older, their testosterone levels are reduced leading to hypogonadism; their semen quality measurements show decreased sperm motility, viability, and semen volume [3]; and greater DNA damage has been observed in infertile men aged  $> 40$  years [4]. In addition, other genetic factors also affect men's fertility: genetic mutations manifested through anomalies and microdeletions of the Y chromosome can cause spermatogenesis failure, and thus lead to male infertility [5].

Lifestyle characteristics can also adversely affect men's semen quality. Lower sperm concentration and decreased total sperm count have been associated with obesity, whilst improved sperm progressive motility (PMot) is associated with eating healthy diets [6]. Moreover, obesity, stress, alcohol abuse, and smoking have

deleterious effects on sperm parameters and sperm DNA fragmentation (SDF) [7–9].

Similarly, environmental pollution, through exposure to chemical or physical agents produced by human activities such as pesticides, solvents and heavy metals, can alter sperm production and trigger hormonal imbalances, which in turn lead to infertility in men [10]. Furthermore, seasonal changes can affect semen quality, where studies have confirmed that men produce higher sperm count during winter or spring than in the summer [11].

Recently, an important emerging factor that has been reported to influence semen quality parameters is the geographical or regional differences. A study in Denmark compared semen concentration of men from a rural area to men from an urban setting, and reported a significantly higher sperm concentration amongst men from the rural area. However, the difference was attributed to sampling procedures rather than the geographical area per se [12]. Similarly, a study in France described significant differences across all seminal characteristics based on the geographical area from which the samples were collected. The seminal volume and total sperm count were lowest in Toulouse, and highest in Caen and Lille. However, sperm motility percentage was highest in Bordeaux and lowest in Tours [13]. Likewise, significant differences in total sperm count were reported amongst semen samples from four European countries (Finland, Denmark, France, and Scotland). Danish men had the lowest sperm concentrations whilst Finnish men had the highest [14]. Such geographical differences in semen characteristics as presented by these studies remain unexplained.

There is a notable lack of epidemiological studies on male infertility in the Middle East and North Africa (MENA) region, despite that the prevalence of infertility was reported to be higher in the MENA region with an incidence of 18.93% [15]. The fifth edition of the WHO semen analysis manual modified its reference values based on samples obtained from men with confirmed fertile status [16]. Nonetheless, the manual has been criticised for not examining samples from different parts of the world including the MENA region. This raises a range of questions regarding the applicability and validity of such new threshold values for MENA-region men.

To bridge this knowledge gap, therefore the present study evaluated the geographical differences in semen characteristics amongst different regions across the globe. Within the State of Qatar, recent major social, economic, and developmental changes have led to a steep increase in the inward migration of non-Qataris from all regions of the world, leading to a great shift in the demographics of the country. Doha has become a multicultural city inhabited by foreign residents from all around the world, where expats constitute ~75% of the general population [17]. Therefore, we compared the results of semen analysis of the residents in Qatar coming from MENA region countries to those of residents coming from other regions of the world (non-MENA countries). The study assessed the relationships between geographical differences and all semen parameters (including SDF), across 13,892 infertile men of 84 diverse nationalities, recruited at a specialised tertiary hospital that represents the main healthcare provider in Qatar.

### Patients and methods

This retrospective study assessed the semen findings of 13 892 infertile men evaluated at the Male Infertility Unit at Hamad Medical Corporation, in Qatar between January 2012 and August 2015. All infertile male patients attending the unit during this period were included in the study. Repeated patients who came for follow-up, and patients who received treatment prior to their semen analysis (e.g. antioxidants, empiric medical therapy, and surgical treatments including varicocelelectomy or seminal tract reconstruction) were excluded. The study was approved by the Institutional Review Board committee at our institute (Protocol No. 16065/16).

Patients were classified into seven regions according to the World Bank classification of countries by region [18]. The sample included patients from the MENA region ( $n = 8799$ ); and from South Asia ( $n = 1166$ ), East Asia and Pacific ( $n = 562$ ), Europe and Central Asia ( $n = 265$ ), Sub – Saharan Africa ( $n = 2981$ ), Latin America and the Caribbean ( $n = 36$ ), and North America ( $n = 83$ ).

Laboratory results for semen analysis and demographic data of all patients were retrieved and collected anonymously from their medical records. Patients from the MENA region ( $n = 8799$ ) were compared with those from the six other regions collectively i.e. non-MENA patients ( $n = 5093$ ) for age, sperm volume, sperm total motility, sperm PMot, abnormal sperm forms (ABF), and SDF. SDF assessment was introduced at our Infertility Unit in 2012 and is only undertaken in select patients with special characteristics and appropriate indications. It is not undertaken for patients with semen analysis showing azoospermia or a sperm count of  $< 5 \times 10^6$  sperm/mL; and it is usually undertaken for cases with expected oxidative stress (e.g. varicocele, pyospermia, obesity), or in cases with history of recurrent abortion or recurrent failure of *in vitro* fertilisation. Therefore, SDF assessment was performed for only 1050 patients, and hence for this particular SDF analysis, we compared 726 MENA with 324 non-MENA patients.

### Semen analysis protocol

Semen samples were collected by masturbation after 3–5 days abstinence from intercourse. The sample was left to liquefy after which analysis of the semen samples was conducted according to WHO 2010 protocols [16].

### SDF protocol

SDF was measured using Halosperm® G2 Test kit (Halotech DNA, SL, Madrid, Spain). This kit determines the degree of DNA damage of a human sperm through sperm chromatin dispersion process, which is responsible for male infertility. This process involves the denaturation and controlled lysis of the sample in an appropriate medium and can be used with both fresh and frozen samples. Sperm with intact DNA produce a dispersion halo as a result of the chromatin released from proteins that can be easily analysed using fluorescence or bright-field microscopy. In contrast, sperm with fragmented DNA will not produce this halo. The technique is as easy as a routine leucocyte count. In line with others, we used the Fernandez protocol, where an SDF level threshold of  $> 30\%$  was taken as high [19].

### Statistical analysis

Each patient was given a code number. Qualitative and quantitative measurements were summarised using frequency with percentage and mean  $\pm$  SD. Descriptive statistics summarised the demographic and clinical characteristics of the patients for each group respectively. For comparisons, the unpaired *t*-test was used for continuous variables, whilst the chi-squared test was used for categorical variables. A  $P < 0.05$  was considered

statistically significant. All statistical analyses were undertaken using the Statistical Package for the Social Sciences (SPSS®, version 19.0; SPSS Inc., IBM Corp., Armonk, NY, USA).

## Results

The 13 892 infertile men recruited in this study represented 84 different countries. Their percentage distribution amongst the regions from which they came from was MENA 63.3%, Sub-Saharan Africa 21.5%, North America 8.4%, East Africa and Pacific 4%, Europe and Central Asia 1.9%, South Asia 0.6%, and Latin America and the Caribbean 0.3%.

The patients' mean (SD) age was 35.7 (0.7) years. For the whole sample, semen parameters revealed a mean (SD) sperm concentration of  $32.3 (0.25) \times 10^6$  sperm/mL, total motility of 45.4% (0.2)%, sperm PMot of 25.1 (0.2)%, and ABF of 79.9 (0.2)%. Overall, 841 patients (6.05%) presented with azoospermia, 3231 (23.3%) with oligozoospermia, 4239 (30.5%) with asthenozoospermia, and 6772 (48.7%) with teratozoospermia. Across 1050 patients, SDF analysis was performed and showed abnormal findings in 333 patients (31.7%).

A comparison of the results of semen analysis between our MENA region and the non-MENA region patients is presented in Table 1. Patients from the MENA were significantly younger than non-MENA and had greater semen volume. However, the non-MENA patients had significantly higher sperm count, total motility and PMot, and lower ABF. SDF analysis showed no statistical difference between the two groups.

Further examination of semen analysis findings showed a significantly higher prevalence of oligozoospermia, asthenozoospermia, and teratozoospermia amongst MENA patients when compared to those from the non-MENA region. The prevalence of normal sperm concentration, normal motility, and normal morphology was lower amongst these MENA patients compared to non-MENA regions. However, azoospermia was more prevalent in non-MENA region patients (Table 2).

**Table 1** Sperm analysis of infertile men: the MENA region compared to non-MENA ( $n = 13,892$ ).

Variable, mean (SE)	MENA $n = 8799$	Non-MENA $n = 5093$	<i>P</i>
Age, years	35.06 (0.09)	36.73 (0.11)	< 0.001
Volume, mL	3.15 (0.02)	2.97 (0.03)	< 0.001
Count, $\times 10^6$ sperm/mL	29.77 (0.30)	36.85 (0.43)	< 0.001
Total motility, %	44.96 (0.26)	47.30 (0.33)	< 0.001
PMot, %	24.60 (0.25)	25.73 (0.33)	< 0.001
ABF, %	80.73 (0.25)	78.54 (0.33)	< 0.001
SDF, %	26.65 (0.69)	27.94 (0.95)	0.287

\* Analysis undertaken for 1050 infertile men with available data (726 MENA, 324 non-MENA).

**Table 2** Detailed sperm analysis of infertile men: the MENA region compared to non-MENA ( $n = 13,892$ ).

	MENA, $n$ (%)	Non-MENA, $n$ (%)	<i>P</i>
<b>Concentration</b>			< 0.001
Azoospermia	530 (6.0)	311 (6.1)	
Oligozoospermia	2331 (26.5)	900 (17.6)	
Normal Concentration	5938 (67.5)	3882 (76.2)	
<b>Total motility</b>			< 0.001
Asthenozoospermia	2282 (27.6)	1116 (23.3)	
Normal Motility	5987 (72.4)	3666 (76.7)	
<b>Morphology</b>			0.001
Teratozoospermia	4382 (49.8)	2390 (46.9)	
Normal Morphology	4417 (50.2)	2703 (53.1)	
<b>SDF*</b>			0.26
Abnormal	316 (43.5)	117 (36.1)	
Normal	410 (56.5)	207 (63.9)	

\* Analysis undertaken for 1050 infertile men with available data (726 MENA, 324 non-MENA).

To assess any temporal trends, we compared the semen analysis differences between MENA and non-MENA patients across different years. Table 3 shows the number of MENA and non-MENA patients across the different years of the study. Throughout the 4 years, the sperm count was constantly significantly less amongst MENA compared to non-MENA patients. In addition, all the other semen parameters showed differences across time. Sperm total motility percentage was generally lower amongst MENA patients across all the years under examination, but there were significant differences between the two groups only in 2014 and 2015. Sperm morphology was also generally of lower quality in MENA patients across all the years, although these differences were significant only in 2013.

We then compared patients aged  $\leq 40$  to those aged  $> 40$  years for the same set of semen parameters. In the  $\leq 40$ -years age-group, the exact same results as for the overall study were reproduced; whilst in the  $> 40$ -years age-group, the same results were again reproduced but with the exception of morphology, which was not significantly different between MENA and non-MENA patients (Table 4).

## Discussion

Geographic variation in semen quality between different regions has been examined over the past few years. However, there are no studies from the MENA area tackling this point. In the present study, we aimed to identify the differences in semen analysis of infertile male patients between two different geographical areas: MENA region vs non-MENA. Our present data revealed that patients from the MENA region had significantly lower quality semen parameter results including count, motility, and morphology compared with non-MENA region patients. This finding was con-

**Table 3** Number of infertile patients by year: the MENA and non-MENA regions.

Year	Infertile patients, <i>n</i>		Total, <i>n</i>
	MENA	Non-MENA	
2012	1582	919	2501
2013	2333	1278	3611
2014	2307	1417	3724
2015	2577	1479	4056

sistent, i.e. consistently observed across the different years of the study, and also across the different age groups. Our present findings are in agreement with others who similarly reported racial differences exist in semen quality at the time of infertility evaluation [20]. It is difficult to attribute such observed differences to a precise cause/s, but several propositions might contribute to explain such observed discrepancies in the quality of semen parameters between MENA and non-MENA infertile men. The causes that can contribute to low semen quality are meshed, interlacing, and difficult to isolate and attribute to.

In terms of diet, the MENA region has observed a radical change in diet during the last few decades, from traditional food consumption habits to more Western food consumption patterns. In recent decades, the dietary choices in the MENA region have dramatically changed from high intake of vitamins, minerals, fruits, vegetables, fibres, and proteins to an increased consumption of processed foods, sugars, fats, animal products, and alcohol [21]. Such changes are mainly due to the widespread introduction of Western fast foods to the MENA markets, in addition to the changes in lifestyle behaviours and globalisation effects. For instance, erosion of traditional Mediterranean diet where e.g. olive oil was a constant feature could negatively influence male fertility, given that olive oil partially counteracts the negative effects of a high-fat diet on sperm quality, by increasing gamete motility, reducing

oxidative stress, and slightly improving mitochondrial respiration efficiency in rats [22]. Likewise, a recent review reported that diets rich in processed meat, soy foods, potatoes, full-fat dairy and total dairy products, cheese, coffee, sugar-sweetened beverages, and sweets have been detrimentally associated with the quality of semen in some studies [23]. These dietary changes have been attributed to the increased prevalence of chronic, non-communicable conditions, metabolic-related diseases, and micronutrient deficiencies [23].

As for physical activity, there is strong evidence indicating that men who have average physical activity levels over sustained periods of 10 min are likely to have better semen quality than men who engage in low or high levels of such activity [24]; and that increased testicular temperature because of body habitus and inactivity impairs spermatogenesis [25]. Residents of the MENA region have experienced a remarkable change in their lifestyle and physical activity levels, with resultant obesity and diabetes that have affected their demographic, socioeconomic, and health status over the past 30 years. MENA populations have reduced physical activity levels and increased prevalence of obesity, mainly due to changes in nutrition habits and improved access to modern facilities that have contributed to sedentary lifestyles [26]. Indeed, it is estimated that ~33% of the MENA population is obese, and another 33% are at a high risk of developing cardiovascular diseases, diabetes, and hypertension [27]. Such increased obesity rates may have largely contributed to the observed reduced sperm quality in MENA countries, particularly as recent evidence suggests that multiple interdependent mechanisms contribute to the damaging effect of obesity on male fertility [25]. In addition, diabetes has been strongly associated with infertility in men [28], and recent reports found that the prevalence of diabetes in the Arabic speaking countries ranged between 4% and 21% [29], with a prevalence of 16.7% in Qatar [30]. Such diabetes prevalence is high compared to the global prevalence, and could be attributed to the high rate of

**Table 4** Semen analysis comparing MENA and non-MENA infertile patients by age.

	Age ≤ 40 years ( <i>n</i> = 10 156)			Age > 40 years ( <i>n</i> = 3736)		
	MENA, <i>n</i> (%)	Non-MENA, <i>n</i> (%)	<i>P</i>	MENA, <i>n</i> (%)	Non-MENA, <i>n</i> (%)	<i>P</i>
<b>Concentration</b>	<i>n</i> = 6565	<i>n</i> = 3545	< 0.001	<i>n</i> = 2234	<i>n</i> = 1548	< 0.001
Azoospermia	371 (5.7)	207 (5.8)		159 (7.1)	104 (6.7)	
Oligozoospermia	1720 (26.2)	619 (17.5)		611 (27.4)	281 (18.2)	
Normal concentration	4474 (68.1)	2719 (76.7)		1464 (65.5)	1163 (75.1)	
<b>Motility</b>	<i>n</i> = 6157	<i>n</i> = 3341	< 0.001	<i>n</i> = 2112	<i>n</i> = 1441	0.04
Asthenozoospermia	1646 (26.7)	724 (21.7)		636 (30.1)	392 (27.2)	
Normal motility	4511 (73.3)	2617 (79.3)		1476 (69.9)	1049 (72.8)	
<b>Morphology</b>	<i>n</i> = 6612	<i>n</i> = 3544	< 0.001	<i>n</i> = 2187	<i>n</i> = 1549	0.151
Teratozoospermia	3196 (48.3)	1578 (44.5)		1186 (54.2)	812 (52.4)	
Normal morphology	3416 (51.7)	1966 (55.5)		1001 (45.8)	737 (47.6)	
<b>SDF (<i>n</i> = 1050)</b>	<i>n</i> = 525	<i>n</i> = 222	0.14	<i>n</i> = 201	<i>n</i> = 102	0.46
Abnormal	215 (40.9)	67 (30.2)		101 (50.2)	50 (49)	
Normal	310 (59.1)	155 (69.8)		100 (49.8)	52 (51)	

consanguineous marriages [31], as well as obesity and sedentary life style in the Arabian Gulf region.

In terms of health literacy, health consciousness and health awareness, people from the Western world seem to generally have a higher sense of awareness of the negative impacts of adopting unhealthy lifestyles and are undertaking more efforts to change their lifestyle behaviours, unlike the populations in the MENA region. A study examining the temporal and regional trends in the prevalence of healthy lifestyles in the USA (1994–2007) revealed a slight increase in the prevalence of healthy lifestyle behaviours over time. This was reflected by citizens having a healthy weight, not smoking, consuming fruits and vegetables, and engaging in physical activity [32].

From the environmental aspect, the MENA region is known to be a large arid region susceptible to impacts from climate change, including the deterioration of water quality, contamination of groundwater aquifers, high temperature increases, reduced precipitation, and salinization of agricultural land. With the absence of strict policy reforms, the MENA nations can do more in terms of protecting their water resources [33]. In addition, the MENA region is also vastly reliant on hydrocarbon resources and has increasing energy and carbon concentrations, which is not the case in other developed countries [33]. A recent report published in 2013 found that CO<sub>2</sub> emissions in the MENA region were higher than the non-MENA, and thus MENA nations seem to be contributing more to environmental pollution [34]. In this case, the population in this region is highly exposed to environmental pollutants and faces increasing temperatures due to the global warming effect, which in turn largely affect the male reproductive system of the population [35].

In terms of consanguinity, the MENA region is characterised by high frequency of consanguineous marriages and a variety of ethnic groups, which reflects a distinct genetic pool of the population. The primary impact of such ‘inbreeding’ is genetic-related diseases with an increase in the incidence of recessive diseases [36]. This could be a strong potential cause of genetic mutations leading to spermatogenesis failure in men. In agreement, others have described a range of new genetic mutations in siblings of consanguineous marriage [37]. Further studies on the common polymorphisms in the MENA region are needed to better understand the potential genetic polymorphism/s that could be inducing SDF [38].

Impacting factors such as the climate, pollution, diet, lifestyle behaviours, chronic diseases, and genetics have previously shown their potentially negative effect on the male reproductive system. Therefore, the results reflect the cultural and environmental differences existing between the MENA region and the non-MENA, which

may lead to disparities in semen quality parameters. We, as others, note that the WHO did not include any country from the MENA region in its reference values for human semen characteristics [16]. The findings of the present study could be a great motive to include them.

### *Limitations*

The study had limitations. A small proportion of the expats who were recruited in Qatar may have been born in and lived all their life in Qatar, in which case such recruits may not have been exposed to the environmental and cultural factors existing in their original countries. Therefore, such residents do not exactly represent their own populations. Whilst such a possibility could represent a minute fraction of the sample, the large number of patients included from each region helps in adjusting any minimal bias that could arise from such a possibility. Our non-MENA group was heterogeneous, limiting our ability to accurately compare the MENA and non-MENA groups in terms of their lifestyle and dietary factors that could influence semen parameters. The retrospective design of the study also has its limitations. Relationships represent associations and not causations; hence interpretation of the findings needs to be cautious. Data about patients’ lifestyle behaviours (e.g. smoking, nutritional patterns, and drinking habits), as well as their occupational and environmental exposure/s would have been beneficial. Likewise, clinical data about risk factors known to be associated with male infertility (e.g. varicocele, hypogonadism, seminal tract obstruction, endocrinopathies, and genetic abnormalities) and other comorbidities (e.g. obesity and diabetes mellitus) would have been useful. Future research would benefit from addressing such limitations.

### **Conclusion**

Our present findings suggest that geographical differences can be associated with different semen quality parameters in infertile men, particularly those of younger age. Such differences are difficult to explain but can be linked to the genetic, lifestyle, demographic, and environmental differences amongst the regions. Future research needs to compare semen quality of homogeneous populations in different geographical areas, in addition to assessing the environmental, biological, and lifestyle factors that may impact the reproductive health of young men.

### **Conflicts of interest**

None.

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