THE POTENTIAL GEOGRAPHICAL VARIATIONS IN SPERMOMGRAM PARAMETERS, STEROID HORMONES, AND ZINC CONCENTRATION AMONG JORDANIAN INFERTILE MALES

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INTRODUCTION

Male infertility is considered as a global public health problem. Despite this, there is a significant lack of studies concerning the effect of geographic factors on male infertility in the Middle East. Previous studies had suggested a geographic heterogeneity of semen parameters among infertile males, but the variation in spermogram techniques applied to some studies prevented common conclusions. Swan et al. utilized a standard protocol applied to different laboratories in different regions of the USA[1]. Their results revealed that men from rural areas have lower sperm counts in comparison to the urban dwellers. Regional differences in semen quality have been reported in Europe[2], India[3], Japan[4], and China[5]. With regard to studies conducted in the Arab regions, a study published in Saudi Arabia used WHO reference values revealed a high prevalence of abnormal semen parameters among Saudi infertile men. In Riyadh city, half of semen samples had single factor abnormality[6]. A study conducted for subfertile Tunisian and Algerian men reported a declining in the semen quality and thus male fertility over past decades[7]. It is obvious to ask why these differences exist and in what time span evolved and led to this heterogeneity in semen parameters. Many studies have found no association between variations in semen quality and location in rural or urban areas, or between cities with a considerable level of air pollution and those with less pollution[1].

However, some other epidemiological studies reported a possible relation between exposure to certain chemical pollutants in certain area and a variation in sperm parameters[8]. Data concerning the role of endocrine system and the decline of semen parameters are variable[9]. Recently, a worldwide decline in semen quality was observed and correlated to some environmental endocrine disruptors (EDCs)[10]. The persistent and continuous exposure EDCs has deleterious effects on the health and the reproductive system by interfering with the synthesis and mechanism of action of male steroid hormones. A variety of these chemicals may affect the reproductive functions causing some developmental anomalies which consequently have direct effects on semen quality[11]. Zinc (Zn) is one of the trace elements essential for normal spermatogenesis and play a critical role as antioxidant defense system. Beside its involvement in several integrated processes associated with hypothalamus-pituitary-gonadal axis, it has potentials effects on the germination, and fertilization[12]. In addition to its importance for the germ cells viability, it is expressed as a functional protein in testicular cells and plays an important role in the meiosis during spermatogenesis[13, 14]. Zinc is present both in spermatozoa and the seminal plasma[15] and its presence directly affects the sperm motility[16]. At low seminal plasma zinc level, a notable reduction in the spermatogenesis and low cellular testosterone[17] was observed.
Due to the prominent lack of studies concerning the epidemiological variation in seminal fluid in the Middle East region and the absence of such studies in Jordan, therefore, semen characterization in relation to various geographical areas was evaluated.

MATERIALS & METHODS

Study Groups

In this retrospective study, three hundred and twenty four infertile men were assessed at the Infertility Laboratory of Medical Hussain City as a part of infertility investigation between October 2016 and May 2017. The Hospital is serving the general population of Amman and referral patients from several other cities of Jordan. All laboratory tests were done with due permission of the ethical committee of the Institute and informed consent was taken from each patient. The patients with a history of hormonal treatment in the last 6 months, any scrotal pathology such as cryptorchidism, previous scrotal surgery such as orchietomy or varicocele ligation, abnormal liver and renal function tests, genital infections, any findings of obstructive azoospermia, or a systemic disease were excluded from the study. The men were asked to complete a questionnaire included detailed information on demography, reproductive history, consumption of tobacco and alcohol, medications, and previous or current diseases.

Seminal Fluid Samples

Semen specimens were collected through masturbation after 2-3 days abstinence. Samples were incubated for 30 min at 37°C for liquefaction. Upon liquefaction, one part of the seminal fluid was subjected to routine semen analysis including; volume, pH, sperm concentration, motility, viscosity, viability and morphology. However, to minimize variations in the assessment of sperm characteristics, samples were analyzed by the same well-trained lab technician using the same instruments and procedures. The other part was centrifuged at 1000 x g for 10 min and the seminal plasma was separated and stored at −70°C for further analyses.

Determination of Seminal Fluid Fructose

A standard Seliwanoff method was used to estimate the concentration of fructose in the seminal plasma. The principle depends on the formation of a pink color in the presence of fructose (ketoses) when heated with resorcinol in the presence of hydrochloric acid (ARCOMEX, Fructose S.F). The intensity of the color is proportional to the fructose concentration and was measured by spectrophotometer at 490 nm. The estima
tion of testosterone, estradiol, and prolactin concentration

Serum levels of testosterone, estradiol, and prolactin were estimated by a competitive chemoluminescent enzyme immunoassay which utilized specific antibody-coated polystyrene beads as a solid phase (IMMULITE 2000, Bio DPC, Los Angeles, CA, USA). Samples were incubated with alkaline phosphatase-labeled regent and the bound label was then quantified using a specific chemoluminescent substrate. Light emission was detected by photomultiplier tube, and the results were calculated for each sample. To determine the concentrations of unknown samples, a standard curve was constructed by plotting the absorbance values against the concentrations of the standards. The normal ranges of testosterone is 262–1593 ng/dl, estradiol <60 pg/ml, and Prolactin 2-18 ng/ml.

Determination of Zn in the seminal plasma

Atomic absorption spectrophotometer (AA 6650 Shimatzu) was used to determined the level of Zn in the seminal plasma. Frozen semen samples were liquefied at room temperature and digested in covered beakers in a fume cupboard with a 1:1 solution of ultrapure nitric acid with moderate heating. All laboratory glassware that has been used was previously treated with 10% nitric acid for 48 hours and rinsed with distilled-deionized water to eliminate possible traces of heavy metals. A standard curve was generated and the concentration of zinc was estimated by comparison with standard values covering different concentration ranges. Aqueous standards for plotting calibration graphs were obtained by serial dilution of stock solutions (1000 pg/ml of nitrates), in addition to the blank that was prepared in a similar fashion.

Statistical analysis

Statistical Package for Social Sciences (SPSS) version 19 was used in which frequency and percentages were used to describe study variables. Variables within the database were converted to four rank cases to make it possible to test two discrete variables using non-parametric statistics. The effects of independent variables were tested using Kruskal-Wallis test.

RESULTS

Seminal fluid parameters

The geographic distribution of the infertile males was demonstrated in Figure 1. The mean ages were 31.30±5.2 years with no significant differences in ages between males from different cities. The mean duration of infertility of the study group was 3.11±2.58 years (Figure 2). The descriptions of seminal fluid parameters for the infertile males are presented in (Table 1). Seminal fluid samples from different cities assessed according to the WHO criteria showed statistically significant differences in the means volume (ml) (3.72 ±2.55) with a range of (0.3-20), sperm density (x10⁹)/24.16 ±33.57 with a range of (1-181), non motile sperm (%) (43.85 ±24.18) with a range of (0-100), germ cells (%) (3.11±4.20) with a range of (0-23), fructose (g/dl) (321.09±92.91) with a range of (162-881), viscosity (0.23±0.45) with a range of (0-2), vitality (%) (59.84±18.79) with a range of (0-93), WBCs (x10⁶/ml) with a range of (1.4 ±2.53) (0-11), morphologically abnormal sperms (%) (78.75 ±34.85) with a range of (0-100). On the other hand, no significant differences were found in progressive motility (grade a+b), sperm tail defects, sperm head defects, and normal morphology. We noticed that Zarka city has the lowest sperm count, fructose level, progressive motility, and the highest non motile sperm percentages. However, Erbid and Ar Ramtha have the lowest semen volume, sperm viability, germ cell percentages, morphology percentages and viscosity.
FIGURE 1. Geographic distribution of infertile male in Jordan

FIGURE 2. Mean duration of male infertility (years).

TABLE 1. Variation in sperm parameters among infertile male lived in varies geographic area

<table>
<thead>
<tr>
<th>City</th>
<th>Sperm count 10⁶/ml</th>
<th>Sperm volume ml</th>
<th>WBCs %</th>
<th>Vialility</th>
<th>Non-prog. Mortany %</th>
<th>Immotile (d) %</th>
<th>Germ cells %</th>
<th>Fructose g/dl</th>
<th>Viscosity cm</th>
<th>Head defect %</th>
<th>Tail defect %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sahab</td>
<td>8.10±9.122</td>
<td>4.90±0.115</td>
<td>0.00±0.00</td>
<td>69.04±12.70</td>
<td>19.00±1.15</td>
<td>47.50±23.6</td>
<td>0.50±0.577</td>
<td>265.0±63.04</td>
<td>0.50±0.577</td>
<td>45.0±5.91</td>
<td>2.50±2.88</td>
</tr>
<tr>
<td>Jerash</td>
<td>28.48±3.93</td>
<td>3.68±1.674</td>
<td>0.77±1.83</td>
<td>56.72±2.67</td>
<td>18.77±12.580</td>
<td>38.23±22.4</td>
<td>2.92±9.23</td>
<td>357.3±64.9</td>
<td>0.00±0.00</td>
<td>71.0±31.28</td>
<td>6.31±4.47</td>
</tr>
<tr>
<td>Mafraq</td>
<td>29.47±3.904</td>
<td>6.50±9.643</td>
<td>0.30±1.34</td>
<td>65.33±17.48</td>
<td>20.67±6.199</td>
<td>59.67±17.8</td>
<td>9.50±7.20</td>
<td>315.5±25.4</td>
<td>0.17±0.389</td>
<td>63.83±30.74</td>
<td>12.17±10.50</td>
</tr>
<tr>
<td>Amman</td>
<td>32.67±52.14</td>
<td>3.33±3.395</td>
<td>1.28±2.88</td>
<td>60.39±7.157</td>
<td>29.67±12.500</td>
<td>41.39±16.04</td>
<td>2.22±7.37</td>
<td>328.4±48.43</td>
<td>0.22±0.422</td>
<td>71.94±27.07</td>
<td>5.78±5.71</td>
</tr>
<tr>
<td>Alghor</td>
<td>18.33±50.65</td>
<td>3.46±1.645</td>
<td>1.56±2.87</td>
<td>38.76±2007</td>
<td>19.67±20.89</td>
<td>22.89±28.7</td>
<td>4.11±6.13</td>
<td>318.6±107.4</td>
<td>0.22±0.428</td>
<td>43.3±40.07</td>
<td>3.78±4.49</td>
</tr>
<tr>
<td>Aljoun</td>
<td>36.55±21.08</td>
<td>2.95±1.789</td>
<td>0.75±1.00</td>
<td>63.50±8.40</td>
<td>31.5±16.37</td>
<td>37.25±14.5</td>
<td>3.0±2.875</td>
<td>344.3±69.68</td>
<td>0.25±0.447</td>
<td>79.88±49.41</td>
<td>8.5±5.29</td>
</tr>
<tr>
<td>Madaba</td>
<td>24.30±18.56</td>
<td>5.10±3.349</td>
<td>1.25±1.75</td>
<td>72.0±6.14</td>
<td>29.00±12.07</td>
<td>38.75±21.8</td>
<td>1.5±1.77</td>
<td>335.0±51.29</td>
<td>0.75±0.463</td>
<td>87.75±61.1</td>
<td>6.0±4.00</td>
</tr>
<tr>
<td>Al Salt</td>
<td>23.95±12.49</td>
<td>4.0±2.025</td>
<td>1.67±1.87</td>
<td>68.67±10.3</td>
<td>26.17±1.10</td>
<td>38.3±7.76</td>
<td>4.03±0.75</td>
<td>309.5±91.5</td>
<td>0.17±0.389</td>
<td>85.17±60.13</td>
<td>6.5±5.29</td>
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<tr>
<td>Zanqa</td>
<td>8.83±15.92</td>
<td>3.68±1.523</td>
<td>2.60±4.088</td>
<td>67.8±17.90</td>
<td>12.0±10.41</td>
<td>77.6±18.4</td>
<td>2.00±1.88</td>
<td>251.4±44.53</td>
<td>0.00±0.00</td>
<td>83.8±3.676</td>
<td>8.8±5.30</td>
</tr>
<tr>
<td>Erbil</td>
<td>9.95±10.47</td>
<td>2.36±1.371</td>
<td>0.50±1.56</td>
<td>62.50±7.9</td>
<td>21.38±15.75</td>
<td>54.13±28.6</td>
<td>1.75±2.408</td>
<td>302.6±57.77</td>
<td>0.63±0.719</td>
<td>58.3±45.83</td>
<td>6.7±5.40</td>
</tr>
<tr>
<td>Ramtha</td>
<td>10.0±11.48</td>
<td>4.40±0.461</td>
<td>5.10±4.03</td>
<td>48.5±8.08</td>
<td>14.50±13.279</td>
<td>73.50±24.8</td>
<td>0.50±0.577</td>
<td>261.0±103.9</td>
<td>0.00±0.00</td>
<td>45.0±51.96</td>
<td>2.0±2.30</td>
</tr>
</tbody>
</table>

**Estimation the level of serum testosterone, estradiol, and prolactin**

The mean level of testosterone, estradiol, and prolactin was 505.0± 65.818, 23.425± 11.36, and 9.846± 12.244, respectively. However, only estradiol showed a significant difference (p=0.043) with respect to different cities. Although there is a significant variation in the levels of the three hormones, their values appeared within the normal ranges (Figure 3).

**Estimation of Zinc level in the seminal fluid**

The detectable mean concentration of Zn in infertile male was 21.26±17.83 mg/dl (10.5- 170.6 mg/dl). However, the differences in zinc levels between different geographic areas appeared to reach a significant value (p=0.045) with a regression value of r = -0.111 (Figure 4). A significant correlation appeared between zinc concentration and plasma estradiol level (r=0.213, p=0.007), WBCs (r=0.274, p=0.001), volume (r=0.320, p=0.001), and tail defect (r= 0.240, p= 0.002).
Potential geographical variations in spermogram

**DISCUSSION**

To understand the likely effects of the geographic heterogeneity on male reproductive status, this research was conducted to study the quality of seminal fluid in different Jordanian cities. A significant variation was observed over different geographic areas in Jordan in particular, sperm count, semen volume, germ cells, and viscosity. In a country like Jordan, which exhibits a heterogeneous population with different socioeconomic status, varied geographic patterns, seasonal climatic conditions, and different lifestyle, it was expected to find some variation in semen analysis results which might influence the individual’s fertility status. The results demonstrate that seminal fluid of infertile male from Zarka, Sahab, Erbid, and Ar Ramtha has the lowest total sperm counts when compared to other cities. These areas resemble the middle (Sahab and Al Zarka), and the
northern part of Jordan (Erbid and Ar Ramtha). Amman and Zarqa are the most urbanized areas in Jordan, while the northern area (Mafraq, Jarash, and Ajloun) and southern area (Karak, Tafiela, Ma'an, and Aqaba) are less urban. Zarqa is located 15 miles (24 km) northeast of Amman and has the second largest population after Amman. It is the most polluted city in Jordan and considered as Jordan's industrial center since it occupied over 50% of Jordanian factories, including: Jordan Petroleum Refinery, steel and pipe factories, Al-Hussein Thermal Power Station, and Assamra wastewater treatment plant. The emissions of air pollutants from industrial activities in Al Zarqa, and Sahab, are the main source of air pollution in these areas, causing degradation of the air quality and adversely impact the public health. Epidemiological studies support the idea that environmental pollutants affect the process of gametogenesis leading to a defect and a drop in the reproductive capacities in exposed populations. Exposure to ambient levels of air pollutants is associated with low birth weight, prematurity, and decreased fertility in males. The reported levels of air pollution in Zarqa were exceeded the Jordanian standard for air quality and some studies proved that the effect of meteorological factors play a great role in influencing the air with gases and dust. Although some studies have found no association between variations in semen quality and location in rural vs. urban areas, or between a highly polluted area and those with less pollution, previous reports identified significant associations with air pollution for less than 24% motile sperm, less than 13% of sperm with normal morphology, and less than 29% of sperm with normal head shape. From the other hand, low sperm count noticed in the middle and northern part of Jordan, might attribute to the high germ cell apoptosis, which was associated with the presence of a significant low percentages of germ cells particularly observed in infertile male from Ar Ramtha and Sahab city (Table 1). Considering other sperm parameters, the mean percentage of sperms with a progressive motility showed a lower percentage mainly in Al Zarka and Ar Ramtha. Moreover, a statistically significant decrease in sperm normal morphology, and fructose concentration also was noticed in Ar Ramtha in comparison to other cities. Ar Ramtha is a transitional zone between the desert and the mountain region that receives the highest precipitation level in Jordan. Groundwater of Ar Ramtha region is locally contaminated, and in some wells the nitrate concentration is greater than 200 mg/l. A study by Els-Walik et al. was carried out to examine the possible adverse reproductive effects of water nitrate pollution on male rats. They find that a significant reduction in the weight of testis followed by a reduction in epididymal sperm number, testosterone, testicular antioxidant components, and superoxide dismutase occur after exposure of the experimental rats to nitrate. Meteorological data for Ar Ramtha indicates that air temperature is increasing at an annual rate of 0.02-0.06 °C/year. One general consequence is the deterioration in the scrotum ability as thermo regulator, which over long time scales may exert an adverse effects on the quality of the producing spermatozoa over, it is suspected that infectious disease may affect reproductive function (Dieterle, 2008). Diagnosis of male genital infection remains difficult and the methods used for detection of these bacteria are not standardized. Although high prevalence of genital infection among men and women has been noticed in both industrialized and developing countries, the relationship between this factor and semen quality decline has never been investigated (LaMontagne et al., 2004; Gdoura et al., 2008). Moreover, it is suspected that infectious disease may affect reproductive function (Dieterle, 2008). Diagnosis of male genital infection remains difficult and the methods used for detection of these bacteria are not standardized. Although high prevalence of genital infection among men and women has been noticed in both industrialized and developing countries, the relationship between this factor and semen quality decline has never been investigated (LaMontagne et al., 2004; Gdoura et al., 2008). Moreover, it is suspected that infectious disease may affect reproductive function (Dieterle, 2008). 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development of sperm, and countering the effects of other trace elements\[33\]. A well-designed follow-up studies are needed to consider the therapeutic effects of zinc supplementation in the treatment of male infertility particularly in Ar Ramtha city. A large number of environmental compounds have been shown to mimic the actions of hormones such as oestrogens, anti-oestrogens, androgens and anti-androgens\[34\]. Studies concerning the geographical variation, confirm the existence of certain environmental factors that might affect the physiology of sex hormones\[35\]. Males from Jordanian cities showed significant differences in the estimated level of steroid hormones. The mean values of testosterone and prolactin are within the normal ranges, however, the mean estimated estradiol value was higher than its normal range and showed a significant increase in Ar Ramtha city (mean=136.889 pg/ ml, p=0.03) (Figure 4). Some studies found that male testosterone level be affected by the grade of urbanization. For example, South African men had higher testosterone levels when living in urban than in rural areas\[36\]. Some geographical differences in the concentrations of serum sex steroids were reported in older men by Kehinde et al.\[37\]. They also reported that a significantly higher level of testosterone in men living in Western industrialized area versus men in pre-industrial area. Furthermore, Asian men had lower levels of glucuronidated androgen metabolites and those living in the Far East of Asia have a considerably lower level than those living in Western countries\[38\].

A limit epidemiological data on regional differences in semen quality from Asia was available. Understanding the causes of potential differences could yield new insights into environmental influences on sex steroid regulation. Steroid hormone also might be affected by trace element particularly Zn. Koithari and Chaudhari\[39\] showed that seminal plasma zinc levels are dependent on endogenous free testosterone. Thus, measurement of seminal plasma trace elements may serve as an accurate parameter to evaluate male fertility. Earlier studies specified some declines in the semen quality in some parts of the world. This seems to be related to geographical variations in a time-related scale\[30\]. However, some studies are not successful in the demonstration of changes in the semen quality among infertile men for a period of more than ten years \[40\]. The global temporal trend in semen quality is still in debate, so we need further studies to assess the effect of environmental factors on the quality of semen. Our study group was relatively small in each area, which might influence the results, affect their validity, and make it difficult to distinguish between a real effect and random variation. However, data obtained from the study should be used to design other confirmatory studies.

**CONCLUSION**

The significant geographic-related variation in semen quality, zinc, and hormonal level observed in this study has important implications with respect to male fertility. However, further studies utilize a large cohort with additional information’s on their occupations, socioeconomic conditions, Nutritional behaviors, lifestyle related factors are secured in order to confirm the findings and to recognize the effect of geographic diversity on the heterogeneity of semen quality.

**ACKNOWLEDGMENTS**

The authors are expressing much appreciation to Al-Balqa Applied University for the financial support of this work.

**Declaration of conflicting interest**

The author declare that there is no conflict of interest.

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