INTERNATIONAL JOURNAL OF SCIENCE AND NATURE

www.scienceandnature.org

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

Hala Ibrahim Al Daghistani*
*Department of Medical Laboratory Sciences, College of Sciences, Al-Balqa Applied University, Al-Salt, 19117 Jordan
*Corresponding Author's hala2002dagh@yahoo.com

ABSTRACT

In this study, the potential geographical differences in semen parameters, serum steroid hormones, and Zinc levels among Jordanian infertile males were investigated. Three hundred and twenty four infertile males distributed in eleven Jordanian cities were enrolled in this study. The study groups were compared in terms of geographical distribution and spermogram parameters including: volume, count, motility grades, morphology, germ cells and fructose. Seminal zinc level was detected by Atomic Absorption Spectrophotometer and serum steroid hormones by a competitive chemoluminescent enzyme immunoassay. Seminal fluid samples showed significant differences between cities in the mean volume (3.72±2.55 ml), sperm count (24.16±33.57 x10^6), morphology of sperms (4.83±4.68 %), non progressive motility (23.46±14.95 %), germ cells (3.11±4.20 %), fructose (321.09±92.91 g/dl), zinc level (21.26±17.83 mg/dl), and viscosity (0.23±0.45 cm). Ar Ranmtha and Al Zarka, particularly, revealed a significant decrease in sperm count, active motility, viability, morphology, germ cells, mean fructose level, and a significant increase in immotile sperm. Estradiol, prolactin, testosterone hormones, and zinc in Ar Ramtha were significantly different from other cities. Geographical differences can affect in one way or another nature of semen in infertile men and might be linked to the regular dietary intake of zinc, and steroid hormones. This study is an introduction to illustrate the geographical diversity to the heterogeneity of semen quality that can be used as an input for future studies.

KEY WORDS: Semen quality, steroid hormones, zinc, male infertility, geographic variation.

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 heterogenicity 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 Hussian 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 written 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 orchiopexy 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 [27]. 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 ×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 [30].

Estimation 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)[31, 32]. 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. Al1 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 ($x10^6$)(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 ($x10^s$/ ml) with a range of (1.4 ±2.53) (0-11), morpho-logicaly 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.

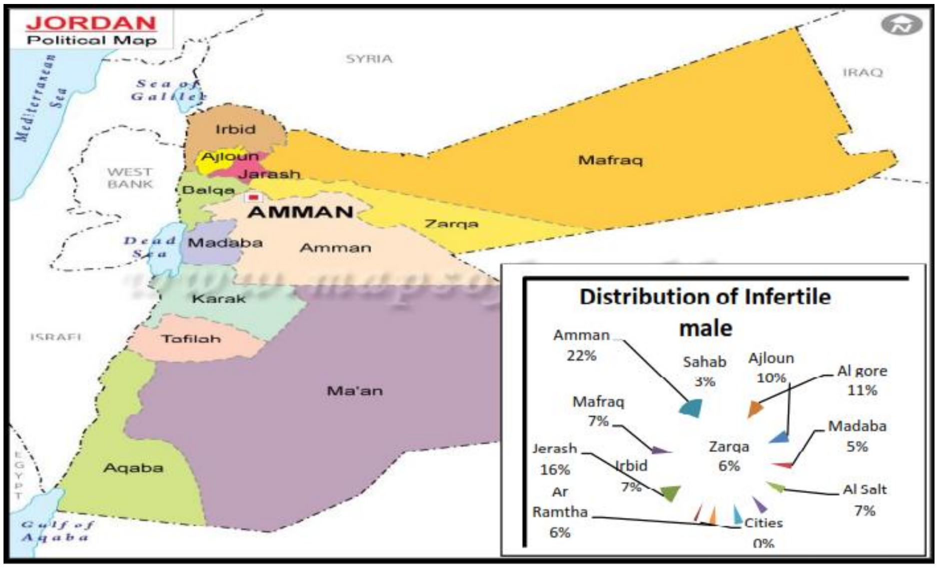

FIGURE 1. Geographic distribution of infertile male in Jordan

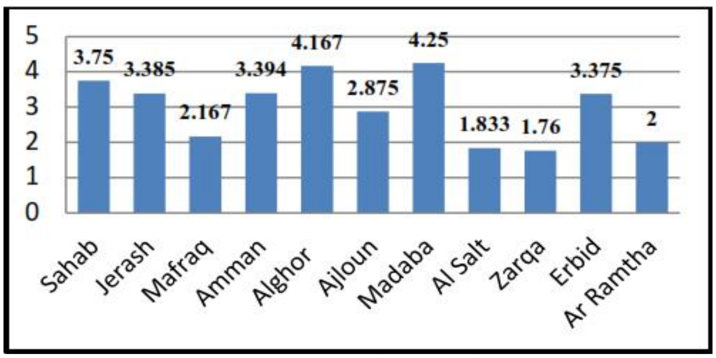

FIGURE 2. Mean duration of male infertility (years).

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

	SF count 10^6/ml Mean ±SD	SF volume ml mean ±SD	WBCs % Mean ±SD	Vibility % Mean ±SD	Non prog. Motility % Mean ±SD	Immotile (d) % mean ±SD	Germ cells % Mean ±SD	Fractose g/dl mean ±SD	Viscosity cm mean ±SD	Head defect % Mean ±SD	Tail defect % Mean ±SD
Sahab	8.10±9.122	4.900±0.115	0.00±.000	69.0±12.70	19.00±1.155	47.50±23.6	0.50±0.577	265.0±6O.04	0.50±0.577	45.0±51.96	2.50±2.88
Jerash	28.48±34.93	3.685±1.674	0.77±1.883	56.77±26.67	18.77±12.580	38.23±24.4	2.92±4.923	357.3±164.9	0.00±0.00	71.0±31.28	6.31±4.47
Mafraq	29.47±30.94	6.500±6.643	3.00±4.134	65.33±17.48	20.67±6.199	59.67±17.8	9.50±7.280	315.5± 25.49	0.17±0.389	63.83±30.74	12.17±10.50
Amman	32.67±52.14	3.333±1.395	1.28±2.288	60.39±7.157	29.67±12.50	41.39±16.04	2.22±2.737	328.8± 48.43	0.22±0.422	71.94±27.07	5.78±5.71
Alghor	18.33±30.65	3.467±1.645	1.56±2.874	38.78±2907	19.67±20.89	22.89±28.7	4.11±4.613	318.6± 107.4	0.22±0.428	43.33±40.07	3.78±4.138
Ajloun	36.55±21.08	2.950±1.789	0.75±1.000	63.50±8.40	31.25±16.37	37.25±14.5	3.0±2.875	344.3± 69.68	0.25±0.447	79.88±9.41	8.50±5.29
Madaba	24.30±18.56	5.10±3.349	1.25±1.753	72.0±6.141	29.00±12.07	38.75±2.18	1.50±1.77	335.0± 51.29	0.75±0.463	87.75±7.611	6.00±4.00
Al Salt	23.95±12.49	4.00±2.025	1.67±1.875	68.67±10.33	26.17±11.01	38.33±7.76	4.0±3.075	309.5±91.59	0.17±0.389	85.17±6.913	6.67±4.45
Zarqa	8.83±15.92	3.680±1.523	2.60±4.088	67.80±17.90	12.00±10.41	77.60±18,4	2.00±1.88	251.4±44.53	0.00±0.00	83.80±3.676	8.80±5.30
Erbid	9.95±10.47	2.363±1.371	0.50±.516	62.50±7.79	21.38±15.75	54.13±28.6	1.75±2.408	302.6±57.77	0.63±0.719	58.38±35.83	6.75±7.40
Ramtha	10.05±11.48	4.40±0.461	5.10±4.503	48.0±8.08	14.50±13.279	73.50±24.8	0.50±0.577	261.0±103.9	0.00±0.00	45.0±51.96	2.00±2.30
P value	**0.028**	**0.04**	**0.012**	**00.1**	**0.003**	**0.005**	**0.023**	**0.022**	**0.03**	**0.001**	**0.01**

Estimation the level of serum testosterone, estradiol, and prolactin

The mean level of testosterone, estradiol, and prolactin was 505.0± 65.818, 23.425± 11.136, and 9.946± 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, but 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 21.261±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).

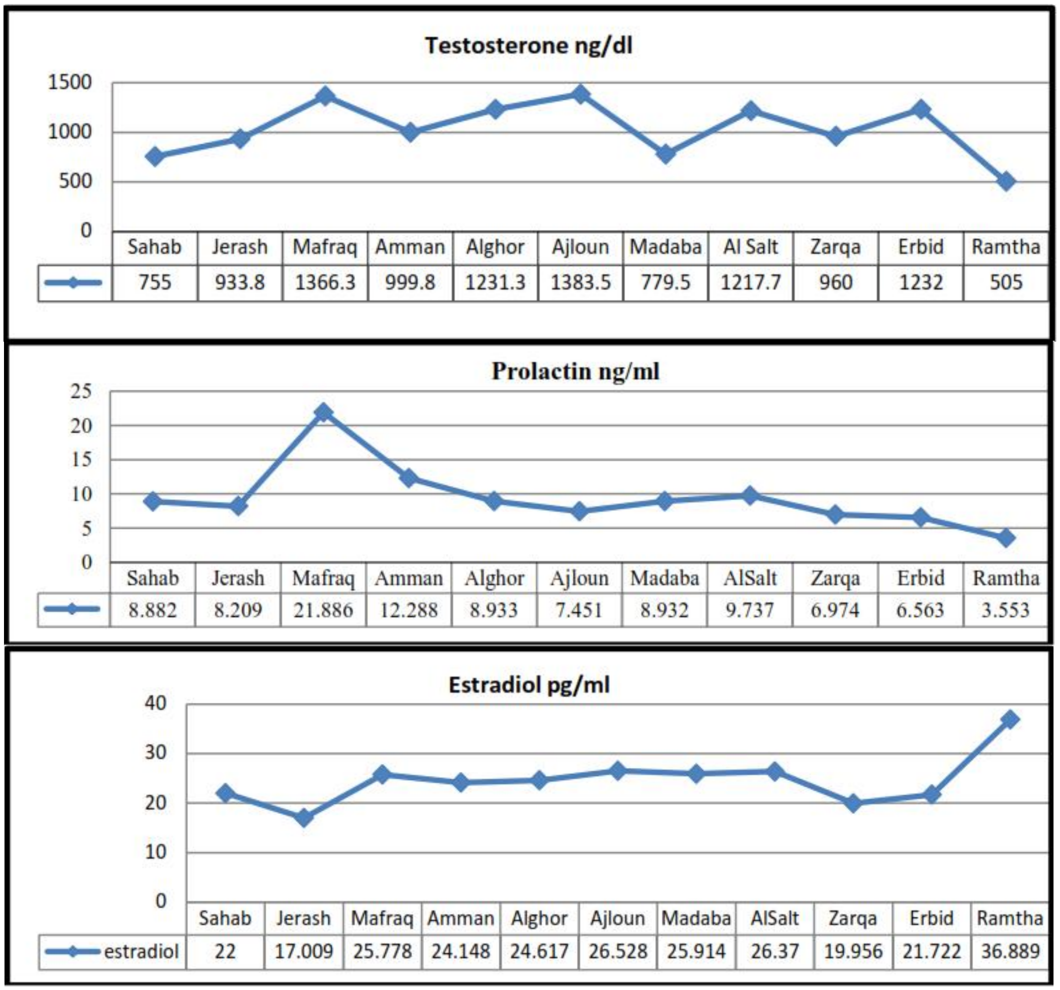

FIGURE 3. Variation in Testosterone (ng/dl), prolactin (ng/ml), and Estradiol (pg/ml) levels among infertile males with respect to different geographic area.

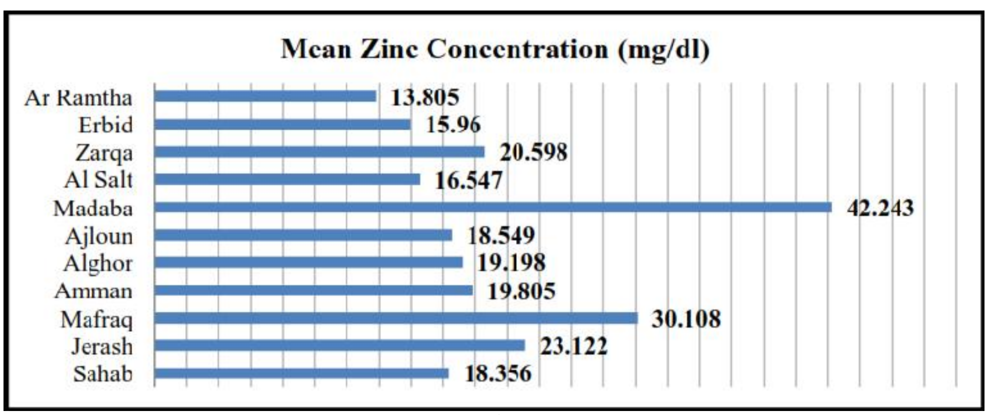

FIGURE 4. Variation in zinc concentration (mg/dl) among infertile male in relation to geographic distribution in Jordan

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 15miles (24 km) northeast of Amman and has the second largest population after Amman. It is the most polluted city in Jordan[22] 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[23]. Exposure to ambient levels of air pollutants is associated with low birth weight, prematurity, and decreased fertility in males[24]. 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[25]. 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[1], 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[26]. 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)[27]. 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-Wakf *et al.* [29] 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[29]. 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 p 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, it is suspected that infectious disease may affect reproductive function (Dieterle, 2008). it is suspected that infectious disease may affect reproductive function (Dieterle, 2008). Declines over time are not well understood. Prenatal exposure t o environmental toxicants, including estro- gens, has been postulated to be the main cause of the Some trace amount of metals are essential for male physiological homeostasis and reproductive function. However, excessive or insufficient concentrations of these metals might induce some toxicity and deficiency symptoms. One of the possible consequences of low zinc concentration is the decrease in the sperm quality [30]. Low zinc level was obviously associated with a decline in the quality of the seminal fluid especially as its affects the testicular function and cause hormonal imbalance[31]. It was not surprising to find that infertile male from Ar Ramtha recorded the lowest level of Zn in their seminal fluid (30.80 mg/dl) in comparison to other cities. It has been reported that seminal plasma Zn level in infertile males was correlated to the derangement of semen parameters, especially sperm motility, volume, count, and viability[32]. Although Zn is found in most types of foods such as red and white meat, fish, and milk, WHO estimates that one-third of the world's population is deficient in zinc. Both zinc and citrate are excreted from the prostate gland as a low-molecular-weight complex; thus, seminal plasma zinc levels are typically reflects the secretive efficiency of the prostate. The decrease in the seminal plasma zinc concentration may result from inadequate intake, absorption and loss problems, or increased demand. Additionally, the commonest worldwide cause is inadequate intake as a result of a diet low in Zn. The decrease in Zn content of the semen may generally affect the semen quality in different ways including; reduction in the antioxidant capacity, atrophy of seminiferous tubules, which directly influence the development of sperm, and counteracting 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. Kothari 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[18]. 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.

REFERENCES

[1]. Swan, S.H., Brazil, C., Drobnis, E.Z. Geographic differences in semen quality of fertile US males. Environ Health Perspect. 2003; 111:414-420.

[2]. Jorgensen N, Andersen AG, Eustache F. Regional differences in semen quality in Europe. Hum Reprod. 2001; 16:1012-9.

[3]. Adiga SK, Jayaraman V, Kalthur G. Declining semen quality among south Indian infertile men: A retrospective study. J Hum Reprod Sci. 2008; 1:15-18.

[4]. Iwamoto T, Nozawa S, Yoshiike M, et al. Semen quality of 324 fertile Japanese men. Hum Reprod. 2006; 21:760-765.

[5]. Gao J, Gao ES, Yang Q. Semen quality in a residential, geographic and age representative sample of healthy Chinese men. Hum Reprod. 2007; 22:477-84.

[6]. Alasmari1 W, Edris F, Albar Z, Gari A, Eskandar M, Al Fageah M, Zawawi S. High Proportion of Abnormal Semen Characteristics among Saudi Infertile Couples. Clinical Medicine and Diagnostics. 2018; 8(1): 14-20.

[7]. Feki NC, Abid N, Rebai A. Semen quality decline among men in infertile relationships: experience over 12 years in the South of Tunisia. J Andro. 2009; l30:541-547.

[8]. Wijesekara GUS, Fernando DMS, Wijerathna S, Bandara N. Environmental and occupational exposure to toxicants and sperm parameters of men investigated for infertility. Sri Lanka Journal of Obstetrics and Gynaecology. 2014; 1:32-36.

[9]. Jeng HA. Exposure to Endocrine Disrupting Chemicals and Male Reproductive Health. Front Public Health. 2014; 2:55-62.

[10]. Rehman S, Usman Z, Rehman S, AlDraihem M, Rehman N, Rehman I, and Ahmad G. Endocrine disrupting chemicals and impact on male reproductive health. Transl Androl Urol. 2018; 7(3):490–503.

[11]. Horan TS, Marre A, Hassold T, Lawson C, Hunt PA . Germline and reproductive tract effects intensify in male mice with successive generations of estrogenic exposure. PLOS. GENETICS. 2017;13(8): e1006980.

[12]. Lee SR. Critical Role of Zinc as Either an Antioxidant or a Prooxidant in Cellular Systems. Oxidative Medicine and Cellular Longevity. 2018; 2018:1-11.

[13]. Kumar N, Singh AK. Role of Zinc in Male Infertility: Review of Literature. Indian Journal of Obstetrics and Gynecology Research. 2016; 3(2):167-171

[14]. Shizuka M, Ohtsuka E. Inoue A. Abnormal spermatogenesis and male infertility in testicular zinc

finger protein Zf p318-knockout mice. Develop Growth Differ. 2016; 58: 600- 608.

[15]. Marzec-Wróblewska, U., KamińsKi, P., ŁaKota P. Influence of Chemical Elements on Mammalian Spermatozoa. *Folia Biologica* (Praha). 2012; 58:7-15.

[16]. Zhao J, Dong X, Hu X. Zinc levels in seminal plasma and their correlation with male infertility: A systematic review and meta-analysis. Sci Rep. 2016; 6: 1-10

[17]. Akinloye O, Abbiyesuku FM, Oguntibeju OO. et al. The impact of blood and seminal plasma zinc and copper concentrations on spermogram and hormonal changes in infertile Nigerian men. 2010; 11(2): 83-98.

[18]. World Health Organization, WHO laboratory manual for the Examination and processing of human semen. 5th ed. 20 Avenue Appia, 1211 Geneva 27, Switzerland, 2010.

[19]. Mann T. Fructose content and fructolysis in semen. Practical application in the evaluation of semen quality. J agrie Sci. 1948; 38: 323.

[20]. Vankrieken L. "IMMULITE reproductive hormone assays: multicenter reference range data," Document zb157-D, Diagnostic Products Corporation, Los Angeles, Calif, USA, 2000.

[21]. Abraham G (ed), Handbook of Radioimmunoassay, Marcel Dekker. New York, NY, USA, 1977.

[22]. Al Smadi BM, Al-Zboon KK, Shatnawi KM. Assessment of Air Pollutants Emissions from a Cement Plant: A Case Study in Jordan, 2009

[23]. Carré J, Gatimel N, Moreau J, Parinaud J, and Léandri R. Does air pollution play a role in infertility?: a systematic review. Environmental Health. 2017; 16:82

[24]. Veras MM, Caldini EG, Dolhnikoff M, Saldiva PH. Air pollution and effects on reproductive-system functions globally with particular emphasis on the Brazilian population. The J Toxicol Environ Health B Crit Rev. 2010 Jan;13(1):1-15

[25]. Odat SA. Diurnal and Seasonal Variation of Air Pollution at Al-Hashimeya Town, Jordan Department of Earth Science and Environment, Fac Environment,Hashemite University, Jordan. JJEES. 2009: 2(1);1- 6.

[26]. Sram RJ, Binkova B, Rossner P, Rubes J, Topinka J, Dejmek J. Adverse reproductive outcomes from exposure to environmental mutagens. Mutat Res. 1999; 428(1-2):203-15.

[27]. Paulina Urriola-Muñoz, Raúl Lagos-Cabré, Ricardo D., Moreno. A Mechanism of Male Germ Cell Apoptosis Induced by Bisphenol-A and Nonylphenol Involving ADAM17 and p38 MAPK Activation. PLoS ONE. 2014; 9(12):e113793.

[28]. El-Wakf AM, Elhabiby SM, El-kholy WM, and El-Ghany EA. Use of Turmeric and Curcumin to Alleviate Adverse Reproductive Outcomes of Water Nitrate Pollution in Male Rats. Nature and Science. 2011; 9(7):229-239

[29]. Abu Sada A, Abu-Allaban M, Al-Malabeh A. Temporal and Spatial Analysis of Climate 406 Change at Northern Jordanian Badia. JJEES. 2015; 7(2):87 - 93

[30]. Anderson JG, Cooney PT, Erikson KM. Inhibition of DAT function attenuates manganese accumulation in the Globus pallidus. Environ Toxicol Pharmacol. 2007; 2:179-184

[31]. Telisman S, Colak B, Pizent A. Reproductive toxicity of low-level lead exposure in men. Environ Res. 2007; 105: 256-266.

[32]. Shquirat WD. Al-Daghistani HI. Hamad AR, et al. Zinc, Manganese and Magnesium in Seminal Fluid and Their Relationship to Male Infertility in Jordan. International Journal of Pharmacy and Medical Sciences. 2013; 3 (1):01-10.

[33]. Jarosz M, Olbert M, Wyszogrodzka G, Młyniec K, and Librowski T. Antioxidant and anti-inflammatory effects of zinc. Zinc-dependent NF-κB signaling. Inflammopharmacology. 2017; 25(1): 11–24.

[34]. De Falco M, Forte M, and Laforgia V. Estrogenic and anti-androgenic endocrine disrupting chemicals and their impact on the male reproductive system. Front. Environ. Sci. 2015; 3:3. doi: 10.3389/fenvs.

[35]. Magee PJ. Rowland IR. Phyto-oestrogens, their mechanism of action: current evidence for a role in breast and prostate cancer. Br J Nutr. 2004; 91:513-531

[36]. 36. Gray PB, Kruger A, Huisman HW, et al. Predictors of South African male testosterone levels: the THUSA study. Am J Hum Biol 2006;18:123-132.

[37]. Kehinde EO, Akanji AO, Memon A, *et al.* Prostate cancer risk: the significance of differences in age related changes in serum conjugated and unconjugated steroid hormone concentrations between Arab and Caucasian men. Int Urol Nephrol 2006; 38: 33- 44.

[38]. 38. Litman HJ, Bhasin S, Link CL, et al. Serum androgen levels in black, Hispanic, and white men. J Clin Endocrinol Metab 2006; 91:4326- 4334

[39]. Kothari RP, Chaudhari AR. Zinc Levels in Seminal Fluid in Infertile Males and its Relation with Serum Free Testosterone. J Clin Diagn Res. 2016; 10(5): CC05–CC08.

[40]. Marimuthu P, Kapilashrami MC, Misro MM. Evaluation of trend in semen analysis for 11 years in subjects attending a fertility clinic in India. Asian J Androl., 2003; 5:221-5.