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STUDY THE RELATIONSHIP BETWEEN TRACE ELEMENTS AND HORMONES AMONG JORDANIAN INFERTILE WOMEN

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ABSTRACT

Background: Infertility is the inability of couples to achieve pregnancy after one year of unprotected intercourse. Different causes are associated with female infertility including ovulation, hormonal failure, tubal damage, in addition unexplained causes of infertility. Therefore, this study was initiated to determine the relationship between serum hormonal profile (follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol β-17, and testosterone. **Methods:** Hormones were detected by Competitive Chemoluminescent Enzyme Immunoassay and trace elements (Zn, Cu, Cr, Mg, Mn, Fe, and Se) measured by atomic absorption in infertile and fertile women. One hundred and thirty eight females including 88(63.8%) infertile females, [50 (56.8%) cases with primary infertility and 38 (43.2%) with secondary infertility], and 50 (36.2%) fertile were enrolled in this study. The duration of infertility was 7.61±4.42 years and the mean ages of the infertile females were (30.68±6.86) years. **Results:** No significant relation was observed between smoking habits and female infertility. The most common cause of female infertility was ovulation and hormonal factors (52.6%), followed by tubal factor (19.2%). However, 28.2% of the females showed no apparent causes of infertility (unexplained infertility). Hormonal study showed that the infertile females exhibit a significant reduction in the mean serum level of FSH, LH, and estradiol as compared to fertile, whereas the mean testosterone level was significantly higher in infertile group. In addition, the mean testosterone, estradiol β-17, and FSH concentrations were higher among non-smoker females. The current study showed a significant decrease in the mean concentration of Zn, Cu, Mg, Fe, Cr and Mn in the serum of infertile females in compared to that observed in fertile. The mean serum concentration of Zn, Cu, Mg, Mn, Fe, Cr, Se, among infertile females was: 795.87ug/L, 1091.89ug/L, 8.78mg/ml, 693,97mg/L, 31.83ng/ml, 86.88ng/ml, and 11.17mg/ml, as compared to 1176.4ug/L, 1713.0ug/L, 14.95mg/ml, 1223.40mg/L, 73.92ng/ml, 124.46ng/ml, and 20.27mg/ml in fertile females, respectively. However, the mean trace elements level in infertile females with primary or secondary causes doesn't show any significant differences. No statistically significant effect of smoking on trace elements concentration in females with infertility. **Conclusion:** a significant increase in testosterone and a significant decrease in estradiol, FSH, LH was observed among infertile female. A significant reduction in Zn, Cu, Mg, Fe, Cr and Se was observed in infertile.

KEYWORDS: follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol β-17, testosterone, Zinc (Zn), Copper (Cu), Chromium (Cr), Magnesium (Mg), Manganese (Mn), Iron (Fe), and Selenium (Se).

INTRODUCTION

Infertility is becoming a spreading phenomenon among women.[1] A high percentage of women infertility causes are related in a way or another to the ages.[2] Between 15 - 30% of couples have no particular explain or reason for their infertility (The Practice Committee of the American Society for Reproductive Medicine (ASRM), 2006). Women over the age of 35 years are about twice more likely to be diagnosed with unexplained and tubal factor infertility, compared with women with age less than 35 years. On the other hand, the diagnosis of ovulatory dysfunction is reduced in women older than 35 years.[2]

Several parameters may play a role in infertility problem such as age, life style, obesity, drugs, physiological problems, psychological stress, transmitted diseases, environmental factors, and biochemical factors such a trace elements, enzymes, hormones, and others. Trace elements can cause infertility by affecting many biological pathways inside the body. Zinc has a role to play in regulating reproductive hormones, in the absence

of sufficient amount of Zn, egg maturation is disrupted. Follicle fluid levels also rely on Zn to transport the egg through the fallopian tubes. Deficiency of Zn has a direct effect on protein synthesis in the cell[4] and is associated with hypogonadism and infertility.[5]

Copper one of the most common mineral imbalances contributing to infertility. This may directly affect infertility rates by lowering progesterone levels, resulting in anovulation, implantation failure or luteal phase defects.[6] Copper has also been shown to block the absorption of many essential minerals that are directly involved with reproductive pathways, especially zinc.

Selenium is essential for growth.[7] Selenium deficiencies may lead to gestational complications, miscarriages and the damaging of the nervous and the immune systems of the fetus.[8] In addition, a low concentration of Se in blood serum in the early stage of pregnancy has been proven to be a predictor of low birth weight of a newborn. Chromium is a vital for cellular structure and for fetal growth.[7] Studies have shown that the necessity of Cr for fetal growth, development and maintaining normal sperm counts.[7]

Mn plays an essential role in a number of physiologic processes and as a main component of some enzymes and an activator of different enzymes.[9] Mn is a potent stimulator of sperm motility through the activation of adenylate cyclase and a high serum Mn levels appeared to have harmful effects on sperm morphology and motility.[9]

Women who do not get sufficient amounts of iron may suffer anovulation (lack of ovulation) and possibly poor egg health, which can inhibit pregnancy at a rate 60% higher than those with sufficient iron stores in their blood.[10]

Hormones are chemical substance produced in the body that controls and regulates the activity of certain cells or organs. The most common cause of ovulation failure (the failure of the ovaries to make an egg) is polycystic ovary syndrome (PCOS).[11] Typically, the polycystic ovary contains many small cysts (fluid filled sacs) or follicles, with a single egg that attempts to maturate to a stage where it is ready to be released from the ovary (ovulation). Due to complex biochemical interactions in the ovaries with PCOS, the development of the follicles is stopped prematurely, resulting in a number of small follicles and no ovulation occurs.[12] Hormones are essential for every activity of life; one of them is reproductive system. Many hormones are involved in the reproductive system in both women and men, such as luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone, and estradiol. Their functions are to regulate pubertal maturation and reproductive processes.[13] Hormonal disturbances were known to affect the ovulation process.[14] Some ovulation disorders include malfunctions of the hypothalamus or pituitary gland, can lead to increase or decrease FSH and LH secretions.[15] This will result in immature eggs production and ovaries being unable to ovulate as it is supposed. Each hormone differs in its chemical structure and route of synthesis with innumerable different steps, so if one substance or link is altered in the chain of hormone synthesis, the hormone may not be produced or may gain or lose its properties. Hormonal dysfunction of the female reproductive system is one of many problems resulting from aberrant disorders of hypothalamic-pituitary-ovarian axis. These relatively common disorders often lead to infertility.[16] Proper evaluation of these disorders involves a multi-dimensional diagnostic approach, with a pivotal contribution from clinical laboratories.[17] In addition, some drugs can elevate levels of prolactin, affects the ovulation, and inhibition of hormones, deficiencies in LH, FSH and elevated prolactin level can affect ovulation.[15]

MATERIALS AND METHODS

Subjects

A total of 138 women (88 infertile and 50 fertile) with ages ranged between 21-44 years old were investigated. Infertile cases were divided into four age groups including: group 1 (21-26), group 2 (27-32), group 3 (33-38), group 4 (39-44) years.

Sample collection

The blood samples were collected from the Al-Amal Maternity Hospital, Amman, Jordan in in vitro fertilization (IVF) department, during the period from Jan. 2014-Augest 2014.

Samples were collected for determination hormones (testosterone, estradiol β-17, FSH LH) and trace elements (Zn, Cu, Mg, Mn, Fe, Cr, and Se) levels. For FSH and LH, blood samples were collected on third day of the cycle, while other hormones were collected during mid cycle 14 – 16 day. Samples were centrifuged to separate the serum at 3000 rpm for 5 minutes. Hemolysis sera were discarded and a fresh specimen was obtained. The serum was stored at -20 ˚C and assays were completed within three days.

Estimation of testosterone, estradiol, LH and FSH concentration.

Serum levels of testosterone, estradiol, LH and FSH were estimated by a competitive chemoluminescent enzyme immunoassay (IMMULITE 2000, Bio DPC, Los Angeles, CA, USA) which utilized specific antibody-coated polystyrene beads as a solid phase (Vankrieken, 2000). After the sample was incubated with alkaline phosphatase-labeled regent, the bound label was then quantified using a specific chemoluminescent substrate and light emission was detected by photomultiplier tube, and the results were calculated for each sample. A standard curve is constructed by plotting absorbance values against concentrations of standards, and the concentrations of unknown samples are determined using this standard curve.

All laboratory tests were done with permission of the ethical committee of the institute and informed written consent was taken from each patient. A standard questionnaire was prepared for the purpose of the research and used the following details concerning: the age, smoking, drugs, diseases, history of family and marriage duration.

Determination of Trace Elements

Determination of standards, samples and blank for copper, zinc, iron, chromium, selenium, magnesium and manganese, were aspirated into Atomic Absorption Spectrophotometer 6000 (Shimadzu, Japan 6000).

Serum samples of Cu, Zn, Fe Mg, Mn, and Cr were diluted (1:5) and determined by Flame Atomic Absorption Spectrophotometer, and for Se levels were determined by Flameless Atomic Absorption Spectrophotometer equipped with graphite furnace. The temperature programming for the heated graphite atomization analysis was set as described in literature (Ashino et al., 2010). The flow of purge gas and the volume of sample injected were selected according to analyze the concentration in order to obtain a response in the linear or non-linear calibration range (Table 1).

Table: 1.Instrument setting for the determination of trace elements in serum.

Trace elements	Wave length	Flow air-acetylene	Slit nm	mA	Injection volume ul.
Fe	213.0	2.0	0.5	4.0	1000
Cu	324.8	1.8	0.5	3.0	1000
Zn	213.9	2.0	0.5	4.0	1000
Cr	359.9	3.0	0.5	5.0	20
Mn	279.5	2.0	0.4	5.0	20
Mg	285.6	2.5	0.5	5.0	20
Se	196.0	furnace	0.3	12.0	20

Statistical Analysis

Statistical Analysis was performed with the SPSS 10.01 (statistical package for social sciences). Data analysis was done using analysis of variance (ANOVA). P-value ≤ 0.05 was used as the level of significance. Descriptive statistics for biochemical features of fertile and infertile women using the range, mean and standard deviation.

RESULTS

One hundred and thirty eight females including 50 (36.2%) fertile and 88 (63.8%) infertile females were enrolled in this study. The duration of infertility was 7.61±4.42 years and the mean ages of the infertile females were (30.68±6.86) years.

Infertile females were divided into two groups based on the main type of infertility. Primary (1˚) infertility, which consists of 50(56.8%) female and secondary (2˚) infertility 38 (43.2%). The most common infertility cause among our study groups was ovulation and hormonal (52.6%), followed by tubal factor (19.2%). However, 28.2% of the females showed no apparent causes of infertility (unexplained infertility) (Table 2).

Table: 2. Numbers and percentages of infertility causes among infertile women.

Causes of infertility	Number (%)	Type of infertility	Age group (years)			
			21-26	27-32	33-38	39-44
Ovulation & hormonal	41(52.6%)	1˚ (23) 2˚ (18)	15	11	9	6
Tubal Factor	15(19.2%)	1˚ (6) 2˚ (9)	2	5	8	0
Unexplained	22(28.2%)	1˚ (11) 2˚ (11)	6	5	4	7
Total	**78**	**1˚ (38) 2˚ (40)**	**23**	**21**	**21**	**13**

Steroid Hormones Estimation

The mean hormone levels among infertile groups in comparison to fertile groups was shown in Table 3. The levels of serum FSH, LH and estradiol concentrations were significantly lower in infertile groups than in the fertile, whereas serum testosterone level was significantly higher in infertile group than in fertile.

Testosterone was elevated in all infertile women (93.1%) when compared to normal upper range. There was a significant differences between infertile and fertile women. Estradiol was decreased in 46.5% of women compared to normal lower range and increased in 9% of the infertile women. LH was decreased in 57.9% of women compared to normal lower range. FSH was decreased in 70.4% of women compared to normal lower rang.

Moreover, the results of hormones revealed a variation in testosterone, estradiol β-17, LH, and FSH among females with primary and secondary infertility (Table 4). These differences did not reach significant values. The mean testosterone, LH and FSH concentrations appeared higher in primary infertility cases than in secondary infertility. However, the mean concentration of Estradiol β-17 was lower among females with primary infertility.

Several factors have been associated with an increased risk for infertility, including age or old of women. The proportion of infertile female population increases with age (Table 5).

Table: 3. Relationship between means hormonal levels, infertile, and fertile women.

Hormones	Fertile females	Infertile females
Testosterone (ng/dl)	$31.63^{b} \pm 2.32$	$124.32^{a} \pm 11.73$
Estradiol β-17 (pg/ml)	$107.74^{a} \pm 13.06$	$41.78^{b} \pm 2.83$
Luteinizing Hormone (Iu/L)	$17.40^{a} \pm 1.41$	$5.35^{b} \pm 0.64$
Follicle-Stimulation Hormone (mIu/ml)	$8.29^{a} \pm 0.84$	$4.13^{b} \pm 0.72$

(Means in rows with the same letter are not significantly different using t-test at 0.05 level).

Table: 4. Relationship between mean serum hormonal levels, infertility type, fertile females.

Hormones	Study cases	Mean ± SE
Testosterone (ng/dl)	Primary	$115.50^{a} \pm 18.58$
	Secondary	$106.13^{a} \pm 12.82$
	Fertile	$31.63^{b} \pm 2.32$
Estradiolβ-17 (pg/mL)	Primary	$40.23^{b} \pm 2.91$
	Secondary	$79.81^{b} \pm 8.45$
	Fertile	$107.74^{a} \pm 13.06$
Luteinizing hormone (IU/L)	Primary	$5.76^{b} \pm 0.80$
	Secondary	$4.95^{b} \pm 1.01$
	Fertile	$17.40^{a} \pm 1.41$
Follicle Stimulating Hormone (mIu/ml)	Primary	$4.73^{b} \pm 1.17$
	Secondary	$3.51^{b} \pm 0.87$
	Fertile	$8.29^{a} \pm 0.84$

(Means within the same column for each hormone with the same letter are not significantly different using Duncan Multiple Range test (DMRT) at 0.05 levels).

Table: 5 Variations in the means hormonal levels among different infertile age groups and fertile females.

Age groups	Hormone ± SE			
	Testosterone	Estradiol	LH	FSH
21-26	$106.1^{a} \pm 0.28$	$118.4^{a} \pm 26.64$	$6.35^{b} \pm 1.39$	$3.09^{b} \pm 1.06$
27-32	$92.02^{a} \pm 0.26$	$95.35^{a} \pm 16.03$	$5.65^{b} \pm 1.24$	$4.39^{b} \pm 1.55$
33-38	$88.78^{a} \pm 0.16$	$77.25^{b} \pm 12.95$	$5.50^{b} \pm 1.23$	$4.90^{b} \pm 1.60$
39-44	$52.46^{a} \pm 0.60$	$44.55^{b} \pm 6.75$	$2.63^{b} \pm 0.43$	$3.81^{b} \pm 0.74$
Fertile	$31.63^{b} \pm 2.31$	$107.7^{a} \pm 13.06$	$17.40^{a} \pm 1.41$	$8.29^{a} \pm 0.84$

(Means within the same + column with the same letter are not significantly different.

Table 6 illustrate the relation between the main causes of infertility and the serum level of hormones. A highest reduction in the mean serum estradiol, LH, and FSH level was observed in unexplained infertility cases as compared to other causes of infertility. However, testosterone, mean level was lower in infertility cases with tubal factors- related infertility.

Table: 6. Relationship between infertility causes and the means hormonal levels in the serum of infertile females.

Infertility Cause	Testostrone (ng/dl) Mean ± SE	Estradiol (Pg/ml) Mean ± SE	LH (Iu/l) Mean ± SE	FSH (mIu/ml) Mean ± SE
Hormone & ovulation	$138.5^{b} \pm 15.9$	$98.5^{a} \pm 14.9$	$5.92^{b} \pm 0.77$	$3.94^{b} \pm 1.82$
Tubal factor	$127.3^{b} \pm 28.2$	$68.80^{b} \pm 11.7$	$7.66^{b} \pm 2.39$	$6.05^{ab} \pm 2.36$
Unexplained	$177.3^{b} \pm 35.2$	$49.41^{c} \pm 9.21$	$4.18^{b} \pm 0.45$	$3.81^{b} \pm 1.29$
Fertile	$31.63^{a} \pm 2.32$	$107.74^{a} \pm 13.06$	$17.40^{a} \pm 1.41$	$8.29^{a} \pm 0.84$

(Means within the same column with the same letter are not significantly different using Duncan Multiple Range test (DMRT) at ≤0.05 levels).

The effect of smoking on the mean hormonal level was shown in Table 7. No significant relationship in the mean testosterone, estradiol, LH and FSH concentrations and smoking habit among infertile females.

Table: 7 Relationship between smoking habit and the means hormonal levels among infertile women.

Hormones	Infertile	
	Non-smoker	Smoker
Testostrone (ng/dl)	102.4[a] ± 0.23	107.6[a] ± 0.8
Estradiol (Pg/ml)	53.12[a] ± 1.51	68.83[a] ± 0.41
Luteinizing hormone (Iu/l)	5.57[a] ± 0.90	5.11[a]± 0.91
Follicle stimulating (mIu/ml)	3.16[a] ± 0.71	5.26[a] ± 1.33

(Means in rows with the same letter are not significantly different using Duncan Multiple Range test (DMRT) at 0.05 level).

Trace Elements Estimation

The current study provides data concerning the levels of Zn, Cu, Mg, Fe, Cr and Mn in the serum of infertile and fertile women. A highly significant decrease in the mean concentration of Zn, Cu, Mg, Fe, Cr, Se and Mn in the serum of infertile females was notice in comparison to that observed for fertile (Table 8). A non-significant reduction in the mean Se concentration among infertile female was observed. The mean serum concentration of Zn, Cu, Mg, Fe, Cr, Se, among infertile females was: 795.87ug/L, 1091.89ug/L, 8.78mg/ml, 693,97mg/L, 31.83ng/ml, 86.88ng/ml, and 11.17mg/ml, respectively as compared to 1176.4ug/L, 1713.0ug/L, 14.95mg/ml, 1223.40mg/L, 73.92ng/ml, 124.46ng/ml, and 20.27mg/ml in fertile females, respectively. However, the mean trace elements levels in infertile females with primary or secondary causes doesn't showed any significant differences (Table 9).

Table: 8. Trace elements mean concentration in infertile and fertile females.

Trace elements	Fertile female Mean± SE	Infertile female Mean± SE
Zn (ug/L)	1176.40[a] ± 39.37	795.87[b] ± 14.95
Cu (ug/L)	1713.00[a] ± 25.31	1091.89[b] ± 30.53
Mg (mg/ml)	14.95[a] ± 0.21	8.78[b] ± 0.22
Fe (mg/L)	1223.40[a] ± 41.48	693.97[b] ± 21.73
Cr (ng/ml)	73.92[a] ± 2.19	31.83[b] ± 1.40
Se (ng/ml)	124.46[a] ± 1.83	86.88[b] ± 2.15
Mn (mg/ml)	20.27[a] ± 0.49	11.17[b] ± 0.42

(Means in rows with the same letter are not significantly different using t-test at 0.05 level).

Table: 9. Relationship between infertile females (primary and secondary), and fertile and the mean concentration of trace elements in the serum.

Trace elements	Study groups	Mean ± SE
Zn (ug/L)	Primary	770.4[b]± 21.76
	Secondary	825.9[b]± 21.32
	Fertile	1176.4[a] ± 39.37
Cu (mg/L)	Primary	1147.6[b] ± 41.09
	Secondary	990.0[c] ± 33.10
	Fertile	1713.0[a] ± 25.31
Mg (mg/ml)	Primary	8.95[b] ± 0.36
	Secondary	8.51[b] ± 0.28
	Fertile	14.95[a]±0.21
Fe (mg/L)	Primary	704.15[b] ± 32.47
	Secondary	703.59[b] ± 28.58
	Fertile	1223.4[a]±41.48
Cr (ng/ml)	Primary	32.67[b] ± 2.06
	Secondary	29.12[b] ± 2.03
	Fertile	73.92[a]±2.19
Se (ng/ml)	Primary	86.28[b] ± 3.08
	Secondary	86.08[b] ± 3.56
	Fertile	124.46[a]±1.83
Mn (mg/ml)	Primary	10.74[b] ± 0.54
	Secondary	11.34[b] ± 0.67
	Fertile	20.27[a]±0.49

(Means within the same column for each hormone with the same letter are not significantly different using Duncan Multiple Range test (DMRT) at 0.05 levels).

Trace elements including Zn(749.03µg/l), Cu(1058.69mg/l), Cr(28.53ng/ml), Se(80.96ng/ml), and Mn(10.35mg/ml) showed a detectable reduction in unexplained infertility cases as compared to fertile females (1176.40µg/l, 1713mg/l, 73.92ng/ml, 124.46 ng/ml, and 20.27mg/ml, respectively) (Table 10). On the other hand, a decrease in the level of both Cu (1058.69mg/l), and Mg (8.52mg/ml) was observed in infertile females with unexplained infertility, ovulation and hormonal problems, respectively, in comparison to fertile females (1713.0 mg/L, and 14.95 mg/ml, respectively).

Table: 10. A comparison between trace elements mean concentration, fertile, and infertile females with different causes of infertility.

Study groups	Zn mean± SE	Cu mean ± SE	Mg mean± SE	Fe mean ± SE	Cr mean ±SE	Se mean ±SE	Mn mean ± SE
Ovulation & hormonal factors	796.83^{c} ±23.16	1109.5^{b} ±42.38	8.52^{b} ±0.30	706.9^{b} ±35.27	30.16^{bc} ±2.10	86.75^{bc} ±3.35	10.93^{b} ±0.62
Tubal factor	867.41^{b}± 30.66	1161.30^{b}± 93.70	9.50^{b}± 0.69	611.69^{c}± 44.74	37.63^{b} ± 2.96	93.12^{b} ± 4.79	12.35^{b}± 0.88
Unexplained infertility	749.03^{c}± 29.90	1058.69^{bc}±76.83	9.19^{b}± 0.54	696.68^{bc}±48.20	28.53^{bc} ± 3.09	80.96^{c} ±3.82	10.35^{b} ± 1.04
Fertile female	1176.40^{a}±39.37	1713.00^{a}±25.31	14.95^{a}± 0.21	1223.40^{a}±41.48	73.92^{a} ± 2.19	124.46^{a} ± 1.83	20.27^{a}± 0.49

(**Means within the same column for each hormone with the same letter are not significantly different using Duncan Multiple Range test (DMRT) at 0.05 level).**

No statistically significant effect of smoking on trace elements concentration in females with infertility (Table 11).

Table: 11. Effect of smoking on trace elements concentration in fertile and infertile females

Trace elements	Infertile female		Fertile female
	Non-smoker	Smoker	
Zn (ug/L)	802.59^{b} ± 19.73	792.90^{b} ± 23.60	1176.40^{a} ± 39.37
Cu (ug/L)	1130.29^{b} ±36.77	1061.90^{b} ± 49.72	1713.00^{a} ± 25.31
Mg (mg/ml)	8.62^{b} ± 0.30	8.96^{b} ± 0.35	14.95^{a} ±0.21
Fe (mg/L)	675.18^{b} ± 31.34	716.23^{b} ± 31.34	1223.40^{a} ± 41.48
Cr (ng/ml)	34.42^{b} ± 1.87	29.19^{b} ± 2.06	73.92^{a} ± 2.19
Se (ng/ml)	90.32^{b} ± 3.09	83.38^{b} ± 3.07	124.46^{a} ± 1.83
Mn (mg/ml)	11.45^{b} ± 0.59	11.01^{b} ± 0.62	20.27^{a}± 0.49

(Means within rows with the same letter are not significantly different using Duncan Multiple Range test (DMRT) at 0.05 level.

DISCUSSION

Infertility is a disease of the reproductive system that affects millions of people globally. Reproductive failure is a major medical issue adversely affecting human health in the 21st century. The results of the present study showed that a high proportion of women aged 21-44 years took part in a program of assisted reproduction, possibly because this is the reproductive age period among women.

It is widely accepted that during the last twenty years, the average age of having children has increased and this is a key factor for infertility. As the age of giving birth is increased, the reproductive capacity is decreased, the ovary becomes less efficient, the frequency of sexual intercourse is decreased and the possibility of chromosomal abnormalities and miscarriage is increased.[19]

Our results showed that 47.6% of the infertility causes were due to hormonal factors in association with the ovulation problems, which often occur as a result of hormonal imbalance or metabolic diseases. It is known that the function of the thyroid gland is directly affected by the relationship of hypothalamic pituitary gland-ovarian hormones. The increased function of the thyroid is likely to cause disorders in menstrual cycle, and an increase or decrease in women's sexual activity, while the decreased function of the gland causes a decrease in sexual activity, and/or increased flow during menstruation, bleeding of the uterus, and more rarely secondary amenorrhea. Also, neoplasms may be the cause of thyroid dysfunction, and disorders of the pituitary gland may cause decreased function of the thyroid.[19,20,21,22]

The most common hormonal problems are pituitary tumors or problems with the development of the pituitary gland leading to a lack of FSH and LH.[23,24] The second common cause of infertility in this study was present in 18.1% of the infertile women, and related to the fallopian tubes which includes any condition affecting the normal

function and anatomy of the fallopian tubes that prevents the meeting of sperm with the ovum and the consequent conception.[19] In our study, it was observed that 25% of the infertile females did not have any apparent cause of infertility. The failure to identify a clear cause of the infertility after a full screening of both partners is defined as unexplained infertility.[21,22]

Other causes that are likely to cause infertility, but not explored in this study are: diseases affecting the function of the ovaries, problems with the uterus or cervix, dietary problems such as excessive increase or decrease in body weight, exposure to radiation, chemical agents, cytotoxic drugs and psychological causes.

Our data reveal a significant increase in the serum level of testosterone in infertile females as compared to fertile. However, 93% of the infertile females showed an elevation in testosterone level. This results is consistent with earlier findings by others[25,26], which stated that there is a connection between hirsutism, PCOS, infertility and high level of testosterone.

Infertility is defined as primary or secondary depending on whether there is previous history of pregnancy or not. Both primary and secondary types of infertility generally share common causes. In this study, it was found that testosterone level increased in primary and secondary infertile when compared to fertile group. Similar results were obtained by Gilling Smith et al., 1997. In addition, testosterone level was increased in relation to infertility causes (ovulation and hormonal, tubal factor, and unexplained infertility) when compared with fertile group. It was reported that the excess effect of testosterone on the body depends on both age and sex.

In females, the majority of testosterone is produced by peripheral conversion of androgen precursor steroids to testosterone, with the remainder produced in the ovaries and adrenal glands. Circulating levels of testosterone fluctuate with the menstrual cycle, and increase during pregnancy. Serum levels of testosterone remain relatively stable during and after menopause.[28] However, polycystic ovary syndrome is the most common cause of hyperandrogenism (increased testosterone levels) in females.[29] This was exactly the results for the detectable increases in the level of testosterone among our infertility cases since most of the infertile females with ovulatory and hormonal disorders have PCOS.

Hormonal imbalance plays an important role in infertility among women. It was observed that there is a significant decrease in the mean serum levels of LH and FSH in the infertile females compared to fertile. Mohan et al. (2010) reported that both LH and FSH are required for follicle development and estrogen production. However, an elevation in prolactin hormone will leads to a decrease in the level of FSH and LH and will cause infertility. Similar result was obtained by Catalano et al. (2002), who mentioned that the increase in infertility rate among females was directly related to increase in hormonal imbalances of FSH and LH, and have an impact on ovulation and menstruation.

Furthermore, another finding was reported by Sasikumar et al. (2014) in which the level of LH and FSH was increased in infertile group when compared with fertile, and Shrim et al. (2003) suggested that the increase in FSH/LH ratio is associated with an inferior outcome in IVF treatment cycles and may be used as an additional predictor for decreased ovarian response. Our results concerning the significant decrease in serum concentrations of FSH, and LH in groups of infertile females with increased levels of testosterone may be due to the negative feedback effect of testosterone on the hypothalamus which in turn causes decrease in the secretion of FSH and LH by the anterior pituitary gland. Gonadotropins production is under the feedback control of sex hormones.[33]

The levels of estradiol, LH and FSH, is significantly decreased in relation with infertility causes (ovulation & hormonal imbalance, tubal factor, and unexplained infertility) when compared with the fertile group. The ovaries convert androgen to estradiol by the granule cells.[34] Estradiol level is usually elevated in mid cycle (cycle day 14-16), which is an indicator of ovulation. For studying estradiol level, the blood samples were collected from women in cycle day 14-16. It was found that estradiol was significantly increased in the control group compared to infertile group. Around 50% of infertile females in this study had a decreased levels of estradiol. This low level of estradiol affects cycle regulation and decrease quality of ovum.[15] In addition, estradiol value was much more less in primary infertility compared to secondary infertility, and both groups have a decreased estradiol level when compared to control group.

Besides the health problems related to the female anatomy, another major factor that causes infertility is smoking.[21] The harmful effects of smoking on health, during the reproductive age of men and women, started to be investigated during the last 20 years. Several studies have highlighted the harmful effects of smoking on fertility in women of reproductive age, as well as the significant reduction in the possibility of a successful outcome after assisted reproduction attempt. In this study, smoking behavior was evaluated for their relationship to serum hormones concentrations in infertile females. No relationship was found between smoking and abnormal hormonal levels. However, Sowers et al., (2000) reported that smokers had the highest testosterone concentrations with decreasing values in nonsmokers ($p = 0.0001$). He speculated that the greater concentrations of testosterone observed among smokers were more likely a product of decreased metabolism rather than increased biosynthesis of the hormone.

The human body requires certain minerals for the overall health and proper functioning of the organ systems. The essential trace elements (Zn, Cu, Fe, Cr, Se, Mn and Mg) were very important for human life, which their imbalance in blood leads to very dangerous diseases. Abnormalities associated with trace elements can be due to specific efficiency from dietary inadequacies and imbalances, or abnormality secondary to other diseases. The data obtained in this study for serum levels of trace elements showed a statistically significant reduction in all trace elements that have been studied including Zn, Cu, Fe, Cr, Se, Mn and Mg in infertile women as compared to fertile.

Studies have reported a conflicting findings as to whether differences exist in serum Zn concentrations between infertile and fertile women.[36,37] However, lower follicular fluid and serum Zn and selenium levels were found in infertile females as compared to fertile.[37] In addition, Zn deficiency can interfere with fertility by disrupting menstrual cycle, due to impairment in the synthesis and secretion of two important hormones FSH and LH, and oral zinc supplementation has been found to correct such impairment [38, 39]. This might explain the significant decreases in FSH and LH among infertile females enrolled in the study.

Another trace element that have been estimated is this study is the copper that has a role in supporting healthy female fertility. Research has revealed a correlation between low copper and magnesium levels with reduced fertility [40]. Low levels of Cu can reduce female fertility by disrupting normal metabolic processes. The low levels of Zn and Cu that were detected in most infertile women are in agreement with the results obtained by others.[39] The imbalance in serum Cu could be the major cause of unexplained infertility.[40]

In the current study, Fe deficiency was detected in infertile females. Iron plays an important role in the infertility mainly those related to ovulatory problems. Jorge et al. (2006) was find that the intakes of iron was inversely related to the risk of ovulatory infertility. Furthermore, Westphal et al., (2004) suggested that the daily iron intake may have an important role in normal ovulation and fertility. Another important trace element was Cr which also detected in a low levels among infertile females. Our results showed a detectable reduction in the mean level of Se among infertile females. However, the deficiency of Se in women might results in ovarian dysfunction and leads to PCOS by interference with thyroid hormone or through increase oxidative stress.[44]

Selenium is an important essential trace element for human biology and health. Increasing evidence suggests that Se plays an important role in normal growth and reproduction in animals and humans. Numerous reports implicate selenium deficiency in several reproductive and obstetric complications including male and female infertility, miscarriage, preeclampsia, fetal growth restriction, preterm labor, gestational diabetes, and obstetric cholestasis [45]. Magnesium and manganese levels were also appeared low levels in infertile women tested in this study. Several enzymes needed to protect a woman's reproductive organs (such as superoxide dismutase) are dependent on the trace elements Zn, Cu, Mg and Mn [46].

In general all trace element under the effect of a smoking show slight increase or decrease in concentration compared to nonsmokers. The scientific literature is filled with evidence on the harmful health effects of carbon monoxide, nicotine, tar, irritants and other noxious gases emitted in tobacco smoke. Attention has been paid to the presence of heavy metals and other toxic in tobacco smoke and their possible effects on biochemical processes in the human body [47].

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